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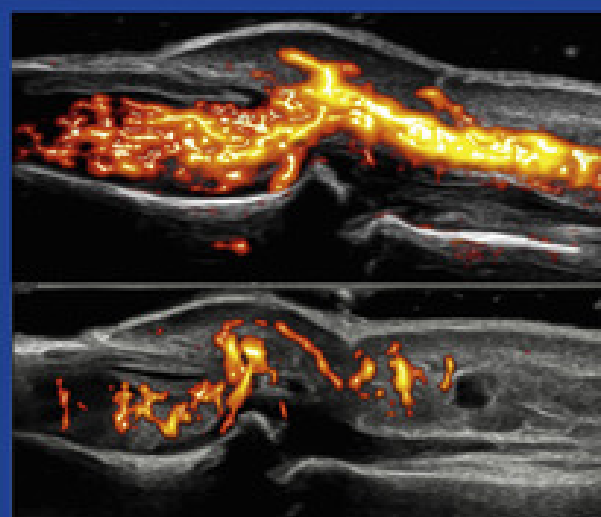
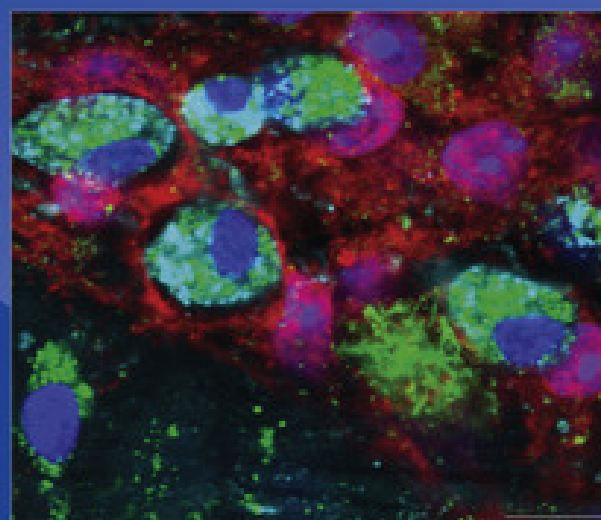
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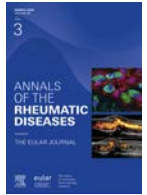
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Recommendations

Development and validation of a EULAR disease activity score in antiphospholipid syndrome

Maria G. Tektonidou^{1,*}, Ricard Cervera², Angela Tincani³,
Guillermo J. Pons-Estel⁴, Savino Sciascia⁵, Jaume Alijotas-Reig⁶, Tadej Avcin⁷,
Maria Laura Bertolaccini⁸, Tiina Jasinski⁹, Francesca Marchiori¹⁰,
Arsene Mekinian¹¹, Pier-Luigi Meroni¹², Vittorio Pengo¹³, Massimo Radin⁵,
Anisur Rahman¹⁴, Guillermo Ruiz-Irastorza¹⁵, Karen Schreiber¹⁶,
Stefka Stoilova¹⁷, Lucas van den Hoogen¹⁸, Ronald van Vollenhoven¹⁹,
Denis Wahl^{20,21}, Tanja Stamm^{22,23}, Robert B.M. Landewé^{24,25},
Michael M. Ward²⁶, EULAR APS Disease Activity Score Collaborators

¹ Rheumatology Unit, First Department of Propaedeutic Internal Medicine, Joint Academic Rheumatology Program, Medical School, National and Kapodistrian University of Athens, Athens, Greece

² Department of Autoimmune Diseases, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Catalonia, Spain

³ Rheumatology and Clinical Immunology Unit-ERN ReCONNET, ASST-Spedali Civili and University of Brescia, Brescia, Italy

⁴ Department of Rheumatology, Grupo Oroño-Centro Regional de Enfermedades Autoinmunes y Reumáticas (GO-CREAR), Rosario, Argentina

⁵ University Center of Excellence on Nephrologic, Rheumatologic and Rare Diseases (ERK-Net, ERN-Reconnet and RITA-ERN Member), University of Turin, Turin, Italy

⁶ Systemic Autoimmune Diseases Unit and Systemic Autoimmune Diseases Research Unit, Vall d'Hebron University Hospital, and Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

⁷ Department of Allergology, Rheumatology and Clinical Immunology, Children's Hospital, University Medical Center Ljubljana, University of Ljubljana, Ljubljana, Slovenia

⁸ Academic Department of Vascular Surgery, School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, United Kingdom

⁹ Estonian Rheumatism Association, Tallinn, Estonia, EULAR PARE, Zurich, Switzerland

¹⁰ EULAR PARE, Zurich, Switzerland

¹¹ Internal Medicine Department, Sorbonne University, Saint Antoine Hospital, CEREMAIIA Reference Center, Paris, France

¹² Immunorheumatology Research Laboratory – IRCCS Istituto Auxologico Italiano, Milan, Italy

¹³ Thrombosis Research Laboratory, University of Padova, Padova, Italy

¹⁴ Division of Medicine, University College London, London, United Kingdom

¹⁵ Biobizkaia Health Research Institute, UPV/EHU, Bizkaia, The Basque Country, Spain

¹⁶ Danish Centre for Expertise in Rheumatology (CeViG), Danish Hospital for Rheumatic Diseases, Sønderborg, Denmark; Institute for Regional Health Research, Odense University, Odense, Denmark; Thrombosis and Haemostasis, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom

¹⁷ Department of Health Care Management, Faculty of Public Health, Medical University of Plovdiv, Plovdiv, Bulgaria

¹⁸ Department of Rheumatology, Radboudumc and Sint Maartenskliniek, Nijmegen, Netherlands

¹⁹ Department of Rheumatology and Clinical Immunology, Amsterdam University Medical Center, Amsterdam Rheumatology Center, Amsterdam, Netherlands

*Correspondence to Prof Maria G. Tektonidou, Rheumatology Unit, First Department of Propaedeutic Internal Medicine, Joint Academic Rheumatology Program, Medical School, National and Kapodistrian University of Athens, Laiko Hospital, Goudi, Athens, Greece.

E-mail addresses: mttektonidou@gmail.com, mttektonidou@med.uoa.gr (M.G. Tektonidou).

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²⁰ Vascular Medicine Department and Reference Center for Rare Systemic Autoimmune and Autoinflammatory Diseases, Nancy University Hospital, Nancy, France

²¹ INSERM DCAC UMR-S 1116, Université de Lorraine, Nancy, France

²² Institute for Outcomes Research, Center for Medical Data Science, Medical University of Vienna, Vienna, Austria

²³ Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Vienna, Austria

²⁴ Department of Rheumatology and Clinical Immunology, Amsterdam University Medical Center, Amsterdam Rheumatology Center (ARC), Amsterdam, Netherlands

²⁵ Zuyderland Medical Center, Heerlen, Netherlands

²⁶ Verier Outcomes Research, Rockville, Maryland, USA

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ABSTRACT

Objectives: To develop and validate a European Alliance of Associations for Rheumatology (EULAR) disease activity score in antiphospholipid syndrome (EAPSDAS).

Methods: Twenty-four Task Force members and an international group of 53 antiphospholipid syndrome (APS) experts/collaborators, 65 patients with primary APS, and 21 healthcare professionals participated. EAPSDAS development proceeded in 4 phases: (i) item generation using a systematic literature review and 2 surveys; (ii) item reduction by rating items on their importance to be included in EAPSDAS and using Delphi methodology; (iii) item scoring based on real-world clinical vignettes and using as criterion standard the physician global assessment (PhysGA); and (iv) validation.

Results: One hundred seventy items representing APS activity were generated, and 140 deduplicated candidate items were rated by participants. Using a $\geq 75\%$ vote threshold and Delphi consensus among Task Force members, 24 items were included in the EAPSDAS thrombotic/microvascular/nonthrombotic (TMN) scale and 6 in the obstetric scale. Item scoring was based on ratings of 3 versions of 60 vignettes with new/worsening manifestations (30 single TMN or obstetric manifestations, 26 combinations of 2 TMN manifestations, 2 inactive cases, 2 testing cases) by physicians. Item scores were calculated as the adjusted mean PhysGA in linear regression analysis. A 1-month time frame was defined for the TMN scale and the entire pregnancy for the obstetric scale. Scores for stable or improved TMN manifestations were also included. High face and content validity, construct validity (internal/external standard), and reliability were demonstrated using real-world clinical vignettes.

Conclusions: Using data-driven and consensus methodology, EAPSDAS was developed and initial validation was performed. Further validation in prospective studies is warranted.

INTRODUCTION

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterised by the presence of antiphospholipid antibodies (aPL) and a broad spectrum of obstetric and thrombotic, microvascular, and non-thrombotic (TMN) clinical features, ranging from mild (eg, livedo reticularis) to major organ (eg, stroke, kidney failure) manifestations, and from single sites to multiple sites of involvement [1–5]. The clinical criteria of APS have been categorised into 5 main domains in the 2023 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria: macrovascular (venous and arterial thrombotic events), microvascular, obstetric, cardiac valve, and haematological [6]. Although not completely understood, the clinical heterogeneity of APS may be driven by a complex interplay between thrombotic and inflammatory mechanisms [7–12].

Newly occurring APS manifestations may be followed by long periods of inactivity, but some manifestations can be refractory or frequently recurring despite the standard anticoagulation and/or antiplatelet treatment [13–16]. Treatments that could decrease the development of new thromboses or pregnancy complications and treatments for microvascular and nonthrombotic manifestations are urgently needed [17–20]. However, testing of new treatments in APS has been hindered by a paucity of suitable outcome measures.

A disease activity score for APS that assesses multiple clinical manifestations would be useful in evaluating response to treatment in clinical trials and monitoring disease activity status in observational studies and clinical practice [21–23]. Disease activity refers to new-onset events (eg, new venous thromboembolism [VTE] rather than postphlebotic syndrome) or reversibility (eg, improvement of thrombocytopenia after treatment), as distinct from damage, which refers to irreversible manifestations of the disease or consequences of its treatment, including permanent organ or tissue injury [24,25]. In contrast, disease severity reflects aspects of both current activity and accumulated damage as well as their impact on an individual's health, functional ability, quality of life, and prognosis.

Our objective was to develop and validate a EULAR disease activity score for APS (EAPSDAS), with the contribution of an international group of APS experts, patients, and healthcare professionals.

METHODS

According to the EULAR standardised operating procedure, a Task Force was assembled after the submission of the project proposal by the convenor (MGT) and its approval by the EULAR Quality of Care Committee (QoC) and the EULAR Council. The Task Force consisted of 24 members from 13 countries: a 5-member Steering Committee (3 adult rheumatologists/APS experts: convenor [MGT], RC, and AT, and 2 fellows [GJP-E,

SS]), 3 methodologists (RBML, TS, MMW), 5 adult rheumatologists, 1 paediatric rheumatologist, 2 vascular medicine physicians, 2 physicians leading large obstetric APS cohorts, 1 immunologist, 2 members of the EMEUNET (Emerging EULAR-NETwork), 1 nonphysician health professional in rheumatology (HPR), and 2 patient research partners from People with Arthritis/Rheumatism (PARE). Five members were included through an open call to EULAR countries via a competitive application process. Task Force members were selected according to their experience in the field, participation in prior task forces, and balance in the geographical distribution. In addition, a multidisciplinary group of 53 experts in APS from 5 continents (Europe, North America, South America, Asia, and Australia/Oceania), designated as ‘Collaborators’ (listed in the Collaborators section), agreed to participate in the project phases. Ninety patients with primary APS and 21 HPRs agreed to participate anonymously in the phase I and II surveys.

Our approach to developing the activity measure was guided by several overarching considerations. First, we defined 2 broad main domains, the TMN domain and the obstetric domain, that would have separate stand-alone scores. This was done because nonpregnant women and men would not be eligible to register scores for obstetric manifestations, and combining scores from obstetric and TMN domains would thereby bias scores against anyone who was not pregnant. Second, because APS manifestations are often episodic events (eg, venous thrombosis, stroke) while only some are more continuous (eg, thrombocytopenia, proteinuria), we planned to create a scale rather than an index. A scale measures intensity based on a hierarchy of items or variables, whereas an index includes ratings of multiple items and adds them together in a single score [26]. For example, the ACR Functional Class [27] and the British Isles Lupus Activity Group-2004 [28] are scales, while the Disease Activity Score 28 [29] and Systemic Lupus Erythematosus (SLE) Disease Activity Index (SLEDAI) [30] are indices. Third, we focused primarily on rating new or worsening manifestations, as these provide the clearest evidence of activity, and secondarily addressed stable or improved manifestations. Fourth, in alignment with other outcome measures, we did not include treatment intensity as an indicator of activity, as doing so could create circularities of reasoning in treatment studies.

Development of the measure proceeded in 4 phases using consensus and data-driven methods: phase I: item generation; phase II: item reduction; phase III: item scoring; and phase IV: testing and validation (Box 1). This project was focused solely on patients with primary APS. A more detailed presentation of the methodology is provided in an accompanying article [31].

Phase I: item generation

In phase I, we aimed to collect an inventory of all items potentially related to disease activity by using (i) a systematic literature review (SLR) to identify features indicating disease activity and their prevalence, and (ii) 2 surveys among experts in APS and patients with primary APS. The first survey asked respondents to describe, in an open-ended format, the ‘concept’ of disease activity in primary APS based on their experience, and the second asked to list all clinical, laboratory, radiological, and histological features that indicate disease activity in primary APS. The SLR and the second survey provided a list of candidate items.

Box 1 Overall methodology for the development and validation of the European Alliance of Associations for Rheumatology (EULAR) antiphospholipid syndrome disease activity score

Phase I: item generation

- Systematic literature review to identify potential features indicating disease activity.
- Survey with open-ended questions to define the concept of disease activity in antiphospholipid syndrome (APS).
- Survey with open-ended questions to describe all clinical, laboratory, radiological, and histological features that indicate disease activity in APS.

Phase II: item reduction

- Elimination of highly related items among those retrieved in phase I.
- Survey to rate items for importance to be included in a disease activity score for APS.
- Delphi method consensus about the items to be included in the EULAR APS disease activity score.

Phase III: clinical vignettes—item scoring

- Development of clinical vignettes based on real-world patient scenarios.
- Survey to rate the degree of the patient’s current disease activity in each clinical vignette, using a 0 to 10 rating scale.
- Linear regression analysis to calculate each item’s scoring.
- Delphi methodology about the time interval for thrombotic manifestations, and the scoring of improved and stable manifestations.

Phase IV: validation

- Face and content validity
- Construct validity: external validity
- Construct validity: internal validity
- Reliability

Phase II: item reduction

In phase II, we reduced the number of items in the list of candidate items as follows: (i) the Steering Committee eliminated similar or closely related items among those reported in the second survey and those retrieved by the SLR, (ii) we created a new (third) survey asking participants (Task Force members/Collaborators, patients, and HPRs) to rate items of the deduplicated list on how important each was for inclusion in a disease activity score for APS, using a 0 to 10 rating scale (0 = not important at all; 10 = extremely important), and (iii) we followed a Delphi method to reach consensus in a 2-day video-conference meeting of the Task Force at which the results of the third survey were discussed and items were voted for inclusion. More than 75% of the Task Force members participated in both days.

The initial threshold for inclusion of an item was an endorsement by >50% of members. This threshold was subsequently changed to ≥75%, by approval of the Task Force members, to reduce the number of items included.

Phase III: item scoring

To develop scores for each item voted by the Task Force, we used clinical vignettes that described real-world patient histories in which a new or worsening disease manifestation occurred over a period of 1 month, a time frame decided after consensus with the Steering Committee and the Task Force group. Vignettes were based on real patient cases from the files of the Steering Committee members. Vignettes included patient demographics, APS history, and other medical history including comorbidities and related medications, current presentation including new/worsening manifestations, and laboratory, imaging, and histologic data. For each item, 3 different patient cases were abstracted for the same manifestation, and 3 vignette versions (A, B, C) were developed. Using 3 versions of clinical cases, patients with the same manifestation but different demographic characteristics and comorbidities were represented, to decrease the likelihood that item scores would be influenced by a given patient's background.

Since 2 different new/worsening manifestations may rarely occur concurrently (within a 1-month period) in APS (eg, arterial thrombosis and thrombocytopenia), a list of potential combinations of 2 items was also developed from those endorsed by the Task Force. We did not test vignettes with 3 or more different new manifestations occurring concurrently, because these were deemed clinically very unusual. Steering Committee members independently reviewed the list of candidate combinations of 2 items and suggested those with moderate-high probability to occur simultaneously; 26 combinations of manifestations that were most likely to co-occur in real life were selected to be included as 'double manifestation' scenarios. Real-world cases with these specific double manifestations were collected from the case files, as described above.

Physicians from the Task Force and the Collaborators were randomly assigned to rate 1 of the 3 vignette versions (A, B, C). Although the 3 sets of clinical vignettes included different single-item cases, the same 26 double-manifestation cases were included in all 3 versions due to their rarity. The criterion standard was the physician global assessment (PhysGA) of the patient's current disease activity using a 0 to 10 rating scale. Participants were asked to rate the level of activity, not severity.

Each item's score was calculated as the adjusted mean PhysGA, based on linear regression analysis that adjusted for physician characteristics: age (35-44/45-54/55-64/65+), gender, geographic region (Europe/North America/Rest of world), rheumatologist vs other specialty, years in practice (0-9/10-19/20+), average number of patients seen monthly (0-4/5-9/10-19/20+), and vignette version (A, B, C). We also examined the concordance of pairwise rankings of all items and the proportion of raters who ranked the items similarly.

We compared the scores of the vignettes with double new or worsening manifestations with the scores of each corresponding single manifestation to determine if summing the item scores was indicated. We also used paired t-tests and/or rank tests to determine if ratings for double manifestations were the approximate sum of the single manifestations, or if these ratings were dominated by the more highly rated single manifestation. Lastly, we tested if the lower-ranked item of the pair was significantly associated with the PhysGA after adjustment for the higher-ranked item in regression analyses. Significant items were retained, and scores for these items received a 'bump-up' when they co-occurred.

Following discussions during the second Task Force meeting on the 20 February 2024, we conducted an additional survey

among the physicians from the Task Force and Collaborators to define the time interval between 2 thrombotic events to be considered as 'new' APS activity, and whether manifestations that were abnormal but stable, or manifestations that were improved but still present, should be scored (Box 1).

Phase IV: testing and validation

Face and content validity

To evaluate the measure's face and content validity, we asked physician experts in APS or SLE and patients with primary APS, not previously involved in the project, to rate each of the 2 EAPSDAS scales (TMN and obstetric) as a whole for relevance and comprehensiveness. Relevance referred to whether the scales' items reflect disease activity in APS, and comprehensiveness referred to whether the scales included diverse manifestations and did not omit critical items.

Construct validity-external standard

We tested the construct validity of the item scores using real-world clinical vignettes, with the PhysGA (0-10 rating scale) as the external standard. Physicians (Task Force members and Collaborators) scored a different vignette version than the one they scored in Phase III. We computed the Spearman correlation between the PhysGA and the items' scores for each physician.

Construct validity-internal standard

This exercise directly compared item scores against each other with regard to the level of disease activity represented by each item. Physicians from the Task Force and Collaborators' group, blinded to item scores, were asked to rank the vignettes from most active to least active, with ties allowed. The item rankings were then correlated with the item scores. This differed from the prior exercise, where each item was rated against an external standard (the PhysGA).

Reliability

Inter-rater reliability of the scale was tested using clinical vignettes. Thirty physicians (half were not previously involved in the project) were asked to apply the EAPSDAS scales to each of 20 clinical vignettes on 16 TMN manifestations and 4 obstetric manifestations. Reliability was measured using intraclass correlations, based on a 2-factor random effects model for agreement.

RESULTS

Phase I: item generation

First survey: concept of disease activity in APS

Responses from 74 Task Force members and Collaborators to the open-ended question about the concept of disease activity in APS were consistent in that the concept was mainly defined by clinical manifestations (71 of 74 responses). The qualifiers of the disease activity concept were new/acute events (30/74), recurrence or worsening (22/74), modifiable with treatment (7/74), and relapse or risk of relapse (4/74). Among the 90 patients who responded to the survey, 25 had incomplete data. Six patients reported that they were unaware or could not explain the meaning of disease activity in APS, and 59 described clinical manifestations/symptoms. Nineteen patients reported on qualifiers of the disease activity concept, including new/acute events (n = 5), worsening events (n = 8), recurrent events (n = 1), and modifiable with treatment features (n = 7).

SLR results and second survey

The SLR yielded 77 items potentially related to disease activity in APS. A detailed presentation of the results of the SLRs is presented in the methodology manuscript published separately [31]. Task Force members/Collaborators (n = 73) nominated 132 items, and patients (n = 59) nominated 74 items.

Phase II: item reduction

Combining items from the SLR and the second survey, 170 items were generated. Similar or highly related items were eliminated to avoid redundancy, which reduced the number of items to 140. In the third survey, participants’ (74 Task Force members and Collaborators, 53 patients and 21 HPRs) ratings of the importance of the 140 items to be included in a disease activity score were calculated separately for each of the 3 groups of participants (physicians, HPRs, and patients). The items rated most highly by all participant groups were catastrophic APS (thrombosis in 3 or more territories in 1 week), pulmonary embolism, arterial thrombosis, deep vein thrombosis, and ischaemic stroke. At the first Task Force meeting, 52 items were voted upon by 50% of participants (Table 1) and 24 items ultimately met the ≥75% vote threshold and were carried forward (Table 2). The Steering Committee subsequently modified some items to increase their specificity: venous thrombosis was divided into provoked and nonprovoked thrombosis, both venous and arterial thrombosis were classified as thrombosis in 1 or 2 or 3 territories, thrombocytopenia was divided into moderate and severe, and APS nephropathy was replaced by APS nephropathy-related acute kidney injury, nephrotic range proteinuria, and non-nephrotic proteinuria [32]. With these changes, 30 single items were included in the list of APS disease activity items.

Phase III: item scoring based on clinical vignettes

In total, 180 real-world patient scenarios (3 versions of 60 vignettes) were sent to 67 physicians, split into 3 groups (each received 1 of 3 vignette versions). Each rater was asked to rate 30 clinical vignettes with 1 new or worsening APS manifestation (24 TMN and 6 obstetric manifestations), 26 vignettes with 2 new APS manifestations, along with 1 vignette with no new thrombotic manifestations, and 1 vignette with a normal pregnancy. Two testing vignettes to orient raters to the task were also included. Two physicians did not score more than 10% of the vignettes, and their responses were omitted. We asked respondents to rate whether the patient described in each vignette was typical of one that they might see in their practice (very typical, somewhat typical, not typical). Seventy-five per cent of TMN manifestations and 91% of obstetric manifestations were rated as very typical or somewhat typical. High consistency of physician ratings of PhysGA was observed (intraclass correlation [95% CI] 0.98 [0.97, 0.99] and 0.99 [0.98, 1.00] for the TMN and obstetric manifestations, respectively). We first compared scores of the vignettes with 2 new manifestations occurring in a 1-month period to the corresponding single-item vignettes. Ratings of these vignettes were not the sum of each single-item rating, but rather were dominated by the more highly rated manifestation in each case. This was universally true for combinations involving either arterial events or venous thrombotic events, which were the most highly rated manifestations, and were also present in many of the other vignettes. Scores of 6 vignettes describing 2 new manifestations concurrently (defined as within 1 month) were significantly

Table 1
Items voted by >50% of Task Force members (ordered by the physician’s mean importance in the third survey)

New or worsening manifestations
Catastrophic APS
Pulmonary embolism
Arterial thrombosis
Deep vein thrombosis
Ischaemic stroke
Splanchnic venous thrombosis
Splanchnic arterial thrombosis
Microangiopathy, microvascular thrombosis
Cerebral venous sinus thrombosis
Radiologic signs of thrombosis
Severe pre-eclampsia/eclampsia
Histologic signs of microthrombi or thrombotic microangiopathy (eg, renal, skin)
Venous thrombosis
APS nephropathy
Myocardial infarction
Thrombocytopenia
Triple antiphospholipid antibody positivity
Intracardiac thrombi
Ophthalmic arterial or venous thrombosis
Foetal death unexplained
Extensive villous ischaemia, villitis, and utero-placental vasculitis, multifocal utero-placental thromboses
Haemolytic anaemia
Persistently high/increase in anti-β2GPI antibody titres
Brain ischaemic lesions on magnetic resonance imaging
Cardiac valve vegetations
Digital ischaemia/gangrene
Skin ulcers
Transient ischaemic attack
HELLP syndrome
Cardiac microvascular disease on magnetic resonance
Intrauterine growth restriction (IUGR)
New recurrent first-trimester miscarriage
Diffuse alveolar haemorrhage
Adrenal haemorrhage
Transverse myelitis
Livedoid vasculopathy
Livedo racemosa
Livedo reticularis
Amaurosis fugax
Noninfectious endocarditis (Libman-Sacks)
Angina
Pulmonary hypertension
Epilepsy/new seizures
Proteinuria
Cognitive/memory impairment
APS nephropathy-related acute kidney injury
Premature birth due to placental insufficiency
Superficial venous thrombosis
Avascular necrosis/osteonecrosis
Cardiac valve thickening
Longitudinal myelitis
Migraine

APS, antiphospholipid syndrome; HELLP, haemolysis, elevated liver enzyme levels, and low platelet levels.

higher than the single manifestation alone, indicating a ‘bump up’ in these scores was justified (Table 3). Among the TMN manifestations, we used paired t-tests to determine if item scores were significantly different between VTE unprovoked or provoked; proteinuria nephrotic or non-nephrotic; and thrombocytopenia moderate or severe, to determine whether these distinctions should be retained or merged. Each pair had significantly different mean ratings, so these were retained as separate items. Item scores based on adjusted means are provided in Table 3.

Table 2
Items voted by ≥75% of Task Force members (ordered by the physician's mean importance in the third survey)

New or worsening manifestations
Catastrophic APS
Venous thromboembolism ^a
Arterial thrombosis ^b
Severe pre-eclampsia/eclampsia
APS nephropathy
Thrombocytopenia
Foetal death unexplained
Haemolytic anaemia
Cardiac valve vegetations including noninfectious endocarditis
Digital ischaemia/gangrene
Skin ulcers
HELLP syndrome
Itrauterine growth restriction (IUGR)
New recurrent first-trimester miscarriage
Diffuse alveolar haemorrhage
Adrenal haemorrhage
Transverse myelitis
Livedoid vasculopathy
Livedo reticularis/livedo racemosa
Pulmonary hypertension
Epilepsy, new seizures
Premature birth due to placental insufficiency
Avascular necrosis/osteonecrosis
Cardiac valve thickening

APS, antiphospholipid syndrome; HELLP, haemolysis, elevated liver enzyme levels, and low platelet levels.

^a Venous thromboembolism (confirmed by imaging) includes pulmonary embolism, deep vein thrombosis, thrombosis/occlusion in splanchnic, renal, cerebral, retinal, and upper extremity veins deep vein thrombosis, pulmonary embolism, and thrombosis/occlusion in splanchnic, renal, cerebral, retinal, and upper extremity veins.

^b Arterial thrombosis (confirmed by imaging) includes thromboses in coronary arteries, cerebral arteries, or peripheral, splanchnic, retinal arteries, and organ infarcts (eg, kidney, liver, or spleen).

Pairwise ranking comparisons

We compared the ratings between pairs of manifestations to examine whether the item scores were consistent with the predominant opinion among physicians. This analysis supported the mean vignette rankings. Of 276 comparisons between all possible pairs of items, 259 comparisons of individual physician rankings were consistent with the ranking of PhysGA means (eg, the item score for unprovoked VTE was higher than the item score for non-nephrotic proteinuria, and 74% of physicians rated unprovoked VTE as higher than non-nephrotic proteinuria, while 18% rated these items the same, and 8% rated proteinuria higher). Seventeen comparisons (6.1%) were inconsistent (eg, mean vignette rating was higher in A vs B, while a higher proportion of physicians rated B higher than A). In 14 of these 17 cases, the inconsistency was the result of rounding of item scores to integers (0 to 10), which made some manifestations ‘equal’ when the mean rating and the pairwise ranking were consistent. Therefore, we omitted rounding and used a scale of 0 to 100. In 3 cases (digital ischaemia/transverse myelitis; avascular necrosis/VTE provoked; avascular necrosis/severe thrombocytopenia), there were slight true discrepancies between the mean ratings and the pairwise comparison.

Time interval for scoring of thrombotic events

Sixty-six physicians (Task Force members and international Collaborators) completed the survey about the time interval between 2 thrombotic events for scoring the second

event as ‘new’ APS activity and the scoring of stable or improved manifestations. Concerning the time interval for scoring an arterial or venous thrombosis as ‘new’, 50% voted for 1 month and 50% voted for greater than 1 month. Voting among the Task Force members was then conducted to decide between 1 month and 3 months; 14 of 18 physicians voted in favour of the 1-month interval for arterial thrombosis and 13 of 18 in favour of the 1-month interval for venous thrombosis.

Scoring of stable or improved manifestations

The majority of physicians who participated in the survey (67%-75%) favoured scoring either stable or improved severe thrombocytopenia and stable or improved haemolytic anaemia. If unchanged, both severe thrombocytopenia and haemolytic anaemia were voted to be scored with the original score. If improved, haemolytic anaemia will be scored with 50% of the original score. If severe thrombocytopenia improves to moderate thrombocytopenia, the score for moderate thrombocytopenia would be used. Moderate thrombocytopenia would be considered ‘improved’ only if normalised, in which case the item will not be scored. If unchanged, moderate thrombocytopenia would be scored with the original score. For skin ulcers and proteinuria, more than 60% voted in favour of scoring when either stable or improved. If unchanged, these items will be scored with the original score (100%). Skin ulcers will be scored with 50% of the original score if improved as judged by the physician. Nephrotic-range proteinuria will be considered improved only if it decreases to the non-nephrotic range, while non-nephrotic proteinuria will be considered improved if it decreases to <500 mg/24 h. Fewer than 60% of respondents voted to retain scores for digital ischaemia/gangrene, seizures, livedoid vasculopathy, and livedo reticularis/livedo racemosa when these items were unchanged (50%-59%) or improved (44%-53%). It was, therefore, decided that these manifestations would only be scored if new or worsened. Scoring of stable or improved aseptic necrosis, pulmonary hypertension, APS nephropathy-related acute kidney injury, valve vegetations, and valve thickening was endorsed by less than 50%, and so these would also only be scored if new or worsened.

In conclusion, the EAPSDAS scales are presented in Table 3. EAPSDAS is composed of 2 separate scales: a scale for TMN manifestations to be used in evaluating all patients with APS, and an obstetric scale to be used in evaluating pregnant patients with a history of APS. Manifestations are scored if they are new (first or relapsing) or worsening, and should be attributed to APS. Other causes should be ruled out. The time frame for the TMN scale is the past 1 month. The time frame for the obstetric scale is any time during the pregnancy. The scoring for bump-up items and the stable or improved manifestations are shown in the corresponding columns.

Phase IV: validation

Face and content validity

Twenty-one experts in APS or SLE and 45 patients with primary APS from 4 continents (Europe, North America, South America, and Asia) not involved in any previous phase of the project, rated the TMN and obstetric scales of the APS disease activity score for face and content validity. For both scales, the majority of raters assessed the relevance and comprehensiveness as very good or adequate (Table 4).

Table 3

Scores for thrombotic, microvascular, and nonthrombotic (TMN) manifestations, and obstetric manifestations attributed to APS (other causes are ruled out)

A. Scores for TMN manifestations.					
Interval = 1 mo					
New or worsening manifestations	Score for new or worsening manifestations	Score for 'bump-up' manifestations (both new or worsening manifestations occurring in a 1-mo period)	Score for 'abnormal but stable' single manifestations	Score for 'improved but still present' single manifestations	TMN-1/TMN-2 items
Thrombosis (arterial or venous) in 3 territories	95				TMN-1
Pulmonary haemorrhage	93				TMN-1
Arterial thrombosis ^a in 2 territories	91				TMN-1
Venous thromboembolism ^b in 2 territories	88				TMN-1
Arterial thrombosis in 1 territory	87				TMN-1
Venous thromboembolism in 1 territory, unprovoked	85				TMN-1
Adrenal haemorrhage	84				TMN-1
Digital ischaemia/gangrene	82				TMN-1
Transverse myelitis	80				TMN-1
Haemolytic anaemia	75		75	37 if haemoglobin levels improved by 50%	TMN-2
Pulmonary hypertension	72				TMN-1
Livedoid vasculopathy	71				TMN-1
APS nephropathy-related nephrotic range proteinuria	71	Nephrotic-range proteinuria and livedo reticularis or livedo racemosa: 81	71	59 if improved to non-nephrotic range; 0 if improved to <500 mg/24 h proteinuria	TMN-2
APS nephropathy-related acute kidney injury	67	APS nephropathy-related acute kidney injury and severe thrombocytopenia: 82			TMN-1
Thrombocytopenia, severe (platelet count: ≤50,000/μL)	66	Severe thrombocytopenia and skin ulcers: 77	66	47 if platelet count improved to 51,000–100,000/μL; 0 if improved to >100,000/μL	TMN-2
Venous thromboembolism in 1 territory, provoked	66				TMN-1
Avascular necrosis (osteonecrosis)	66				TMN-1
Cardiac valve vegetation	65				TMN-1
Skin ulcers	64	Skin ulcers and livedo reticularis or livedo racemosa: 70 Skin ulcers and nephrotic range or non-nephrotic proteinuria: 76	64	32 if improved 50% by physician judgement	TMN-2
Seizures/epilepsy	61				TMN-1
APS nephropathy-related non-nephrotic proteinuria (>500 mg/24 h)	59	APS nephropathy-related non-nephrotic proteinuria and moderate or severe thrombocytopenia: 76	59	0 if <500 mg/24 h proteinuria	TMN-2
Cardiac valve thickening	50				TMN-1
Thrombocytopenia, moderate (platelet count: 51–100,000/μL)	47		47	0 if platelet count >100,000/μL	TMN-2
Livedo reticularis or livedo racemosa	39				TMN-1
No new or worsening manifestations	0				TMN-1
B. Scores for obstetric manifestations					
Interval = pregnancy; score = highest rated manifestation that occurred during the pregnancy					
New manifestation					Score
HELLP syndrome					85
Eclampsia or severe pre-eclampsia					82
Foetal death					72
Premature birth due to placental insufficiency (with or without severe features):					72
Intrauterine growth restriction					67
New recurrent first-trimester miscarriage					46
No pregnancy complications					0

APS, antiphospholipid syndrome; HELLP, haemolysis, elevated liver enzyme levels, and low platelet levels.

Grey colour, 'not scored' items; TMN-1, items not scored if they are stable or improved; TMN-2, items scored if they are stable or improved.

Provoked venous thromboembolism is a venous thromboembolism that occurs in the setting of a transient risk factor (major or minor) or a persistent risk factor.

^a Arterial thrombosis (confirmed by imaging) includes thromboses in coronary arteries, cerebral arteries, or peripheral, splanchnic, retinal arteries, and organ infarcts (eg, kidney, liver, or spleen).

^b Venous thromboembolism (confirmed by imaging) includes pulmonary embolism, deep vein thrombosis (legs or arms), thrombosis/occlusion in splanchnic, renal, cerebral, or retinal veins.

Table 4
Face and content validity appraisals by physicians and patients

Rater	Scale	Feature	Judgement			
			Very good (%)	Adequate (%)	Doubtful (%)	Inadequate
Physicians N = 21	TMN scale	Relevance	62	33	5	0
	TMN scale	Comprehensiveness	81	14	5	0
	Obstetric scale	Relevance	90	5	5	0
	Obstetric scale	Comprehensiveness	76	24	0	0
Patients N = 45	TMN scale	Relevance	84	16	0	0
	TMN scale	Comprehensiveness	74.5	25.5	0	0
	Obstetric scale	Relevance	87.5	12.5	0	0
	Obstetric scale	Comprehensiveness	85	15	0	0

TMN, thrombotic, microvascular, nonthrombotic manifestations.

Construct validity-external standard

Sixty physicians from the Task Force group and Collaborators, all blinded to the EAPSDAS scale scores, were invited to participate in the construct validity exercise, which involved rating 30 clinical vignettes for overall APS activity. Fifty-four physicians replied (90%); 2 had incomplete data. Correlations of the physicians' ratings (PhysGA) with the APS disease activity item scores were high for both the TMN scale (median Spearman $r = 0.74$) and the obstetric scale (median Spearman $r = 0.88$) (Table 5). Differences among raters were not attributable to differences in scoring any particular item.

Construct validity-internal standard

Fifty-five physicians, blinded to item scores, completed a survey in which they placed each vignette in rank order of APS activity (highest to lowest), separately for TMN vignettes and obstetric vignettes. These rankings were highly correlated with item scores of each scale (median Spearman $r = 0.79$ for the TMN scale; median Spearman $r = 0.91$ for the obstetric scale) (Table 5).

Reliability

When tested by applying the EAPSDAS to 20 clinical vignettes, inter-rater reliability (by intraclass correlations) was 0.82 (95% CI 0.72, 0.92) for the TMN scale and 0.97 (95% CI 0.92, 1.00) for the obstetric scale.

Implementation of EAPSDAS

In the implementation of EAPSDAS, the observation time for the TMN scale would be divided into monthly intervals. The score for each month is the score of the highest-rated item that is new/worsened in that month. For example, if a patient developed in a 1-month period unprovoked VTE (rated as 85) and

non-nephrotic proteinuria (rated as 59), the score for this month would be 85 (the VTE would count (highest rated), but the proteinuria would not contribute). The only exception is the 6 double manifestation combinations (bump-up items), which have their own scores, which are not the sum of each of the 2 single manifestations (Table 3).

In the context of a clinical trial or observational study having a defined time frame, the maximum rating achieved for each patient in any monthly interval over the entire observation time would be their 'maximum' TMN score, while their average TMN score would be the average of each monthly score over the observation time. The maximum score provides an indication of the highest or peak level of activity over the observation interval. The average score gives an indication of the persistence of activity, analogous to an area under the curve.

No average score exists for the obstetric scale since only a single event may occur during the pregnancy (eg, foetal loss). For the TMN scale, some items are scored only once (eg, thrombosis), while others are scored multiple times (eg, proteinuria), which may distort the average score. Therefore, the Task Force members voted (unanimously) that average scores be calculated separately for the items scored if they were stable/improved (TMN-2 items) and items scored only when new/worsening (TMN-1 items) (Table 3). The scores of TMN-1 and TMN-2 items are not added or combined; they are independent outcomes. In total, there are 3 maximum scores (TMN-1, TMN-2, Obstetric) and 2 average scores (TMN-1, TMN-2). Some hypothetical cases that demonstrate the average and maximum scoring are shown in Table 6. Although we acknowledge that multiple different manifestations over a short time are not necessarily common in APS, more than 1 manifestation per patient was included for purposes of illustration.

The definitions for the items of the EAPSDAS scales are presented in Supplementary Text S1 (Glossary). Most are based on

Table 5
Construct validity

	Scale	Number of physician raters	Median (25th, 75th)
External standard	TMN scale	52	0.74 (0.64, 0.81)
	Obstetric scale	52	0.88 (0.77, 0.96)
Internal standard	TMN scale	55	0.79 (0.70, 0.83)
	Obstetric scale	55	0.91 (0.81, 0.98)

TMN, thrombotic, microvascular, nonthrombotic manifestations.

Spearman correlations between item scores and physician global assessment (external standard) and between item scores and rank order of vignettes by activity (internal standard).

Table 6
Hypothetical cases for scoring the EAPSDAS

Age/sex	Scale	Clinical and laboratory manifestations												Maximum score	Average score
		Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12		
51 yo man		–	Unprovoked VTE (one territory)	–	–	–	–	–	–		Moderate				thrombocytopenia
Moderate															thrombocytopenia
Moderate															thrombocytopenia
	TMN-1	0	85	0	0	0	0	0	0	0	0	0	0	85	85/12 = 7.08
	TMN-2	0	0	0	0	0	0	0	0	0	47	47	47	47	$47 \times 3 = 141$ 141/12 = 11.75
	Obstetric	–	–	–	–	–	–	–	–	–	–	–	–	–	N/A
32 yo woman		–	Pregnancy	Pregnancy	Pregnancy	Pregnancy	Foetal death	Provoked VTE	–	–	–	–	–	–	
	TMN-1	0	0	0	0	0	0	66	0	0	0	0	0	66	66/12 = 5.5
	TMN-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Obstetric	0	0	0	0	0	72	0	0	0	0	0	0	72	N/A
19 yo woman		–	LR (new)	LR	LR; digital ischaemia	LR; digital ischaemia	LR; digital ischaemia	LR; digital ischaemia	LR; digital ischaemia	LR	LR; pregnancy	LR; first-trimester miscarriage	LR		
	TMN-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	TMN-2		39	0	82	0	0	0	0	0	0	0	0	82	$39 + 82 = 121$ 121/12 = 10
	Obstetric	0	0	0	0	0	0	0	0	0	0	46	0	46	N/A
36 yo man		–	Nephrotic range proteinuria*	Nephrotic range proteinuria	Nephrotic range proteinuria	Non-nephrotic proteinuria	Non-nephrotic proteinuria	Non-nephrotic proteinuria	Non-nephrotic proteinuria	Non-nephrotic proteinuria	Non-nephrotic proteinuria	Non-nephrotic proteinuria	–		
	TMN-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	TMN-2	0	71	71	71	59	59	59	59	59	59	59	0	71	$71 \times 3 + 59 \times 7 = 626$ 626/12 = 52.1
	Obstetric	–	–	–	–	–	–	–	–	–	–	–	–	–	N/A
47 yo woman		–	–	Stroke	–	–	–	Stroke	Haemolytic anaemia	Haemolytic anaemia	Haemolytic anaemia	–	–	–	
	TMN-1	0	0	87	0	0	0	87	0	0	0	0	0	87	$87 \times 2 = 174$ 174/12 = 14.5
	TMN-2	0	0	0	0	0	0	0	75	75	75	0	0	75	$75 \times 3 = 225$ 225/12 = 18.75
	Obstetric	–	–	–	–	–	–	–	–	–	–	–	–	–	N/A
39 yo man	Clinical	–	–	Arterial thrombosis in 2 territories	–	–	–	–	Skin ulcer (one)	Skin ulcer	Skin ulcer	Skin ulcer	Skin ulcer	–	
											improved 50% by physician judgement	improved 50% by physician judgement	improved 50% by physician judgement		
	TMN-1	0	0	91	0	0	0	0	0	0	0	0	0	91	91/12 = 7.58
	TMN-2	0	0		0	0	0	0	64	64	32	32	32	64	$64 \times 2 + 32 \times 3 = 229$ 229/12 = 18.66
	Obstetric	–	–	–	–	–	–	–	–	–	–	–	–	–	N/A
46 yo man				Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening	Cardiac valve thickening		
	TMN-1		0	0	0	0	0	0	0	0	0	0	0	0	0
	TMN-2	0	0	50	0	0	0	0	0	0	0	0	0	50	50/12 = 4.1
	Obstetric	–	–	–	–	–	–	–	–	–	–	–	–	–	N/A

APS, nephropathy-related nephrotic range proteinuria; EAPSDAS, EULAR antiphospholipid syndrome disease activity score; LR, livedo reticularis; N/A: not applicable; TMN, thrombotic, microvascular, nonthrombotic manifestations; VTE, venous thromboembolism; yo, year old.

Assume each patient is observed for 12 months. No average score exists for the obstetric scale. Average TMN score is the average of each monthly score over the observation time.

the glossary developed for the 2023 ACR/EULAR APS classification criteria. Instructions about the scoring of items and the implementation of EAPSDAS are presented in [Supplementary Text S1](#) (instructions for the EAPSDAS implementation).

DISCUSSION

Primary APS is a complex disorder with a wide variety of clinical manifestations and heterogeneity in response to standard treatments. Developing outcome measures in primary APS is crucial for assessing disease status, treatment response, and outcomes in clinical practice, observational studies, and clinical trials. Using consensus and data-driven methods, a EULAR disease activity score was developed, and initial validation was performed.

We followed the main methodology phases used in other disease activity scores in rheumatic diseases, including item generation, item reduction, item scoring based on clinical vignettes, and validation [33–35]. A primary goal and a strength of the project was using a combination of evidence-based data from an SLR, a Delphi consensus methodology, and multiple surveys. A major strength is the participation of an international multidisciplinary group of APS experts, patients with primary APS, as well as nonphysicians HPRs familiar with APS. Another strength is the contribution of the above international participants in defining the concept of disease activity in APS in the first open-ended question, as well as the consistent reporting of ‘new or worsened events’ and the ‘ability to change’, which differentiate disease activity from disease damage.

Since the first descriptions of APS clinical characteristics, including mainly macrovascular thrombosis and adverse events in pregnancy, various microvascular, cardiac, and haematological manifestations of APS have been recognised and recently incorporated in the new ACR/EULAR APS classification criteria [6]. Although development of the EAPSDAS began before the new APS classification criteria were published, all the above manifestations and others, previously classified as noncriteria APS manifestations [36], as well as transverse myelitis [37], seizures [38], aseptic necrosis [39], pulmonary hypertension [40], and haemolytic anaemia [41], have been retrieved by the SLR and the second open-ended survey aimed to generate all potential manifestations related to disease activity. This is also reflected by the high percentage of physicians and patients who graded the comprehensiveness and relevance of the EAPSDAS items as very good/adequate in the face and content validity exercise.

Another strength of the project is the use of real-world patient cases for the item scoring and the validation of EAPSDAS. Since the derivation of the EAPSDAS was mainly based on the PhysGA, estimates on one set of case profiles might differ if different sets of case profiles were used. To minimise bias, we included 3 versions of clinical vignettes for each item, randomly assigned to each of the 3 groups of participants. The 3 versions differed in patient demographics: APS history, general medical history, and current presentation.

The upward skew of the item scores is likely the result of 2 processes. First, these items were those that survived a multistep selection process that focused on identifying manifestations that were highly specific for APS activity. Less specific manifestations, which might have scored in the range of 0 to 30, would have been removed at earlier stages. Second, we interpret the distribution as reflecting ratings of activity rather than severity. If severity was the dominating factor, we would expect thrombosis in 3 territories (ie, catastrophic APS) to be rated much higher

than any other item, and yet scores of all acute thrombotic manifestations are clustered together. Similarly, if severity was the determining factor, we might expect livedo reticularis/livedo racemosa to be rated much lower. This scoring indicates that new events, including less serious manifestations such as new or worsening livedo reticularis/livedo racemosa, were accorded somewhat high scores by the raters, likely because these were interpreted to represent new activity.

EAPSDAS differs from other outcome measures, eg, SLEDAI, in that an interval between 2 events was defined to confirm that the second event should be counted as a new APS activity rather than a continuation of the previous activity. For the obstetric items, the interval is the entire pregnancy period, and the score is the highest-rated manifestation during pregnancy. The interval for TMN manifestations is 1 month. Thus, scores of items in different organ systems are not added together to arrive at the final score if the manifestations occur over 1 month; rather, the highest-rated item, which reflects the highest degree of APS activity attained in that interval, is used. The only exception is the 6 ‘bump-up’ manifestations, which are scored differently. During a predefined observation time (eg, 12 or 24 months in a clinical trial), the ‘maximum’ score for the TMN manifestations will be the highest score in any monthly interval, while the average of each monthly score during this period will be the average score. Another difference from other scores is the lack of a dichotomous (all-or-none) approach to score variables. In EAPSDAS, new/worsening events, abnormal but stable (eg, persistent moderate thrombocytopenia or non-nephrotic proteinuria) manifestations, or manifestations that are improved but still present are scored differently. In addition, a separate average score is calculated for the items in the TMN scale scored when stable/improved, from those not scored if stable/improved.

The choice between a numerical score and an ordinal measure reflects the competing priorities of reliability and sensitivity to change of the measure. Ordinal measures are typically highly reliable but often less sensitive to smaller changes because patients need to switch to different categories for the score to register a change, which requires a more substantial shift in their condition. This is in contrast to numerical scores that can detect smaller changes. Given the motivation to develop a score for use as an endpoint in clinical trials, where sensitivity to change is important, we decided that a numerical score would be more useful.

Antiphospholipid antibodies (aPL), including newly positive aPL, lupus anticoagulant, high anti- β 2GP1 antibodies, high anti-cardiolipin antibodies, aPL triple positivity, and antiprothrombin antibodies, were among the items nominated in the item generation phase but excluded in subsequent surveys and rounds of voting by Task Force members on the most important items to include in the activity measure. Similarly, C-reactive protein, erythrocyte sedimentation rate, D-dimers, prothrombin fragments, complement split products, and other laboratory measures were excluded during the voting process. The stage at which each item was omitted is presented in the accompanying methodology paper [31].

Other potential equally likely or more likely causes should be excluded, and only items attributed to APS should be scored. EAPSDAS manifestations are differentiated from disease damage, even those that are persistent (eg, thrombocytopenia or proteinuria), considering that they represent activity and are potentially reversible. In addition to face and content validity, we examined construct validity using 2 separate comparisons: with the PhysGA as an external standard and with item ranks as an internal standard. Both results supported the validity of the

item scores. In all validation exercises, physicians and patients with APS from different centres and countries across 5 continents participated. We also found high inter-rater reliability when scores were tested using real-world clinical vignettes.

We used the PhysGA as the reference standard for item scores, which may be viewed as a limitation. However, PhysGA has been validated in disease activity scores for other systemic autoimmune disorders [33,34], and we found a high consistency of physician ratings of PhysGA on the initial vignette survey. Another potential limitation is that although different surveys were designed for patients and the introduction and questionnaire were presented in patient-friendly language, a glossary for each manifestation was not provided. This is because we considered that APS manifestations would be known as a standard medical term rather than as a descriptive presentation of the terms. In the surveys asking for item ratings, the following statement was included ‘If you do not know a specific item, please do not rate it’. Concerning language barriers, the first 2 open-ended questions were answered in English but also in different languages. The face and content validity exercises were translated into multiple languages.

EAPSDAS use as a primary outcome in future clinical trials on new treatments for the classical (thrombotic and obstetric) and nonclassical (microvascular, nonthrombotic) APS manifestations may help to better assess the response to therapy. The latter manifestations usually do not respond to the standard anticoagulation therapy. In addition, recurrences of classical thrombotic or obstetric manifestations often occur despite adequate anticoagulant/antiplatelet treatment. The lack of response or recurrence to standard anticoagulation treatment may relate to the involvement of inflammation, in addition to or independently of thrombotic mechanisms in APS [7–12]. With the contribution of high-throughput ‘omic’ technologies, new pathways involving adaptive and innate immunity and the corresponding therapeutic targets are being explored [42–50]. Given that new treatment targets and drugs are emerging, using EAPSDAS as an endpoint in future clinical trials in primary APS constitutes a major implementation plan.

We anticipate that the EAPSDAS can be used to evaluate APS activity in trials of new treatments in one of 2 ways. In the case of trials that enrol patients with diverse active manifestations (eg, APS-nephropathy-related nephrotic-range proteinuria, severe thrombocytopenia, etc.), we would expect an efficacious treatment to result in lower average EAPSDAS scores over the trial period compared to placebo. In APS, preventive trials may be particularly important. In these cases, patients with currently inactive disease but with a history of recurrent events would be enrolled and treated with either a new treatment or placebo, and followed for new activity based on EAPSDAS.

In conclusion, an APS disease activity scale that quantifies the level of disease activity was developed. We used a rigorous evidence-based and consensus-based approach with special attention to face, content, internal and external construct validity, and reliability. Future plans include prospective validation of the score in multicentre and multiethnic APS cohorts, with PhysGA, and patient-reported quality of life as the reference standards.

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Contributors

MGT was convenor, and was responsible for study conception and design, supervision of the project, preparation of surveys, acquisition of data, analysis and interpretation of data, and manuscript writing. RC and AT (Steering Committee members) were responsible for study conception, acquisition of data, and interpretation of data. GJP-E and SS (Steering Committee members/Fellows) were responsible for systematic literature review, acquisition of data, analysis of some data, and interpretation of data. TS was responsible for study design and supervision of methodology. RBML was responsible for study design, supervision of methodology, and interpretation of data. MMW was responsible for study design, supervision of methodology, analysis of data, and interpretation of data.

The Task force members participated in project surveys (data acquisition), discussions during the Task force meetings, and the Delphi methodology. MR, LVDH (EMEUNET members), and KS also contributed to the systematic literature review. The Collaborators participated in project surveys (data acquisition). All Task Force members and Collaborators reviewed critically the manuscript for important intellectual content and approved the submitted version.

MGT had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests

MGT reports grants to the institution were provided by AbbVie, Boehringer Ingelheim, DEMO, Faran, GSK, and UCB, and MGT has participated in advisory boards from GSK, Lilly, and UCB. AT has participated in advisory boards from UCB and Galapagos. VP has received lecture fees from Werfen group, Milan, Italy. TS has received grant support from Roche and personal fees from AbbVie, Roche, Sanofi, Takeda, and Novartis, outside the submitted work. RBML has received consulting fees from AbbVie, BMS, Celgene, Eli-Lilly, Galapagos, Gilead, Glaxo-Smith-Kline, Janssen, Merck, Novartis, Pfizer, Roche, and UCB and is Director of Rheumatology Consultancy BV. MMW worked as consultant to Advanced Clinical LLC. RC, GJP-E, SS, JA-R, TA, MLB, TJ, FM, AM, P-LM, MR, AR, GR-I, KS, SS, LVDH, RVV, and DW report no conflict of interest.

Patient consent for publication

Not applicable.

Ethics approval

This study involves human participants and was approved by the Laiko General Hospital Institutional Review Board (protocol number 148, 14-03-22); the Ethics Committee of Centro Regional de Enfermedades Autoinmunes y Reumáticas, Rosario, Argentina; and the Ethics Committee AOU Città della Salute e della Scienza di Torino (approval number 0126695, 31/10/2023, file number 386/2023) for a shared project with the University of Brescia.

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Data availability statement

All data relevant to the study are included in this article, and the accompanying methodology article is published simultaneously.

ORCID iDs

Maria G. Tektonidou <https://orcid.org/0000-0003-2238-0975>
 Ricard Cervera <https://orcid.org/0000-0001-6085-492X>
 Angela Tincani <https://orcid.org/0000-0003-4355-9333>
 Guillermo J. Pons-Estel <https://orcid.org/0000-0002-0647-929X>
 Savino Sciascia <https://orcid.org/0000-0003-1266-9441>
 Jaume Alijotas-Reig <https://orcid.org/0000-0002-9212-3619>
 Tadej Avcin <https://orcid.org/0000-0001-8409-9092>
 Maria Laura Bertolaccini <https://orcid.org/0000-0002-8623-139X>

139X

Francesca Marchiori <https://orcid.org/0000-0001-7961-6788>
 Arsene Mekinian <https://orcid.org/0000-0003-2849-3049>
 Pier-Luigi Meroni <https://orcid.org/0000-0002-3394-1451>
 Vittorio Pengo <https://orcid.org/0000-0003-2064-6071>
 Massimo Radin <https://orcid.org/0000-0003-1941-2606>
 Anisur Rahman <https://orcid.org/0000-0003-2346-4484>
 Guillermo Ruiz-Irastorza <https://orcid.org/0000-0001-7788-1043>

1043

Karen Schreiber <https://orcid.org/0000-0003-0660-7690>
 Stefkia Stoilova <https://orcid.org/0000-0002-3327-0491>
 Lucas van den Hoogen <https://orcid.org/0000-0002-6380-7800>
 Ronald van Vollenhoven <https://orcid.org/0000-0001-6438-8663>

8663

Denis Wahl <https://orcid.org/0000-0003-4846-6793>
 Tanja Stamm <https://orcid.org/0000-0003-3073-7284>
 Robert B.M. Landewé <https://orcid.org/0000-0002-0577-6620>
 Michael M. Ward <https://orcid.org/0000-0003-1857-9367>

Appendix

EULAR APS Disease Activity Score Collaborators: Nancy Agmon-Levin, Paula Alba, Ales Ambrozic, Olga Amengual, Mary-Carmen Amigo, Zahir Amoura, Danieli Andrade, Laura Andreoli, Tatsuya Atsumi, Cristina Belizna, Eloisa Bonfa, Ware Branch, Antonio Cabral, Cecilia Chighizola, Hannah Cohen, Fabrizio Conti, Nathalie Costedoat-Chalumeau, Mark Crowther, Maria J Cuadrado, David D' Cruz, Philip de Groot, Andrea Doria, Thomas Dörner, Katrien Devreese, Doruk Erkan, Bahar Artim Esen, Gerard Espinosa, Raquel Ferrer Oliveras, Paul Fortin, David Garcia, Maria Gerosa, Bill Giannakopoulos, Jean-Christophe Gris, Munther Khamashta, Jason Knight, Roger Levy, Michael Lockshin, Chary Lopez-Pedraza, Marta Mosca, Thomas Ortel, Michelle Petri, Guilherme Ramires de Jesús, Jacob Rand, Robert Roubey, Jane Salmon, Antonio Serrano, Surya V Seshan, Yehuda Shoenfeld, Elisabet Svenungsson, Imad Uthman, Carlos Vasconcelos, Rohan Willis, Stéphane Zuily.

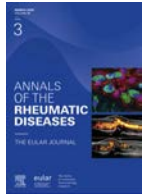
Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.10.006.

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Viewpoint

Antiobesity medications in rheumatology. Quo vadis?

Niki Kyriazi¹, Konstantinos D. Vassilakis², Amalia Bakiri², Alexios Iliopoulos³, George E. Fragoulis^{1,2,4,*}

¹ Rheumatology Department, “Evangelismos” Hospital, Athens, Greece

² Rheumatology Department, Joint Academic Rheumatology Program, First Propedeutic and Internal Medicine Clinic, Athens, Greece

³ Rheumatology Department, “NIMTS” Hospital, Athens, Greece

⁴ School of Infection and Immunity, University of Glasgow, Glasgow, UK

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ABSTRACT

Obesity is a well-recognised comorbidity in the context of rheumatic and musculoskeletal diseases (RMDs), adversely affecting disease-related outcomes. Adipose tissue, through immunological mechanisms, induces a low-grade inflammatory state; as a result, there has been growing interest in evaluating the potential role of antiobesity drugs in the management of RMDs. Although they were initially approved for type 2 diabetes mellitus, obesity, and their cardiorenal associations, there is increasing evidence that glucagon-like peptide-1 receptor agonists (GLP-1 RAs) in particular may exert immunomodulatory and anti-inflammatory effects in the setting of RMDs. In this viewpoint, we discuss current data regarding the effects of GLP-1 RAs on several conditions, including osteoarthritis, systemic lupus erythematosus, rheumatoid arthritis, fibromyalgia, and osteoporosis. We also highlight ongoing studies, which appear to be promising. Furthermore, we propose that these drugs could be administered to difficult-to-manage cases or in people at an increased risk of developing RMDs (like obese psoriatic patients) or even as adjunctive therapy, considering also the cost barrier that exists in most countries. Preliminary findings are encouraging; however, as most of the available evidence is limited to a small sample size, large-scale randomised controlled trials are needed to evaluate also the long-term safety of these drugs throughout the spectrum of rheumatic disorders.

OBESITY AND ANTI-OBESITY DRUGS

Obesity is defined as excessive fat deposition in different parts of the body or organs. Although it is classically defined as a body mass index (BMI) ≥ 30 kg/m², the term ‘clinical obesity’ was recently introduced. It refers to obesity-related organ or tissue dysfunction, limitation of daily activities, and includes assessment of body fat (where tools are available) or measurement of other anthropometric characteristics (eg, waist circumference) in addition to BMI [1,2].

For obesity management, in addition to a calorie-restricted diet and regular physical activity, pharmacotherapy can be

added not only to achieve but also to maintain weight loss [3].

There are 6 pharmaceutical agents currently available and US Food and Drug Administration approved for weight management, which act by decreasing appetite, enhancing satiety, and delaying gastric emptying: semaglutide and liraglutide (glucagon-like peptide-1 receptor agonists [GLP-1 RAs]), orlistat (gastric and pancreatic lipase inhibitor), phentermine-topiramate (norepinephrine agonist/ γ -aminobutyric acid agonist and glutamate antagonist), naltrexone-bupropion (opioid receptor antagonist/dopamine and norepinephrine reuptake inhibitor), and recently, tirzepatide

*Correspondence to Dr. George E. Fragoulis, Rheumatology Department, Joint Academic Rheumatology Program, First Propedeutic and Internal Medicine Clinic, Athens, Greece.

E-mail address: geofragoul@yahoo.gr (G.E. Fragoulis).

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(gastric inhibitory polypeptide/glucagon-like peptide-1 [GLP-1] dual agonist) [4].

OBESITY IN RHEUMATIC AND MUSCULOSKELETAL DISEASES

The spectrum of rheumatic and musculoskeletal diseases (RMDs) includes inflammatory and crystal-induced arthritides, connective tissue diseases (eg, systemic lupus erythematosus [SLE]), degenerative joint diseases (eg, osteoarthritis [OA]), and conditions related to bone metabolism (eg, osteoporosis) and chronic pain (eg, fibromyalgia).

It has been demonstrated that there is a complex interplay between obesity and RMDs, especially inflammatory arthritides. Besides, obesity is one of the most common comorbidities in the setting of psoriatic arthritis (PsA) and, to a lesser extent, in rheumatoid arthritis (RA) and axial spondyloarthritis (SpA) [5], with the respective figures being about 40% for PsA and 10% to 15% for RA, SpA and SLE [6,7]. It is also evident that obese people living with inflammatory arthritis exhibit lower odds of achieving or maintaining favourable therapeutic outcomes [8]. The reasons behind this are not entirely clear. It was established that there is an interaction between the immune system and the adipose tissue. Visceral fat can produce proinflammatory cytokines (eg, interleukin [IL]-17 and tumour necrosis factor [TNF]- α) and adipokines (leptin, resistin, visfatin, and adiponectin) that have both anti-inflammatory and proinflammatory properties [9]. Therefore, adipose tissue is not an ‘innocent bystander’ but an immunologically active player [10]. Hence, obesity is considered a chronic low-grade inflammatory state.

WHAT IS THE POTENTIAL ROLE OF GLP-1 RAs IN THE MANAGEMENT OF RMDs?

GLP-1 RAs demonstrate a wide range of benefits. Beyond their importance in cardiorenal and metabolic outcomes, there is also

growing evidence of their immunomodulatory and anti-inflammatory effects in nonmetabolic diseases [11]. Repurposing of these drugs, or at least their introduction as an add-on treatment in RMDs, seems to be a likely scenario in the near future, delineating the connection between different disciplines.

The risk of all-cause mortality and major adverse cardiovascular events in patients with immune-mediated inflammatory diseases and type 2 diabetes mellitus is lower with the administration of GLP-1 RAs compared with dipeptidyl peptidase-4 inhibitor exposure, based on a recently published cohort study [12]. An overview of the accumulating evidence, presented below, of the potential benefit of GLP-1 RAs in RMDs is depicted in the Figure and summarised in the Table.

OA

The negative impact of obesity (mechanical overload and low-grade systemic inflammation) in OA is well established. Although it is classified as a noninflammatory disorder, in the early stages, inflammation of the synovium, mediated by cytokines and prodegradative mediators, can occur [13].

There are studies that highlight the beneficial effects of GLP-1 RAs in the management of OA. Immunohistochemistry revealed that GLP-1 receptor protein is expressed in both the synovial membrane and chondrocytes of human OA knee joints [14]. Based on this evidence, the efficacy of liraglutide on pain perception and inflammatory response in OA was assessed in animal models. A significant dose-dependent increase in paw withdrawal threshold was detected in OA mouse models that received intra-articular injections of liraglutide compared with saline-treated mice; the efficacy persisted until the end of the experiment. Furthermore, it was observed that the IL-1 β -induced proinflammatory mediators were diminished, and cartilage breakdown was prevented following liraglutide treatment [14].

Pain management was also evaluated in the STEP (Semaglutide Treatment Effect in People with Obesity) 9 trial, in which

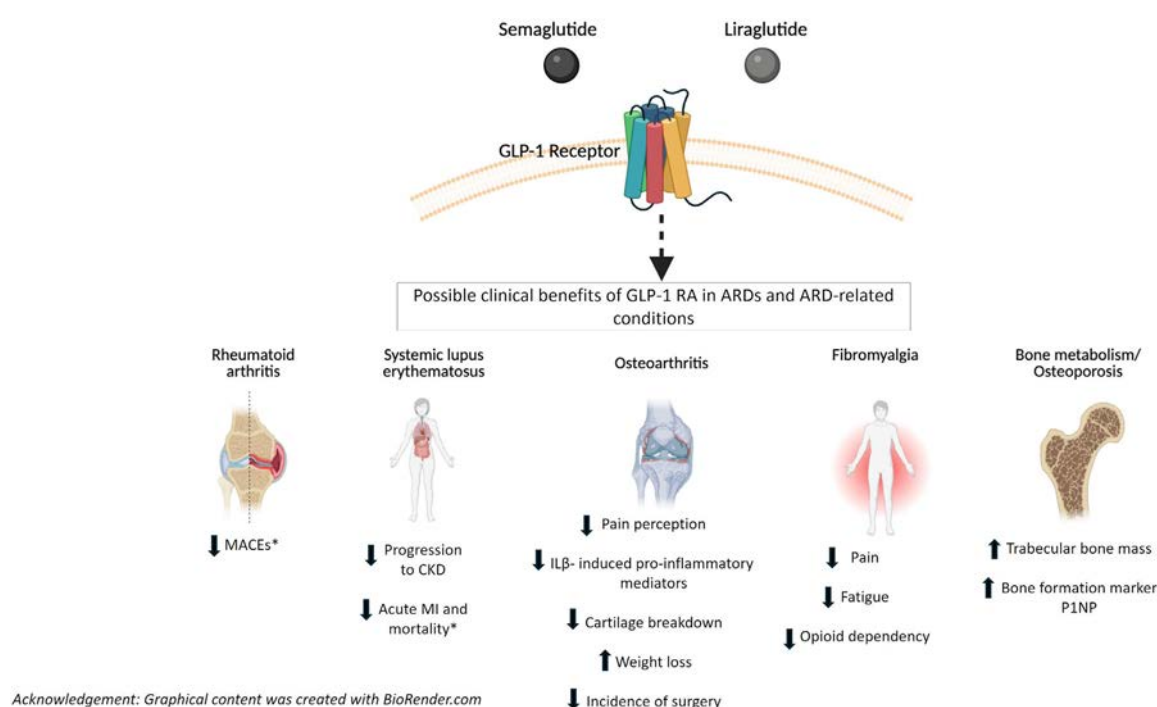


Figure. Potential clinical benefits of glucagon-like peptide-1 receptor agonists (GLP-1 RAs) in autoimmune rheumatic diseases (ARDs) and ARD-related conditions. CKD, chronic kidney disease; IL-1 β , interleukin-1 β ; MACE, major adverse cardiovascular event; MI, myocardial infarction; P1NP, N-terminal propeptide of type 1 procollagen. *Data were retrieved from abstracts.

Table
Studies on antiobesity drugs in rheumatic diseases

Study group	Intervention	Study objective	Main results
MIA OA mice model	IA liraglutide injections	Anti-inflammatory and analgesic effects of liraglutide	Decrease in pain-related behaviour
Obese individuals (knee OA)	Semaglutide 2.4 mg vs placebo group	Percentage change in weight and WOMAC score	Weight and WOMAC score both improved in the semaglutide group
Diabetic individuals with OA	GLP-1 RA group vs control group	Incidence of knee surgery	Lower incidence of knee surgery in the GLP-1 RA group
Overweight/obese individuals (knee OA)	Liraglutide 3 mg/d vs placebo group	Efficacy and safety of liraglutide	Weight loss, no difference in KOOS
SLE patients	GLP-1 RAs	Outcomes of GLP1-RAs in SLE	Not associated with increased disease flares
SLE nephritis patients	GLP-1 RAs vs SGLT-2 inhibitors	Effect of GLP-1 RAs compared with SGLT-2 inhibitors	Lower risk of CKD, MI, and mortality in GLP-1 RAs patients
RA patients	GLP-1 RAs with/without JAK inhibitor	Role of GLP-1 RAs against CV adverse effects	Lower risk of acute coronary syndromes, DVT, and overall arterial CV events
Fibromyalgia patients	GLP-1 RAs users vs GLP-1 RAs naive	Impact of GLP-1 RAs on fibromyalgia's opioid use, fatigue, and pain	Reduction of symptom burden

CKD, chronic kidney disease; CV, cardiovascular; DVT, deep vein thrombosis; GLP-1 RA, glucagon-like peptide-1 receptor agonist; IA, intra-articular; JAK, Janus kinase; KOOS, Knee injury and Osteoarthritis Outcome Score; MI, myocardial infarction; MIA, monoiodoacetate; OA, osteoarthritis; RA, rheumatoid arthritis; SGLT-2, sodium-glucose cotransporter-2; SLE, systemic lupus erythematosus; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

407 individuals with moderate knee OA were enrolled. They were randomised to receive once-weekly subcutaneous semaglutide (2.4 mg) or placebo, together with a low-calorie diet and adjusted physical activity. The mean change in both body weight from baseline and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score was significantly improved at week 68 in the semaglutide group [15].

Furthermore, the potential use of GLP-1 RAs as disease-modifying drugs for knee OA was studied by the Shanghai Osteoarthritis Cohort, involving 1807 patients with both knee OA and type 2 diabetes. The group that was exposed to GLP-1 RAs experienced greater weight loss and a lower incidence of knee surgery than the control group. Statistically significant differences were also detected between the 2 groups on the WOMAC total and pain subscale scores [16].

By contrast, in another study in which 156 patients were randomised to receive liraglutide 3 mg/d or placebo, after an 8-week diet, results were less encouraging; only one of the primary endpoints (changes in body weight), but not the other (a change in the Knee injury and Osteoarthritis Outcome Score [KOOS]), was met [17].

SLE

There is some evidence regarding the use of GLP-1 RAs in SLE patients. After presentation of a case of drug-induced lupus secondary to semaglutide administration [18], a retrospective study showed that GLP-1 RAs were not associated with flares of the disease after 6 to 10 months of use, although these patients experienced a significant decrease in BMI [19]. Nevertheless, these findings require careful interpretation due to the small sample size and the absence of a control group. In another recent cohort study of patients with lupus nephritis (n = 1034), the use of GLP-1 RAs correlated with a reduced risk of progression of chronic kidney disease, acute myocardial infarction, and mortality in comparison with patients on sodium-glucose cotransporter-2 (SGLT2) inhibitors (n = 1375). These data are published only as an abstract thus far; therefore, it should be interpreted with caution as some information, like previous treatments administered for lupus nephritis, is lacking [20].

RA

Current data on RA are limited. Recent evidence from Mendelian randomisation analysis revealed that increased GLP-1 receptor gene expression is significantly associated with a reduced risk of several autoimmune diseases. In the case of RA, it was detected that higher GLP-1 receptor gene expression was associated with a 27% lower odds for RA development [21]. Moreover, a recent retrospective cohort analysis of RA patients on Janus kinase (JAK) inhibitors divided into 2 groups, each cohort comprising 2449 patients (one with concurrent GLP-1 RAs use and the other only on JAK inhibitors), showed that those receiving GLP-1 RAs demonstrated an approximately 35% lower risk of acute coronary syndrome ($p < .001$), a 31% lower risk of deep venous thrombosis ($p < .05$), and about a 33% lower incidence of arterial cardiovascular events ($p < .001$) [22]. Certainly, further studies with larger populations are needed to clearly elucidate the role and the effect of GLP-1 RAs in patients with RA.

Fibromyalgia

Despite the limited data (published in abstract form thus far), positive results for fibromyalgia were reported. Analysing data from the TriNetX network, 2 groups were evaluated: group 1, patients diagnosed with fibromyalgia who have used GLP-1 RAs at least twice on different occasions (n = 46,409), and group 2, patients with fibromyalgia without a recorded use of GLP-1 RAs (n = 716,185). It was shown that the use of GLP-1 RAs was connected to significantly reduced pain, fatigue, and opioid dependency [23].

Osteoporosis

The relation between GLP-1 and bone metabolism may elucidate the impact of GLP-1 RAs on bone quality, too. Treatment with liraglutide in healthy obese women, after a weight loss period, showed that those who received the drug demonstrated an increase in bone formation marker N-terminal propeptide of type 1 procollagen (P1NP) by 16% vs the control group ($p < .05$) [24]. In addition, a positive effect of GLP-1 RAs on bone health

was found in ovariectomised mice, which received liraglutide and exenatide, resulting in improvement of trabecular bone mass (but not on cortical bone) in comparison with the saline group [25].

However, plasma P1NP was not affected after semaglutide administration in patients with increased fracture risk; in this group, enhanced bone resorption was recorded, which was attributed to concurrent weight loss [26].

WHEN AND BY WHOM COULD GLP-1 RAS BE USED?

Since obesity appears to influence both the onset and treatment response across most RMDs, one could consider GLP1-RAs as a useful aid. Given the cost, as in most countries, GLP-1 RAs are currently covered only for type 2 diabetes patients. A relevant question is: which patients would be more suitable to receive these drugs, and whether the latter can act as a disease-modifying treatment in this context. Considering the accumulating data for difficult-to-manage/refractory cases of inflammatory arthritis, these patients could be the right candidates. Also, in an effort to intercept disease, people at high risk for developing RMDs, such as pre-RA or obese psoriasis patients with relevant risk factors, could have some benefit [27,28]. Finally, it is not irrational to hypothesise that these drugs might have immunomodulatory effects that would enhance/modify the action of classic disease-modifying antirheumatic drugs. However, studies involving larger populations are warranted to confirm their long-term safety.

FUTURE STUDIES AND PERSPECTIVES

At this time, 9 antiobesity drugs are undergoing phase 3 clinical trials either by targeting the GLP-1 receptor alone (orforglipron, semaglutide, ecnoglutide, and TG103), in combination with a GLP-1 receptor agonist and amylin (CagriSema, Novo Nordisk), by acting as agonist on both GLP-1 and glucagon receptors (mazdutide and survodutide), or acting as triple agonists (GLP-1, glucose-dependent insulinotropic peptide, and glucagon receptor [retatrutide]) [29]. Despite their effect on weight control, their broader systemic impact is being investigated across a variety of clinical conditions. The concomitant use of ixekizumab with tirzepatide in obese/overweight adults with active PsA or psoriasis is being studied in phase 3 clinical trials to show the efficacy and safety of this drug combination not only for weight management but also for improvement of psoriatic disease (NCT06588296 and NCT06588283). This viewpoint discusses the accumulating evidence that antiobesity drugs have beneficial effects on a variety of RMDs. In addition to their effect on cardiovascular events as mentioned before, GLP-1 RAs are undergoing trials for metabolic liver disease, kidney disease, and Alzheimer's disease [4]. It is evident that the antiobesity drugs expected to enter the market in the coming years may transform the management of several chronic diseases.

Therefore, the answer to 'quo vadis', cannot be easily given. Nonetheless, the preliminary findings seem to be encouraging, opening new avenues for the development of novel therapeutic strategies for the management of RMDs.

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Competing interests

All authors declare they have no competing interests.

CRedit authorship contribution statement

Niki Kyriazi: Writing – original draft, Investigation, Data curation. **Konstantinos D. Vassilakis:** Writing – original draft, Data curation. **Amalia Bakiri:** Writing – original draft, Data curation. **Alexios Iliopoulos:** Writing – review & editing, Conceptualization. **George E. Fragoulis:** Writing – review & editing, Investigation, Conceptualization.

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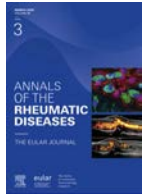
Orcid

George E. Fragoulis: <http://orcid.org/0000-0003-4932-7023>

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Rheumatoid arthritis

Difficult-to-treat rheumatoid arthritis: systematic review and meta-analysis on global prevalence

Wenhui Xie^{1,2}, Tong Chen^{1,2}, Shiyu Xiao^{3,4}, Hong Huang^{1,2}, Zhuoli Zhang^{1,2,*}¹ Department of Rheumatology and Clinical Immunology, Peking University First Hospital, Beijing, China² National Clinical Research Center for Skin and Immune Diseases, Beijing, China³ Department of Gastroenterology, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China⁴ Clinical Immunology Translation Medicine Key Laboratory of Sichuan Province, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China

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ABSTRACT

Objectives: Despite significant advances in targeted therapies, difficult-to-treat rheumatoid arthritis (D2T-RA) remains a major clinical challenge. This study aimed to systematically evaluate the global prevalence of D2T-RA using the European League Against Rheumatism (EULAR) definition and to quantify the proportion attributable to persistent inflammatory refractory rheumatoid arthritis (RA).

Methods: We comprehensively searched PubMed, EMBASE, and Cochrane Library (up to April 2025) for observational studies reporting the prevalence of D2T-RA according to the EULAR definition. Subgroup analyses were performed to explore heterogeneity sources. Random-effects meta-analysis of proportions using generalised linear mixed models was conducted to calculate pooled prevalence rates and 95% CI.

Results: Twenty-three studies with 27,987 patients with RA from 13 countries were included. The global pooled prevalence of D2T-RA was 11.7% (95% CI: 9.5%–14.3%). The pooled prevalence of D2T-RA was 10.9% (95% CI: 8.2%–14.2%) in overall RA cohorts and 13.2% (95% CI: 9.1%–18.8%) in studies restricted to biologic and target synthetic disease-modifying antirheumatic drugs (b/tsDMARDs)-treated populations. D2T-RA prevalence was stable in the region, RA criteria, and study setting; significant variations only existed in the enrolled period (pre-2020 vs post-2020: 9.86% [95% CI: 7.6%–12.7%] vs 14.3% [95% CI: 10.3%–19.6%]; $P = .08$). Moreover, about half of patients with D2T-RA exhibited evidence of persistent inflammatory refractory RA (pooled proportion: 47.1%, 95% CI: 33.3%–61.4%). Prevalence decreased sharply with stricter definitions: D2T(3)-RA (≥ 3 b/tsDMARD failures): 4.3% (2.7%–6.7%) and D2T(4)-RA (≥ 4 failures: 1.6% (0.6%–4.3%).

Conclusions: Based on available global data, approximately 12% of patients with RA meet standard D2T-RA criteria, with nearly half exhibiting persistent inflammatory disease. Stricter definitions with failure of ≥ 3 b/tsDMARD identify the small group of patients for whom innovative treatments or strategies are needed.

*Correspondence to Dr. Zhuoli Zhang, Department of Rheumatology and Clinical Immunology, Peking University First Hospital, Beijing, China.

E-mail address: zhuoli.zhang@126.com (Z. Zhang).

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Despite significant advances in targeted therapies, difficult-to-treat rheumatoid arthritis (D2T-RA) remains a major clinical challenge. Current studies report highly variable D2T-RA prevalence estimates, ranging from 5% to 28%.
- D2T-RA has been proposed to classify these patients into persistent inflammatory refractory rheumatoid arthritis (PIRRA) or noninflammatory refractory rheumatoid arthritis (NIRRA) categories. To date, no comprehensive meta-analysis has synthesised the global pooled prevalence of European League Against Rheumatism-defined D2T-RA and quantified the proportion attributable to PIRRA.

WHAT THIS STUDY ADDS

- In this study of 23 unique studies that include 27,987 patients with RA, the pooled prevalence of D2T-RA was 11.7% (95% CI: 9.5%–14.3%).
- About half of patients with D2T-RA exhibited evidence of PIRRA (pooled proportion: 47.1%, 95% CI: 33.3%–61.4%).
- Prevalence decreased sharply with stricter definitions: D2T(3)-RA (≥ 3 biologic and target synthetic disease-modifying antirheumatic drugs failures): 4.3% (2.7%–6.7%) and D2T(4)-RA (≥ 4 failures): 1.6% (0.6%–4.3%).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- D2T-RA represents a significant and not uncommon clinical challenge, imposing a substantial public health burden. Timely recognition should trigger intensified monitoring and prompt, tailored intervention strategies.
- This precise phenotypic characterisation of D2T-RA is essential to guide truly individualised therapeutic decisions.
- This evidence will directly support clinical practice by informing trial designs, guideline updates, and health policy decisions aimed at improving management of D2T-RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease affecting 0.2% to 1% of adults globally [1–3]. Management of RA has been revolutionised by treat-to-target strategies and advanced therapeutics, including biologic and target synthetic disease-modifying antirheumatic drugs (b/tsDMARDs). These approaches have significantly improved clinical outcomes, enabling higher rates of remission and low disease activity [4].

In clinical practice, a considerable subset of patients exhibits persistent treatment resistance, failing multiple therapeutic classes and sustaining high disease activity. To address this challenge, the 2021 European League Against Rheumatism (EULAR) guidelines proposed the first standardised definition of difficult-to-treat RA (D2T-RA) with established 3-core criteria [5]. Subsequent studies report highly variable D2T-RA prevalence estimates, ranging from 5% to 28% [6–12]. This heterogeneity stems from methodological limitations with single-centre designs, population diversity with different disease characteristics, and geographical disparities with differing comorbidity burdens. Importantly, the definition of the EULAR D2T-RA is broad and heterogeneous, as elevated disease activity scores may stem not only from persistent synovitis but also from noninflammatory pain mechanisms. Recently, it has been proposed to classify these patients into persistent inflammatory refractory RA (PIRRA) or noninflammatory refractory RA (NIRRA) categories, based on the presence or absence of objective evidence of active inflammation [13,14]. To date, no comprehensive meta-analysis has synthesised global evidence on these topics. This

systematic review aims to determine the global pooled prevalence of EULAR-defined D2T-RA and quantify the proportion attributable to PIRRA.

METHODS

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [15]. The protocol was prospectively registered at the International Prospective Register of Systematic Reviews with registration number CRD420251081574.

Search strategy and selection criteria

A comprehensive literature search was conducted across PubMed, EMBASE, and Cochrane Library from 1 January 2021 to 1 April 2025 without language restrictions. The final systematic literature search across all databases was conducted on 1 April 2025. The search strategy combined Medical Subject Headings and free-text terms for RA ('rheumatoid arthritis') and difficult-to-treat status ('difficult-to-treat', 'difficult-to-manage', 'treatment-resistant', 'refractory', 'D2T-RA'), with full Boolean syntax provided in [Supplementary Appendix S1](#). The literature search was executed by 1 author (WX), and an academic librarian validated the strategy. Additional sources included the electronic search by manually reviewing reference lists of all included studies and relevant review articles for potentially eligible studies. Conference abstracts from the EULAR, American College of Rheumatology (ACR) Convergence (2021–2024) were screened for potentially relevant reports.

Eligibility criteria required: (1) adult RA populations (≥ 18 years) with well-defined diagnostic criteria, (2) application of EULAR D2T-RA definition; (3) observational studies (cohort, cross-sectional) reporting D2T-RA prevalence or sufficient data for calculation with or without its subtype PIRRA. Exclusion criteria encompassed studies lacking explicit RA or D2T-RA criteria, case-control studies exploring the D2T-RA predictors, case reports, nonhuman studies, and paediatric populations. When duplicate reports from identical source populations were identified, we retained the most comprehensive dataset with the largest sample size to prevent data overlap. Search results were imported into EndNote X8 (Clarivate Analytics) for the management of records and removal of duplicates. Two authors (WX and TC) independently screened titles/abstracts and reviewed full texts of potentially eligible articles. Discrepancies were resolved through consensus discussion.

Data extraction

A predesigned, standardised data extraction form in Microsoft Excel was piloted and used for data collection from the included studies. Two investigators (WX and TC) independently extracted data: (1) general study characteristics: first author, publication year, geographic region, study design, data source, and study period; (2) population characteristics: demographics (sample size, age, sex distribution, body mass index, comorbidities), RA-specific parameters: (RA duration, RA diagnostic method, serostatus, prior DMARDs/biologics, disease activity); (3) outcome metrics: D2T-RA ascertainment, prevalence, and PIRRA proportion.

Risk-of-bias assessment

Two investigators (WX and TC) conducted methodological evaluations using the 11 items recommended by the Agency for

Healthcare Research and Quality methodology checklist to assess the quality of cross-sectional studies. The total score ranged from 0 to 11, and a score ≥ 8 was considered to represent high quality [16,17]. Quality of cohort studies was evaluated using the Newcastle-Ottawa scale, with a total maximum score of 9, and the studies with scores of 7 or higher were considered high quality [18].

Data synthesis and analysis

All analyses were performed with R software (version 3.6.0), with a 2-sided P value $< .05$ considered statistically significant. The primary outcome was the prevalence of D2T-RA, which was defined according to the EULAR 2021 definition [5]. The second outcome included the proportion of PIRRA in D2T-RA and the prevalence of D2T-RA according to stricter definitions (failure of ≥ 3 or ≥ 4 b/tsDMARDs). There was no consensus on the diagnosis of PIRRA, which was generally considered as meeting EULAR D2T-RA criteria with evidence of objective inflammation (eg, ultrasound synovitis) [13]. Random-effects models using generalised linear mixed models were used for prevalence pooling with Clopper-Pearson intervals [17,19,20]. The generalised linear mixed model is advocated as a statistical approach that better accounts for within-study variation, with the assumption of a binomial likelihood for individual study events [21,22]. Heterogeneity across studies was quantified by I^2 and τ^2 statistics (I^2 : 0%-25% represents low heterogeneity, 25%-50% moderate heterogeneity, 50%-75% substantial heterogeneity, 75%-100% high heterogeneity). Subgroup analyses of D2T-RA prevalence were conducted to explore the source of heterogeneity according to year of publication (before vs after 2023), geographic regions (Europe/America vs Asia), study design (cross-sectional vs cohort), study setting (monocentre vs multicentre), median enrolment period (pre-2020 vs post-2020; grouping will be determined by the predominant enrolment period for studies spanning 2020), RA ascertainment (exclusively 2010 EULAR/ACR criteria vs others), median sample size (>1000 vs <1000). Studies were stratified by the median enrolment year (2020) to explore temporal trends. This cutoff was selected primarily to achieve balanced group sizes for comparative analysis. According to the enrolment criteria of the included studies, we additionally stratified the D2T-RA prevalence assessment into 2 distinct cohorts: (1) the overall RA population (regardless of treatment status with b/tsDMARDs, including patients naïve to b/tsDMARDs), and (2) specifically among patients exposed to b/tsDMARDs. In subgroup analyses of this meta-analysis, a P value of $< .1$ was considered statistically significant [23,24]. Due to the frequent reporting of disease characteristics exclusively in the D2T-RA subgroup without corresponding data for the overall enrolled population, meaningful subgroup analyses of D2T-RA prevalence across different disease features (eg, age, RA duration, comorbid conditions such as fibromyalgia) were precluded. Sensitivity analyses assessed robustness through leave-one-out methods. Publication bias was evaluated via funnel plots, Egger's test and Begg's test.

RESULTS

Study selection and characteristics

The systematic search identified 1,658 unique records, with 23 studies comprising 27,987 patients with RA meeting

inclusion criteria after screening (Fig 1) [6–12,25–40]. Detailed reasons for the exclusion of articles are in [Supplementary Appendix S2](#). All included studies were published between 2021 and 2025, with geographical distribution showing 17 (40.5%) from Europe, 15 (35.7%) from North America, and 10 (23.8%) from Asia ([Supplementary Table S1](#)). [Figure 2](#) illustrates the geographical distribution of the included studies and their respective sample sizes of total patients and patients with D2T-RA. The map demonstrates a concentration of studies and larger sample sizes in Europe and North America, while highlighting the notable underrepresentation of other regions, particularly Africa and South America. Study designs included 18 cross-sectional analyses (78.2%) and 5 cohort studies (21.8%), all utilising the 2021 EULAR D2T-RA diagnostic criteria. All studies were peer-reviewed publications except 1 conference proceedings [10]. While 22 studies were published in English, 1 utilised non-English languages [8]. For RA ascertainment, the 1989 ACR criteria and/or 2010 EULAR/ACR criteria were applied in all included studies, except 1 study using International Classification of Diseases codes with a positive predictive value $>90\%$ [34]. For population treatment background, 13 studies reported overall RA populations while 10 focused on b/tsDMARD-exposed cohorts [8,9,27,30–32,34–37]. There were 4 studies that reported the results based on overall RA populations and b/tsDMARD-exposed cohorts. The sample sizes of the included studies varied substantially, ranging from 243 to 6,040 participants, with the reported number of D2T-RA cases ranging from 27 to 450 cases. Demographic, clinical characteristics, and outcome metrics of the study populations were summarised ([Supplementary Tables S2, S3](#)). Methodological quality assessment showed all studies were judged as high quality, and the detailed assessment of the risk of bias for each domain is presented in [Supplementary Tables S4 and S5](#).

D2T-RA prevalence

There were 21,454 patients from 18 cross-sectional studies, while 5 cohort studies provided longitudinal data from 6,533 patients with RA with a median follow-up of 3 to 5 years. Of the total 27,987 patients with RA, 3,119 patients were identified as having D2T-RA, with a pooled D2T-RA prevalence of 11.7% (95% CI: 9.5%–14.3%) with substantial heterogeneity ($I^2 = 94\%$) ([Fig 3](#)). Sensitivity analysis using the leave-one-out method showed that the pooled prevalence remained stable, ranging from 11% to 12% ([Supplementary Fig S1](#)). This consistency indicates our findings were not driven by any single study. Funnel plots were symmetrical, and Egger's test ($P = .75$) and Begg's test ($P = .15$) indicated no significant publication bias ([Supplementary Fig S2](#)).

In subgroup analysis, the D2T-RA prevalence was 11.8% (95% CI: 9.4%–14.8%) in 18 cross-sectional studies and 11.2% (95% CI: 6.8%–18.0%) in 5 cohort studies, respectively ($P = .85$) ([Table, Supplementary Fig S3](#)). D2T-RA prevalence was numerically higher in b/tsDMARD-treated populations (13.2% [9.1%–18.8%]) vs all populations (10.9% [8.2%–14.2%]), without a statistical difference ($P = .41$) ([Table, Supplementary Fig S4](#)). D2T-RA prevalence was generally stable in other subgroup analyses, including region, RA criteria, study location, and sample size; significant variations only existed in the enrolled period (pre-2020 vs post-2020:

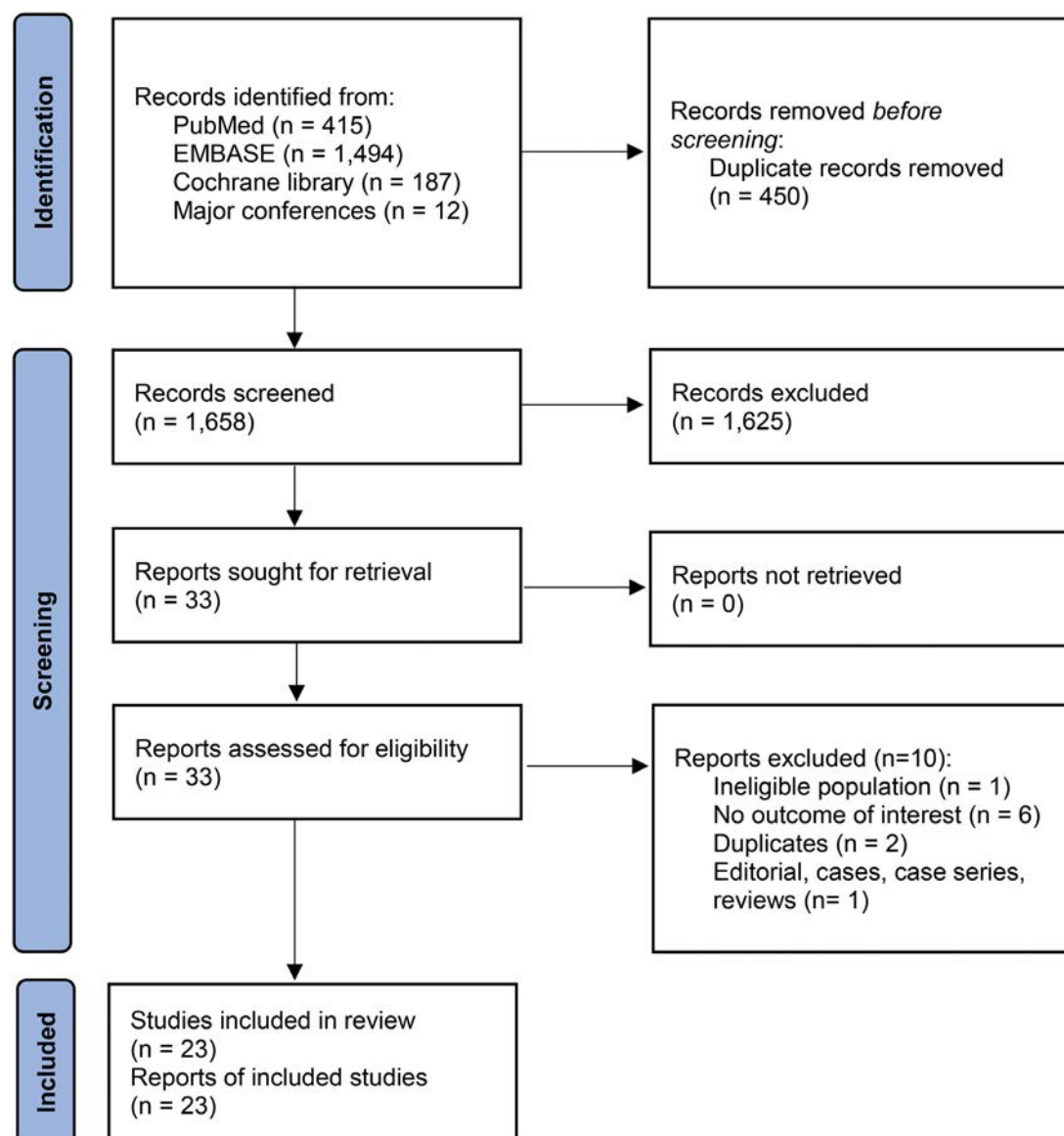


Figure 1. PRISMA flow diagram of the study selection process. PRISMA, Reporting Items for Systematic Reviews and Meta-Analyses.

9.9% [95% CI: 7.6%-12.7%] vs 14.3% [95% CI: 10.3%-19.6%]; $P = .08$) (Table, Supplementary Fig S5).

PIRRA and D2T-RA with stricter criteria

There were 5 studies reporting the proportion of PIRRA and NIRRA [6,12,25,32,38]. The specific definition used to classify PIRRA across the 5 included studies is provided in Supplementary Table S6, with 3 studies utilising ultrasound-defined inflammation [12,32,38]. There was a total of 319 patients with D2T-RA identified from 3,516 patients with RA. Of the 319 D2T-RA, there were 152 patients classified as PIRRA, commonly judged by ultrasound synovitis. The pooled proportion of PIRRA was 47.1% (95% CI: 33.3%-61.4%) in the D2T-RA population, with corresponding NIRRA proportion of 52.9% (Fig 4).

There were 4 [6,7,9,32] and 3 studies [6,7,32] defining more refractory RA through modification of the EULAR criteria—replacing the ≥ 2 b/tsDMARD failure threshold with

≥ 3 or ≥ 4 failures while maintaining other criteria. The pooled prevalence estimates from included studies were: D2T(3)-RA (≥ 3 b/tsDMARD failures): 4.3% (95% CI 2.7%-6.7%), and D2T(4)-RA (≥ 4 failures): 1.6% (95% CI: 0.6%-4.3%) (Fig 5).

DISCUSSION

This meta-analysis provides the first robust global prevalence estimate of D2T-RA using standardised EULAR criteria, establishing a pooled prevalence of 11.7%. Our result consolidates evidence from contemporary large-scale registries and resolves longstanding clinical ambiguity stemming from historically variable estimates (5%-30%). The 12% prevalence of D2T-RA underscores its substantial clinical burden, demanding proactive management strategies. Our data also provided the first reliable benchmark for healthcare resource planning and trial design. Subgroup analyses revealed a significantly higher prevalence of D2T-RA in studies conducted post-2020 (14.3% vs 9.9%

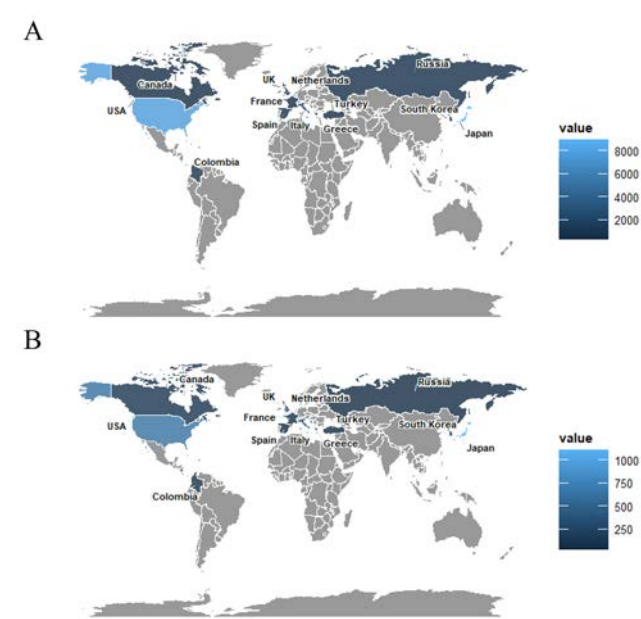


Figure 2. Geographical distribution of included studies according to (A) number of total patients, (B) number of patients with difficult-to-treat rheumatoid arthritis.

pre-2020). While improved treatment access (eg, biosimilars, tsDMARDs) likely contributed to enabling more patients to attempt multiple drug classes and meet EULAR criteria, other factors may also play important roles. These include the well-documented global rise in RA incidence and prevalence over recent decades [1,3], more consistent and precise application of

the EULAR definition in newer studies designed specifically for this purpose. Notably, prevalence remained consistent across geographic regions and healthcare settings, supporting D2T-RA as a universal clinical entity. In addition, constrained reporting of population characteristics in primary studies precluded comprehensive subgroup analyses of disease-modifying factors. This is particularly consequential given established, yet inconsistent evidence suggesting that lifestyle factors (eg, smoking, obesity) and comorbidities (eg, fibromyalgia, depression) may influence D2T-RA prevalence, warranting further investigation in future studies.

A key finding of our study is the near-equal distribution of inflammatory (PIRRA, 47.1%) and noninflammatory (NIRRA, 52.9%) phenotypes. The results underscore that nearly half of patients meeting D2T-RA criteria do not have driven persistent inflammatory activity, and this phenotypic distinction carries significant clinical implications. In general, patients classified as PIRRA exhibit demonstrably higher inflammatory burden, evidenced by significantly greater median swollen joint counts, disease activity scores, and C-reactive protein levels [32]. Imaging examination, such as ultrasound, could help stratify the NIRRA and PIRRA phenotypes. This distinction mandates a paradigm shift in management: patients with PIRRA benefit from treatment escalation (eg, tsDMARDs), whereas NIRRA requires comorbidity management and nonpharmacologic interventions, rather than further immunosuppression. In addition, some researchers have advocated for more stringent definitions of D2T-RA, such as failure in ≥ 3 or ≥ 4 classes of b/tsDMARDs [6,7]. Our study observed stricter definitions (≥ 3 or ≥ 4 b/tsDMARD failures) identified smaller refractory subgroups (4.3% and 1.6%, respectively), highlighting that most

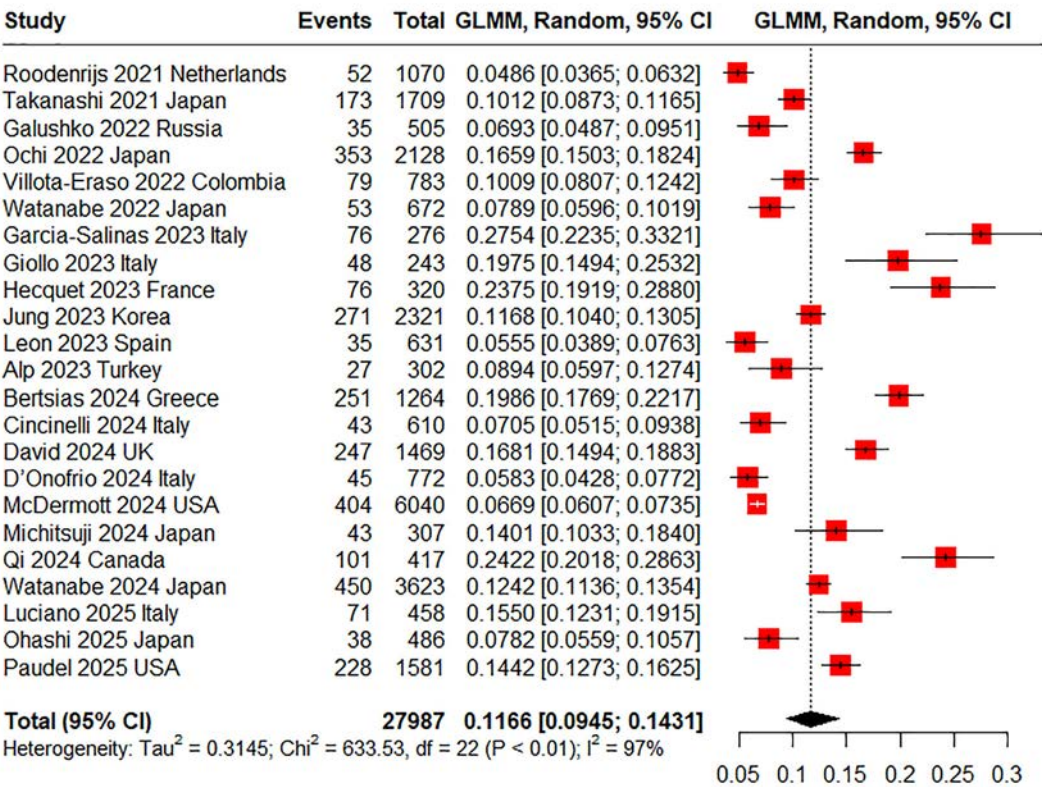


Figure 3. Forest plot of pooled prevalence of difficult-to-treat rheumatoid arthritis in population with rheumatoid arthritis. GLMM, generalised linear mixed models.

Table
Subgroup analysis of difficult-to-treat rheumatoid arthritis prevalence according to the study characteristics

	No. study	Pooled estimate (95% CI)	I ² (%)	P _{between group}
All	23	11.7% (9.5%-14.3%)	97	/
Publication year				
Before 2023	12	11.3% (8.2%-15.3%)	96	.74
After 2023	11	12.1% (9.2%-15.8%)	97	
Region				
Europe or America	15	12.1% (8.9%-16.2%)	98	.62
Asia	8	11.1% (9.2%-13.2%)	90	
Study design				
Cross-sectional	18	11.8% (9.4%-14.8%)	97	.85
Cohort	5	11.2% (6.8%-18.0%)	97	
Setting				
Monocentre	15	12.1% (9.1%-15.8%)	96	.64
Multicentre	8	11.0% (8.2%-14.5%)	97	
Method of rheumatoid arthritis ascertainment				
2010 criteria	13	12.6% (9.1%-17.4%)	96	.35
Others	10	10.5% (8.6%-12.8%)	96	
Enrolled period				
Before 2020	12	9.9% (7.6%-12.7%)	97	.08
After 2020	11	14.3% (10.3%-19.6%)	94	
Sample size				
>1000	9	11.8% (8.9%-15.4%)	98	.94
<1000	14	11.6% (8.6%-15.5%)	95	
Population treatment ^a				
All population	15	10.9% (8.2%-14.2%)	96	.41
b/tsDMARDs-treated	12	13.2% (9.1%-18.8%)	99	

b/tsDMARDs, biological and targeted synthetic disease-modifying antirheumatic drugs.

^a Four study contained the data on all population and b/tsDMARDs-treated patients.

patients achieve control before exhausting therapeutic options. In fact, the ≥ 4 failure criterion likely underrepresents the clinically relevant D2T-RA population due to systemic barriers: drug access limitations prevent many patients globally from reaching later-line therapies. Consequently, EULAR definition with ≥ 2 mechanism-class failures offers greater clinical utility by capturing patients with significant unmet needs irrespective of absolute drug exposure.

The principal strength of this meta-analysis lies in being the first to synthesise global evidence on the prevalence of D2T-RA using standardised EULAR criteria, encompassing 27,987 patients across 23 studies. Beyond quantifying the pooled prevalence, we provide pioneering data on the phenotype distribution of PIRRA and NIRRA. The robustness of these findings is further evidenced by sensitivity analyses showing $<2\%$ prevalence fluctuation across methodological scenarios. However, several limitations merit consideration. First, the predominance of cross-sectional study designs, while optimal for estimating point prevalence, limits our ability to infer causal relationships between

risk factors and the subsequent development of D2T-RA. The retrospective nature of most included studies also introduces a potential for misclassification and confounding bias, as data collection was not originally designed for this specific purpose. Second, our data were limited to 13 countries, leading to a scarcity of available quality data from many areas worldwide, most notably Africa and South America, alongside limited studies from parts of Asia. This gap underscores an urgent ethical and scientific need to conduct epidemiologic studies of D2T-RA in these underrepresented regions to improve geographical coverage. Third, substantial heterogeneity reflects both clinical diversity and methodological variability in applying EULAR criteria. Constrained reporting of baseline characteristics of the whole population precluded comprehensive subgroup analyses. Last, a notable limitation of this analysis is the absence of a universally accepted definition for the PIRRA phenotype [13,41]. Although all included studies employed classification strategies based on the core principle of objective inflammation, the specific operational criteria varied. This heterogeneity may introduce

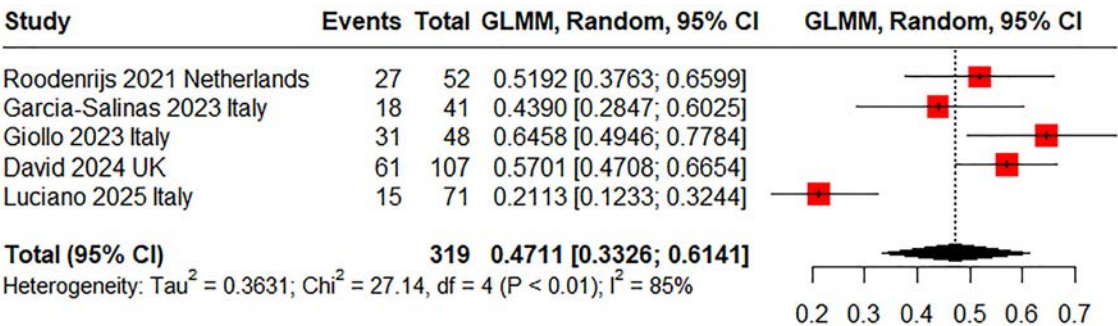


Figure 4. Forest plot of pooled proportion of persistent inflammatory refractory rheumatoid arthritis among patients with difficult-to-treat rheumatoid arthritis. GLMM, generalised linear mixed models.

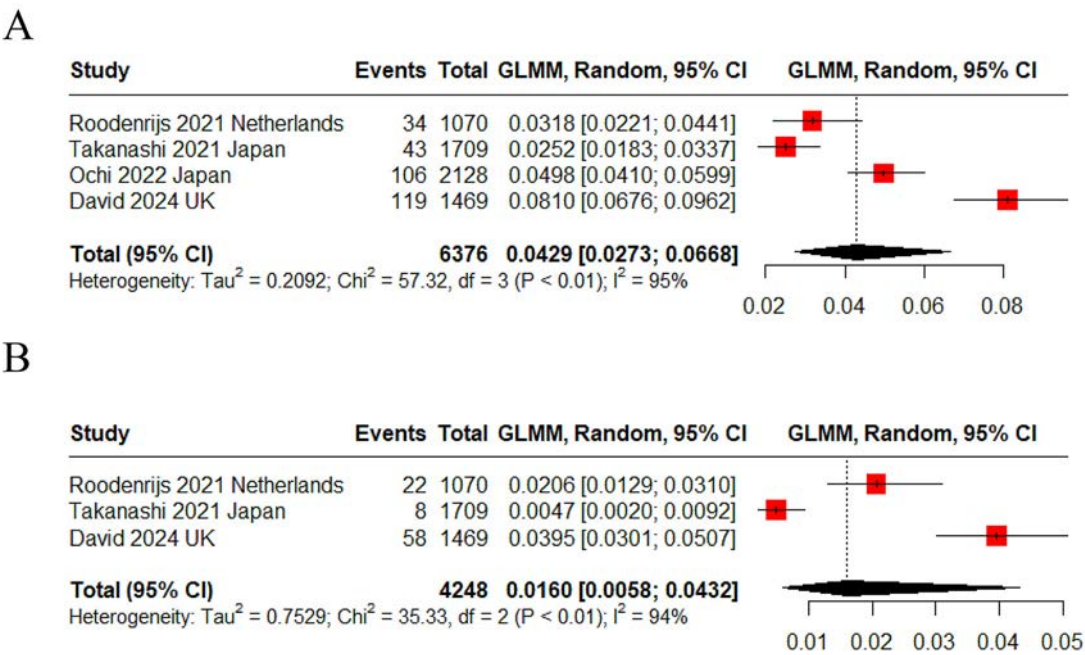


Figure 5. Forest plot of pooled prevalence of difficult-to-treat rheumatoid arthritis using stricter failure criteria (A) ≥ 3 b/tsDMARDs failures, (B) ≥ 4 b/tsDMARDs failures. b/tsDMARDs, biological and targeted synthetic disease-modifying antirheumatic drugs; GLMM, generalised linear mixed models.

misclassification bias and affect estimate precision, highlighting the need for validated consensus criteria in future studies.

CONCLUSION

This meta-analysis establishes an 11.7% global prevalence of EULAR-defined D2T-RA, with nearly half of cases driven by persistent inflammation (PIRRA phenotype). Implementing phenotype-guided management is therefore essential. Stricter criteria (≥ 3 b/tsDMARD failures) identify the small group of patients (4.6% prevalence) for whom innovative treatments or strategies are needed.

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Competing interests

All authors declare they have no competing interests.

CRediT authorship contribution statement

Wenhui Xie: Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tong Chen:** Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Shiyu Xiao:** Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Hong Huang:** Supervision, Software, Methodology, Funding acquisition, Formal analysis, Data curation. **Zhuoli Zhang:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Data curation, Conceptualization.

Patient consent for publication

Patients were not involved in the study design, conduction, development or dissemination of the results.

Ethical approval

All data for this study were obtained from existing publications and ethical approval was not required for this research.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

All relevant data are reported in the manuscript and appendix.

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ar.2025.10.007](https://doi.org/10.1016/j.ar.2025.10.007).

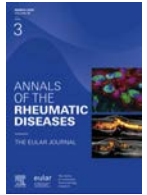
Orcid

Wenhui Xie: <http://orcid.org/0000-0002-3881-0266>

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Rheumatoid arthritis

Inflammation rather than anticitrullinated protein antibodies is associated with cardiovascular mortality in RA: insights from rheumatoid arthritis and coronary artery disease cohorts

Veerle F.A.M. Derksen¹, Jane Lindschou², Per Winkel², Graciela E. Delgado³, Emma C. de Moel¹, Tom W.J. Huizinga¹, Winfried März³, Janus C. Jakobsen^{2,4}, Annette H.M. van der Helm - Van Mil^{1,5}, René E.M. Toes¹, Sofia Ajeganova⁶, Christian Gluud^{2,4}, Jan Leipe^{3,7}, Diane van der Woude^{1,*}, on behalf of the CLARICOR Trial Group and the LURIC Study Group

¹ Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

² Copenhagen Trial Unit, Centre for Clinical Intervention Research, The Capital Region, Copenhagen University Hospital–Rigshospitalet, Copenhagen, Denmark

³ Vth Department of Medicine (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

⁴ Department of Regional Health Research, The Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

⁵ Department of Rheumatology, Erasmus Medical Centre, Rotterdam, The Netherlands

⁶ Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden

⁷ Division of Rheumatology, Department of Internal Medicine I, University Medical Centre Schleswig-Holstein, Kiel, Germany

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ABSTRACT

Objectives: Anticitrullinated protein antibodies (ACPAs) are associated with increased mortality in patients with rheumatoid arthritis (RA). Previous data suggest ACPAs might be associated with worse disease outcomes in patients without RA with coronary artery disease (CAD). Therefore, we investigated ACPA prevalence and its association with mortality in patients with CAD without RA. Furthermore, the role of systemic inflammation in the relation between ACPAs and mortality was investigated in RA.

Methods: The prevalence of ACPAs in patients with CAD without RA was investigated in 2 CAD cohorts (LUDwigshafen Risk and Cardiovascular Health n = 2189 patients and 656 controls; CLARithromycin for patients with stable CORonary heart disease n = 959 patients) using a commercial enzyme-linked immunosorbent assay. Multivariable Cox proportional hazards models were performed to investigate the association between ACPAs and all-cause mortality. In 2 RA cohorts (Early Arthritis Clinic [EAC] n = 764; Better Anti-rheumatic Farmaco-therapy [BARFOT] n = 794), joint models were applied to investigate the role of C-reactive protein (CRP) on the association between ACPAs and (cardiovascular) mortality.

*Correspondence to Prof. Diane van der Woude, Rheumatology Department, Leiden University Medical Center, Leiden, The Netherlands.

E-mail address: dvanderwoude@lumc.nl (D. van der Woude).

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CLARICOR Trial Group members: Hans-Jörn Kolmos, Gorm Boje Jensen, Erik Kjølner, Jens Kastrup, Ahmad Sajadieh, Per Winkel, Janus C. Jakobsen, Jane Lindschou and Christian Gluud

LURIC Study Group members: Graciela E. Delgado, Marcus Kleber, Jan Leipe, and Winfried März

Jan Leipe and Diane van der Woude contributed equally to this work.

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Results: Average follow-up time in the cohorts ranged between 8.2 and 11.8 years. In both CAD cohorts, ACPA prevalence was low (0.9% and 4.6%), and no association was found between seropositivity and all-cause mortality. In patients with RA, the association between ACPA positivity and all-cause mortality was no longer significant after adjustment for CRP. In contrast, CRP was significantly associated with all-cause and cardiovascular mortality in RA (indirect effect hazard ratio [95% CI]: EAC 1.24 [1.14–1.34], BARFOT 1.33 [1.24–1.42]).

Conclusions: ACPA prevalence is not increased in patients with CAD without RA. In RA, the association between ACPA positivity and increased (cardiovascular) mortality was primarily explained by CRP. This highlights the impact of chronic inflammation on cardiovascular outcomes in RA.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Anticitrullinated protein antibodies (ACPAs) are specific for rheumatoid arthritis (RA), and a relationship between ACPAs and increased cardiovascular mortality has been described in patients with RA. However, 2 previous studies have suggested an increased prevalence of ACPAs in patients with coronary artery disease (CAD) without RA, and in these patients, ACPAs were associated with worse disease outcomes.

WHAT THIS STUDY ADDS

- We found that ACPA prevalence was not increased in 2 large cohorts of patients with CAD without RA, and no association between ACPA seropositivity and mortality was identified. In RA, the association between ACPAs and all-cause mortality seems to be explained by higher C-reactive protein (CRP) levels over time observed in ACPA-seropositive patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Inflammation might be more important for increased all-cause and cardiovascular mortality rates in RA than the presence of ACPAs in itself, making therapies targeting continuous inflammation key to improving long-term outcomes for patients with ACPA-positive RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a prevalent autoimmune disease characterised not only by chronic synovial inflammation, but also by an increased risk of cardiovascular disease (CVD) [1,2]. Epidemiological evidence indicates that patients with RA have a 43% increased risk of myocardial infarction and a 55% increased risk of experiencing any major adverse cardiac event compared to the general population [3,4]. Approximately half of this excess risk appears to be attributable to traditional cardiovascular risk factors [5], some of which are also risk factors for the development of RA, such as smoking [6] and obesity [7]. About one-third of the excess cardiovascular risk in RA is thought to result directly from the disease itself [5].

Systemic inflammation is known to contribute to accelerated atherosclerosis and increased cardiovascular events [8–10]. However, there are also indications that RA-specific autoantibodies, especially anticitrullinated protein antibodies (ACPAs), might play a direct role in the increased incidence of CVD in patients with RA. Several disease-specific autoantibodies can be detected in the serum of patients with RA, including rheumatoid factor (RF), ACPAs and antibodies against carbamylated proteins (anti-CarP) [11]. ACPAs are routinely determined in clinical practice and are specific for RA. These autoantibodies are detected in approximately two-thirds of patients with RA and can be present in serum years before the onset of arthritis [12].

Although their role in RA pathophysiology remains incompletely understood, ACPAs are believed to contribute to chronic inflammation, as ACPAs are able to activate the complement system [13] and trigger Fc-receptors leading to proinflammatory cytokine production [14]. Several studies have indicated that ACPAs may be independently associated with increased all-cause mortality in patients with RA, particularly from cardiovascular causes [15–18], although findings remain inconsistent [1,19–23]. Histological studies have identified citrullinated proteins within atherosclerotic plaques [24] and experimental data demonstrated that ACPAs can activate platelets via FcγRIIa-dependent pathways [25]. Taken together, these findings might indicate that ACPAs can contribute directly to the increased cardiovascular risk observed in RA.

There is also evidence that ACPAs may augment cardiovascular risk in individuals who do not have RA. Two clinical studies reported increased ACPA seropositivity in non-RA cohorts: 10.4% (15/144) of patients with coronary artery disease (CAD) compared to 3.8% (11/288) of healthy controls were ACPA-positive in 1 cohort [26], and 11% (29/275) of patients with ST-elevation myocardial infarction (STEMI) were seropositive compared with 2% (3/160) in healthy controls in another cohort [27]. In the latter study, ACPAs were independently associated with worse long-term mortality in patients with STEMI during a median follow of 9.4 years [27]. Notably, none of the 29 ACPA-positive patients developed RA during follow-up. These findings point to a potential direct role for ACPAs in cardiovascular risk in individuals without RA, but require confirmation in larger well-characterised cardiovascular cohorts. Furthermore, it remains unclear whether other RA-associated autoantibodies such as RF or anti-CarP are also present in patients with CAD, and how the presence of autoantibodies is related to cardiovascular mortality in both non-RA and RA populations. Especially, anti-CarP is of interest in this context, as carbamylation has been found in atherosclerotic plaques and increased systemic protein carbamylation was associated with a higher risk of major adverse cardiac events in a population at risk for CVD [28].

To address these questions, we examined the prevalence of ACPAs and other RA-associated antibodies in 2 large, independent non-RA CAD cohorts and evaluated the association between seropositivity and all-cause mortality. In parallel, 2 early RA cohorts were analysed to assess whether systemic inflammation plays a role in the relationship between ACPAs and all-cause or cardiovascular mortality. We hypothesised that the observed association between ACPAs and adverse cardiovascular outcomes in RA might (partly) reflect underlying inflammatory burden instead of a consequence of autoantibody presence by itself.

METHODS

Study design

For the analyses reported here, we used baseline laboratory data, patient demographics and characteristics, and mortality

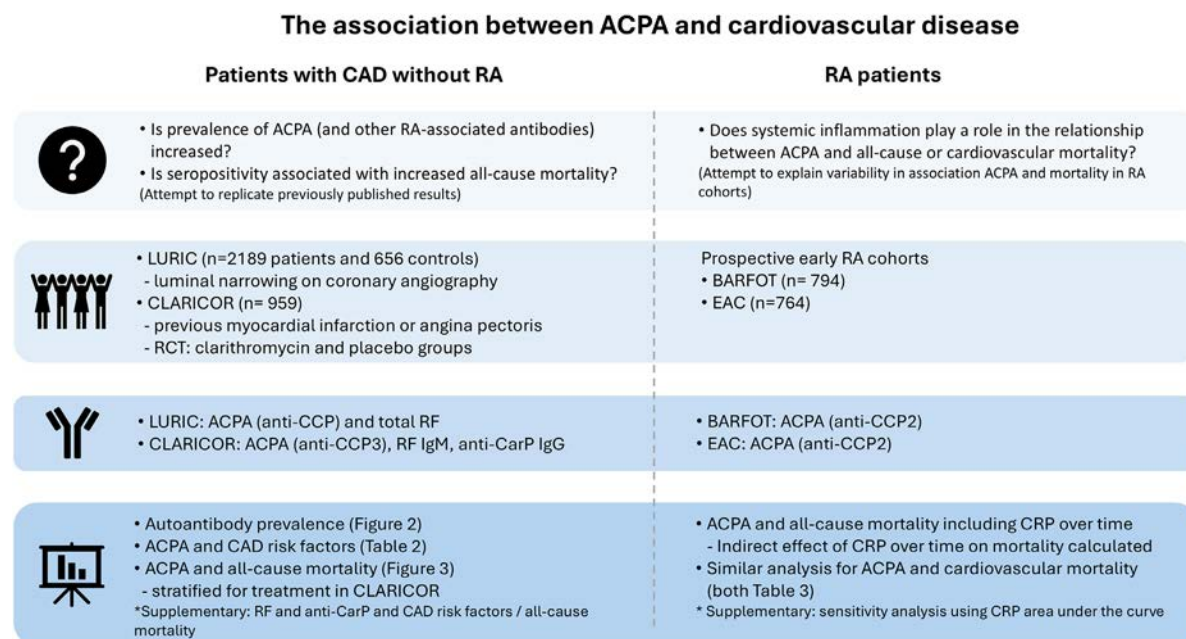


Figure 1. Overview of the study design. Research question, cohorts, autoantibody measurements and analyses are stated in both patients with CAD without and with RA. ACPA, anticitrullinated protein antibody; anti-CarP, antibodies against carbamylated proteins; BARFOT, Better Anti-rheumatic Farmaco-therapy; CAD, coronary artery disease; CCP, cyclic citrullinated peptide; CLARICOR, CLARithromycin for patients with stable CORonary heart disease; CRP, C-reactive protein; EAC, Early Arthritis Clinic; LURIC, LUDwigshafen Risk and Cardiovascular Health; RA, rheumatoid arthritis; RCT, randomised controlled trial; RF, rheumatoid factor.

data collected from 2 large cardiovascular and 2 RA cohorts (see cohort descriptions below). For the cardiovascular cohorts, participants with a reported comorbidity of RA were excluded based on the data available to allow more accurate comparisons between the cardiovascular and RA cohorts. In all 4 original studies, written informed consent was obtained from all participants, and the studies were approved by local ethics committees [29–32]. An overview of the methodology is provided in Figure 1.

CAD non-RA cohorts

To investigate the prevalence of ACPAs and other autoantibodies and their association with mortality in non-RA populations with CAD, autoantibodies were measured specifically for the current study in stored baseline samples from 2 large independent cohorts of patients with CAD: the CLARithromycin for patients with stable CORonary heart disease (CLARICOR) trial (959 patients analysed) [32] and the LUDwigshafen Risk and Cardiovascular Health (LURIC) study (2189 patients and 656 controls analysed) [29].

CLARICOR is a Danish, randomised multicentre trial that enrolled patients with a previous diagnosis of acute myocardial infarction or angina pectoris between 1993 and 1999 (detailed information is described elsewhere [32]). Enrolled patients were randomised to receive clarithromycin or a placebo for a period of 2 weeks between October 1999 and April 2000 [32]. A total of 959 randomly selected patients from both the intervention and control arms were analysed. Ten years follow-up data on cardiovascular outcomes, comorbidities, and mortality were obtained from the Danish National Patient Register, which records diagnoses using the International Statistical Classification of Diseases) with a coverage of almost 100%.

The LURIC study is a German prospective cohort study that enrolled patients with CAD defined as luminal narrowing on angiography between 1997 and 2000 (detailed information is described elsewhere [29]). Clinical indications for angiography

were suspected CAD based on symptoms such as acute chest pain or noninvasive tests consistent with myocardial ischaemia. Follow-up mortality data for this cohort were available until 2010. Although severe chronic disease was an exclusion criterion in LURIC [29], additional screening using baseline comorbidity- and medication data, as well as self-reported (incomplete) follow-up comorbidity surveys, was performed to identify and exclude RA cases from the analyses reported here, leading to the exclusion of 29 patients with CAD and 4 controls. Of the included participants, 2189 patients with CAD and 656 controls (family members and patients who underwent angiography, but did not have CAD) were tested for ACPAs and RF.

RA cohorts

To evaluate the role of systemic inflammation in the relation between ACPAs and mortality in RA, data from 2 prospective early RA cohorts were examined: the Leiden Early Arthritis Clinic (EAC) [30] and the Better Anti-rheumatic Farmaco-therapy (BARFOT) cohort [31]. In both cohorts, antirheumatic drug treatment was initiated by the treating rheumatologist based on the accepted treatment strategies at that time.

In the EAC, 764 patients with early RA, enrolled between 1993 and 2010, were studied (detailed information is described elsewhere [30]). Seventy-nine patients were excluded due to missing data on smoking status. In EAC, between 1993 and 1995 patients were initially treated with nonsteroidal anti-inflammatory drugs. In 1996 to 1998, hydroxychloroquine or sulfasalazine were used early in the disease course, and from 1999 onwards, treatment with methotrexate (MTX) was initiated. C-reactive protein (CRP) levels were measured annually for up to a maximum of 10 years. All-cause mortality data were available through April 2012, while cause-specific mortality could only be assessed through 2008 due to changes in privacy legislation, limiting the scope of the cardiovascular mortality subanalyses to 575 patients [15].

The Swedish BARFOT cohort included 803 patients with newly diagnosed early RA recruited between 1993 and 1999. Nine patients were excluded because of missing smoking status, 6 patients because of missing causes of death. In BARFOT between 1993 and 1996, most patients were initially treated with mild disease-modifying antirheumatic drugs such as sulfasalazine or auranofin, and from 1996 onwards, most patients received early treatment with MTX. CRP was measured at baseline and at follow-up visits at 6, 12, 24, 60, and 96 months. Mortality data were available until December 2010 (detailed information is described elsewhere [31]).

Autoantibody measurements

In CLARICOR, ACPAs were measured in stored baseline serum using the QUANTA Lite cyclic citrullinated peptide (CCP) 3 immunoglobulin (Ig)G enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics). Positivity was calculated according to the manufacturer's instructions using the standard-curve method. RF IgM and anti-CarP IgG were determined using validated in-house ELISAs, and 78 healthy individuals from the Leiden area aged 20 to 70 years were taken along as controls to determine the cut-off (Supplementary Methods S1). In LURIC, ACPA and RF positivity were assessed in samples (either heparin plasma, citrated plasma, or acidified citrated plasma [Stabilyte]) using the Alinity i anti-CCP IgG and Alinity c total RF assays (Abbott), according to manufacturer protocols. In both EAC and BARFOT, ACPAs were measured at baseline using the anti-CCP2 ELISA (Euro-Diagnostica) with a cut-off >25 units.

Statistical analysis

In CLARICOR and LURIC, baseline characteristics and risk factors were compared between autoantibody-positive and autoantibody-negative patients using the appropriate statistical tests (chi-square, Fisher's exact, Student's *t* test, or Mann-Whitney *U* test). Associations between autoantibodies and all-cause mortality were evaluated using Kaplan-Meier curves and log-rank tests. Furthermore, Cox proportional hazards analyses with adjustment for age and sex were performed, with the autoantibody-negative patient group as reference. In CLARICOR, all-cause mortality analyses were performed separately for the clarithromycin and placebo groups, as previous research indicated higher mortality in the clarithromycin group [32]. A meta-analysis based on a random-effects model was performed using R package metafor. The small number of antibody-positive patients prohibited meaningful analyses concerning cause-specific mortality outcomes.

In EAC and BARFOT, previous analyses showing an association between ACPA positivity and both higher all-cause and cardiovascular mortality [15] were extended to explore the role of systemic inflammation on this relation. CRP repeatedly measured over time was used as a proxy for inflammation. As depicted in Supplementary Figure S1, inflammation might be a potential mediating factor in the association between ACPAs and mortality in RA. The relationship between ACPAs and CRP trajectories was analysed using linear mixed-effects models (LMMs) with random intercept and random slope using restricted maximum likelihood estimation. In all analyses, CRP is log-transformed. Joint models, which incorporate both an LMM and a Cox regression model, were employed to simultaneously use the repeatedly measured CRP values for each patient and the time-to-event (mortality) data, with the Cox model adjusted for age, sex, smoking status, and year of

inclusion as proxy of changed treatment strategy over time, in line with previous analyses [15]. Mediation effects were assessed by calculating the indirect effect of CRP on mortality in these joint models [33–35]. As a sensitivity analysis, a joint model with a nonweighted cumulative association structure was calculated, using the CRP area under the curve. Hazard ratios (HRs) with 95% CIs are reported. Further details are provided in Supplementary Methods S2.

RESULTS

Baseline demographics and mortality outcomes across patient populations in all CAD and RA cohorts are shown in Table 1. The mean follow-up time was approximately 9 years. Mortality rates during follow-up ranged from 16% (105/656) in the LURIC control group to nearly 39% (372/959) in CLARICOR.

Autoantibody prevalence in patients with CAD

In CLARICOR, 4.6% (44/959) of patients with CAD without RA tested positive for ACPAs (Fig 2A), which is lower than the 10% to 11% ACPA seropositivity described previously in other patients with CAD populations [26,27]. RF IgM and anti-CarP were detected in 8.6% (82/959) and 3.0% (26/959) of patients, respectively (Fig 2B,C). In contrast to what is commonly observed in patients with RA [36], dual autoantibody positivity was rare in patients with CAD without RA with only 5 patients being ACPAs and RF double positive, and no patients who were ACPAs and anti-CarP double positive. No triple-positive patients with CAD were identified.

In the LURIC cohort, 0.9% (19/2189) of patients with CAD and 0.3% (2/656) of controls were positive for ACPAs (Fig 2D) and 1.1% (24/2189) of patients with CAD and 0.6% (4/656) of controls were positive for RF (Fig 2E). There were no significant differences in the frequency of autoantibody positivity between patients with CAD and controls (anti-CCP: *P* = .19; RF: *P* = .38). Dual autoantibody positivity was low with only 2 patients with CAD being both RF- and ACPA-double positive.

To investigate whether traditional cardiovascular risk factors might be less present in patients with CAD who are ACPA-positive compared to seronegative patients with CAD, for example, due to differences in underlying atherosclerotic processes, cardiovascular risk factors at baseline were compared between ACPA-positive and ACPA-negative individuals in both CLARICOR and LURIC. Age, sex, smoking status, diagnosis of hypertension, low-density lipoprotein (LDL) levels, and percentage of patients with previous myocardial infarction did not significantly differ between ACPA-positive and ACPA-negative patients in either cohort (Table 2). Similar findings were observed for RF (CLARICOR and LURIC, Supplementary Tables S1, S2, respectively) and anti-CarP (CLARICOR only, Supplementary Table S3), with the exception of RF-positive individuals in LURIC being significantly older than RF-negative individuals. Overall, autoantibody-positive and autoantibody-negative patients were comparable concerning cardiovascular risk factors.

Autoantibodies and mortality in patients with CAD

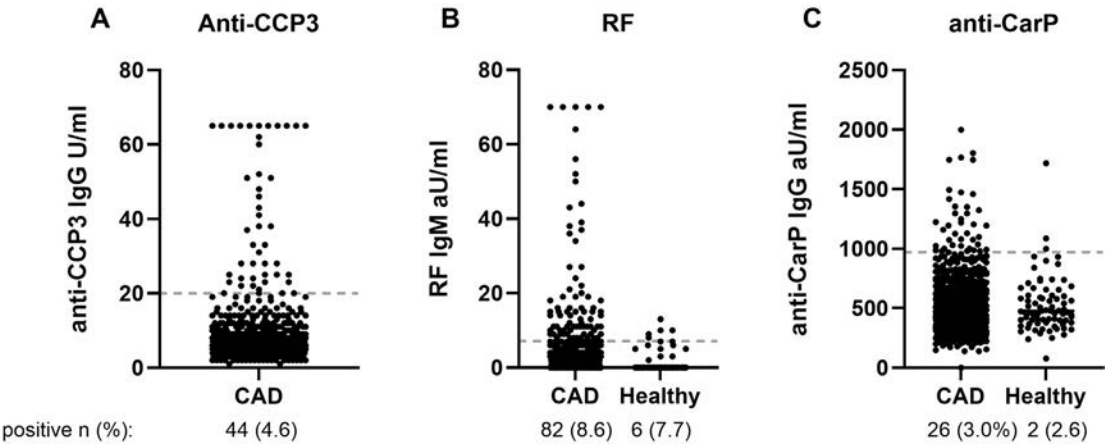
Kaplan-Meier curves for all-cause mortality showed no differences between ACPA-positive and ACPA-negative patients in the clarithromycin and placebo arms of CLARICOR (Fig 3A,B) and in LURIC (Fig 3C) (log-rank tests not significant). Age- and sex-adjusted Cox regression analyses confirmed these findings

Table 1
Demographic data at baseline and information on deaths during follow-up in coronary artery disease and rheumatoid arthritis cohorts

Demographics	CAD cohorts			RA cohorts	
	CLARICOR	LURIC		BARFOT	EAC
	CAD (n = 959)	CAD (n = 2189)	Controls (n = 656)	RA (n = 794)	RA (n = 764)
Baseline					
Age (y), mean ± SD	65.3 ± 10.4	63.6 ± 10.0	58.3 ± 12.1	57.5 ± 15.6	56.8 ± 15.7
Female, n (%)	293 (30.6)	532 (24.3)	313 (47.7)	524 (66.0)	514 (67.3)
Smoking ever, n (%)	814 (84.9)	1503 (68.7)	319 (48.6)	469 (59.1)	415 (54.3)
BMI in kg/m ² , mean ± SD	-	27.5 ± 3.9	27.5 ± 4.4	25.4 ± 5.0	25.8 ± 3.9
Myocardial infarction, n (%)	630 (65.7)	1157 (52.9)	-	-	-
Follow-up					
Follow-up (y), mean ± SD	8.2 ± 2.9	8.7 ± 3.1	9.5 ± 2.4	11.8 ± 3.7	9.0 ± 4.8
Death, n (%)	372 (38.8)	694 (31.7)	105 (16.0)	228 (28.7)	137 (17.9)
Death from cardiac cause, n (%)	170 (17.7)	394 (18.0)	49 (7.5)	141 (17.8)	40 (5.2)

BARFOT, Better Anti-rheumatic Farmaco-therapy; BMI, body mass index; CAD, coronary artery disease; CLARICOR, CLARithromycin for patients with stable CORonary heart disease; EAC, Early Arthritis Clinic; LURIC, LUDwigshafen Risk and Cardiovascular Health; RA, rheumatoid arthritis.

CLARICOR



LURIC

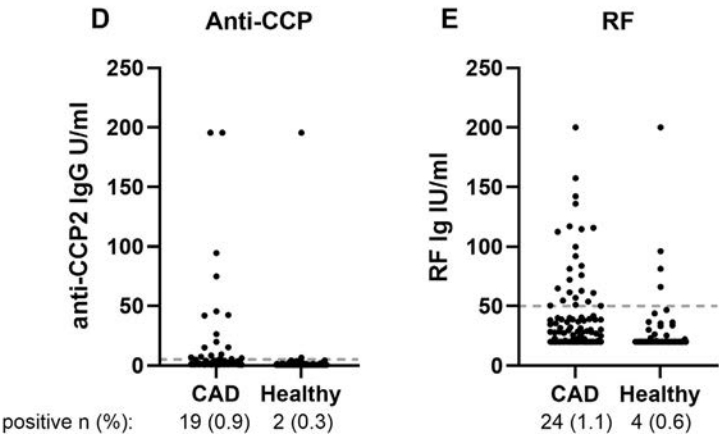


Figure 2. Autoantibody measurements in CAD cohorts. CLARICOR: (A) anti-CCP3 IgG in patients with CAD only, (B) RF IgM in patients with CAD and Leiden healthy controls, (C) anti-CarP IgG in patients with CAD and Leiden healthy controls. LURIC: (D) anti-CCP IgG and (E) total RF in patients with CAD and LURIC controls. Dashed lines represent assay cut-offs. anti-CarP, antibodies against carbamylated proteins; CAD, coronary artery disease; CLARICOR, CLARithromycin for patients with stable CORonary heart disease; CCP, cyclic citrullinated peptide; Ig, immunoglobulin; LURIC, LUDwigshafen Risk and Cardiovascular Health; RF, rheumatoid factor.

(CLARICOR clarithromycin group HR 0.94 [95% CI: 0.52-1.68]; CLARICOR placebo group HR 0.63 [95% CI: 0.26-1.53]; LURIC HR 0.87 [95% CI: 0.39-1.94]; [Supplementary Table S4](#)), which were pooled using meta-analysis (HR 0.84 [95% CI: 0.55-1.27]).

Similar results were obtained for RF and anti-CarP in CLARICOR ([Supplementary Fig S2A-D](#)), which were further confirmed with adjusted Cox regression analyses ([Supplementary Table S4](#)). In the LURIC study, RF-positive patients displayed worse survival

Table 2
Cardiovascular risk factors at baseline in CLARICOR and LURIC by ACPA status

Patient characteristics	CLARICOR			LURIC		
	anti-CCP3– (n = 915)	Anti-CCP3 + (n = 44)	P value	Anti-CCP– (n = 2170)	anti-CCP + (n = 19)	P value
Age (y), mean ± SD	65.2 ± 10.4	67.1 ± 9.6	.20	63.6 ± 10.0	62.7 ± 11.1	.72
Female, n (%)	281 (30.7)	12 (27.3)	.75	527 (24.3)	5 (26.3)	.79
Smoking ever, n (%)	775 (84.7)	39 (88.6)	.62	1489 (68.6)	14 (73.7)	.82
Clarithromycin group, n (%)	452 (49.4)	28 (63.6)	.091	-	-	-
BMI (kg/m ²), mean ± SD	-	-	-	27.5 ± 3.9	25.9 ± 3.7	.073
Hypertension, n (%)	371 (40.5)	19 (43.2)	.85	1623 (74.8)	15 (78.9)	.80
LDL cholesterol in mmol/L, median (IQR)	2.5 (2.1-3.0)	2.5 (2.0-3.1)	.90	2.9 (2.4-3.5)	2.8 (2.3-3.2)	.36
Previous myocardial infarction, n (%)	597 (65.2)	33 (75.0)	.24	1144 (52.7)	13 (68.4)	.26

ACPA, anticitrullinated protein antibody; BMI, body mass index; CLARICOR, CLARithromycin for patients with stable CORonary heart disease; CCP, cyclic citrullinated peptide; LDL, low-density lipoprotein; LURIC, LUDwigshafen Risk and Cardiovascular Health.

In CLARICOR, ACPA is measured using anti-CCP3 tests, in LURIC using anti-CCP tests.

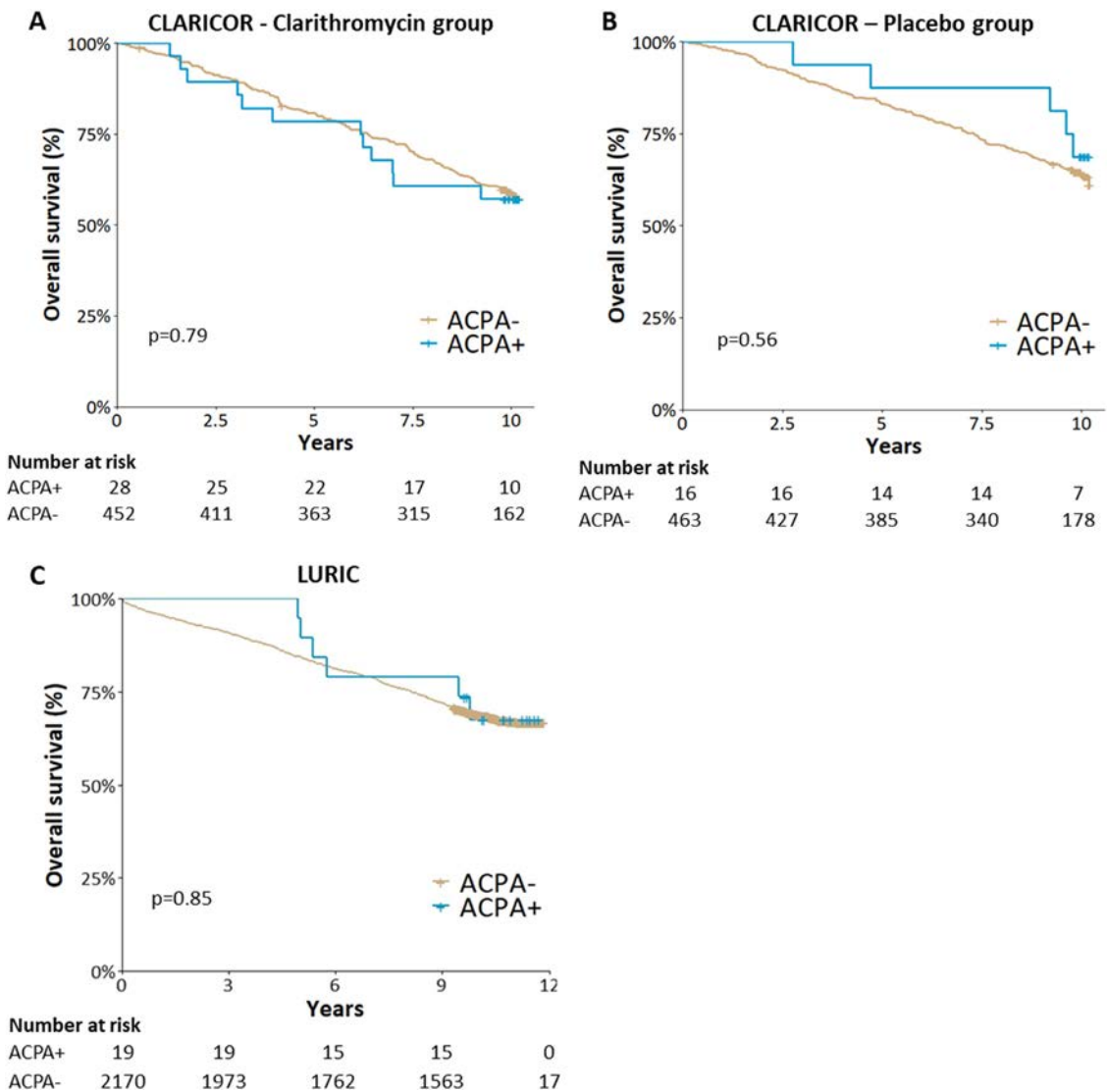


Figure 3. Association between ACPA positivity and all-cause mortality depicted using Kaplan-Meier curves. (A) CLARICOR clarithromycin treatment group, (B) CLARICOR placebo group, (C) LURIC. P values calculated with log-rank tests. Number of patients at risk in both strata over time is listed below each graph. ACPA, anticitrullinated protein antibody; CLARICOR, CLARithromycin for patients with stable CORonary heart disease; LURIC, LUDwigshafen Risk and Cardiovascular Health.

compared to their RF-negative counterparts (log-rank test $P = .011$; [Supplementary Fig S2E](#)), but after correction for age and sex this difference was no longer significant (HR 1.40 [95%

CI: 0.81-2.43]; [Supplementary Table S4](#)). Based on these results, it seems that the presence of ACPAs does not associate with worse all-cause mortality in patients with CAD.

Table 3
Association between ACPA and mortality in 2 RA cohorts

Model	HR (95% CI) in EAC	HR (95% CI) in BARFOT
All-cause mortality		
Adjusted Cox regression	1.66 (1.17-2.37)	1.50 (1.13-1.99)
Joint model including CRP	1.22 (0.70-2.17)	1.14 (0.73-1.75)
Cardiovascular mortality		
Adjusted Cox regression	1.57 (0.82-2.99)	2.10 (1.46-3.01)
Joint model including CRP	0.92 (0.31-2.53)	1.65 (0.95-2.92)

ACPA, anticitrullinated protein antibody; BARFOT, Better Anti-rheumatic Farm-aco-therapy; CRP, C-reactive protein; EAC, Early Arthritis Clinic; HR, hazard ratio; RA, rheumatoid arthritis.

HRs with 95% CI for ACPA in both adjusted Cox regression (without CRP) and joint models (including CRP) in EAC and BARFOT are given. Outcome was either all-cause mortality or cardiovascular death. Survival analysis was adjusted for age, sex, inclusion period and smoking status.

ACPAs, CRP, and mortality in patients with RA

In contrast to the results in the CAD cohorts, ACPA positivity has been associated with increased all-cause mortality in multiple cohorts of patients with RA [15,18,37]. This raises the question whether the increase in mortality rates in ACPA-positive patients with RA can be solely attributed to the presence of autoantibodies or whether other factors, such as systemic inflammation, are involved. If so, CRP over time, as a proxy for systemic inflammation, could be a mediator in the association between ACPAs and mortality in patients with RA (as depicted in [Supplementary Fig S1](#)). To investigate this hypothesis, we reanalysed data from 2 early RA cohorts, the EAC and the BARFOT cohort. During follow-up, 137/764 patients (17.9%) died in EAC and 228/794 (28.7%) in BARFOT. Multivariable Cox models on the association between ACPAs and all-cause mortality, including age, sex, smoking status, and year of inclusion as covariates, were repeated and provided results consistent with previous findings (EAC HR 1.66 [95% CI: 1.17-2.37]; BARFOT HR 1.50 [95% CI: 1.13-1.99]; [Table 3](#)) [15]. Furthermore, CRP over time was significantly associated with ACPA positivity (EAC $\beta = 0.13$ [95% CI: 0.08-0.18]; BARFOT $\beta = 0.16$ [95% CI: 0.12-0.20]), a prerequisite for CRP to be a potential mediator. When CRP was added using a joint model, the association between ACPAs and all-cause mortality was attenuated and became nonsignificant (EAC HR 1.22 [95% CI: 0.70-2.17]; BARFOT HR 1.14 [95% CI: 0.73-1.75]; [Table 3](#), for complete models see [Supplementary Tables S5, S6](#)). In contrast, there was a significant indirect effect of logCRP on mortality (EAC HR 1.24 [95% CI: 1.14-1.34]; BARFOT HR 1.33 [95% CI: 1.24-1.42]). Thus, the association between ACPAs and all-cause mortality found earlier in BARFOT and EAC is most likely largely explained by CRP.

Analyses were repeated using cardiovascular mortality instead of all-cause mortality as the outcome measure. A total of 40/575 patients (7.0%) in EAC and 141/788 (17.9%) in BARFOT died from cardiovascular causes. A significant association between ACPA positivity and cardiovascular mortality was observed in BARFOT (HR 2.10 [95% CI: 1.46-3.01]), but not in EAC (HR 1.57 [95% CI: 0.82-2.99]) in multivariable Cox regression. Adjustment for CRP in the joint model reduced the HRs, now being nonsignificant in both cohorts (EAC HR 0.92 [95% CI: 0.31-2.53]; BARFOT HR 1.65 [95% CI: 0.95-2.92]; [Table 3](#), for complete models see [Supplementary Tables S7, S8](#)). The calculated indirect effect for logCRP over time on cardiovascular mortality was

significant in both cohorts (EAC HR 1.45 [95% CI: 1.27-1.66]; BARFOT HR 1.42 [95% CI: 1.31-1.54]). Sensitivity analysis using the cumulative CRP model yielded similar results ([Supplementary Table S9](#)).

DISCUSSION

In this study, the relationship between ACPA positivity and mortality was investigated in 2 large cohorts of patients with CAD without RA as well as in 2 cohorts of patients with RA. In contrast to previous findings [26,27], the prevalence of ACPAs in individuals with CAD without RA was not significantly higher compared to controls without CAD. In both CLARICOR and LURIC, the presence of ACPAs was not associated with worse all-cause mortality in patients with CAD. Additional analyses investigating other RA-associated autoantibodies similarly showed no significant association between RF and anti-CarP and mortality in patients with CAD without RA. Regarding RA, on the other hand, multiple studies have reported a significant association between ACPA positivity and increased all-cause mortality [15,17,18,37]. However, using joint modelling analyses, we found that the HR for the effect of ACPAs on all-cause and cardiovascular mortality became nonsignificant after the addition of CRP over time, while increased CRP levels were significantly associated with mortality. Thus, high inflammation over time seems to be a more important factor for mortality and CVD in patients with RA than the presence of ACPAs in itself.

The prevalence of ACPAs in patients with CAD in LURIC and in CLARICOR was lower than the 10% to 11% ACPA positivity previously described in 2 other CAD non-RA cohorts [26,27]. There might be several explanations for this disparity. Test characteristics might vary between different commercial assays, but this seems unlikely to fully explain the different findings. Differences in size and inclusion criteria between cohorts may further explain differences in ACPA frequencies and outcomes. For example, in CLARICOR, 959 patients with a previous diagnosis of myocardial infarction or angina pectoris were included, a diagnosis which could have been established several years before, while 1 of the previous studies included 275 patients at the time of STEMI diagnosis [27]. In the other study by Cambridge et al [26], 3052 healthy middle-aged men without clinical CAD were included with a follow-up of 5 years, whereas in LURIC 2189 patients with CAD were included. Moreover, using the Danish National Patient Register, we could reliably exclude patients with a concomitant diagnosis of RA from the patients with CAD in CLARICOR, whereas in the other studies, there might have been unidentified patients with RA left in CAD groups, skewing the results. The low number of patients with CAD without RA positive for multiple RA-related autoantibodies further supports the successful exclusion of patients with RA from our investigation.

In both RA cohorts, chronic inflammation seemed to play a more important role in the increased all-cause and cardiovascular mortality risk in ACPA-positive patients with RA than the presence of ACPAs in itself. It is known that systemic inflammation can lead to atherosclerosis and CAD via increased oxidative stress and endothelial dysfunction [1,2,8,9]. Our findings are in line with previous reports that found that the risk of cardiovascular events was higher in patients with RA with higher disease activity scores (DAS) [38,39] and increased CRP levels [40,41]. ACPA positivity might reflect a disease state in which there is more ongoing inflammation, explaining why multiple studies have reported a significant association between ACPA positivity and increased all-cause mortality before [15,17,18,37].

Early intense treatment in RA was shown to normalise excess mortality rates in ACPA-negative patients with RA, but in ACPA-positive patients, mortality rates are still higher than in the general population [37]. Hence, therapies specifically targeting the continuous underlying inflammation in ACPA-positive RA might be the key to improve long-term outcomes. Several studies investigated the effect of intensifying immunosuppressive therapy on cardiovascular risk in RA, including both seropositive and seronegative patients, based on surrogate endpoints. In the treatment of early aggressive rheumatoid arthritis (TEAR) trial, total cholesterol increased after treatment initiation, regardless of treatment regimen (MTX monotherapy, MTX and etanercept duo therapy or MTX, sulfasalazine, and hydroxychloroquine triple therapy), and was inversely correlated with DAS and CRP [42]. Over time, patients receiving triple therapy had higher levels of the favourable high-density lipoprotein cholesterol and lower levels of LDL cholesterol (LDL-C) compared to patients on other treatments [42]. Addition of the interleukin-6 receptor blocker tocilizumab in patients treated with MTX led to a reduction of inflammation and other vascular risk surrogate markers such as fibrinogen, but was also associated with elevations of total cholesterol and LDL-C [43]. In contrast, a more recent study showed that in patients with RA with moderate disease activity on MTX, both add-on therapy with tumour necrosis factor α inhibitors or with sulfasalazine and hydroxychloroquine led to a reduction in arterial inflammation based on ^{18}F -fluorodeoxyglucose positron emission tomography/computed tomography scans [44]. In summary, these studies using surrogate endpoints for cardiovascular risk do not allow a definitive conclusion regarding the long-term effect of specific RA treatment strategies on clinical CVD.

Our study has several limitations. The number of identified ACPA-positive patients with CAD was limited despite using large CAD non-RA cohorts, which restricted analyses, for example, on cardiovascular mortality. Furthermore, there was no uniformity in ACPA tests among cohorts and differences in specificity and sensitivity might have led to differences in positivity rates. Except for LURIC, no population-specific control groups were available. Although in CLARICOR patients with RA were excluded from the CAD non-RA group with high certainty, comorbidity registration over time was less thorough in LURIC and depended on chart review of medication at inclusion and survey data on comorbidity and medication use over time. The low number of LURIC patients that are positive for more than 1 RA-specific autoantibody indicates this approach was successful, but the possibility that a few patients with RA might have remained in the analyses cannot be excluded. Moreover, in both RA cohorts, CRP was measured on a yearly basis, providing only a rough estimate of cumulative inflammation over time. In the CAD cohorts, there was no information about inflammation over time available. In the EAC cohort cause-specific mortality data were only available for a subset of patients because of changes in privacy regulations, thereby decreasing the power of the analyses on cardiovascular mortality in this cohort.

Despite these drawbacks, our study also has considerable strengths. Two large CAD cohorts including follow-up data were used, making this, to the best of our knowledge, the most extensive study into the prevalence of ACPAs in the non-RA CAD population to date. In addition to ACPAs, we also evaluated RF and anti-CarP, which have also been indicated to potentially impact CVD and mortality [15,45]. Furthermore, findings in CAD cohorts were complemented with analyses of 2 large early RA cohorts with well-documented CRP and mortality data. Advanced joint modelling techniques, which are only rarely used in the field of

rheumatology despite its appealing name, were applied to adjust mortality analyses in these patients with RA for CRP, a time-varying covariate, to optimise the modelling of inflammation over time. In this manner, we feel this study provides the most comprehensive answer to date on the question of whether ACPAs are associated with CVD in individuals with and without RA.

Taken together, our research shows that ACPA positivity is not associated with CVD in patients without RA. The association between ACPA positivity and CVD in RA, described before by others and in our cohorts, is likely explained by the higher inflammation over time in seropositive patients with RA. Therefore, in contrast to seronegative RA, in patients with ACPA-positive RA, the ongoing chronic inflammatory process seems to be especially critical in the development of CVD and is therefore an important therapeutic target to improve long-term outcomes for these seropositive patients with RA.

Competing interests

None.

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Contributors

All authors meet the 4 criteria for authorship. All authors contributed to study design/data acquisition/analyses, were involved in drafting or critically revising the manuscript, approved the final version for publication and agree to ensure that any questions related to accuracy or integrity of the work are appropriately investigated and resolved.

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Patient consent for publication

All participants provided written informed consent.

Ethics approval

All studies were performed in concordance with the Declaration of Helsinki, approved by the relevant local medical ethical committee.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data are available upon reasonable request. For the R code for the analyses, or the statistical analysis plan drafted before starting this study, please contact Veerle Derksen. Deidentified participant data belong to the respective study groups: LURIC group (prof Winfried März), CLARICOR trial group (prof Christian Glud), the BARFOT group (Dr Sofia Ajeganova), and the EAC (prof Annette van der Helm - Van Mil). This data can only be used in collaboration with the study groups.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ard.2025.11.023.

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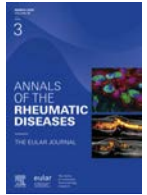
Veerle F.A.M. Derksen: <http://orcid.org/0000-0002-8246-7055>

Diane van der Woude: <http://orcid.org/0000-0001-8121-5879>

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Rheumatoid arthritis

METTL1-mediated internal m⁷G methylation of cathepsin B mRNA promotes synovial aggression in rheumatoid arthritis

Simin Chen¹, Kai Sun¹, Chenxi Peng¹, Yu Kuang¹, Shuoyang Zhang¹, Ruiru Li¹, Huijuan Hu¹, Suling Liu¹, Fan Su¹, Qian Qiu¹, Liuqin Liang¹, Ligang Jie², Youjun Xiao¹, Hanshi Xu^{1,*}

¹ Department of Rheumatology and Immunology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong Province, China

² Department of Rheumatology, Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong Province, China

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ABSTRACT

Objectives: Recent studies show that methyltransferase-like 1 (METTL1)-mediated internal messenger ribonucleic acid (mRNA) N⁷-methylguanosine (m⁷G) modification has a unique role in cancer metastasis. Here, we aimed to uncover the role of METTL1-mediated internal mRNA m⁷G in controlling fibroblast-like synoviocytes' (FLSs') functions in rheumatoid arthritis (RA).

Methods: FLSs were separated from patients with active established RA. Western blot, immunohistochemistry, and immunofluorescence were used to measure protein expression in synovium. The Boyden chamber was used to detect cell migration and invasion. m⁷G RNA immunoprecipitation sequencing was performed to seek the potential target of METTL1. Dual-luciferase reporter gene assay was used to investigate the m⁷G-dependent regulation of cathepsin B (*CTSB*) by METTL1. The protein translation efficiency was detected by polysome profiling. *METTL1* heterozygous knockout or intra-articular injection of *METTL1* short hairpin ribonucleic acid adenovirus (Adv-shRNA-*METTL1*) was used to inhibit arthritis in RA models.

Results: We observed increased levels of *METTL1* and internal mRNA m⁷G in FLSs and synovial tissues from patients with RA. *METTL1* knockdown or overexpression decreased or increased the migration and invasion of RA FLSs. Synovial *METTL1* level was positively correlated with the disease activity score on 28 joints-erythrocyte sedimentation rate scores in patients with RA. *METTL1* knockdown *in vivo* mitigated the severity of arthritis in RA animal models. Mechanistically, we probed that METTL1 promotes the aggressive action of RA FLSs through regulating the translation efficiency of the internal mRNA m⁷G modification of *CTSB*. *CTSB* knockdown also suppressed the aggression of RA FLSs.

Conclusions: Our findings reveal an important role of METTL1-mediated internal mRNA m⁷G modification in promoting synovial aggression of RA, suggesting that METTL1 might be a potential target for therapy of RA, even other dysregulated FLS-associated diseases.

*Correspondence to Dr. Hanshi Xu, Department of Rheumatology and Immunology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

E-mail address: xuhanshi@mail.sysu.edu.cn (H. Xu).

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Simin Chen, Kai Sun, and Chenxi Peng contributed equally.

Drs. Hanshi Xu, Youjun Xiao, and Ligang Jie have made substantial contributions to this work and should be considered co-corresponding authors.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Activated rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLSs) drive persistent synovial inflammation, exhibit resistance to apoptosis, and display hyperplasia and aggressive behaviour. However, the underlying molecular mechanism of activation remains unclear, and targeted therapies are currently lacking.
- Methyltransferase-like 1 (METTL1)-mediated m⁷G transfer ribonucleic acid (tRNA) modification plays a critical role in the pathogenesis of multiple human disorders, including cancers and autoimmune diseases.
- The involvement of the METTL1/tryptophan-aspartic acid (WD) repeat domain 4 (WDR4) complex in regulating internal messenger ribonucleic acid (mRNA) m⁷G modification has been suggested; however, the role of METTL1-mediated internal mRNA m⁷G modification in autoimmune diseases, such as RA, remains unknown.

WHAT THIS STUDY ADDS

- We demonstrate that elevated synovial METTL1-mediated m⁷G modification enhances the migration and invasion of RA FLSs.
- We also show that *in vivo* attenuation of *METTL1*, achieved either through heterozygous deletion or localised knockdown in the joint, leads to improved bone erosion in RA animal models.
- Our findings show that METTL1 drives synovial inflammation and bone erosion by regulating m⁷G modifications on cathepsin B mRNA.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This work will expand the understanding of epigenetic dysregulation in RA FLSs pathological activation through mRNA modifications, identifying promising new targets for therapeutic intervention.

INTRODUCTION

Rheumatoid arthritis (RA), a systemic autoimmune disease characterised by chronic synovial inflammation and joint damage, is one of the main causes of disability and loss of function. Over the past 20 years, prominent advancements have been made in the treatment of RA, particularly with the application of biological agents and small molecule targeted drugs, which have significantly improved the prognosis of RA; however, a considerable number of patients have no or less response to current treatment [1], which indicates our limited understanding of the cellular mechanisms that promote rheumatoid synovial inflammation and joint damage [2].

Fibroblast-like synoviocytes (FLSs), a main type of tissue-resident cells in the joint, play a critical role in modulating synovial homeostasis and joint destruction in RA [3–5]. Activated RA FLSs result in a persistent synovial inflammatory process and present resistance to apoptosis, hyperplasia, and aggressive behaviour. Interestingly, recent studies indicate that RA FLSs exhibit different subtypes with distinct functions [5–7]. Based on their anatomical localisation within synovial tissue, RA FLSs can be further subdivided into lining layer and sublining layer subsets. Specifically, PDPN⁺THY1[−] FLSs are distributed within the lining layer, whereas PDPN⁺THY1⁺ FLSs are localised to the sublining layer. Lining FLSs are mainly responsible for driving bone and cartilage destruction, while sublining FLSs primarily fuel inflammatory processes. These 2 subsets constitute

anatomically distinct and functionally heterogeneous cell populations, underscoring their independent roles in synovial tissue architecture and pathogenic mechanisms [4], as well as the complexity of RA synovitis. While systemic disease-modifying anti-rheumatic drugs (DMARDs) remain the cornerstone of treatment, directly targeting these resilient, locally destructive FLS populations has emerged as a crucial strategy to achieve better control over both inflammation and joint destruction in RA [8]. However, how FLSs exhibit stable activation and become imprinted aggressors in RA is still to be defined. Increased evidence has shown that epigenetic modifiers, including RNA modification and noncoding RNA, are involved in rheumatoid pathogenesis and provide new insight into the RA therapy [9–15]. For example, m⁶A demethylase ALKBH5 and fat mass and obesity-associated protein (FTO) have been reported to be increased in FLSs and synovial tissues from patients with RA, which promotes inflammation and aggressive behaviour of RA FLSs [12,13].

Among many types of RNA modifications, N⁷-methylguanosine (m⁷G), one of the most conserved modifications occurring in transfer ribonucleic acid (tRNA), ribosomal ribonucleic acid (rRNA), messenger ribonucleic acid (mRNA) cap, is critical for controlling mRNA splicing, export, and translation. Methyltransferase-like 1 (METTL1), formed a complex with tryptophan-aspartic acid (WD) repeat domain 4 (WDR4), is the primary known ‘writer’ protein that catalyses m⁷G methylation in mammals [16,17]. Previous studies focus on the important role of METTL1-mediated m⁷G tRNA modification in several human disorders, including cancers and autoimmune diseases [17–21]. Recently, accumulating evidence indicates that METTL1 is identified in some cell lines, such as cancer cells and neural stem cells [22,23]. Accordingly, METTL1/WDR4 complex is also involved in regulating mRNA internal m⁷G modification [22]. However, to date, the role of METTL1-mediated internal mRNA m⁷G modification in autoimmune diseases including RA is still unknown.

In the present study, we determined the role of METTL1-mediated internal mRNA m⁷G modification in controlling functions of RA FLSs and explored its underlying mechanisms. Moreover, we investigated the effect of *METTL1* heterozygous deletion or intra-articular injection of *METTL1* short hairpin ribonucleic acid adenovirus (Adv-shRNA-*METTL1*) on the severity of arthritis in models of RA.

METHODS

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

See other methods in online [supplemental materials](#).

RESULTS

Elevated METTL1-mediated m⁷G modification in FLSs and synovial tissues from patients with RA

We initiated our investigation by measuring the levels of m⁷G in mRNA derived from FLSs of patients with RA and healthy controls (HCs). The results showed significantly increased levels of m⁷G modification in the mRNA from RA FLSs compared to HC FLSs, as demonstrated by m⁷G dot blot analysis (Fig 1A, [Supplementary Fig S1A–C](#)). To further elucidate the underlying causes of the elevated m⁷G modification in RA FLSs, we assessed

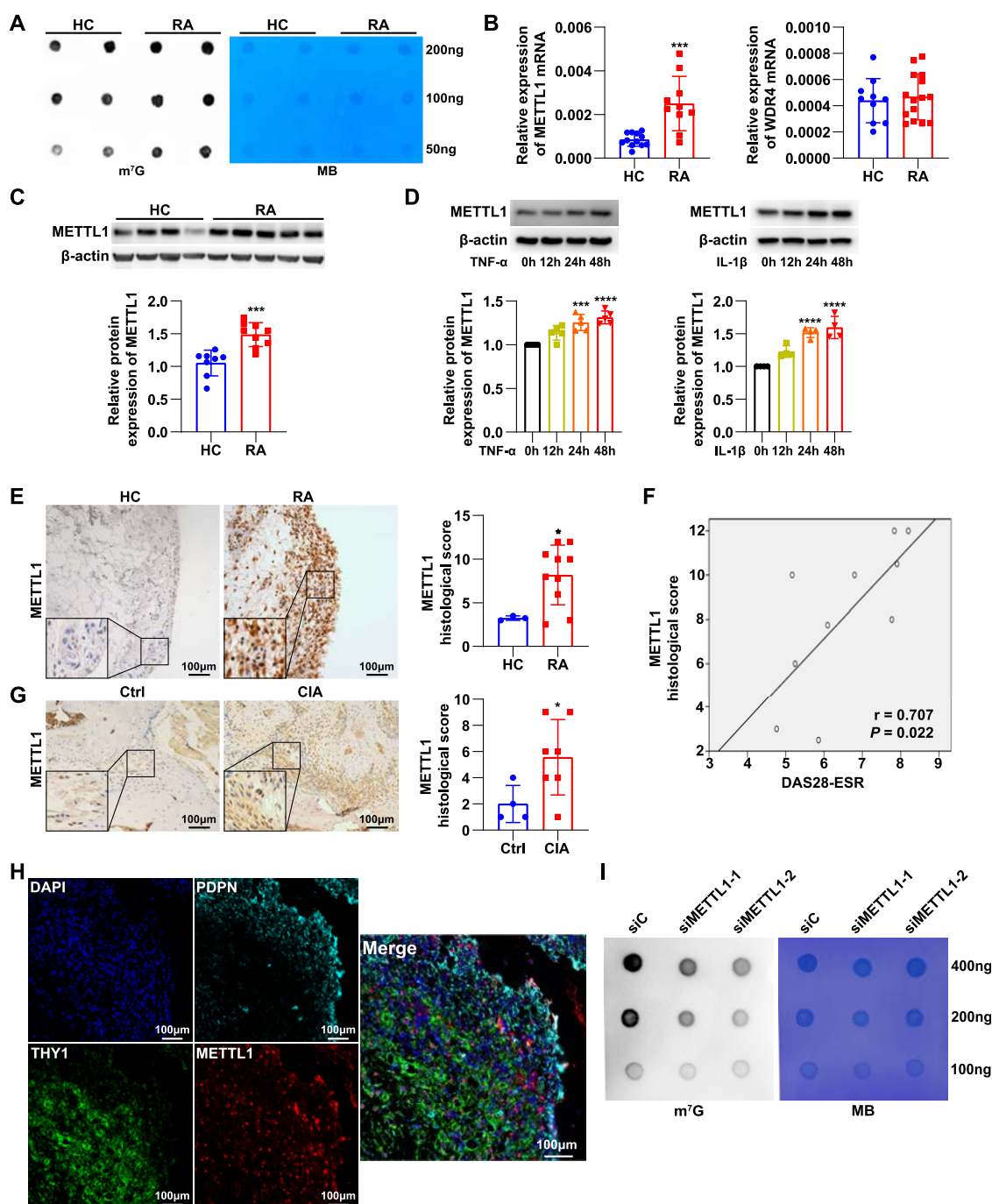


Figure 1. Increased m⁷G levels and *METTL1* expression in FLSs and synovium from patients with RA. (A) The internal m⁷G levels of mRNA in RA FLSs and HC FLSs were determined by m⁷G dot blot. Methylene blue staining (as loading control). (B) RT-qPCR analysis of *METTL1* and *WDR4* in FLSs from RA and HC groups. The graphic shows the quantitative analysis. (C) Western blot analysis of *METTL1* in FLSs from RA and HC groups. The graphic shows the quantitative analysis (lower panel). (D) *METTL1* protein expression in RA FLSs treated with TNF-α (10 ng/mL) or IL-1β (10 ng/mL) for 0 hour, 12 hours, 24 hours, and 48 hours. The graphic shows the quantitative analysis (lower panel). (E) The expression of *METTL1* in human synovial tissues was detected by IHC staining (original magnification, × 200). (F) Correlation analysis between IHC scores of synovial *METTL1* and DAS28-ESR in patients with RA. (G) The expression of *METTL1* in murine synovial tissues was determined by IHC staining (original magnification, × 200). (H) Multiplex immunofluorescence for DAPI, PDPN, THY1, and *METTL1* in synovium from patients with RA. PDPN and THY1 served as markers to identify FLSs within synovial tissues (original magnification, × 200). (I) Effect of *METTL1* knockdown on internal m⁷G levels of mRNA was detected by m⁷G dot blot assays. The IHC and immunofluorescence staining with isotype control and negative control are shown in [Supplementary Figure S1A–C](#). Data are presented as the means ± SD from at least 3 independent experiments. * $P < .05$, *** $P < .001$, **** $P < .0001$ vs HC, Ctrl or 0 hour. CIA, collagen-induced arthritis; Ctrl, control; DAPI, 4′ 6-diamidino-2-phenylindole; DAS28-ESR, disease activity score on 28 joints-erythrocyte sedimentation rate; FLS, fibroblast-like synoviocyte; HC, healthy control; IHC, immunohistochemistry; IL, interleukin; MB, methylene blue; *METTL1*, methyltransferase-like 1; m⁷G, N⁷-methylguanosine; mRNA, messenger ribonucleic acid; PDPN, podoplanin; RA, rheumatoid arthritis; RT-qPCR, real-time quantitative polymerase chain reaction; THY1, thymocyte differentiation antigen 1; TNF, tumour necrosis factor; WDR4, tryptophan-aspartic acid (WD) repeat domain 4.

the mRNA expression levels of the components of the m⁷G methyltransferase complex, including the methyltransferase METTL1 and the cofactor WDR4. Our findings revealed a marked upregulation of *METTL1* mRNA expression in RA FLSs compared to HC FLSs, while no significant difference was observed in *WDR4* expression (Fig 1B). Subsequently, we validated the protein level of *METTL1*, confirming its increased expression in RA FLSs (Fig 1C). Moreover, stimulation with inflammatory cytokines tumour necrosis factor (TNF)- α and interleukin (IL)-1 β promoted the expression of *METTL1* (Fig 1D). We further examined *METTL1* expression in the synovium from patients with RA and HC subjects, and found that *METTL1* expression was increased in RA synovial tissues compared to HC subjects (Fig 1E). More importantly, *METTL1* expression in the synovium from patients with RA correlated with RA disease activity as measured by disease activity score on 28 joints-erythrocyte sedimentation rate (Fig 1F). Additionally, we observed elevated *METTL1* levels in the synovium of collagen-induced arthritis (CIA) mice (Fig 1G). Using multiplex immunofluorescence analysis of synovial tissues from patients with RA, we identified that *METTL1* was predominantly expressed in PDPN⁺THY1⁺ FLSs in the lining layer and PDPN⁺THY1⁺ FLSs in the sublining layer (Fig 1H). Finally, we found that *METTL1* knockdown using siRNA downregulated m⁷G levels in RA FLSs, suggesting that *METTL1* is a key regulator of m⁷G modification (Fig 1I). In light of these findings, this study will focus on the regulatory role of *METTL1* in the aberrant activation of RA FLSs.

METTL1 promotes the migration and invasion of RA FLSs

To evaluate the role of *METTL1* in regulating functions of RA FLSs, we employed an integrated experimental approach that combined gain-of-function (overexpression) and loss-of-function (knockdown) strategies (Supplementary Fig S2A–F). As shown in Figure 2, *METTL1* knockdown, facilitated by either siRNA (Fig 2A) or shRNA (Fig 2B), significantly impeded the migration and invasion of RA FLSs. In contrast, lentivirus-mediated *METTL1* overexpression (Fig 2C) potently enhanced the migratory and invasive capabilities of RA FLSs. Notably, when RA FLSs were infected with a lentivirus carrying a catalytically inactive *METTL1* mutant, their migration and invasion capacities remained comparable to those of the control group (Fig 2C). Collectively, these results indicate that *METTL1* exerts potent regulatory control over the aggressive phenotypes of RA FLSs, and this regulation is critically dependent on m⁷G modification. Actin cytoskeleton reorganisation and pseudopodia formation are essential for cell migration and invasion. We investigated pseudopodia formation in RA FLSs and observed a significant decrease in pseudopodia formation following *METTL1* knockdown, by using immunofluorescence staining for F-actin and vinculin (Fig 2D and Supplementary Fig S3A). Moreover, matrix metalloproteinases (MMPs) are required to facilitate RA FLSs invasion and cartilage degradation. Our results revealed that *METTL1* knockdown led to a decreased expression of *MMP9*, while having negligible effects on the expression levels of *MMP1* and *MMP3* (Fig 2E). We further found that *METTL1* knockdown reduced the secretion of *MMP9* in RA FLSs (Fig 2F).

To investigate the role of *METTL1* in the proliferation of RA FLSs, we employed the 5-ethynyl-2'-deoxyuridine (EdU) assay to evaluate cell growth in an *in vitro* culture system. The knockdown of *METTL1* did not result in any significant changes in the proliferative capacity of RA FLSs, as illustrated in Supplementary Figure S3B. Furthermore, we examined the effect of

METTL1 knockdown on the apoptosis of RA FLSs by utilising the Annexin V/PI double staining technique in conjunction with flow cytometry analysis. Notably, the percentage of apoptotic cells in the *METTL1* knockdown group was comparable to that of the control group, indicating that the knockdown of *METTL1* does not influence the apoptosis of RA FLSs (Supplementary Fig S3C). Inflammatory cytokines play a crucial role in synovial inflammation. We also investigated the role of *METTL1* in the expression of inflammatory cytokines in RA FLSs. The results revealed that *METTL1* knockdown did not affect TNF- α -induced expression of *IL-1 β* , *IL-6*, and *IL-8* (Supplementary Fig S3D).

CTSB is a potential target of METTL1 in RA FLSs

We next investigated the underlying mechanisms for *METTL1*-mediated m⁷G modification in regulating the migration and invasion of RA FLSs. We performed m⁷G RNA immunoprecipitation sequencing (m⁷G-RIP-seq) analysis among RA FLSs with *METTL1* shRNA (sh-*METTL1*) and RA FLSs with control shRNA (Ctrl). Our findings revealed that a 'GA'-enriched motif, indicative of internal m⁷G modification, was detected in both control and *METTL1*-knockdown RA FLSs (Fig 3A). Notably, the majority of m⁷G modification peaks was localised within the 3' untranslated region (3' UTR) and coding sequences (CDSs) region of mRNA transcripts (Fig 3B).

Subsequent differential methylation analysis identified 41 genes with altered m⁷G modification patterns between the *METTL1* knockdown and control groups. Of these genes, 14 genes exhibited hypermethylation, while 27 genes showed hypomethylation (Fig 3C). Given that *METTL1* is the pivotal m⁷G methyltransferase in RA FLSs, we focused on the hypomethylated genes following *METTL1* depletion for further investigation. Among these 27 hypomethylated targets, thymosin beta 10 (*TMSB10*) and cathepsin B (*CTSB*) emerged as the top 2 genes with the most significant fold change differences.

Previous researches have established that *METTL1* predominantly regulates translation rather than the mRNA stability of m⁷G-modified transcripts [22,24]. Our experimental findings revealed that *METTL1* knockdown reduced the *CTSB* protein level (Fig 3E), while *TMSB10* protein expression remained unchanged (Fig 3D). Since *CTSB* is a secreted protein, we further evaluated whether *METTL1* knockdown influences its secretion. We demonstrated that *METTL1* knockdown reduced the secretion of *CTSB* in RA FLSs (Supplementary Fig S4A). Notably, despite the decrease in *CTSB* protein, the abundance of *CTSB* mRNA remained unaltered (Fig 3F). These data implicate *CTSB* as a principal downstream effector of *METTL1*-mediated m⁷G modification in regulating functions of RA FLSs.

We further confirmed the role of *METTL1* in the m⁷G modification of *CTSB* mRNA. The m⁷G-RIP-seq analysis revealed that following *METTL1* knockdown, the m⁷G modification level in the 5' UTR of *CTSB* mRNA was significantly decreased (Fig 3G). In accordance with the m⁷G-RIP-seq data, the m⁷G-RIP-quantitative polymerase chain reaction (m⁷G-RIP-qPCR) results demonstrated a marked reduction in the m⁷G modification level of *CTSB* mRNA in the si-*METTL1* group (Fig 3H). Collectively, these findings strongly suggest that *METTL1* positively regulates the m⁷G modification of *CTSB* mRNA.

To further verify whether *METTL1* is involved in the regulation of *CTSB* expression in an m⁷G modification-dependent manner, we conducted a dual-luciferase reporter gene assay targeting the m⁷G modification site (chr8: 11868073) located in the 5' UTR of *CTSB*. Our results indicated that the knockdown of *METTL1* significantly reduced the luciferase activity of the

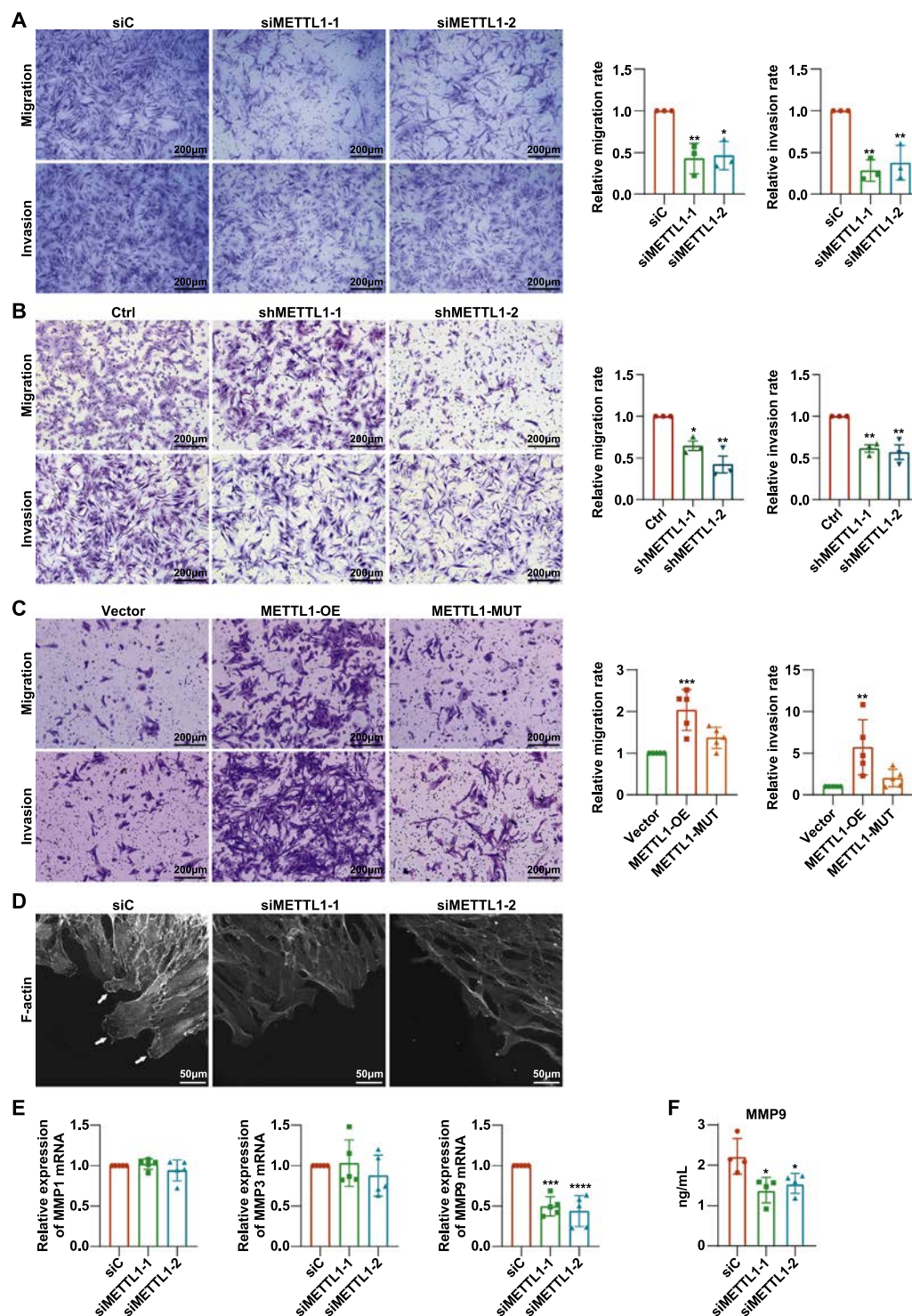


Figure 2. Functional impact of METTL1 modulation on migration and invasion of RA FLSs. (A, B) *METTL1* knockdown inhibited the migration and invasion of RA FLSs, detected by transwell assays (original magnification, $\times 100$). RA FLSs were transfected with *METTL1* siRNA (siMETTL1-1, siMETTL1-2) (A) or infected with *METTL1* shRNA lentivirus (shMETTL1-1, shMETTL1-2) (B). (C) *METTL1* overexpression promoted the migration and invasion of RA FLSs, detected by transwell assays (original magnification, $\times 100$). RA FLSs were infected with *METTL1* overexpressed lentiviruses (*METTL1*-OE) or *METTL1* mutant lentiviruses (*METTL1*-MUT). (D) Effect of *METTL1* knockdown on lamellipodia formation in RA FLSs (original magnification, $\times 400$). The arrows indicate lamellipodia formation. (E) Effect of *METTL1* knockdown on MMPs expression in RA FLSs, measured by RT-qPCR. (F) ELISA was performed to measure MMP9 secretion levels in RA FLSs. Data are presented as the means $\pm$ SD from at least 3 independent experiments. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$ vs siC, Ctrl or Vector. ELISA, enzyme-linked immunosorbent assay; FLS, fibroblast-like synoviocyte; METTL1, methyltransferase-like 1; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; RT-qPCR, real-time quantitative polymerase chain reaction; shRNA, short hairpin ribonucleic acid; siC, siRNA control.

wild-type *CTSB* (*CTSB*-5' UTR-WT), whereas no significant effect was observed on the mutant *CTSB* (*CTSB*-5' UTR-MUT) (Fig 3I).

Considering the METTL1 influence on the protein level of *CTSB* in RA FLSs as mentioned above, we explored the

intricate molecular mechanism through which METTL1 governs the protein expression of *CTSB*. Our initial step involved conducting an experiment aimed at assessing the influence of METTL1 on the stability of the *CTSB* protein. Our data clearly revealed that the knockdown of *METTL1* had no

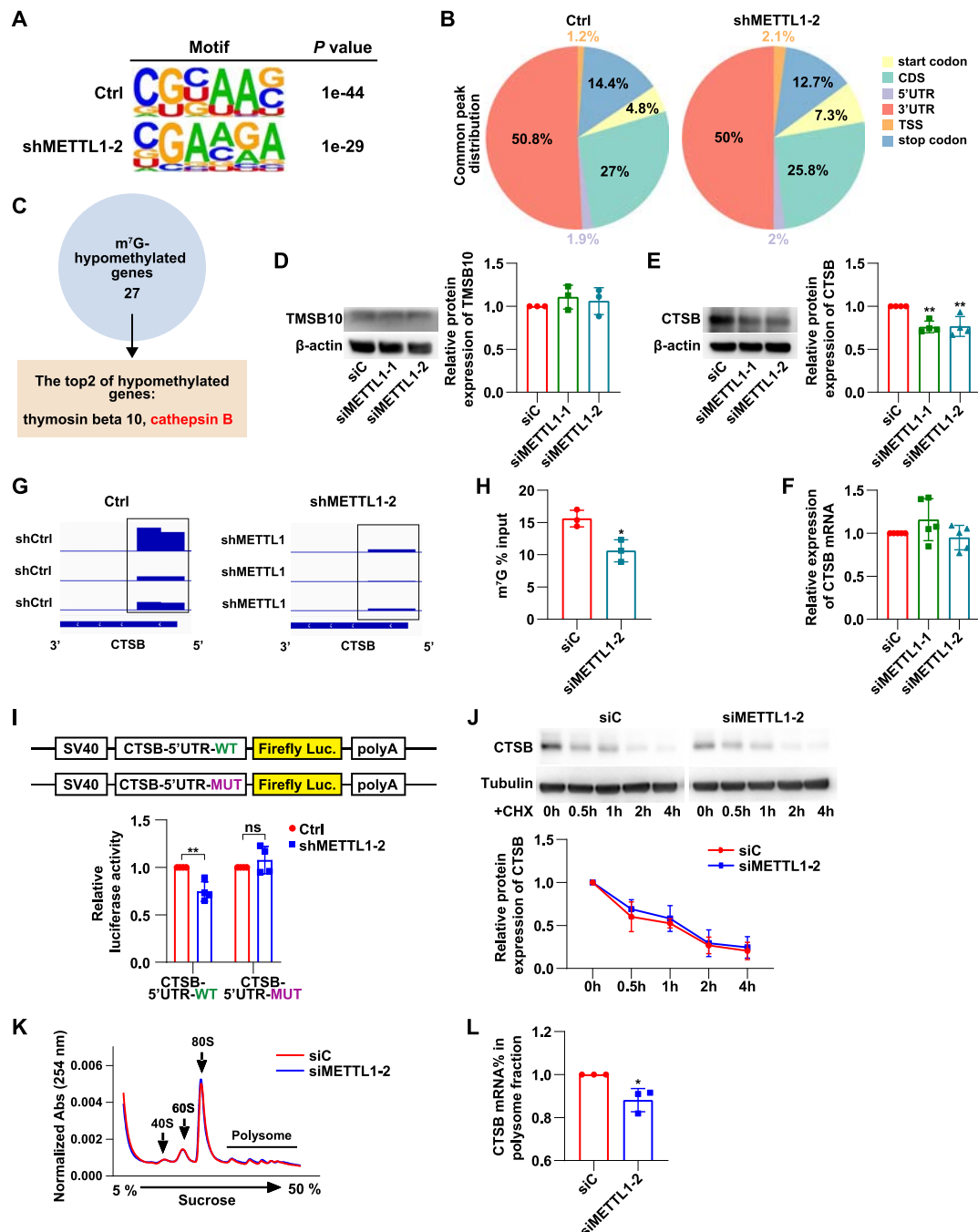


Figure 3. Characterisation of m⁷G modification and identification of METTL1 target in RA FLSs. (A) The m⁷G peak-associated motifs within shMETTL1 and Ctrl groups in RA FLSs. (B) Proportional allocation of m⁷G peaks across transcriptomic regions within shMETTL1 and Ctrl groups in RA FLSs. (C) Hypomethylated transcripts identified via m⁷G-RIP-seq following METTL1 knockdown. (D, E) Protein expression of TMSB10 (D) and CTSSB (E) in siMETTL1- or siC-treated RA FLSs was determined by Western blot. (F) Transcript levels of CTSSB in siMETTL1- or siC-treated RA FLSs were determined by RT-qPCR. (G) CTSSB mRNA m⁷G modifications within shMETTL1 and Ctrl groups in RA FLSs were displayed using IGV. (H) The m⁷G levels of CTSSB in siMETTL1- or siC-treated RA FLSs were determined by m⁷G-RIP-qPCR analysis. (I) The expression of WT and MUT CTSSB in the METTL1-knockdown RA FLSs was determined by luciferase reporter assays. (J) RA FLSs were treated with cycloheximide (CHX, 100 µg/mL) for 0.5, 1, 2, and 4 hours. CTSSB protein expression was determined by Western blot. (K) Polysome profiling showing the effect of METTL1 knockdown on global translation efficiency in RA FLSs. (L) The enrichment of CTSSB mRNA from the polysome fractions upon siMETTL1 treatment was quantified by RT-qPCR. Data are presented as the means ± SD from at least 3 independent experiments. **P* < .05, ***P* < .01 vs siC or Ctrl. Abs, absorbance; CDS, coding sequence; CTSSB, cathepsin B; FLS, fibroblast-like synoviocyte; IGV, Integrative Genomics Viewer; METTL1, methyltransferase-like 1; m⁷G-RIP-seq, m⁷G RNA immunoprecipitation sequencing; mRNA, messenger ribonucleic acid; MUT, mutant; RA, rheumatoid arthritis; RT-qPCR, real-time quantitative polymerase chain reaction; TMSB10, thymosin beta 10; TSS, transcription start site; UTR, untranslated region; WT, wild-type.

influence on the degradation rate of the CTSSB protein (Fig 3J), suggesting that the CTSSB protein stability remained unperturbed by the absence of METTL1. Second, we further explored how METTL1 impacts the translation process of the CTSSB protein. Polysome profiling analysis revealed that

METTL1 depletion did not result in any significant alterations in the global efficiency of protein translation (Fig 3K). However, we observed a notable reduction in the percentage of CTSSB mRNA in polysome fractions following METTL1 knockdown (Fig 3L). Collectively, these results suggest that

METTL1 selectively modulates the translation efficiency of *CTSB*, while not influencing the global translation efficiency in RA FLSs.

Currently, Quaking protein (QKI) is recognised as the sole reader protein for the internal m⁷G modification of mRNA [25]. Therefore, we selected QKI as a potential m⁷G reader protein for *CTSB*. However, *QKI* knockdown did not significantly affect the protein expression level of *CTSB* (Supplementary Fig S5A,B). This indicates that there are also multiple reader proteins in the internal m⁷G modification of mRNA. We will seek the novel reader protein(s) that modulate the internal mRNA m⁷G modification of *CTSB* in the future.

CTSB modulates migration and invasion of RA FLSs

To clarify whether *CTSB* serves as a mediator in the influence of METTL1 on the migration and invasion of RA FLSs, we first measured the expression levels of *CTSB* at both the mRNA and protein levels. Our data demonstrated that the mRNA (Fig 4A) and protein (Fig 4B) expression of *CTSB* were increased in RA FLSs compared to HC FLSs. We further determined that treatment with TNF- α or IL-1 β resulted in an increase in *CTSB* levels (Supplementary Fig S6A,B).

Next, we explored the role of *CTSB* in the migration and invasion of RA FLSs. Both siRNAs (si*CTSB*-1 and si*CTSB*-3) substantially inhibited *CTSB* expression and were used for the subsequent experiments (Fig 4C, D). Our results revealed that the knockdown of *CTSB* exerted a significant inhibitory effect on the migration and invasion of RA FLSs (Fig 4E). Moreover, the outcomes of F-actin immunofluorescence staining indicated that the formation of pseudopodia was substantially reduced in RA FLSs following *CTSB* knockdown (Fig 4F). Additionally, we found that *CTSB* knockdown led to a notable suppression of *MMP9* expression, as evidenced by Figure 4G. However, *CTSB* knockdown had minimal effects on the proliferation, apoptosis, and viability of RA FLSs (Supplementary Fig S7A-C).

Finally, we carried out rescue experiments involving the overexpression of *CTSB* (Fig 4H, I) in the context of *METTL1* knockdown. As shown in Figure 4J, the knockdown of *METTL1* resulted in the inhibition of migration and invasion of RA FLSs. Conversely, the concurrent overexpression of *CTSB* effectively counteracted this inhibitory effect, restoring the migratory and invasive potential of RA FLSs.

METTL1 suppression in vivo improved the severity of arthritis in RA models

To assess the *in vivo* impact of *METTL1* inhibition on arthritis progression, we developed 2 distinct RA animal models: rats with CIA and mice with delayed-type hypersensitivity arthritis (DTHA).

We first employed the CIA, a classic model of RA, to evaluate the *in vivo* effect of *METTL1* inhibition on RA. The adenovirus particles stably expressing *METTL1*-shRNA (Adv-*METTL1*) or control shRNA (Adv-Ctrl) were generated and then administered via intra-articular injection (2×10^8 PFU per injection) into the ankles of Wistar rats with CIA on days 8 and 15 (Fig 5A,B, Supplementary Fig S8A) postimmunisation. As illustrated in Figure 5C, the rats with CIA exhibited pronounced joint swelling, corroborated by characteristic imaging and pathological manifestations of arthritis, thereby validating successful model establishment. Notably, compared to the Adv-Ctrl-treated group, the rats treated with Adv-*METTL1* exhibited significant

amelioration of arthritis symptoms, which was evidenced by decreased paw volume, reduced ankle joint circumference, and lower arthritis score (Fig 5D). Imaging analyses further revealed a marked reduction in the area of bone erosion in Adv-*METTL1*-treated rats. Micro-CT quantitative analysis demonstrated that the bone surface to bone volume ratio was significantly lower in the Adv-*METTL1*-treated group than in the Adv-Ctrl-treated group (Fig 5E). Histopathological assessment indicated that *METTL1* knockdown substantially mitigated synovial tissue inflammation, suppressed bone erosion, and decelerated cartilage degradation (Fig 5F). Furthermore, immunofluorescence staining analysis demonstrated a simultaneous downregulation of *METTL1* and *CTSB* expression levels in the synovial tissues of Adv-*METTL1*-treated rats (Fig 5G).

To gain a more comprehensive understanding of the *in vivo* biological function of *METTL1*, we employed the CRISPR/Cas9 technology to establish a *METTL1* gene knockout model in C57BL/6J strain mice. While homozygous deletion of *METTL1* (*METTL1*^{-/-}) leads to lethality, *METTL1* heterozygous knockout (*METTL1*^{+/-}) mice were selected for subsequent experimental investigations (Fig 6A, B; Supplementary Fig S9A,B, Supplementary Fig S10A). Considering the DTHA model exhibits an exceptional induction efficiency (nearly 100%) in C57BL/6J mice, we chose this model to conduct a more in-depth validation of the *in vivo* function of *METTL1* (Fig 6C). Our data revealed that arthritis symptoms in *METTL1*^{+/-} group mice were markedly alleviated, with a reduction in arthritis severity score and attenuation of tarsal thickness, suggesting *METTL1* haploinsufficiency conferred significant protection against arthritis progression (Fig 6D, E). Moreover, histopathological staining results of ankle joint tissues demonstrated that the levels of synovial inflammation, bone erosion, and cartilage destruction in *METTL1*^{+/-} group mice were all substantially reduced compared to those in wild-type group mice (Fig 6F). Meanwhile, immunohistochemistry staining analysis showed that the expression of *CTSB* was downregulated within the synovial tissues of *METTL1*^{+/-} mice (Fig 6G). Collectively, these results strongly indicate that partial deletion of *METTL1* can effectively impede the progression of arthritis in DTHA mice.

DISCUSSION

In the present study, we demonstrated elevated levels of *METTL1* and internal mRNA m⁷G modification in synovial tissues and FLSs from patients with RA. We further determined that *METTL1* knockdown decreased the m⁷G levels of mRNA and inhibited migration, invasion, and expression of *MMP9* in RA FLSs. Interestingly, *METTL1* suppression *in vivo* improved the severity of arthritis in models of RA. Mechanistically, we explored that *METTL1* modulates the aggression of RA FLSs by upregulating the translation efficiency of internal m⁷G-modified *CTSB* mRNA. Our findings suggest that increased synovial *METTL1*-mediated internal mRNA m⁷G modification of *CTSB* promotes synovial aggression and joint destruction in RA.

As one of the most conserved modifications of RNA, m⁷G is important in modulating nucleosides, impacting mRNA export, splicing, and translation. Previous reports indicate that *METTL1*-mediated m⁷G tRNA modification plays an important role in regulating cell processes and contributes to the pathogenesis of some human diseases [17–21]. However, increasing evidence shows that m⁷G is also revealed to occur in internal mRNA, mainly in CDS, 5' UTR, and 3' UTR, which has critical roles in pathological and physiological processes of several cell lines [22,24]. For instance, *METTL1*-mediated internal mRNA

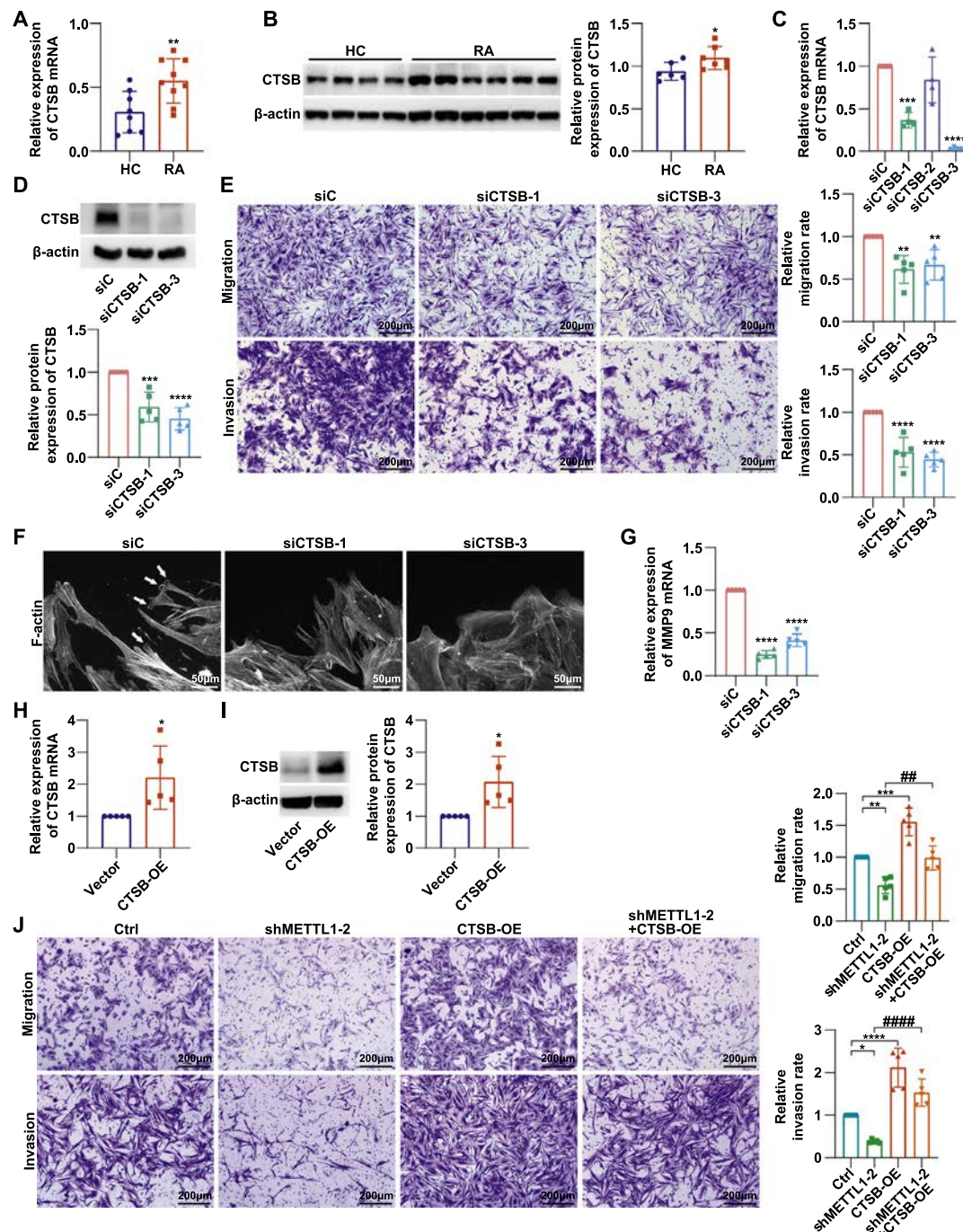
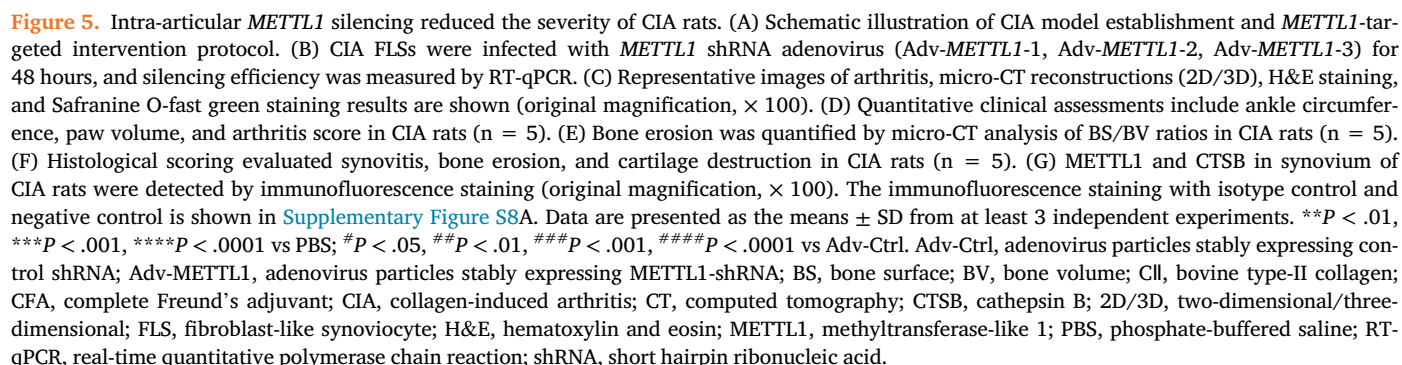


Figure 4. Effect of *CTSB* knockdown on migration and invasion of RA FLSs. (A–B) *CTSB* mRNA and protein levels in FLSs were quantified by RT-qPCR (A) and immunoblotting (B), respectively. (C, D) RA FLSs were transfected with *CTSB* siRNA for 48 hours, and silencing efficiency was measured by RT-qPCR (C) and Western blot (D). (E) Effect of *CTSB* knockdown on the migration and invasion of RA FLSs. The migration and invasion of RA FLSs were tested using transwell assays (original magnification, $\times 100$). (F) Effect of *CTSB* knockdown on lamellipodia formation in RA FLSs (original magnification, $\times 400$). The arrows denote lamellipodia formation. (G) Effect of *CTSB* knockdown on *MMP9* expression in RA FLSs, measured by RT-qPCR. (H, I) RA FLSs were infected with *CTSB* overexpressed lentiviruses and control lentiviruses (Vector) for 4 days, and then *CTSB* expression was measured by RT-qPCR (H) and Western blot (I). (J) Effect of *CTSB* overexpression on the *METTL1* knockdown-induced reduction of migration and invasion of RA FLSs, detected by transwell assay (original magnification, $\times 100$). Data are presented as the means $\pm$ SD from at least 3 independent experiments. $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$ vs HC, siC, Vector or Ctrl; $##P < .01$, $####P < .0001$ vs shMETTL1. CTSB, cathepsin B; FLS, fibroblast-like synoviocyte; HC, healthy control; METTL1, methyltransferase-like 1; MMP, matrix metalloproteinase; mRNA, messenger ribonucleic acid; RA, rheumatoid arthritis; RT-qPCR, real-time quantitative polymerase chain reaction.

m⁷G is enriched in the CDS and 3' UTR regions upon oxidative stress and heat shock [24]. METTL1-mediated m⁷G modification within mRNA promotes the stability and translation of *Sptbn2* mRNA in hippocampal neurogenesis [23]. However, the role of internal mRNA m⁷G methylome in autoimmune diseases remains to be defined. In this study, we showed that elevated levels of *METTL1* and internal mRNA m⁷G promote the

migration and invasion of RA FLSs. More interestingly, we determined that intra-articular injection of *METTL1* shRNA in rats with CIA and heterozygous deletion of *METTL1* in mice with DTHA improved arthritis symptoms and synovial inflammation, bone erosion, and cartilage destruction. Our data suggest that an increased level of METTL1-mediated internal mRNA m⁷G contributes to the synovial aggression and joint destruction in RA.



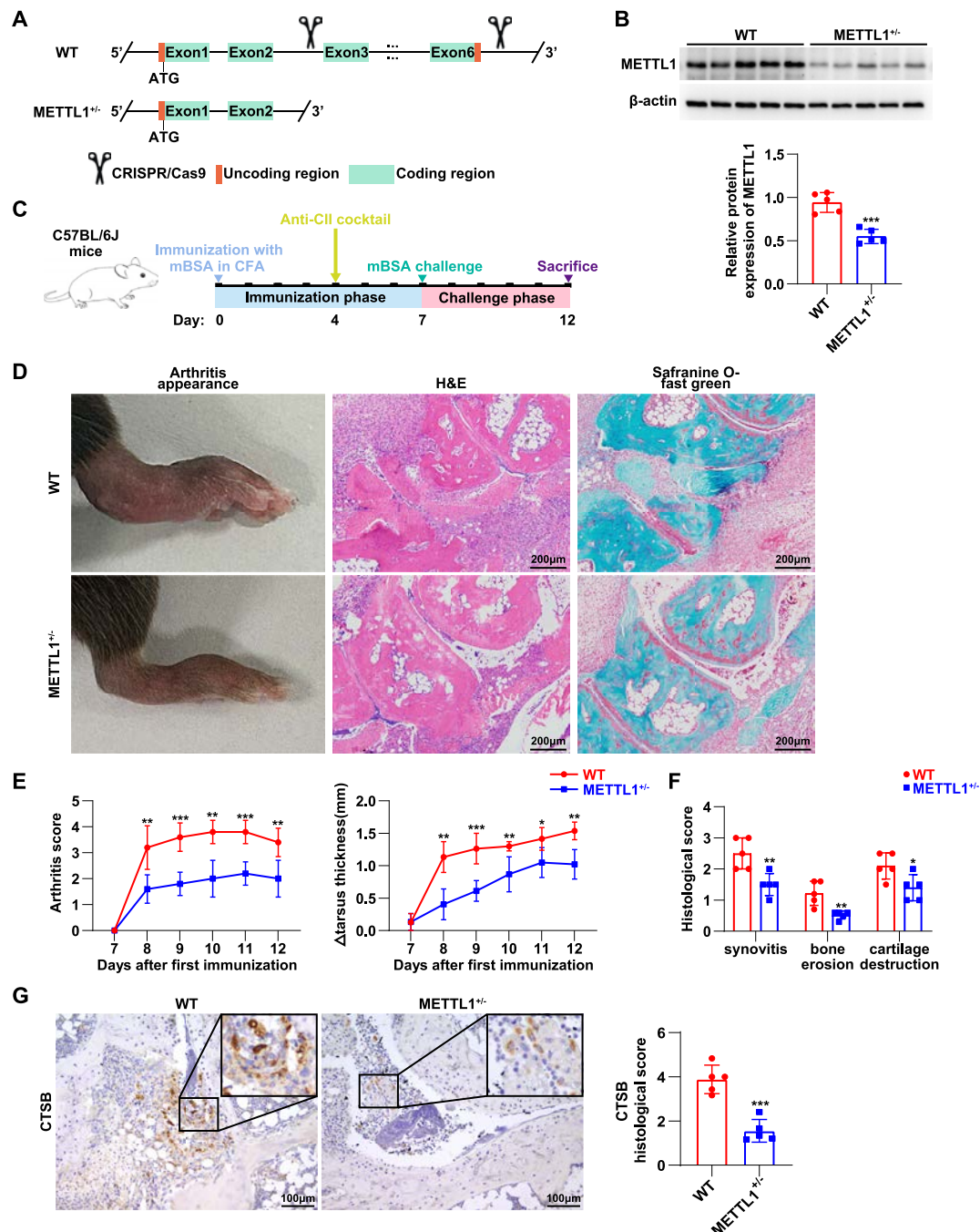


Figure 6. Therapeutic effect of *METTL1* haploinsufficiency in DTHA mice. (A) Schematic representation of *METTL1* heterozygous knockout mice generation. (B) The knockout efficiency of mice was identified by Western blot using synovial tissues. (C) Schematic illustration of DTHA model establishment. (D) Representative images of arthritis, H&E staining, and Safranin O-fast green staining results are shown (original magnification, $\times 100$). (E) Quantitative clinical assessments include arthritis score and paw swelling (Δ thickness) in DTHA mice ($n = 5$). (F) Histological scoring evaluated synovitis, cartilage damage, and bone erosion in DTHA mice ($n = 5$). (G) CTSSB expression in synovium of WT mice and *METTL1*^{+/-} mice was detected by IHC staining (original magnification, $\times 200$). The IHC staining with isotype control and negative control is shown in [Supplementary Figure S10A](#). Data are presented as the means $\pm$ SD from 5 independent experiments. * $P < .05$, ** $P < .01$, *** $P < .001$ vs WT. anti-CII, anti-collagen II; CFA, complete Freund's adjuvant; CTSSB, cathepsin B; DTHA, delayed-type hypersensitivity arthritis; H&E, hematoxylin and eosin; IHC, immunohistochemistry; mBSA, albumin methylated from bovine serum; METTL1, methyltransferase-like 1; WT, wild-type.

We next investigated how METTL1-mediated internal mRNA m⁷G modification controls the aggressive behaviour of RA FLSs using analysis of m⁷G-RIP-seq. We identified *CTSSB* as one of the direct targets of METTL1 and further demonstrated that METTL1-mediated m⁷G methylome regulates *CTSSB* expression by increasing translation efficiency. Previous reports indicate that METTL1 promotes protein expression through regulating m⁷G tRNA or microRNA modification [26,27]. However, recent studies show that METTL1 controls mRNA translation by

directly regulating internal mRNA m⁷G methylation [22,24,28]. In our study, we demonstrated that METTL1-mediated m⁷G modification directly regulates *CTSSB* expression using a luciferase reporter assay. Furthermore, we explored that *METTL1* knockdown reduced *CTSSB* translation efficiency, but did not affect its mRNA stability. Therefore, our data indicate METTL1-mediated internal mRNA m⁷G modification promotes *CTSSB* expression by upregulating its mRNA translation efficiency.

CTSB, a member of the cysteine protease family, is an important element of the lysosome, and plays a critical role in modulating cellular pathological processes [29]. Increased level of CTSB is observed under various pathological conditions such as cancer, inflammation, and infection [30,31]. Previous studies indicate that CTSB has important roles in controlling tumour growth, migration, invasion, metastasis, and angiogenesis, and is considered as a potential target for anti-cancer therapy [32,33]. Herein, we found that *CTSB* expression was increased in FLSs and synovial tissues from patients with RA, and knockdown of *CTSB* suppressed migration, invasion, and *MMP9* expression in RA FLSs. Consistent with our study, previous reports indicate that *CTSB* expression is elevated in FLSs and synovial tissues in patients with RA, and *CTSB* inhibition suppresses migration of RA FLSs [34,35]. We further found that overexpression of *CTSB* reversed the inhibitory effect of silencing *METTL1* on the migration and invasion of RA FLSs. *METTL1* heterozygous deletion or intra-articular injection of Adv-shRNA-*METTL1* also reduced synovial expression of CTSB in RA animal models. Collectively, these results indicate that elevated *METTL1* promotes aggressive behaviour of RA FLSs by upregulating the translation efficiency of internal m⁷G-modified *CTSB* mRNA. Previous studies have shown that CTSB can directly degrade extracellular matrix (ECM) proteins [36,37] or act indirectly by activating MMPs [38], thereby facilitating tumour cell metastasis. Therefore, it is plausible that CTSB plays an important role in RA joint destruction through the direct or indirect degradation of ECM components or the regulation of MMP secretion. However, we do not completely rule out the possibility that other unidentified molecules mediate the *METTL1* functions. It is also needed to investigate how CTSB regulates the aggressive action of RA FLSs.

Our results show that increased *METTL1* promotes the aggression of RA FLSs by boosting the internal mRNA m⁷G modification of *CTSB*. The *METTL1* may be a novel potential therapeutic target for future therapy of RA.

Competing interests

The authors have declared no conflicts of interest.

CRediT authorship contribution statement

Simin Chen: Resources, Methodology, Conceptualization. **Kai Sun:** Methodology, Data curation. **Chenxi Peng:** Methodology, Data curation. **Yu Kuang:** Methodology. **Shuoyang Zhang:** Resources, Data curation. **Ruiru Li:** Methodology. **Huijuan Hu:** Methodology. **Suling Liu:** Methodology. **Fan Su:** Resources, Data curation. **Qian Qiu:** Methodology, Data curation. **Liuqin Liang:** Conceptualization. **Ligang Jie:** Conceptualization. **Younjun Xiao:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Hanshi Xu:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

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Patient consent for publication

Not applicable.

Ethics approval

This study involves human participants and was approved by IEC for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University (no. [2023]038). Participants gave informed consent to participate in the study before taking part. This study involves animal subjects and was approved by Animal Experimental Ethics Committee in Sun Yat-sen University (no. SYSU-IACUC-2021-000332, no. SYSU-IACUC-2024-002440).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The m⁷G-RIP-seq data from this study have been deposited to GEO series: GSE299961. Other data are available upon reasonable request. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.09.010.

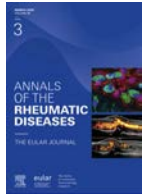
Orcid

Simin Chen: <http://orcid.org/0000-0002-7348-6238>

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Psoriatic arthritis

Ultrasound for assessing disease activity and structural damage in psoriatic arthritis: the UPsA scores from a multicentre study by the Italian Society of Rheumatology

Alen Zabotti^{1,*}, Matteo Piga^{2,3}, Marco Canzoni⁴, Cristina Di Nicola⁵, Davide Rozza⁵, Greta Carrara⁵, Nicola Boffini⁶, Valentina Picerno⁷, Ivan Giovannini¹, Alberto Floris^{2,3}, Nicola Cabas¹, Nicola Farina⁶, Garifallia Sakellariou⁸, Pietro Leccese⁷, Giovanni Zanframundo^{8,9}, Alessandra Bortoluzzi^{10,11}, Ettore Silvagni^{10,11}, Bernd Raffener¹², Silvia Zanoni¹², Simone Parisi¹³, Maria Chiara Ditto¹³, Orazio De Lucia¹⁴, Sara Zandonella Callegger¹⁵, Luca Idolazzi¹⁶, Fabiana Figus¹⁷, Lorenzo Dagna⁶, Roberta Ramonda¹⁸, Salvatore D'Angelo⁷, Alberto Cauli^{2,3}, Luca Quartuccio¹, Carlo Alberto Scirè^{5,19}, Annamaria Iagnocco¹⁷, UPSTREAM study collaborators

¹ Department of Medicine, Rheumatology Institute, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy

² Rheumatology Unit, AOU Cagliari, Cagliari, Italy

³ Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy

⁴ Simple Departmental Operational Unit Rheumatology (UOSD), Nuovo Regina Margherita Hospital, ASL Roma-1, Rome, Italy

⁵ Epidemiology Research Unit, Italian Society for Rheumatology, Milan, Italy

⁶ UnIRAR Ospedale San Raffaele, Milano, Milan, Italy

⁷ Rheumatology Department of Basilicata, San Carlo Hospital, Potenza, Italy

⁸ Department of Internal Medicine and Therapeutics, Università di Pavia, Pavia, Italy

⁹ Division of Rheumatology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

¹⁰ Rheumatology Unit, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

¹¹ Azienda Ospedaliera Universitaria S. Anna, Cona (Ferrara), Italy

¹² Department of Rheumatology, Teaching Hospital of the Paracelsus Medical University, Central Hospital of Bolzano (ASAA-SABES), Bolzano, Italy

¹³ Rheumatology Unit, Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Turin, Italy

¹⁴ Department of Rheumatology, UOC Clinica Reumatologica, ASST Gaetano Pini-CTO, Milan, Italy

¹⁵ Department of Rheumatology, Teaching Hospital of the Paracelsus Medical University, Brunico Hospital (ASAA-SABES), Brunico, Italy

¹⁶ Section of Rheumatology, University of Verona, Verona, Italy

¹⁷ Academic Rheumatology Center, DSCB - Università di Torino, Turin, Italy

¹⁸ Rheumatology Unit, Department of Medicine-DIMED, University of Padova, Padova, Italy

¹⁹ Department of Medicine and Surgery, Bicocca University, Monza, Italy

*Correspondence to Dr. Alen Zabotti, Rheumatology Institute, Department of Medicine, University of Udine, Udine, Italy.

E-mail address: zabottialen@gmail.com (A. Zabotti).

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ABSTRACT

Objectives: As part of the ultrasound in psoriatic arthritis (PsA) treatment (Ultrasound in Psoriatic Arthritis Treatment-NCT03330769) study, 2 ultrasound scores for PsA (UPsA)—the UPsA activity score and the UPsA damage score—were developed and internally validated to assess musculoskeletal inflammation and structural damage in PsA. In addition, a simplified UPsA activity score (sUPsA) was also derived to enhance feasibility and applicability in routine clinical practice.

Methods: Baseline and 6-month data from patients with PsA across 19 Italian centres were analysed. Clinical evaluations included joint counts, enthesitis, dactylitis, and patient-reported outcomes. Ultrasound assessments, performed by trained sonographers, covered 42 joints, 36 tendons, 12 entheses, and 2 bursae. Factor analysis was used to derive composite scores (range 0–10). Construct validity was assessed by Spearman's correlations with clinical variables, and sensitivity to change was evaluated using the standardised response mean (SRM).

Results: A total of 312 patients with PsA were enrolled. The mean UPsA activity score was 3.7 (SD 1.86), correlating with Disease Activity for Psoriatic Arthritis ($r_s = 0.42$), 68-tender joint count ($r_s = 0.31$), and 66-swollen joint count ($r_s = 0.45$; all $P < .001$). The UPsA damage score averaged 4.1 (SD 2.26), correlating with the modified Sharp–van der Heijde score ($r_s = 0.36$, $P < .001$). The UPsA activity score showed moderate sensitivity to change overall (SRM = 0.63) and high responsiveness in patients achieving minimal disease activity (SRM = 1.03). The sUPsA retained 90% of the information from the full score while substantially improving feasibility.

Conclusions: The UPsA scores underwent internal validation and demonstrated responsiveness, representing valuable tools to assess PsA activity and damage

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Psoriatic arthritis (PsA) is a complex condition requiring precise assessment of inflammation and damage. While musculoskeletal ultrasound provides a comprehensive evaluation of multitarget lesions, existing sonographic composite scores (eg, five targets power doppler for psoriatic disease (5TPD), PsA-Son) are rarely used due to limited evidence and practical constraints. Validated, multidimensional ultrasound scores are needed to better capture disease activity and damage.

WHAT THIS STUDY ADDS

- This study introduces and internally validates the ultrasound scores for PsA (UPsA) activity and damage scores, which correlate strongly with clinical indices of disease activity and structural damage. The activity score shows moderate-to-high sensitivity to change, offering a standardised tool for evaluating musculoskeletal inflammation in patients with PsA.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The UPsA scores can improve precision in evaluating PsA inflammation and damage in clinical trials, and—with the simplified score—enhance feasibility for disease monitoring in clinical practice. They pave the way for incorporating ultrasound-based assessments into trials and clinical care, promoting broader use of this imaging modality in PsA management.

INTRODUCTION

Psoriatic arthritis (PsA) is a heterogeneous disease that typically manifests in various clinical forms, often overlapping with each other. Synovitis, enthesitis, and dactylitis are the hallmark peripheral manifestations, but they can be accompanied by other features such as peritendinitis, osteitis, subcutaneous oedema, and axial involvement [1]. This diversity in

presentation makes diagnosis and treatment complex, as patients may exhibit multiple symptoms simultaneously, mimicking other types of arthritis and each requiring tailored therapeutic approaches [2,3]. Despite the availability of numerous therapies, around half of the patients in clinical practice and only 30% to 40% of patients enrolled in clinical trials achieve remission [4,5]. The assessment of remission is typically based on clinimetric indices that are patient-centred and, in addition to objective measures of inflammation, include subjective evaluations from the patient through patient-reported outcomes (PROs) [2,6,7]. For this reason, in everyday clinical practice, there is an effort to dichotomise nonresponder patients into those with persistent inflammation and those without clear inflammation. Ultrasound can undoubtedly be considered a potential objective tool for evaluating musculoskeletal inflammation, as it has higher sensitivity compared to physical examination [8]. In this scenario, comprehensive sonographic composite scores are essential for monitoring disease activity and detecting the progression of structural damage in PsA. A recent systematic literature review identified only 2 ultrasound scores specifically developed for PsA—the 5 five target power Doppler (5TPD) score and the PsA-Sonographic score (PsA-Son) [8–10]. While both demonstrated good sensitivity in detecting inflammation and showed feasibility, their use in clinical practice and clinical trials is extremely limited, partly due to insufficient evidence in the scientific literature. In recent years, the Outcome Measures in Rheumatology (OMERACT) initiative has led several efforts to develop standardised imaging-based scoring systems to quantify inflammation and damage in PsA. These include the Psoriatic Arthritis Magnetic Resonance Imaging Score for hand assessment and, more recently, the magnetic resonance imaging whole-body score for inflammation in peripheral joints and entheses [11,12]. In parallel, the OMERACT Ultrasound Working Group has refined consensus definitions and scoring systems for key elementary lesions, such as synovitis, enthesitis, and tenosynovitis, thereby establishing

ultrasound as a reliable outcome measurement instrument in clinical trials [13]. Moreover, the international rheumatology community has dedicated specific efforts to apply ultrasound for the study of hallmark PsA features, particularly dactylitis and enthesitis, to improve their characterisation and standardisation across studies [14–16]. The Ultrasound in Psoriatic Arthritis Treatment (UPSTREAM) study is a prospective observational cohort study developed by the Musculoskeletal Ultrasound Study Group of the Italian Society of Rheumatology (SIR) with the goal of identifying clinical and ultrasound predictors of treatment response in patients with PsA who have clinically active peripheral joint disease starting a new treatment, with therapeutic changes made in accordance with routine clinical practice [17]. Within the framework of the UPSTREAM study, we developed 2 ultrasound scores for PsA (UPsA), generated from the ultrasound characteristics of the patients enrolled, to quantify disease activity and damage in patients with active peripheral PsA and primarily intended for use in clinical trials. In addition, we also developed a simplified UPsA score (sUPsA), specifically focused on assessing sonographic disease activity, designed to enhance feasibility and applicability in routine clinical practice.

METHODS

The extended UPSTREAM protocol (V.1.3, 28 November, 2016) has been previously published and registered at clinical-trial.gov (NCT03330769) [17]. Ethical and governance approval for this study was obtained from the IRB/IEC Comitato Etico Lazio 1 (Prot. N. 198 02-02-2017) and subsequently from the IRB/IEC of each participating centre. The study is now complete and closed at all participating centres.

Patients were enrolled at the discretion of the treating investigator among adults (>18 years) who fulfilled the Classification Criteria for Psoriatic Arthritis (CASPAR) for PsA [18], presented with at least 1 swollen and tender peripheral joint, were not in minimal disease activity at baseline, and required a therapeutic change. The decision to start a new treatment (NSAIDs, intra-articular corticosteroids, csDMARDs, bDMARDs, or tsDMARDs) was made in accordance with routine clinical practice and independently from ultrasound results. Patients unable to adhere to the study protocol were excluded. The UPsA activity and damage scores were generated using baseline clinical and sonographic variables. For the sensitivity-to-change analysis of the generated scores, baseline data and 6-month follow-up data were used. Clinical data (including adverse events and concomitant medications) were entered into the REDCap system provided by the SIR [19]. The data system includes password protection and internal quality checks, such as automatic range checks, to identify inconsistent, incomplete, or inaccurate data [19].

Patient and public involvement

This study was conducted within UPSTREAM, a strategic observational project of the SIR. The SIR acted as the scientific promoter, owned the study data, and provided full financial support, including resources such as centralised reading of radiographic images. The project and this study were developed by the SIR Musculoskeletal Ultrasound Study Group under the supervision of a steering committee composed of 5 rheumatologists (AZ, MP, MC, CAS, AI). The online calculator for the UPsA scores was developed by the SIR and is freely accessible

under a Creative Commons Attribution–NonCommercial–NoDerivatives 4.0 International License. It is not copyrighted and is intended exclusively for noncommercial use, with no financial interest or benefit for either the authors or the Society. A formal patient and public involvement process was not implemented. However, SIR routinely integrates patient perspectives and feedback into its broader research planning and strategic initiatives. No direct patient codesign, consultation, or partnership was undertaken in the conception or conduct of this specific study.

Clinical assessment

Demographics, detailed medical history, comorbidities, laboratory results, and past and ongoing medications were recorded at baseline. Clinical assessments were performed by a rheumatologist (clinical assessor), who was blinded to the sonographic data. The 68-tender joint count (68-TJC) and the 66-swollen joint count (66-SJC) were performed to evaluate peripheral joint involvement. Enthesitis was assessed using the Leeds Enthesitis Index (LEI), and 4 additional bilateral enthesal sites (quadriceps insertion patella, plantar fascia, inferior pole patella, and tibial tubercle), thus ensuring consistency with the ultrasound variables collected. Dactylitis will be evaluated using the tender dactylitis count on 20 digits; the clinician squeezes the swollen fingers with moderate pressure and documents the patient's response.

The Bath Ankylosing Spondylitis Disease Activity Index was used to assess axial involvement. Patient global assessment, patient pain assessment, and physician global assessment were scored using 0 to 100 visual analogue scale. Cutaneous psoriatic lesion burden was evaluated by calculating the body surface area. Physical function and health-related QoL were assessed using the Health Assessment Questionnaire and the Psoriatic Arthritis Impact of Disease 12-item questionnaire, respectively. The Disease Activity Index for Psoriatic Arthritis (DAPSA) score was calculated.

Ultrasound assessment

The first step towards the development of sonographic scores to be used in the UPSTREAM study was a qualitative research process aimed at identifying anatomical sites considered most relevant by rheumatologists with expertise in PsA and musculoskeletal ultrasound. To this end, a web-based ranking exercise was performed among 20 experts to evaluate the relative importance of different structures [20]. The candidate sites included in the survey were derived from a previously published systematic literature review [8], and the final ranking informed the selection of joints, tendons, entheses, and bursae incorporated into the UPSTREAM protocol [17]. The evaluation included 42 joints, 36 tendons (26 for the assessment of tenosynovitis and 10 for the assessment of peritendinitis), 12 entheses, and 2 bursae, for a total of 92 structures assessed. [Supplementary Material S1](#) details the anatomical sites and the lesions to be collected in greyscale (GS) and power-Doppler (PD), categorised by activity and damage. Ultrasound examinations were performed at baseline, on the same day of the clinical assessment after the treatment choice was taken, and again at the 6-month follow-up. Sonographers, who were blinded to clinical data and met the inclusion criteria specified in the published protocol, carried out both GS and PD sonography using a high-end ultrasound system

with multifrequency linear transducers (including a high-frequency probe of at least 14 MHz). The reliability assessment among sonographers was performed on static images using a reliability exercise, with a kappa statistic value ≥ 0.7 compared to a ‘gold-standard’ scorer, which was required for inclusion in the study as a sonographer.

Conventional radiography assessment

Baseline radiographs of the hands and feet were taken, and the modified Sharp–van der Heijde score (mSVH score) was evaluated by a rheumatologist with over 30 years of experience in X-ray interpretation, blinded to both clinical and sonography data.

Statistical analyses

Descriptive data are provided according to data type. Continuous variables are presented as mean $\pm$ SD or median and IQR. Absolute and relative frequencies are reported for categorical variables. All statistical analyses were performed using R software, V.3.4.2 (R Foundation for Statistical Computing).

Construction of UPsA scores

For the construction of UPsA scores, the following 4 steps were performed:

1. Lesion score calculation

For each individual, site scores were averaged between the right and left sides, and the resulting values were summed to obtain the total lesion score. The UPsA activity score includes the assessment of synovitis (GS/PD, score 0–3), tenosynovitis (GS/PD, score 0–3), peritendinitis (GS/PD, score 0–1), bursitis (GS/PD, score 0–1), and active enthesitis (score 0–1). The UPsA damage score evaluates the presence or absence of joint erosion, bone proliferation, tendon tear, enthesophyte formation, calcification, and irregularities in the bone profile of entheses.

2. Normalisation

The total lesion scores were normalised by Box-Cox transformations. Transformed lesion scores are indicated by [lesion score]_{bc}.

3. Factor analysis

Factor analysis (FA) is a statistical technique used to identify underlying structures in a dataset [21]. It is often employed to simplify data and reduce dimensionality (Supplementary Material S2). The factor loadings indicate the contribution of each variable to the respective factors, with only variables with significant weights retained for further analysis. Loadings ≥ 0.25 were considered meaningful in this setting. Each factor is a linear combination of the meaningful underlying lesion scores. Discriminant validity of the extracted factors was evaluated with the factor correlation matrix. Reliability was assessed by examining whether the items defining each factor were internally consistent, as indicated by Cronbach’s alpha and construct reliability (CR). In addition, convergent validity was examined through the average variance extracted (AVE). For simple and concrete constructs, a single-item measure may be modelled as a directly observed variable, without requiring internal consistency reliability (Supplementary Material S2).

4. Final score calculation

Final score calculation: individual factor scores were calculated by combining normalised variables weighted by their respective loadings [22]. These factors were summed to derive a composite score, which was subsequently scaled between 0 and 10 using a min–max linear transformation, where 0 corresponds to the complete absence of inflammation or damage (depending on the score used) and 10 indicates the maximum burden detectable across all assessed sites.

Construct validity and sensitivity to change

Spearman’s correlation (r_s) between the newly developed sonographic scores and clinical variables usually associated with activity and damage in patients with PsA was used to investigate the construct validity of the UPsA scores.

To assess the sensitivity to change, the standardised response mean (SRM) was used [23]. SRM is a commonly used effect size measure in clinical and scientific research to evaluate the responsiveness of a scale or instrument to change over time. It is defined as the mean change in scores divided by the SD of the change scores. The following is a general guideline for interpreting SRM magnitudes: SRM < 0.20 : minimal responsiveness (small effect), SRM = 0.20–0.50: low responsiveness (small to moderate effect), SRM = 0.50–0.80: moderate responsiveness (moderate effect), and SRM > 0.80 : high responsiveness (large effect).

Development of the sUPsA score

To derive the sUPsA score, patients were stratified at baseline into 4 clinically relevant subsets: the enthesitic subset, defined by the presence of at least 1 tender enthesis according to the LEI; the dactylitic subset, defined by the presence of at least 1 dactylitic digit; and the oligo/monoarticular and polyarticular subsets. A patient could be classified into more than 1 subset, with the exception of the oligo/monoarticular and polyarticular groups, which were mutually exclusive by definition. Within each subset, variable selection was performed using the Least Absolute Shrinkage and Selection Operator regression [24]. This approach allowed us to identify the variables with the highest predictive value in relation to the full score. For each clinical subset, the variables retained were those most strongly associated with the complete score. All variables adding predictive power within the subset were retained in the final score. Importantly, the mathematical structure of the reduced score was preserved to mirror that of the complete score.

RESULTS

Study sample characteristics

Between February 2017 and March 2020, a total of 312 patients with PsA (153 women, 49.7%) were enrolled in the UPSTREAM study across 19 Italian centres. The study involved 41 rheumatologists overall. Among them, 22 acted as sonographers and 19 as clinical assessors. The group of sonographers included specialists with a mean age of 39.1 years (SD 8). Their median experience in the ultrasound assessment of PsA was 8 years (IQR 7–12). Interobserver reliability, assessed through the kappa coefficient, showed a mean value of 0.81 (SD 0.08).

The patients had a mean (SD) age of 52.8 (13.4) years and a median disease duration of 3.6 years (IQR: 1.1–9.6 years). The median (IQR) of tender joints was 6 (3–13), while the median of

swollen joints was 2 (1-5), with a mean DAPSA (SD) of 26.3 (13.7). Among the enrolled patients, 133/312 (42.6%) had LEI >0, and 61/312 (19.6%) patients presented with at least 1 finger or toe affected by dactylitis. The full set of baseline characteristics of the enrolled patients is reported in Table 1. At 6 months after enrolment, 251 of the 312 patients (80.4%) remained in active follow-up, while 17 of the 61 losses (27.8%) were attributable to the COVID-19 pandemic.

At the sixth month, 76 out of 251 (30.3%) patients had achieved MDA. The mean duration of the ultrasound examination, obtained from the average of 5 ultrasound exams conducted by the 4 rheumatologists with the highest number of enrolments, was 36.2 (±7.9) minutes. The distribution of sites affected by imaging, detected inflammatory involvement, is reported in Supplementary Material S3.

UPsA activity score

After evaluating the applicability of FA, 5 factors were obtained by choosing variables with meaningful loadings (≥0.25). (Supplementary Tables S1,S2 in Supplementary Material S2). Each factor represents a new dimension of the disease activity, summarising the underlying initial lesion variables and reducing their numerosity. The first factor contains synovitis PD_{bc}, peritendinitis PD_{bc}, and GS_{bc}; the second encompasses synovitis PD_{bc}, tenosynovitis PD_{bc}, and GS_{bc}; the third is represented by bursitis PD_{bc} and GS_{bc}, and the fourth by synovitis PD_{bc} and GS_{bc}. Finally, the fifth factor is only composed of active enthesitis_{bc}. The final UPsA activity score is: [(peritendinitis-GS_{bc} + peritendinitis-PD_{bc} + synovitis-PD_{bc}) × 1.88] +

[(tenosynovitis-GS_{bc} + tenosynovitis-PD_{bc} + Synovitis-PD_{bc}) × 1.72] + [(bursitis-GS_{bc} + bursitis-PD_{bc}) × 1.61] + [(synovitis-GS_{bc} + synovitis-PD_{bc}) × 1.46] + [active enthesitis_{bc} × 1.00]. The UPsA activity score ranges from 0 to 10. At the baseline, the mean (SD) UPsA activity score was 3.72 (1.86), and the distribution of the activity score is shown in Figure 1A.

An oblique rotation was performed, and the factor correlation matrix indicated generally low-to-moderate correlations among factors (range = 0.003-0.36). Although some factors shared modest associations, none of the correlations were high enough to suggest redundancy, confirming that factors were clearly differentiated.

Internal consistency was evaluated, with satisfactory Cronbach's alpha coefficients for all factors (≥ 0.7). In addition, CR and AVE were assessed, with all values falling within recommended thresholds (Supplementary Material S2).

Table 1
Baseline demographic and clinical characteristics of the study population

Baseline data (n = 312)	
Age, mean (SD)	52.8 (13.4)
Age, median (IQR)	54 (45-63)
Sex (female), n (%)	153 (49.7)
BMI, mean (SD)	26.3 (4.4)
BMI, median (IQR)	25.8 (23.2-28.8)
Disease duration (y), mean (SD)	7.1 (9.3)
Disease duration (y), median (IQR)	3.6 (1.1-9.6)
Disease duration (≥2 y), n (%)	196 (63.6)
HAQ, mean (SD)	0.8 (0.6)
HAQ, median (IQR)	0.8 (0.2-1.1)
TJC 68, mean (SD)	9.5 (8.8)
TJC 68, median (IQR)	6 (3-13)
SJC 66, mean (SD)	3.8 (4)
SJC 66, median (IQR)	2 (1-5)
PGA, mean (SD)	58.6 (22.9)
PGA, median (IQR)	64 (40.5-77)
VAS pain, mean (SD)	59.1 (24.2)
VAS pain, median (IQR)	63 (40-80)
DAPSA, mean (SD)	26.3 (13.7)
DAPSA, median (IQR)	24 (16.4-33.1)
PSAID12, mean (SD)	42.7 (21.3)
PSAID12, median (IQR)	43 (25.8-61)
LEI >0, n (%)	133 (42.6)
Dactylitis >0, n (%)	61 (19.6)
BSA ≥3, n (%)	50 (16.4)
CRP, mean mg/L (SD)	0.9 (3)
CRP, median mg/L (IQR)	0.1 (0-0.5)

BMI, body mass index; BSA, body surface area; CRP, C-reactive protein; DAPSA, disease activity in psoriatic arthritis; HAQ, Health Assessment Questionnaire; LEI, Leeds Enthesitis Index; PGA, patient global assessment; PSAID, Psoriatic Arthritis Impact of Disease; SJC, swollen joints count; TJC, tender joints count; VAS, visual analogue scale.

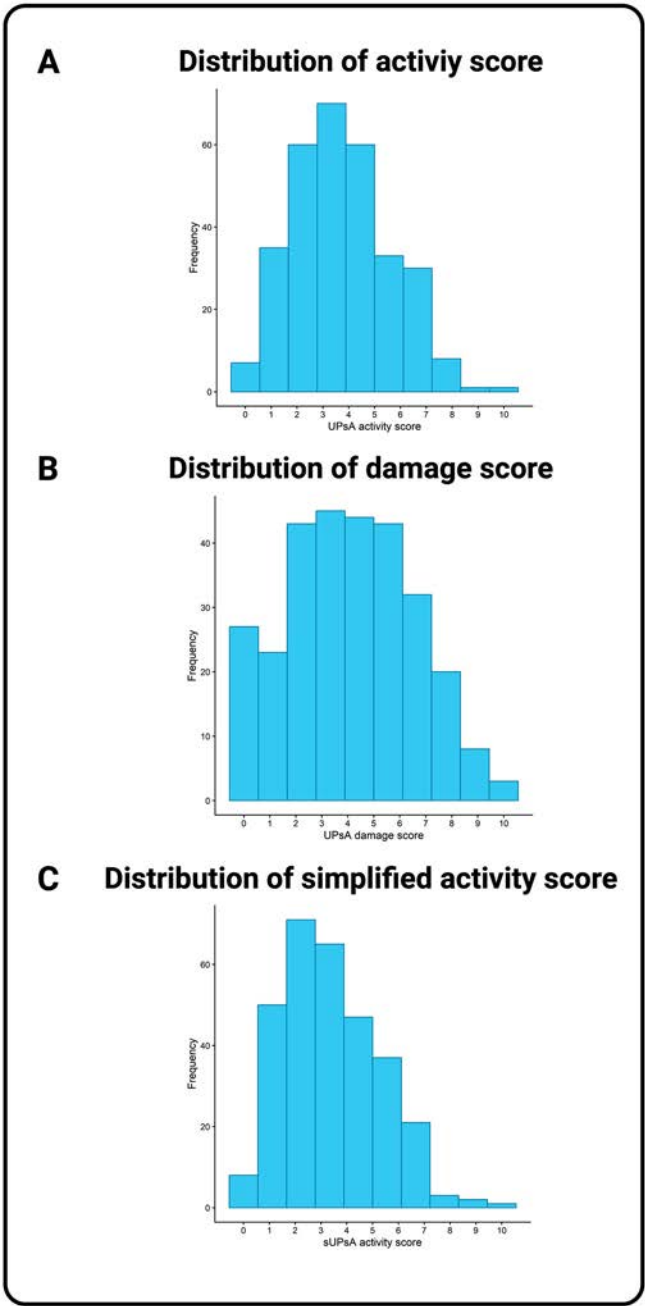


Figure 1. Distribution of the UPsA activity score (A), damage score (B), and simplified UPsA score (C). UPsA, ultrasound scores for PsA.

Communality values were consistently high (>0.70), confirming that the extracted factors adequately represented the variables (Supplementary Material S2).

The Link calculator for the UpSA activity score is available here (Calculator UPsA activity score, <https://craldn.shinyapps.io/appsh/>) (Fig 2).

UPsA damage score

After evaluating the applicability of FA, 5 factors were identified, each with specific loadings as presented in (Supplementary Tables S1 and S2 in Supplementary Material S2). As regards the UPsA damage score, the first factor includes enthesophyte_{bc}, calcification_{bc}, and irregular enthesal bone profile_{bc}; the second contains bone proliferation_{bc} and enthesophyte_{bc}; the third is represented by joint erosion_{bc}; and the fourth by tendon tear_{bc}. Finally, the fifth factor is only composed of enthesal erosion_{bc}. The final UPsA damage score is: $[(\text{enthesophyte}_{bc} + \text{enthesal calcification}_{bc} + \text{irregular enthesal bone profile}_{bc}) \times 1.62 + [(\text{bone proliferation}_{bc} + \text{enthesophyte}_{bc}) \times 1.44] + [(\text{joint erosion}_{bc}) \times 1.04] + [\text{tendon tear}_{bc} \times 1.01] + [\text{enthesal erosion}_{bc} \times 1.00]$. The UPsA damage score ranges from 0 to 10. At the baseline, the mean (SD) UPsA damage score was 4.1 (2.26), and the distribution of the activity score is shown in Figure 1B.

An oblique rotation was performed, and the factor correlation matrix indicated generally low-to-moderate correlations among factors (range = 0.14–0.35). The correlation between

the first and second factors ($r = 0.51$) was higher than the others; nevertheless, enthesophytes were retained, reflecting 2 distinct dimensions: enthesopathy and proliferative activity.

Internal consistency was satisfactory, with satisfactory Cronbach's alpha coefficients for all factors (≥ 0.7). In addition, CR and AVE were assessed, with all values falling within recommended thresholds (Supplementary Material S2). Communality values were consistently high (>0.70), confirming that the extracted factors adequately represented the variables (Supplementary Material S2).

The Link calculator for the UpSA damage score is available here (Calculator UPsA damage score, <https://craldn.shinyapps.io/apps/>) (Fig 2).

Internal validation of the sonographic activity and damage scores

Both scores demonstrated good construct validity based on their correlations with clinical variables (Table 2). The UPsA activity score showed moderate correlations with key measures of disease activity, particularly 66-SJC ($rs = 0.45$, $P < .001$) and DAPSA ($rs = 0.42$, $P < .001$), and weaker but significant correlations with 68-TJC ($rs = 0.31$, $P < .001$), dactylitis count ($rs = 0.34$, $P < .001$), and C-reactive protein (CRP) ($rs = 0.20$, $P < .001$). In contrast, the UPsA damage score correlated moderately with the modified Sharp–van der Heijde score ($rs = 0.36$, $P < .001$), while showing only weak correlations with a few clinical variables such as TJC ($rs = 0.19$, $P = .0008$) and LEI ($rs = 0.13$,

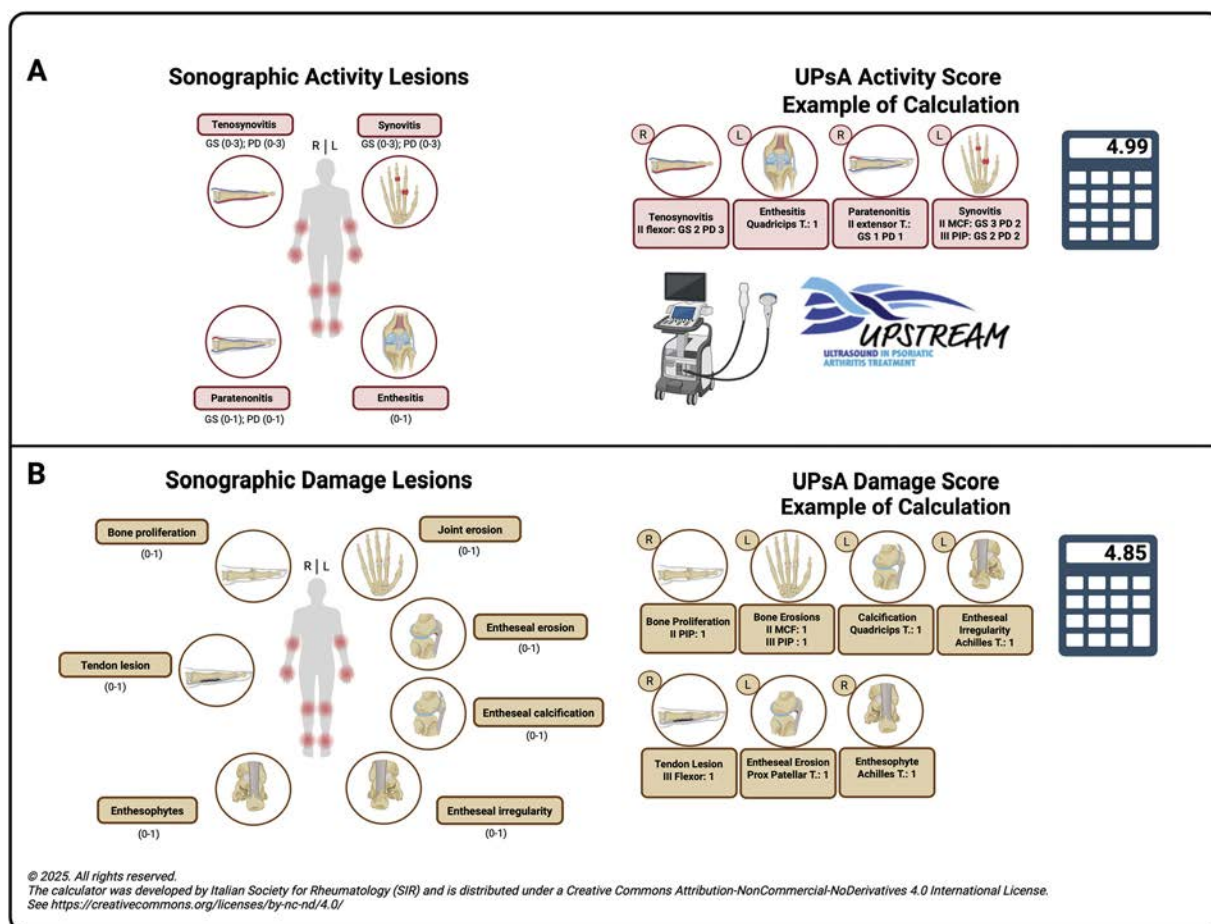


Figure 2. Ultrasound lesions of activity (A) and damage (B) contributing to the UPsA scores, with an example of calculation and a QR code linking to the calculation tool. GS, greyscale; MCP, Metacarpophalangeal (joint); PD, power-Doppler; PIP, Proximal Interphalangeal (joint); UPsA, ultrasound scores for PsA.

Table 2

Correlation between clinical variables and UPsA activity and damage scores and simplified UPsA score

Features	UPsA activity score		UPsA damage score		Simplified UPsA score	
	Spearman's correlation	P value	Spearman's correlation	P value	Spearman's correlation	P value
ESR	0.24	<.001	0.06	.31	0.22	<.001
CRP	0.202	<.001	0.12	.03	0.25	.04
TJC 68	0.312	<.001	0.19	.0008	0.29	<.001
SJC 66	0.45	<.001	0.04	.41	0.37	<.001
Dactylitis count	0.339	<.001	−0.01	.75	0.31	<.001
LEI	−0.04	.534	0.13	.02	0.04	.48
PhGA	0.255	<.001	0.04	.45	0.21	<.001
PGA	0.22	<.001	−0.12	.03	0.2	<.001
VAS pain	0.25	<.001	−0.05	.37	0.24	<.001
HAQ	0.28	<.001	0.10	.08	0.24	<.001
BASDAI	0.28	<.001	0.14	.01	0.23	<.001
PSAID12	0.22	<.001	0.10	.07	0.17	.002
DAPSA	0.42	<.001	0.14	.02	0.28	<.001
mSharp–van der Heijde score	−0.05	.6	0.36	<.001	0.04	.6

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; DAPSA, The Disease Activity Index for Psoriatic Arthritis; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; LEI, Leeds Enthesitis Index; PGA, patient global assessment; PhGA, physician global assessment; PSAID, Psoriatic Arthritis Impact of Disease; SJC, swollen joints count; TJC, tender joints count; UPsA, ultrasound scores for PsA; VAS, visual analogue scale.

$p < 0.05$ is considered significant and shown in bold.

$P = .02$) (Table 2). In addition, for descriptive purposes, we compared UPsA activity score categories (low, medium, high) with DAPSA values, as shown in Supplementary Figure S1 (inside Supplementary Material S4), and UPsA damage scores with the mSVH scores (Supplementary Fig S2 inside Supplementary Material S4). This categorisation is exclusively meant for illustration, and its cutoffs are not clinically validated.

Sensitivity to change of the UPsA activity score

The sensitivity-to-change analysis for the UPsA activity score was performed on 218 of these patients (69.8% of the total cohort and 86.9% of those who completed the 6-month follow-up) who had complete ultrasound data available at both baseline and 6 months. The mean UPsA activity score at baseline was $3.83 (\pm 1.96)$, while the mean score at 6 months was $2.6 (\pm 1.93)$, showing a significant reduction in disease activity as assessed by ultrasound ($P < .001$) (Fig 3A). The SRM was 0.62 (95% CI: 0.54–0.80), suggesting that the score has moderate sensitivity to change. In the 72/218 (35.3%) patients who achieved MDA at 6-month follow-up, the SRM was 1.03 (95% CI: 0.89–1.41), demonstrating a high sensitivity to change (Fig 3B). For comparison, the DAPSA demonstrated an SRM of 0.93 (95% CI: 0.81–1.09) in the overall cohort (Supplementary Material S5).

Simplified UPsA score

The simplified UPsA activity score markedly reduced the number of collected variables, decreasing from 80 in the full version to 46 in the simplified form, corresponding to a 42.5% reduction. The variables retained in the simplified score are highlighted in light blue (see Supplementary Material S1). The correlation between the simplified and the complete UPsA activity score was strong (Spearman's $r = 0.89$, $P < .001$), confirming that the simplified model retained almost 90% of the information captured by the extended version (Fig 4). The sUPsA activity score showed significant correlations with key measures of disease activity, including 66-SJC ($rs = 0.37$, $P < .001$), DAPSA ($rs = 0.28$, $P < .001$), 68-TJC ($rs = 0.29$, $P < .001$), dactylitis count ($rs = 0.31$, $P < .001$), CRP ($rs = 0.25$,

$P = .04$), and erythrocyte sedimentation rate ($rs = 0.22$, $P < .001$) (Table 2).

At baseline, the mean (SD) sUPsA was 3.40 (1.85), which was highly comparable to the corresponding value of the full UPsA activity score [3.72 (1.86)], and the distribution of sUPsA is shown in Figure 1C. At the 6-month follow-up, the mean sUPsA decreased to 2.4 (1.8), indicating a significant reduction in ultrasound-detected disease activity ($P < .001$) (Fig 3C). The SRM was 0.53 (95% CI: 0.40–0.68), reflecting a moderate sensitivity to change. Among the 72 of 218 patients (35.3%) who achieved MDA at 6 months, the SRM increased to 0.92 (95% CI: 0.70–1.19), demonstrating a high sensitivity to change. The Link calculator for the sUPsA score is available here (Calculator sUPsA Activity score, <https://craldn.shinyapps.io/appshh/>).

DISCUSSION

The target of therapy with DMARDs in PsA is to abrogate inflammation [2], and the achievement of this target is generally associated with a state of remission according to clinimetric indices, particularly MDA and DAPSA. Musculoskeletal ultrasound is undoubtedly a more sensitive tool than clinical examination for identifying inflammation and can detect the precise location of the inflammatory abnormalities [8]. Despite these unique features, it is not currently included in composite indices of disease activity, which are generally composed of clinical examination and PROs [25]. However, although clinical studies have not yet provided clear evidence of the added value of ultrasound compared to clinical examination, its affordability, accessibility, and lack of radiation exposure have made it a widely used tool in many rheumatology clinics. In this study, we developed and internally validated 2 multidimensional scores to assess musculoskeletal inflammation (UPsA activity score) and damage (UPsA damage score) in PsA with active peripheral involvement, together with a simplified activity score for easier use in clinical practice. The full scores are mainly intended for clinical trials.

Several steps contributed to the development of the scores: a previously published systematic literature review of musculoskeletal ultrasound in PsA provided the scientific background and laid the foundation for a web-based ranking exercise among

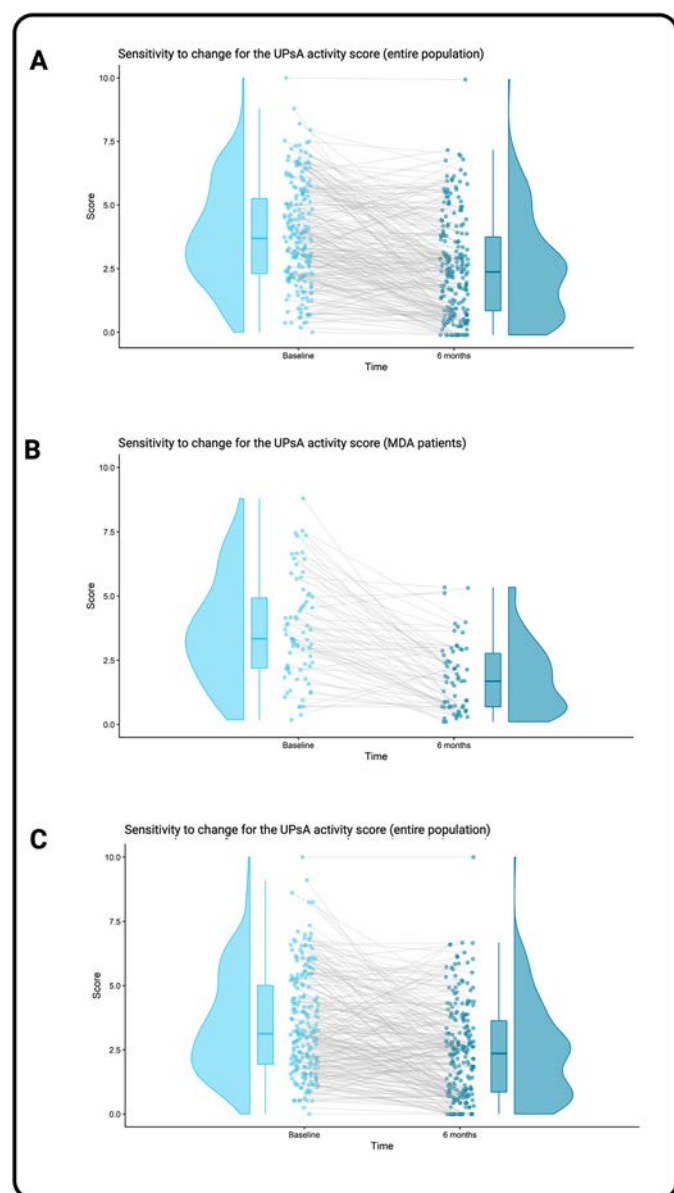


Figure 3. The sensitivity-to-change analysis for the UPsA activity (A), damage (B) and sUPsA activity scores (C). MDA, minimal disease activity; sUPsA, simplified UPsA activity score; UPsA, ultrasound scores for PsA.

20 rheumatologists to define the anatomical sites considered relevant for the management of PsA [20]. This led to the creation of the sonographic protocol used in the UPSTREAM study. Using baseline data from patients enrolled in UPSTREAM and applying a methodology previously used in the development of

the Disease Activity Index for Reactive Arthritis (DAREA)/DAPSA score [26], we created an activity score and a damage score. Consistent with the multifaceted nature of PsA, some lesions (such as synovitis) contributed to more than one factor, reflecting the clinical overlap between inflammatory processes (synovitis, tenosynovitis, and peritendinitis) rather than statistical redundancy. This approach ensured that the UPsA factors captured distinct but pathophysiologically related dimensions of inflammation and structural damage, while maintaining statistical independence and internal consistency. These scores demonstrated internal construct validity, with statistically significant correlations of weak-to-moderate magnitude with clinical variables of activity and damage. In our cohort, UPsA activity related significantly to clinical indices in the weak-to-moderate range, while UPsA damage correlated significantly and in the weak-to-moderate range with structural damage on radiographs assessed by the mSVH score. These magnitudes are consistent with the literature in inflammatory arthritis, where ultrasound metrics and clinimetric indices only partially overlap and strong correlations are uncommon. For example, the APPRAISE OMERACT–EULAR study in rheumatoid arthritis reported no meaningful correlation between sonographic Global OMERACT–EULAR Synovitis Score (GLOESS) scores and DAS28-derived outcomes—indicating that imaging and clinical composites capture complementary aspects of disease activity [27]. Taken together, these findings support the view that clinical examination, clinimetric indices, and ultrasound may assess different dimensions of PsA activity, and that the UPsA scores should be interpreted alongside clinical examination and PROs as added value to qualify inflammation and damage at the individual-patient level. Additionally, the UPsA activity score demonstrated moderate-to-high sensitivity to change at the 6-month follow-up. However, the sensitivity to change of the UPsA damage score could not be defined, as the 6-month timeframe is too short to detect changes in joint damage assessed by ultrasound. The methodology that guided the development of the score is undoubtedly a strong point. Equally significant was the nationwide participation, involving 22 sonographers and 19 clinicians from 19 centres across Italy.

Following external validation in independent cohorts, the UPsA scores would be of particular interest for application in clinical trials to evaluate their performance as alternative, ultrasound-based multidimensional instruments of inflammation, to which a joint-damage component can be paired [28].

This study has limitations. First, the UPsA scores underwent development and internal validation only; external validation in independent cohorts and settings is needed to confirm generalisability and refine interpretability and thresholds. Second, the prospective multicentre observational design in routine care, with enrolment focused on clinically active peripheral PsA

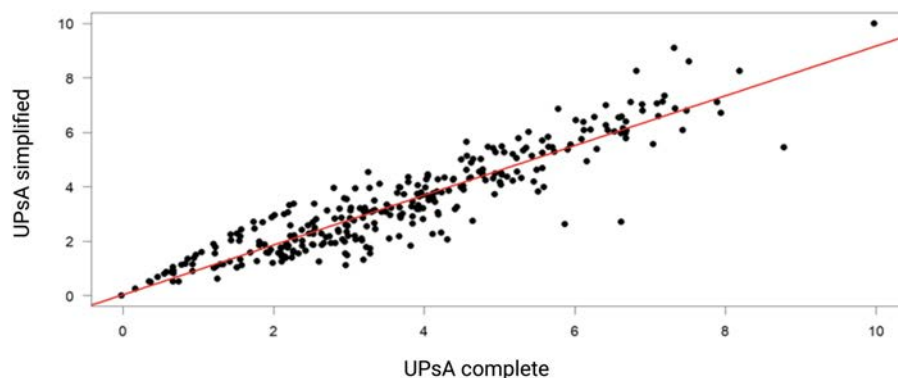


Figure 4. Correlation between the simplified (sUPsA) and the complete UPsA activity score (Spearman's $r = 0.89$, $P < .001$). sUPsA, simplified UPsA activity score; UPsA, ultrasound scores for PsA.

starting new treatment, may limit generalisability and leave residual confounding.

In conclusion, the UPsA scores introduce a novel ultrasound-based approach to assessing joint inflammation and damage in PsA. In this multicentre study, the UPsA scores showed construct validity and sensitivity to change, complementing traditional clinical indices. Furthermore, to enhance feasibility in routine care, we have also developed a simplified version aimed at easier implementation, while the primary focus of the present work remains a tool for research and trials. Overall, these findings are encouraging and suggest promising prospects for future application and refinement, including integrated clinic–imaging workflows to support treatment-response assessment and patient stratification.

CONTRIBUTORS

AZ, MP, MC, CAS, and AI contributed to the conceptualisation of the study. Methodology was developed by AZ, MP, MC, CAS, AI, CDN, GC, and DR. Data curation was performed by DR, CDN, and GC, while formal analysis was conducted by DR, CDN, GC, and CAS. The original draft was written by AZ, and the manuscript was reviewed and edited by AZ, MP, and MC. Project administration was carried out by AZ, MP, MC, CAS, and AI. Funding was acquired by AI. Supervision was carried out by all authors, and all authors participated in the investigation and contributed resources, and have read and approved the final manuscript.

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Competing interests

The authors declare no conflicts of interest related to this study.

Patient consent for publication

Written informed consent for publication was obtained from the patients.

Ethics approval

The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the local Institutional Review Board / Ethics Committee, and all participants provided written informed consent prior to inclusion.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

All study data are stored at the Research Center of the Italian Society for Rheumatology (Centro Studi della Società Italiana di Reumatologia). Data are available for consultation upon justified request addressed to both the President of the Italian Society for Rheumatology and the Coordinator of the Research Center.

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ard.2025.10.032](https://doi.org/10.1016/j.ard.2025.10.032).

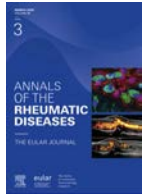
Orcid

Alen Zabotti: <http://orcid.org/0000-0002-0573-464X>

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Psoriatic arthritis

CD3 + immune cell endotypes are associated with PsA disease phenotype and response to advanced therapy: an integrated mass cytometry and proteomics cohort study

Lihi Eder^{1,2,3,*}, Steven Dang^{1,3}, Stephane M. Caucheteux², Xianwei Li⁴, Sydney Thib¹, Darshini Ganatra^{5,6}, Christopher Ritchlin⁷, Igor Jurisica^{5,8,9}, Vincent Piguet^{1,2,3}, Liqun Diao⁴, Vinod Chandran^{2,3,5,6,10}

¹ Women's College Research Institute, Women's College Hospital, Toronto, Ontario, Canada

² Department of Medicine, Temerty Faculty of Medicine, University of Toronto, Ontario, Canada

³ Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada

⁴ Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, Ontario, Canada

⁵ Schroeder Arthritis Institute, Krembil Research Institute, Toronto, Ontario, Canada

⁶ Gladman Krembil Psoriatic Arthritis Program, UHN, Toronto, Ontario, Canada

⁷ Department of Allergy, Immunology and Rheumatology, University of Rochester, Rochester, New York, USA

⁸ Departments of Medical Biophysics and Computer Science, and Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

⁹ Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia

¹⁰ Department of Laboratory Medicine and Pathobiology, Temerty Faculty of Medicine, University of Toronto, Ontario, Canada

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ABSTRACT

Objectives: This study aims to identify circulating cellular immune cell endotypes in psoriatic arthritis (PsA), assess their association with treatment response, and explore key biological pathways linked to these immune profiles.

Methods: Using mass cytometry, we analysed CD3+ immune cell populations in patients with PsA initiating targeted therapies. Hierarchical clustering identified immune cell endotypes, and their associations with clinical features and treatment response were assessed using generalised estimating equation models. Proteomic profiling via an aptamer-based assay compared differentially expressed proteins across clusters, followed by pathway analysis. Imaging mass cytometry analysis was performed to characterise T cell subsets in synovial tissue samples.

Results: We analysed blood samples from 40 treatment periods involving 34 patients and identified 3 immune clusters (C); C1: 'memory CD4+ T cell endotype' – associated with higher sonographic musculoskeletal inflammation and peri-articular bone formation, and poorer response to therapy; C2: 'Nonclassical T cell endotype'—exhibited lowest levels of musculoskeletal inflammation; and C3: 'Terminal effector/Th1 cell endotype'—linked to higher sonographic peri-articular inflammation. The immune endotypes remained relatively stable 3 months post-treatment. The 'memory CD4+ T cell endotype' was characterised by deregulation of immune-related biological pathways, including several intracellular signalling pathways, the most

*Correspondence to Dr. Lihi Eder, Women's College Research Institute, Women's College Hospital, Toronto, Ontario, Canada.

E-mail address: lihi.eder@wchospital.ca (L. Eder).

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notable being WNT signalling. CD4⁺ cells with memory and effector phenotypes were abundant in the synovial tissue sample from patients with PsA.

Conclusions: Heterogeneity in circulating immune cell profiles is associated with PsA clinical features and therapeutic response. The results underscore the potential of immune cell phenotyping to improve prognosis in PsA, which could inform personalised treatment strategies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Immune heterogeneity likely plays a role in the pathogenesis and clinical expression of psoriatic arthritis (PsA). However, the relationship between specific cellular immune endotypes and the therapeutic response to targeted treatments remains incompletely understood.

WHAT THIS STUDY ADDS

- Through integrated analysis of mass cytometry and proteomic data, this study identified a CD4⁺ memory T cell-dominant immune endotype associated with dysregulation of several intracellular signalling pathways, the most notable being the WNT signalling pathway. Patients with PsA exhibiting this immune profile demonstrated reduced responsiveness to targeted therapies and were more likely to develop a ‘difficult-to-treat’ disease phenotype.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Immune cell phenotyping has the potential to refine prognostic stratification in PsA by identifying individuals with immunologic resistance to current targeted therapies. Early detection of such endotypes may inform treatment decisions and support a more aggressive immunomodulatory approach in selected patients.

INTRODUCTION

Psoriatic arthritis (PsA) is a clinically and genetically heterogeneous disease, contributing to suboptimal outcomes with fewer than half of patients achieving a state of minimal disease activity [1–4]. Some patients remain treatment-refractory despite multiple therapies and are classified as ‘difficult-to-treat’ (D2T) PsA [5,6].

While previous studies have identified heterogeneous associations between immune profiles and clinical features of PsA [7–9], it remains unclear whether distinct immune endotypes are linked to clinical manifestations or treatment response. Prior studies focused on selected immune cell populations without comprehensive immune endotype profiling and rarely integrated clinical data and treatment response, often comparing PsA to healthy controls [10–13]. As a result, it remains unclear whether comprehensive immune cell profiling can identify clinically relevant immune endotypes in PsA.

The objectives of this exploratory study were to: (1) identify cellular immune endotypes using unsupervised cluster analysis and assess their associations with PsA clinical features and treatment response; and (2) characterise key biological pathways linked to these immune endotypes through integrated mass cytometry and proteomic analysis.

METHODS

Setting and population

Consecutive patients with PsA initiating targeted therapy for active peripheral disease were prospectively recruited. They

underwent clinical assessment, musculoskeletal ultrasound, and blood sample collection (whole blood and serum) at baseline and 3 months posttreatment (Supplementary S1). Patients were then followed within the University of Toronto PsA cohort, with longitudinal therapeutic response data systematically collected. Twenty individuals without psoriasis or arthritis, with similar age, sex, and race distributions, were recruited from hospital staff as controls. The study was approved by the Research Ethics Board of Women’s College Hospital, and all participants provided written informed consent.

Clinical and ultrasound assessments

A rheumatologist assessed all patients using a standardised protocol (Supplementary S2). Treatment response was evaluated by the American College of Rheumatology (ACR) 20 response and changes in the Disease Activity Index for PsA (DAPSA) score from baseline. D2T PsA was defined as an inadequate response to ≥ 2 targeted therapies [6], and its prevalence was assessed from baseline through 24 months after treatment initiation.

To objectively quantify inflammation and structural damage in peripheral tissues, musculoskeletal ultrasound was performed by a single assessor (LE) before and 3 months after treatment initiation, following a standardised protocol (Supplementary S3) [14]. Intrarater reliability of ultrasound showed an intraclass correlation coefficient of 0.90 across all domains [14].

Whole blood staining and mass cytometry acquisition

A detailed description of mass cytometry procedures can be found in Supplementary S4. Whole blood samples were stained for cell surface markers using the Maxpar Direct Immune Profiling Assay panel (Standard BioTools Inc.) that includes 30 dry metal-conjugated antibodies, as per the manufacturer’s instructions.

Samples were acquired using a Helios mass cytometer (Standard BioTools). The Maxpar Direct Immune Profiling Assay Template was used for the acquisition. Automated immune cell quantification was performed using Maxpar Pathsetter v3.0 to eliminate unwanted events and identify and label 37 major immune cell populations based on predefined cellular markers. This automated approach eliminates the variability associated with manual gating [15].

Mass cytometry data analysis

We then used the quantitative data of the annotated 37 immune cell populations in each sample for an unsupervised hierarchical cluster analysis with a focus on immune endotypes rather than on individual immune cell populations.

Our initial unsupervised hierarchical cluster analysis produced only two clusters distinguished by their neutrophil/lymphocyte ratio, with limited variability in T cells. This likely reflects the dominant presence and variability of granulocytes, which can obscure more subtle immune features, such as variability signals from less frequent T cell subsets in high-

dimensional analyses, thereby masking biologically meaningful distinctions.

To refine our approach, we then focused on CD3+ cells, which are better phenotyped by our panel, and repeated clustering using 16 cell populations among CD3+ cells from patients with PsA. The clustering algorithm was then applied to the control samples.

Associations between the 3 identified clusters and clinical, sonographic parameters, and treatment response parameters were evaluated using generalised estimating equation (GEE) models [16] to account for inpatient correlations. No corrections for multiple testing were applied due to the study's exploratory nature.

Proteomic pathway enrichment analysis

Serum samples from 30 individual patients with PsA, collected concurrently with the whole blood samples used for mass cytometry, were analysed to identify differentially expressed proteins (DEPs) and pathways associated with the 3 identified immune endotypes (clusters).

Samples were analysed using the SomaScan 7K assay (SomaLogic), an aptamer-based proteomics platform [17]. Raw relative fluorescence units from the analysis values were standardised and normalised [18]. The panel measured 7289 human protein aptamers, corresponding to 6402 unique proteins. For proteins with multiple aptamers, we selected the aptamer with the lowest *P*-value and highest fold change. Proteomic data processing was conducted in *RStudio* using the *somadataIO* package (v6.1.0). The data were converted into an expression set, and relative fluorescent units values were log2-transformed before batch effect assessments were performed using multidimensional scaling plots.

The proteomic analysis used a 2-step approach: DEPs were first identified among patients with PsA classified into cluster 1 vs cluster 2 and vs cluster 3 without multiple testing correction (nominal *P* value < .05) using the *limma* package (v3.58.1). This step was followed by pathway enrichment analysis, performed using literature-curated pathways from pathDIP v5 (<https://ophid.utoronto.ca/pathDIP>) where false discovery rate (FDR) correction was applied [19]. The enriched pathways were derived from the pathDIP matrix, enabling the construction of a protein-pathway network, which was visualised using Network Analysis, Visualization, & Graphing TORonto v3 [20].

This two-step approach prioritised discovery of biologically relevant pathways rather than individual biomarkers, which is often justified in exploratory proteomics with limited sample size [21,22]. While this may increase false positives at the protein level, the application of FDR correction at the pathway level minimises false positive discoveries in the final biological interpretation.

Imaging mass cytometry

Imaging mass cytometry (IMC) was performed on synovial tissue samples from 4 patients with PsA and 1 osteoarthritis control to characterise memory and effector T cell subsets. The methodology has been described in detail previously [23]. Briefly, we analysed a panel of antibodies targeting T cell subsets and functional markers (Supplementary S5). Data acquisition was conducted using a Helios time-of-flight mass cytometer coupled to a Hyperion Imaging System (Standard Biotools). Laser ablation generated an intensity map of the target proteins across the region of interest. For image processing, pixel classification, and single-cell segmentation, *ome.tif* images were exported to QuPath. Cell detection/object classification was

performed using DNA and Vimentin staining to identify the nucleus and plasma membrane [24]. Pixel classification training was performed on all antibody staining. Cell position and cell/signal measurements were exported to CytoMAP for further analysis. Clustering, 2D dimensionality reduction, heatmap, and phenotype annotation were performed in CytoMAP [25].

RESULTS

Blood samples collected from 34 patients with PsA during 40 treatment periods, and from 20 controls, were analysed. The following advanced therapies were used: 21 IL-17 inhibitors (i) (ixekizumab 15; secukinumab 5, bimekizumab 1), 16 TNFi (certolizumab 10, adalimumab 5, golimumab 1), and 3 JAKi (tofacitinib 3).

Immune cell endotype characterisation

Unsupervised cluster analysis identified 3 distinct immune endotypes (clusters) among CD3+ cells which were labelled based on the dominant cell populations (Fig 1A-E, Supplementary S6). Cluster 1 (*N* = 12): characterised by elevated levels of central memory and effector memory T cells, particularly within the CD4+ subset, defining a 'memory CD4+ T cell endotype'. Cluster 2 (*N* = 15): Characterised by the $\gamma\delta$ T cells and MAIT/NKT cells ('Nonclassical T cell endotype'). This cluster had higher levels of naïve T cells, particularly in the CD8+ compartment. Cluster 3 (*N* = 13): Defined by an enrichment of CD4+ and CD8+ terminal effector T cells and Th1 cells, forming a 'Terminal Effector/Th1 endotype'.

Imaging and clinical features across immune endotypes

The identified immune endotypes exhibited distinct sonographic PsA features (Fig 2A). The 'memory CD4+ T cell endotype' displayed the most active inflammation, with the highest total sonographic inflammation scores driven primarily by higher synovitis and enthesitis scores. This cluster also showed higher peri-articular and enthesal new bone formation scores. In contrast, the 'Nonclassical T cell endotype' had the mildest presentation, characterised by the lowest sonographic inflammation scores and a low physician global assessment. The 'Terminal effector/Th1 endotype' represented an intermediate phenotype, marked by prominent periarticular inflammation, as evidenced by the highest peritendon inflammation scores.

No significant differences were observed in baseline clinical measures of disease activity and in C-reactive protein (CRP) levels (Table 1). Several demographic differences were observed across the clusters. The 'memory CD4+ T cell endotype' was more prevalent in older patients and associated with a later onset of PsA. The 'Nonclassical T cell endotype' had the earliest onset of PsA, and patients were younger. The 'Terminal effector/Th1 endotype' was most frequently observed among non-White patients.

All 3 immune endotypes were observed in both patients with PsA and controls, with numerical differences in their distribution (Fig 2B). Most controls (55%) were classified as 'Nonclassical T cell endotype', while only 15% fell into the 'Memory CD4+ cell endotype' which was more prevalent among patients with PsA.

Treatment response across immune endotypes

Patients in the 'CD4+ memory cell endotype' were less likely to achieve an ACR20 response compared to the other two endotypes (odds ratio: cluster 1 vs 2 = 0.15 [0.02, 0.93]; cluster

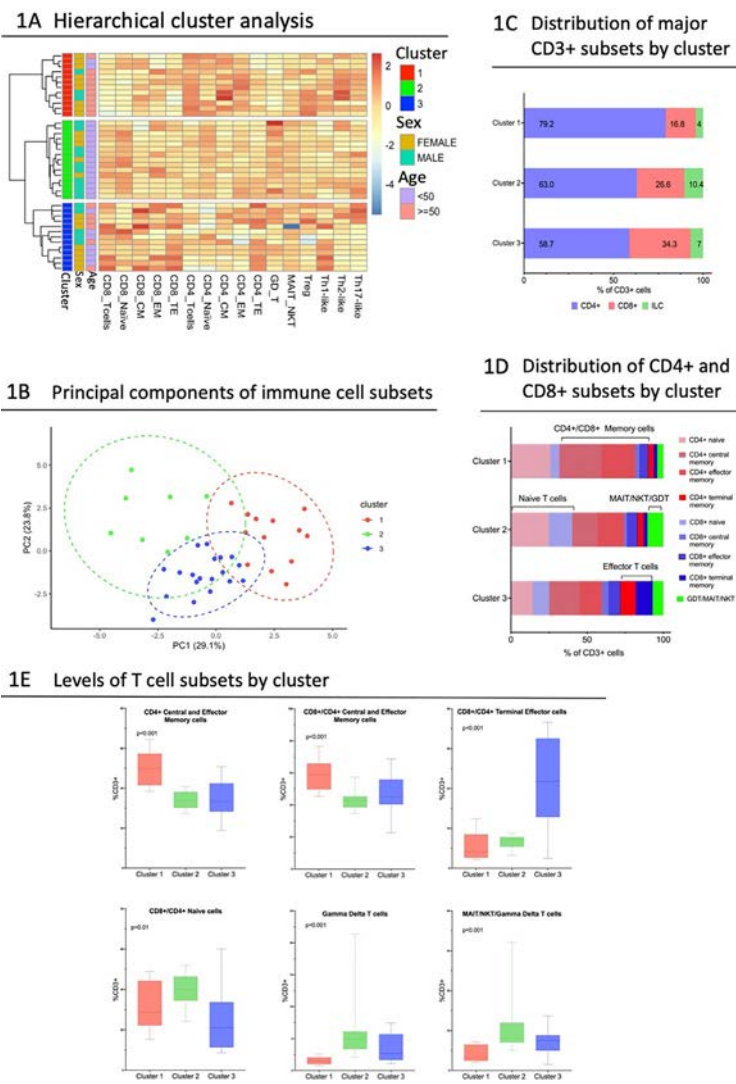


Figure 1. Characteristics of the immune clusters (endotypes). (A) Hierarchical cluster analysis identified 3 clusters. Cluster 1: CD4 + memory cell endotype; cluster 2: nonclassical T cell endotype; cluster 3: terminal effector/Th1 endotype. (B) Principal components of immune cell subsets among CD3 + by cluster. (C) Distribution of CD4 +, CD8 +, and nonclassical T cells among CD3 + cells by cluster. (D) Distribution of CD4 + and CD8 + subsets among CD3 + cells by cluster. (E) Comparison of the levels of selected T cell subsets by cluster. CM, central memory; EM, effector memory; TE, terminal effector.

1 vs 3 = 0.09 [0.01, 0.68], Table 2). Consistently, this endotype was associated with lower reduction in DAPSA score (β : cluster 1 vs 2 = 12.3 [5.7, 18.9]; cluster 1 vs 3 = 13.9 [7.7, 19.9]). These associations remained statistically significant after adjustment for age and race (data not shown). Associations between individual immune cell populations and treatment response parameters are shown in Figure 3 and Supplementary S7. While our study was not powered to assess treatment response by drug class, drug class distribution across immune endotypes was similar (Fig 3A). The proportion of patients meeting the D2T criteria progressively increased within the ‘CD4 + memory cell endotype’, reaching two-thirds of this group 2 years after treatment initiation (Fig 3B).

Immune clusters remained stable 3 months posttreatment (31 paired samples), with only 1 patient reclassified from cluster 3 to cluster 2. Consistent with these findings, no significant changes were observed in individual cell populations (Supplementary S7-9). A significant reduction in granulocytes and an increase in lymphocytes were observed 3 months posttreatment (data not shown).

Proteomic pathway analysis across immune endotypes

To identify key biological pathways characterising the ‘CD4 + memory cell endotype’, proteomic analysis compared cluster 1 with clusters 2 (285 DEPs) and 3 (349 DEPs) (Fig 4A).

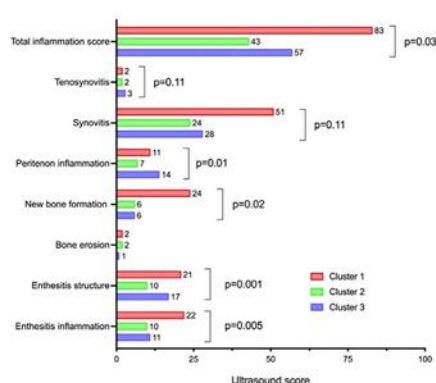
Based on these DEPs, we identified several enriched pathways associated with the ‘CD4 + memory cell endotype’, including intracellular signalling pathways, such as WNT, RAS, PI3K-AKT-mTOR, and MAP kinase, as well as platelet activation and VEGF signalling, as shown in the protein interaction network map (Fig 4B, Supplementary S10, S11).

The WNT signalling pathway appeared most distinct to the ‘CD4 + memory cell endotype’, as it contained the highest number of DEPs unique to this pathway, whereas other pathways shared multiple proteins. Several WNT-related proteins, including DKK2, WNT5b, ROR2, FZD7, FZD8, and FZD2, were upregulated in this endotype and positively correlated with disease activity measures, including DAPSA and sonographic enthesitis scores (Fig 4C). Interestingly, these biomarkers were negatively correlated with CRP and positively correlated with peri-articular and enthesal osteoproliferation.

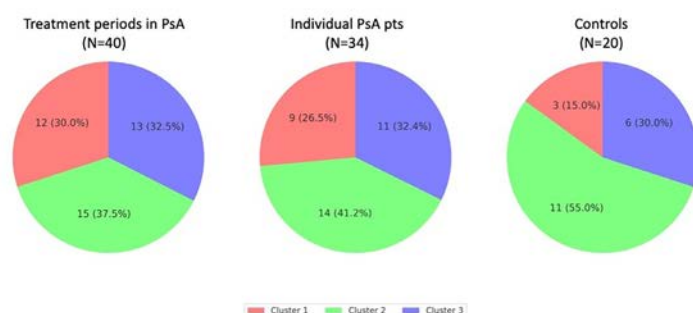
IMC of synovial T cells

IMC analysis of synovial tissue focused on T cell populations (Fig 5). As expected, T cells were present in PsA samples but scarce in the control. Unsupervised cell clustering identified 24 unique populations, including 3 CD4 + clusters and 1 CD8 + T cell cluster (Fig 5B). Two CD4 + clusters expressed CD45RO, a memory T cell marker. One (C16) coexpressed activation markers (HLA-DR, CCR6) and CCR7, consistent with central memory T

2A. Ultrasound scores by immune cluster



2B. Distribution of PsA and controls by cluster



cells. The other (C14) had higher CD45RO but lower activation markers. The CD8⁺ cluster (C13) lacked memory cell markers. Two patients (PsA3 and PsA4) had matched blood and IMC data and were classified within the ‘Nonclassical T cell-predominant endotype’ associated with better treatment response. Their synovial tissue showed fewer CD4⁺ memory/effector T cells and more CD8⁺ cells (Fig 5D).

Figure 2. (A) Ultrasound scores patients by cluster with differences evaluated with GEE models. (B) The frequencies (%) of the three clusters among individual treatment periods (N = 40), individual patients with PsA (N = 34), and controls (N = 20). GEE, generalised estimating equation; PsA, psoriatic arthritis.

DISCUSSION

Our study provides new insights into the heterogeneity of circulating immune cell endotypes in PsA and their potential clinical relevance. Specifically, we identified a CD4⁺ central and effector memory cell-predominant endotype that was associated with more active sonographic inflammation, greater structural

Table 1
Clinical characteristics of the immune endotype*

	Individual participant at first visit [‡] (N = 34)	Cluster 1* N = 12	Cluster 2* N = 15	Cluster 3* N = 13	P value [†]
Age (y)	43.6	60.4	39	45	<.001
Sex: female	18 (52.9)	9 (75)	5 (33.3)	8 (62)	.17
Race: White	24 (70.6)	11 (91.7)	13 (86.7)	5 (38.5)	.02
BMI (kg/m ²)	26.5	30.7	26.5	25	.16
Age at diagnosis of psoriasis (y)	29	51	24	35	<.001
Age at diagnosis of PsA (y)	40	56	33	44	<.001
Duration of PsA (y)	2.7	3.8	3.7	1.6	.97
PASI	2.5	2.6	1.5	3.4	.11
DAPSA	22.6	27.8	22.5	22.2	.40
Physician global PsA activity (0-10)	4	5	3	5	.008
Tender joint count (0-68)	5	6.5	4	6	.63
Swollen joint count (0-66)	3	3.5	2	6	.047
Dactylitis (%)	13 (38.3)	3 (25)	7 (46.7)	3 (23.1)	.45
Enthesitis (%)	18 (52.9)	7 (58.3)	9 (60)	5 (38.5)	.35
CRP (mg/L)	3.5	1.3	4.4	3.7	.61
Damaged joints (%)	10 (29.4)	5 (41.7)	3 (20)	6 (46.2)	.54
Patient pain (0-10)	4	6.5	5	4	.01
Patient global (0-10)	5	6.5	4	4	.03
HAQ (0-3)	0.50	0.75	0.62	0.25	.36
Prior exposure to biologics (%)	15 (44.1)	8 (66.7)	7 (46.7)	6 (46.2)	.46

BMI, body mass index; CRP, C-reactive protein; DAPSA, disease activity in PsA; HAQ, health assessment questionnaire; PASI, psoriasis area and severity index; PsA, psoriatic arthritis.

* Medians and frequencies (%) of the characteristics of the participants of the 40 treatment periods contributed by 34 individuals.

[†] P values were calculated using generalised estimating equation (GEE) regression models to compare differences across the 3 clusters, accounting for within-subject correlation from individuals contributing more than one treatment period.

[‡] Characteristics of 34 individual participants at the beginning of their first treatment period.

Table 2
GEE regression model of the association between immune endotype and the probability of achieving ACR20 response and change in DAPSA score and change in total ultrasound score from baseline

Variable	ACR20 response OR (95% CI)	Change in DAPSA* β (95% CI)	US score change β (95% CI)
Cluster 1 vs cluster 2	0.15 (0.02, 0.93) $P = .035$	12.3 (5.7, 18.9) $P < .001$	8.6 (-5.8, 23) $P = .22$
Cluster 1 vs cluster 3	0.09 (0.01, 0.68) $P = .016$	13.9 (7.7, 19.9) $P < .001$	4.4 (-11.2, 19.9) $P = .56$

ACR20, American College of Rheumatology; DAPSA, disease activity in psoriatic arthritis; OR, odds ratio.

* The difference in DAPSA at 3 months minus DAPSA at baseline.

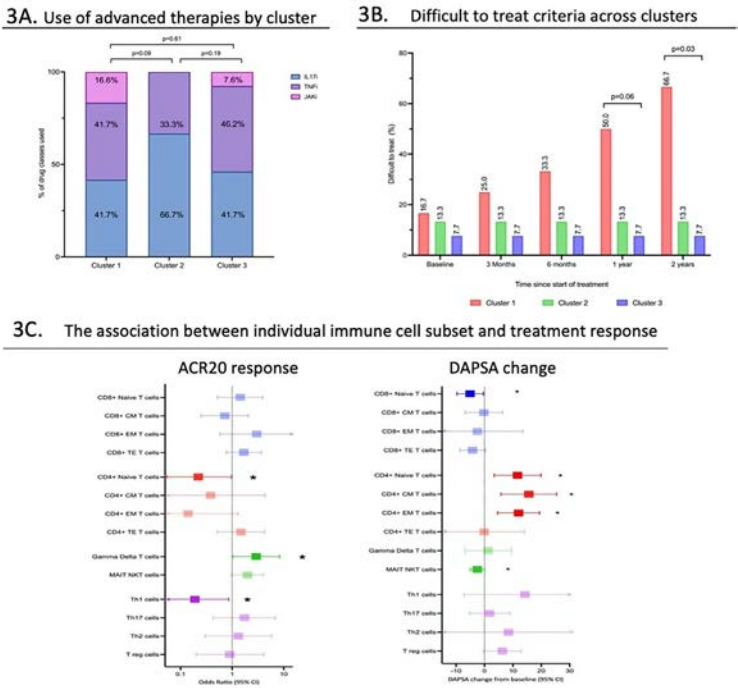


Figure 3. (A) The use of IL-17, TNF, and JAK inhibitor in each cluster. (B) Proportion of patients meeting difficult-to-treat criteria at baseline, 3, 6, 12, and 24 months after treatment initiation by cluster. (C) Forest plots of the odds ratio and β coefficient and their 95% CIs from the GEE regression model assessing the association of individual immune cell population and ACR20 response and DAPSA change, respectively. *Indicates $P < .05$. ACR20, American College of Rheumatology; DAPSA, Disease Activity Index for PsA; DEP, differentially expressed protein; GEE, generalised estimating equation.

joint damage, and poorer response to advanced therapies. This endotype was associated with a distinct proteomic profile marked by dysregulation of several intracellular signalling pathways, most notably WNT.

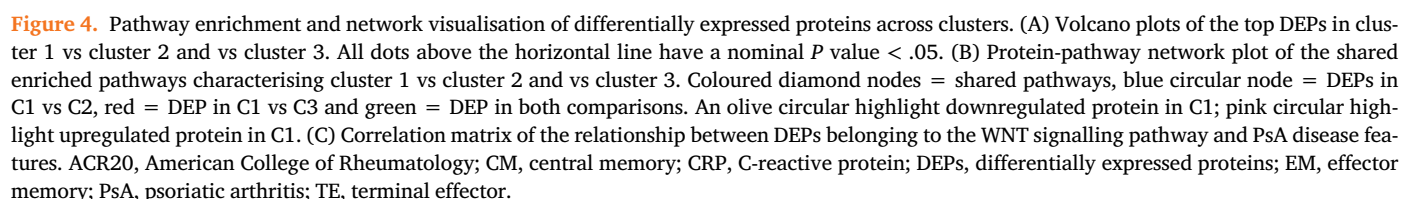
Recent mass cytometry studies in PsA have highlighted differences in innate lymphoid cells, and CD4+ /CD8+ memory and effector cells between PsA, psoriasis and controls [12,26,27], underscoring the involvement of both adaptive and innate immune compartments. Using flow cytometry, Skougaard et al [28] defined four circulating immune phenotypes in PsA. One phenotype, characterised by higher levels of Th17 and activated CD4+ memory Tregs, was associated with longer disease duration and higher activity, while another, dominated by Th1 and naïve Tregs, was associated with shorter disease duration. Although methodological differences preclude direct comparison between our findings and those of Skougaard et al [28], both studies emphasise the role of immune endotypes, particularly those defined by CD4+ memory vs naïve T cell ratios, in PsA heterogeneity.

The role of memory T cells in PsA has primarily been studied in the context of skin-resident memory CD8+ cells and their potential link to arthritis susceptibility [29–31]. However, limited data exist on their association with PsA clinical phenotype and therapeutic response. Our study demonstrated that an

immune profile dominated by CD4+ memory T cells was associated with more severe sonographic inflammation, as well as with greater structural damage. Notably, these patients were less likely to respond to targeted therapies, with more meeting D2T criteria after 2 years. The proteomic profile of this ‘CD4+ memory cell endotype’ showed dysregulation in several intracellular signalling pathways, including WNT and vascular pathways, both previously implicated in PsA pathogenesis [32–34].

While our study was not designed to establish a causal relationship between memory T cells and PsA, several mechanisms may underlie their association with disease outcomes. Memory T cells have a prolonged lifespan and mount a rapid recall response to antigens, producing a broad array of proinflammatory cytokines that sustain chronic inflammation [35]. Polyfunctional proinflammatory CD4+ T cells have been identified in PsA synovial tissue and correlate with DAPSA scores [36]. Their capacity to produce multiple cytokines simultaneously and their reliance on alternative survival pathways may contribute to reduced susceptibility to targeted therapies.

Our proteomic analysis identified key biological pathways associated with the ‘CD4+ memory cell endotype’, revealing predominant enrichment of intracellular signalling pathways, and downstream effector mechanisms, such as vascularisation, platelet activation, and epithelial-mesenchymal transition. The



WNT signalling has been implicated in PsA, with WNT proteins and their inhibitors, such as DKK-1 and sclerostin, acting as effector molecules that enhance osteoblast activity and promote new bone formation [33,38]. The 'CD4+ memory T cell endotype' was associated with increased new bone formation at both articular and enthesal sites, potentially driven by

Older age was associated with the ‘CD4+ memory cell endotype’. Ageing is associated with immunosenescence, which is characterised by chronic inflammation (‘inflammaging’), a decline in naïve T cells, expansion of memory T cells, and impaired tissue repair capacity [41,42]. Studies have shown that older patients with PsA show greater sonographic inflammation.

a

b

c

d

Figure 1: Single-cell RNA-seq analysis of synovial tissue cells. Panel a shows a t-SNE plot of 10,000 cells with 15 clusters. Panel b shows a heatmap of marker gene expression across these clusters. Panel c shows a heatmap of cell cycle phase marker expression across the same clusters. Panel d shows a heatmap of cell cycle phase marker expression across the same clusters, with a color scale from -3 to 3.

A t-SNE plot titled "All Cells" showing the distribution of cells in a 2D space defined by "time Cells 1" (x-axis) and "time Cells 2" (y-axis). The plot is color-coded by cell type, with a color bar on the right ranging from 0 (blue) to 25 (red). Three clusters are labeled: "CD8+ T cells" (red/orange), "Effector T cells" (yellow/green), and "CM T cells" (blue/cyan).

Stacked bar chart showing the number of cells per image (median) for three T cell populations: All/CD8 T Cells (green), All/Central Memory (red), and All/Effector T Cells (blue). The y-axis ranges from 0 to 10. The x-axis has four categories. The first category has the highest count (~10 cells), followed by the second (~5 cells), the third (~2 cells), and the fourth (~1 cell).

CM, central memory; PsA, psoriatic arthritis.

Our study has several limitations. First, it is an exploratory study with a relatively small sample size, which may not fully capture the immune cell and clinical heterogeneity of PsA. Given the small sample size and exploratory nature, with a primary goal of hypothesis generation and pathway prioritisation no

In summary, our study suggests that heterogeneity in circulating immune cell profiles is associated with PsA clinical features and therapeutic response. A 'CD4⁺ memory cell endotype' was associated with more active sonographic inflammation, greater structural damage, and a poorer response to advanced therapies. This endotype was linked with a distinct proteomic signature, characterised in part by dysregulation of intracellular signalling including the WNT signalling pathway. These findings underscore the potential of immune cell phenotyping to improve our understanding of PsA pathogenesis; further research is needed to determine the predictive clinical value of these biomarkers.

Contributors

LE was responsible for conceptualisation, data curation, funding acquisition, formal analysis, supervision, and writing – original draft. SD was responsible for conceptualisation, formal analysis, visualisation, and writing – review and editing. SMC and XL were responsible for conceptualisation, formal analysis, visualisation, and writing – review and editing. ST was responsible for project administration, investigation, and writing – review and editing. DG was responsible for data curation, investigation, project administration, and writing – review and editing. CR, VP, VC, and IJ were responsible for conceptualisation, methodology, and writing – review and editing. LD was responsible for conceptualisation, formal analysis, and writing – review and editing.

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Competing interests

LE has received research and educational grants from Abbvie, Pfizer, Novartis, Eli Lilly, UCB, J&J, Fresenius Kabi, and has participated on the advisory board and received speaker honoraria from Abbvie, Pfizer, Novartis, UCB, J&J, Fresenius Kabi, Eli Lilly, BMS, and her partner is employed by Standard BioTools. VP has received grants from AbbVie, Bausch Health, Boehringer Ingelheim, Bristol Myers Squibb, Celgene, Eli Lilly, Incyte, Janssen, LEO Pharma, L'Oréal, Novartis, Organon, Pfizer, Sandoz, and Sanofi, has received payment or honoraria for speaking engagement from Sanofi, has participated on an advisory board for LEO Pharma, Novartis, Sanofi, Union Therapeutics, Abbvie and UCB, and has received equipment donation from L'Oréal. VC has received advisory board honoraria from Abbvie, BMS, Eli Lilly, Fresenius Kabi, J&J, Novartis, and UCB, and their spouse is employed by AstraZeneca. The remaining authors have no conflicts to declare.

Patient consent for publication

Not applicable.

Ethics approval

The study was approved by the Research Ethics Board of Women's College Hospital.

Provenance and peer review

Not commissioned, externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.10.011.

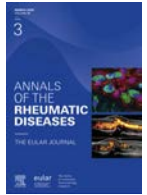
Orcid

Lihi Eder: <http://orcid.org/0000-0002-1473-1715>

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Axial spondyloarthritis

Evaluation of the placebo and treatment effect overtime in randomised clinical trials evaluating the efficacy of biologics in axial spondyloarthritis: systematic review and meta-analysis

Léa Perrot^{1,*}, Anna Molto^{2,3}, Caroline Paris¹, Robinson Gravier Dumonceau⁴, Maxime Dougados⁵, Thao Pham¹

¹ Aix Marseille University, Rheumatology Department, Sainte Marguerite Hospital, APHM, Marseille, France

² Université Paris Cité, Service de Rhumatologie, Hôpital Bichat, APHP.Nord, Paris, France

³ Université Paris Cité and Université Sorbonne Paris Nord, Inserm, INRAE, Centre for Research in Epidemiology and Statistics (CRESS), Paris, France

⁴ Équipe QuantIM, UMR SESSTIM 1252 INSERM/IRD/Université AMU, Marseille, France

⁵ Rheumatology Department, Cochin Hospital, APHP.Centre, Paris, France

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ABSTRACT

Objectives: To evaluate temporal trends in treatment efficacy and placebo responses in randomized controlled trials in axial spondyloarthritis (axSpA).

Methods: We conducted a systematic review of RCTs in MEDLINE up to July 2024 (PROSPERO: CRD42024563776). Eligible trials compared tumour necrosis factor inhibitors (adalimumab, certolizumab pegol, etanercept, infliximab, golimumab) or interleukin-17 inhibitors (bimekizumab, ixekizumab, secukinumab) versus placebo in axSpA. Two reviewers independently selected studies and extracted data. Primary outcomes were temporal trends in placebo response for Assessment of SpondyloArthritis International Society 20% and 40% response criteria (ASAS20/40), axSpA Disease Activity Score (ASDAS) Clinically Important Improvement, and ASDAS Inactive Disease (ASDAS-ID) at each trial's primary endpoint. Random-effects meta-regressions estimated annual changes in treatment effects (odds ratios) and in arm-specific response rates, adjusted for age, sex, radiographic status, region, route of administration, baseline Bath Ankylosing Spondylitis Disease Activity Index score, baseline C-reactive protein, and disease duration.

Results: Thirty-one RCTs ($n = 6437$) were included. Treatment effect declined over time for ASAS20 (-5% per year, $P < .001$) and ASAS40 (-3.7% , $P = .04$), driven by increased placebo response (ASAS20: $+1\%$ per year, $P < .0001$) and a decline in treatment response for ASAS40. In contrast, ASDAS-based treatment effects were stable, despite a small increase in placebo ASDAS-ID ($+0.19\%$ per year, $P < .05$).

Conclusions: ASAS20/40 treatment effects have declined over time in axSpA trials, consistent with a fading of reported effectiveness, whereas ASDAS-based endpoints remain stable, indicating greater robustness to placebo effects and temporal trends.

*Correspondence to Dr Léa Perrot, 270 Boulevard Sainte-Marguerite, 13009 Marseille, France.

E-mail address: lea.perrot2@gmail.com (L. Perrot).

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WHAT IS ALREADY KNOWN ON THIS TOPIC

Placebo responses have increased over time in rheumatoid arthritis and psoriatic arthritis trials, but no study has assessed this phenomenon or its impact on treatment effect in axial spondyloarthritis (axSpA).

WHAT THIS STUDY ADDS

This study reveals a temporal attenuation in treatment effects based on Assessment of SpondyloArthritis International Society (ASAS) 20% and 40% responses in axSpA trials, driven by increased placebo response rates for ASAS20. In contrast, axSpA Disease Activity Score (ASDAS)-based treatment effects remained stable, suggesting greater robustness to temporal trends.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

These findings support using more stringent disease activity criteria such as ASDAS, which incorporates biomarkers and appears less susceptible to the temporal decline in treatment effect.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory condition that primarily affects the axial skeleton, as well as involving the peripheral joints, entheses, and extramusculoskeletal organs such as the eyes, skin, and gastrointestinal tract (eg, uveitis, psoriasis, and inflammatory bowel disease) [1].

Nonsteroidal anti-inflammatory drugs remain the treatment cornerstone, but the advent of tumour necrosis factor inhibitors (TNFis) in the early 2000s marked a significant breakthrough in treatment. TNFis have demonstrated substantial efficacy in improving pain, disability, and quality of life for patients with both radiographic (r-) and nonradiographic (nr-) axSpA in placebo-controlled randomised trials [2–5]. More recently, interleukin 17 inhibitors (IL-17is) have broadened the biological treatment armamentarium for axSpA [6–8].

Despite these advances, recent axSpA randomised controlled trials (RCTs) appear to show smaller treatment effects compared with earlier trials [5,7]. One explanation could be that treatment is initiated at an earlier stage of the disease, perhaps in patients with less severe disease than in the first RCTs, owing to better knowledge of the disease and earlier diagnosis [9]. Another possibility is an increasing placebo effect over time. Similar increases in placebo effect have been observed in rheumatoid arthritis (RA) for outcomes such as American College of Rheumatology (ACR) criteria for 50% (ACR50) and 70% (ACR70) response and in psoriatic arthritis (PsA) for ACR for 20% response [10,11].

To date, no study has specifically examined how placebo and treatment responses have evolved over time in axSpA trials. Understanding these temporal trends is essential to accurate interpretation of RCT results and to improve trial design.

We aimed to evaluate temporal changes in treatment effects of TNFis and IL-17is in axSpA RCTs and to assess trends over time in response rates in placebo and active treatment arms.

METHODS

Ethical considerations

The study adhered to the guidelines for preferred reporting items for systematic reviews and meta-analyses [12]. The

systematic review was registered with the International Prospective Register of Systematic Reviews (registration number: CRD42024563776).

Literature review

The Population, Intervention, Comparator, and Outcome (PICO) characteristics were as follows: (P) adults with r-axSpA or nr-axSpA; (I) biologic disease-modifying antirheumatic drugs (TNFis or IL-17is); (C) placebo as comparator; and (O) efficacy outcomes as defined below in the section, *Assessed efficacy criteria*.

A systematic literature review was conducted by 2 independent reviewers (LP, CP) using the MEDLINE database with a language restriction to English. To be selected, studies had to be double-blinded clinical trials, with at least one inert (placebo) group and at least one active (TNFi or IL-17i) group (ie, adalimumab, certolizumab pegol, etanercept, infliximab, golimumab, bimekizumab, ixekizumab, secukinumab) and including patients with r-axSpA or nr-axSpA, without time restriction. Trials evaluating targeted synthetic disease-modifying antirheumatic drugs were excluded because their oral administration and more recent introduction could have biased the interpretation of temporal trends [13]. To assess the risk of bias, only studies with full text available were included. Abstracts without full-text articles were excluded from the analysis. If articles were derived from the same RCT, they were considered duplicates, and we kept only the version with the most patients, the longest follow-up, and the most detailed efficacy results. Two independent reviewers analysed the full texts to confirm final inclusion.

The following search string was used: ('Spondyloarthritis'[Mesh] OR 'Axial Spondyloarthritis'[Mesh] OR 'Ankylosing Spondylitis'[Mesh]) AND ('Adalimumab'[Mesh] OR 'Etanercept'[Mesh] OR 'Golimumab'[Mesh] OR 'Infliximab'[Mesh] OR 'Certolizumab Pegol'[Mesh]) OR ('Secukinumab'[Mesh] OR 'Ixekizumab'[Mesh] OR 'Bimekizumab'[Mesh]).

Study selection, data extraction, and assessment of risk of bias

The 2 reviewers examined all titles and abstracts to identify eligible studies fulfilling the inclusion criteria, followed by full-text review if retained. The risk of bias for the included RCTs was assessed using the Cochrane Risk of Bias 2.0 tool (Supplementary Table S1).

Data extraction was performed from July 10, 2024 and focused on the main characteristics of the studies including: year of publication, age, sex, r-axSpA status, route of administration, baseline Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score, C-reactive protein (CRP, mg/L), mean duration of disease since diagnosis and mechanism of action (TNFi or IL-17i), sample size, time of evaluation for the primary endpoint, and efficacy outcomes: Assessment of SpondyloArthritis International Society (ASAS) 20 response, ASAS40 response, axSpA Disease Activity Score (ASDAS) Clinical Important Improvement (ASDAS-CII) response and ASDAS Inactive Disease (ASDAS-ID) response. When a single study reported outcomes separately for distinct patient subgroups (eg, r-axSpA vs nr-axSpA), each subgroup was included as a separate study in the meta-analysis.

Geographic region of recruitment was assigned using the method described by Bechman et al. [10] based on countries reported in each trial. Studies were categorised as follows: Europe (EU), North America (NA), South America (SA), Asia (AS), Australia (AUST), and worldwide for studies involving

recruitment from all 5 regions. No distinction was made between Western and Eastern Europe. We used a pragmatic aggregate data meta-analysis approach, relying on reported summary statistics from each study.

Assessed efficacy criteria

The primary outcome of interest was efficacy response, assessed by binary response ASAS20, ASAS40, BASDAI50, ASDAS-CII, ASDAS major improvement (ASDAS-MI) and ASDAS-ID measured at the primary endpoint of each study. We predefined that only efficacy outcomes reported in at least 10 RCTs would be considered for analysis.

An ASAS20 response is defined as an improvement of $\geq 20\%$ from baseline and absolute improvement from baseline of at least 1 on a 0 to 10 scale in at least 3 of the following 4 domains: patient global, total back pain, function (Bath Ankylosing Spondylitis Functional Index, BASFI), and inflammation (average of the last 2 questions of the BASDAI concerning morning stiffness) without worsening ($\geq 20\%$ and ≥ 1 unit) in the remaining domain [14]. An ASAS40 response is defined as an improvement $\geq 40\%$ in the same parameters without worsening in the remaining one [15]. A BASDAI50 response is defined as an improvement of at least 50% or an absolute improvement of ≥ 2 units (on a 0–10 scale) in the BASDAI [16]. The ASDAS is a composite index combining patient-reported outcomes (pain, stiffness, global assessment, peripheral symptoms) and objective biomarkers (CRP or erythrocyte sedimentation rate) to assess disease activity in axSpA. ASDAS-CII is defined by a reduction ≥ 1.1 points from baseline, ASDAS-MI by a reduction ≥ 2.0 points from baseline, and ASDAS-ID as an ASDAS score < 1.3 [17].

In this study, the active group refers to patients who received TNFi or IL-17i treatments, while the placebo group includes those who were administered the inactive substance. The treatment effect was evaluated using odds ratios (ORs), comparing the proportion of responders in the active and placebo groups at the primary endpoint of each study.

Data analysis

The data from these RCTs were used to perform a meta-regression of proportions within each study arm (placebo and active) to evaluate the temporal evolution of response rates, incorporating the year of publication as a moderator. Random-effects models were used to give a more conservative estimate of the effect, allowing for any heterogeneity among studies. Inverse-variance weighting was applied to reflect study-level uncertainty.

Meta-regression models were fitted using restricted maximum likelihood estimation to ensure robust parameter estimates. The linearity assumption was assessed visually by weighted scatterplots. Forest plots were constructed to present individual study estimates and the overall pooled response rates under a random-effects model with 95% CIs.

We calculated ORs comparing proportion of active and placebo response for each outcome to estimate the treatment effect, using the inverse-variance method. Adjustments were made based on age, sex, radiographic status of axSpA, geographical recruitment, route of administration, baseline BASDAI score, baseline ASDAS score, CRP, disease duration, prior exposure to biologic therapy (naïve vs exposed), and biologic mode of action (TNFi vs IL-17i).

Heterogeneity was quantified by the I^2 parameter. If I^2 was $> 50\%$, identification of outlying studies with influence on the

overall effect size was performed using 3 methods: Baujat plot, screening for externally studentised residuals, and leave-one-out analysis. A study was considered an outlier if it was identified by the 3 methods. It was definitely excluded from the meta-analysis if its suppression led to a gain of at least 10 points of heterogeneity for the I^2 parameter. Egger's regression test and visual inspection of funnel plots for asymmetry were used to assess publication bias. All analyses were performed using R software, version 4.3.

RESULTS

Study characteristics

A search of the MEDLINE database identified 3478 studies. After the exclusion of noninterventional trials and articles in languages other than English, 639 articles were retained. After reading all abstracts, 69 articles were selected, and after full reading of the articles, 31 articles (and 6437 patients) were finally included. The flow chart of the search is presented in [Supplementary Figure S1](#).

Twenty-eight of 31 studies were judged to be at low risk of bias across all domains. Three studies were rated as having some concerns in the domain of selective reporting. Full details of the risk-of-bias assessment are provided in [Supplementary Table S1](#).

Twenty-one and 10 RCTs assessed TNFi and IL-17i drug efficacy versus placebo, respectively. Included articles were published between 2002 and 2023. Twenty-seven RCTs were multicentric, involving more than 6 centres. Recruitment was predominantly based in Europe ($n = 28$ including 6 RCTs in EU only) and North America ($n = 21$ including 2 RCTs in NA only). Most publications concerned patients with r-axSpA, whereas only 7 RCTs exclusively included patients with nr-axSpA.

All efficacy outcomes reported across the included RCTs and the number of trials reporting each are detailed in [Supplementary Table S2](#). The median time of assessment of the primary outcome was 15 weeks (range from 6 to 28 weeks). [Table 1](#) [2–8,18–41] lists the characteristics of the included studies.

Baseline patient characteristics

The mean $\pm$ SD number of patients included in the placebo and the active arms was 87.9 ± 45.4 and 111.4 ± 66.8 , respectively. The baseline disease activity characteristics in each group are detailed in [Supplementary Table S3](#).

The route of administration was similar between placebo and active treatment groups across all studies, with 5 RCTs using intravenous administration and 26 using subcutaneous administration. However, the composition of the placebo was not described in any of the studies. Biologic-naïve patients were included in 21 of the RCTs.

Temporal trends in baseline BASDAI, ASDAS, CRP levels, and disease duration in both placebo and active groups are presented in [Supplementary Figure S2](#).

Efficacy outcomes

[Table 2](#) presents the pooled response rates for ASAS20, ASAS40, ASDAS-ID, and ASDAS-CII in placebo and active treatment groups, while [Supplementary Figure S3](#) shows the corresponding forest plots for each endpoint. In addition, [Supplementary Figure S4](#) provides a graphical representation of these pooled response rates.

Table 1
Characteristics of the included studies

Author	Year of publication	Drug name	No. of patients in placebo group	No. of patients in drug group	axSpA status	Recruitment country	Efficacy criteria measured
Gorman et al. [18]	2002	Etanercept	20	20	Radiographic	NA	ASAS20, ASDAS-CII, ASDAS-ID
Braun et al. [19]	2002	Infliximab	35	35	Radiographic	EU	ASAS20
Davis et al. [20]	2003	Etanercept	139	138	Radiographic	NA, EU	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
Brandt et al. [21]	2003	Etanercept	17	16	Radiographic	EU	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
Calin et al. [2]	2004	Etanercept	39	45	Radiographic	EU	ASAS20
van der Heijde et al. [3]	2005	Infliximab	78	201	Radiographic	NA, EU	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
van der Heijde et al. [22]	2006	Etanercept	51	155	Radiographic	EU	ASAS20, ASAS40
van der Heijde et al. [23]	2006	Adalimumab	107	208	Radiographic	NA, EU	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
Haibel et al. [24]	2008	Adalimumab	24	22	Nonradiographic	EU	ASAS20, ASAS40, ASDAS-ID
Inman et al. [25]	2008	Golimumab	78	138	Radiographic	NA, EU, AS	ASAS20, ASDAS-CII, ASDAS-ID
Inman et al. [26]	2010	Infliximab	37	39	Radiographic	NA	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
Sieper et al. [4]	2013	Adalimumab	94	91	Both	NA, EU	ASAS20, ASAS40
Dougados et al. [27]	2014	Etanercept	106	109	Nonradiographic	EU, SA, AS	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
Dougados et al. [28]	2014	Etanercept	48	42	Both	EU	ASAS20, ASAS40
Bao et al. [29]	2014	Golimumab	105	108	Radiographic	AS	ASAS20
Landewé et al. [30]	2014	Certolizumab	107	111	Radiographic	NA, EU, SA	ASAS20, ASAS40
Sieper et al. [31]	2014	Infliximab	52	106	Both	EU, AS	ASAS20, ASAS40
Huang et al. [32]	2014	Adalimumab	115	229	Radiographic	AS	ASAS20, ASAS40
Sieper et al. [5]	2015	Golimumab	100	98	Nonradiographic	NA, EU, AS	ASAS20, ASAS40
Sieper et al. [33]	2017	Secukinumab	74	72	Radiographic	Worldwide	ASAS20, ASAS40, ASDAS-CII, ASDAS-ID
Braun et al. [34]	2017	Secukinumab	122	125	Radiographic	NA, EU, SA, AS	ASAS20, ASAS40
Pavelka et al. [35]	2017	Secukinumab	76	74	Radiographic	NA, EU, SA	ASAS20, ASAS40, ASDAS-CII
Deodhar et al. [36]	2018	Ixekizumab	104	114	Radiographic	NA, EU, SA, AS	ASAS20, ASAS40, ASDAS-ID
van der Heijde et al. [6]	2018	Ixekizumab	87	81	Radiographic	NA, EU, AS	ASAS20, ASAS40, ASDAS-ID
Kivitz et al. [37]	2018	Secukinumab	117	116	Radiographic	NA, EU	ASAS20, ASAS40
Deodhar et al. [38]	2019	Certolizumab	158	159	Nonradiographic	NA, EU, AS AUST	ASAS40, ASDAS-ID
Deodhar et al. [39]	2019	Golimumab	103	105	Radiographic	Worldwide	ASAS20, ASAS40
Deodhar et al. [40]	2020	Ixekizumab	105	96	Nonradiographic	NA, EU, SA, AS	ASAS40
Huang et al. [41]	2020	Secukinumab	153	305	Radiographic	EU, AS	ASAS20, ASAS40
Deodhar et al. [7]	2021	Secukinumab	186	185	Nonradiographic	NA, EU	ASAS20, ASAS40
Baraliakos et al. [8]	2023	Bimekizumab	126	128	Nonradiographic	NA, EU, AS	ASAS20, ASAS40, ASDAS-ID
Baraliakos et al. [8]	2023	Bimekizumab	111	121	Radiographic	NA, EU, AS	ASAS20, ASAS40, ASDAS-ID

AS, Asia; ASAS20, Assessment of SpondyloArthritis International Society 20% response; ASAS40, Assessment of SpondyloArthritis International Society 40% response; ASDAS-CII, Axial Spondyloarthritis Disease Activity Score Clinical Important Improvement response; ASDAS-ID, ASDAS Inactive Disease response; AUST, Australia; EU = Europe, NA, North America; SA, South America; Worldwide = Asia, Australia, Europe, North America, and South America.

Detailed results of the heterogeneity analysis are available in [Supplementary Table S4](#).

ASAS20 response

Treatment effect. The pooled OR for ASAS20 response was 3.24 (95% CI: 2.69–3.91). A significant temporal decline in the treatment effect was observed, with a 5% decrease in OR per year ($P < .001$).

Temporal trend in ASAS20 response in placebo group and active treatment group. [Figure A](#) depicts the temporal trends for ASAS20 response rates for both placebo and active groups. Over time, placebo response rates increased, whereas active response rates showed a slight decline.

In the nonadjusted model, publication year was significantly associated with an increase in ASAS20 response rates in the placebo group ($\beta = 0.99$; 95% CI: 0.5–1.46; $P < .001$), corresponding to a 1% increase per year. Female sex was positively

Table 2
Pooled response rates for efficacy outcomes in placebo and active treatment groups

Outcome	Group	Pooled response rate (95% CI)
ASAS20	Placebo	0.32 (0.28-0.36)
	Active	0.62 (0.58-0.65)
ASAS40	Placebo	0.18 (0.15-0.22)
	Active	0.46 (0.41-0.50)
ASDAS-ID	Placebo	0.04 (0.02-0.05)
	Active	0.21 (0.18-0.24)
ASDAS-CII	Placebo	0.14 (0.11-0.19)
	Active	0.46 (0.41-0.51)

ASAS20, Assessment of SpondyloArthritis International Society 20% response; ASAS40, Assessment of SpondyloArthritis International Society 40% response; ASDAS-CII, Axial Spondylitis Disease Activity Score Clinical Important Improvement response; ASDAS-ID, ASDAS Inactive Disease response.

associated with higher placebo response rates. In the placebo group, the ASAS20 response rates were significantly influenced by 2 variables; a higher percentage of female participants was associated with an increase in response rates ($\beta = 0.27$; 95% CI: 0.02-0.5; $P = .02$) while longer disease duration was linked to a significant decrease in response rates ($\beta = -1.67$; 95% CI: -0.74 to -2.6 ; $P < .001$).

No significant association was found between publication year and response rates in the active treatment group

($\beta = -0.28$; 95% CI: -0.81 to 0.26 ; $P = .3$). No other covariates were significantly associated with treatment response.

After adjustment, the association between publication year and placebo response remained significant ($\beta = 0.65$; 95% CI: 0.1-1.29; $P = .03$), while it remained nonsignificant for the active group ($\beta = 0.31$; 95% CI: -0.99 to 0.88 ; $P = .48$).

Assessments of heterogeneity are presented in [Supplementary Figure S5](#). A sensitivity analysis excluding the Sieper et al. [31] study reduced heterogeneity (I^2 from 69% to 52%) without altering the significance of the results.

ASAS40 response

Treatment effect. The pooled OR for ASAS40 response was 3.79 (95% CI: 3.06-4.68). A significant temporal decline in the treatment effect was observed, with a 3.7% decrease in OR per year ($P = .04$).

Temporal trend in ASAS40 response in placebo group and active treatment group. [Figure B](#) illustrates the temporal trends for ASAS40 response rates. Placebo response rates remained stable, whereas active response rates declined over time.

In the placebo group, no significant association was found between publication year and ASAS40 response ($\beta = 0.02$; 95% CI: -0.4 to 0.51 ; $P = .58$). A higher percentage of female participants was associated with increased placebo response ($\beta = 0.16$; 95% CI: 0.01-0.32; $P = .02$). Intravenous administration was associated with lower response rates ($\beta = -8.41$; 95% CI: -15.3 to -1.48 ; $P = .01$).

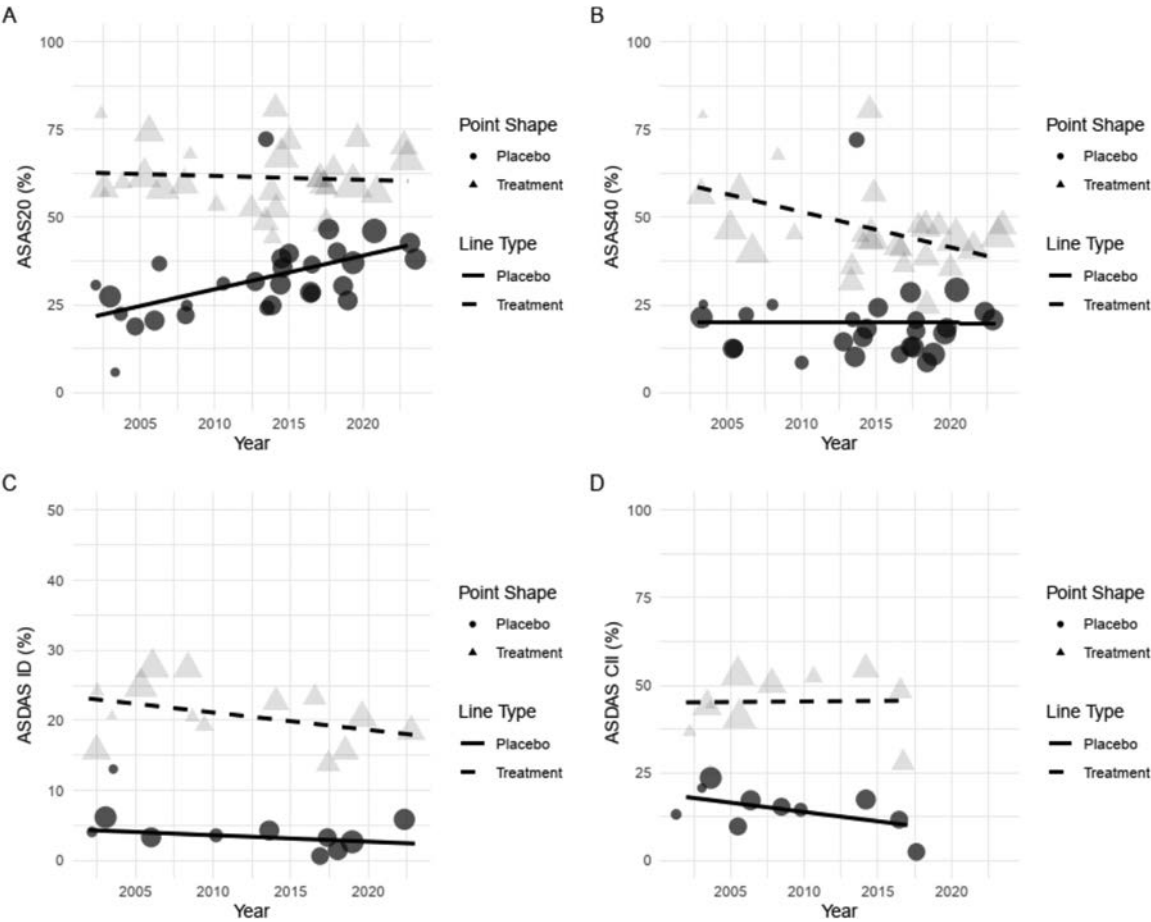


Figure. Efficacy response between 2002 and 2023. (A) Adjusted ASAS20 response rates in placebo and active treatment groups. (B) Adjusted ASAS40 response rates in the placebo and active treatment groups. (C) Adjusted ASDAS-ID response rates in the placebo and active treatment groups. (D) Adjusted ASDAS-CII response rates in the placebo and active treatment groups. ASAS20, Assessment of SpondyloArthritis International Society 20% response; ASAS40, Assessment of SpondyloArthritis International Society 40% response; ASDAS-CII, Axial Spondyloarthritis Disease Activity Score Clinical Important Improvement response; ASDAS-ID, ASDAS Inactive Disease response.

In the active treatment group, publication year was significantly associated with a decline in ASAS40 response ($\beta = -0.73$; 95% CI: -1.53 to -0.3 ; $P < .005$). After adjustment, publication year remained associated with a decrease in ASAS40 response in the active group ($\beta = -0.1$; 95% CI: -1.7 to -0.03 ; $P < .05$).

Assessments of heterogeneity are presented in [Supplementary Figure S5](#). A sensitivity analysis excluding Sieper et al. [31] reduced heterogeneity (I^2 from 89% to 64%) but did not change the significance of the results.

ASDAS-ID response

Treatment effect. The pooled OR for ASDAS-ID response was 5.92 (95% CI: 3.79–9.24). No significant temporal change in the treatment effect was observed ($P = .83$). This result is illustrated in [Supplementary Figure S6A](#).

Temporal trend in ASDAS-ID response in placebo group and active treatment group. [Figure C](#) shows the temporal trends for ASDAS-ID response rates. Placebo response increased slightly, while active treatment response decreased over time.

A small but significant increase in ASDAS-ID response rates over time in the placebo group ($\beta = 0.19$; 95% CI: 0.05–0.33; $P < .05$), corresponding to a 0.19 percentage point increase per year. Longer disease duration was associated with lower placebo response rates ($\beta = -0.33$; 95% CI: -0.48 to -0.1 ; $P < .05$). After adjustment, disease duration remained significantly associated with lower placebo response ($\beta = -0.18$; 95% CI: -0.3 to -0.05 ; $P < .05$).

In the active treatment group, no significant association was found between publication year and ASDAS-ID response ($\beta = -0.28$; 95% CI: -0.8 to 2.1 ; $P = .17$), and a higher baseline BASDAI was associated with a lower response ($\beta = -7.68$; 95% CI: -14.1 to -0.35 ; $P = .01$).

Assessments of heterogeneity are presented in [Supplementary Figure S7](#). A sensitivity analysis excluding the Davis et al. [20] and Sieper et al. [33] studies reduced heterogeneity (I^2 from 89% to 64%), revealing a significant association with the publication year.

ASDAS-CII response

Treatment effect. The pooled OR for ASDAS-CII response was 4.12 (95% CI: 3.06–5.55). No significant temporal change in the treatment effect was observed ($P = .56$). This result is illustrated in [Supplementary Figure S6B](#).

Temporal trend in ASDAS-CII response in placebo group and active treatment group. [Figure D](#) presents the temporal trends for ASDAS-CII response rates, showing a slight decline in placebo response and stable active treatment response over time. No significant association was found with the publication year in the placebo group ($\beta = 0.19$; 95% CI: -0.64 to 1.02 ; $P = .65$).

In the active treatment group, biologic-naïve patients had higher ASDAS-CII response rates ($\beta = 8.96$; 95% CI: 1.27–16.6; $P = .02$) although no significant association with the publication year was demonstrated ($\beta = 0.71$; 95% CI: -0.15 to 1.58 ; $P = .10$).

Assessments of heterogeneity are presented in [Supplementary Figure S7](#). A sensitivity analysis excluding Sieper et al. [33] did not reduce heterogeneity (I^2 from 54% to 59%) or impact coefficient significance.

Assessment of publication bias

Funnel plots were produced for each outcome and study arm to visually explore potential publication bias ([Supplementary Fig S8](#)). Significant funnel plot asymmetry was detected in the

placebo arms for ASAS20, ASDAS-ID, and ASDAS-CII and in the active arm for ASAS40. Results are detailed in [Supplementary Table S5](#).

DISCUSSION

In this meta-analysis of RCTs in axSpA, we assessed temporal trends in treatment effect using ASAS20, ASAS40, ASDAS-CII, and ASDAS-ID responses. We found that the treatment effect declined significantly over time for ASAS20 and ASAS40, with estimated yearly reductions of 5% and 3.7%, respectively. However, no significant temporal change was observed for ASDAS-based outcomes.

A first explanation for the decline in the OR for ASAS20 is the progressive increase in the placebo effect over time. These results are consistent with trends observed in other chronic inflammatory diseases such as RA and PsA. In RA, a significant increase in placebo response rates was observed for both ACR50 ($\beta = 0.39$; 95% CI: 0.04–0.75; $P = .03$) and ACR70 ($\beta = 0.17$; 95% CI: 0.02–0.32; $P = .02$) over the last 2 decades [10]. Similarly, a recent analysis in PsA has reported comparable findings [11]. We also identified some factors associated with temporal placebo response. Female sex was associated with an increased placebo response in these RCTs based on ASAS20 and ASAS40 criteria, echoing findings from RCTs in hand osteoarthritis [42]. Conversely, a longer duration of disease was associated with a lower placebo response with the ASAS20 and ASDAS-ID criteria, possibly due to cumulative experience of patients with different treatments. However, prior exposure to biologics did not significantly influence placebo response in our analysis.

We examined whether factors previously reported to be associated with placebo response also influenced the temporal evolution of placebo response in our study, but none were significant. Baseline disease activity was not associated with the temporal trend placebo response, which contrasts with findings in neuropathic pain trials (diabetic neuropathy and postherpetic neuralgia), where higher initial pain intensity was linked to stronger placebo effects [43]. Similarly, recruitment geography did not significantly impact placebo response in our study, unlike findings in PsA where higher placebo responses were reported in studies with greater recruitment from low-income countries [11].

Additionally, the route of administration of the placebo (intravenous vs subcutaneous) did not appear to influence response rates in our analysis. In contrast, in cardiology, stronger placebo effects have been reported in angina trials when invasive procedures such as pacemaker implantation were compared with oral placebo [44].

These results suggest that the reasons for the decline in treatment effect differ between ASAS20 and ASAS40. For ASAS20, the decrease is mostly explained by an increase in placebo response over time. For ASAS40, placebo response stayed stable, and the decline is mainly due to lower responses in the active treatment group. This may be due to a change in patient characteristics. In our study, CRP levels seem to decrease over time (see [Supplementary Fig S2](#)), whereas patient-reported measures such as BASDAI increased. These trends may reflect changes in the patient populations enrolled in more recent trials. In the early years of biologic disease-modifying antirheumatic drug development, trials often included patients with long-standing, severe, and highly inflammatory disease who had been waiting for effective treatment. As these patients were progressively treated and no longer entered clinical trials, subsequent studies

included more heterogeneous populations, often with lower inflammatory activity.

With earlier diagnosis of axSpA, fewer patients with radiographic disease are included and more patients present with nr-axSpA, who have lower CRP levels than radiographic patients [45]. However, in our adjusted analyses, neither baseline CRP nor radiographic status showed a significant association with the evolution of response rates over time, in both placebo and treatment groups.

Some degree of diagnostic misclassification may also have occurred, but it would likely affect all efficacy outcomes similarly and is not expected to explain the divergent trends between ASAS20 and ASAS40 [46]. This divergence may rather reflect a different sensitivity to nonspecific effects; expectations can drive a 20% improvement but not a 40% one if inflammation persists or is absent, which may explain the increasing placebo response only for ASAS20.

Beyond the mechanisms previously discussed, our findings may also be consistent with the phenomenon known as ‘fading of reported effectiveness,’ described by Gehr et al. [47]. This concept refers to a progressive decline in reported treatment effects over time in RCTs often attributed to increasing placebo responses or changes in patient characteristics [47]. Our findings regarding ASAS20 and ASAS40 suggest that a fading of reported effectiveness may also be occurring in axSpA that, to our knowledge, has not been described.

In contrast to the ASAS-based outcomes, we did not observe any significant change in the treatment effect over time for ASDAS-ID and ASDAS-CII. This suggests that these criteria may be more temporally robust and less susceptible to variations linked to evolving trial design, patient characteristics, or placebo effects. This may be partly explained by the fact that ASAS criteria are entirely patient-reported, whereas ASDAS criteria include objective measures such as CRP level, which may contribute to preserving the stability of the treatment effect over time. Similar trends have been observed in other domains, such as cardiology, where in trials of angina and hypertension, patient-reported measures such as reduced chest pain were strongly influenced by placebo, while stringent measures such as exercise tolerance or ejection fraction showed less variation [48].

However, this interpretation should be made with caution. The lower number of studies reporting ASDAS outcomes and the more stringent thresholds of ASDAS-CII and ASDAS-ID may also limit the ability to detect temporal changes and may contribute to a floor effect, which could partly explain the observed stability.

These findings have important implications for future RCTs in axSpA. The observed decline in treatment effect with ASAS responses over time highlights the need to prioritise more stringent endpoints, such as ASDAS criteria, which appear less sensitive to temporal changes and increasing placebo responses.

Certain limitations must be acknowledged. The potential risk of publication bias remains a concern, as studies with strong placebo responses and nonsignificant treatment effects may be less likely to be published. As all analyses were conducted using aggregated data, the observed associations between response rates and study patient characteristics may be subject to ecological bias, which prevents the evaluation of individual phenomena such as regression to the mean. Additionally, our research was limited to the MEDLINE database and was conducted by the investigators rather than a professional librarian, which may have resulted in missing some eligible studies. However, screening the reference lists of included articles did not reveal any additional trials.

In summary, we observed a progressive decline in treatment effect over time for ASAS20 and ASAS40 criteria, consistent with a fading of reported effectiveness, whereas ASDAS-based endpoints remained stable, suggesting greater resistance to placebo influences and temporal trend. These findings emphasise the importance of employing stringent evaluation criteria, such as ASDAS, that incorporate biologic measures and seem less affected by the decline in the treatment effect over time. This not only provides a more reliable assessment of treatment efficacy but also underscores the robustness of axSpA-specific evaluation tools in minimising the impact of placebo. Future RCT designs should carefully consider these factors to enhance the reliability of trial outcomes.

CRediT authorship contribution statement

Léa Perrot: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Anna Molto:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Caroline Paris:** Investigation, Data curation, Conceptualization. **Robinson Gravier Dumonceau:** Methodology, Formal analysis, Data curation. **Maxime Dougados:** Writing – review & editing, Validation, Supervision. **Thao Pham:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization.

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Competing interests

All authors declare they have no competing interests.

Patient consent for publication

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Supplementary materials

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Orcid

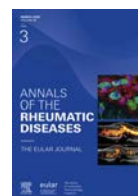
Léa Perrot: <http://orcid.org/0000-0002-0547-6695>

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Systemic lupus erythematosus

B cell depletion and BAFF receptor blockade with belimumab (VAY736) for the treatment of moderate-to-severe systemic lupus erythematosus: a phase 2 randomised, double-blind, placebo-controlled trial with subsequent open-label treatment

Nancy Agmon-Levin¹, Stanislav Ignatenko², Alexander Gordienko³, Josefina Cortés-Hernández⁴, Pongthorn Narongroeknawin⁵, Katarzyna Romanowska-Próchnicka⁶, Nan Shen⁷, Hana Ciferská⁸, Masanari Koderá⁹, Wei J Cheng-Chung^{10,11}, Piotr Leszczynski¹², Joung-Liang Lan¹³, Eduardo Mysler¹⁴, Rafal Wojciechowski¹⁵, Tunde Tarr¹⁶, Elena Vishneva¹⁷, Yi-Hsing Chen¹⁸, Yuko Kaneko¹⁹, Stephanie Finzel²⁰, Alberta Hoi²¹, Masato Okada²², Ajchara Koolvisoot²³, Shin-Seok Lee²⁴, Dai Lie²⁵, Hiroshi Kaneko²⁶, Bernadette Rojkovich²⁷, Lingyun Sun²⁸, Eugeny Zotkin²⁹, Jean-Francoise Viallard³⁰, Berta Paula Magallares López³¹, Swati Aashish Ghanshani³², Carol Lau³³, Alexandre Avrameas³⁴, Carole Sips³⁴, Stephen John Oliver^{34,*}

¹ Zabudowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel Aviv Univ, Tel Aviv, Israel

² Charité Research Organisation GmbH, Berlin, Germany

³ Department of Hospital Therapy, SM Kirov Military Medical Academy, St Petersburg, Russia

⁴ Lupus Unit, Rheumatology Department, Vall d'Hebron Hospitals, Barcelona, Spain

⁵ Rheumatic Disease Unit, Department of Internal Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand

⁶ Department of Biophysics, Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland

⁷ Shanghai Institute of Rheumatology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

⁸ Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine, Charles University, Prague, Czech Republic

⁹ Department of Dermatology, Japan Community Healthcare Organization, Chukyo Hospital, Nagoya, Japan

¹⁰ Department of Allergy, Immunology & Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan

¹¹ Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

¹² Department of Internal Medicine and Metabolic Disorders, Poznan University of Medical Sciences, Poznan, Poland

¹³ Rheumatology and Immunology Center, China Medical University Hospital, Taichung, Taiwan

¹⁴ Organizacion Medica de Investigacion, Buenos Aires, Argentina

¹⁵ Department of Rheumatology and Systemic Connective Tissue Diseases, University Hospital No. 2, Bydgoszcz, Poland

¹⁶ Division of Clinical Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

¹⁷ LLC Family Clinic, Yekaterinburg, Russia

*Correspondence to Dr. Stephen Oliver, Novartis Pharma AG, Basel, Switzerland.

E-mail address: stephen.oliver@novartis.com (S.J. Oliver).

Handling editor Josef S. Smolen.

- ¹⁸ Division of Allergy, Immunology and Rheumatology, Taichung Veterans General Hospital, Taichung, Taiwan
- ¹⁹ Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan
- ²⁰ Department of Rheumatology and Clinical Immunology, University Medical Centre Freiburg, Freiburg, Germany
- ²¹ Department of Rheumatology, Monash Health, Monash University, Melbourne, VIC, Australia
- ²² Immuno-Rheumatology Center, St. Luke's International Hospital, Tokyo, Japan
- ²³ Division of Rheumatology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
- ²⁴ Division of Rheumatology, Department of Internal Medicine, Chonnam National University Medical School and Hospital, Gwangju, South Korea
- ²⁵ Department of Rheumatology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China
- ²⁶ Division of Rheumatic Disease, National Centre for Global Health and Medicine, Tokyo, Japan
- ²⁷ Department of Rheumatology and Physiotherapy, Polyclinic of the Hospitaller Brothers of St. John of God, Semmelweis University, Budapest, Hungary
- ²⁸ Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China
- ²⁹ VA Nasonova Research Institute of Rheumatology, Moscow, Russia
- ³⁰ Department of Internal Medicine, CHU de Bordeaux, Hôpital Haut-Lévêque, Pessac, France
- ³¹ Department of Rheumatology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
- ³² Novartis Healthcare Pvt. Ltd, Hyderabad, India
- ³³ Novartis Pharmaceuticals Corp, East Hanover, NJ, USA
- ³⁴ Novartis Pharma AG, Basel, Switzerland

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ABSTRACT

Objectives: B cell depletion or B cell activating factor (BAFF) blockade has shown benefits in systemic lupus erythematosus (SLE). We compared ianalumab, a monoclonal antibody targeting BAFF receptor (BAFF-R)-expressing B cells to lyse B cells and block BAFF-R, with placebo for SLE treatment combined with standard therapies.

Methods: Patients with active SLE were randomised (1:1) to monthly subcutaneous ianalumab 300 mg or placebo. The primary outcome was a composite of SLE Responder Index (SRI)-4 at week 28 in patients successfully achieving corticosteroid (CS) tapering criteria. Patients subsequently received open-label (OL) ianalumab until week 48, followed by exploratory assessments at week 52 and off-treatment to week 68. Safety monitoring continued until B cell recovery. This report describes interim analyses conducted on the week 68 dataset.

Results: Sixty-seven patients were randomised and received blinded treatments until week 28. The primary composite endpoint was more frequently achieved with ianalumab vs placebo: 15/34 (44.1%) vs 3/33 (9.1%), with responses sustained to week 52 and replicated by placebo transitioned to OL ianalumab: 15/33 (45.5%) and 13/32 (40.6%). Positive treatment effects were consistently observed across other lupus disease activity outcomes (SRI-6, Definition of Remission in SLE, Lupus Low Disease Activity State, flare reduction, and CS use) at week 28, with clinical benefits until weeks 52 and 68. Ianalumab was not associated with increased serious adverse events or serious infections. Nonserious local injection site reactions occurred more frequently with ianalumab.

Conclusions: At week 28, reduced disease activity was observed in patients with SLE receiving ianalumab plus standard therapies compared with those receiving standard therapies alone, with sustained benefits with further treatment until 1 year, which was well tolerated.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disease that causes organ damage and is associated with an increased mortality [1,2]. Despite advances in SLE treatment, many patients continue to have active or relapsing-remitting disease [3] for whom chronic management with corticosteroids (CS) exacerbates organ damage. As complete remission is rarely achieved, treatment guidelines advocate for achieving a low disease activity state, thereby minimising CS use, avoiding severe flares, and slowing disease progression [4,5].

Targeted biological treatments currently approved for patients with active, treatment-resistant SLE include blockade of soluble B cell activating factor (sBAFF) with belimumab [6–8]

or type I interferon receptor with anifrolumab [9,10]. Although these therapies demonstrated clinical efficacy in their respective trials, many treated patients continued to have disease activity, CS usage, and severe flares. Thus, there remains a substantial need to develop more effective treatments for achieving and maintaining a low disease activity state.

B cell depletion has been a therapeutic target for lupus over 2 decades [11]. Clinical experience with this modality in lupus has largely been derived from off-label use of the anti-CD20 monoclonal antibody (mAb) rituximab despite failure of 2 large, placebo-controlled trials to demonstrate efficacy in these disease populations [12,13]. Improved clinical efficacy through more effective B cell depletion was recently shown in patients with lupus nephritis using obinutuzumab, a CD20-targeted, humanised mAb having enhanced antibody-dependent, cellular

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Depletion of B cells is an effective treatment for autoimmune diseases such as lupus, with more effective B cell depletion being associated with improved clinical responses.
- Another established therapeutic target for lupus is the B cell activating factor (BAFF), which is essential for B cell proliferation and survival. Elevated BAFF levels present in patients with lupus can confer protection against direct depletion of autoreactive B cells and drive disease relapse.
- Clinical investigations of combination therapy with BAFF blockade and B cell depletion in lupus have shown synergistic benefits, including reducing B cell counts, serum autoantibody levels, and incidence of disease flares.

WHAT THIS STUDY ADDS

- This lupus trial with ianalumab evaluated the targeting of BAFF receptor (BAFF-R)-expressing B cells for depletion by antibody-mediated cellular cytotoxicity, along with concurrent BAFF-R signalling blockade.
- The results show that this approach is well tolerated with positive treatment effects, meeting its primary objective against placebo for added treatment on top of standard of care, as well as across a range of secondary and exploratory outcomes. These findings were supplemented by open-label treatment, which provided evidence for further clinical and laboratory-associated benefits with ianalumab exposure up to 1 year.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Multiple efficacy signals generated by this study support therapeutic benefits from targeted depletion of the B cell subset expressing BAFF-R, adding to a growing body of evidence in lupus that effective B cell depletion, coupled with BAFF-R signalling blockade, can lead to effective reductions in disease activity, flares, and steroid exposure.

cytotoxicity (ADCC) through afucosylation of the Fc segment [14,15].

Ianalumab (VAY736) is a novel, fully human mAb that depletes B cells through ADCC, enhanced by Fc afucosylation to maximise Fcγ receptor binding [16]. By targeting B cells via the B cell activating factor (BAFF) receptor (BAFF-R), ianalumab also blocks BAFF:BAFF-R signalling, which is essential for B cell proliferation and survival [8]. Benefits of this dual mechanistic approach of sustained B cell depletion and BAFF-R blockade with ianalumab have been demonstrated in 2 early trials of patients with Sjögren's disease [17,18]. The larger, dose-finding study confirmed that 300 mg monthly dosing, predicted to completely block BAFF-R signalling over the dosing interval, was most effective in reducing disease activity and increasing stimulated salivary flow [17].

This phase 2 study was conducted in patients with moderate-to-severe SLE on stable background medication with CS tapering to examine the efficacy, safety, and tolerability of ianalumab and explore these outcomes under longer treatment duration and posttreatment (PT) conditions.

METHODS

Study design

This is a multicentre, randomised, parallel-group, double-blind (DB) trial (NCT03656562) consisting of 2 separate,

placebo-controlled treatment cohorts for ianalumab and iscalimab (anti-CD40 mAb), conducted in 28 sites across 14 countries in Europe, South America, Australia, and Asia. Study treatment was administered as add-on to existing stable, standard of care (SoC) therapy for SLE. This report is limited to results from the ianalumab treatment cohort; iscalimab cohort data will be published separately. The trial was conducted in accordance with the protocol, the International Conference on Harmonisation guideline for Good Clinical Practice, and applicable local regulations as well as with the Declaration of Helsinki. All patients provided written informed consent before study participation. The protocol and informed consent were approved by local ethics committees before study initiation.

After a 4-week screening period, patients were randomised (1:1) using web-based, interactive response technology to receive either 300 mg ianalumab or placebo every 4 weeks. Stratification factors included high disease activity (Systemic Lupus Erythematosus Disease Activity Index [SLEDAI] ≥ 10) and presence of autoantibodies against extractable nuclear antigens (ENAs), a marker previously associated with reduced clinical responses in lupus patients treated with rituximab [19]. To provide timely availability of ENA autoantibody data for patient stratification at time of enrolment, ENA autoantibody testing was performed during patient screening, with all tested ENA antibodies (SS-A, SS-B, Jo-1, Scl-70, Sm, Sm/RNP, Rib P, Pm-Scl, U1-RNP) treated similarly to determine seropositivity (yes or no). Doses were administered as 2 subcutaneous injections of ianalumab (150 mg lyophilizate reconstituted in 1 mL sterile water) or placebo (provided as liquid in vial). All patients received 75 to 80 mg methylprednisolone intravenously approximately 1 hour before injection (day 1 and at the start of open-label [OL] treatment) to minimise systemic reactions from rapid lysis of circulating B cells.

The study consisted of 2 treatment periods (Fig 1). The DB period compared ianalumab to placebo for 28 weeks, followed by an OL period of 24 weeks duration consisting of continued ianalumab 300 mg every 4 weeks for the active arm (IAN-OL), and transition of placebo-arm patients to start receiving ianalumab 300 mg every 4 weeks (PBO-OL). Following week 52 outcome assessments, patients entered a PT period for the measurement of safety and efficacy up to week 68. A subsequent PT period was used to collect safety data on patients until B cell recovery, triggering end of study (EoS).

Starting at week 4, patients underwent a protocol-guided CS taper to ≤ 5 mg/d prednisolone (or equivalent CS), or less than or equal to baseline dose by week 16, whichever was lower, and maintained within these limits until week 28. Prednisolone > 5 mg/d was permitted if clinically indicated but could not exceed 30 mg/d, be taken for > 2 days within a 4-week period, or within 2 weeks of the week 28 primary outcomes assessment.

Entry criteria

Eligible patients included adults aged 18 to 75 years, with SLE diagnosis of ≥ 6 months' duration. Patient screening was based on the presence of antinuclear antibody $\geq 1:80$ as assessed by the central laboratory, excluding HEp2 cell fluorescence patterns atypical for SLE (ie, nuclear membrane, centromere; internal data), followed by the required presence of ≥ 4 of the 11 American College of Rheumatology SLE criteria [20,21]; active disease, defined by a SLEDAI-2000 (SLEDAI-2K) [22] score of ≥ 6 , and a British Isles Lupus Assessment Group 2004 index (BILAG-2004) [23] of ≥ 1 'A' in either the mucocutaneous or musculoskeletal domain or 1 'B' grade in either the

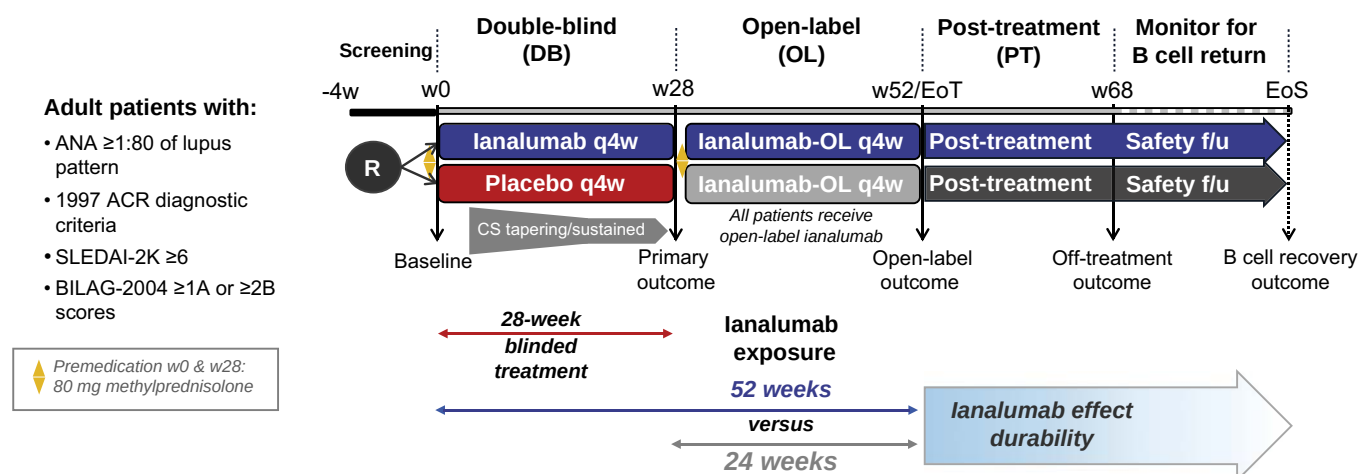


Figure 1. Study design. After a screening period for up to 28 days, patients were blindly randomized in a 1:1 ratio to receive SC administration of either ianalumab 300 mg or placebo every 4 weeks. On day 0, patients underwent baseline assessments, followed by pre-medication with 75 to 80 mg IV methylprednisolone 1 hour before the first exposure to the study medication. Study visits thereafter occurred every 4 weeks up to the final blinded assessment at week 28, after which all patients again received premedication with IV methylprednisolone before receiving OL ianalumab 300 mg every 4 weeks until week 48, followed by EoT assessments at week 52. Patients returned for additional study assessments at weeks 60 and 68, followed by less frequent monitoring for safety until the B cell recovery criteria were achieved (EoS). ACR, American College of Rheumatology; ANA, antinuclear antibody; BILAG-2004, British Isles Lupus Assessment Group-2004; CS, corticosteroids; EoS, end of study; EoT, end of treatment; f/u, follow-up; IV, intravenous; OL, open-label; q4w, every 4 weeks; R, randomization; SC, subcutaneous; SLEDAI-2K, Systemic Lupus Erythematosus (SLE) Disease Activity Index-2000; w, study week.

mucocutaneous or musculoskeletal domain and ≥ 1 'A' or 'B' in a second domain [23–26].

Background therapy allowed at baseline included stable CS (≤ 30 mg/d prednisone equivalent for ≥ 8 weeks before randomisation and stable for ≥ 2 weeks before randomisation) and/or antimalarials, thalidomide, and no more than one of the following: methotrexate or imidazole derivative (eg, azathioprine) or mycophenolic acid derivative.

Key exclusion criteria were recent use of B cell-depleting therapies or other biologics, prior malignancies, severe lupus renal disease, or active chronic infections. Patients with core hepatitis B antibody were not excluded if (1) negative for hepatitis B surface antigen (HBsAg) and viral DNA, (2) receiving antiviral prophylaxis, and (3) monitored throughout the study (HBsAg and hepatitis B virus DNA).

Endpoints

Patients were assessed for safety and efficacy markers on day 1 before drug administration, followed by assessments every 4 weeks and study drug treatment to week 52, with further monitoring every 4 weeks for safety and every 8 weeks for efficacy until week 68. Following week 68 assessments, patients achieving B cell recovery criteria, defined as $\geq 80\%$ of baseline or ≥ 50 cells/mL, proceeded to the EoS visit within 4 weeks. Otherwise, patients remained in the study for periodic safety monitoring until meeting recovery criteria, at which time efficacy outcomes were assessed again at EoS (results to be reported after study completion by all patients).

The primary composite endpoint at week 28 was the proportion of patients who achieved SLE Responder Index (SRI)-4 status [27] and successfully met and maintained protocol-defined CS-tapering requirements. For exploratory purposes, this composite outcome was reapplied during the OL period to identify SRI-4 responders at week 52 who had also met CS-tapering requirements of ≤ 5 mg/d prednisolone by week 40 and

maintaining within these limits to week 52. Patients who received CS outside of protocol-specified limits or required rescue therapies such as addition or increases of disease-modifying antirheumatic drugs (DMARDs), were classified as nonresponders for the composite outcome.

Additional secondary and exploratory disease activity assessments included Physician's Global Assessment (PhGA), performed using a 100-mm visual analogue scale (VAS), ranging from 'no disease activity' to 'maximal disease activity' for all aspects affected by the patient's disease, and SRI-6 [28], Lupus Low Disease Activity State (LLDAS) [29], and the Definition of Remission in Systemic Lupus Erythematosus (DORIS) [30]. For SRI, LLDAS, and DORIS derivations, the PhGA data were converted to a 0 to 3 scale by dividing by 33.33. The occurrence of moderate or severe flares [24,31] was assessed as number of patients experiencing flares, defined using BILAG-2004 score as severe flare: an A score in any system due to items that are new or worse, or moderate flare: ≥ 2 B scores due to items that are new or worse. The degree of CS use was captured both as number of patients achieving $\geq 50\%$ dose reduction compared with baseline and as those achieving ≤ 5 mg/d prednisolone.

Patient-reported outcomes (PROs) assessed at major time points included the Functional Assessment of Chronic Illness Therapy-Fatigue Scale (FACIT-F) [32,33], Short Form Health Survey-36 (SF-36), and Patient Global Assessment (PtGA) of disease using a VAS of 100 mm, ranging from 'no disease activity' to 'severe disease activity'.

Study conduct and entry of patient data into electronic case report forms by site personnel were independently monitored by Novartis staff. Lupus disease activity assessments (BILAG-2004, SLEDAI-2K, and PhGA) were electronically managed using iBLIPS v9.0 (ADS-Limathon Ltd), with quality control of data entries by the vendor performed on an ongoing, weekly basis in a blinded manner.

Standard laboratory assessments were performed at a centralised laboratory. Pharmacodynamic biomarkers (cellular, serum,

and transcriptomics) included circulating immune cells (TBNK, measuring T cells, B cells, natural killer cells) and B cell subsets, sBAFF, complement levels, immunoglobulins (Igs), and autoantibodies.

Safety evaluations

Safety assessments included adverse event (AE) monitoring, physical examination, electrocardiogram findings, vital signs evaluations, blood chemistry, haematology, urinalyses, and specific monitoring for serious or unusual infections.

All AEs and serious AEs (SAEs) were described using a standardised coding dictionary, Medical Dictionary for Regulatory Activities version 23.0, with groupings by system organ class and preferred term.

Statistical analysis

The primary composite endpoint was analysed using a Bayesian logistic regression model with treatment group, ENA status, and baseline SLEDAI-2K score as fixed effects. Posterior estimates were obtained using weakly informative priors. The efficacy criteria were considered met when there was >90% posterior probability that the responder rate with ianalumab was better than that with placebo and the estimated mean effect on ianalumab responder rate was >15% that of placebo.

All patients receiving ≥ 1 doses of study treatment were included in the primary analysis, where early dropouts and those exceeding permitted CS doses were considered nonresponders. With a sample size of 30 patients per treatment, there was 84% chance that the efficacy criteria would be met, assuming a 25% true difference in the responder rate in favour of ianalumab and a 17% placebo responder rate, as reported in a similar trial [28].

Subjects were analysed according to the study treatment received. Categorical data are presented as frequencies and percentages. Continuous data are presented as median, minimum, and maximum.

RESULTS

Recruited patients

From the 190 patients screened into the study between December 19, 2018, and January 31, 2022, 67 were recruited into the ianalumab treatment cohort and randomised to receive either placebo (n = 33) or ianalumab (n = 34) (Supplementary Fig S1).

Baseline characteristics were balanced between the groups for demographic features, disease activity, and background therapy. Most patients were female (>80%), with a median age of approximately 40 years, were seropositive for autoantibodies against double-stranded DNA (anti-dsDNA) and ENAs and received SoC treatment with CS, antimalarials, and DMARDs (Table 1).

During the 28-week DB treatment period, 2 randomised patients (1 in each group) were prevented from attending study visits due to local pandemic lockdown measures, requiring treatment discontinuation either for protocol nonadherence or patient's/investigator's decision, but all other patients completed DB treatment. Study treatment discontinuation in the OL period occurred for 5 patients (14.7%) in the IAN-IAN-OL arm, including 2 patients (5.9%) for AEs, and in 2 patients (6.1%) in the PBO-IAN-OL arm, including 1 patient (3%) for AE.

Table 1

Demographics, disease features, and medication use at baseline

Characteristic	Ianalumab n = 34	Placebo n = 33
Demographics		
Age, y, median (range)	42.0 (25-70)	39.0 (18-57)
Female, n (%)	32 (94.1)	27 (81.8)
Weight, kg, median (range)	63.8 (48.2-136.0)	58.0 (41.0-130.0)
Race, n (%)		
White	25 (73.5)	21 (63.6)
Asian	9 (26.5)	12 (36.4)
Disease activity		
Time from diagnosis, y, median (range)	8.3 (0.6-34.3)	7.8 (0.8-50.7)
SLEDAI-2K score		
Median (range)	10.5 (6-32)	10.0 (4-21)
≥ 10 , n (%)	20 (58.8)	20 (60.6)
BILAG 2004 score, median (range)	20.5 (9-45)	17.0 (9-29)
PhGA score, mm, median (range)	61.0 (27-81)	58.0 (33-84)
PtGA score, mm, median (range)	57.0 (3-94)	55.0 (0-87)
Disease markers		
ENA positive, n (%)	29 (85.3)	27 (81.8)
Anti-dsDNA, IU/mL		
Median (range)	111.4 (6.2-2667.1)	93.8 (6.2-647.3)
Seropositive (>75), n (%)	18 (52.9)	19 (57.6)
C3 complement (mg/dL)		
Median (range)	90.3 (39-142)	85.2 (29.5-144)
C4 complement (mg/dL)		
Median (range)	11.3 (2.5-38.9)	14.8 (2.5-46)
Pharmacodynamic markers, median (range)		
CD19+ B cell counts, cells/mL	97.0 (11.0-517.0)	66.0 (3.0-472.0)
Serum BAFF, pg/mL	1105.8 (579.5-3314.6)	1147.8 (550.8-3390.0)
IgG, g/L	14.3 (6.9-24.9)	14.1 (7.8-28.0)
IgA, g/L	3.1 (0.1-7.3)	2.8 (1.3-5.4)
IgM, g/L	0.9 (0.1-2.3)	0.7 (0.1-2.8)
SLE medication		
Corticosteroids		
Patients taking CS, n (%)	27 (79.4)	29 (87.9)
Median, mg/d (range)	10.0 (0-30)	10.0 (0-27.5)
Immunosuppressants, n (%)		
Antimalarials	30 (88.2)	30 (90.9)
Methotrexate	7 (20.6)	12 (36.4)
Azathioprine	16 (47.1)	10 (30.3)
Mizoribine	0	1 (3.0)
Mycophenolic acid	5 (14.7)	6 (18.2)
derivatives		

BAFF, B cell activating factor; BILAG, British Isles Lupus Activity Group's Disease Activity Index; CD, cluster of differentiation; C4, complement component 4; CS, corticosteroid, defined as a prednisolone-equivalent dose; C3, complement component 3; dsDNA, double-stranded DNA; ENA, extractable nuclear antigen; Ig, immunoglobulin; PhGA, Physician's Global Assessment; PtGA, Patient's Global Assessment; SLE, systemic lupus erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

Discontinuations in the PT period occurred in 4 patients in the IAN-IAN-OL arm (11.8%) and 9 patients in the PBO-IAN-OL arm (27.3%), with none for AEs.

Efficacy outcomes

Global disease activity measures

At the end of the DB period (week 28), analyses of the primary variable (SRI-4 response under tapered CS) showed a mean treatment difference of 34.5% (90% credible interval: 19%-49%), and the posterior probability that the responder rate was higher with ianalumab than with placebo was >99%.

Median treatment differences for all SRI-4 components are presented in Table 2 and Supplementary Figure S2. At week 28, there were more responders with ianalumab than with placebo for the primary variable (15 vs 3), the CS-taper component (21 vs 14), and the SRI-4 component (24 vs 10). These increases were maintained in the OL period and into the PT period. Similar increases occurred in the placebo group after switching to OL, which were also retained in the PT period (Table 2, Fig 2).

Consistent with the primary composite endpoint, a greater percentage of patients receiving ianalumab than those receiving placebo met the criteria at week 28 for an SRI response requiring a 6-point reduction in SLEDAI-2K scores (50.0% vs 21.2%) as well as for LLDAS (17.6% vs 9.1%) and DORIS (11.8% vs 3.0%), signifying low disease activity and remission, respectively (Table 2, Fig 2). These global disease activity responses were maintained or increased as patients from both arms moved into the OL period to week 52 and the PT period to week 68. Post hoc analysis of responder rates for LLDAS and DORIS without applying criteria for concomitant medication use revealed minimal differences from rates obtained under full criteria (Supplementary Fig S3), indicating that achieving these responder outcomes was not primarily driven by CS usage.

Corticosteroid use

A higher number of ianalumab-treated patients achieved a sustained oral CS reduction target at week 28 than patients receiving placebo (Table 2). Among patients taking >5 mg/d CS at baseline, a higher proportion on ianalumab compared with placebo achieved ≥50% reductions at week 28 in CS and a daily CS dose of ≤5 mg (66.7% vs 21.7% and 52.4% vs 26.1%, respectively). Further CS reductions occurred for patients in both study arms in the OL and PT periods up to week 68, along with increasing numbers of patients who were without any CS use, although some patients continued on prednisolone dosed up to 20 mg/d over these study periods (Table 2).

Flare incidence

During the DB period, fewer ianalumab-treated patients experienced any moderate or severe flare compared to those receiving placebo: 3 (8.8%) vs 10 (30.3%) patients, respectively, with a similar pattern observed for the individual category of severe flare: 3 (8.8%) vs 8 (24.2%), respectively. No additional patients in the ianalumab arm experienced flares during the OL period. Flares were also reduced in placebo-arm patients once transitioned to the OL period (Table 2, Supplementary Fig S4). Flare incidence remained low for all treated patients during the PT period to week 68.

PROs

Although scores did not differ significantly at week 28 for the PROs, numerical changes observed for PtGA, FACIT-F, and SF-36 physical components were in the direction of a positive treatment effect (Table 2, Supplementary Fig S5).

Laboratory markers

Ianalumab-treated patients showed increases in complement component 3 and 4 (C3 and C4) concentrations from baseline compared to those on placebo, evident by week 8 (Fig 3A, B), with further increases throughout the DB period to week 28. Complement concentrations progressively improved upon continued treatment with ianalumab into the OL period to week 52 that persisted in the PT period to week 68. Lesser increases in C3 and C4 levels occurred in PBO-IAN-OL patients (Fig 3A, B).

Similar treatment response patterns were observed for normalisation of complement concentrations for patients having low baseline values (Table 2).

In patients with detectable anti-dsDNA and anti-C1q antibodies at baseline, greater reductions in serum antibody levels and higher sero-reversion rates were observed with ianalumab than with placebo at week 28 (Table 2, Fig 3C, D). Further reductions occurred by week 52 in IAN-IAN-OL patients and to a lower degree in PBO-IAN-OL patients, with these changes persisting into the PT period up to week 68 (Table 2, Fig 3C, D). Similar ianalumab treatment effects were observed for autoantibody levels targeting ENAs, with results shown for U1-RNP and Smith antigens as 2 of the more prevalent of these autoantibodies with baseline detectable levels in the study patients (Fig 3E, F).

Pharmacodynamics

To evaluate the degree and durability of B cell depletion by ianalumab, absolute numbers of circulating CD19⁺ B cell counts were assessed at baseline and again at the end of each study period (Table 2, Supplementary Fig S6). Ianalumab induced a rapid and sustained depletion in circulating B cells throughout the treatment periods. In ianalumab-treated patients assessed at week 28 (n = 19), B cells were depleted to ≤3 cells/mL. Similar rates of B cell depletion were observed at week 52 for patients in the IAN-IAN-OL and PBO-IAN-OL arms (n = 21 tested for each arm), except for an outlier B cell count for one IAN-IAN-OL patient who had earlier withdrawn from the study treatment. Evidence for partial B cell reconstitution after 16 weeks of treatment cessation was seen among patients tested at week 68 (IAN-IAN-OL: n = 19; PBO-IAN-OL: n = 21). Similar depletion patterns were also observed across B cell lineage subsets (reported separately). No change or minor increases were noted for non-B cell lymphocytes, including CD3⁺ T cells (CD4⁺ and CD8⁺) and natural killer (NK) cells (CD56⁺, CD16⁺; Supplementary Fig S7). Following ianalumab treatment, sBAFF levels increased from baseline and remained so until week 68 (Table 2). Decreases in serum Ig levels occurred with ianalumab exposure (Table 2, Supplementary Fig S8). In the ianalumab-treated group, 38.7% and 44.8% of patients had IgM levels below the lower limit of normal (LLN) at weeks 28 and 52, respectively. Reductions in IgG levels were comparatively more limited, with mean levels having reduced ~20% from baseline; only 1 patient's IgG concentrations fell below normal limits (week 68, 5.47 g/L; LLN ≤5.65 g/L) after receiving ianalumab dosing during both the DB and OL periods. Overall reductions in IgA levels were similar to those observed for IgG.

Safety data

Placebo-controlled safety data were limited to the 28-week DB period (Table 3). Fewer patients reported ≥1 AEs with ianalumab (25 [73.5%]) than with placebo (29 [87.9%]). The proportion of patients with mild or moderate AEs was comparable between ianalumab (70.6%, mild; 23.5%, moderate) and placebo arms (72.7%, mild; 24.2%, moderate). SAEs reported were limited to 1 (2.9%) in the ianalumab arm and to 2 (6.1%) patients in the placebo arm. Local and systemic injection-related reactions were observed in ianalumab-treated patients and placebo patients during the DB and OL periods. All events were of mild-to-moderate severity, and none of them were serious, life threatening, or led to treatment discontinuation. No allergic hypersensitivity reactions were reported. No increase in infection rates was observed, and opportunistic infections or deaths

Table 2
Efficacy outcomes at the end of each study period

Outcome	DB (weeks 0-28)		OL (weeks 29-52)		PT (weeks 53-68)	
	IAN n = 34	PBO n = 33	IAN-IAN-OL n = 33	PBO-IAN-OL n = 32	IAN-IAN-OL n = 32	PBO-IAN-OL n = 30
Primary response, n (%)						
SRI-4 + CS-taper target	15 (44.1)	3 (9.1)	15 (45.5)	13 (40.6)	ND	ND
CS-taper target met	21 (61.8)	14 (42.4)	22 (66.7)	18 (56.2)	ND	ND
SRI-4	24 (70.6)	10 (30.3)	24 (72.7)	23 (71.8)	22 (68.7)	21 (70.0)
Other composite outcomes, n (%)						
SRI-6	17 (50.0)	7 (21.2)	21 (63.6)	19 (59.3)	18 (56.2)	17 (56.6)
LLDAS	6 (17.6)	3 (9.1)	13 (39.4)	7 (21.9)	14 (43.8)	7 (23.3)
DORIS	4 (11.8)	1 (3.0)	9 (27.3)	4 (12.5)	10 (31.3)	4 (13.3)
Outcomes, median change from BL (min, max)						
PhGA	−35 (−67, 7)	−20 (−56, 10)	−43 (−78, 12)	−35 (−82, 4)	−43 (−78, −2)	−31 (−77, 11)
SLEDAI-2K	−6 (−26, 3)	−2 (−10, 5)	−6 (−32, 13)	−6 (−12, 2)	−6 (−32, 11)	−6 (−12, 2)
BILAG-2004	−12 (−31, 16)	−8 (−17, 5)	−15 (−32, 20)	−9 (−28, 0)	−13 (−32, 6)	−9 (−27, 5)
BILAG flares, n (%)						
Moderate or severe flare	3 (8.8)	10 (30.3)	1 (3.0)	4 (12.5)	2 (6.3)	3 (10.0)
Severe flare	3 (8.8)	8 (24.2)	1 (3.0)	4 (12.5)	1 (3.1)	1 (3.3)
CS use (all patients)						
Median, mg/d (range)	5.0 (0, 18)	7.0 (0, 23)	0.0 (0, 20)	0.5 (0, 15)	0.0 (0, 17)	0.0 (0, 11)
CS use (subgroup BL >5 mg/d)						
Pts with >5 mg/d at BL, N	21	23	21	23	20	21
Reduction of ≥50% from BL, n (%)	14 (66.7)	5 (21.7)	19 (90.5)	13 (56.5)	19 (95.0)	16 (76.2)
Achieving ≤5 mg/d, n (%)	11 (52.4)	6 (26.1)	18 (85.7)	14 (60.9)	19 (95.0)	16 (76.2)
CS use (pts on CS = 0 mg), n (%)	4 (11.8)	5 (15.2)	20 (60.6)	16 (50.0)	25 (78.1)	23 (76.7)
PROs, median change from baseline (min, max)						
PtGA	−11.0 (−76, 13)	−10.5 (−54, 64)	−18.0 (−57, 20)	−24.0 (−53, 71)	−16.5 (−63, 17)	−17.5 (−47, 34)
FACIT-F	3.0 (−25, 24)	1.5 (−11, 17)	7.0 (−20, 27)	5.0 (−21, 23)	3.5 (−16, 25)	3.5 (−4, 22)
SF-36 (physical)	3.560 (−6.19, 20.43)	1.690 (−6.12, 17.28)	3.690 (−7.52, 21.16)	7.145 (−19.10, 20.57)	3.885 (−12.98, 19.26)	5.050 (−8.41, 22.61)
SF-36 (mental)	0.530 (−20.55, 12.23)	2.370 (−21.87, 24.00)	1.160 (−24.10, 24.20)	2.205 (−16.10, 15.55)	0.475 (−22.21, 25.55)	4.805 (−20.39, 18.70)
PD markers (median)						
Patients tested, N	19	21	21	21	19	21
CD19+ B cells, cells/mL (range)	1.0 (0.0-3.0)	81.0 (2.0-486.0)	1.0 (0.0-73.0) ^a	1.0 (0.0-4.0)	12.0 (0.0-338.0)	5.0 (0.0-287.0)
sBAFF, pg/mL	3119.8	1177.4	3429.0	3260.2	2144.8	2220.8
Pts with <LLN Ig, n (%)						
IgG <5.65 g/L	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)
IgM <0.4 g/L	12 (38.7)	8 (28.6)	13 (44.8)	14 (45.2)	12 (46.2)	15 (48.4)
IgA <0.7 g/L	2 (6.9)	0 (0)	4 (13.8)	0 (0)	3 (10.7)	0 (0)
Loss of seropositivity						
Anti-dsDNA, n/N (%)	8/20 (40.0)	3/18 (16.7)	12/20 (60.0)	10/20 (50.0)	12/20 (60.0)	8/20 (40.0)
Anti-C1q, n/N (%)	2/18 (11.1)	2/20 (10.0)	6/18 (33.3)	5/20 (25.0)	7/18 (38.9)	4/20 (20.0)
Normalised complement						
C3, n/N (%) of BL <LLN	4/17 (23.5)	3/20 (15.0)	5/17 (29.4)	7/20 (35.0)	6/17 (35.3)	8/20 (40.0)
C4, n/N (%) of BL <LLN	6/14 (42.9)	1/8 (12.5)	6/14 (42.9)	2/8 (25.0)	6/14 (42.9)	3/8 (37.5)

anti-dsDNA, autoantibodies against double-stranded DNA; BILAG-2004, British Isles Lupus Assessment Group-2004; BL, baseline; CD, cluster of differentiation; C4, complement component 4; CS, corticosteroid, prednisolone dose equivalent; C3, complement component 3; DB, double-blind period; DORIS, Definition of Remission in Systemic Lupus Erythematosus; dsDNA, double-stranded DNA; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; IAN, ianalumab; Ig, immunoglobulin; LLDAS, Lupus Low Disease Activity State; LLN, lower limit of normal; OL, open-label; PBO, placebo; PD, pharmacodynamics; PhGA, Physician's Global Assessment; PRO, patient-reported outcome; PT, posttreatment; PtGA, Patient's Global Assessment; sBAFF, soluble B cell activating factor; SF-36, Short Form Health Survey-36; SLEDAI-2K, Systemic Lupus Erythematosus (SLE) Disease Activity Index-2000; SRI, Systemic Lupus Erythematosus Responder Index.

CS dose is prednisolone or equivalent; SRI-4 + CS-taper target, SRI-4 responders who also met criteria for a sustained oral CS dose reduction to target; severe flare equals ≥1 new BILAG-A domain score, moderate flare defined as ≥2 BILAG-B domain scores that are new or worse. n/N represents the number of patients (n) observed at the indicated visit time, with a measured outcome within the group (N) being tested. Patients meeting study visit criteria for a moderate and a severe flare are counted only once irrespective of the number of flares at that visit. Reference ranges: anti-dsDNA, seropositive >75 IU/mL; anti-C1q, seropositive >15 IU/mL; C3 LLN <90 mg/dL; C4 LLN <16 mg/dL.

^a Elevated outlier B cell counts include patients who discontinued the study treatment in the OL period.

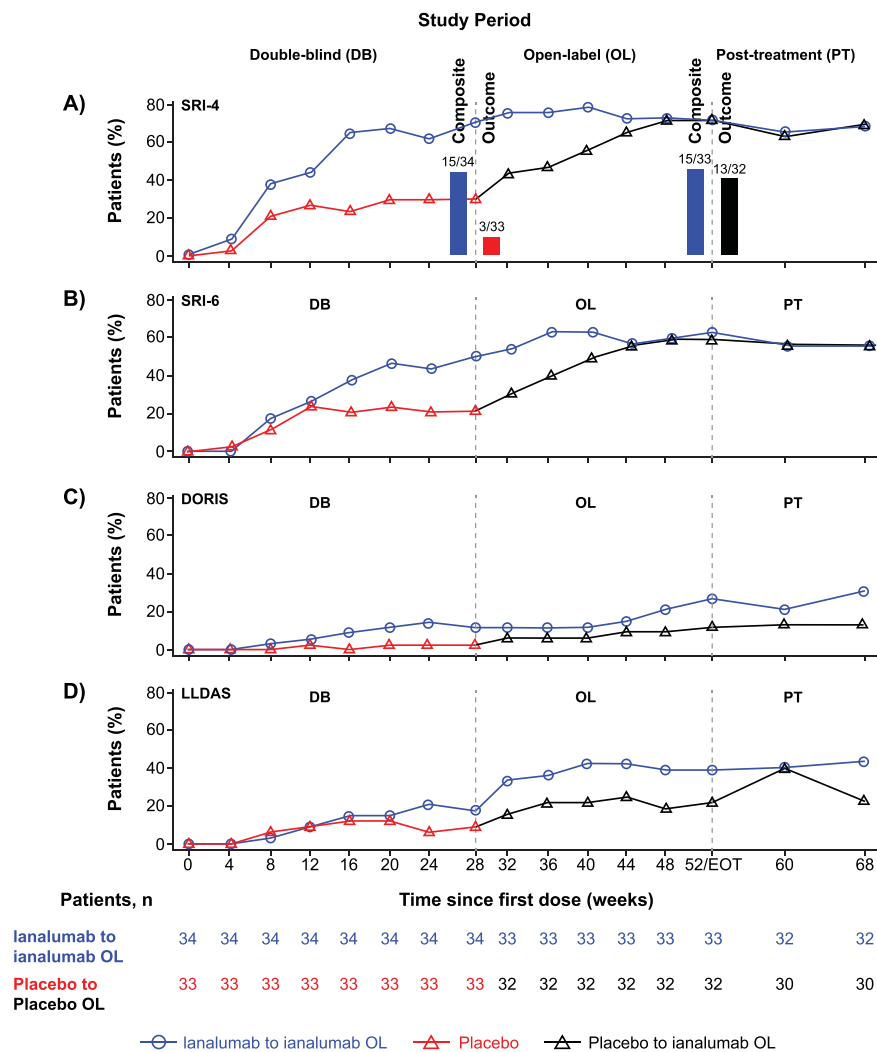


Figure 2. Time course of the selected efficacy outcomes. Efficacy outcomes over the 3 reported study periods: double-blind (DB) weeks 0 to 28, open-label (OL) weeks 29 to 52, and posttreatment (PT) weeks 53 to 68. (A) SRI-4 response with overlay bar graphs showing composite measure SRI-4 in patients also meeting CS-tapering goals at week 28 (primary outcome) and again at week 52 (exploratory outcome); (B) SRI-6 response; (C) DORIS response; and (D) LLDAS response. Ianalumab-to-ianalumab OL (blue), placebo (red), placebo-to-ianalumab OL (grey). ‘Patients’ represents the number of patients who responded to the SRI-4, SRI-6, DORIS, or LLDAS criteria. DB, double-blind; DORIS, Definition of Remission in Systemic Lupus Erythematosus; LLDAS, Lupus Low Disease Activity State; OL, open-label; PT, post-treatment phase; SRI, Systemic Lupus Erythematosus Responder Index.

were not observed. SAE incidence was low, and no infection SAE was reported in ianalumab-exposed treated patients. Over the 28-week DB period, 4 SAEs were reported for patients in the placebo arm vs 1 SAE reported in the ianalumab arm. There were more patients on ianalumab than on placebo with reduced neutrophil counts reaching common terminology criteria grade 3 (4 vs 2, respectively), some of whom had grade 1 or 2 neutropenia at baseline. Grade 3 neutropenia was usually short-lived and did not progress to grade 4 or lead to infections.

From week 28, all patients switched to OL ianalumab, resulting in 2 arms that differed in total ianalumab exposure (13 vs 6 doses). There were no relevant changes in the safety profile between DB and OL periods. Collection of both safety and efficacy endpoints continued to week 68. Thereafter, safety parameters were monitored less frequently until patients met the B cell recovery criteria. Common AEs by preferred term affecting ≥ 3 patients ($\sim 10\%$) in the PT and safety follow-up periods were similar between groups, with nasopharyngitis (3 vs 2 patients) and COVID-19 (3 vs 1 patients) in the IAN-IAN-OL arm and the PBO-IAN-OL arm, respectively (Table 3). Few patients experienced an SAE (2 vs 2 patient, 1 patient with 2 SAEs). None of the SAEs in the 2 study arms were considered treatment-related.

DISCUSSION

This study was designed to assess whether targeted depletion of BAFF-R⁺ B cells by ianalumab, together with BAFF-R

signalling blockade, could reduce disease activity, CS use, and flare incidence in patients with active lupus despite SoC therapy. The results at week 28 revealed that ianalumab was superior to placebo in achieving the primary composite endpoint of SRI-4 status in patients who also met CS-tapering requirements. Benefits of ianalumab were also observed across the different global disease activity measures, including SRI-4 and SRI-6 responses, LLDAS, and DORIS attainment, as well as upon moderate or severe disease flare.

Treatment effects with ianalumab compare favourably against results published for belimumab (Belimumab International Study [BLISS]-52, BLISS-76) [6,7,34] and anifrolumab (Treatment of Uncontrolled Lupus via the Interferon Pathway [TULIP]-1, TULIP-2) [9,10,35]. However, differences in study design and treatment duration make any direct comparisons of these phase 2 results for ianalumab preliminary. Moreover, few ianalumab-treated patients reached treat-to-target objectives of DORIS and LLDAS by week 28, although this number increased by week 52 with further exposure to OL ianalumab. These preliminary results require confirmatory evidence generated from larger phase 3 trials of longer, blinded treatment duration.

CS usage was also reduced in the ianalumab cohort, with more patients achieving or maintaining ≤ 5 mg/d prednisolone, a goal in line with current treatment guidelines to reduce chronic CS exposure [4]. PRO measures showed consistent, although nonsignificant, changes towards benefit with ianalumab. Laboratory findings revealed improvements in biomarkers

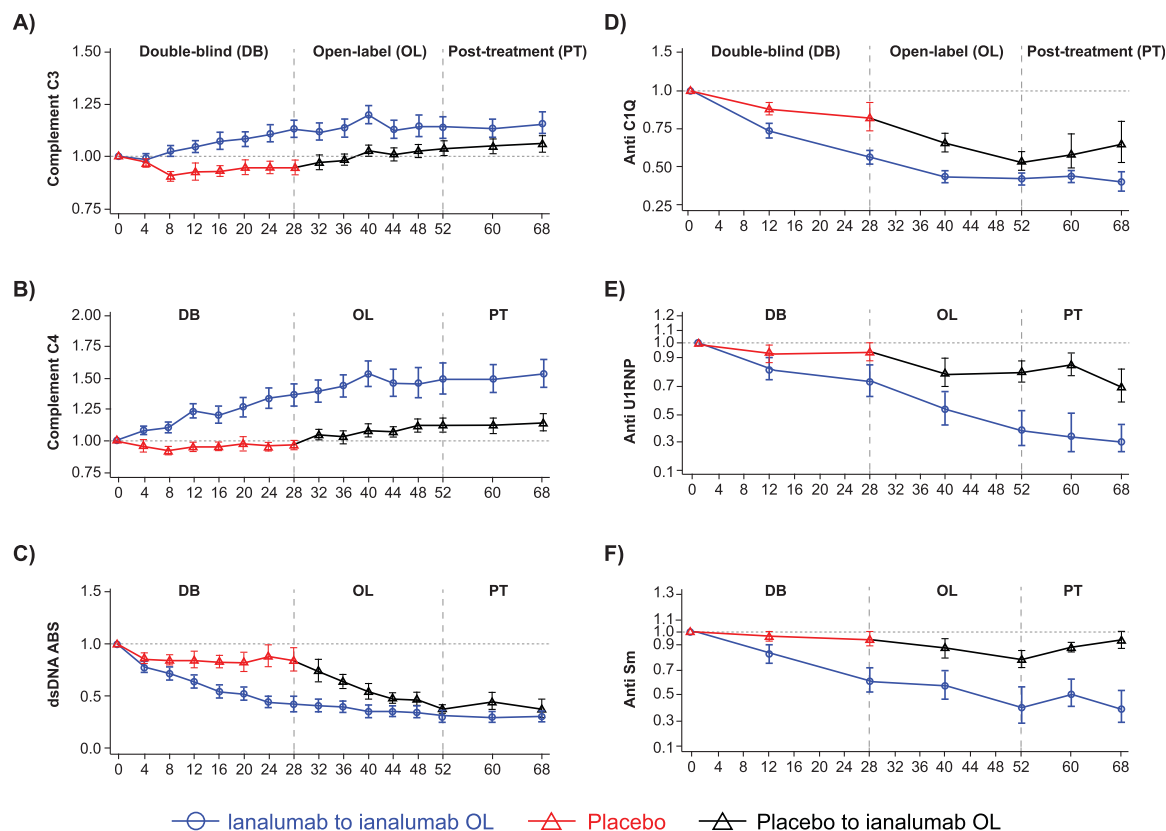


Figure 3. Time course of the selected biomarkers. Analyses provided as the ratio to geometric mean at baseline for biomarker analyses over the 3 reported study periods: double blind (DB) weeks 0 to 28, open-label (OL) weeks 29 to 52, and post treatment (PT) weeks 53–68. Ianalumab to ialanumab OL (blue), placebo (red), and placebo to ialanumab OL (gray). Serum complement levels for (A) C3 and (B) C4; patients with baseline serum levels above the negative threshold for autoantibodies against (C) dsDNA, (ialanumab, $n = 26$; placebo, $n = 26$) and (D) C1q (ialanumab, $n = 23$; placebo, $n = 27$), and (E) for U1-RNP (ialanumab, $n = 12$; placebo, $n = 12$) and (F) Sm (ialanumab, $n = 9$; placebo, $n = 7$). Assays: dsDNA, Quanta Lite, Inova Diagnostics, range 12.300 to 1000.0 IU/mL, negative <30 , borderline 30 to 75 IU/mL, positive >75 IU/mL; C1q, Bühlmann Laboratories AG, Ref. EK-AC1, range 5 to 340 IU/mL, negative ≤ 15 IU/mL, positive >15 IU/mL; U1-RNP, Sm, Biosynex/Theradiag MX 117, 1/201 dilution, negative <40 AU/mL, positive ≥ 40 AU/mL. C1q, complement component 1q; DB, double-blind; dsDNA, anti-double-stranded DNA; OL, open-label; PT, post-treatment phase; U1-RNP, U1 ribonucleoprotein particle; Sm, Smith.

associated with lupus disease activity, including increases in complement levels and reductions in serum autoantibodies. Thus, although limited in number of participants and treatment duration, the multiple efficacy signals generated by this study consistently support a therapeutic effect for ialanumab.

Difficulties in establishing clinical efficacy for rituximab in lupus have been attributed partly to the wide variability in depth of B cell depletion achieved by rituximab in this population, with incomplete depletion linked to poorer clinical responses [36,37]. Subsequent clinical investigations identified a range of factors contributing to reduced rituximab effectiveness for B cell depletion, including genetic or acquired deficiencies that can exist in one or more rituximab effector mechanisms, including ADCC, complement-dependent cellular cytotoxicity, and antibody-dependent cellular phagocytosis [38]. Rituximab also appears less effective in autoimmune diseases when blood and tissue levels of BAFF are elevated before treatment or undergo further elevations after B cell depletion, suggesting high BAFF levels protect against the depletion of autoreactive B cells and drive disease relapses [19,39,40]. To mitigate the effects of elevated BAFF levels in lupus patients treated with rituximab, investigators have tested combination therapy of rituximab followed by belimumab [41–43]. Although major endpoints for these studies were missed, the combination therapy did show synergistic benefits for disease flare risk and resulted in lower B cell counts and serum

autoantibody levels. In this study, B cell depletion in ialanumab-treated patients over the DB, OL, and PT periods was accompanied by fewer and low-severity flares despite 3-fold elevations in BAFF levels in response to B cell depletion, effects that are consistent with ialanumab-targeted BAFF-R blockade.

Laboratory biomarkers from interventional clinical trials can detect changes in the underlying pathobiology and show durability of the identified effects after treatment cessation. For example, improved clinical outcomes are linked to reductions in serum autoantibodies in response to treatment [19,44], whereas disease relapse after rituximab treatment was associated with parallel increases in anti-dsDNA antibodies and sBAFF [40]. In this study, reductions in autoantibody levels associated with ialanumab included antibodies against dsDNA and C1q, as well as against ENAs such as U1-RNP and Sm—autoantibodies typically resistant to rituximab treatment unless combined with belimumab [44,45]. Moreover, these observed changes in autoantibody levels were progressive over the period of ialanumab exposure, with further reductions observed for patients in the IAN-OL arm at week 52 compared to those at week 28, or when compared to PBO-OL arm patients at week 52 with ialanumab exposure limited to the 6-month OL period. Improvements in laboratory markers of disease activity were also maintained in the PT period to week 68, suggesting disease modification effects. Consistent with its mechanisms of action, ialanumab was also associated with reductions in serum Igs,

Table 3
Patients with any AE, common AEs, and any SAE during each period

During DB period	Ianalumab, n (%) n = 34	Placebo, n (%) n = 33
Any AE	25 (73.5)	29 (87.9)
Infection and infestations SOC	17 (50.0)	18 (54.5)
Common AEs (≥3 patients)		
Injection site reaction	9 (26.5)	1 (3.0)
Nasopharyngitis	7 (20.6)	7 (21.2)
Upper respiratory infection	3 (8.8)	1 (3.0)
Headache	3 (8.8)	1 (3.0)
SAEs	1 (2.9)	4 (12.1)
Renal impairment	1 (2.9)	0
Herpes zoster	0	1 (3.0)
Ovarian cyst	0	1 (3.0)
Pyelonephritis	0	1 (3.0)
<i>Salmonella</i> bacteraemia	0	1 (3.0)
During OL period	IAN-IAN-OL, n (%) n = 33	PBO-IAN-OL, n (%) n = 32
Any AE	24 (72.7)	27 (84.4)
Infection and infestations SOC	13 (39.4)	9 (28.1)
Common AEs (≥3 patients):		
Injection site reaction	6 (18.2)	12 (37.5)
Nasopharyngitis	5 (15.2)	1 (3.1)
Blood IgM decreased	3 (9.1)	0
Insomnia	0	3 (9.4)
SAEs	3 (9.1)	1 (3.1)
Acute myocardial infarction	1 (3.0)	0
Spinal compression fracture	1 (3.0)	0
Vertigo positional	1 (3.0)	0
Jaw fracture	0	1 (3.1)
During PT period	IAN-IAN-OL, n (%) n = 32	PBO-IAN-OL, n (%) n = 30
Any AE	15 (46.9)	16 (53.3)
Infection and infestations SOC	9 (28.1)	7 (23.3)
Common AEs (≥3 patients)		
Nasopharyngitis	3 (9.4)	2 (6.7)
COVID-19	3 (9.4)	1 (3.3)
SAEs	2 (6.3)	2 (6.7)
Pancreatitis ^a	1 (3.1)	0
Meniscus injury	1 (3.1)	0
Spinal stenosis	0	1 (3.3)
CNS vasculitis	0	1 (3.3)

AE, adverse event; CNS, central nervous system; DB, double-blind period; IAN, ianalumab; MedDRA, Medical Dictionary for Regulatory Activities; OL, open-label period; PBO, placebo; PT, posttreatment period; SAE, serious adverse event; SOC, system organ class.

AEs overall, common AEs by preferred term (in ≥3 patients), and SAEs overall and by preferred term according to MedDRA v23.0, unless indicated otherwise as SOC.

^a The pancreatitis SAE occurred twice in the same patient.

disproportionately affecting IgM levels. Importantly, low IgG levels were rare, limited to 1 patient having IgG less than LLN at week 68. There were no infections reported associated with low Ig levels.

Ianalumab was well tolerated by patients, with no discontinuation during the DB period or dose modification for safety reasons. Over the DB period, patients with any AE, infections, or neutropenia were not more frequent with ianalumab than with placebo. No serious infection SAE was observed with ianalumab, compared to 3 serious infection SAEs reported in the placebo arm (herpes zoster, *Salmonella* bacteraemia, and pyelonephritis). The single SAE for ianalumab, reported within the DB period of renal impairment, was due to a hospitalisation to facilitate 24-hour urine collection following clinic visit findings of proteinuria by urine dipstick; the condition spontaneously resolved within days without an identified aetiology. The increased AE frequency for ianalumab over placebo was limited to injection site reactions, upper respiratory tract infection, and headache.

Although this study was not powered to detect rare safety signals, the safety and tolerability profile observed in this lupus patient population was comparable to the reported experience with the same ianalumab dosing regimen in patients with primary Sjögren's disease [17].

No further increase in susceptibility to infection was detected with extended ianalumab treatment up to 1 year. Zoster infection under ianalumab treatment was limited to 1 mild case occurring during the DB period that resolved within 7 days. An SAE of central nervous system vasculitis occurred in a PBO-IAN-OL patient nearly 1 year after the last ianalumab treatment. This trial, which spanned the COVID-19 pandemic period, had only 9 confirmed SARS-CoV-2 infections identified in patients, both unvaccinated and vaccinated, and for whom infections occurred either under study treatment or during the PT follow-up. All COVID-19 cases were of mild-to-moderate severity that fully resolved without complication or need for hospitalisation. This relatively limited susceptibility to infection may be due in part to ianalumab-targeted depletion of BAFF-R⁺ B cells, without evidence of reduced numbers of circulating T cells and NK cells in the treated patients (Supplementary Fig S7) or downregulation of NK-cell or T-cell activity in the whole blood transcriptome (data not shown).

In conclusion, ianalumab was well tolerated in this SLE trial, with consistent benefits seen across multiple clinical and laboratory measures. These observed outcomes require confirmation in larger trials of longer duration, currently ongoing in SLE (NCT05639114 and NCT05624749) and lupus nephritis (NCT05639114).

CRedit authorship contribution statement

Nancy Agmon-Levin: Writing – review & editing, Investigation. **Stanislav Ignatenko:** Writing – review & editing, Investigation. **Alexander Gordienko:** Writing – review & editing, Investigation. **Josefina Cortés-Hernández:** Writing – review & editing, Investigation. **Pongthorn Narongroeknawin:** Writing – review & editing, Investigation. **Katarzyna Romanowska-Próchnicka:** Writing – review & editing, Investigation. **Nan Shen:** Writing – review & editing, Investigation. **Hana Ciferská:** Writing – review & editing, Investigation. **Masanari Kodera:** Writing – review & editing, Investigation. **Wei J Cheng-Chung:** Writing – review & editing, Investigation. **Piotr Leszczynski:** Writing – review & editing, Investigation. **Joung-Liang Lan:** Writing – review & editing, Investigation. **Eduardo Mysler:** Writing – review & editing, Investigation. **Rafal Wojciechowski:** Writing – review & editing, Investigation. **Tunde Tarr:** Writing – review & editing, Investigation. **Elena Vishneva:** Writing – review & editing, Investigation. **Yi-Hsing Chen:** Writing – review & editing, Investigation. **Yuko Kaneko:** Writing – review & editing, Investigation. **Stephanie Finzel:** Writing – review & editing, Investigation. **Alberta Hoi:** Writing – review & editing, Investigation. **Masato Okada:** Writing – review & editing, Investigation. **Ajchara Koolvisoot:** Writing – review & editing, Investigation. **Shin-Seok Lee:** Writing – original draft, Investigation. **Dai Lie:** Writing – review & editing, Investigation. **Hiroshi Kaneko:** Writing – review & editing, Investigation. **Bernadette Rojkovich:** Writing – review & editing, Investigation. **Lingyun Sun:** Writing – review & editing, Investigation. **Eugeniy Zotkin:** Writing – review & editing, Investigation, Conceptualization. **Jean-Francoise Viallard:** Writing – review & editing, Investigation. **Berta Paula Magallares López:** Writing – review & editing, Investigation. **Swati Aashish Ghanshani:** Writing – review & editing, Writing –

original draft, Visualization, Formal analysis, Data curation. **Carol Lau:** Writing – review & editing, Writing – original draft. **Alexandre Avrameas:** Writing – review & editing, Writing – original draft. **Carole Sips:** Writing – review & editing, Writing – original draft. **Stephen John Oliver:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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Contributors

The protocol was designed by Novartis Pharma AG. Data management and statistical analysis were performed by Novartis staff. The manuscript was written by SJO, CS, SAG, and AA. All authors participated in the conduct of the study and critically reviewed the manuscript for completeness, accuracy, and content and approved the revised, final version in writing before submission. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Competing interests

WJC reports consulting or advisory fees, funding grants, and speaking and lecture fees from AbbVie Inc, Bristol-Myers Squibb Company, Eli Lilly and Company, and Pfizer. Additionally, WJC received funding grants from Amgen Inc, GlaxoSmithKline Inc, Novartis, Sun Pharma, and UCB Pharma; speaking and lecture fees from AstraZeneca, Chugai Pharmaceutical Co Ltd, Janssen Pharmaceuticals, and Eisai Inc; consulting or advisory fees with Celgene LLC, Chugai Pharmaceutical Co Ltd, Eisai Inc, GlaxoSmithKline Inc, Janssen Pharmaceuticals Inc, Novartis, Sanofi-Aventis, and UCB Pharma; paid expert testimony from Janssen Pharmaceuticals Inc; and board membership or involvement with TSH Taiwan. EM reports consulting or advisory fees, funding grants, paid expert testimony, and speaking and lecture fees from AbbVie Inc; funding grants from Alpine Immunology, Amgen Inc, AstraZeneca, Bristol-Myers Squibb Company, Eli Lilly and Company, GlaxoSmithKline Inc, Hi Bio, Novartis, Pfizer, Roche, and Sanofi; and speaking and lecture fees from Amgen Inc, AstraZeneca, GlaxoSmithKline Inc, Pfizer, and Sanofi. EM also reports consulting or advisory fees with AstraZeneca, GlaxoSmithKline Inc, Hi Bio, and Sanofi. YHC reports consulting or advisory fees and speaking and lecture fees from Novartis Pharma AG, AbbVie Ltd, AstraZeneca, and Pfizer; consulting or advisory fees with CSL Behring, Sanofi, Astellas Pharma Inc, and Thermo Fisher Scientific Inc; speaking and

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Patient consent for publication

Not applicable.

Ethics approval

The study was approved by institutional review boards and independent ethics committees of participating countries and was conducted in accordance with the International Conference on Harmonisation guideline for Good Clinical Practice and applicable local regulations as well as with the Declaration of Helsinki. All patients provided written informed consent before study participation. The protocol and informed consent were approved by local ethics committee before study initiation.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The datasets generated and analysed for this study are not publicly available. Novartis will review requests for data from qualified external researchers for scientific merit. All patient-level data must obscure patient identity to respect patient privacy and conform to applicable laws and regulations. Any requests should be made to the corresponding author.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Supplementary materials

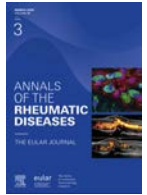
Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ard.2025.11.015](https://doi.org/10.1016/j.ard.2025.11.015).

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Systemic lupus erythematosus

Low-dose belimumab reduced risk of flares in patients with systemic lupus erythematosus: a multicentre, randomised, double-blind, placebo-controlled trial

Fangfang Sun¹, Huijing Wang¹, Danting Zhang¹, Nan Shen¹, Sheng Chen¹, Ting Li¹, Weiguo Wan², Sheng-Ming Dai³, Shuang Ye^{1,*}

¹ Department of Rheumatology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

² Department of Rheumatology, Huashan Hospital, Fudan University, Shanghai, China

³ Department of Rheumatology and Immunology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

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ABSTRACT

Objectives: The efficacy of low-dose belimumab for disease flare prevention in patients with lupus with low disease activity was evaluated (NCT04515719).

Methods: In this 52-week, randomised, placebo-controlled trial, patients who had a Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score of ≤ 6 , with no A and ≤ 1 B on British Isles Lupus Assessment Group, and who had prednisone ≤ 20 mg/d at screening were enrolled and randomly assigned 1:1 to intravenous 120 mg belimumab or placebo (saline). The primary outcome was disease flares within 52 weeks. The trial was terminated prematurely on 10 April 2022 when Renji Hospital was designated as a COVID-19 referral centre during the Shanghai lockdown.

Results: Overall, 90.5% and 86.1% of patients in the belimumab ($n = 116$) and placebo ($n = 115$) arms completed the study within a follow-up of 31 ± 16.1 weeks. The primary endpoint was met. In total, 7.8% of patients receiving low-dose belimumab had disease flares, significantly lower than that in the placebo group (19.1%) ($P = .012$; difference 11.3%; 95% CI 1.8%–20.9%). Kaplan-Meier curves also demonstrated lower cumulative flare rates in the belimumab arm ($P = .011$, Hazard Ratio (HR), 0.38; 95% CI 0.17–0.83). Glucocorticoid-sparing effects were comparable (Δ prednisone from baseline, -1.55 ± 10.11 mg/d vs -0.91 ± 7.43 mg/d, $P = .075$). SELENA-SLEDAI was reduced by 0.62 ± 2.14 in the belimumab group; however, it increased by 0.12 ± 2.80 in the placebo group ($P = .009$). Any adverse events were comparable (61.2% vs 64.3%). Only 4 (3.4%) and 6 (5.2%) severe adverse events occurred in 2 groups, respectively.

Conclusions: Treatment with low-dose belimumab helped reduce the risks of disease flares in Chinese patients with systemic lupus erythematosus at low disease activity. Belimumab was generally well-tolerated.

*Correspondence to Dr. Shuang Ye, Department of Rheumatology, South Campus, Shanghai Renji Hospital, Shanghai, China.

E-mail address: ye_shuang2000@163.com (S. Ye).

Fangfang Sun, Huijing Wang and Danting Zhang contributed equally.

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Belimumab, a humanized monoclonal antibody targeting BlyS (B-lymphocyte stimulator), is the first FDA-approved biological agent for active lupus patients at the standard 10 mg/kg dose. The pivotal BLISS-52 trial also indicated clinical benefits at a lower 1 mg/kg dose.
- Evidence showed possible association between elevated BlyS levels and an increased risk of disease flares.

WHAT THIS STUDY ADDS

- In this multicenter, randomized, placebo-controlled trial involving Chinese SLE patients with low disease activity, a low dose (~2 mg/kg) of belimumab significantly reduced the risk of disease flares within 52 weeks compared to placebo.
- The low-dose belimumab group demonstrated lower cumulative flare rates and a more significant reduction in SELENA-SLEDAI scores.
- Immunopharmacological effects were corroborated by a higher anti-dsDNA seroconversion rate and a significant reduction in IgG levels in the belimumab group.
- Low dose belimumab was generally well-tolerated in this Chinese patient population.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The efficacy and safety of low dose belimumab in SLE patients with low disease activity in this multicenter, randomized, placebo-controlled trial could repurpose it as a flare prevention strategy with potential cost and safety advantages in this population.
- The significant reduction in flare risk, as well as other clinical and immunopharmacological benefits, suggest low dose belimumab as a potential disease-modifying agent, meriting further trials to confirm its long-term impact on organ damage and prognosis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex chronic autoimmune disorder typically manifested as remission-relapse patterns in its course. Although evidence supported that the attainment of remission or low disease activity (LDA) confers a lower risk of lupus flare [1,2], a low-grade lupus activity population with a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score lower than 6 with an average score of 2 to 4 still incurs a 30% to 40% annual flare rate in real-world settings [3,4]. Each flare event, unfortunately, increases the disease burden and doubles the risk of damage accrual, making the secondary prevention of lupus flare a key task yet largely uncharted by the contemporary randomized, controlled trials.

Belimumab, a humanised monoclonal antibody targeting BlyS (B-lymphocyte stimulator), is the first Food and Drug Administration-approved biological agent for patients with active lupus with scores of at least 6 on the Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) [5–7]. Administration of 10 mg/kg belimumab intravenously in combination with standard of care (SOC) was proved to be effective in reducing disease activity, as measured by Systemic Lupus Erythematosus Responder Index-4 (SRI-4), in several randomized, controlled trials and real-world observational studies [5,6,8,9]. Notably, a lower dose of 1 mg/kg also demonstrated clinical benefit in the BLISS-52 (Belimumab in Subjects with Systemic Lupus Erythematosus [BLISS]-52) trial when evaluating both

SRI-4 and lupus low disease activity state (LLDAS) achievement [1,6]. Furthermore, belimumab treatment was associated with a reduction in flares in both BLISS-52 and BLISS-76, an effect particularly evident in long-term extension data from the BLISS-76 (Belimumab in Subjects with Systemic Lupus Erythematosus [BLISS]-76) trial [1,5,6].

By using this plausible active lupus treatment, we aimed to repurpose it for patients with low-grade activity (SELENA-SLEDAI ≤ 6) and to find support for low-dose belimumab as a flare prevention tool in the management of such a population. The rationale is supported by reports linking elevated BlyS levels to an increased risk of flares, even if the exact mechanisms remain elusive [10,11]. To validate this hypothesis, we conducted this multicentred, randomised, placebo-controlled trial to evaluate the efficacy of low-dose belimumab in terms of flare prevention in Chinese patients with low-grade SLE.

METHODS

Study design and participants

This investigator-initiated, multicentre, randomised, double-blind, RCT was carried out at Rheumatology centres in Shanghai, China (Renji Hospital South Campus, Renji Hospital West Campus, Huashan Hospital, Sixth People's Hospital). Eligible patients were adults (age ≥ 18 years old) who met The 2012 SLICC (Systemic Lupus International Collaborating Clinics) criteria for SLE [12] and had low-grade disease activity defined as the SELENA-SLEDAI score [13] of ≤ 6 at screening, with no British Isles Lupus Assessment Group (BILAG) [14] A domain scores and no more than one B organ domain score. All participants received a stable treatment of no more than 20 mg/d prednisone, and/or hydroxychloroquine, and/or immunosuppressive agents (azathioprine, mycophenolate, methotrexate, ciclosporin, tacrolimus, leflunomide) for at least 30 days. All participants willing to join the trial signed a written informed consent.

Key exclusion criteria were as follows: (i) hepatic dysfunction (alanine aminotransferase >2 times the upper normal limit of normal); (ii) renal dysfunction (estimated creatinine clearance rate <60 ml/min); (iii) current pregnancy or breastfeeding; (iv) participants were not allowed to use cyclophosphamide within the preceding 6 months or B-cell targeted therapy (eg, rituximab or a BlyS inhibitor) within the past year; (v) herpes zoster infection within 3 months before screening; (vi) participants with a history of malignancy within the last 5 years (except for adequately treated nonmelanoma skin cancer or cervical carcinoma *in situ*); (vii) active infection at screening (such as hepatitis B defined by positive for hepatitis B surface antigen, HIV, tuberculosis, etc).

The study was approved by the Ethics Committee of all participating centre. Any serious adverse event (SAE) with detailed information about date, severity, causal relationship to the medication, and the clinical management was reported to the principal investigator and ethics committee within 24 hours. The trial was registered on ClinicalTrials.gov (NCT04515719).

Randomisation and masking

Participants were randomly assigned in a 1:1 ratio to receive either a fixed dose of 120 mg (~2 mg/kg) belimumab or placebo (normal saline) on the background of SOC, stratified by clinical centre using permuted blocks of size 4. The randomisation sequence was computer-generated, and allocation concealment was ensured through sealed, opaque envelopes.

Participants, investigators responsible for disease assessments and follow-up, and personnel conducting the statistical analyses remained blinded to group allocation. An unblinded pharmacy specialist prepared the investigational infusions according to the predetermined randomisation sequence. The diluted belimumab solution in saline was indistinguishable from the placebo (normal saline) in terms of visual characteristics, container size, and colouration. An emergency hotline available 24 hours a day and 7 days a week was established for reporting SAEs for all the participants. Unblinding was permitted only if the clinical management of an SAE necessitated knowledge of the treatment allocation.

Belimumab was administered intravenously at a fixed dose of 120 mg at week 0, week 2, and week 4, followed by every 4 weeks up to week 48. The placebo group received the same volume of normal saline at the same time points. The final efficacy and safety evaluation was predefined at 52 weeks. The trial treatment could be discontinued based on the severity of adverse events and the investigators' clinical judgement.

All the participants received the standard care of lupus, which could include prednisone (≤ 20 mg/d), and/or hydroxychloroquine, and/or immunosuppressive agents (eg, mycophenolate mofetil, azathioprine, cyclosporine, tacrolimus, methotrexate, leflunomide).

The doses of prednisone and immunosuppressive agents could be adjusted downwards according to participants' disease activity and the physicians' decision. In the event of disease exacerbation, immunosuppressive therapy could be intensified according to the severity of the lupus flare.

Outcomes and follow-up visits

The primary endpoint measure was the proportion of participants who experienced a disease flare within 52 weeks, as defined by SELENA-SLEDAI flare index [13,15]. Mild-to-moderate flares are defined as one or more of the following criteria: (i) a change in SELENA-SLEDAI score of 3 points or more (but not to more than 12); (ii) new or worsening rashes, cutaneous vasculitis, nasopharyngeal ulcers, serositis, arthritis, or fever attributable to lupus; (iii) increase in prednisone, but less than 0.5 mg/kg/d; (iv) added nonsteroidal anti-inflammatory drugs or hydroxychloroquine; and (v) ≥ 1 increase in Physician Global Assessment (PGA) (0–3). Severe flares are defined as one or more of the followings: (i) change in the SELENA-SLEDAI score to more than 12; (ii) new or worsening systemic manifestations requiring doubling or an increase in dosage of prednisone to greater than 0.5 mg/kg/d or hospitalisation, including central nervous system involvement, vasculitis, nephritis, myositis, thrombocytopenia (platelet count $< 60 \times 10^9$ cells/L), or haemolytic anaemia (haemoglobin level < 70 g/L or decrease in haemoglobin level > 30 g/L); (iii) increase in dosage of prednisone to greater than 0.5 mg/kg/d; (iv) added cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil, or hospitalisation for lupus activity; and (v) increased PGA to > 2.5 .

Secondary outcomes included the proportions of mild-to-moderate flares and severe flares, time to first disease flare, changes in prednisone dose, changes in SELENA-SLEDAI score from baseline to the last visit, and the percentage of patients achieving prednisone-free status successfully within 52 weeks. Safety issue was collected until 4 weeks after the last infusion.

Exploratory outcomes included changes in complement and immunoglobulin (Ig) levels, anti-ds-DNA positivity, and sensitivity analysis.

Sample size estimation and statistical analysis

The target sample size was calculated as 167 patients per group, accounting for an estimated 10% drop-out rate, yielding a total of 334 participants. However, the COVID-19 lockdown in Shanghai, China (March 2022 to June 2022) severely hindered patient recruitment and follow-up as participants were unable to attend site visits for endpoint assessments, leading to major deviation or permanent discontinuation of treatment. Consequently, the trial was terminated prematurely on 10 April 2022 when Renji Hospital, South campus, was designated as a COVID-19 referral centre during the Shanghai lockdown. Before the database lock, a revised power calculation was performed based on the actual enrolments and the observed effect sizes of the predefined primary endpoint. With an alpha level of 0.05 and the enrolment of 231 patients, the trial retained 90% power to detect a 10-percentage-point difference between groups in the half-year flare rates and 75% power to detect a 7.5-percentage-point difference, assuming an annual flare rate of 30% to 40% in the placebo group based on our previous 'Met Lupus' trial enrolling a similar population [3,16].

The primary endpoints were conducted in the modified intention-to-treat population, which included all the eligible randomised patients who received at least 1 dose of study treatment. The per-protocol population (PPP) excluded patients with major protocol violations. Sensitivity analyses were performed in the PP population and in a subset excluding participants with less than 3 months of follow-up to enhance the robustness of the findings.

Between-group comparisons were made using 2-sample *t*-tests for continuous variables, and Fisher's exact test for categorical variables, as appropriate. Time-to-flare analysis was performed using Kaplan-Meier curves and the log-rank test. A 2-sided *P* value of 0.05 was considered statistically significant.

All the analyses were conducted using SPSS software (version 29.0), Prism Graphpad software (version 10.5.0), and R software (version 4.5.1; <http://www.Rproject.org>).

Ethical considerations

The clinical trial was conducted in compliance with the Good Clinical Practice Guidelines, Declaration of Helsinki, and Chinese clinical trial regulations. All substantial protocol amendments were submitted to and approved by the respective ethics committees of the participating centres. Any SAEs were reported to the principal investigator and the ethics committees within 24 hours, with detailed information including the date of occurrence, severity, assessed relationship to study drug, and clinical management measures taken.

Role of the funding source

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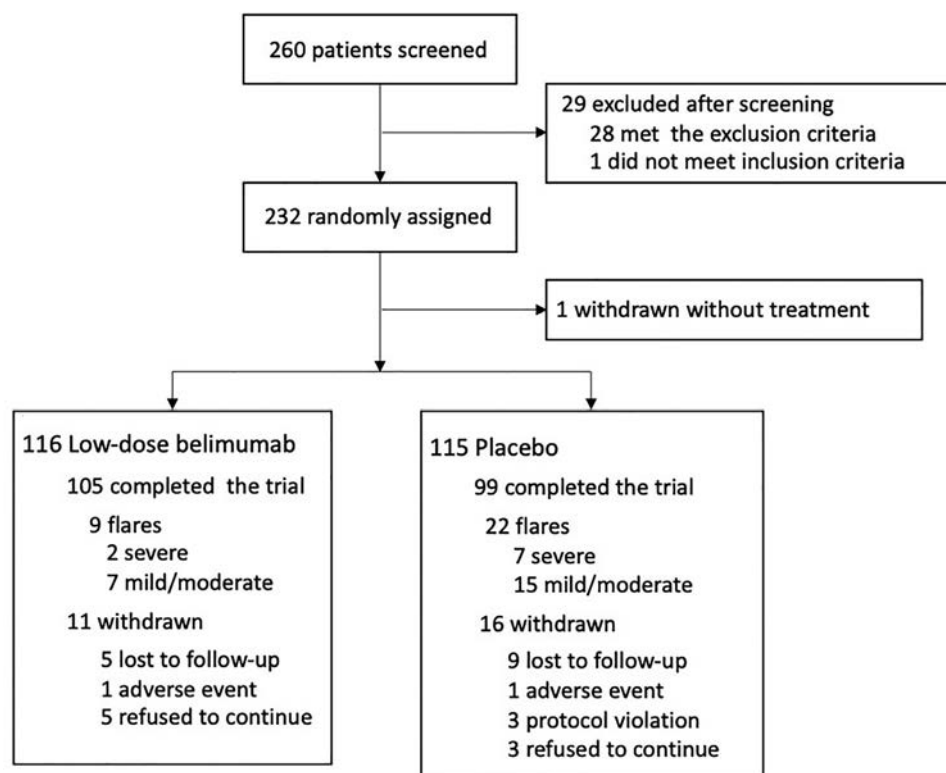


Figure 1. Flow chart.

Table 1
Baseline characteristics of participants from 2 arms

	Belimumab (n = 116)	Placebo (n = 115)
Age (y)	35.84 (9.93)	35.50 (10.00)
Gender (female)	112 (96.6%)	101 (87.8%)
SLE duration (y)	8.39 (6.36)	7.56 (5.62)
Laboratory results		
WBC $\times 10^9/L$	5.40 (1.74)	5.81 (1.99)
Haemoglobin (g/L)	125.50 (18.13)	128.00 (14.86)
PLT $\times 10^9/L$	207.03 (79.12)	208.61 (61.42)
Positivity of anti-dsDNA	63 (54.3%)	62 (53.9%)
C3 (g/L)	0.81 (0.22)	0.82 (0.24)
C4 (g/L)	0.14 (0.08)	0.15 (0.06)
Hypocomplementemia	78 (67.2%)	78 (67.8%)
IgG (g/L)	15.85 (5.34)	15.10 (4.69)
IgA (g/L)	2.78 (1.26)	2.98 (1.35)
IgM (g/L)	1.00 (0.85)	0.93 (0.69)
Disease activity		
PGA (0-3)	0.18 (0.20)	0.18 (0.23)
SELENA-SLEDAI score	2.68 (1.72)	2.49 (1.74)
Organ manifestations	29 (25.0%)	26 (22.6%)
Mucocutaneous	17 (14.7%)	8 (7.0%)
Haematological	9 (7.8%)	14 (12.2%)
Renal	5 (4.3%)	6 (5.2%)
Treatment		
Prednisone (mg/d)	7.11 (4.02)	7.03 (4.65)
Hydroxychloroquine	109 (93.1%)	103 (89.6%)
Immunosuppressants	57 (49.1%)	63 (54.8%)
Azathioprine	6 (5.2%)	5 (4.3%)
Calcineurin inhibitor	8 (6.9%)	9 (7.8%)
Leflunomide	4 (3.4%)	2 (1.8%)
Mycophenolate	27 (23.3%)	27 (23.5%)
Mycophenolate + tacrolimus	1 (0.9%)	1 (0.9%)
Methotrexate	11 (9.5%)	19 (16.5%)

dsDNA, anti-double stranded DNA; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; PGA, physician global assessment; PLT, platelet; SLE, systemic lupus erythematosus; SELENA-SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index; WBC, white blood cells.

RESULTS

Between March 2021 and April 2022, a total of 260 patients were screened, and 232 eligible patients were enrolled in the study. One participant withdrew the informed consent before treatment initiation. The remaining 231 patients were randomly assigned to receive either low-dose belimumab (n = 116) or placebo (n = 115). In the belimumab group, 5 patients were lost to follow-up, 5 patients withdrew consent, and 1 discontinued the medication due to adverse events. In the placebo group, 16 patients discontinued the trial (Fig 1).

Of 231 patients, 213 were female; their mean age was 35.7 ± 9.9 years, mean disease duration was 8.0 ± 6.0 years, and mean SELENA-SLEDAI score at screening was 2.6 ± 1.7 . A total of 55 participants (23.8%) had mild organ involvement meeting the BILAG inclusion criteria. Baseline characteristics, including age, disease duration, SELENA-SLEDAI score, and concomitant therapies, were balanced between groups (Table 1), except for a higher proportion of females in the belimumab group (96.6% vs 87.8%). Mean baseline prednisone doses were 7.1 ± 4.1 mg (belimumab) and 7.0 ± 4.7 mg (placebo), respectively. At baseline, 3 patients in the belimumab group and 11 patients in the placebo group were steroid-free. The use of hydroxychloroquine (belimumab vs placebo: 93.1% vs 89.6%) and immunosuppressive agents (belimumab vs placebo: 49.1% vs 54.8%) were similar between groups (Table 1).

By 4 April 2022, in a mean follow-up of 31 weeks, the primary endpoint was met. In total, 7.8% (9/116) of patients receiving low-dose belimumab on the basis of SOC had disease flares within follow-up, which was significantly lower than that in patients receiving placebo, ie, 19.1% (22/115) ($P = .012$; difference 11.3%; 95% CI 1.8%–20.9%; Fig 2A).

Regarding the secondary outcomes, the rate of severe flares in the belimumab group was numerically lower than that in the placebo group (1.7% vs 6.1%, $P = .102$), despite being nonsignificant (Fig 2B). Manifestations of disease flares in both arms

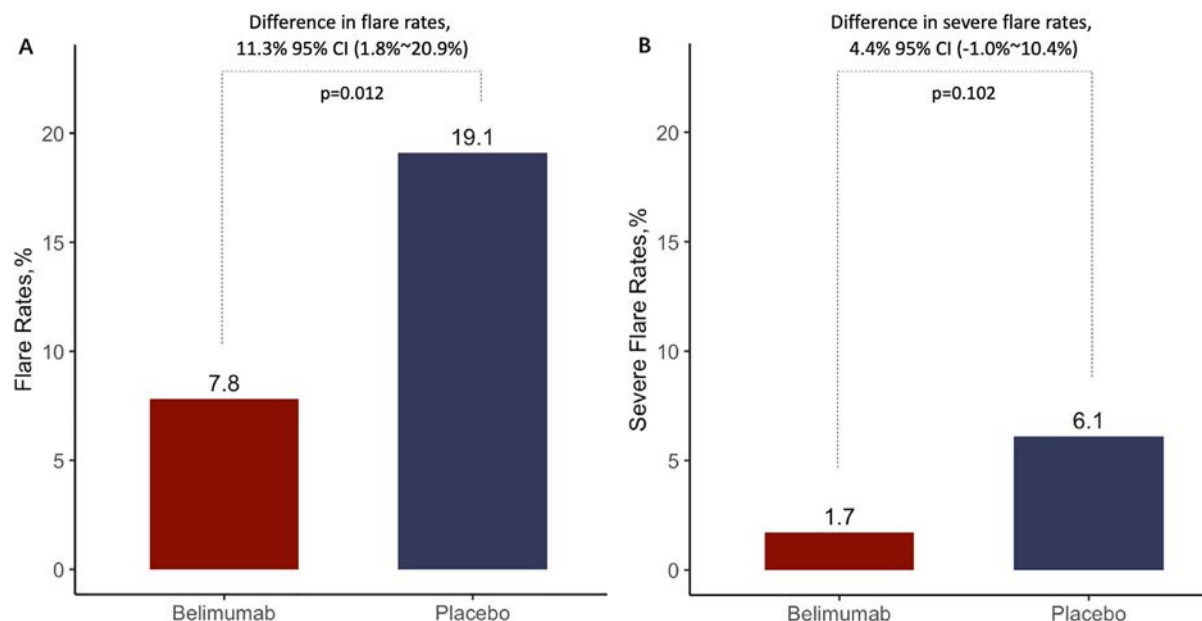


Figure 2. The difference in overall disease flare rate (A) and severe flare rate (B) between participants receiving low-dose belimumab and placebo on the basis of SOC. SOC, standard of care.

were listed in [Supplementary Table S1](#). Kaplan-Meier curve also demonstrated much lower cumulative flare rates in patients receiving belimumab plus SOC ($P = .011$; HR 0.38; 95% CI 0.17–0.83), indicating its protective efficacy against disease flares ([Fig 3A](#)). Patients assigned to the low-dose group tended to have lower cumulative severe flares than those in the placebo group, albeit no significance was observed in 2 arms ($P = .092$; HR 0.28; 95% CI 0.06–1.36) ([Fig 3B](#)).

Glucocorticoid-sparing effect was similarly observed in 2 groups (Δ prednisone from baseline, 1.55 ± 10.11 mg/d vs 0.91 ± 7.43 mg/d, $P = .075$) ([Fig 4A](#)). No significant difference was observed between the groups in the proportion of participants who achieved steroid withdrawal during follow-up (belimumab 10/113 vs placebo 4/104, $P = .17$). However, the SLENA-SLEDAI score was decreased by 0.66 ± 2.14 in the belimumab group, but increased by 0.12 ± 2.80 in the placebo group, with a significant between-group difference ($P = .009$) ([Fig 4B](#)).

During follow-up, daily glucocorticoid use and changes in steroid dosage from baseline at each visit are demonstrated in [Supplementary Figure S1A,B](#). Disease activities, as assessed by PGA and SLEDAI scores at each visit from baseline to 12 months, are shown in [Supplementary Figure S2A,B](#). At the 12-month time point, the SLEDAI score was significantly lower in the low-dose

belimumab group compared to the placebo group. No significant difference was observed in PGA changes between the 2 groups.

At baseline, anti-double stranded DNA (dsDNA) antibody positivity was observed in 63 (54.3%) participants in the belimumab and 62 (53.9%) in the placebo group. Seroconversion of anti-dsDNA antibody from positive to negative occurred in 13 (20.6%) patients in the belimumab group, which was significantly higher than that in the placebo group (6.5%, $P = .034$).

Changes in complement levels, including C3 and C4, throughout the study are illustrated in [Supplementary Figure S2C,D](#). At baseline, hypocomplementemia, defined by C3 lower than 90 mg/dl or C4 lower than <10 mg/dL, was observed in 78 patients from each arm. At 12 months, the increase in C4 was more pronounced in the belimumab group than in the placebo group. However, no significant difference was detected in the correction of hypocomplementemia between the 2 groups.

During follow-up, the incidence of any adverse events was comparable between the low-dose belimumab group (71 patients, 61.2%) and the placebo group (73 patients, 64.3%). The proportion of patients experiencing SAEs was also similar (belimumab vs placebo: 4 [3.4%] vs 6 [5.2%]). SAEs in the belimumab group consisted of 2 severe flares requiring hospitalisation, 1 case of pneumonia and 1 asthma attack, while in the placebo group, SAEs included 3 severe flares, 2 cases of

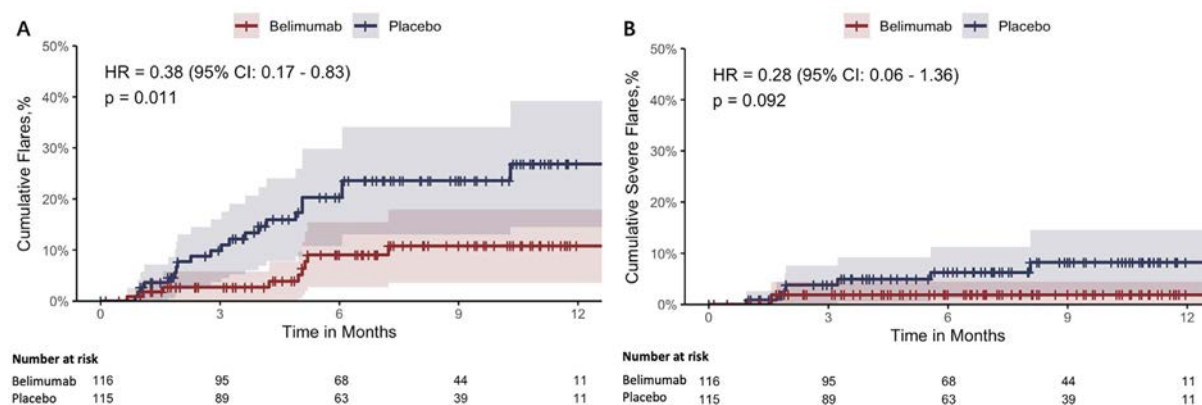


Figure 3. Kaplan-Meier curves demonstrated cumulative flare rates (A) and severe flare rates (B) of participants from 2 arms.

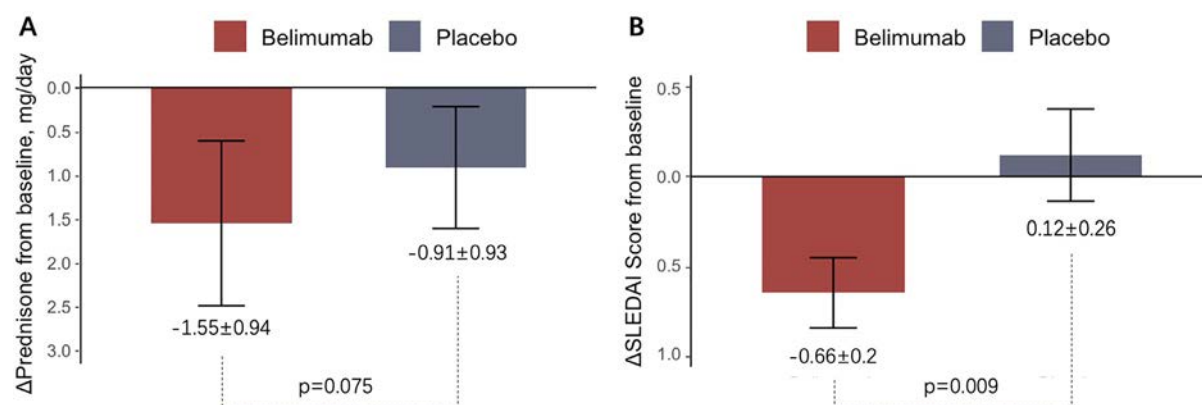


Figure 4. The difference of prednisone dosages (A) and SLEDAI scores (B) from baseline to last visit was demonstrated. Error bars represented the SE of the means. SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

gastroenteritis and 1 case of lumbar spinal tuberculosis confirmed by biopsy (Table 2). Treatment discontinuation due to adverse events occurred in 3 (2.6%) belimumab recipients and 6 (5.2%) placebo recipients.

Infections were the most common adverse events, occurring in 45 patients (38.8%) in the belimumab group and 41 patients (35.7%) in the placebo group. Upper respiratory infections were the most frequently reported, followed by gastroenteritis and urogenital tract infections. Most infections were mild, and patients were able to continue treatment after symptom resolution. No COVID-19 cases were reported in either group. Infusion reactions were rare and mild, occurring in 3 (2.6%) belimumab-treated patients and 4 (3.5%) placebo-treated patients, respectively. Other adverse events such as headache, hypertension, abdominal discomfort, rash, and arthralgia were observed at comparable frequencies in 2 arms. Liver injury occurred in 3 (2.6%) participants in the belimumab and 4 (3.5%) patients in the placebo group, all of which were mild with liver enzymes elevated by less than 2 times the upper normal limit. Five patients receiving belimumab had leukopenia, which was similar to that in the placebo arm ($n = 4$). Osteonecrosis was

reported in 2 patients in the belimumab arm and was considered unrelated to the study drug.

Changes in immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) levels over time are demonstrated in Supplementary Figure S3. Significantly greater reductions in IgA and IgM were observed in the belimumab group at multiple time points (IgA: belimumab vs placebo, 3 month 2.64 ± 1.31 g/L vs 3.02 ± 1.26 g/L, 6 month 2.58 ± 1.29 g/L vs 3.17 ± 1.46 g/L, 9 month 2.52 ± 1.11 g/L vs 3.06 ± 1.35 g/L; IgM: belimumab vs placebo, 9 month 0.68 ± 0.41 g/L vs 0.96 ± 0.83 g/L, 12 month 0.68 ± 0.46 g/L vs 0.98 ± 0.96 g/L). Although IgG levels did not differ significantly at individual visits, the overall change from baseline to the last visit showed a decrease of 0.72 ± 2.64 g/L in the belimumab group compared to an increase of 0.94 ± 2.65 g/L in the placebo group ($P < .001$). Nevertheless, severe hypogammaglobulinemia (IgG < 2 g/L) was not observed in either group. There were no reports of psychiatric events, deaths, or malignancies.

To increase the robustness of the results, sensitivity analyses were carried out in the PPP and in patients with more than 3 months of follow-up as exploratory endpoints. The primary endpoint was still met (Supplementary Table S2). An exploratory binomial logistic regression analysis, adjusted for the observed imbalance in gender distribution, indicated that low-dose belimumab remained significantly associated with a reduced risk of flares (Odds Ratio (OR) 0.36, 95% CI 0.15–0.83, $P = .019$) (Supplementary Table S3).

DISCUSSION

SLE is a chronic systemic autoimmune disease characterised by recurrent flares that contribute to progressive organ damage and increased morbidity. While current therapies primarily target active disease, strategies for preventing flares in patients with low-grade activity remain an unmet need.

Over the past decade, novel SLE treatments, targeting B-cell (eg, belimumab) and type I interferon pathways, have demonstrated significant success in reducing lupus disease activity as gauged by SRI-4 or BILAG-based Composite Lupus Assessment [17] in patients with active SLE [18–22]. These advances have shifted the treatment paradigm towards a ‘treat-to-target’ approach, aiming for remission (clinical SLEDAI = 0) or LDA (SLEDAI ≤ 4) with a daily prednisone dose of less than 5 mg [23]. Consistent with the recently proposed definition of disease modification in SLE, ideal therapeutic strategies should fulfil several criteria, including reduction of disease activities

Table 2

Adverse events in 2 arms

	Belimumab (n = 116)	Placebo (n = 115)
Adverse event	71 (61.2%)	73 (64.3%)
Severe adverse event	4 (3.4%)	6 (5.2%)
Discontinuations due to adverse events	3 (2.6%)	6 (5.2%)
Infections	45 (38.8%)	41 (35.7%)
Upper respiratory infections	29 (25.0%)	25 (21.7%)
Skin infections	3 (2.6%)	3 (2.6%)
Gastroenteritis	7 (6.0%)	8 (7.0%)
Urogenital tract infection	6 (5.2%)	7 (6.1%)
Gingivitis	2 (1.7%)	1 (0.9%)
Pneumonia	3 (2.6%)	2 (1.7%)
Admission to hospital due to infections	3 (2.6%)	2 (1.7%)
Headache	3 (2.6%)	1 (0.9%)
Hypertension	2 (1.7%)	2 (1.7%)
Abdominal discomfort	3 (2.6%)	4 (3.5%)
Infusion reactions	3 (2.6%)	4 (3.5%)
Rash	10 (8.6%)	8 (7.0%)
Arthralgia	6 (5.2%)	8 (7.0%)
Elevated liver enzyme	3 (2.6%)	4 (3.5%)
Leukopenia	5 (4.3%)	4 (3.5%)
Osteonecrosis	2 (1.7%)	0 (0%)

(preferably achieving remission or LDA), prevention of disease flares, and tapering of steroid and other immunosuppressive agents—all of which may ultimately mitigate organ damage and improve long-term prognosis [24]. Nevertheless, studies specifically designed to prevent lupus flares are scarce and few trials designated flare rate as a primary endpoint.

Belimumab, an anti-BLyS monoclonal antibody, was the first Food and Drug Administration-approved biologic agent for the treatment of active lupus. Both randomised trials and real-world evidence have consistently demonstrated its efficacy in reducing disease activities in active lupus population [5–7]. However, in clinical practice, the majority of patients with SLE exhibit low-grade disease activity, yet still experience a substantial annual flare rate, as shown in our previous randomised trials and cohort studies [3,4,9,16]. Many of these patients, even in the absence of overt systemic manifestations, continue to display serological activity. Conventional therapies often fall short in effectively preventing flares and maintaining disease quiescence in this large subgroup. Given that the 10 mg/kg dose of belimumab is approved for patients with active disease, we hypothesised that belimumab, the first biological agent approved for SLE, might also benefit patients with low-grade activity. Supporting this notion, a 1 mg/kg dose of belimumab was effective in the BLISS-52 trial in terms of SRI-4 response and flare prevention [6]. Although BLISS-76 did not meet its primary SRI-4 endpoint, the 1 mg/kg dose still contributed to reductions in SLEDAI scores, glucocorticoid use, and disease incidence [5]. A post-hoc analysis further suggested that 1 mg/kg belimumab could help attain LLDA compared with placebo [1]. In the present trial, we used a fixed dose of 120 mg belimumab, approximately equivalent to 2 mg/kg for Chinese patients with lupus, to minimise drug wastage.

This rigorously designed multicentre RCT provides for the first time that low-dose belimumab (120 mg) significantly reduces the risk of disease flares in patients with SLE with LDA (SELENA-SLEDAI ≤ 6), supporting its potential role as a flare prevention and multifaceted disease-modifying agent. The study met its primary endpoint, with a significantly lower flare rate in the belimumab group compared to placebo (7.8% vs 19.1%; $P = .012$). This corresponds to a 66% relative risk reduction, consistent with evidence linking elevated BLyS levels to flare susceptibility and extends prior findings on belimumab's efficacy in active SLE [10]. Additional benefits observed in the belimumab group included a modest improvement in SELENA-SLEDAI scores (change from baseline: -0.66 ± 2.14 vs $+0.12 \pm 2.80$; $P = .009$) and a trend towards greater steroid-sparing (change in prednisone: -1.55 ± 10.11 mg/d vs -0.91 ± 7.43 mg/d; $P = .075$). These results validate the rationale of using lower belimumab doses for flare prevention in patients with low-grade activity – a strategy with potential cost and safety advantages. Immunopharmacological effects were further supported by a higher rate of anti-dsDNA seroconversion (20.6% vs 6.5%; $P = .034$) and a significant reduction in IgG levels (-0.72 g/L vs $+0.94$ g/L in placebo; $P < .001$) in the belimumab group. Previous post-hoc analyses indicated that serologically active patients respond better to the 10 mg/kg dose. Further investigation of biomarkers to predict the responses to low-dose belimumab in lupus patients with low-grade disease activity is also warranted. Last but not least, the safety profile of low-dose belimumab was favourable and comparable to placebo, with no new safety signals emerging.

The study has several limitations. First and foremost, the trial was terminated prematurely due to the COVID-19 lockdown in Shanghai, reducing the planned enrolment from 334 to 231

patients. Nevertheless, an ad-hoc power analysis confirmed that the study retained 90% power to detect a 10% absolute risk reduction in 6-month flare rate. In parallel, the relatively short follow-up (mean 31 weeks) precluded assessment of long-term outcomes, such as damage accrual (SLICC-SDI (SLICC/ACR Damage Index)) [25], or medium-term outcomes, such as steroid-free status. Lack of a standardised tapering protocol for glucocorticoid and immunosuppressants in this trial also contributed to this limitation. Furthermore, recruitment was limited to centres in Shanghai, which may affect the generalisability of our findings. Finally, gender imbalance (more females in the belimumab arm) occurred, though sensitivity analysis with adjustment upheld the primary result.

In conclusion, this trial provides the first RCT evidence that low-dose belimumab reduces flares in patients with SLE with low-grade disease activity. By repurposing an approved therapy at a lower dose, we offer a practical strategy to prevent lupus flares. Future studies should confirm these findings in larger multiethnic cohorts with longer follow-up.

CRediT authorship contribution statement

Fangfang Sun: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Huijing Wang:** Formal analysis, Data curation. **Danting Zhang:** Methodology, Investigation, Formal analysis. **Nan Shen:** Supervision. **Sheng Chen:** Supervision. **Ting Li:** Supervision. **Weiguo Wan:** Supervision. **Sheng-Ming Dai:** Supervision. **Shuang Ye:** Supervision, Conceptualization.

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Competing interests

All authors declare they have no competing interests.

Patient consent for publication

Not applicable.

Ethics approval

The study was approved by the Ethic Committee of all participating centers.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Deidentified participants' data will be available upon reasonable request to the corresponding author.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.10.010.

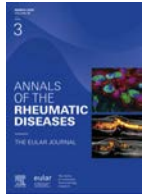
Orcid

Fangfang Sun: <http://orcid.org/0000-0003-2570-4576>

Shuang Ye: <http://orcid.org/0000-0002-5257-5768>

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Antiphospholipid syndrome

Clinical and biologic predictors of thrombosis in persistently antiphospholipid antibody-positive patients: Prospective analysis of the International APS ACTION Clinical Database and Repository (‘Registry’)

Jonathan Thaler¹, Michael Parides¹, Danieli Castro Oliveira de Andrade², Diana Paredes Ruiz³, Maria G. Tektonidou⁴, Vittorio Pengo⁵, Savino Sciascia⁶, Cecilia Nalli⁷, Guilherme de Jesús⁸, Paul R. Fortin⁹, Maria Efthymiou¹⁰, H. Michael Belmont¹¹, Michelle Petri¹², Ricard Cervera¹³, Leslie Skeith¹⁴, Tatsuya Atsumi¹⁵, Chary López-Pedrerá¹⁶, Yu Zuo¹⁷, D. Ware Branch¹⁸, Rohan Willis¹⁹, Nina Kello²⁰, Zhuoli Zhang²¹, Esther Rodriguez Almaraz²², Bahar Artim-Esen²³, Jose Pardos-Gea²⁴, Guillermo Pons-Estel²⁵, Giulia Pazzola²⁶, Hui Shi²⁷, Ali Duarte-Garcia²⁸, Medha Barbhuiya¹, Cecile Yelnik²⁹, Pierluigi Meroni³⁰, Robert Roubey³¹, Maria Laura Bertolaccini³², Hannah Cohen³³, Jacob H. Rand³⁴, Doruk Erkan^{1,*}

¹ Hospital for Special Surgery, New York, New York, USA

² University of Sao Paulo, Sao Paulo, Brazil

³ Biobizkaia Health Research Institute, Barakaldo, Spain

⁴ National and Kapodistrian University of Athens, Athens, Greece

⁵ Padova University Hospital, Padova, Italy

⁶ ASL Città Di Torino and University of Turin, Turin, Italy

⁷ University of Brescia, Brescia, Italy

⁸ Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

⁹ CHU de Quebec-Universite Laval, Quebec, QC, Canada

¹⁰ University College London, London, UK

¹¹ New York University Langone Medical Center, New York, New York, USA

¹² Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

¹³ Hospital Clinic Barcelona, Barcelona, Catalonia, Spain

¹⁴ University of Calgary, Calgary, Alberta, Canada

¹⁵ Hokkaido University Hospital, Sapporo, Japan

¹⁶ IMIBIC/Reina Sofia Hospital, University of Cordoba, Cordoba, Spain

¹⁷ University of Michigan, Ann Arbor, Michigan, USA

¹⁸ University of Utah and Intermountain Healthcare, Salt Lake City, Utah, USA

¹⁹ University of Texas Medical Branch, Galveston, Texas, USA

²⁰ Northwell Health, Great Neck, New York, USA

²¹ Peking University First Hospital, Beijing, China

*Correspondence to Dr. Doruk Erkan, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021.

E-mail address: erkand@hss.edu (D. Erkan).

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²² Hospital Universitario 12 de Octubre, Madrid, Spain²³ Istanbul University School of Medicine, Istanbul, Turkey²⁴ Vall d'Hebron University Hospital Barcelona, Spain²⁵ Centro Regional de Enfermedades Autoinmunes y Reumaticas del Grupo Orono (GO-CREAR), Rosario, Argentina²⁶ Rheumatology Unit, Azienda USL IRCCS di Reggio Emilia, Reggio Emilia, Italy²⁷ Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China²⁸ Mayo Clinic, Rochester, Minnesota, USA²⁹ Lille University, Lille, France³⁰ Department of Clinical Science and Community Health, University of Milan, Milan, Italy³¹ University of North Carolina, Chapel Hill, North Carolina, USA³² King's College London, London, UK³³ University College London Hospitals NHS Foundation Trust, London, UK³⁴ Weill Cornell Medical Center, New York, New York, USA

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ABSTRACT

Objectives: There is a lack of high-quality data to inform risk-stratified long-term thrombosis prevention strategies in patients with persistently positive antiphospholipid antibodies (aPL). We aimed to determine independent clinical and biologic predictors of thrombosis among persistently aPL-positive patients.

Methods: Patients positive for aPL according to the Revised Sapporo Classification Criteria are eligible for inclusion in the Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) Registry. Registrants with at least 1 year of follow-up were included in this study. We fit Cox proportional hazards models to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) for independent predictors of thrombosis.

Results: In unadjusted analyses, based on 1067 patients with a mean follow-up of 4.43 years (4,727 person-years), history of thrombosis, hematologic disease (autoimmune haemolytic anaemia and/or thrombocytopenia), microvascular disease, obesity, renal disease, sedentary lifestyle, baseline anticoagulant use, and family history of early cardiovascular disease occurred more frequently ($P < .05$) among patients with new thrombosis ($n = 93$) than among those without new thrombosis ($n = 974$). After adjustment, independent predictors of new thrombosis were history of thrombosis (HR 2.34, 95% CI 1.14 to 4.81, $P = .02$) and hematologic disease (HR 1.95, 95% CI 1.19 to 3.18, $P = .01$); there was a trend for history of microvascular disease ($P = .06$) and obesity ($P = .08$).

Conclusions: In this prospective analysis, history of thrombosis and hematologic disease each conferred an approximately twofold increased risk of new thrombosis in persistently aPL-positive patients. These findings can guide future clinical trial designs and inform patient management decisions.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Among patients with persistently positive antiphospholipid antibodies (aPL), a history of thrombosis is widely regarded to confer risk for future thrombosis. Independent predictors of thrombosis are otherwise largely unknown.

WHAT THIS STUDY ADDS

- This is the first large-scale, international, prospective, time-to-event analysis of independent predictors of thrombosis among persistently aPL-positive patients. Our findings show that a history of hematologic disease (defined as autoimmune haemolytic anaemia and/or otherwise unexplained thrombocytopenia with platelet nadir $< 100,000/\mu\text{L}$) and a history of thrombosis each confer an approximately twofold increased risk of thrombosis.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- These results may impact shared decision making discussions among persistently aPL-positive patients and providers regarding primary and secondary thrombosis prevention and may help guide future clinical trial designs.

INTRODUCTION

Antiphospholipid antibodies (aPL) target phospholipid-binding plasma proteins and increase the risk of thrombosis [1]. Commonly used tests to detect aPL are anticardiolipin (aCL) IgG/M/A antibodies, anti- β_2 -glycoprotein-I ($\alpha\beta_2\text{GPI}$) IgG/M/A antibodies, and the lupus anticoagulant (LA) test; one or more persistently positive aPL test is necessary but not sufficient to diagnose antiphospholipid syndrome (APS), which is a systemic autoimmune disorder that can occur in isolation or in the setting of another systemic autoimmune disorder such as systemic lupus erythematosus (SLE) [1].

Long-term thrombosis prevention strategies in aPL-positive patients are still debated. Those with persistent aPL-positivity and no history of thrombosis occasionally receive long-term low-dose aspirin for primary thrombosis prevention, despite the lack of support from prospective studies or randomized clinical trials [2]. Those with both persistent aPL-positivity and a history of thrombosis are typically considered to have APS and are generally treated with anticoagulation for secondary thrombosis prevention; however, there are no risk-stratified studies to guide the duration (short- vs long-term) of anticoagulation among patients

with APS [3]. Overall, there is a major need in APS research for well-designed large-scale prospective studies that investigate the role of risk factors for thrombosis among aPL-positive patients to improve thrombotic risk stratification—and ultimately inform management decisions—among these patients [3].

Antiphospholipid Syndrome Alliance For Clinical Trials and International Networking (APS ACTION) is an international research network created to further our understanding of APS through large scale, multicentre studies [4]. One of the specific aims of this collaborative effort is to better define the natural history of persistently aPL-positive patients. To this end, an international prospective registry was created in 2012 enrolling persistently aPL-positive patients (with or without aPL-related clinical events) as defined by the Revised Sapporo Classification Criteria [1]; all patients must have been tested for aPL within one year prior to enrolment [5].

The APS ACTION registry data have been the subject of several descriptive analyses of thrombosis risk since 2012 [6–11]. As of 2023, based on approximately 4500 patient-years of follow-up, risks of first and recurrent thrombosis were 1.00 and 2.13 per 100 patient-years, respectively. Obesity, renal failure, and direct oral anticoagulant use were more common in APS patients with recurrent thrombosis than in those without; smoking history was more common in patients with first thrombosis than in those without [6]. Interpretation of those associations, however, was limited by the likely influence of confounding and multiplicity.

The aim of this study was to prospectively identify independent demographic, clinical, and laboratory risk factors for thrombosis among persistently aPL-positive patients enrolled in the APS ACTION registry by conducting a multivariable time-to-event analysis using Cox proportional hazards modelling.

METHODS

Patients between 18 and 75 years old who are positive for aPL according to the Revised Sapporo Classification Criteria (last tested within 1 year prior to enrolment) are eligible for inclusion in the APS ACTION registry [1,5]. Patients both with and without a history of thrombosis, eg, obstetric APS or asymptomatic aPL positivity (eg, identified as part of the workup for lupus or an elevated activated partial thromboplastin time [aPTT]), are eligible for inclusion. Informed consent is obtained at the time of registry enrolment. Data are collected at enrolment and every 12 ± 3 months via the web-based application REDCap and include demographics, aPL-related clinical characteristics (vascular and obstetric events; microvascular disease defined as livedoid vasculopathy, pulmonary haemorrhage, adrenal haemorrhage, myocardial disease, and/or aPL nephropathy; haematologic disease defined as persistent anaemia with haemolysis and a positive direct antiglobulin test [ie, autoimmune haemolytic anaemia], and/or otherwise unexplained thrombocytopenia with platelets <100,000/μL; other autoimmune disease such as SLE; aPL-related medications including use of anticoagulants, antiplatelet agents, and immunomodulatory medications; and cardiovascular disease (CVD) risk factors including hypertension, obesity, hyperlipidaemia, renal disease [glomerular filtration rate < 60 mL/min/1.73 m² or nephrotic syndrome], smoking, diabetes, sedentary lifestyle [less than 30 minutes of exercise per day of any intensity level], family history of early CVD [myocardial infarction in a first-degree relative before age 55 in men and 65 in women]), and laboratory values (aPL profile, with aCL and aβ₂GPI positivity defined based on a threshold of ≥ 40 ELISA units and LA positivity defined based on the International Society

on Thrombosis and Haemostasis [ISTH] 2020 guidelines [12]; SLE-related autoantibodies; and complement levels). All data collected and registrant baseline characteristics have been well described [5]. At the time of any new thrombosis during the prospective follow-up, the concomitant CVD and venous thromboembolism risk factor data are also collected.

Given the known tendency of aPL test results to fluctuate over time, patients with one or more negative results for a given aPL test (eg, aβ₂GPI IgG < 40 ELISA units) prior to registry entry were still considered ‘positive’ for that test at baseline if both of the following criteria were met: (a) there were 2 positive results checked at least 12 weeks apart and (b) the most recent result was positive.

APS ACTION registrants with at least 1 year of follow-up were included in this prospective cohort analysis; patients with no or less than 1 year of follow-up were excluded. The outcome of thrombosis was ascertained prospectively based on patient report and confirmed with chart review as well as the results of appropriate imaging studies when available. Thrombotic events included macrovascular arterial events (eg, myocardial infarction and cerebrovascular accident), macrovascular venous events (eg, venous thromboembolism and hepatic vein thrombosis), and catastrophic APS (CAPS). New onset microvascular disease did not qualify as a thrombotic event for the purposes of this analysis.

For the purpose of calculating the incidence rate of new thrombosis (ie, thrombosis during prospective follow-up), we divided the cohort into those with versus without a history of thrombosis at the time of registry entry. As has been done in prior interim analyses, a separate thrombosis incidence rate was calculated for each of these groups given they are treated as distinct populations in clinical practice (ie, thrombotic APS versus nonthrombotic APS or aPL positive only) [6–11]. For the purposes of the rest of the analysis, including multivariable modelling, the entire cohort was analysed together regardless of thrombosis history as our event number was relatively small and power therefore limited. We compared the baseline characteristics of those with versus without new thrombosis. Student *t* test or Wilcoxon rank sum tests were used to compare continuous variables, whereas chi-squared or Fisher exact tests were used to compare categorical variables. *P* values < .05 were considered significant. We then conducted a time-to-event analysis, with parameters as follows: (a) time origin: registry enrolment; (b) target event: thrombosis; (c) censor if: death unrelated to thrombosis, withdrawal from study, or loss to follow-up; and (d) follow-up endpoint: most recent study follow-up appointment.

We fit Cox proportional hazards regression models to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) for predictors of thrombosis among the entire cohort. Covariates included in multivariable models included a prespecified set of candidate risk factors (age, gender, anticoagulant use, antiplatelet use, systemic autoimmune disease [aside from APS], and aPL profile) along with those found to be significant in univariate analysis. We generated Kaplan–Meier plots for variables found to be significantly independently associated with thrombosis. Joint hot deck imputation was performed on missing covariate data. All analyses were performed using SAS version 9.4 (SAS Institute, Cary NC).

Patient and public involvement statement

Patients and the public were not directly involved in the design, conduct, reporting, or dissemination plans of this research.

RESULTS

As of May 2024, 1196 persistently aPL-positive patients with or without APS by Revised Sapporo APS classification criteria were enrolled, of whom 1067 (89%) had at least 1 year of follow-up. Forty-five patients died during follow-up (11 fatal thromboses and 34 nonthrombosis deaths) and 135 patients were lost-to-follow-up or withdrew from the study. Comparing the baseline demographic and clinical characteristics of those who were lost to follow-up, withdrew, or died from causes other than thrombosis to those of the rest of the cohort demonstrated that renal disease, sedentary lifestyle, aCL IgM positivity, and a β_2 GPI IgM positivity were more common in the former; hydroxychloroquine use was less common (Supplementary Table S1). All other measured characteristics were similar between these 2 groups.

Mean follow-up (enrolment to first new thrombosis or most recent follow-up) was 4.43 years (4,727 person-years [p-y]) overall, 4.36 years (1890 p-y) among those without a history of thrombosis at enrolment ($n = 381$), and 4.46 years (3405 p-y) among those with a history of thrombosis at enrolment ($n = 686$) (Table 1).

Based on 18 first new events among patients without a history of thrombosis and 75 first new events among patients with a history of thrombosis, new thrombosis incidence was 0.95 per 100 p-y among patients without a history of thrombosis and 2.20 per 100 p-y among patients with a history of thrombosis. These 93 new events, ie, endpoint-defining events in the survival analysis, included 52 arterial, 39 venous, 1 CAPS, and 1 macrovascular thrombosis not otherwise specified. Of note, 19 of these 93 patients (20%) experienced at least 1 additional new thrombosis (15 arterial and 12 venous) during follow-up which did not contribute to the survival analysis.

In unadjusted analyses, history of thrombosis, haematologic disease, and microvascular disease, as well as baseline obesity, renal disease, sedentary lifestyle, anticoagulation status, and family history of early cardiovascular disease were all more common ($P < .05$) among patients with new thrombosis ($n = 93$) than among those without new thrombosis ($n = 974$) (Table 1).

After multivariable adjustment, independent predictors of first new thrombosis included history of thrombosis (HR = 2.34, 95% CI 1.14 to 4.81, $P = 0.02$; Fig 1) and haematologic disease (HR = 1.95, 95% CI 1.19 to 3.18, $P = .01$; Fig 2); there was a trend for history of microvascular disease (HR = 1.74; 95% CI 0.98 to 3.10, $P = .06$) and obesity (HR = 1.54, 95% CI 0.96 to 2.48, $P = .08$) (Table 2).

In a subgroup analysis of patients with a history of haematologic disease, univariate analysis demonstrated a significant association between thrombocytopenia and thrombosis ($P = .005$) but not between autoimmune haemolytic anaemia and thrombosis ($P = .85$) (Table 1). A multivariable model including thrombocytopenia and autoimmune haemolytic anaemia (in place of the broader category of haematologic disease) and controlling for the same covariates as in Table 2 yielded similar results to univariate analysis (thrombocytopenia: HR = 0.52, 95% CI 0.33 to 0.81, $P = .004$; autoimmune haemolytic anaemia: HR = 0.98, 95% CI 0.40 to 2.43, $P = .97$).

To address whether the severity of thrombocytopenia may correlate with the risk of thrombosis, we completed an ad hoc analysis of the risk of thrombosis among those with a baseline platelet count less than the commonly accepted lower limit of normal (150,000/ μ L), stratifying according to the following baseline platelet count categories: 0 to 49,000/ μ L, 50,000 to

99,000/ μ L, and 100,000 to 149,000/ μ L. We also examined the relationship between risk of thrombosis and platelet count with the latter treated as a continuous variable. As is shown in supplementary Tables 2 and 3, we found no statistically significant association of thrombocytopenia severity with thrombosis risk, with the caveat that these ad hoc analyses were uncontrolled (ie, univariate).

DISCUSSION

In this prospective time-to-event analysis of persistently aPL-positive individuals, history of thrombosis and history of haematologic disease (defined as persistent autoimmune haemolytic anaemia or otherwise unexplained thrombocytopenia with platelet nadir $< 100,000/\mu$ L) were each found to confer an approximate 2-fold increased risk of thrombosis, independent of age, gender, anticoagulation and antiplatelet medication use, concomitant autoimmune diseases, and several cardiovascular disease risk factors.

History of thrombosis is already widely regarded to be a significant risk factor for future thrombosis, not only among aPL-positive individuals but also in the general population, as demonstrated both in the literature and by the widespread use of long-term therapeutic anticoagulation among many such patients (including among those with persistently positive aPL who are considered to have thrombotic APS) [13–17]. However, the finding that aPL-associated haematologic disease confers increased risk for thrombosis among aPL-positive patients is novel. Based on our results, this increased risk is similar to that conferred by a history of thrombosis.

Both autoimmune haemolytic anaemia and immune thrombocytopenia increase thrombotic risk among the general population [18–22]; as such, our findings are not entirely surprising. Nevertheless, the likely increased baseline risk of thrombosis in persistently aPL-positive patients may translate to a larger impact of hematologic disease on the absolute risk of thrombosis among these patients than in the general population. The fact that the hazard ratio for history of hematologic disease was similar to that for history of thrombosis does suggest the possibility that a history of aPL-associated hematologic disease may warrant a more aggressive approach to thrombosis prevention (as history of thrombosis does). However, we believe that further prospective data are necessary to establish the exact nature and magnitude of the relationship between hematologic disease and thrombosis among aPL-positive patients in order to optimally inform management decisions regarding interventions such as therapeutic anticoagulation or immunomodulation for primary thrombosis prevention. Questions about management decisions must additionally take into account the fairly low absolute risk of thrombosis in aPL-positive patients, which has been demonstrated in our prospective cohort both in this and prior analyses [6–11].

Given the relatively small event number and therefore limitations on number of covariates included in our models [23,24], we defined hematologic disease *a priori* to include either autoimmune haemolytic anaemia or otherwise unexplained thrombocytopenia. One could ask whether increased risk of thrombosis conferred by hematologic disease is driven by thrombocytopenia, autoimmune haemolytic anaemia, or both, ie, grouping them together may introduce confounding. Our exploratory analyses, both univariate and multivariate, suggested that thrombocytopenia is more likely to be independently associated with thrombosis than autoimmune haemolytic anaemia, although there were far more patients in our cohort with

Table 1

Baseline characteristics of 1067 aPL-positive individuals from the APS ACTION registry with or without new thrombosis during follow-up

	No thrombosis during follow-up n = 974	Thrombosis during follow-up n = 93	P value
Demographics			
Mean age $\pm$ SD (years)	46.5 $\pm$ 13.4	43.8 $\pm$ 14.6	.11
Female	724 (74%)	65 (70%)	.34
Race			.73
White	676 (69%)	62 (67%)	
Other	227 (23%)	25 (27%)	
Unknown	71 (7%)	6 (7%)	
Concomitant systemic autoimmune disease	462 (47%)	40 (43%)	.41
Systemic lupus erythematosus	321 (33%)	28 (30%)	.58
Other ^a	141 (15%)	12 (13%)	.68
APS-related past medical history			
<i>Macrovascular thrombosis</i>	<i>611 (63%)</i>	<i>75 (81%)</i>	< .01
<i>Microvascular disease</i> ^b	<i>84 (9%)</i>	<i>17 (18%)</i>	< .01
<i>Hematologic disease</i> ^c	<i>205 (21%)</i>	<i>30 (32%)</i>	.01
Autoimmune Haemolytic Anaemia	57 (6%)	5 (5%)	.85
Thrombocytopenia	168 (17%)	27 (29%)	< .01
Pregnancy morbidity ^d	193 (20%)	21 (23%)	.53
Cardiac valve disease ^e	89 (9%)	12 (13%)	.24
aPL Tests			
Lupus anticoagulant (LA) positive	754 (79%)	76 (82%)	.52
aCL ^f IgG $\geq$ 40 ELISA units	410 (44%)	47 (52%)	.12
aCL IgM $\geq$ 40 ELISA units	180 (21%)	12 (14%)	.14
a β_2 GPI ^g IgG $\geq$ 40 ELISA units	343 (38%)	36 (41%)	.49
a β_2 GPI IgM $\geq$ 40 ELISA units	162 (20%)	13 (16%)	.43
Mean duration of aPL positivity $\pm$ SD (years) ^h	5.8 $\pm$ 5.6	6.2 $\pm$ 5.4	.27
aPL profiles			.64
Triple aPL positive	280 (34%)	28 (35%)	
LA positive but not triple positive	420 (51%)	42 (52%)	
LA negative with a β_2 GPI IgG and/or aCL IgG	82 (10%)	9 (11%)	
LA negative with a β_2 GPI IgM and/or aCL IgM (no IgG)	49 (6%)	2 (3%)	
Anticoagulants (current use)			.01
<i>Warfarin or other vitamin K antagonist</i> [*]	<i>505 (52%)</i>	<i>57 (61%)</i>	
<i>Low-molecular-weight heparin</i>	<i>41 (4%)</i>	<i>4 (4%)</i>	
<i>Direct oral factor Xa inhibitor</i>	<i>25 (3%)</i>	<i>7 (8%)</i>	
<i>No anticoagulation</i>	<i>403 (41%)</i>	<i>25 (27%)</i>	
Other medications (current use)			
Antiplatelet (eg, aspirin, clopidogrel)	485 (50%)	37 (40%)	.07
Hydroxychloroquine	456 (47%)	44 (47%)	.93
Statin	242 (25%)	22 (24%)	.8
Cardiovascular disease (CVD) risk factors			
Hypertension on medication	312 (32%)	32 (34%)	.64
Diabetes on medication	58 (6%)	8 (9%)	.31
Hyperlipidaemia on medication	243 (25%)	20 (22%)	.46
Family history of early CVD ⁱ	115 (12%)	19 (20%)	.02
Obesity ^j	242 (25%)	34 (37%)	.01
Chronic kidney disease ^k or nephrotic syndrome	42 (4%)	9 (10%)	.02
Smoking history (any)	338 (35%)	34 (37%)	.72
Sedentary lifestyle ^l	354 (37%)	46 (50%)	.01
Number of CVD risk factors			.22
0	290 (30%)	20 (22%)	
1	290 (30%)	26 (28%)	
2	205 (21%)	23 (25%)	
3 or more	189 (19%)	24 (26%)	

^a Includes rheumatoid arthritis, Sjogren syndrome, systemic sclerosis, inflammatory muscle disease, vasculitis, spondylarthritis, juvenile idiopathic arthritis, sarcoidosis, polymyalgia rheumatica, and/or 'SLE-like' disease meeting 3 out of eleven 1997 ACR SLE Classification Criteria.

^b Based on the 2023 ACR/EULAR APS Classification Criteria (CC) microvascular domain items; largely livedoid vasculopathy, pulmonary haemorrhage, and/or biopsy-proven aPL-associated nephropathy.

^c Persistent anaemia with haemolysis and a positive direct antiglobulin test (ie, autoimmune haemolytic anaemia) and/or otherwise unexplained thrombocytopenia with platelets < 100,000/ μ L.

^d Based on the Revised Sapporo APS CC pregnancy morbidity definitions.

^e Based on the Revised Sapporo APS CC Committee Definitions.

^f Anticardiolipin antibody.

^g Anti- β_2 -glycoprotein-I antibody.

^h Time from the first positive aPL test (determined by site-specific investigators) to enrolment.

ⁱ Myocardial infarction in a first-degree relative before age 55 in men or age 65 in women.

^j BMI $\geq$ 30 kg/m².

^k GFR < 60 mL/min/1.73 m².

^l Less than 30 minutes of daily exercise (any intensity).

^{*} Among patients taking warfarin at baseline who experienced thromboses during follow up (n = 57) and in whom prospective INR data was available (n = 41), 15 (34%) had an INR < 2.0 based on the last INR recorded prior to the thrombotic event, and warfarin was on hold for an additional 3 patients (5%) due to a recent bleed.

ACR, American College of Rheumatology; aPL, antiphospholipid antibody; APS, antiphospholipid syndrome; BMI, body mass index; CC, classification criteria; EULAR, European Alliance of Associations for Rheumatology; GFR, glomerular filtration rate; INR, international normalised ratio; SLE, systemic lupus erythematosus.

Bolded and italicized p values demonstrate statistical significance.

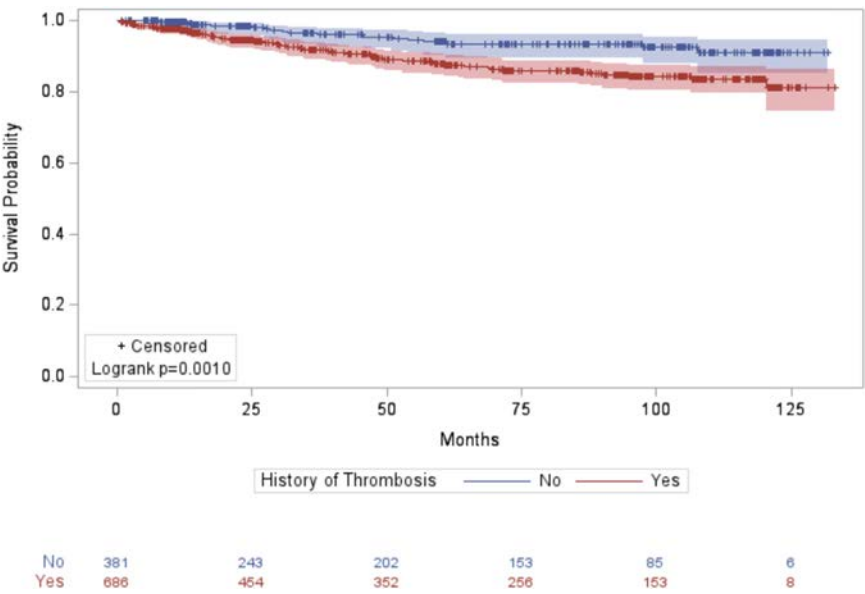


Figure 1. Thrombosis-free survival by history of thrombosis in 1067 aPL-positive individuals from the APS ACTION registry. aPL, antiphospholipid antibodies; APS ACTION, Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking.

thrombocytopenia ($n = 195$) than with autoimmune haemolytic anaemia ($n = 62$), thus limiting our ability to examine the latter as a risk factor for thrombosis. There was no trend towards significance in the association of autoimmune haemolytic anaemia with thrombosis; the percentage of patients with autoimmune haemolytic anaemia was nearly identical among those with vs without new thrombosis (5.4% vs 5.9%, respectively).

Given the fundamental role of platelets in clotting (ie, as pro-coagulant factors), one might find it paradoxical that a low platelet count would independently associate with an increased risk for thrombosis. In fact, prior data support this paradoxical notion, including work by Camera et al [25] demonstrating an activated platelet phenotype in APS marked by tissue factor-positive platelet leukoaggregates; activated platelets (ie, in the aggregates) could be ‘sequestered’ with a reduction of the circulating (ie, measured) number, but at the same time may be responsible for an increased thrombophilic phenotype [26].

We found that both a history of aPL-associated microvascular disease and obesity trended towards significance as independent risk factors for thrombosis. Existing data support the hypothesis that aPL-associated microvascular disease in particular may be a marker for an ongoing thromboinflammatory state and therefore could confer significant risk for thrombosis. A recent APS

ACTION registry analysis of a small number of patients showed that the thromboinflammatory protein signature shared by all APS subtypes (characterised by enrichment of coagulation, complement, innate immune, and adaptive immune pathways) is particularly pronounced in those with a microvascular phenotype [27]. Similarly, Erkan et al [28] found disproportionately elevated levels of cell-bound complement activation products in those with aPL-associated microvascular disease compared to those with other aPL/APS subtypes (eg, aPL positivity without associated clinical manifestations). Future larger-scale analyses of APS ACTION registry may further elucidate the extent to which microvascular disease predicts future thrombosis.

Regarding aPL profile, LA positivity, double aPL-positivity, triple aPL-positivity, and IgG isotype (in cases of aCL and/or $\alpha\beta_2$ GPI positivity) are considered to confer an especially high thrombosis risk [29,30]. These findings were not replicated in our analysis, in which aPL profile was not associated with risk of thrombosis. There are a few reasons why we may have failed to detect such an association, if it exists. First, unlike most of the baseline and follow-up data, aPL profile was ascertained retrospectively based on a combination of patient report and chart review at the time of enrolment (local centre-reported data). Some but not all patients had available core laboratory baseline

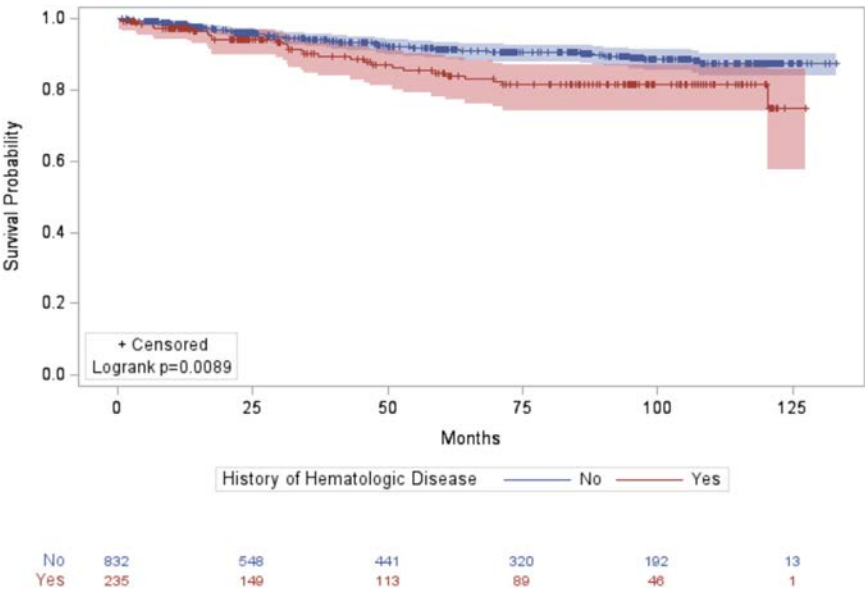


Figure 2. Thrombosis-free survival by history of hematologic disease in 1067 aPL-positive individuals from the APS ACTION registry. aPL, antiphospholipid antibodies; APS ACTION, Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking.

Table 2
Predictors of thrombosis in 1067 aPL-positive individuals from the APS ACTION registry

	Hazard Ratio (95% CI)	P value
Age	0.99 (0.97–1.00)	.11
Male	1.16 (0.71–1.89)	.56
Anticoagulant use ^a	0.94 (0.48–1.84)	.85
Antiplatelet use	0.81 (0.49–1.34)	.42
Family history of early cardiovascular disease ^b	1.41 (0.78–2.56)	.26
Chronic kidney disease ^c or nephrotic syndrome	1.83 (0.83–4.05)	.14
Obesity ^d	1.54 (0.96–2.48)	.08
Sedentary lifestyle ^e	1.13 (0.71–1.78)	.61
Systemic autoimmune disease	0.84 (0.53–1.34)	.47
Macrovascular thrombosis	2.34 (1.14–4.81)	.02
Microvascular disease ^f	1.74 (0.98–3.10)	.06
Haematologic disease ^g	1.95 (1.19–3.18)	.01
aPL profile		.43
Triple aPL positive	1.43 (0.33–6.23)	
LA ^h positive but not triple positive	1.38 (0.33–5.88)	
LA negative with a β 2GPI ⁱ IgG and/or aCL ^j	2.47 (0.52–11.62)	
IgG		
LA negative with a β 2GPI IgM and/or aCL IgM (no IgG)	1 (referent group)	

^a Binary variable (yes/no); results were not substantively different when individual anticoagulants were considered (as in Table 1).

^{b–j} See Table 1 for definitions.

a β 2GPI, anti- β 2-glycoprotein-I; aCL, anticardiolipin antibody; aPL, antiphospholipid antibody; APS, antiphospholipid syndrome; CI, confidence interval; IgG, immunoglobulin G; IgM, immunoglobulin M; LA, lupus anticoagulant.

Bolded and italicized p values demonstrate statistical significance.

aPL data, limiting our ability to repeat the analysis using core laboratory data. Additionally, very few registrants were negative for LA, limiting our ability to draw conclusions about LA-negative patients or to determine the extent of the association between LA positivity and thrombosis. Increased availability of APS ACTION core laboratory data in the future may allow for more robust analysis of the relationship between aPL profile and thrombosis risk.

We chose to define our primary end point as macrovascular thrombosis; for several reasons (delineated below), new onset microvascular disease did not constitute thrombosis for the purposes of this analysis. First, existing data suggest that microvascular APS seems to represent a distinct clinical and mechanistic phenotype; and traditional thrombosis risk factors are thought less likely to play a role in the development of microvascular disease, eg, livedoid vasculopathy [31,32]. Additionally, the ascertainment of microvascular disease presents practical challenges as it is often asymptomatic or can be chronic without a clear ‘event’ to denote. We plan to conduct a separate analysis of those with prospective microvascular events in the future.

Direct oral anticoagulants (DOACs) such as apixaban and rivaroxaban are regarded as likely inferior to warfarin for secondary prevention of thrombosis in APS, especially for triple aPL-positive patients with a history of arterial stroke. Similarly, the use of antiplatelet agents such as clopidogrel or aspirin is not the current standard of care for secondary thrombosis prevention in patients with thrombotic APS [33]. Our registry data demonstrate that, in practice, some APS patients with a history of thrombosis have been treated with DOACs or antiplatelet agents. Although the observational nature of this study limited our ability to establish causality and to control for all potential confounders, the registry represents valuable ‘real-world’ data supporting the fact that there are many variables involved in decisions about

anticoagulation and antiplatelet therapy, many of which are not necessarily related to aPL-positivity.

For the purposes of this analysis, it was not feasible to determine the precise nature and intensity of anticoagulation immediately prior to events in all patients with thrombotic events. In the future, this information could add valuable insight to our findings. We did, however, conduct a descriptive subgroup analysis of available INR values preceding new thrombotic events among patients taking warfarin at baseline who experienced thromboses during follow up (Table 1 footnote); we found subtherapeutic INR preceding thrombosis in approximately one-third of patients. Although these descriptive data should be interpreted cautiously, they do suggest the possibility that subtherapeutic anticoagulation or temporary interruption could have contributed to these breakthrough events and could partly explain why baseline warfarin use did not demonstrate an independent protective effect on thrombosis risk. Collinearity between the anticoagulation variable and prior thrombosis history may also have contributed to this finding in the multivariable model.

To our knowledge, this was the first large-scale, international, prospective, time-to-event analysis of predictors of thrombosis among persistently aPL-positive patients. Key strengths of our study include the prospective ascertainment of covariates and outcomes (thrombosis). Additionally, thrombosis-related deaths were able to be differentiated from nonthrombotic deaths, allowing for inclusion of patients with potentially more severe clinical manifestations in the current analysis. Although generalizability of our results may be limited somewhat by referral bias to centres experienced in APS, the APS ACTION registry includes an overall demographically diverse patient population with respect to sex, race, and geographical region.

Our study has several limitations. First, despite the large size of the registry, the number of patients with at least one thrombosis during follow-up was relatively small, limiting the scope and nature of our analysis. The low event number limited the number of covariates included in our multivariable models and necessitated that those with and without a history of thrombosis at the time of registry entry be combined into a single analysis; confounding effects of covariates that were excluded from our models cannot be ruled out. Second, for patients with early terminations (those who were either lost to follow-up or died), we relied on centre-reported data and systematically collected the reasons for early terminations. Reliance on investigator-reported causes of death introduces the chance of misclassification or underreporting, impacting the accuracy of identified main causes of death; that is, there was no systematic analysis of death certificates given the multicentre international nature of the study. Acknowledging the potential for under- or misreporting of thrombotic events in this setting, we believe that several factors ultimately contribute to overall strong outcome ascertainment, including the prospective nature of the registry, the strict and standardised definition of thrombosis (including the requirement for imaging confirmation), and periodic database monitoring and cleaning (ie, for completeness and accuracy). Third, we noted a few differences in the demographic and clinical characteristics of those who withdrew, were lost-to-follow-up, or died vs the rest of the cohort (including prevalence of renal disease, sedentary lifestyle, aCL IgM positivity, a β 2GPI IgM positivity, and hydroxychloroquine use), introducing the possibility of informative censoring, which could have introduced bias into our calculation of overall thrombosis risk and our survival analysis. However, these differences lose statistical

significance after adjusting for multiplicity (given the number of covariates compared). Fourth, the time origin of our time-to-event analysis (date of enrolment in the registry) could be questioned as it is not a variable with a clear biologic correlate. In other words, enrolment in the registry was not required to coincide with an event such as thrombosis or new positivity of an aPL test which had previously been monitored and negative. We did, however, include ‘disease duration’ (defined as the date of the first recorded aPL-positive test) as a covariate in univariate analysis and in several exploratory multivariable models, and it was not shown to be associated with thrombosis or to meaningfully change our models. Fifth, our analysis was also limited by coding and collection of data as defined at the outset of the APS ACTION registry in 2012. Thus, we were able to analyse many but not all covariates, especially concomitant cardiovascular disease and venous thromboembolism risk factors, in a manner consistent with the most recent 2023 APS classification criteria (for example, results could have differed if thrombocytopenia had been defined based on an upper platelet limit of 130,000/ μL per the 2023 classification criteria rather than 100,000/ μL) [34].

The prospective nature of this analysis is one of its primary strengths. However, the retrospective data collected at baseline (ie, at registry entry) carries the same limitations as in any retrospective analysis. For example, it was not feasible to collect precise, verifiable information on all previous anticoagulant and antiplatelet medications for all enrollees, and as such it was only baseline (ie, ‘current’) anticoagulant and antiplatelet medications that were recorded. Nevertheless, we did account for history of prior thromboses as well as several other covariates such as cardiovascular comorbidities which, one could argue, are more likely to be independently associated with thrombosis than prior medications from a mechanistic standpoint. As a whole, the retrospective baseline data recorded at study entry was verified in the patient’s medical record. Given the variable availability, nature, and extent of documentation in patients’ medical records, we did not prespecify a standard ‘lookback time’ for the purposes of the baseline data collection (eg, including only medical history from less than 10 years prior to registry entry). Therefore, potential bias introduced by differences in electronic medical record keeping among patients cannot be excluded.

The APS ACTION Registry is still active. Longer follow up times (ie, higher event numbers) will be required in order to conduct increasingly sophisticated analyses going forward which we hope to ultimately demonstrate a more complete picture of the risk and protective factors for thrombosis in aPL-positive patients. For example, in the future we plan to analyse recurrent thromboses during follow up (not included in this survival analysis, in which first prospective thrombosis was the primary end point), and to examine variables such as medications (particularly anticoagulant, antiplatelet, and immunomodulatory agents) as time-varying covariates. Analysis of platelet count as a time-varying covariate will allow us to examine thrombocytopenia (and its severity) as a candidate marker of disease activity among aPL-positive patients.

In conclusion, in this first of its kind, large-scale, international prospective analysis, history of thrombosis and hematologic disease each conferred an approximately twofold increased risk of new thrombosis in persistently aPL-positive patients. These findings can guide future clinical trial designs and inform patient management decisions. Future registry analyses using standardised APS ACTION core laboratory aPL results, and with longer follow-up times, time-dependent

variables, and larger numbers of events, will help better define predictors of thrombosis in aPL-positive patients.

CRedit authorship contribution statement

Jonathan Thaler: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Michael Parides:** Methodology, Formal analysis. **Danieli Castro Oliveira de Andrade:** Writing – review & editing, Investigation. **Diana Paredes Ruiz:** Writing – review & editing, Investigation. **Maria G. Tektonidou:** Writing – review & editing, Investigation. **Vittorio Pengo:** Writing – review & editing, Investigation. **Savino Sciascia:** Writing – review & editing, Investigation. **Cecilia Nalli:** Writing – review & editing, Investigation. **Guilherme de Jesús:** Writing – review & editing, Investigation. **Paul R. Fortin:** Writing – review & editing, Investigation. **Maria Efthymiou:** Writing – review & editing, Investigation. **H. Michael Belmont:** Writing – review & editing, Investigation. **Michelle Petri:** Writing – review & editing, Investigation. **Ricard Cervera:** Writing – review & editing, Investigation. **Leslie Skeith:** Writing – review & editing, Investigation. **Tatsuya Atsumi:** Writing – review & editing, Investigation. **Chary López-Pedrerá:** Writing – review & editing, Investigation. **Yu Zuo:** Writing – review & editing, Investigation. **D. Ware Branch:** Writing – review & editing, Investigation. **Rohan Willis:** Writing – review & editing, Investigation. **Nina Kello:** Writing – review & editing, Investigation. **Zhuoli Zhang:** Writing – review & editing, Investigation. **Esther Rodriguez Almaraz:** Writing – review & editing, Investigation. **Bahar Artim-Esen:** Writing – review & editing, Investigation. **Jose Pardos-Gea:** Writing – review & editing, Investigation. **Guillermo Pons-Estel:** Writing – review & editing, Investigation. **Giulia Pazzola:** Writing – review & editing, Investigation. **Hui Shi:** Writing – review & editing, Investigation. **Ali Duarte-Garcia:** Writing – review & editing, Investigation. **Medha Barbhaiya:** Writing – review & editing, Investigation. **Cecile Yelnik:** Writing – review & editing, Investigation. **Pierluigi Meroni:** Writing – review & editing, Investigation. **Robert Roubey:** Writing – review & editing, Investigation. **Maria Laura Bertolaccini:** Writing – review & editing, Investigation. **Hannah Cohen:** Writing – review & editing, Investigation. **Jacob H. Rand:** Writing – review & editing, Supervision, Methodology. **Doruk Erkan:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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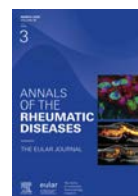
Orcid

Jonathan Thaler: <http://orcid.org/0009-0003-7320-8427>
Doruk Erkan: <http://orcid.org/0000-0001-7216-677X>

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Systemic sclerosis

RUNX1 is expressed in a subpopulation of dermal fibroblasts and is associated with disease severity of systemic sclerosis

Rezvan Parvizi^{1,2}, Zhiyun Gong¹, Helen C. Jarnagin^{1,2},
 Diana M. Toledo^{1,2}, Tamar R. Abel^{1,2}, Dillon Popovich^{1,2},
 Madeline J. Morrisson^{1,2}, Tammara A. Wood¹, Sasha Shenk³,
 Mrinal K. Sarkar⁴, Olesya Plazyo⁴, Poulami Dey^{4,5}, Anthony Coon⁴,
 Jake M. Erba⁴, Lam C. Tsoi⁴, Pei-Suen Tsou⁵, Monique E. Hinchcliff⁶,
 Dinesh Khanna⁵, Jonathan A. Garlick³, Johann E. Gudjonsson^{4,5},
 Patricia A. Pioli⁷, Michael L. Whitfield^{1,2,*}

¹ Department of Biomedical Data Science, Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

² Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA

³ Department of Basic and Clinical Translational Sciences, Tufts University School of Dental Medicine, Boston, MA, USA

⁴ Department of Dermatology, University of Michigan, Ann Arbor, MI, USA

⁵ Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

⁶ Section of Rheumatology, Allergy and Immunology, Department of Medicine, Yale School of Medicine, New Haven, CT, USA

⁷ Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

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ABSTRACT

Objectives: The activation of runt-related transcription factor 1 (RUNX1) in fibroblasts has been implicated in wound healing and fibrosis; however, the role of RUNX1 in the fibrotic progression of the autoimmune disease systemic sclerosis (SSc) remains known.

Methods: Leveraging gene expression, genome-wide DNA methylation, and single-cell resolution data of SSc skin and fibroblast, we analysed the impact of RUNX1 dysregulation in SSc dermal fibrosis. RUNX1 function was subsequently assessed using siRNA, pharmacologic inhibition, and CRISPR knockout in 2-dimensional and 3-dimensional fibroblast cultures.

Results: Analysis of gene expression in multiple cohorts demonstrated an association between the severity of dermal fibrosis and the expression levels of RUNX1 in the skin of patients with SSc. Epigenomic analyses of methylation identified hypomethylated 5-Cytosine-phosphate-Guanine-3 (CpG) sites proximal to the RUNX1 gene, implicating their potential role in the increased expression of RUNX1. Analysis of single-cell RNA-seq data from skin biopsies of individuals with SSc revealed that RUNX1 is higher in subpopulations of fibroblasts enriched in SSc, which are believed to contribute to fibrosis. RUNX1 CRISPR knockout resulted in reduced alpha smooth muscle actin expression. Inhibition of RUNX1 activity caused a reduction in fibroblast activation, contraction, extracellular matrix components, and proliferation rates, including a reduction in *SFRP4*, *LUM*, and *COL1A1*.

Conclusions: This study is the first to demonstrate a potential role for RUNX1 in the pathogenesis of SSc dermal fibrosis. RUNX1 is associated with more severe SSc fibrosis and is associated with a subpopulation of dermal fibroblasts implicated in fibrosis.

*Correspondence to Dr. Michael L. Whitfield, Dartmouth College Geisel School of Medicine, Lebanon, NH, USA.

E-mail address: michael.whitfield@dartmouth.edu (M.L. Whitfield).

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Dysregulation of dermal fibroblast activity leads to excessive collagen production and deposition, causing scar tissue formation and fibrotic lesions in systemic sclerosis (SSc).
- Transcription factors play a key role in driving functional diversity among dermal fibroblasts in SSc, and understanding their dysregulation in SSc fibrosis is critical for the development of new therapeutic strategies.
- Runt-related transcription factor 1 (RUNX1) is a member of the core-binding factor family that regulates proliferation, differentiation, and cell survival in multiple cell lineages.
- Current understanding of the role of RUNX1 in dermal fibroblasts and its extent of involvement in SSc dermal fibrosis is very limited.

WHAT THIS STUDY ADDS

- Our observations indicate a potential association between the severity of dermal fibrosis and *RUNX1* expression levels in SSc skin.
- *RUNX1* is enriched in SSc-specific fibroblast subpopulations.
- We identify hypomethylated CpG sites near the *RUNX1* gene, which suggests their potential involvement in the upregulation of *RUNX1* in SSc dermal fibroblasts.
- Inhibition of RUNX1 activity by Ro5-3335, knockdown by siRNA, and RUNX1 knockout resulted in statistically significant changes in extracellular matrix components.
- Inhibition of RUNX1 activity caused a reduction in fibroblast contraction and proliferation rates.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The discovery of unique associations between disease-specific fibroblast subpopulations and RUNX1 provides the foundation for therapeutic targeting of RUNX1 in SSc.

INTRODUCTION

Dermal fibrosis is a major clinical manifestation of systemic sclerosis (SSc) [1,2], which is the result of uncontrolled deposition of extracellular matrix (ECM) by fibroblasts residing in the skin [3]. As dermal fibrosis progresses, patients with SSc experience skin thickening, distortion, and tightening that can limit joint movement and cause significant discomfort [4]. The collective crosstalk between dermal transcriptional patterns, signalling pathways, and epigenetic alterations contributes to fibrotic activation in SSc [3,5]. The modified Rodnan Skin Score (mRSS) is commonly used to quantify the degree and progression of dermal fibrosis and the severity of SSc. There are 2 clinical types of the disease, based on the extent of skin involvement: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc) [4]. We have developed a molecular classification system based on gene expression changes observed in lesional and nonlesional skin biopsies. This classification categorises patients with SSc into 4 subtypes: inflammatory, fibroproliferative, normal-like, and limited [6]. Through network analyses of transcription factor (TF) activity, we previously identified the *runt-related transcription factor 1* (*RUNX1*) as a key regulator in SSc but its function in SSc was not characterised [7].

RUNX1 is a member of a family of DNA-binding TFs that partners with a constitutively expressed core-binding factor subunit- β to form an active heterodimer that regulates the expression of downstream genes. The *RUNX1* gene is located on chromosome 21 and encodes 3 isoforms (a, b, and c) that differ in their N-terminal coding sequences [8]. Expression of *RUNX1* is tightly regulated by transcriptional, translational, and posttranslational

mechanisms. Significantly, several studies have documented crosstalk between RUNX1 and profibrotic signalling pathways, including transforming growth factor- β (TGF- β) [9], Nuclear Factor κ B (NF- κ B) [10], and Wnt signalling [11]. While the importance of RUNX1 in most haematopoietic cell lineages has been characterised in detail [12], its role in fibroblasts and dermal fibrosis more broadly remains poorly understood.

RUNX1 is overexpressed in a range of human cancers that are characterised by fibrosis [13–16], including gliomas. There is a direct association between *RUNX1* levels, tumour grade, and the expression of ECM-related genes such as *Fibronectin 1* (*FN1*), *collagen type IV alpha 1 chain* (*COL4A1*), and *Lumican* (*LUM*), which are implicated in the pathogenesis of glioblastoma [17]. While RUNX1 is involved in wound healing, as demonstrated by a recent spatial multiomic analysis showing high RUNX1 activity in inner wound mechanofibrotic fibroblasts that differentiate into a strongly profibrotic cellular subset [18], it is also implicated in pathological fibrosis [19,20]. Additionally, *RUNX1* promotes the development of pulmonary fibrosis and pulmonary arterial hypertension [21,22] and mediates cardiac fibrosis following myocardial infarction [20,23,24]. Synovial biopsies from patients with rheumatoid arthritis and plaque biopsies from patients with atherosclerosis also identified *RUNX1* as a key upregulated TF [25], providing further support for RUNX1 in the pathogenesis of rheumatological and vascular disease.

In this study, we demonstrate for the first time *RUNX1* expression in a subpopulation of dermal SSc fibroblasts and investigate its role in regulating SSc disease severity. Using publicly available skin transcriptional datasets and analysing data from the skin of 200 patients with SSc, we characterise *RUNX1* expression patterns and epigenetic profile, then elucidate the effect of RUNX1 inhibition on ECM regulation and fibroblast function to establish a comprehensive understanding of its role in SSc dermal fibrosis.

METHODS

A detailed description of the experimental methods, computational procedures, data processing, and statistical analyses can be found in the supplemental material.

RESULTS

RUNX1 expression is significantly higher in the skin of patients with diffuse SSc and is associated with a TGF- β fibroblast gene signature

Our prior bioinformatic analyses predicted a role for the *RUNX1* TF in the inflammatory subset of SSc patients [7]. To validate and extend these findings, we analysed a larger cohort of skin biopsies from individuals with SSc and healthy controls to examine the distribution of *RUNX1* expression and to further investigate its correlation with disease severity [7]. Analysis of gene expression data showed significantly increased expression of *RUNX1* in lesional forearm skin biopsies from 86 individuals diagnosed with lcSSc and dcSSc [26]. *RUNX1* was also elevated in samples of nonlesional skin (samples collected from the flank of patients with SSc compared to healthy control nonlesional skin, Fig 1A), suggesting that the disease-specific activation of *RUNX1* is shared between early fibrotic skin and prelesional/prefibrotic skin biopsies. *RUNX1* expression was highest among individuals diagnosed with early dcSSc and decreased over the course of 3 years (Fig 1B, Supplementary Fig S1). Increased expression of *RUNX1* was subsequently confirmed in 5

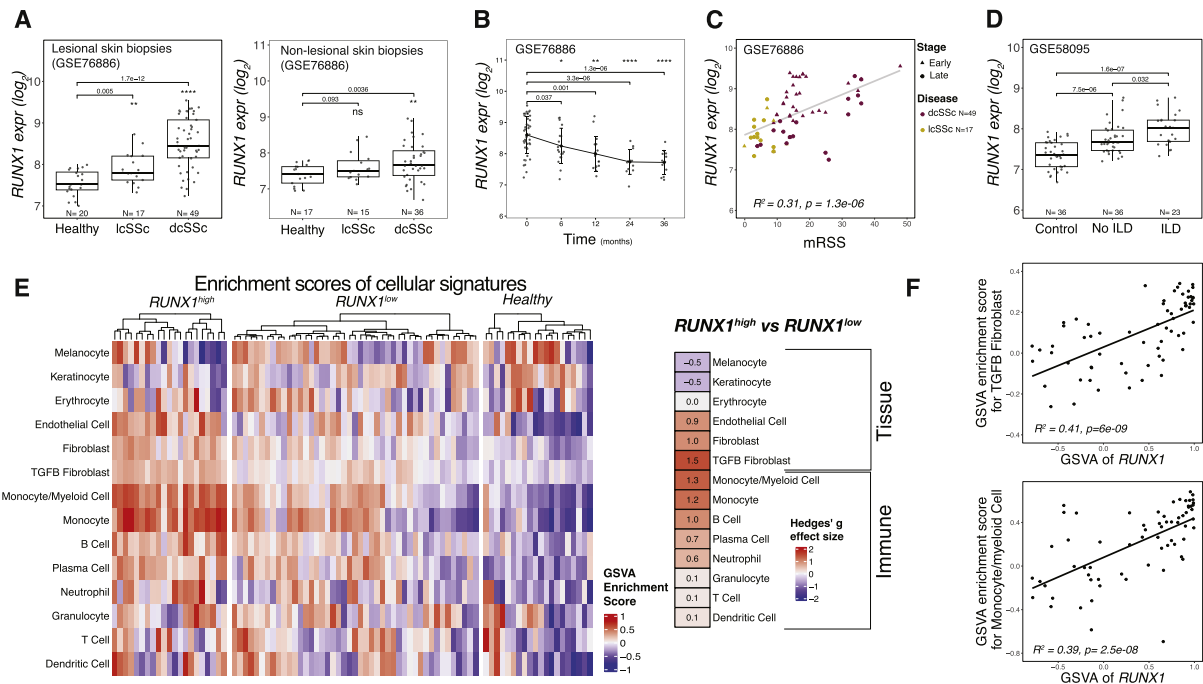


Figure 1. *RUNX1* expression is significantly higher in the skin of patients with dcSSc and is associated with TGF- β fibroblast and myeloid cell signatures. (A) *RUNX1* expression rate in forearm (lesional) skin biopsies of dcSSc (N = 49), lcSSc (N = 17), and healthy (N = 20) individuals and in flank (nonlesional) skin biopsies of dcSSc (N = 36), lcSSc (N = 15), and healthy (N = 17) individuals. (B) The expression rate of *RUNX1* over the course of 3 years (data presented for 0, 6, 12, 24, and 36 months). (C) Correlation between *RUNX1* expression and mRSS skin score at baseline for both lcSSc (yellow) and dcSSc (red) (N = 66); early-stage patients are shown as a triangle and late-stage patients as a circle. (D) *RUNX1* expression rate in healthy donors (N = 36), patients with ILD (N = 23), and patients without ILD (N = 36) at baseline. (E) GSEA enrichment scores of main cellular signatures in healthy (N = 20), patients with *RUNX1*^{high} (N = 21), and patients with *RUNX1*^{low} (N = 45). Hedge's g effect size of *RUNX1*^{high} vs *RUNX1*^{low} is presented in the graph. (F) Correlation between the TGF- β fibroblast and monocyte and myeloid cell signatures with *RUNX1*. dcSSc, diffuse cutaneous SSc; GSEA, gene set variation analysis; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; mRSS, modified Rodnan Skin Score; *RUNX1*, runt-related transcription factor 1; TGF- β , transforming growth factor- β .

additional publicly available datasets (GSE9285, GSE32413, GSE125362, GSE97248, and GSE58095), totalling more than 120 individuals with SSc [6,27–31] (Supplementary Fig S2A–E, Supplementary Table S1). At baseline, individuals with early disease (defined as biopsies taken less than 2 years since first onset of non-Raynaud's symptoms) exhibited higher levels of *RUNX1* expression compared with those with a disease duration of greater than 2 years (Supplementary Fig S1). A significant positive correlation was found between *RUNX1* expression and mRSS in 2 separate cohorts [29,31] ($r^2 = 0.31$, $P = 1.3e-06$; $r^2 = 0.25$, $P = 4.9e-05$) (Fig 1C, Supplementary Fig S2F). *RUNX1* was highest among patients with SSc with a higher skin score at the site of biopsy (Supplementary Fig S2G) and those with interstitial lung disease (ILD) (Fig 1D) [7], indicating a potential association between disease severity and *RUNX1* expression levels.

We next set out to identify the cell-type-specific signalling pathways with which *RUNX1* expression was most closely associated. We performed gene set variation analysis (GSEA) to calculate the enrichment scores of fibroblasts, endothelial cells, keratinocytes, and immune cells [32] for 86 individuals at baseline (Supplementary Table S2). Patients with SSc with a high mRSS score (greater than 10) were divided into 2 groups: *RUNX1*^{high}, defined as *RUNX1* expression over 1 SD above the mean, and *RUNX1*^{low}. The GSEA scores with the strongest enrichment in patients with *RUNX1*^{high} SSc were the TGF- β -activated fibroblast and monocyte/myeloid cell gene signatures (Fig 1E). Hedge's g effect size was used to identify the magnitude of the difference between groups and confirmed strong enrichment of

these pathways (effect sizes of 1.5 and 1.3, respectively). Similarly, evaluation of the Pearson's correlation between the major cellular signatures and *RUNX1* demonstrated that TGF- β -activated fibroblast ($r^2 = 0.41$, $P = 6e-09$) and monocytes/myeloid gene signatures ($r^2 = 0.39$, $P = 2.5e-08$) had the highest correlations with *RUNX1* mRNA expression (Fig 1F).

RUNX1 is expressed in a subpopulation of SSc-associated fibroblasts

To identify the fibroblast subpopulation(s) expressing *RUNX1*, we analysed publicly available single-cell RNA sequencing (scRNA-seq) data from 12 patients with dcSSc and 10 matched healthy controls (GSE138669) [33]. The skin biopsies in this study were collected from dorsal midforearm, and a single-cell suspension was created through enzymatic digestion. Figure 2A [33] shows the major cell types in a uniform manifold approximation and projection (UMAP) plot after data processing. The total *RUNX1* aggregate expression was then calculated and displayed in each sample. While heterogeneity exists between the patients, *RUNX1* was more highly expressed in dcSSc samples than in healthy control skin samples, in accordance with bulk gene expression analyses (Fig 2B). Notably, the patient with SSc exhibiting the highest *RUNX1* expression levels was in the early stage of the disease (duration of ~10 months; donor ID: SC189).

To examine *RUNX1* expression in the fibroblast subpopulations, the total fibroblast populations were clustered into 10 groups (clusters 0–9). As demonstrated in Figure 2C,D, *RUNX1*

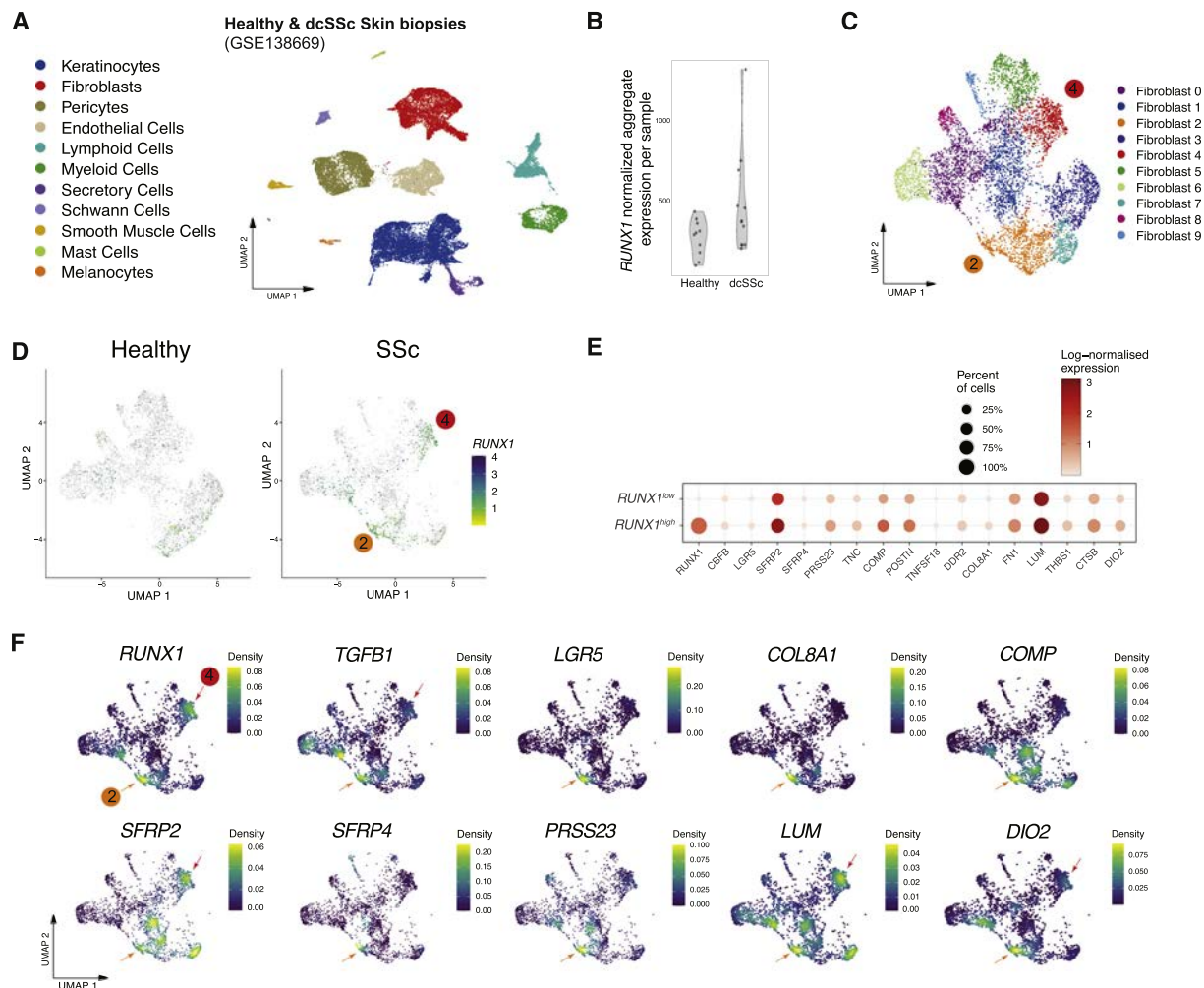


Figure 2. Enrichment of *RUNX1* in SSc-specific fibroblast subpopulations. (A) UMAP projection of cell types identified in the Tabib et al., [33] scRNA-seq of forearm skin biopsies. (B) *RUNX1*-normalised aggregate expression of 10 samples from healthy donors and 12 from patients with dcSSc. (C) UMAP projection of 10 fibroblast subpopulations (clusters 0–9). Two fibroblast populations of 2 and 4 are marked, which are enriched in SSc samples. (D) Feature plots of *RUNX1* expression in healthy and SSc fibroblasts. Two *RUNX1*-expressing fibroblast clusters are marked with their respective numbers. (E) The log-normalised expression rate of main differentially expressed genes between *RUNX1*^{high}- and *RUNX1*^{low}-expressing SSc fibroblasts. (F) Density plots of *RUNX1* and major SSc-relevant markers within SSc fibroblast subpopulations. Arrows indicate clusters 2 and 4 of SSc-specific subpopulations of fibroblasts. dcSSc, diffuse cutaneous SSc; *RUNX1*, runt-related transcription factor 1; scRNA-seq, single-cell RNA sequencing; SSc, systemic sclerosis; UMAP, uniform manifold approximation and projection.

was enriched in SSc-enriched subpopulations 2 and 4. Comparison of *RUNX1*^{high}- with *RUNX1*^{low}-expressing single cells showed that the fibroblasts with high *RUNX1* expression levels also highly expressed fibrotic genes, including *FN1*, *LUM*, *POSTN*, *Cartilage Oligomeric Matrix Protein (COMP)*, *COL8A1*, and *TNC* (Fig 2E). Analysis of markers identified in SSc-associated fibrotic fibroblasts from prior studies shows that *RUNX1* is expressed in *LGR5*⁺ and *TGF-β1*⁺ cells (Fig 2F). *LGR5*⁺ fibroblasts are associated with ECM degradation and skin remodeling/assembly genes [34]. Moreover, *RUNX1*-expressing fibroblasts are *SFRP2*⁺/*SFRP4*⁺/*Col8A1*⁺, indicating that these cells are myofibroblast progenitors [33]. Consistent with this result, these subpopulations of fibroblasts also express high levels of *COMP*, *PRSS23*, *LUM*, and *DIO2*.

RUNX1 regulation is associated with the activation of TGF-β1 signalling in SSc fibroblasts

To assess the regulation of *RUNX1* by the TGF-β1 signalling pathway, we investigated the expression of *RUNX1* in fibroblasts after exposure to exogenous TGF-β1. TGF-β1 treatment of 3 isolated healthy fibroblasts significantly increased the expression

of *RUNX1* at the protein level (Fig 3A). We then analysed a previously generated DNA microarray dataset (National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO): GSE12493) consisting of 2 independent SSc fibroblast cell lines, 1 healthy control fibroblast cell line (isolated in parallel), and 1 normal human dermal (NHD) fibroblast cell line obtained from American Type Culture Collection (ATCC), treated with 50 pM TGF-β1 [35] (Fig 3A). As shown in Figure 3B, *RUNX1* expression was significantly induced by TGF-β1 (relative to vehicle alone treatment) as early as 4 hours after treatment, peaked at 12 hours, and remained elevated until the experimental endpoint at 24 hours. Differential gene expression analyses comparing SSc fibroblasts collected after 12 hours of TGF-β1 treatment with baseline showed significantly elevated levels of *RUNX1* and *CBFB* mRNA (Fig 3C). Analysis of the differentially expressed pathways using Reactome showed that the top pathways associated with high *RUNX1* expression in SSc fibroblasts after TGF-β1 exposure were ‘ECM organisation’, ‘signalling by TGF-β family members’, and ‘IL4 and IL13 signalling’ (Fig 3D).

To investigate whether inhibition of TGF-β would reduce *RUNX1* expression in SSc skin, we analysed data from a clinical

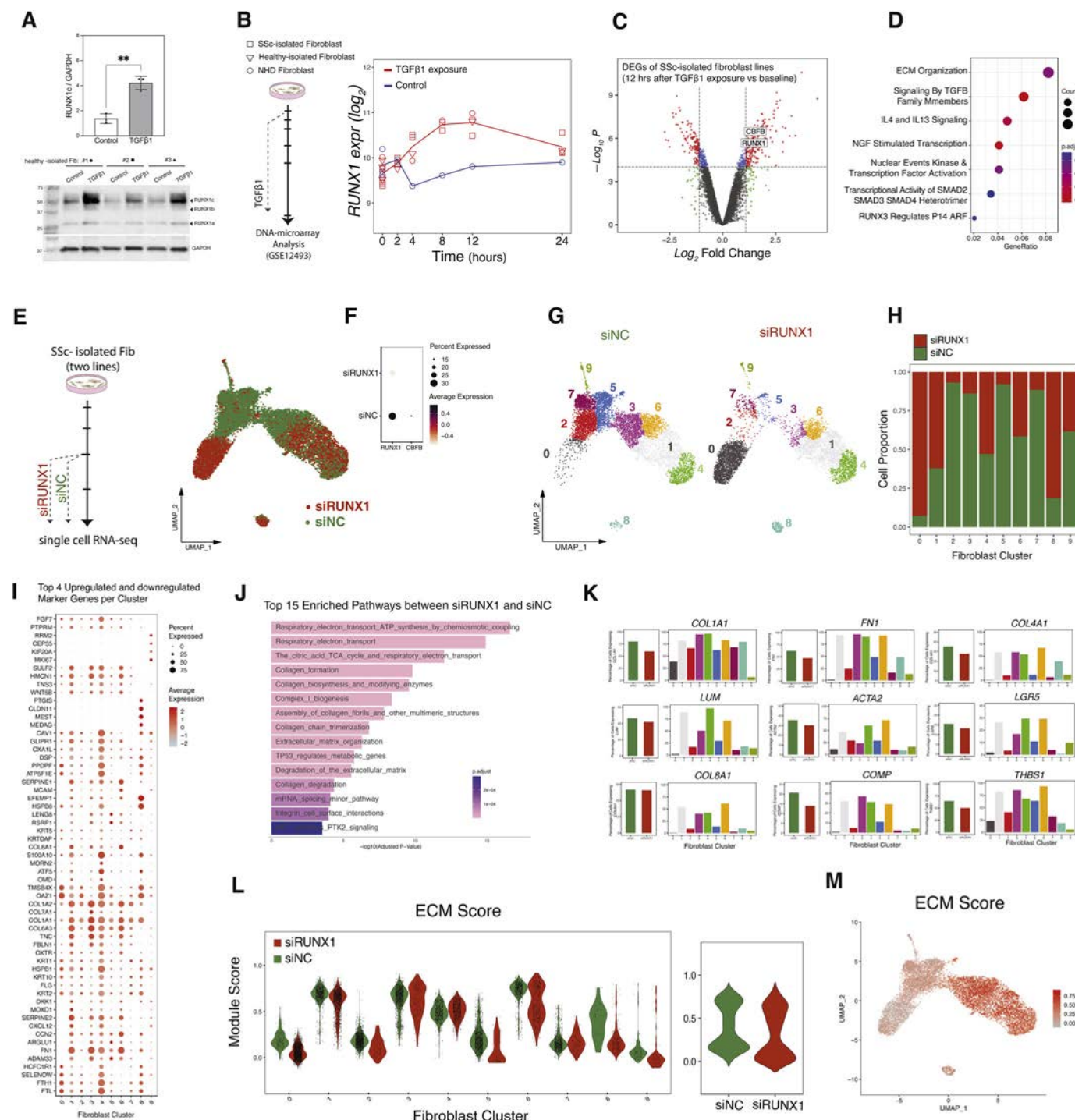


Figure 3. TGF-β1 increases the *RUNX1* expression in SSc fibroblasts and inhibition of *RUNX1* reduces ECM markers. (A) Western blot of 3 isolated fibroblasts lines treated with TGF-β1. The blot shows all isoforms of RUNX1a, b, and c that are overexpressed under the TGF-β1 stimulation. (B) Schematic graph illustrating the timeline for the culture and TGF-β1 treatment of dcSSc-isolated fibroblasts, matched healthy-isolated fibroblasts, and normal human dermal (NHD) fibroblast cells. (C) Line graph showing *RUNX1* expression rate in samples treated with TGF-β1 (in red) vs control (in blue) for the 24 hours after exposure. (D) Volcano plot of differentially expressed analysis of the 2 SSc-isolated fibroblast lines at 12 hours after exposure vs the baseline. (E) The pathway analysis of Reactome gene sets shows the biological pathways and processes that are significantly represented within top DEG genes of SSc-isolated fibroblast lines 12 hours after TGF-β1 treatment vs the baseline. Data from B to D were obtained through publicly available data of GSE12493. (F) Schematic graph showing 2 lines of SSc-isolated fibroblasts treated with siRNA against RUNX1 (siRUNX1) and nontargeting control siRNA (siNC). (G) UMAP projection and dot plot of *RUNX1* and *CBFB* of the single-cell RNA-seq data. (H) UMAP of 10 fibroblast clusters (0-9) for siRUNX1 and siNC. (I) Cell proportion of siRUNX1 and siNC per cluster. (J) Top 4 upregulated and downregulated marker genes per cluster. (K) Top 15 enriched pathways that are significantly represented across siRUNX1 and siNC (K) Bar plots showing the percentage of cells expressing *COL1A1*, *FN1*, *COL4A1*, *LUM*, *ACTA2*, *LGR5*, *COL8A1*, *COMP*, and *THBS1* per condition (red: siRUNX1, green: siNC) or per cluster. (L) Module score for extracellular matrix organisation pathway per cluster and per condition. (M) Feature plot of the ECM module score. dcSSc, diffuse cutaneous SSc; DEG, differentially expressed gene; ECM, extracellular matrix; RUNX1, runt-related transcription factor 1; siRUNX1, siRNA targeting RUNX1; SSc, systemic sclerosis; TGF-β, transforming growth factor-β; UMAP, uniform manifold approximation and projection.

trial of fresolimumab conducted by Rice et al [30]. Fresolimumab is a human monoclonal antibody that binds to and inhibits all 3 isoforms of TGF- β (TGF- β 1–3). In this study, participants were divided into 2 treatment arms: 1 that received 2 low-dose infusions of fresolimumab and another that received 1 high-dose infusion. Midforearm skin biopsies were collected at baseline and 3, 7, and 24 weeks after treatment. While no significant changes were observed in samples analysed at 24 weeks post-treatment, expression of *Thrombospondin 1* (*THBS1*), which was used as a biomarker of TGF- β pathway activation, was decreased 3 and 7 weeks after treatment in patients who received high-dose fresolimumab. *RUNX1* expression decreased with fresolimumab treatment, paralleling *THBS1* expression (Supplementary Fig S3A). Analysis of the TGF- β fibroblast gene signature at baseline and 3 weeks after a single high-dose treatment with fresolimumab showed that TGF- β -associated genes had decreased expression (Supplementary Fig S3B). Consistent with inhibition of *RUNX1* and *THBS1*, expression of *CBFB*, *CCL2*, *COMP*, *TGFB1*, *ACTA2*, *COL4A1*, and *FN1* was attenuated in 5 of 7 patients that received high-dose fresolimumab (Supplementary Fig S3C). Collectively, these data imply that TGF- β signalling regulates the expression of *RUNX1* in SSc skin.

Inhibition of *RUNX1* reduces the ECM signature

To investigate the mechanism by which *RUNX1* activity regulates SSc fibroblast activation, we isolated single-cell suspensions from SSc fibroblasts treated with siRNA targeting *RUNX1* (si*RUNX1*) and a nontargeting control (siNC) as input for scRNA-seq (Fig 3E, Supplementary Table S3). Quality-controlled single-cell data yielded 7544 si*RUNX1* and 5738 siNC cells, with an average of 1387 genes and 2768 transcripts per cell. Consistent with single-cell transcriptomic analyses of skin biopsies, we selected highly variable genes and performed UMAP-based dimensionality reduction and clustering (Fig 3E, Supplementary Fig S4A). As indicated in Figure 3F, *RUNX1* and *CBFB* expression were markedly reduced following knockdown (KD) in si*RUNX1*-transfected cells. Subsequent clustering analysis identified 10 distinct fibroblast subpopulations (Fig 3G,H). In contrast to fibroblast subpopulations observed in skin tissue, these clusters exhibited reduced functional heterogeneity, as reflected by the top differentially expressed markers in each cluster (Fig 3I). Notably, clusters 0 and 8 were predominantly composed of si*RUNX1*-derived cells (Fig 3H).

Cell cycle analysis revealed a decrease in the G2/M phase genes in si*RUNX1* cells compared to siNC transfectants (Supplementary Fig S4B). Moreover, the small proliferative cluster 9 was primarily composed of siNC fibroblasts (Supplementary Fig S5) and is identified with cell cycle markers such as *KIF20A*, *CEP55*, and *MKI67* (Fig 3I). This suggests that *RUNX1* is essential for fibroblast proliferation *in vitro*. Differential expression analysis between si*RUNX1* and siNC fibroblasts identified a substantial number of differentially expressed genes in the *RUNX1* KD samples. Functional pathway analysis of the top 15 upregulated and downregulated pathways demonstrated significant alterations in collagen synthesis, degradation, assembly, and ECM organisation (Fig 3J, Supplementary Table S4). Accordingly, key molecules involved in ECM and fibroblast activation, including *ACTA2* (alpha smooth muscle actin [α -SMA]), *COL1A1*, *COL4A1*, *FN1*, *LUM*, *COL8A1*, *COMP*, and *LGR5* were all expressed at a lower percentage in si*RUNX1* fibroblasts, particularly in clusters 0 and 8 compared with clusters 3, 4, and

6 (Fig 3K), suggesting *RUNX1* promotes myofibroblast transition.

To illustrate the capacity for ECM involvement by different fibroblast clusters and the effect of *RUNX1* KD, we compared the module score based on the gene set from the ECM organisation pathway in the Reactome database as well as other significantly altered pathways such as collagen formation, Tricarboxylic Acid Cycle (TCA) cycle, fibroblast activation and migration (Fig 3L,M, Supplementary Fig S4C,D). Fibroblasts transfected with si*RUNX1* exhibited a decreased ECM module score compared with siNC fibroblasts, with the most pronounced reductions observed in clusters 0 and 8 (Fig 3L,M). This suggests that reduced *RUNX1* activity via siRNA downregulates collagen gene expression, disrupts ECM organisation, and reduces the activated fibroblast subpopulations.

Epigenetic dysregulation of *RUNX1* in SSc fibroblasts

To evaluate the epigenetic state of *RUNX1* in SSc fibroblasts, we conducted genome-wide DNA methylation profiling using Illumina's Infinium Methylation EPIC array to characterise fibroblasts in either 2-dimensional (2D) culture or in a 3-dimensional (3D) self-assembled (SA) skin-like tissue model culture [36,37]. Clinical characteristics of the patients with dcSSc and healthy donors are provided in Supplementary Tables S3 and S5. Two skin biopsies were collected from each donor, with 1 biopsy taken from forearm skin (lesional) and the other from flank skin (nonlesional). DNA was extracted from fibroblasts in 2D culture or in 3D SA tissues. The methylation EPIC array was used to assess cytosine methylation at over 850,000 CpG sites across the genome. Once processed, data were filtered based on the most variated methylated sites; 592 CpG sites passed this filter and were hierarchically clustered by beta values (Fig 3A, Supplementary Table S6). The beta value shows the degree of DNA methylation at a specific genomic locus, where 0 represents completely unmethylated CpG sites and 1 represents completely methylated CpG sites.

Samples clustered based on the most variable probes and were grouped primarily by the type of culture (ie, 2D or 3D) and then by the type of sample (ie, SSc or healthy) (Fig 4A). In 1 set of samples, differences were noted in several CpG sites when comparing fibroblasts from lesional vs nonlesional skin isolated from the same donor, suggesting that anatomic, site-specific differences in methylation patterns exist in isolated cells. To determine if the method of cell culture (2D vs 3D) significantly impacts the epigenetic landscape of SSc or healthy donor fibroblasts, we performed probe-wise differential methylation analysis between SSc and healthy samples in each culture type using the limma package. As shown in Figure 4B, 34,126 CpGs were found to be significantly hypomethylated in 2D cultured fibroblasts and 42,821 CpGs were hypermethylated in SSc samples relative to healthy controls. In 3D culture, 26,314 and 32,352 CpGs were hypomethylated and hypermethylated, respectively, in SSc samples compared with healthy controls. Among all significant CpGs (hypo- or hypermethylated), 40,106 were common between the 2D and 3D culture, which suggests that the culture system may affect cytosine methylation rates in fibroblasts (Fig 4B). However, it is possible that these differences in CpG methylation may not translate to altered gene regulation and pathway activity. Therefore, we used the top significant probes to analyse the enrichment of Reactome pathways using the methylGSA package, which accounts for the number of CpGs per gene. As shown in Figure 4C, there were significant pathway differences between SSc fibroblasts and healthy control cells in both

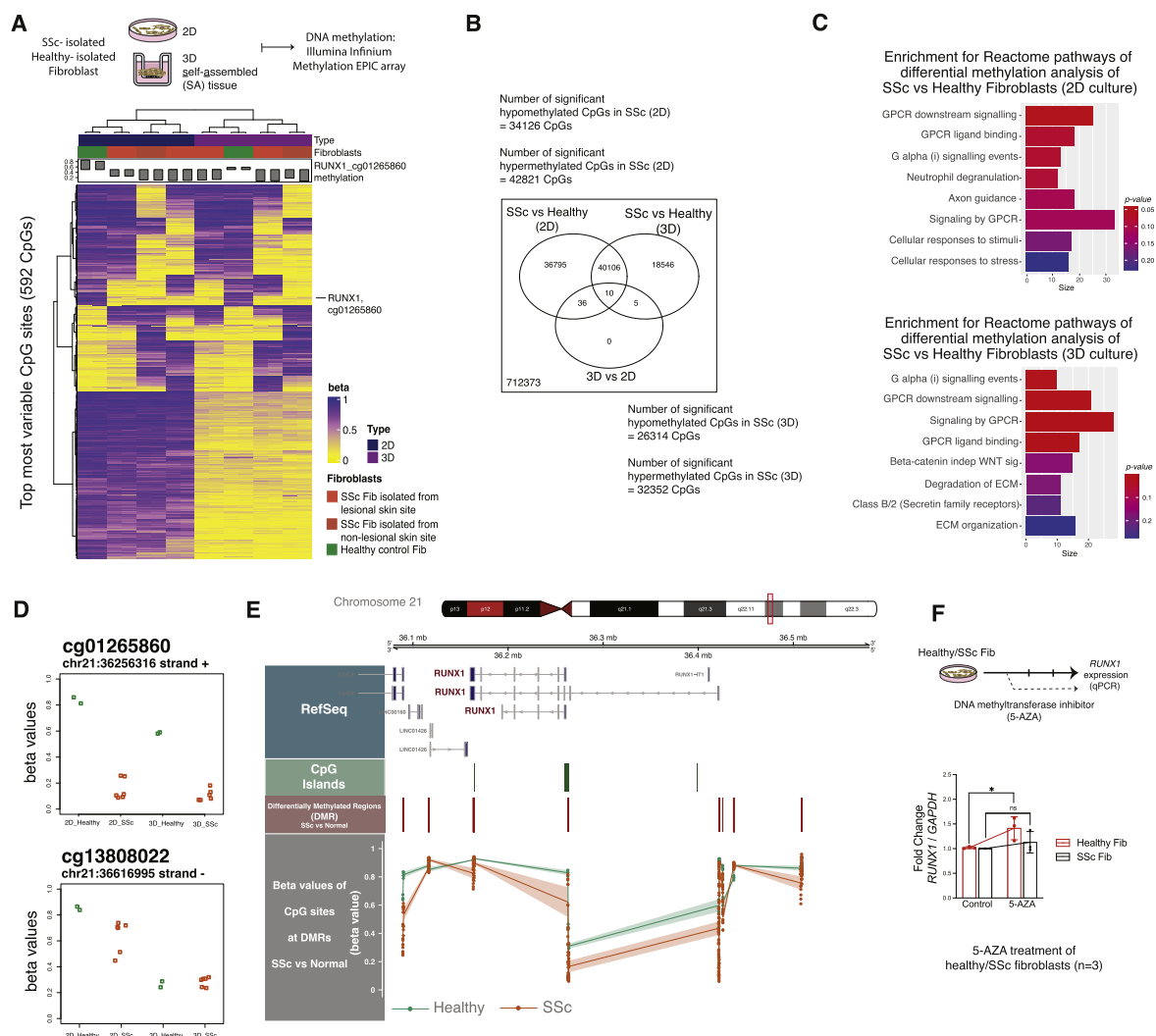


Figure 4. *RUNX1* is hypomethylated in SSc fibroblasts. (A) DNA methylation profile of 2D- and 3D-cultured fibroblasts isolated from patients with dcSSc or healthy donors, created using Illumina's Infinium Methylation EPIC array. Heatmap shows top 592 methylated CpG sites, with blue/yellow gradient of beta values. The bar plot on top shows the *RUNX1* beta value that is labelled within the heatmap, showing that *RUNX1* is hypomethylated in dcSSc samples. (B) Result of paired-wise differentially methylated CpGs and the number of significant CpGs in each group. (C) Pathway enrichment analysis of Reactome gene sets using the top significant CpGs identified in (B) for each 2D and 3D culture. (D) The beta values of representative CpGs in *RUNX1* locus in 2D and 3D SSc and healthy conditions. (E) *RUNX1* locus on chromosome 21 and common CpG islands in green. The differentially methylated regions (DMRs) are identified between SSc and healthy samples are shown in red. The beta values corresponding to the CPGs at DMRs for SSc (in orange) and healthy (in green). (F) *RUNX1* expression rate in isolated healthy and SSc fibroblasts (n = 3) treated with DNA methyltransferase inhibitor, 5-Aza-2'-deoxycytidine (5-AZA) for 72 hours. dcSSc, diffuse cutaneous SSc; *RUNX1*, runt-related transcription factor 1; SSc, systemic sclerosis; 3D, 3-dimensional; 2D, 2-dimensional. G-Protein Coupled Receptor (GPCR).

2D and 3D culture, while no significant pathway differences were identified as a result of differences in culturing conditions.

Significantly, analysis of the most highly variable CpG sites identified 1 CpG annotated at the *RUNX1* genomic locus (*cg01265860*, located on the positive/sense strand of chr21:36256316), ie, hypomethylated in all SSc fibroblasts compared to healthy control fibroblasts. Moreover, we also found additional probes—including *cg09019048* and *cg10011479* on the sense strand and *cg13808022* on the antisense strand—that were located on the *RUNX1* locus and were also hypomethylated (Fig 4D, Supplementary Fig S5). Hypomethylation in a gene on the sense strand is often associated with increased gene expression. While methylation on the antisense strand may not directly impact the expression of the gene, it can play a regulatory role potentially through effects on RNA stability or processing.

Next, the differentially methylated regions (DMRs) between SSc and healthy fibroblasts were analysed to identify proximal

CpGs that are concordantly differentially methylated (Supplementary Table S7). Figure 4E shows the *RUNX1* locus on chromosome 21 using the hg19 reference genome. The *RUNX1* gene is indicated and common CpG islands are shown (Fig 4E). Several DMRs were evident between SSc and healthy control fibroblasts, and analysis of the average beta values of CPGs surrounding *RUNX1* demonstrated that the SSc samples were hypomethylated at almost all the sites compared to healthy controls (Fig 4E). For validation, we treated 3 healthy and 3 SSc-isolated fibroblasts with a DNA methyltransferase inhibitor, 5-Aza-2'-deoxycytidine (5-AZA). This commonly results in passive DNA demethylation over successive cell divisions and reactivation of epigenetically silenced gene. After 72 hours, we observed a significant upregulation of *RUNX1* expression in healthy fibroblasts (Fig 4F), suggesting that this gene is epigenetically repressed under normal conditions and can be reactivated upon DNA demethylation. There was no change in the gene

expression in SSc fibroblasts consistent with the hypomethylated state of *RUNX1* in these cells. Taken together, our data suggest that the significantly elevated *RUNX1* expression observed in patients with SSc is due to epigenetic dysregulation, and DNA methylation plays a regulatory role in controlling *RUNX1* expression in fibroblasts.

RUNX1 is required for fibroblast activation, proliferation, and contraction

To determine the effect of *RUNX1* deletion in fibroblasts, we generated the *RUNX1* knockout (KO) in human dermal fibroblasts using a CRISPR/Cas9 (Associated Protein 9) system

(Supplementary Fig S6). Validation of the KO clone was performed by sequencing and western blotting to confirm loss of *RUNX1* protein. *RUNX1* protein was eliminated in CRISPR-generated *RUNX1* KO fibroblast and maintained at the same level under the TGF- β 1-induced condition (Fig 5A). We evaluated the expression of α -SMA, a key marker of myofibroblast activation, using immunofluorescence staining. We observed a significant reduction in α -SMA expression in the *RUNX1* KO fibroblasts compared to wild-type. This decrease was further corroborated by quantitative measurements of α -SMA protein levels as well as *ACTA2* gene expression, both of which showed consistent down-regulation in the KO cells. Importantly, this reduced expression of α -SMA protein and *ACTA2* mRNA was maintained even under

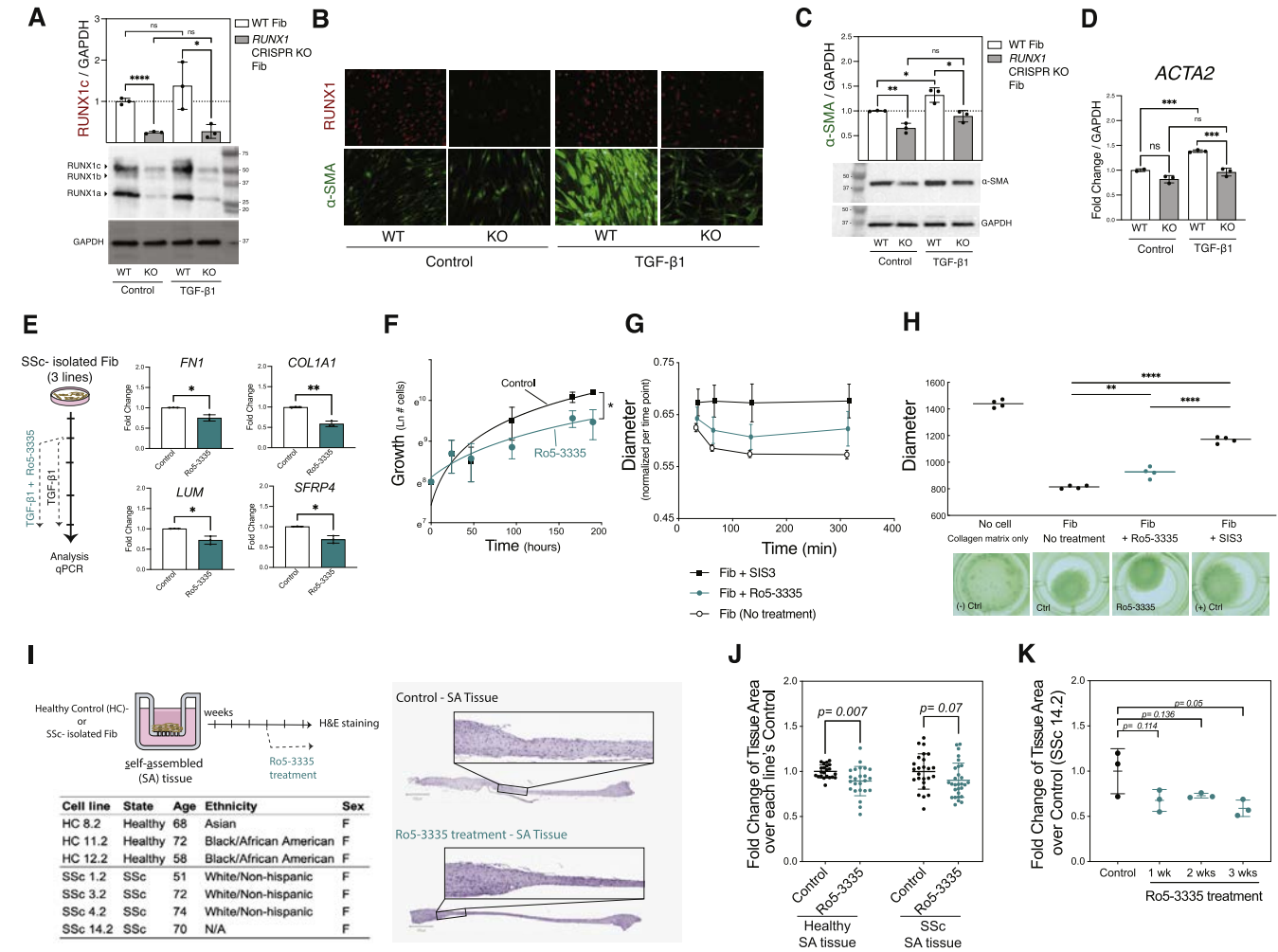


Figure 5. *RUNX1* contributes to fibroblast activation, proliferation, and contraction. (A) *RUNX1* western blot of CRISPR-generated *RUNX1* KO and wild-type (WT) fibroblasts under the TGF- β 1 stimulation vs control. *RUNX1* isoforms of a, b, and c were marked in the blot by arrows. (B) α -SMA and *RUNX1* IF staining of KO and WT fibroblasts under the TGF- β 1 stimulation vs control. (C) α -SMA western blot of KO and WT fibroblasts under the TGF- β 1 stimulation vs control. (D) *ACTA2* mRNA expression of KO and WT fibroblasts under the TGF- β 1 induction vs control. (E) Fold change expression of *FN1*, *COL1A1*, *LUM*, and *SFRP4* in TGF- β 1-induced SSc fibroblasts treated with Ro5-3335 compared to control (3 lines of SSc fibroblasts, 2 replicates each). (F) Proliferation curve of normal human dermal (NHD) fibroblasts in the presence and absence of Ro5-3335. (G,H) The 3D collagen contraction assays, fixed (G) and floating (H) models, of NHD fibroblasts treated with Ro5-3335 (4 replicates for each condition). SIS3 (SMAD3 inhibitor) was used as positive control that significantly eliminates the contraction ability of fibroblasts. Negative control is collagen matrix with no fibroblasts. The overhead pictures represent 1 replicate for each condition. (I) 3D self-assembled (SA) tissue constructs from the healthy- and SSc-isolated fibroblast lines with donors' clinical characteristics. H&E staining of representative untreated and Ro5-3335-treated tissues. (J) Tissue area fold change of each cell line over the control for healthy and SSc SA tissues. Data from 3 healthy and 4 SSc lines, 3 replicates per line, repeated in 2 independent sets. (K) Change in area of an SSc-isolated SA tissue when treated for 1, 2, or 3 weeks with Ro5-3335 compared to control (Student's *t* test *P* value: ** .001-.01, **** < .0001 in GraphPad Prism v9). α -SMA, alpha smooth muscle actin; H&E, haematoxylin and eosin; KO, knockout; *RUNX1*, runt-related transcription factor 1; SSc, systemic sclerosis; TGF- β , transforming growth factor- β ; Clustered Regularly Interspaced Palindromic Repeats (CRISPR), Smad Family Member 3 (SMAD3); Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Not Applicable (N/A), Quantitative Polymerase Chain Reaction (QPCR), Quantitative Polymerase Chain Reaction, Immunofluorescence (IF).

stimulation with TGF- β 1 (Fig 5B–D). These findings suggest that the *RUNX1* KO attenuates the fibrotic response mediated by TGF- β 1.

The compound Ro5-3335 has been reported to directly interact with *RUNX1* and its heterodimeric partner CBF β , repressing *RUNX1*/CBF β -dependent transactivation in reporter assays [38]. Therefore, we hypothesised that Ro5-3335 would attenuate SSc fibroblast activation and contraction by inhibiting *RUNX1* activity. To test this, we treated 3 lines of TGF- β 1-activated SSc fibroblasts with Ro5-3335. As shown in Figure 5E, the levels of ECM component genes such as *FN1*, *COL1A1*, *LUM*, and *SFRP4* were significantly reduced by 20 μ M Ro5-3335. Other concentrations of Ro5-3335 were tested on several markers, confirming that the effect is concentration dependent (Supplementary Fig S7). Furthermore, Ro5-3335 reduced the proliferation rate of fibroblasts over the course of 3 days (Fig 5F). Inhibition of *RUNX1* activity by Ro5-3335 also significantly reduced the ability of NHD fibroblasts to contract collagen gel matrices (Fig 5G,H).

Next, we assessed the effect of *RUNX1* inhibition on dermal matrix formation using the 3D SA tissue model (Fig 5I). This *in vitro* SSc model recapitulates the fibrotic SSc phenotype present in the skin of patient with SSc as previously determined by histology and transcriptomics [36,37]. We observed that *RUNX1* inhibitor, Ro5-3335, reduced the total area of tissues in healthy ($n = 3$) and most SSc lines (3 of 4 lines) (Fig 5J,K). Furthermore, time-course analysis demonstrated a sustained reduction in matrix formation after 1, 2, and 3 weeks of *RUNX1* inhibition compared to the control (Fig 5G). Collectively, these results demonstrate that *RUNX1* regulates key ECM gene expressions, fibroblast cell proliferation, matrix formation, and contractility in a collagen gel matrix and indicates an important role for *RUNX1* as a driver of pathogenic SSc fibroblast activation.

DISCUSSION

Efforts to fully characterise SSc dermal fibroblast heterogeneity and translational states are limited by tissue scarcity, anatomic site-specific differences, and a paucity of reliable fibroblast subset markers [39]. Defining SSc-specific fibroblast regulatory programmes that drive fibrosis, or conversely promote regeneration, is essential for achieving favourable healing outcomes. This work establishes *RUNX1* as a key player in the regulation of SSc fibroblast activation. We demonstrated that *RUNX1* is enriched in both the lesional and nonlesional skin of patient with SSc, with the highest expression in patients with dcSSc. Because *RUNX1* is aberrantly expressed in both lesional and nonlesional skin, *RUNX1* may be a driver of SSc pathogenesis in a prelesional state [32]. Additionally, higher expression of *RUNX1* is correlated with increased disease severity in patients with SSc, as evidenced by a greater risk for elevated mRSS and the presence of ILD.

Konkimalla et al [40] have recently shown that *RUNX1* is a key driver of alveolar fibroblast transitional states in multiple lung-injury mouse models, and that loss of *Runx1* decreases pulmonary levels of ECM components and their assembly. However, the mechanisms by which *RUNX1* controls the expression of ECM components in TGF- β -activated fibroblasts are not yet clear. In this regard, TGF- β signalling is known to enhance fibrogenesis and to stimulate the production of ECM components, including collagen, fibrillin, laminin, fibronectin, proteoglycans, and elastin [41–43]. Previous studies have shown that *RUNX1* is essential for mesenchymal stem cell proliferation and myofibroblast differentiation, and associates with the SMAD-

dependent TGF- β signalling pathway to directly bind promoters and enhancers of multiple cell-state-specific genes related to cell proliferation and migration [9,44–46]. Consistent with these results, we demonstrated that the inhibition of *RUNX1* via Ro5-3335 reduced dermal fibroblast proliferation, contraction, and 3D *in vitro* tissue matrix formation. Recognising that Ro5-3335 may have off-target effects, we used multiple methods to eliminate *RUNX1* function. Collectively, these data provide additional support for *RUNX1* as an integral driver of fibroblast activation in both 2D culture and a more biologically relevant 3D tissue culture.

Moreover, *RUNX1* upregulation is a hallmark of several other fibrotic diseases. For example, *RUNX1* is expressed in human retinal microvascular endothelial cells in proliferative vitreoretinopathy [47]. In agreement with our findings, inhibition of *RUNX1* activity resulted in a significant reduction of retinal lesion size and reduced migration, proliferation, and tube formation of human retinal microvascular endothelial cells *in vitro* [47–49]. Overexpression of *RUNX1* is associated with enhanced liver fibrosis [50], and *RUNX1* inhibition significantly mitigates pathology in models of pulmonary fibrosis [22]. *RUNX1* binds to the transcriptional coactivator Yes-Associated Protein 1 (YAP), a key regulator in the Hippo signalling pathway, which has a role in differentiation and homeostasis of myofibroblast and the endothelial-to-mesenchymal-transitioning fibroblasts in SSc [51,52].

We have also demonstrated that *RUNX1* is hypomethylated in the SSc-derived fibroblast genome, indicating that the increased *RUNX1* expression observed in patients with SSc is likely due to dysregulation at the epigenetic level. 5-AZA treatment increased expression in healthy but not SSc fibroblasts consistent with these methylation results. Using single-cell multiomic analysis, we showed that *RUNX1* is enriched in a subpopulation of fibroblasts in an SSc-derived self-assembled skin-equivalent (saSE) tissue model at levels similar to those observed in SSc skin biopsies [53]. Using a single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq), this study identified a fibroblast subpopulation with elevated TF motifs for *RUNX1* and *RUNX2*, together with *SMAD3*, *SMAD5*, and *NFKB1* [53]. This population was also characterised by increased expression of multiple collagens and profibrotic genes, including *POSTN* and *LUM* [53]. In light of DNA methylation and enriched chromatin accessibility data, which indicate higher TF motif accessibility, these observations imply that SSc fibroblasts are more susceptible to activation and differentiation under the influence of *RUNX1*.

Recent scRNA-seq from SSc skin biopsies identified distinct dermal SSc dermal fibroblast subsets [33,34,51,54]. An increased proliferation of *SFRP2*^{hi}*PRSS23*⁺ fibroblasts, which represent a putative myofibroblast progenitor population, with *RUNX1*^{high} activity was identified in SSc skin [33]. Notably, dysregulation of associated genes in the *Wnt* signalling pathway, *SFRP2* and *SFRP4* (*Secreted Frizzled-Related Protein 2 and 4*), has been implicated in the pathogenesis of fibrosis disease [55]. Another scRNA-seq and scATAC-seq study identified a distinct SSc-specific subpopulation of fibroblasts termed as *LGR5*⁺-scleroderma-associated fibroblasts [34]. Our data demonstrated that *RUNX1*^{high} fibroblasts are *LGR5*⁺, suggesting that *RUNX1* is an SSc fibroblast TF that contributes to the altered phenotype and function of these cells. Another study by Ma et al [51] identified 2 major clusters of *SFRP*⁺ fibroblasts and *COL8A1*⁺ fibroblasts. *COL8A1*⁺ fibroblasts were located in the deeper dermis of SSc skin and expressed high levels of *ACTA2*, *SFRP4*, *POSTN*, and *PRSS23*, and also had the highest ECM score. We show here

that *RUNX1* is activated in *COL8A1*⁺ cells. Regardless of the variation among the fibroblast subpopulations reported in the different cohorts, *RUNX1* expression is consistently linked to either the myofibroblast or SSc-specific fibroblast or progenitor population.

This study combines human skin data from multiple platforms and cohorts, ranging from bulk microarray to scRNA-seq, to validate our findings at different levels of resolution. Leveraging gene expression, DNA methylation, and single-cell data of SSc fibroblasts, we discovered unique associations between disease-specific subpopulations of fibroblasts and *RUNX1*, which may serve as a potential novel therapeutic target for SSc. As *RUNX1* activity is tissue specific, our study is limited by the identification of the direct target genes in SSc fibroblasts. A limitation of our study is the use of only 2 to 3 biological replicates in the inhibition assays. Given the significant heterogeneity of *RUNX1* expression observed across patients, a larger sample size is needed to capture the full biological variability. Additionally, our work in part relied on Ro5-3335, a compound with low potency and specificity; as such, results obtained with this inhibitor cannot be directly extrapolated to the clinical setting. Continued development of next-generation *RUNX1* inhibitors with improved pharmacological properties is essential. Finally, future research should incorporate more advanced pre-clinical models, including *in vivo*, and patient-derived systems to thoroughly investigate *RUNX1*'s role and assess whether the inhibition of *RUNX1* is sufficient to block disease progression and reverse established dermal fibrosis.

Contributors

Conceptualisation was performed by RP, PAP, and MLW. Data curation was carried out by RP, ZG, HCJ, DMT, TRA, PD, JEG, and MLW. Formal analysis and visualisation were conducted by RP, ZG, and HCJ. Funding acquisition involved RP, HCJ, TRA, PD, MJM, JAG, PAP, and MLW. The investigation was undertaken by RP, ZG, HCJ, DMT, TRA, MJM, MKS, AC, LCT, JME, P-ST, MEH, DK, JAG, JEG, PAP, and MLW. Experimental resources were provided by RP, DMT, TRA, DP, MJM, TAW, SS, MKS, AC, LCT, JME, P-ST, MEH, DK, JAG, JEG, PAP, and MLW. Supervision was provided by MLW. The original draft was written by RP, and the review and editing were completed by RP, ZG, HCJ, MJM, TRA, DP, PAP, and MLW. All authors reviewed and approved the final manuscript.

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Competing interests

MLW has received consulting and research funding from Bristol-Myers Squibb, Boehringer Ingelheim, Corbus Pharmaceuticals, Celdara Medical, LLC, and UCB Biopharma for systemic sclerosis research. All other authors declare no competing interests.

Patient consent for publication

Ethics approval

Written informed consent was obtained from all participants.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

DNA methylation data have been deposited into the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) at Accession Number GSE311858. Single cell data for *RUNX1* siRNA knockdowns have been deposited under Accession Number GSE312591.

Supplementary materials

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Orcid

Rezvan Parvizi: <http://orcid.org/0000-0001-8650-1225>

Zhiyun Gong: <http://orcid.org/0000-0001-8657-4387>

Helen C. Jarnagin: <http://orcid.org/0000-0002-7017-1409>

Diana M. Toledo: <http://orcid.org/0000-0002-7501-8286>

Tamar R. Abel: <http://orcid.org/0000-0002-6519-6898>

Dillon Popovich: <http://orcid.org/0000-0002-3015-4030>

Madeline J. Morrisson: <http://orcid.org/0009-0004-4035-1136>

1136

Monique E. Hinchcliff: <http://orcid.org/0000-0002-8652-9890>

Jonathan A. Garlick: <http://orcid.org/0009-0008-9500-6435>

Johann E. Gudjonsson: <http://orcid.org/0000-0002-0080-0812>

Patricia A. Pioli: <http://orcid.org/0000-0001-6896-7259>

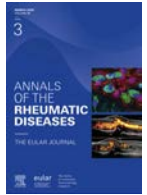
Michael L. Whitfield: <http://orcid.org/0000-0002-0862-6003>

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Myositis

Frequent genetic alterations in myositis autoantigen genes in cancer-associated dermatomyositis

Ana Belén Moreno-Cárdenas^{1,2}, Javier Ros³, Albert Gil-Vila⁴, Debayan Datta¹, Roberta Fasani⁵, Garazi Serna⁵, Jose Jimenez⁵, Ernesto Trallero-Araguás⁶, Cristina Viaplana⁷, Julia Lostes³, José César Milisenda^{8,9}, Josep Maria Grau-Junyent^{8,9}, Raquel Lopez-Perez^{10,11}, Iago Pinal-Fernández^{12,13}, Paolo Nuciforo⁵, Rodrigo A. Toledo^{1,2,*}, Albert Selva-O'Callaghan⁴

¹ Biomarkers and Clonal Dynamics Group, Vall d'Hebron Institute of Oncology, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

² Centro de Investigacion Biomedica en Red de Cáncer (CIBERONC), Institute of Health Carlos III (ISCIII), Madrid, Spain

³ Medical Oncology Department, Vall d'Hebron University Hospital, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

⁴ Systemic Autoimmune Diseases, Vall d'Hebron General Hospital, Departament de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain

⁵ Molecular Oncology Group, Vall d'Hebron Institute of Oncology, Barcelona, Spain

⁶ Rheumatology Department, Vall d'Hebron General Hospital, Barcelona, Spain

⁷ Oncology Data Science Group, Vall d'Hebron Institute of Oncology, Barcelona, Spain

⁸ Muscle Research Unit, Internal Medicine Service, Hospital Clinic de Barcelona, Barcelona, Spain

⁹ CIBERER, IDIBAPS, University of Barcelona, Barcelona, Spain

¹⁰ Radiomics Group, Vall d'Hebron Institute of Oncology, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

¹¹ Radiology Department, Vall d'Hebron University Hospital, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

¹² Muscle Disease Unit, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland, USA

¹³ Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

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ABSTRACT

Objectives: One-third of patients diagnosed with dermatomyositis harbour an occult cancer, a rare condition named cancer-associated dermatomyositis (CAD). Emerging evidence suggests that genetic alterations in autoantibody-related genes give rise to neoantigens and specific autoantibodies that initiate an autoimmune response characteristic of CAD. This study evaluated the prevalence of such genetic alterations and elucidated molecular mechanisms that may underlie their role in triggering autoimmunity.

Methods: Fifteen patients with CAD were included in the study. We conducted genomic and transcriptomic analyses of available leukocyte (germline), muscle, skin, and tumour samples. T-cell receptor (TCR) repertoire profiling and multiplex immunophenotyping were performed to characterise immune responses in tumoural and nontumoural tissues.

Results: Somatic mutations and loss of heterozygosity (LOH) in autoantibody-related genes as tripartite motif containing 33, tripartite motif containing 66, *MORC family CW-type zinc finger 3 (MORC3)*,

* Correspondence to Dr. Rodrigo A. Toledo.

E-mail address: rtoledo@vhio.net (R.A. Toledo).

Ana Belén Moreno-Cárdenas and Javier Ros are co-first authors.

Rodrigo A. Toledo and Albert Selva-O'Callaghan are co-last authors.

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Chromodomain Helicase DNA Binding Protein 4, and IFIH1, interferon-induced helicase C domain-containing protein 1—corresponding to known myositis-specific autoantibodies Anti-transcriptional intermediary factor 1 gamma, anti-nuclear matrix protein 2, (anti-transcriptional intermediary factor 1 gamma (anti-TIF1 γ), anti-nuclear matrix protein 2 (anti-NXP2), anti-Mi2, antimelanoma differentiation-associated gene 5 protein), were detected in the majority of tumours from patients with CAD (75%). Tumour-specific missense mutations gave rise to highly immunogenic neoantigens, while LOH resulted in enriched allelic expression not observed in nontumoural lesions (skin and muscle). Immunophenotyping revealed a clonally expanded lymphocyte population within tumours, but not in nontumoural lesions, suggesting a distinct cellular immune response.

Conclusions: We detected highly frequent genetic alterations in autoantibody-related genes, supporting their role in the CAD pathogenic mechanisms. Moreover, our findings suggest that distinct TCR repertoire and immune signatures between tumoural and nontumoural tissues may underlie divergent cancer and dermatomyositis outcomes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Cancer-associated dermatomyositis (CAD) can occur in one-third of patients diagnosed with dermatomyositis.
- It has been suggested that genetic alterations in the tumours from patients with CAD may generate neoantigens and the production of specific autoantibodies, triggering a typical immune response.

WHAT THIS STUDY ADDS

- First comprehensive genomic and immunological analysis of patients with CAD (N = 15) using multiple OMICs technologies.
- Majority (75%) of CAD tumours exhibited genetic alterations in genes linked to myositis-specific autoantibodies (including *TRIM33*, *MORC3*, chromodomain helicase DNA binding protein 4, *IFIH1*).
- T-cell receptor analysis showed distinct clonal lymphocyte patterns in tumours vs nontumoural tissues, indicating a unique immune response.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Tumour mutations in myositis-specific genes represent a mechanism shared across several autoantibody groups.
- These results provide a foundation for future research and support the exploration of immunotherapy strategies for patients with CAD.

INTRODUCTION

Idiopathic inflammatory myopathies (IIM) encompass a heterogeneous group of autoimmune disorders impacting multiple organs and systems, including muscles, skin, lungs, and joints [1]. Dermatomyositis (DM), a prominent phenotype within IIM, is notably associated with cancer. Epidemiological studies define cancer-associated myositis as the occurrence of both cancer and myositis within a 3-year period [2]. The exact mechanisms linking cancer and myositis remain unclear; however, previous research in DM and related autoimmune diseases, such as scleroderma, suggests that somatic mutations in the tumours of these patients may contribute to disease pathogenesis [3–5].

Early studies on patients with paraneoplastic systemic sclerosis, characterised by inflammation in the skin and other organs driven by DNA-directed RNA polymerase III subunit RPC1 (anti-RPC1) autoantibodies, suggest a molecular mechanism whereby tumour cells somatic mutations in the *POLR3A* gene, which encodes the RPC1 protein, lead to the formation of highly immunogenic neoantigens [3]. These neoantigens trigger the production of antibodies that cross-react with nontumoural tissues, leading to autoimmune manifestations characteristic of the disease. In

addition to *POLR3A* mutations, other tumours in patients with anti-RPC1-related scleroderma, however, did not exhibit somatic mutations; instead, they showed loss of heterozygosity (LOH) in the *POLR3A* locus [3], a well-known phenomenon that results in the loss of the mutated allele—and consequently, the loss of expression of the associated neoantigen—thereby promoting immune evasion. Since this foundational study, advancements in understanding the genetic and immunological mechanisms underlying paraneoplastic diseases have remained limited. We recently reported that tumours from patients with cancer-associated dermatomyositis (CAD) and anti-TIF1 γ autoantibodies harboured somatic mutations and LOH in the TIF1 γ -related gene, tripartite motif containing 33 (*TRIM33*) [4]. However, the prevalence of such alterations in *TRIM33*—as well as in other genes encoding known myositis autoantigens, including *TRIM66*, *MORC3*, chromodomain helicase DNA binding protein 4 (*CHD4*), and *IFIH1* (corresponding to the anti-NXP2, anti-Mi-2, and anti-melanoma differentiation-associated gene 5 protein [MDA5] autoantibodies, respectively)—is still poorly characterised. Moreover, no comprehensive multiomics studies have been conducted in CAD, and the underlying molecular mechanisms driving disease pathogenesis remain largely unexplored.

Immune checkpoint inhibitors (ICIs) have changed the landscape of cancer, mainly in melanoma and nonsmall cell lung cancer among other tumours. These monoclonal antibodies activate the immune system increasing the immune reaction against tumour cells. Patients with autoimmune disorders are currently excluded from receiving such therapies to avoid potential immune-related adverse events (irAEs) and worsening of the preexisting autoimmune condition. However, clinical data show ICIs as efficacious antitumor therapy to patients with common autoimmune disorders while causing frequent but only mild and controllable irAEs [6,7]. Despite the extensive review of clinical and therapeutic data and outcome, the molecular basis of these clinically relevant questions remains largely unexplored and an in-depth investigation into the underlying molecular features that could drive response and/or irAEs remain lacking.

In this study, we aim to build upon our previous findings by performing integrated genomic, transcriptomic, and immunophenotypic analyses of both tumoural and nontumoural tissues from patients with CAD.

METHODS

Patients

Fifteen patients diagnosed with CAD at Vall d'Hebron University Hospital were included in the study. The diagnosis of DM was based on both the European Alliance of Associations for

Rheumatology / American College of Rheumatology (EULAR/ACR) criteria (score >95) [8] and the European Neuromuscular Centre (ENMC) criteria [9]. Serum samples were tested for anti-MDA5, anti-small ubiquitin-like modifier activating enzyme (anti-SAE), anti-Mi2, and anti-NXP2 autoantibodies using a commercial immunoblot assay (Euroimmun, Lübeck, Germany), and for anti-TIF1 γ using an in-house blot/Enzyme-Linked Immunosorbent Assay (ELISA) previously validated by immunoprecipitation [10].

Biological samples

A total of 38 biological samples from the 15 patients with CAD were available for multi-OMICS profiling and immunophenotyping. These included 15 tumour samples, 6 muscle lesions, and 2 skin lesions and 15 germline DNA. From these specimens, we generated whole-exome sequencing data (N = 33). Additionally, a subset of samples underwent whole-genome sequencing (N = 8), RNA sequencing (N = 6), and immunophenotyping (N = 12) for comprehensive immunogenomic profiling (Fig 1; Supplementary Table S1).

Bioinformatics

Whole-exome DNA sequencing

Whole-exome library preparation and sequencing were performed as described previously [11,12] using the ImmunoID NeXT platform. DNA was sequenced in paired-end format using NovaSeq instrumentation (Illumina) to a coverage depth of $\geq 300\times$. Alignment to the human genome (hs37d5) was performed using Burrow-Wheeler Aligner [13] (BWA v. 0.6.2) and Picard (<http://broadinstitute.github.io/picard>, v.1.74), with

quality score recalibration via the Genome Analysis Toolkit (GATK v3.1).

Whole-genome sequencing (WGS)

WGS data were analysed using the SAREK pipeline (v3.3.2) as part of the nf-core framework [14]. Briefly, alignment was performed against the GRCh38 reference genome using BWA (v0.7.17-r1188) and variant calling was performed using GATK (v4.4.0.0).

Somatic mutation detection

Core single-nucleotide variants (SNVs) and SNV quality metrics were generated using GATK's HaplotypeCaller6. SNVs were stratified by their false-positive likelihood using the variant quality score recalibration module (GATK), in addition to in-house SNV accuracy software. Miscalled variants were omitted using in-house SNV accuracy software, which employs a model informed by genomic context and sequence alignment data. MuTect [15] was used to call somatic SNVs and indels, and VarDict [16] was used to call small somatic insertions or deletions (indels) less than 50 bp. Combined somatic SNV and indel calls were then analysed through a set of rigorously validated filters incorporating coverage, read quality, and positional features, along with the likelihood of presence in normal tissue.

We calculated the *P* value for observing the number of somatic mutations detected or a higher count using a Poisson-binomial distribution Discrete Fourier Transform and Convolutional Fast Fourier Transform (DFT-CFFTDFT-CFFT), with individual mutation probabilities derived from the somatic genetic variants identified in the 10,967 tumour samples of the Cancer Genome Atlas (TCGA) PanCancer Atlas Study [17].

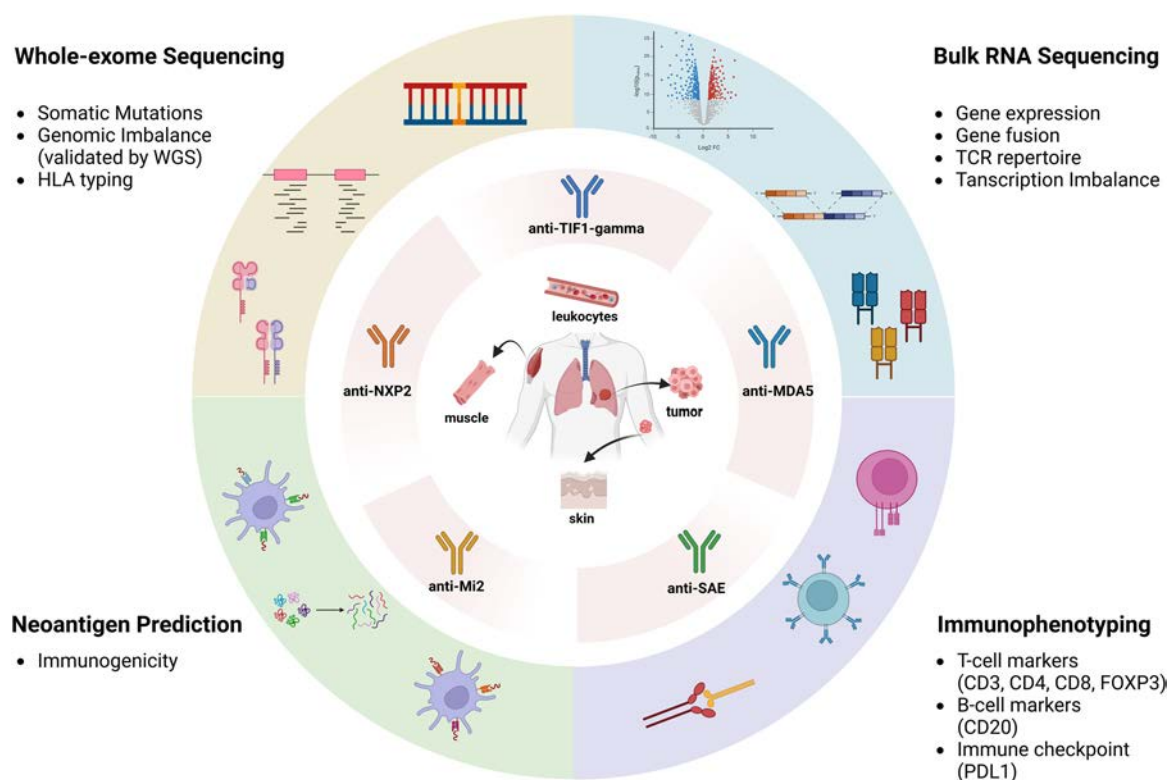


Figure 1. Schematic representation of the research methodology employed to elucidate the genomic, transcriptomic, and immune profiles of patients afflicted with cancer-associated DM characterised by the presence of dermatomyositis-specific autoantibodies (TIF1 γ , Mi2, NXP2, SAE, and MDA5). This comprehensive analysis encompassed the examination of germline DNA, tumour, skin, and muscle DNA/RNA samples through whole-exome sequencing (WES), bulk RNA sequencing, and immunofluorescence immunophenotyping techniques. DM, dermatomyositis; MDA5, melanoma differentiation-associated gene 5 protein; TCR, T-cell receptor; WGS, whole-genome sequencing.

Copy number analysis and clonality estimation

Allele-specific copy number analysis of tumors (ASCAT) (v3.1.3) was used to identify which regions of the tumour genome have gained or lost gene copies, while adjusting for tumour purity and ploidy. This algorithm accurately dissects allele-specific copy number profiles in solid tumours by accounting for tumour cellularity and nonaberrant (normal) cell admixture [18]. As a result, it allows for precise detection of genomic gains, losses, copy number-neutral events, and LOH.

Clonality of somatic mutations was assessed by calculating the cancer cell fraction (CCF), which indicates whether a given mutation is present in all tumour cells (clonal) or only in a subset (subclonal). This distinction provides valuable insights into the evolutionary dynamics and potential aggressiveness of the tumour. CCF was estimated using the formula:

$CCF = VAF / (P \cdot CT + (1 - P) \cdot CN)$, where *VAF* is the variant allele frequency, *P* is tumour purity, *CT* is the copy number at the mutation site in the tumour, and *CN* is the corresponding copy number in normal tissue (typically assumed to be 2 for autosomes). Variants with $CCF > 0.9$ were considered clonal, whereas those below this threshold were classified as subclonal.

Typing and LOH of HLA loci

Human Leukocyte Antigen (HLA) typing up to 4 places was carried out via the ImmunoID NeXT Platform, which employs probes targeting the Major Histocompatibility Complex (MHC) reference sequences in the GRCh38 human reference genome. HLA LOH was called using DASH (Deletion of Allele-Specific HLAs), a machine-learning-based algorithm [19].

Neoantigen prediction

Nonsynonymous somatic DNA variants (SNVs, indels, and fusions) were used to call putative neoantigens, which were then verified using transcriptomic data. For each putative neoantigen, all peptides containing the event were generated; binding was assessed for each Class I HLA using NetMHC [20] and NetMHCpan [21], and immunogenicity scores were calculated as described in Calis et al [22].

RNA sequencing

Whole transcriptome sequencing of tumour RNA was performed as described in Abbott et al. Briefly, RNA sequencing was performed to a coverage depth of ≥ 192 million reads, with augmentation of T-cell receptor (TCR) regions (100-fold). Ribosomal reads were excluded, and transcripts were aligned using STAR [23]. Transcript expression was calculated and normalised with the aid of Salmon [24], and gene expression was derived using a proprietary tool, Expressionist (Personalis, Inc).

TCR sequencing

TCR β clones were profiled using the ImmunoID NeXT RepertoireID. RepertoireID captures CDR3 regions and boosts TCR region transcriptome sequencing to over 100 times the standard ImmunoID NeXT transcriptome. TCR clones were identified using MiXCR. Clones containing frameshift mutations or premature stop codons in the CDR3 sequence were omitted, along with clones with alignment scores below 70 for V or J sequences.

Immunophenotyping

Next-generation immunohistochemistry was used for the analysis and quantification of different T cell populations (regulatory, helper, cytotoxic, and total T cells) as previously published [25]. Antibodies and protocols are shared in Supplementary Table S1. Visiopharm was used to quantify each

subpopulation, and ImageJ was used to assign virtual colours to the different stainings and merge them, providing images of the different biomarkers and their coexpressions (cyan/green = CD3+ FOXP3-CD8+; Blue = CD3+ FOXP3-CD4+; Red/Pink = CD3+ FOXP3+).

RESULTS

Patient population

The median age of the cohort was 63 years (ranging from 35 to 88), and 10 out of 15 patients were female (66.7%). Regarding tumour type, 6 patients (40%) were diagnosed with thoracic malignancies (5 small-cell lung cancer [SCLC], and 1 thymic carcinoma), 3 patients with gynaecological malignancies (1 squamous cervix carcinoma, 1 cervix adenocarcinoma, and 1 ovarian serous carcinoma), and 3 patients with gastrointestinal malignancies (1 colonic neuroendocrine tumour-NET, 1 rectum adenocarcinoma, and 1 stomach adenocarcinoma). Two patients presented with breast cancer, and 1 patient presented nasopharyngeal carcinoma (Supplementary Table S1). Two patients had localised tumours, and 1 patient was diagnosed with DM in a malignancy relapse. Data on patients' cancer diagnoses, immunological profiles, and the timing of DM onset in relation to cancer diagnosis and treatment are summarised in Table 1.

Tumours from patients with Cancer-associated myositis (CAM) harbour genetic events in common cancer drivers

Among the 12 patients with available genetic analysis, 5 patients were diagnosed with SCLC and the other tumour types were thymic carcinoma, squamous cervix carcinoma, nasopharyngeal carcinoma, colonic neuroendocrine tumour, stomach adenocarcinoma, breast cancer, and ovarian serous carcinoma (Table 1). Our analyses revealed a spectrum of mutations, copy number alterations (CNA), and gene fusions (Supplementary Fig S2) in cancer-associated genes listed on the OncoKB platform [26]. Mutations in the *TP53* tumour suppressor gene were the most frequently observed genetic events, followed by alterations in genes such as *CSMD3*, *NPIP15*, *EP400*, *KMT2C*, and *KMT2D*, among others (Supplementary Fig S1A). Tumour mutation burden exhibited substantial variability, with a mean of 229.3 ± 86.86 nonsynonymous somatic mutations.

Tumours from patients with CAM harbour highly frequent genetic events in autoimmune antibody-associated genes

Genetic alterations in members of the C-VI subgroup of tripartite motif (TRIM) genes, specifically *TRIM24* (TIF1 α), *TRIM28* (TIF1 β /KAP1), *TRIM33* (TIF1 γ), and *TRIM66* (TIF1 δ), which all feature a C-terminal tandem plant homeodomain and bromodomain module [27], were the most common, as follows. The tumour sample from patient DM8, who was diagnosed with SCLC and detectable TIF1 γ circulating antibodies, had a somatic mutation in *TRIM33* (p.Y1072), a mutation previously linked to CAM [4] (Fig 2B). Also, patient DM16, who was diagnosed with stomach adenocarcinoma and detectable TIF1 γ circulating antibodies, had another somatic mutation in *TRIM33* (p.R440C). Tumours from other patients with anti-TIF1 γ autoantibodies (DM1, DM12, DM17, DM18, and DM19) presented evidence of LOH in the *TRIM33*, *TRIM24*, and *TRIM66* genes (Fig 3, Table 2), which together with the *TRIM33*, *TRIM24*, and *TRIM28* forms the transcriptional intermediary factor 1 (TIF1) family proteins [28] (Supplementary Fig S3).

Table 1

Demographic and clinical features of patients with CAD

Patient	Sex	MSA	MAA	Age at DM onset	Type of cancer	Time from cancer to IIM (months) ^a	DM treatment ^b	Oncology treatment ^b
DM1	F	TIF1g	ANA	49	Squamous cervix carcinoma	+ 24	No	CBDCA-VP16-radiotherapy
DM2	M	MDA5	ANA	52	Nasopharyngeal carcinoma	−4	PDN, TAC	Cisplatin-fluorouracil-Cetuximab, anti-PD-1-radiotherapy
			Ro52					
			PM-Scl-75					
DM3	M	SAE	ANA	68	Colonic neuroendocrine tumour	0	PDN, IVIG	No
DM4	F	Mi2	ANA	35	Small-cell lung cancer	0	No	CBDCA-VP16, CBDCA-paclitaxel, docetaxel-radiotherapy
DM5	F	TIF1g	ANA	63	Small-cell lung cancer	−6	PDN, MTX, IVIG	CBDCA-VP16-radiotherapy
			Ro52					
DM6	F	TIF1g	ANA	41	Cervix adenocarcinoma	0	No	No Cisplatin (
			AMA					<i>CDDP</i>)-paclitaxel-radiotherapy
DM8	F	TIF1g	ANA	61	Small-cell lung cancer	0	No	No
								<i>CBDCA-VP16</i>
DM10	F	SAE	No	44	Rectum adenocarcinoma	+ 33	No	FOLFOX, capecitabine-bevacizumab, FOLFIRI-aflibercept
DM11	F	TIF1g	ANA	35	Breast adenocarcinoma	+ 12	No	Mastectomy, fluorouracil,-doxorubicin-cyclophosphamide, paclitaxel, docetaxel-bevacizumab
DM12	M	TIF1g	ANA	64	Thymic carcinoma	+ 48 ^c	PDN	CBDCA-paclitaxel
			Ro60					
DM15	M	NXP2	ANA	66	Small-cell lung cancer	−1	PDN	No
								<i>CBDCA-VP16 then anti-PD-1, topotecan, CBDCA-VP16</i>
DM16	F	TIF1g	dsDNA	79	Gastric adenocarcinoma	−3	PDN, MMF	No
DM17	F	TIF1g	No	77	Breast (February 21)	+ 11	No	Radiotherapy + tumourectomy
					Uterus serous carcinoma (September 22)			CBDCA-paclitaxel
DM18	F	TIF1g	No	88	Ovarian serous carcinoma	0	No	No
DM19	M	TIF1g	No	64	Small-cell lung cancer	−3	PDN, MMF	No
								<i>CBDCA-VP16-antiPDL1-DLL3-CD3 antibody</i>

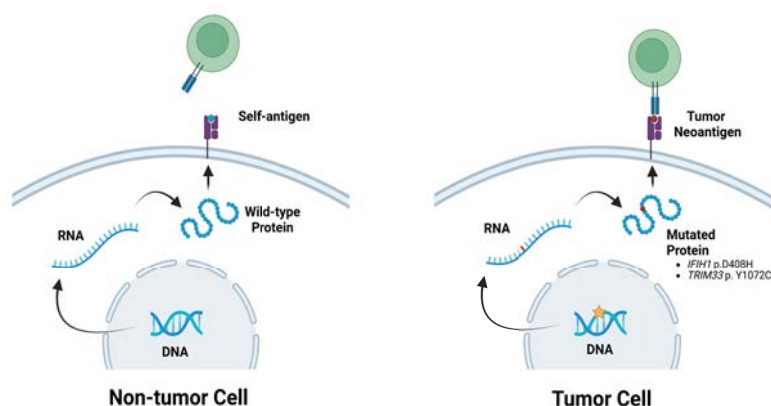
AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; CBDCA, carboplatin; DM, dermatomyositis; F, female; IHC/IF, immunohistochemistry/immunofluorescence; IIM, idiopathic inflammatory myopathies; IVIG, intravenous immunoglobulins; M, male; MAA, myositis-associated antibodies; MDA5, melanoma differentiation-associated gene 5 protein; MMF, mycophenolate mofetil; MSA, myositis-specific antibodies; MTX, methotrexate; PDN, prednisone; TAC, tacrolimus; VP16, etoposide; WES, whole-exome sequencing.

^a Negative (−) results refer to IIM diagnosed before cancer, and positive (+) results refer to IIM diagnosed after the diagnosis of cancer.

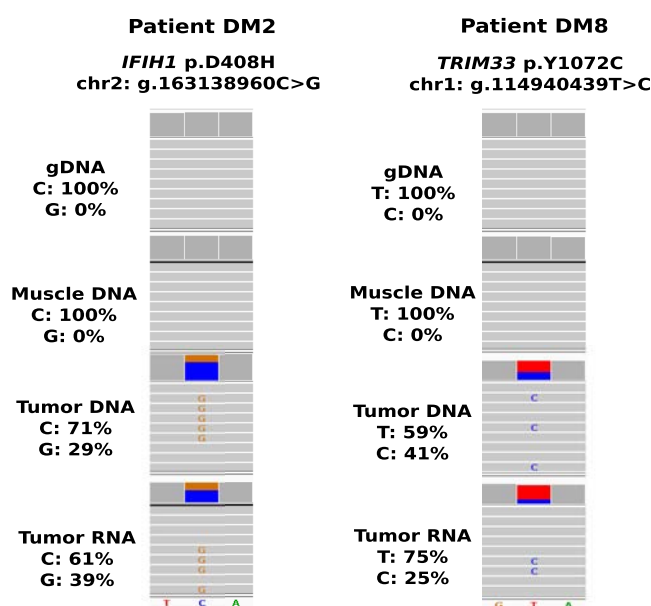
^b Refers to treatment before sample collection (blood, muscle, skin, or tumour). In *italics* treatment received after sample collection.

^c This patient had active cancer at DM onset.

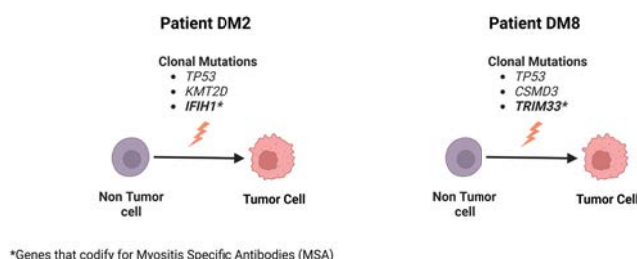
A.



B.



C.



*Genes that codify for Myositis Specific Antibodies (MSA)

Figure 2. Somatic mutations are present in genes that codify for myositis-specific antibodies (MSA). (A) In our proposed hypothetical model, somatic mutations appear in the tumorigenesis process. These mutations might lead to an alternative RNA sequence that is translated into a mutated protein. Proteins containing the aberrant sequence of amino acids are presented and immune cells recognise them as tumour neoantigens. (B) Patient DM2 and DM8 harbour somatic mutation in genes that codify for MSA (*IFIH1* and *TRIM33*, respectively), but are not present in muscle sample nor gDNA. (C) Patient DM2 and DM8 show clonal mutations containing among them, mutations in genes that codify for myositis-specific antibodies (*IFIH1* and *TRIM33*, respectively). DM, dermatomyositis; gDNA, genomic DNA; *IFIH1*, interferon-induced helicase C domain-containing protein 1; MSA, myositis-specific antibodies; *TRIM33*, tripartite motif containing 33.

Patient DM15, who presented anti-NXP2 autoantibodies, was found to have a somatic mutation in *MORC3*, the gene encoding the NXP2 protein, and LOH in Microorchidia family CW-type zinc finger 2 (*MORC2*) (Fig 4), a gene closely related structurally to *MORC3* (Fig 3, Table 1). Similarly, patient DM4, with anti-Mi2 autoantibodies presented focal amplifications in chromosome 12 that contains the *CHD4* gene, coding for Mi2b (Fig 3). Patient DM3, with anti-SAE autoantibodies, presented a gain in the whole chromosome 19, the genomic region that contains the gene UMO1 Activating Enzyme Subunit 1 (*SAE1*) (Fig 3).

Lastly, patient DM2, who tested positive for antibodies against MDA5, was diagnosed with nasopharyngeal carcinoma that exhibited a previously unreported missense somatic mutation (p.D408H) in the gene encoding interferon-induced helicase C domain-containing protein 1 (*IFIH1*), which is responsible for producing MDA5 (Fig 2B).

In stark contrast, the frequency of somatic mutations in *TRIM33*, *IFIH1*, and *MORC3* was approximately 10-fold higher in DM-associated tumours compared to large, publicly available pan-cancer datasets that are not enriched for DM. Specifically, mutations in these genes were present in 33.3% of DM-related tumours vs 3.4% of non-DM-associated tumours (368 of 10,967 samples) across 32 genomic studies in the 2Portal database (<https://www.cbioportal.org/>). Both Fisher's exact test and binomial statistical analyses demonstrated a significant enrichment of these mutations in the DM-associated cohort relative to the pan-cancer background (odds ratio = 14, $P < .001$).

Consistently, the frequency of these DM-related mutations remained low across multiple tumour types in additional pan-cancer datasets not enriched for patients with DM. For example, in the very large Cosmic catalogue of somatic mutations in cancer (COSMIC) database, their combined

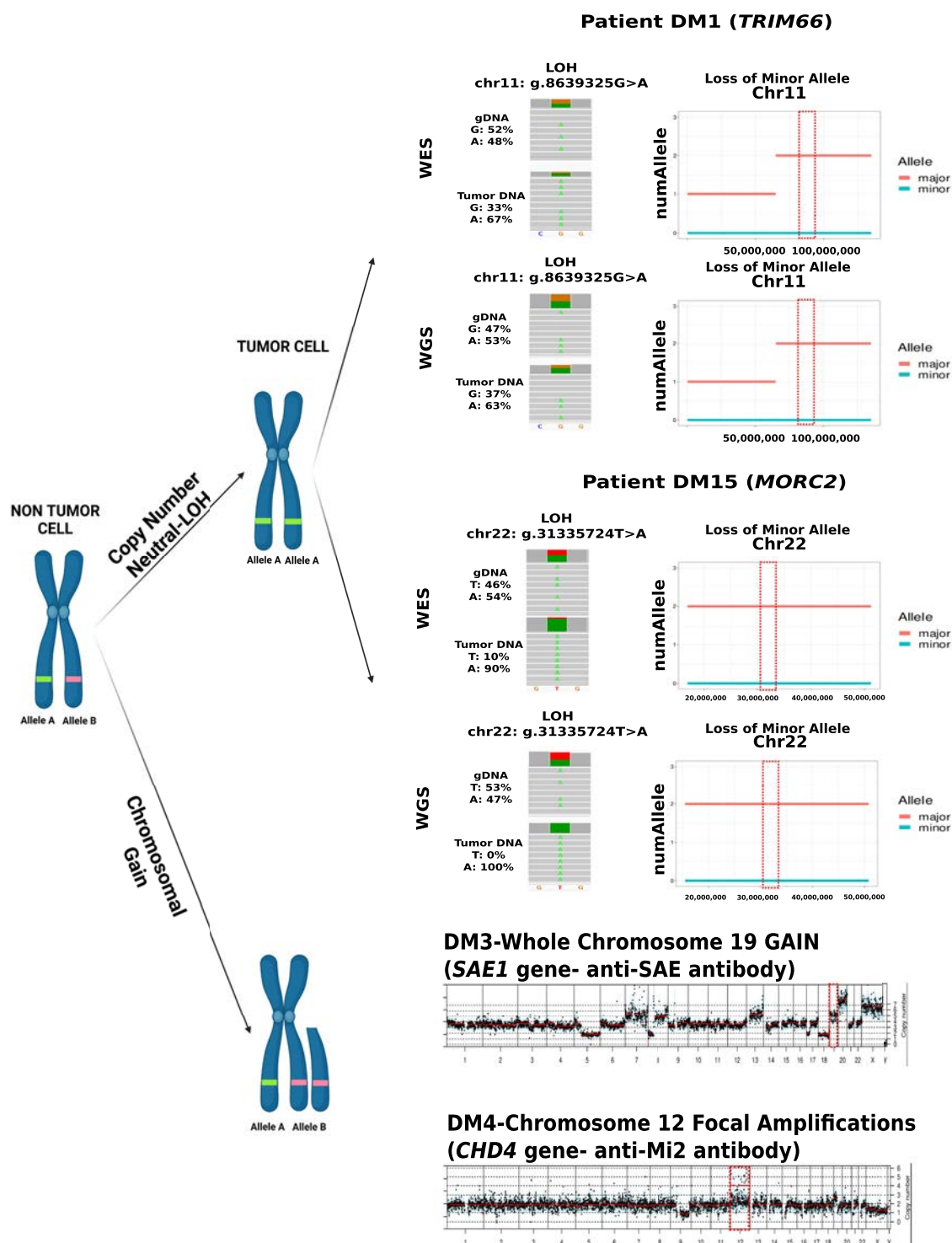


Figure 3. Tumours from cancer-associated patients with DM harbour loss of heterozygosity (LOH) and focal or whole chromosome alterations. Patients DM1 and DM15 showed copy number neutral -LOH with the loss of the minor allele and duplication of the major allele in regions that contain the sequences that codify for *TRIM66* in patient DM1 and *MORC2* in patient DM15. Patient DM3 had a whole chromosome 19 gain, which contains the *SAE1* gene that codifies for SAE antibody and patient DM4 showed focal amplifications in chromosome 12, which contains the *CHD4* gene, and that codifies for Mi2 antibody. DM, dermatomyositis; *TRIM33*, tripartite motif containing 66; Mi1, no extension; *MORC2*, Microrchidia family CW-type zinc finger 2; *SAE1*, *SUMO1* Activating Enzyme Subunit 1; Chr 11, Chromosome 11.

mutation frequency was only 0.96% (4001 out of 416,935 tumour samples). Notably, mutation of the *CHD4* gene, which encodes the Mi-2 autoantigen, showed a specific enrichment in uterine carcinoma in the cBioPortal dataset. In contrast, no somatic mutation enrichment signal was detected for any of the other DM-associated genes across non-DM-related tumour types.

Similar patterns were seen for somatic CNA; however, data on the frequency of allele-specific LOH were not available in large pan-cancer datasets. As an indirect assessment of LOH in DM-associated genes, we compared the mutant allele frequencies (MAF) between the 2 cohorts. A high somatic MAF is indicative of LOH. The average MAF of tumours from the DM cohort (Figs 2-4) was 0.75 (75%), suggestive of frequent LOH, whereas

Table 2

Frequent somatic genomic alterations in genes encoding myositis-specific antibodies (MSA) genes in tumours from patients with CAD

Patient ID	tumour type	Myositis-specific antibodies (MSA)	Somatic alterations in CAD-related genes	Genomic position	Protein change	Predicted neoantigen immunogenicity (NetMHC: strong binder ≤ 0.5 ; weak binder ≥ 2)
DM1	Squamous cervix carcinoma	anti-TIF1- γ	TRIM66 (LOH) (validated by WGS)	TRIM 66 chr11: g.8633339G>A; g.8639325G>A; g.8642218C>T; g.8644530T>G; g.8644563_8644566del-CATA; g.8646763G>C; g.8660117C>G; g.8661751C>T; g.8662516T>C; g.8664454A>G; g.8669694C>T; g.8671547G>A; g.8693405G>A	—	—
DM2	Nasopharyngeal carcinoma	anti-MDA5	IFIH1 (clonal mutation)	IFIH1 chr2: g.163138960:C>G	p. D408H	— .3966
DM3	Colonic NET	anti-SAE	Whole chromosome 19 gain	Whole chromosome 19 gain	—	—
DM4	Small-cell lung cancer	anti-Mi2	CHD4 focal amplification	—	—	—
DM5	Small-cell lung cancer	anti-TIF1- γ	Not detected	—	—	—
DM8	Small-cell lung cancer	anti-TIF1- γ	TRIM33 (clonal mutation)	TRIM33 chr1: g.114940439: T>C	p. Y1072C	.0055
DM12	Thymic carcinoma	anti-TIF1- γ	Not detected	—	—	—
DM15	Small-cell lung cancer	anti-NXP2	MORC2 (LOH) (Validated by WGS); MORC3 (mutation)	MORC2 chr22: g.31323900G>C; g.31329056G>C; g.31329954A>G; g.31333631T>C; g.31335724T>A; g.31336149G>A; g.31342376G>C; g.31342594C>T; g.31354653G>A; g.31359374_31359375insTT; MORC3 chr21: g.37706088delT	—	—
DM16	Gastric adenocarcinoma	anti-TIF1 γ	TRIM33 (mutation)	TRIM33 chr1: g.114427279G>A	p. R440C	—0.0056
DM17	Breast	anti-TIF1 γ	TRIM33 (LOH), TRIM24 (LOH), TRIM66 (LOH)	TRIM33 chr1: g.114392790-114511203 TRIM24 chr7: g.138460259-138589996 TRIM66 chr11: 8612037-8683231	—	—
DM18	Ovarian serous carcinoma	anti-TIF1 γ	TRIM33 (LOH), TRIM24 (LOH), TRIM66 (LOH)	TRIM33 chr1: g.114392790-114511203 TRIM24 chr7: g.138460259-138589996 TRIM66 chr11: g.8612037-8683231	—	—
DM19	Small-cell lung cancer	anti-TIF1 γ	TRIM33 (LOH), TRIM24 (LOH)	TRIM33 chr1: g.114392790-114511203 TRIM24 chr7: g.138460259-138589996	—	—

CAD, cancer-associated dermatomyositis; CHD4, chromodomain helicase DNA binding protein 4; DM, dermatomyositis; IFIH1, interferon-induced helicase C domain-containing protein 1; TRIM 24, tripartite motif containing 24; TRIM 33, tripartite motif containing 33; TRIM 66, tripartite motif containing 66; WGS, whole-genome sequencing.

Patient DM15

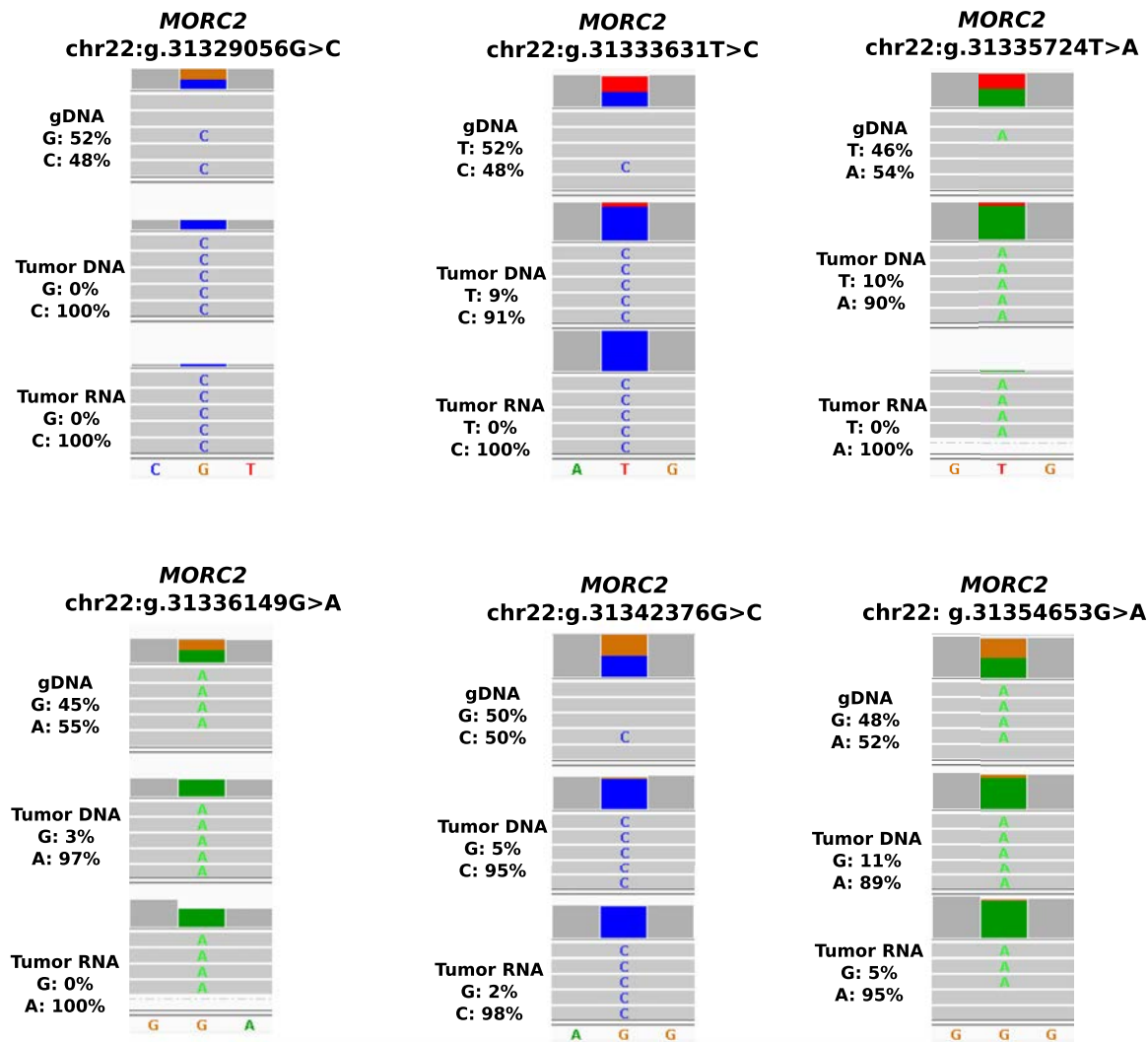


Figure 4. Patient DM15 showed a copy number neutral -LOH in *MORC2*, gene that codifies for Microorchidia CW-type zinc finger protein 2. Allelic fractions in gDNA are approximately 50%-50%, while in tumour DNA, only one allele is present. This leads to a transcriptional imbalance that is evident in the RNA from the tumour. gDNA, genomic DNA; LOH, loss of heterozygosity.

the average MAF in 1128 non-DM tumours was significantly lower (31%, t-test $P < .001$), suggesting that LOH is rare in this context. Specifically, only 2.5% of non-DM tumours exhibited a MAF higher than 0.59, which was the minimum observed among DM-associated tumours (Supplementary Table S2).

To further assess the likelihood of observing somatic mutations in autoantigen-encoding genes among patients with CAD, we compared the observed mutation frequency with the expected background mutation risk. Across the analysed cases, somatic mutations were identified in 4 out of 12 tumours in genes corresponding to known autoantibodies. Using reported baseline mutation frequencies for each gene, we calculated a cumulative P value of 1.3×10^{-5} .

Clonality of somatic mutations in cancer-associated DM-related genes

To determine when the *IFIH1* and *TRIM33* mutations occurred during tumorigenesis, we calculated the CCF based on variant allele frequency, copy number, and ploidy, as detailed in the Methods section. The CCF values for both *IFIH1* and *TRIM33* mutations were 1,

strongly indicating that these mutations arose early, likely during tumour initiation. These findings are consistent with the co-occurrence of common driver mutations such as *TP53*, *CSM3*, and *KMT2D*, all of which also exhibited CCF values greater than 0.9, in line with their role in early tumorigenesis (Supplementary Table S1).

Analysis of genetic events in paired paraneoplastic lesions and tumours

We conducted whole-exome sequencing (WES) on genomic DNA (gDNA) and paired tumour and muscle paraneoplastic lesions in 6 patients with CAD. Employing a stringent MAF cutoff of 5%, we detected somatic mutations in all sequenced muscle samples. The median number of mutations was 9.2 (ranging from 5 to 15) and a MAF ranging from 5% to 44% (Supplementary Fig S4A). The number of mutations observed in affected muscle lesions was significantly lower than that in tumours, with the number of mutations in tumours ranging from 297 to 2309 and MAFs from 5% to 98% (Supplementary Fig S4A). Importantly, no genetic events at the mutation or gene level were shared between

paired tumour and muscle lesions from the same patient, except for a single instance where the *FAM47B*, a gene with largely unknown functions, carried mutations in both the tumour (p.P25L, MAF = 32%) and muscle (p.H246P, MAF = 6%) (Supplementary Fig S4B).

HLA typing

HLA typing at the germinal level was performed on all patients, and there was no enrichment of a specific typing in our cohort (Supplementary Fig S5A–B). Somatic HLA allele-specific copy number was also analysed, and one tumour showed evidence of LOH in the tumour sample from patient DM3 (Supplementary Fig S5C).

Immune profiling in tumours

We characterised tumour-infiltration lymphocytes in 10 tumours by sequential multiplexed immunohistochemistry and grouped lesions into different categories according to the density of immune cells and the pattern of infiltration (Fig 5A, Supplementary Table S1). Two tumours were defined as ‘hot/inflamed’ based on a substantial intratumoural and peritumoral infiltration of T cells; 4 tumours were classified as ‘excluded’, indicating the presence of a considerable number of T cells at the interface between the tumour and peritumoral stroma but relatively few within the tumours themselves; another 4 tumours, all SCLCs, were categorised as ‘desert’, revealing a very low or complete absence of T cells both outside and inside the tumours (Fig 5B–D).

Immune profiling in skin paraneoplastic lesions

Immunophenotyping and RNAseq deconvolution analyses unveiled elevated infiltration of immune cells in the 2 skin lesions available for the study, from patients DM5 and DM10 (Fig 5B,C and 6A–F).

TCR-seq repertoire in tumours and skin paraneoplastic lesions

In our RNAseq data analysis, we observed significant variability in the TCR repertoire across both skin lesions and tumours. Approximately, half of the samples exhibited increased clonality, while the other half displayed a multitude of clones with relatively low frequencies (Fig 6A). Particularly striking was the divergent TCR repertoire in the paired skin lesion and tumour of patient DM5. The skin lesion exhibited a plethora of TCR clones (N = 106) with notably low frequencies (average 0.009, range 8e-5–0.049), whereas the tumour demonstrated a markedly lower number of TCR clones (N = 50) but with considerably higher frequencies (average 0.02, range 0.001–0.284). Importantly, 6 TCRs were shared between the skin and the tumour. Remarkably, all 6 of these TCRs were substantially enriched in the tumour, ranging from 4.16 to 45.35 times (Fig 6B–D).

DISCUSSION

This study reveals a high prevalence of genetic alterations in tumour cells affecting genes that encode DM-specific autoantigens in patients with CAD. These genetic alterations are exceedingly rare in databases like TCGA [17] and GENIE [29], suggesting that they could be specific to CAD. These findings suggest that such tumour-specific alterations may play a central

role in initiating the autoimmune response, potentially through the generation of neoantigens that drive the production of myositis-specific autoantibodies. In addition, the identification of distinct immunogenomic profiles and tumour-infiltrating lymphocyte patterns provides further insight into the immunopathogenesis of CAD and supports a model of paraneoplastic autoimmunity driven by early oncogenic events.

In total, genetic alterations in CAD-related genes were highly frequent (75%), strongly suggesting that such genetic alterations may serve as the initiating pathogenic basis of the disease. This finding parallels the high frequency of *POLR3A* mutations and LOH observed in tumours from patients with scleroderma paraneoplastic disease and anti-RPC1 antibodies (75%) [3].

Interestingly, we identified somatic mutations in autoantigens corresponding to the detected autoantibodies in patient groups not typically associated with cancer, with anti-MDA5 serving as a notable example. Although most patients with anti-MDA5 DM are not paraneoplastic and our cohort overall did not demonstrate a strong association between anti-MDA5 autoantibodies and cancer, this particular patient exhibited a clear cancer association and harboured a mutation in the relevant autoantigen.

These findings suggest that distinct sources of molecular mimicry may give rise to the same autoantibody. We propose that in most patients from cancer-unrelated groups, such as anti-MDA5, autoantibodies emerge through molecular mimicry triggered by noncancer sources, including viral or bacterial antigens. By contrast, in a minority of patients—such as the case described here—they may instead result from tumour-derived mutations that generate neoantigens. For autoantibodies strongly associated with malignancy, such as anti-TIF1 γ , the balance of mechanisms may be shifted towards tumour-derived sources, translating into an epidemiological association with cancer.

Based on the results in this study and previous research from our group and others, the current model of CAD disease suggests that tumour neoantigens induce myositis-specific antibodies (MSA) against the mutated neoantigen that then cross-react with the unmutated antigens found in other tissues, such as skin or muscle [10]. Consistent with this model, we identified somatic genetic events in CAD-specific genes such as *TRIM33* and *TRIM66* within the tumours. Importantly, our study extends this paradigm beyond anti-TIF1 γ -positive patients by reporting, for the first time, a somatic mutation in the *IFIH1* gene, which encodes the MDA5 protein, in a patient positive for anti-MDA5 antibodies (patient DM2, Table 1), as well as a somatic mutation in *MORC3* in a patient with anti-NXP2 autoantibodies. The new mutation in anti-MDA5 is predicted to result in the overproduction of IFN- γ according to conformational and functional studies of MDA5 protein. It is worth noting that gain-of-function mutations in the *IFIH1*^{MDA5} gene cause type 1 interferonopathies [30]. Our findings underscore the potential significance of *IFIH1*^{MDA5} mutations in triggering autoimmunity and in the development of CAD.

Moreover, recently published findings indicate that certain autoantibodies in myositis can be internalised into living cells [31,32], where they disrupt the function of their respective autoantigens, providing further evidence for a self-perpetuating cycle of the disease, particularly in patients with CAD. In this cycle, somatic mutations in tumours generate neoantigens, which trigger the production of myositis-associated autoantibodies by the immune system. These autoantibodies subsequently are internalised, targeting the corresponding wild-type antigens in affected tissues and disrupting the function of their

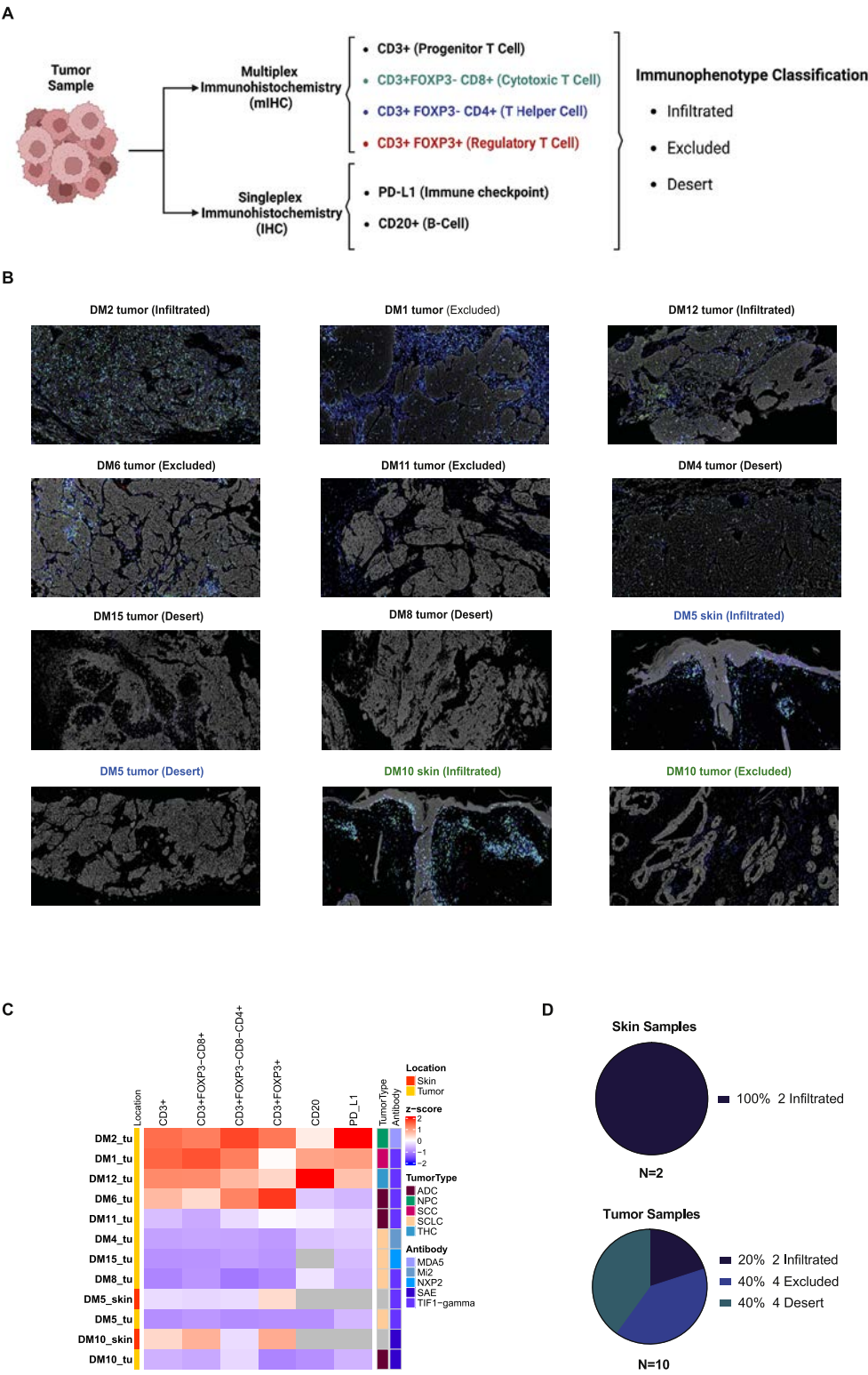


Figure 5. Immunophenotyping analyses for assessing immune infiltration status in tumour and skin samples from patients with cancer-associated DM. (A and B) Immunofluorescent and immunohistochemistry analyses from 10 tumour and 2 skin samples from cancer-associated patients with DM using next-generation immunohistochemistry. Immunophenotyping results of different markers, including CD3 + (T cell progenitor), CD3 + FOXP3– CD8 + (cytotoxic T Cell); CD3 + FOXP3– CD4 + (T helper cell); CD3 + FOXP3 + (regulatory T cell); CD20 + (B cell); PD-L1 (immune checkpoint) from patients with cancer-associated DM. (C) Density values and combined positive score for different markers obtained by multiplex Immunohistochemistry are shown. (D) Overview of the immunophenotyping results showing high infiltration in skin lesions and variable infiltration in the tumours. Patients are classified according to their detected autoantibody, tumour type, and a loss of heterozygosity in the HLA gene. DM, dermatomyositis; MDA5, melanoma differentiation-associated gene 5 protein; NPC, nasopharyngeal carcinoma; SCC, squamous cervix carcinoma; SCLC, small-cell lung cancer; THC, thymic carcinoma.

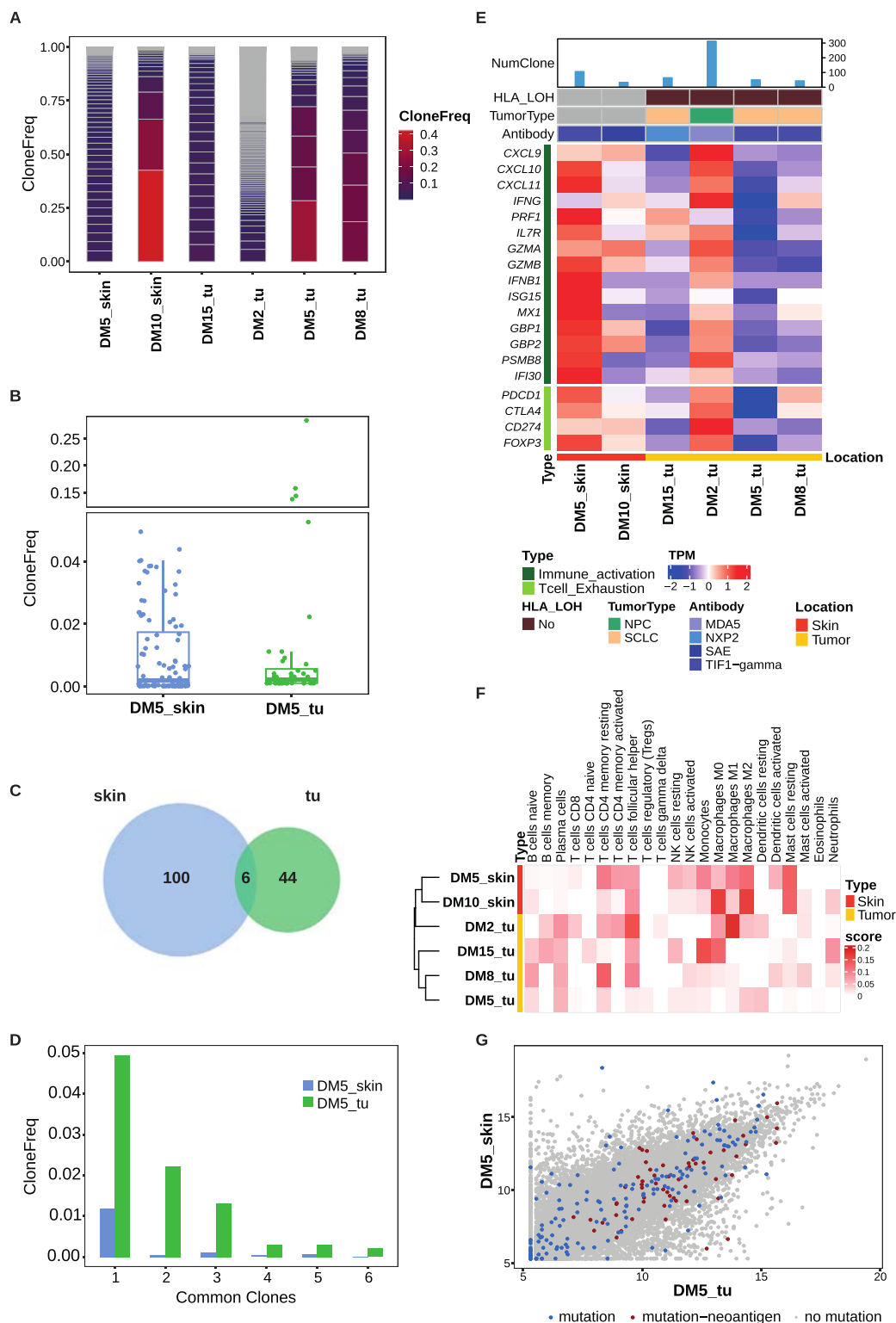


Figure 6. RNAseq-based analyses of TCR repertoire, transcriptional immune-signature, and predicted neoantigens in skin and tumour samples from cancer-associated patients with DM. (A) TCR alpha clone number and representation within 2 skin and 4 tumour samples. (B) Comparison of numbers and the proportion of the TCR clones in paired tumour and skin lesion samples of patient DM5. (C) Venn diagram showing the number of TCR clones exclusive to the skin lesion or tumour, as well as the ones shared. (D) Proportion of the 6 shared TCR clones found by the skin lesion and tumour of patient DM5. (E) RNAseq TPM scores for genes involved in immune activation and T-cell Exhaustion. Patients' auto antibody, tumour type, and status HLA loss of heterozygosity are provided. Vertical bars indicate the number of TCR alpha clones for each sample. (F) Quantification of different cell fractions from RNAseq data from skin and tumour samples from cancer-associated patients with DM using CIBERSORT. (G) Concordance of expression of mutated and nonmutated genes in paired skin lesion and tumour samples from patient DM5. Gene expression raw counts were transformed using the variance stabilising transformation from the DeSeq2 package [30]. A scatter plot of the normalised gene expression values is presented, and Pearson correlation coefficient is indicated. Genes mutated in the tumour are coloured in blue and, among them, those with high binding to the HLA and predicted to be neoantigens are coloured in red. Neoantigens were called based on the NetMHC HLA Rank threshold – for Class I HLA binding, ‘Strong’ binders have NetMHC HLA Rank score ≤ 0.5 (see the Methods section). DM, dermatomyositis; MDA5, melanoma differentiation-associated gene 5 protein; NPC, nasopharyngeal carcinoma; SCLC, small-cell lung cancer; TCR, T-cell receptor.

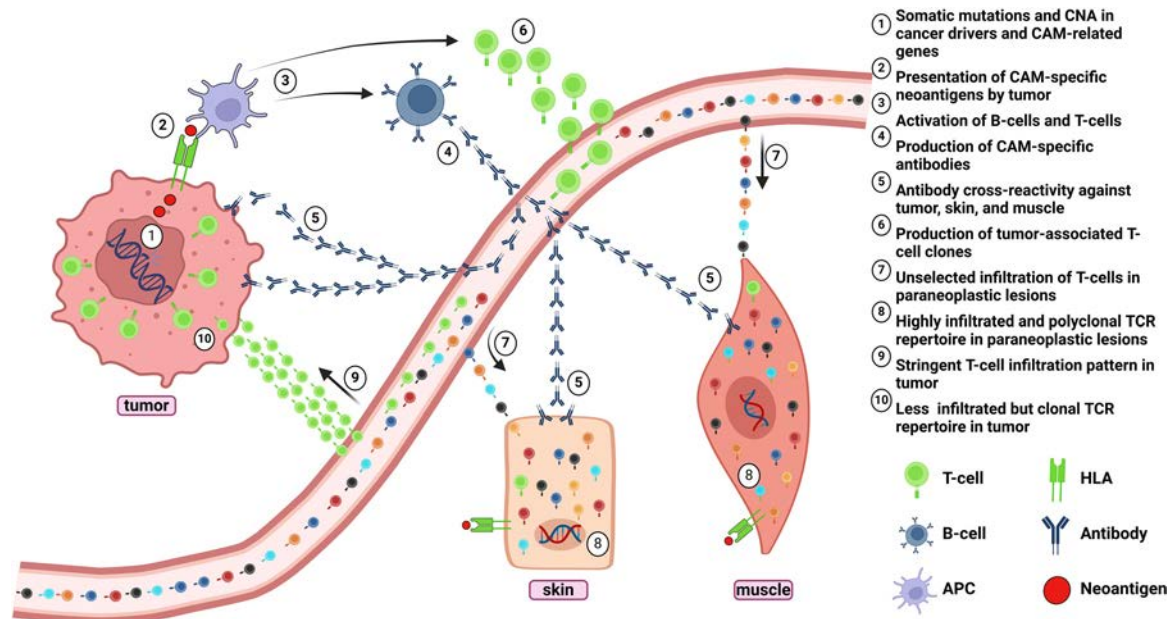


Figure 7. Hypothetical model showing sequential immuno-genetic events underlying the mechanism of the cancer-associated dermatomyositis paraneoplastic disease, as follows. (1) Occurrence of somatic mutations and CNA in cancer-associated DM-related genes such as *IFIH1* (interferon induced with helicase C domain 1); TRIM33 (tripartite motif containing 33); TRIM66 (tripartite motif containing 66); CHD4 (chromodomain helicase DNA binding protein 4); TRIM24 (tripartite motif containing 24); MORC2 (MORC family CW-type zinc finger 2); MORC3 (MORC family CW-type zinc finger 3). (2) Presentation of cancer-associated DM-specific neoantigens by tumour. (3) Activation of B-cells and T cells. (4) Production of cancer-associated DM-specific autoantibodies, such as TIF1 γ , Mi2, NXP2, SAE, and MDA5, depending on the tumour mutation. (5) Antibody cross-reactivity against tumour, skin and muscle. (6) Expansion of tumour-associated T-cell clones. (7) Unselect infiltration of a broad variety of T cells in skin, and muscle paraneoplastic lesions. (8) Polyclonal TCR repertoire and low TCR clonality in skin and muscle paraneoplastic lesions. (9) Stringent T-cell infiltration pattern in the tumour. (10) High TCR clonality in tumours from patients with cancer-associated DM. CNA, copy number alterations; DM, dermatomyositis; MDA5, melanoma differentiation-associated gene 5 protein; NTCR, T-cell receptor.

respective autoantigens, thereby contributing to the characteristic autoimmune manifestations of the disease.

Our immunophenotyping analysis revealed heterogeneous immune infiltration patterns in tumours from patients with CAD, using T-cell (CD4, CD8, FOXP3), B-cell (CD20), and Programmed death-ligand 1 (PDL1) markers. As typical of autoimmune contexts, paraneoplastic lesions predominantly exhibited an ‘inflamed’ phenotype with dense immune infiltration (Fig 5B). TCR sequencing further demonstrated minimal clonal overlap between tumours and paraneoplastic lesions, suggesting a more selective T-cell infiltration in the tumour than in areas affected with paraneoplastic autoimmunity (Fig 6A–D and 7).

SCLC is an aggressively progressing cancer that is frequently associated with various paraneoplastic syndromes [33,34]. ICIs have gained approval for use in both first- and second-line treatments for SCLC [35,36]. Distinct immune profiles have been observed depending on the type of paraneoplastic syndrome. Notably, neurologic paraneoplastic syndromes were associated with higher CD3+ T-cell infiltration (88% vs 33%) and improved overall survival compared to endocrinologic cases, even under similar treatment regimens [37]. In our small cohort of 4 patients with SCLC with CAD, tumours showed ‘cold’ immune phenotypes, lacking immune infiltration and PDL1 expression.

Despite the risk of flare and irAEs, patients with preexisting common autoimmune disease can experience clinical benefit from ICI [38,39]. However, it is currently unclear whether patients with rare autoimmune diseases, such as CAD, could benefit from these treatments in a safe manner. Notwithstanding the limited number of patients included in our study, our immunophenotyping, RNAseq, and TCRseq data reveal that the immunogenomic profiles of the paraneoplastic lesions and tumours in the patients do not seem to greatly overlap but rather are

composed of mutually exclusive somatic mutations and diverse TCR clones (Figs 5 and 6). This indicates that the autoimmune response to the muscle and skin, while likely triggered by genetic events at the tumour site, has evolved separately over time (Fig 7). Taking into consideration the distinct immune profiles, we observed in tumour and nontumoural tissues, patients with CAD could possibly benefit from the anticancer benefits of ICI without necessarily eliciting a major flare in the paraneoplastic lesions at least in some cases.

The present study, while offering valuable insights, must be interpreted within the context of its inherent limitations. Although this represents the largest cohort analysed at the molecular level to date, the modest sample size of 15 patients limits statistical power and constrains the generalisability of the findings. In addition, the expected mutation rates were derived from large publicly available datasets rather than sequencing data drawn from an identical epidemiologic background. Furthermore, the heterogeneous nature of the cancer types associated with CAD presents another natural limitation. The diverse range of cancer types, each with distinct molecular profiles and clinical characteristics, may introduce substantial variability in the results. Autoantibodies against TRIM24, TRIM28, and TRIM66—all members of the TIF1 family sharing relevant structural homology with TRIM33—were not tested, and these specificities were therefore excluded from the analysis assessing the association between cancer and myositis. The significance of these variants in relation to immune responses remains undetermined. Future research with larger independent cohorts of patients will be needed to validate our findings and generalise the observed outcomes.

In conclusion, the current study reports the largest cohort of patients with CAM with available multiomics data described to date. The results define tumour mutations and/or genetic

imbalance in genes that codify for antigenic proteins, leading to MSA as the genetic basis of CAD. Furthermore, our molecular findings demonstrating distinct immune profiles in tumour and nontumoural tissues may support future investigations into the use of ICI in patients with CAD. The comprehensive data analysis presented here reveals that tumours and paraneoplastic lesions in patients with CAD may harbour divergent genetic and immunological characteristics.

These findings provide novel insights into the interplay between autoimmunity and tumour immunity in CAD, suggesting that while specific antigens may act as common triggers for the autoimmune response, the overall immunogenomic landscapes of these conditions remain largely distinct.

This growing body of evidence supports the role of somatic mutations as potential initiators of autoimmune disease and may guide future investigations into the use of ICIs as a therapeutic strategy for patients with autoimmune disorders.

Competing interests

All authors declare they have no competing interests.

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Contributors

JR, AG-V, ET-A, RAT, and AS-OC conceived and designed the study. ABM-C, JV, AG-V, RF, GS, JJ, ET-A, JCM, JMG-J, IP-F, PN, RAT, CV, JL and AS-OC participated in sample collection and data acquisition. ABM-C, RF, GS, PN, and RAT conducted experiments. ABM-C, DD, and RAT performed bioinformatics analyses and generated figures. All authors contributed to the interpretation of data. RL-P conducted imaging and radiological analyses. RAT wrote the manuscript, and ABM-C, JV, AG-V, DD, ET-A, RL-P, PN, and AS-OC were actively involved in the review and editing process. All authors reviewed and approved the final manuscript.

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Patient consent for publication

Not applicable.

Ethics approval

The study was approved by the Ethics Commission of Vall d'Hebron University Hospital, Barcelona, Spain (PR (AG) 301/2017). The research was conducted in accordance with the Declaration of Helsinki and local data protection laws. Patients included in the study gave informed consent to the use of their clinical data and serum and tumour samples for research purposes. All data provided are anonymised in line with applicable laws and regulations.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The FASTQ files corresponding to the WES and RNAseq data from tumour, skin, and muscle samples analysed in the paper have been deposited in the European Genome-phenome Archive (EGA, <https://ega-archive.org>) under study ID EGAS50000001367 (pending) and dataset ID EGAD50000001977 (pending) with appropriate measures for controlled access (DUO: 0000020, DUO: 0000021). Clinical and molecular data for the patients are available in [Supplementary Table S1](#).

Code availability

The codes for the pipelines that were used to processing, postprocessing, filtering, validating and the immunogenomics characterisation analysis are available at the different <https://github.com> entries listed in [Supplementary Table S1](#).

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ar.2025.10.018](https://doi.org/10.1016/j.ar.2025.10.018).

Orcid

Javier Ros: <http://orcid.org/0000-0002-8137-5415>

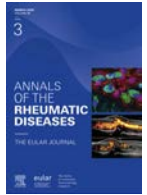
Albert Gil-Vila: <http://orcid.org/0000-0003-2786-2009>

Albert Selva-O'Callaghan: <http://orcid.org/0000-0003-2823-9761>

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Vasculitis

Spatial profiling of giant cell arteritis tissues reveals immune heterogeneity and potential predictors of glucocorticoid response

Cecilia Ansalone^{1,*}, Samuel McAllister¹, Ethan S. Pickerill², Lin Zhang², David C. Gemperline², Annie Peacock¹, Ishita Gupta¹, Dominic McGovern³, Victoria Kellior¹, Evelyn Qian¹, Aysin Tulunay Virlan¹, Maria Laura Vieri¹, Holly Leslie⁴, Yoana Doncheva⁴, Claire Kennedy Dietrich⁴, Sylvia Wright³, Paul Cauchi³, Timothy Beckman³, Lisa Hutton³, John Cole¹, Nigel B. Jamieson⁴, Isabella H. Wulur², Ajay Nirula⁵, Iain B. McInnes¹, Robert J. Benschop², Carl S. Goodyear¹, Neil Basu^{1,**}

¹ School of Infection and Immunity, University of Glasgow, Glasgow, UK

² Eli Lilly and Company, Indianapolis, IN, USA

³ NHS Greater Glasgow and Clyde, Glasgow, UK

⁴ School of Cancer Sciences, University of Glasgow, Glasgow, UK

⁵ Lilly Biotechnology Center, San Diego, CA, USA

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ABSTRACT

Objectives: Giant cell arteritis (GCA) is an immune-mediated vasculitis of large- and medium-sized arteries that can lead to systemic symptoms and irreversible vision loss. Glucocorticoids (GCs) remain the primary treatment but fail to induce sustained remission (SR) in approximately half of patients, resulting in disease relapses and significant treatment-related toxicity. We aimed to identify tissue-based markers at disease onset capable of distinguishing patients who later achieve SR from those who do not (non-remission [NR]) under GC monotherapy.

Methods: Using spatial biology techniques, we performed a comprehensive analysis of GCA-affected arterial tissues obtained at disease onset, correlating molecular profiles with clinical trajectory. We compared gene expression and immune cell populations between SR and NR groups, corroborated key findings by immunohistochemistry, and evaluated the diagnostic performance of identified biomarkers.

Results: Patients with NR exhibited an upregulation of extracellular matrix remodelling (ECM) and T cell activation pathways, reflecting persistent inflammation and fibrotic-like responses. By contrast, SR cases were distinguished by an enrichment of immunoglobulin G-producing plasma cells in the adventitia, which correlated with increased macrophage infiltration in the intima. Quantitative analyses suggested that combining plasma cell and macrophage markers could accurately predict GC responsiveness.

*Correspondence to Dr. Cecilia Ansalone, School of Infection and Immunity, College of Medical, Veterinary & Life Sciences, Glasgow, UK.

**Correspondence to Prof. Neil Basu, School of Infection and Immunity, College of Medical, Veterinary & Life Sciences, Glasgow, UK.

E-mail addresses: Cecilia.ansalone@glasgow.ac.uk (C. Ansalone), neil.basu@glasgow.ac.uk (N. Basu).

Carl S. Goodyear and Neil Basu contributed equally.

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Conclusions: Our findings reveal an immunopathologic signature in GCA at diagnosis, characterised by plasma cell-rich infiltrates associated with favourable outcomes, and ECM- and T cell-associated inflammation enriched in GC-refractory cases. These insights could foster a precision medicine approach to GCA management, enabling patient stratification for GC-sparing therapies and optimising patient outcomes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Approximately half of patients with giant cell arteritis (GCA) fail to achieve sustained remission on glucocorticoids alone.
- Tissue-based predictors of glucocorticoid response remain poorly defined.

WHAT THIS STUDY ADDS

- This is the first study to spatially resolve transcriptomic and immune cell profiles across arterial wall layers in GCA, revealing compartment-specific immunopathological and molecular features linked to treatment outcome.
- Responders (sustained remission) show a strong plasma cell signature in the adventitia, whereas non-responders exhibit broader inflammatory activation including extracellular matrix remodelling and T cell-related pathways.
- The combined presence of plasma cells in the adventitia and macrophages in the media/intima has the potential to predict response to glucocorticoids.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- These findings support the feasibility of tissue-based stratification in GCA and highlight spatial profiling as a promising tool for precision medicine.
- The dataset offers a valuable resource for mechanistic studies of GCA immunopathology and encourages prospective validation of candidate tissue biomarkers to guide both trial design and treatment decisions.

INTRODUCTION

Giant cell arteritis (GCA) is a prevalent immune-mediated inflammatory disease (IMID) affecting predominantly medium- and large-sized arteries, most notably the branches of the carotid artery. International guidelines now recommend advanced imaging modalities as the first-line investigations for suspected GCA, yet temporal artery biopsies (TABs) remain the gold-standard confirmatory investigation, providing the highest diagnostic specificity and a rich source of tissue-based information on disease pathogenesis [1–3]. TABs often demonstrate distinct, layer-specific changes within the inflamed vessel wall. Lymphohistiocytic and granulomatous infiltrates typically localise in the adventitia or extend across the vessel wall, with intimal hyperplasia and internal elastic lamina disruption contributing to luminal narrowing that may lead to profound ischaemic complications, such as irreversible vision loss [4].

Despite the central role of high-dose glucocorticoids (GCs) in preventing acute vision loss, up to half of patients fail to maintain remission when GC dosages are tapered, necessitating re-escalation and posing a risk for significant GC-related toxicities (eg, diabetes, osteoporosis, infections) [1,5,6]. While adjunctive immunosuppressants such as methotrexate or tocilizumab (an interleukin-6 [IL-6] receptor inhibitor) can reduce GC exposure, 20% to 50% of patients still experience relapses, and concerns over the safety and cost of these therapies limit their broader clinical use [7].

Given the clinical and pathophysiological heterogeneity of GCA, there is a growing rationale for a precision medicine approach aimed at tailoring therapies based on individual disease biology [8]. Although successful implementation of precision medicine has been most clearly demonstrated in oncology—where gene expression signatures and molecular markers routinely guide treatment selection—such approaches are increasingly being explored in IMIDs. Precision-based approaches have gained traction in rheumatoid arthritis, where tissue-based pathotypes have been proposed [9]—yet practical challenges, including limited access to tissue, have impeded widespread adoption, and wider clinical translation remains limited to research settings. In GCA, TABs are part of routine practice, offering a valuable opportunity to define disease subtypes through direct assessment of the vascular immune microenvironment.

Earlier attempts to identify tissue predictors of GC response in GCA used classical histology, targeted immunostaining, or bulk molecular profiling. These approaches have shown inconsistent reproducibility and limited sensitivity [10–12]. Bulk transcriptomics averages signals from all cell subsets, potentially obscuring critical differences in less abundant but biologically relevant compartments. Targeted immunostaining provides spatial context but often focuses on only a few markers. In contrast, emerging spatial biology platforms generate an unbiased, high-dimensional map of gene or protein expression within intact tissues, allowing resolution of the complex multicellular landscape. This approach has already illustrated the complex and varied immunopathological landscape of GCA across each arterial layer, highlighting layer-specific immune responses and the potential to identify novel therapeutic targets [13].

We applied advanced spatial biology tools to comprehensively profile GCA-affected arterial tissues, focusing on layer-specific and cell type-specific signatures. The primary objective of our study was to characterise the immune pathways influencing differential treatment responses in GCA, with the overall goal of informing precision medicine strategies that could reduce reliance on GCs. Additionally, we explored potential biomarkers that might predict patient outcomes at disease onset.

METHODS

Study design summary

This retrospective study examined archived formalin-fixed, paraffin-embedded (FFPE) TABs from patients evaluated for suspected GCA. One-year outcomes classified patients as sustained remission (SR), or non-remission (NR) based on the 2018 European Alliance of Associations for Rheumatology (EULAR) recommendation for management [6]. SR was defined as the absence of clinical signs or symptoms attributable to active vasculitis, normalisation of erythrocyte sedimentation rate and C-reactive protein, and maintenance of remission for at least 12 months while receiving ≤ 5 mg/d of prednisolone. NR cases had elevated acute-phase reactants or clinical relapse requiring GC escalation or therapy change; non-GCA controls (CTRL) were also included (n = 4). In total, 4 CTRL, 12 NR, and 15 SR cases

Table 1
Participant characteristics

	Non-GCA controls (CTRL) (n = 4)	GCA non-remission (NR) (n = 12)	GCA sustained remission (SR) (n = 15)
Age	62.2 ± 15.4	73.0 ± 8.7	75.8 ± 5.5
Sex	1 (3)	2 (10)	7 (8)
Days on GCs	9.5 ± 5.3	9.6 ± 6.3	7.8 ± 4.7
Prebiopsy GCs	485.0 ± 162.8	487.5 ± 353.1	431.0 ± 254.5
Baseline CRP	61.7 ± 17.4	125.0 ± 78.6	99.4 ± 56.8
Baseline ESR	41.8 ± 10.3	84.3 ± 30.7	75.1 ± 35.6

CRP, C-reactive protein in milligrams per litre (mg/L); ESR, erythrocytes sedimentation rates in millilitres per hour; GCs, glucocorticoids; GCA, giant cell arteritis.
Prebiopsy GCs are the cumulative dose of glucocorticoids given prebiopsy in mg. Sex refers to biological sex, as recorded in clinical records, and is presented in the format of males (females). All values are mean ± SD.

passed all quality checks (Table 1) and were used across the different platforms, as specified in the Results sections and figure legends. Ethical approval was granted by West of Scotland REC (22/WS/0020). All included GCA cases met predefined histopathological criteria for active arteritis, and blinded histological scoring revealed no statistically significant differences between SR and NR groups (Supplementary Fig S1). The majority of these samples were profiled by spatial transcriptomics using the GeoMx Digital Spatial Profiler. Differentially expressed genes (Benjamini-Hochberg adjusted *P* value [*P*-adj] <.05, |log2FC| >0.5) were identified using DESeq2, followed by pathway enrichment (ClusterProfiler), and immune deconvolution (CIBERSORTx). A smaller subset of GeoMx-profiled cases with available serial sections underwent multiplex tissue imaging using PhenoCycler/CODEX. In parallel, independent samples that were not used for CODEX were employed for validation by immunohistochemistry and immunofluorescence. Two-way analysis of variance was used to compare cell frequencies derived from tissue imaging. Biomarker performance was assessed by receiver operating characteristic curves, employing repeated cross-validation and bootstrapping to derive 95% confidence intervals (CIs) at 90% sensitivity. Detailed protocols—covering specimen retrieval, patient inclusion and exclusion criteria, histology, sample preparation, data acquisition, normalisation, and statistical analyses—are provided in the Supplementary Methods.

RESULTS

Spatial transcriptomic analysis reveals gene expression divergence based on clinical response at each arterial layer

To detect and characterise gene expression within topographical regions of the arterial tissue—adventitia, media, and intima—polygonal regions of interest (ROIs) were selected based on vessel morphology in GCA cases, including both NR and SR groups, as well as non-GCA control tissues (n = 8 NR, n = 11 SR, and n = 4 CTRL) (Fig 1A,B). Fold vs fold-change scatter plots comparing transcript expression between SR and NR groups, relative to controls, revealed a strong positive correlation (Spearman’s correlation coefficient = 0.7–0.8), reflecting broad transcriptional similarity between SR and NR cases when compared to non-GCA controls (Fig 1C). There were similarities across the most differentiating genes in the adventitia, media, and intima, including immunoglobulin, antigen presenting, and extracellular matrix (ECM) genes. For instance, key upregulated genes that clustered in the bottom-left quadrant across all layers (Fig 1C) included immunoglobulin G (IgG) and A (IgA) genes, such as *IGHG1*,

IGHG2, *IGHG3*, *IGHG4*, and *IGHA1* (Fig 1C). Notably, while these immunoglobulin-related genes were significantly upregulated in both NR and SR groups in comparison to controls, their expression levels varied between the 2 groups and in each vessel layer, with the highest expression in the adventitia layer of SR cases (Fig 1D, top panel). Moreover, a subset of genes was uniquely upregulated in NR cases vs controls, including *CCL2*, *COL6A1*, and *VCAN*, with *VCAN* displaying significant elevation, particularly in the intima of NR cases. Conversely, SR-specific upregulation vs controls was observed for genes like *SPP1*, which was specifically enriched in the intima, and *CCL3*, which was upregulated across all layers (Fig 1D, bottom panel).

Adventitia as the primary site of immune and structural divergence in GCA tissue pathology in NR and SR trajectories

Given the observed differences in the magnitude and distribution of gene expression between NR and SR groups across arterial layers, we next focused on direct comparisons between NR and SR within each discrete compartment. The adventitia layer exhibited the highest number of differentially expressed genes (DEGs), with 197 DEGs identified, compared to 70 in the media and 61 in the intima (Supplementary Fig S2). This indicated that the adventitia was the principal region of variation between the 2 clinical groups. In particular, 102 genes were upregulated in the adventitia of NR, while 95 were upregulated in SR (Fig 2A). Pathway enrichment analysis of upregulated genes in NR revealed numerous significantly enriched pathways across all 3 arterial layers (Supplementary File S1–S3), many of which were involved in regulating ECM synthesis and degradation, smooth muscle contraction, and structural integrity. In particular, pathways such as ‘focal adhesion’ and ‘degradation of the ECM’ were highly prominent in the adventitia (Fig 2B,C) and mirrored in the media and intima by similar pathways (Supplementary Fig S3a and e). Consistently upregulated structural genes (eg, *ACTA2*, *ACTG2*, *TAGLN*) and ECM genes (eg, *COL1A1*, *COL1A2*, *COL6A1*, *COL6A2*) suggested an inflammation-driven fibrotic response and active tissue remodelling across all the layers of NR cases. Additionally, ‘regulation of chemotaxis’ was also among the top enriched pathways in NR adventitia and included *CCL2*, which has been previously associated with clinical relapse and persistence of disease [11].

Pathway enrichment of upregulated genes in SR showed 4 significant pathways in the adventitia and 2 in the media and intima (Fig 2D, Supplementary Fig S3c and g, and Supplementary File S1–S3). The top pathway in the SR adventitia was ‘positive regulation of T cell activation’, characterised by upregulated genes that overlapped with those in the ‘B-lymphocyte

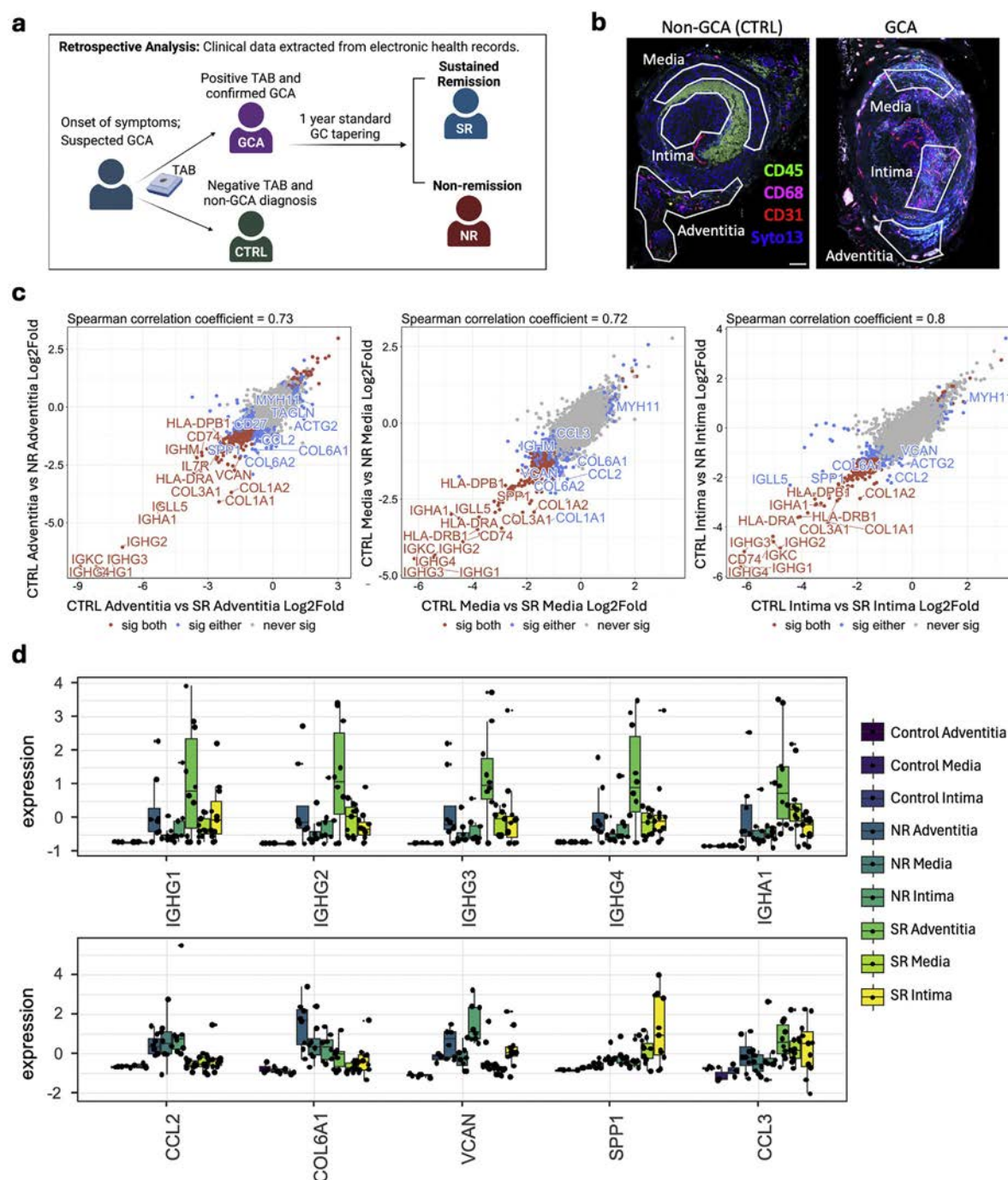


Figure 1. Spatial transcriptomic analysis of arterial layers reveals overlapping and divergent gene expression between GCA clinical response groups (NR and SR) and controls. Spatial transcriptomic analysis was performed on GCA cases, including non-remission (NR) and sustained remission (SR) groups, compared to non-GCA control tissues (CTRL) ($n = 8$ NR, $n = 11$ SR, and $n = 4$ CTRL) across the adventitia, media, and intima layers. (a) Schematic of the study design, outlining sample selection and grouping. (b) Representative images of temporal artery biopsy (TAB) staining (CD45 in green, CD68 in magenta, CD31 in red, and Syto13 in blue) with corresponding regions of interest (ROIs) selected for adventitia, media, and intima layers, delineated by white continuous lines. Pale green staining observed in the CTRL section reflects autofluorescence from red blood cells. (c) Fold-change vs fold-change scatter plots comparing transcript expression in SR and NR groups relative to controls, for adventitia (left), media (middle), and intima (right) ROIs. Each gene is represented by a dot. Log2 fold changes are displayed on the x- and y-axes. Genes significantly upregulated in both comparisons (adjusted $P < .05$, absolute log2 fold change > 0.5) are highlighted in red, significant in only 1 comparison in blue, and nonsignificant genes in grey. When comparing NR to CTRL, there were 515 (423 upregulated), 344 (336 upregulated), and 336 (304 upregulated) DEGs in adventitia, media, and intima, respectively; while for SR vs CTRL, the corresponding numbers were 768 (590 upregulated), 488 (413 upregulated), and 423 (362 upregulated). (d) Box plots of selected genes that are either upregulated in both comparisons (top panel) or only in 1 comparison (bottom panel). Expression levels are row-scaled into z-scores. Each dot represents an independent case, with the line indicating mean expression. DEG, differentially expressed genes.

cell line' pathway (Fig 2D,E). Notably, *CD27*—a marker of memory B cells—was enriched in the SR adventitia, indicating a more accentuated B cell signature in this group. Furthermore, in the media layer of SR, there were a few cathepsin genes that

clustered together (ie, *CTSB*, *CTSH*, and *CTSS*), while in the intima there were genes related to oxidative stress responses and metabolism (ie, *CYBB*, *HSF1*, and *MT1G*) (Supplementary Fig S3g,h). Among these, *CTSS*, *CHIT1*, and *CYBB* are highly

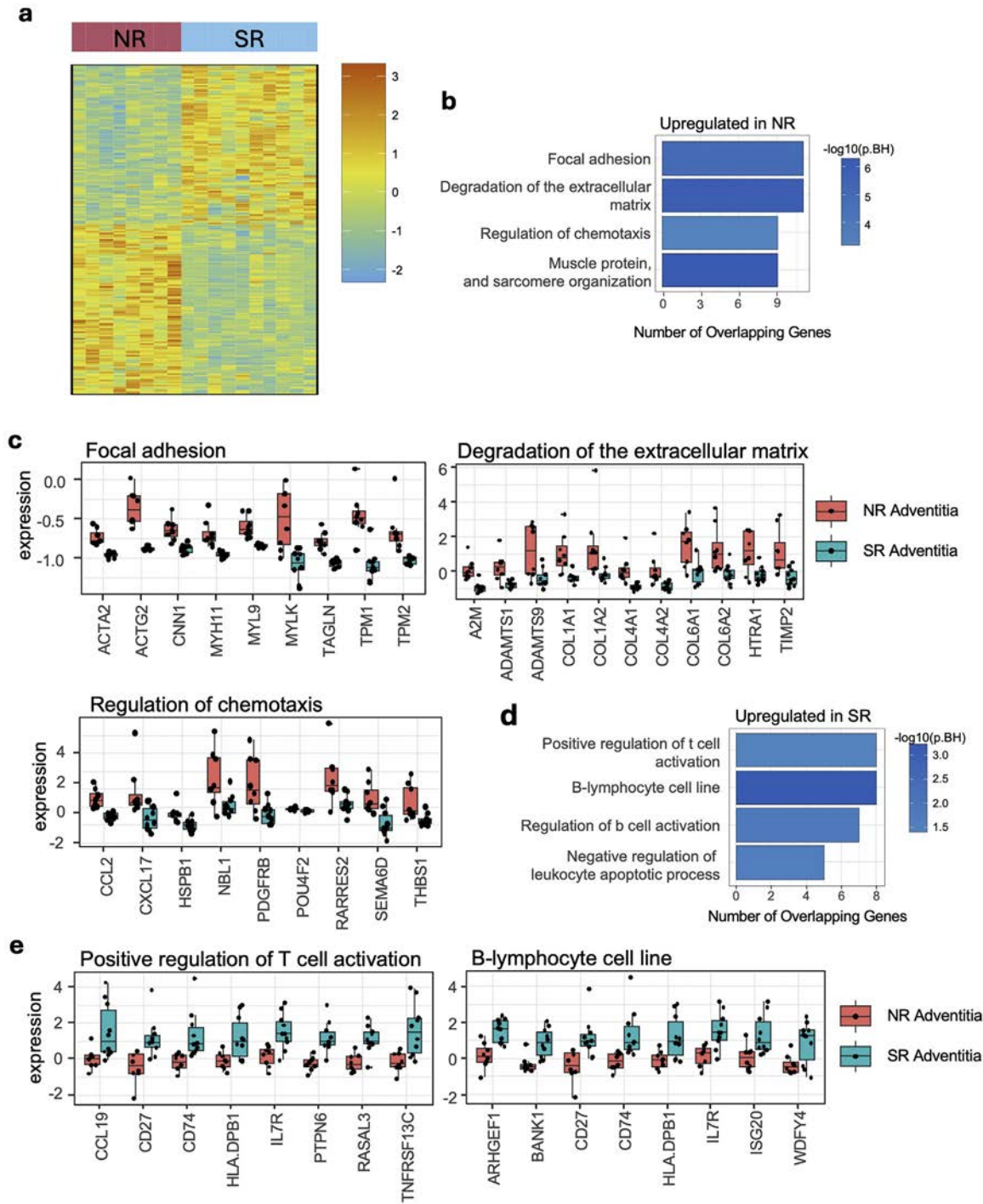


Figure 2. Pathway enrichment analysis of differentially expressed genes (DEGs) in the adventitia layer highlights molecular divergence between sustained remission (SR) and non-remission (NR) cases. (a) Heatmap of significantly DEGs between NR and SR, with colour intensity reflecting expression levels (blue: low, red: high). Data are row-scaled to z-scores. (b–e) STRING Pathway enrichment analysis using DEGs in the adventitia layer between NR and SR. (b) Bar chart of selected pathways significantly upregulated in the NR adventitia layer, and (c) boxplots showing the expression of genes from the top 3 NR-upregulated pathways in both NR and SR groups. (d) Bar chart of significantly upregulated pathways in the SR adventitia layer, and (e) boxplots of gene expression from the top 2 SR-upregulated pathways in both NR and SR groups. In (b) and (d), pathways are listed on the y-axis and bars indicate numbers of genes associated with a given pathway and coloured by significance (adjusted *P* value, *p.BH*). In (c) and (e), expression values are row-scaled to z-scores, with each dot representing an independent case. The line indicates the mean expression.

expressed by activated macrophages, particularly in response to inflammatory stimuli, and their upregulation could indicate an activated macrophage phenotype in the SR media and intima layers.

In summary, molecular differences between NR and SR highlight distinct immune pathways, particularly in the adventitia, that may influence clinical outcomes.

Molecular characterisation of adventitial immune infiltrates highlights a more diverse inflammatory signature in NR cases

Lack of response remains a hallmark of GCA treatment limitations, as persistent inflammation (especially in the adventitia) and fibrosis lead to vascular complications [14]. Therefore, characterising the immune pathways that persist at disease

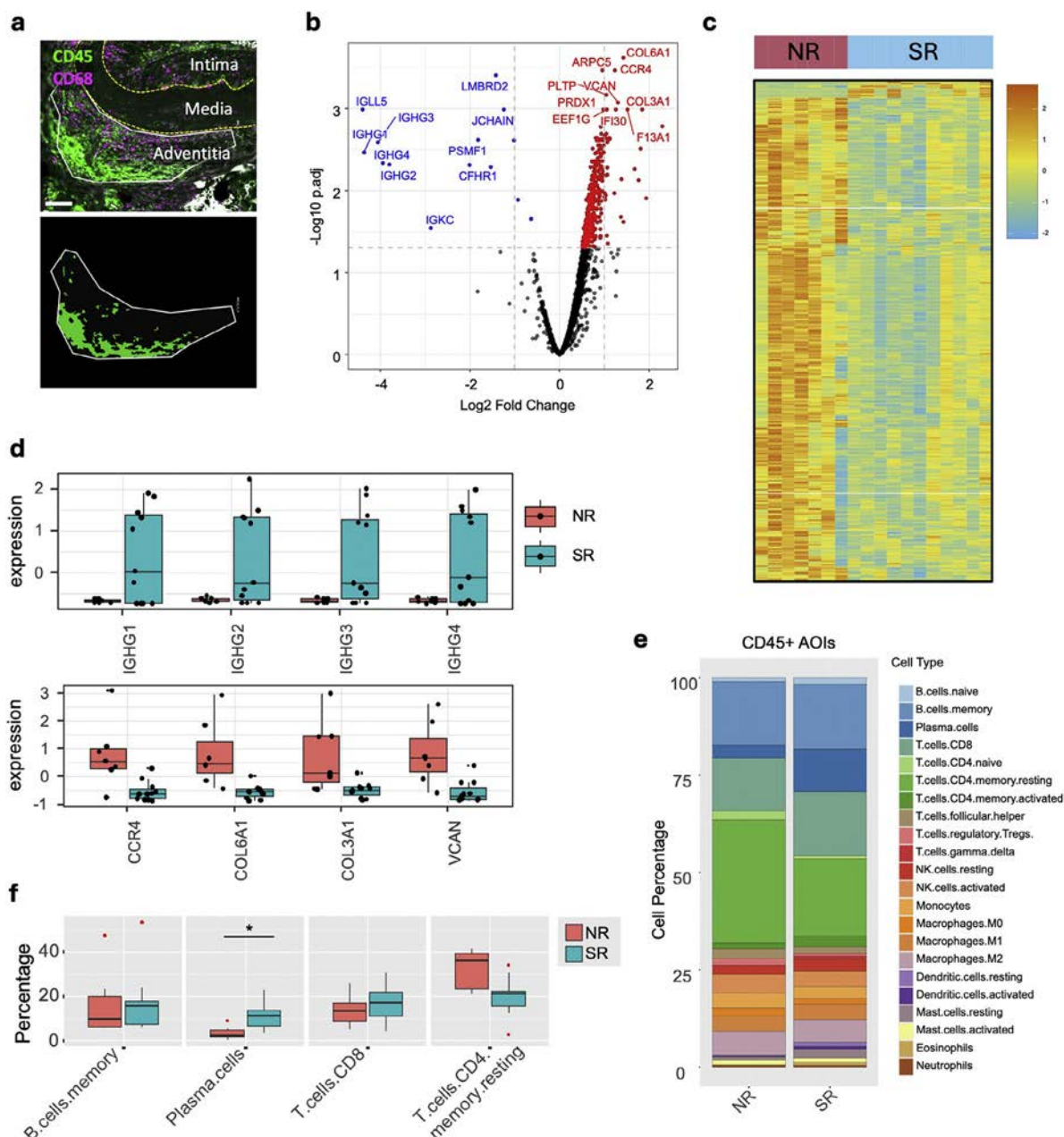


Figure 3. Distinct molecular signatures in adventitial immune infiltrates at disease onset indicate differences in immune cell composition between sustained remission (SR) and non-remission (NR) cases. Spatial transcriptomic analysis of CD45+ areas of interest (AOIs) within adventitial regions of interest (ROIs). (a) Representative images of TAB staining (top image; green for CD45+ leukocytes and magenta for CD68+ macrophages) and corresponding area selection of an CD45+ AOI for spatial transcriptomic analysis (bottom image). The yellow dotted line delineates the vessel layers (adventitia, media, and intima); the thin continuous white line outlines the adventitia ROI selected; and the green mask within indicates the CD45+ AOI selected for profiling. White bar = 100 μ m. (b–d) Differential expression analysis between NR and SR CD45+ AOIs. Statistical significance was calculated using the Wald test as described in DESeq2 (adjusted P value < .05). (b) Volcano plot of significantly differentially expressed genes (DEGs) either upregulated in NR (red) or upregulated in SR (blue) CD45+ AOIs, with the top 10 genes labelled. (c) Heatmap visualising the difference in expression values for all significant genes determined between the differential expression analysis between NR and SR. (d) Box plots of the expression of selected genes significantly upregulated in SR (top) and NR (bottom). Expression levels have been row-scaled into z-scores. Each dot represents an independent case, with a line indicating the mean expression. (e, f) Cell deconvolution analysis using the LM22 cell annotation matrix. (e) Each colour corresponds to the average percentage value for each cell type, in each condition. (f) Boxplots of the top 4 enriched cell types. Statistical analysis was done using 2-way analysis of variance and Šidák's multiple comparisons test to compare NR to SR. $* = P \leq .01$. TAB, temporal artery biopsy; LM22, leukocyte gene signature matrix of 22 immune cell types.

onset and are associated with subsequent relapse despite initial, however minimal, GC treatment can provide insights into the mechanism of incomplete response and guide the development of more targeted therapies. Given that immune infiltrates are often abundant in the adventitia—particularly in early or focal lesions—and that this layer showed the most robust molecular differences between NR and SR, we directly profiled immune infiltrates in the adventitia, where sufficient cellularity could be

consistently captured. We selected CD45 as a marker for leukocytes to accurately guide the masking of areas of interest (referred to as CD45+ areas of interest [AOIs] within ROIs at the adventitia and/or at the adventitia-media junction ($n = 7$ NR, $n = 11$ SR) (Fig 3A).

There were 421 DEGs in CD45+ AOI between NR and SR, with 407 upregulated in NR and only 14 in SR (Fig 3B,C and Supplementary File S4). Among these 14 genes, 6 were

immunoglobulin genes and included all the IgG heavy chain genes (Fig 3B, D). Cell deconvolution analysis revealed that, consistent with the upregulation of IgG genes, the plasma cell (PC) molecular signature was significantly enriched in SR cases (Fig 3E,F). The signature associated with other major immune cell types, including memory B cells and T cell subgroups, was comparable in SR and NR.

Among all immune cell subsets, ‘T cell CD4 memory resting’ accounted for the largest molecular signature in NR, followed by CD8 T cells and memory B cells (Fig 3E). Consistent with the ‘T cell CD4 memory resting’ signature in NR, *CCR4* was among the most upregulated genes. *CCR4* is a marker for various T cell subsets, including effector memory T cells, and is involved in directing T cell migration and function. Although CD45⁺ AOs may include minor stromal signal due to spatial spillover, immune transcripts predominated, supporting the use of the LM22 matrix for deconvolution. The detection of ECM-related transcripts (eg, *VCAN*, *COL6A1*, *COL3A1*) in NR AOs likely reflects their marked upregulation and proximity to immune infiltrates, consistent with patterns observed in the full adventitia layer (Fig 3B–D).

These data indicate that the molecular profile of immune infiltrates at disease onset differs based on the subsequent clinical trajectory. In particular, a specific and narrowed PC signature characterises SR cases, while NR have a more diverse transcriptional landscape, with upregulation of genes that point to the key involvement of ECM organisation and T cells.

Adventitial immune infiltrates in NR cases associate with tissue remodelling and Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling

To further understand the molecular signature of NR leukocyte milieu, we performed pathway enrichment analysis for the DEGs upregulated in NR, using gene ontology (GO) enrichment analysis and STRING (Search Tool for the Retrieval of Interacting Genes/Proteins database) (Fig 4A,B). Among the DEGs, over 70 genes were associated with translational initiation and ribosomal activity, suggesting elevated protein synthesis. To avoid skewing the analysis, we excluded these genes from the pathway enrichment, allowing us to focus on other biologically relevant processes and pathways. Among the most significant pathways in the GO analysis, there were inflammatory pathways such as ‘actin filament organisation’, ‘positive regulation of cell adhesion’, ‘leukocyte and myeloid migration’, ‘response to type II interferon’, and ‘regulation of T cell activation’ (Fig 4A and Supplementary File S5). These pathways were reflected in the STRING enrichment analysis such as ‘T-lymphocytes’, ‘regulation of actin filament organisation’, and ‘T cell receptor signalling pathway’ (Fig 4B and Supplementary File S6). Consistently, in the STRINGdb network analysis, there were nodes associated with chemokine signalling pathway, degradation of ECM, smooth muscle contraction, and interferon signalling. There were also 2 orderly clusters associated with glycolysis and cellular response to stress (eg, *COX6C*, *COX6B1*, *COX7C*, *COX8A*), alongside with metabolism of protein and RNA, indicating a highly activated cellular state (Fig 4C and Supplementary Files S7,S8).

Further interrogation of the STRINGdb network revealed complex nodes associated with monocytes (*CD14*) and T cells (*CD247*), suggesting that these are in a central role in the NR pathology. Further evaluation of the *CD14* node revealed association with *SPI1* (encoding for the transcription factor PU.1) and *IKKB* (encoding the IKK β protein) (Fig 4C). The former is a key

regulator of macrophage differentiation, and the latter is downstream of the canonical NF- κ B signalling pathway, promoting expression of proinflammatory cytokines. T cell-related pathways were identified as well, including IL-12 signalling, with *JAK1* and *STAT4* as key upregulated genes in NR (Fig 4C,D and Supplementary Files S7,S8). IL-12 promotes Th1 cell differentiation from naïve CD4⁺ T cells and stimulates the production of interferon-gamma (IFN- γ or type II IFN), which is a hallmark cytokine of the Th1 response. Conversely, *JAK1* was also associated with *INFR2* (encoding for interferon gamma receptor 2) as part of the IFN- γ signalling (Fig 4C). This latter pathway also related to the antigen presentation genes *HLA-DRA* and *HLA-DPA1*, which clustered with genes involved in proteolysis (*CTSB*, *CTSC*, *CTSL*) and degradation of ECM (*MMP9*, *COL1A2*, *COL3A1*, *COL6A1*, *COL6A2*, *COL6A3*) (Fig 4C and Supplementary Files S7, S8). Although GO and STRING analyses highlighted overlapping biological processes, some divergence in pathway outputs was observed, reflecting differences in their underlying enrichment strategies and interpretive scope.

In summary, our data indicate that areas occupied by immune infiltrates in NR TABs display a molecular signature of contractile and metabolic stress, protein proteolysis, and ECM degradation, alongside IFN- γ and JAK1/STAT4 signalling. These pathway enrichment patterns likely reflect both immune-intrinsic transcriptional activity and the complex cellular milieu of the inflammatory microenvironment.

Immune cell distribution based on protein expression reveals increased adventitial PCs and intimal macrophage infiltration in SR cases

Given the diverse immune landscape of NR and SR as revealed by spatial gene expression analysis, we hypothesised that differences in the composition and distribution of immune cells across arterial layers could distinguish the two clinical trajectories a priori. To investigate this, we further characterised the distribution and abundance of major immune cell types, as well as vascular smooth muscle cells (VSMCs) and endothelial cells for structural context, within each arterial layer based on protein marker expression. Sequential tissue sections from a smaller cohort of NR, SR, and control samples (n = 5 NR, n = 6 SR, n = 4 CTRL) were analysed using CODEX technology and k-Means clustering (Supplementary Fig. S4a, b).

Compared to controls, both NR and SR samples exhibited a significantly higher number of CD45⁺ leukocytes, accompanied by a reduction in alpha-smooth muscle actin (α SMA)⁺ VSMCs (Supplementary Fig S4c), indicating a similar extent of immune infiltration and medial damage. A trained random forest algorithm further classified CD45⁺ leukocytes into CD3 ϵ ⁺ lymphocytes (interpreted as T cells in this tissue context), CD20⁺ B cells, and CD68⁺ macrophages (Fig 5A and Supplementary Fig S4a,b). Quantification of these immune cell subsets across specific ROIs, defined for the adventitia, media, and intima, revealed that T cells and macrophages were the most abundant cell subsets overall (Supplementary Fig S3d).

Macrophage distribution differed between NR and SR tissues, with SR cases having higher numbers in the intima than NR (Fig 5B,C and Supplementary Fig S4d). These findings were consistent with the upregulation of the *CD14* gene observed in NR adventitial immune infiltrates (Fig 4C), as well as with the presence of macrophage-associated genes in the SR intima (Supplementary Fig S3g,h). Additionally, CD138⁺ PCs were significantly overrepresented in the adventitia of SR cases compared to NR

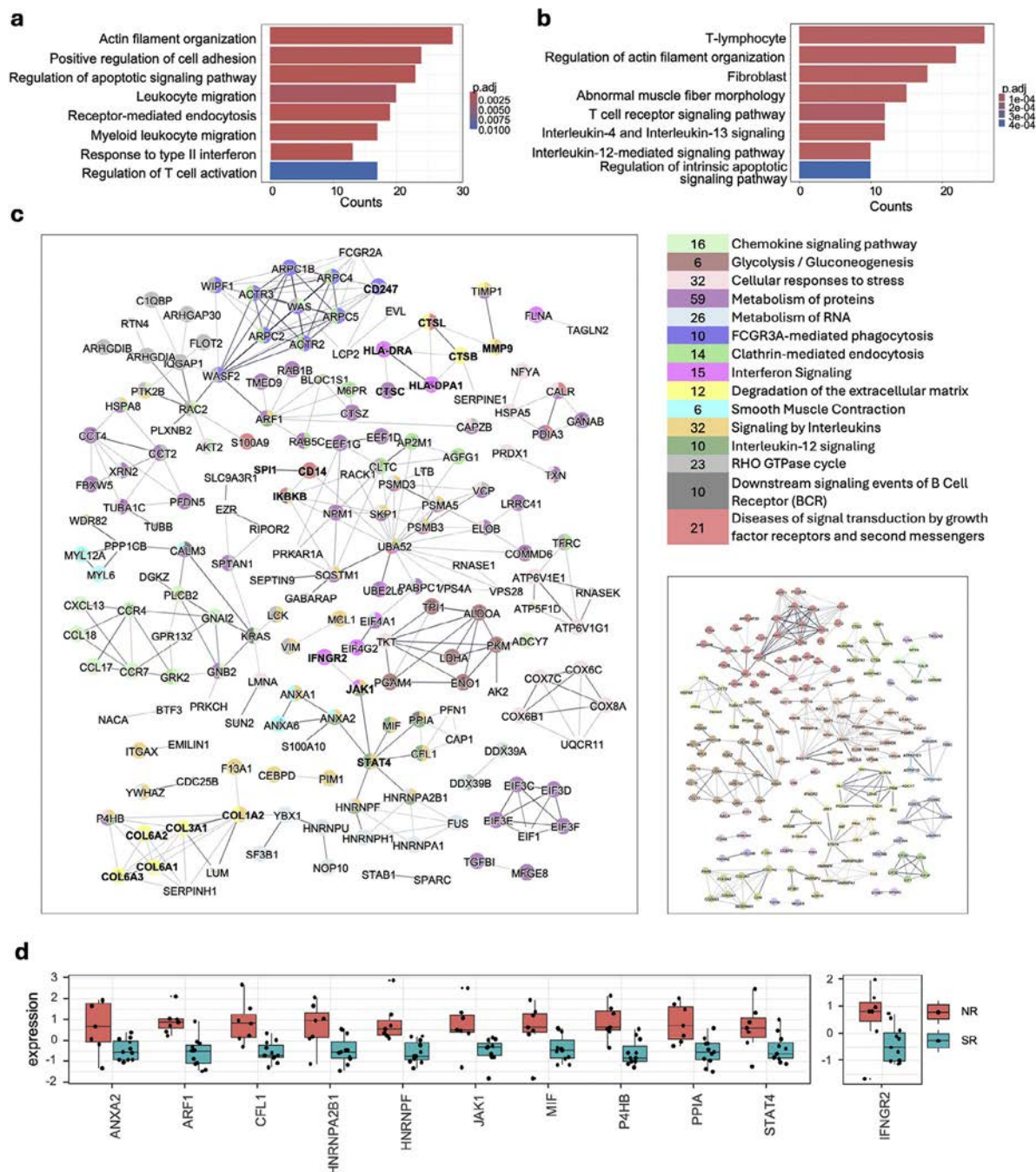


Figure 4. Interferon (IFN) signalling and JAK/STAT are enriched in the immune infiltrates of cases that do not respond to glucocorticoids. Significantly upregulated DEGs in NR CD45 + ROI were analysed for pathway analysis and protein interaction networks. Selected significantly upregulated pathways identified via (a) GO enrichment and (b) STRING pathway analysis. Bars indicate the numbers of genes associated with a given pathway and coloured by significance (adjusted *P* value, *P*-adj). (c) STRING protein interaction network analysis based on active interactions in curated databases. Line thickness indicates the strength of data support. Twenty-two clusters were identified using k-means clustering (bottom-right diagram) and coloured based on KEGG and Reactome pathway associations (main diagram). Disconnected nodes in the network are hidden. Numbers in the legend are the number of genes belonging to that pathway. Genes associated with ribosomal RNA or subunits were not included in the analysis. (d) Boxplots of selected DEGs belonging to the ‘interleukin-12-mediated signalling pathway’, with the addition of *IFNGR2*, in NR and SR. Expression levels have been row-scaled into z-scores. Each dot represents an independent case, with a line indicating the mean expression. JAK/STAT, Janus kinase/signal transducer and activator of transcription; DEGs, differentially expressed genes; GO, gene ontology; NR, non-remission; ROI, regions of interest; SR, sustained remission; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; KEGG, Kyoto Encyclopaedia of Genes and Genomes; *IFNGR2*, interferon gamma receptor 2.

cases (Fig 5B,C). This observation aligned with spatial transcriptomic data, which showed preferential expression of immunoglobulin genes in the SR adventitia layer (Fig 1D). Further histological analysis confirmed the presence of IgG in GCA cases but not in controls (Supplementary Fig S5a). The extent of IgG staining corresponded with the density of PCs in the tissue (Supplementary Fig S5b). IgG staining localised around areas of immune infiltration in the adventitia and

granulomatous lesions in the media and intima (Supplementary Fig S5a-c). Specifically, CD68 + macrophages and giant cells in the media and intima exhibited strong IgG staining (Supplementary Fig S5c,d). This observation suggests an interplay between these 2 compartments in SR tissue pathology, further supported by a significant correlation between the total number of PCs and the cumulative number of macrophages in the media and intima across all cases ($R^2 = 0.5884$, $P = .0008$; Fig 5D).

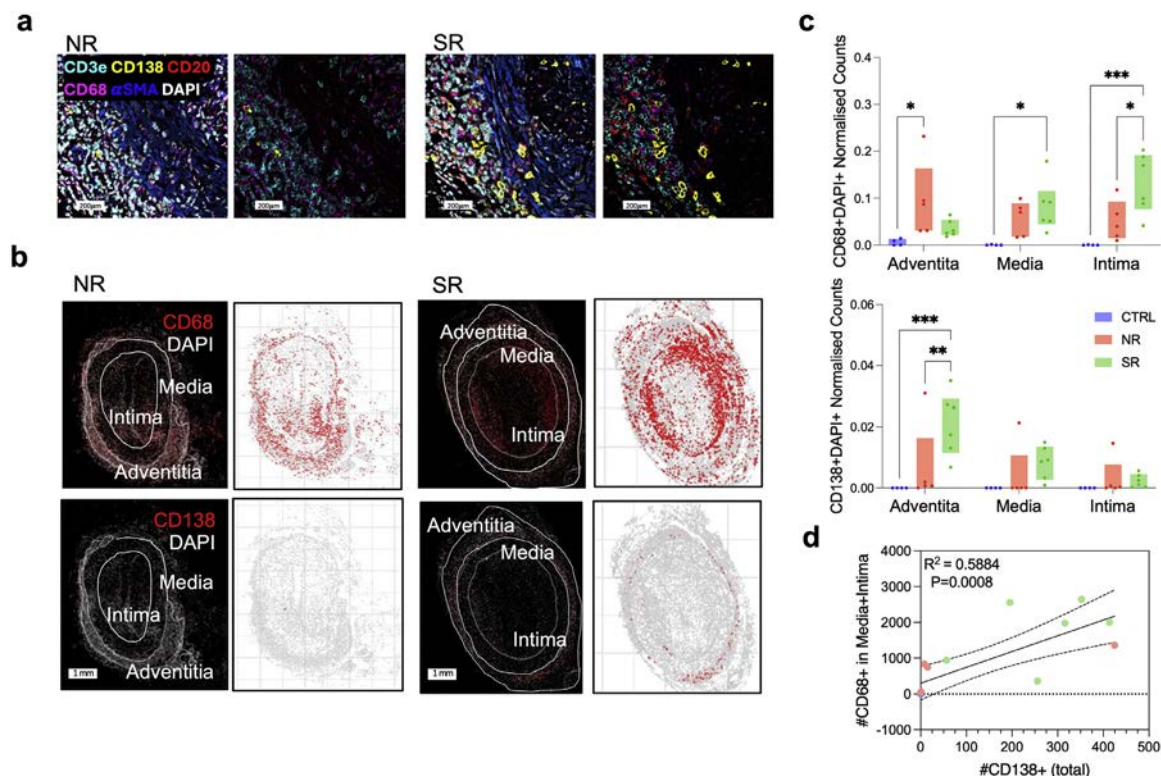


Figure 5. Sustained remission (SR) cases have higher numbers of adventitial plasma cells and intimal macrophages. TABs were stained for protein immunophenotyping using the CODEX technology. $n = 4$ for CTRL, $n = 5$ for NR, and $n = 6$ for SR. (a) Representative tissue immunofluorescence staining showing staining for CD3e (cyan), CD138 (yellow), CD20 (red), CD68 (magenta), α SMA (blue), and DAPI (white) in the adventitia of NR and SR. Scale bar = 200 μ m. (b) ROIs corresponding to the 3 vessel layers—adventitia, media, and intima—were manually traced (white lines), and within these CD68+ and CD138+ cells were identified and counted. Representative sections showing the actual staining for CD138 (red) and DAPI (white) on the left-hand side and the corresponding computational identification of CD138+ cells used for quantification on the right-hand side. Scale bar = 1 mm. (c) Box plots of normalised counts per total cells per ROI of CD68+ macrophages (top) and CD138+ plasma cells (bottom) in controls (CTRL), NR and SR. Statistical analysis was done using 2-way analysis of variance and Tukey's multiple comparisons test. $**P \leq .01$; $***P \leq .001$. (d) Simple linear regression showing the correlation between the absolute numbers of CD68+, calculated as media + intima cumulative counts, and the total numbers of CD138+ in adventitia + media + intima. (c, d) Each dot represents the average calculated on 2 different sections of each case. NR, non-remission; ROI, regions of interest; SR, sustained remission; TAB, temporal artery biopsy, α -SMA, alpha-smooth muscle actin; DAPI, 4',6-diamidino-2-phenylindole.

PCs and intimal macrophages as potential biomarkers for predicting treatment response

To confirm local IgG production by PCs, colocalisation of IgG and CD138 was assessed. IgG+ PCs accounted for $40.5\% \pm 12.0\%$ ($n = 16$ sections) of total CD138+ PCs and were present across all 3 vessel layers. However, in the adventitia, they were uniquely organised in cellular clusters (Fig 6A).

To assess the potential of PCs and macrophages as a biomarker, we transitioned to a standard and generalisable immunostaining technique to quantify CD138+ PCs and macrophages in an independent cohort of TAB samples ($n = 6$ NR and $n = 8$ SR). Our analysis confirmed that SR cases had significantly higher numbers of PCs compared to NR cases (Fig 6B), as well as had higher infiltration of macrophages in the media and intima layer (Fig 6C and Supplementary Fig S6).

Furthermore, the diagnostic performance of the number of PCs (#CD138+ PCs), the amount of macrophage infiltration in the media and intima (intimal+medial M ϕ area), and the combined score of these 2 parameters were evaluated using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), all with 95% confidence intervals. With sensitivity fixed at 90%, the combined score demonstrated high specificity (median 83.3%), a median PPV of 87.8%, and an NPV of 86.2%, positioning it as the most reliable predictor, particularly within the 0.5 to 0.75 specificity range. In contrast, both

#CD138+ PCs and intimal+medial M ϕ area alone showed lower median specificity (31.7% and 3.3%, respectively), suggesting limitations in accurately identifying true negatives (Fig 6D and Table 2).

These findings highlight the potential of PCs and macrophages present at disease onset as key components for distinguishing clinical outcomes in GCA, suggesting their utility as biomarkers to aid in patient stratification.

DISCUSSION

This study represents the first application of spatial biology technologies to characterise temporal artery inflammation in GCA in relation to treatment response trajectories. Our findings are broadly consistent with recent transcriptomic studies characterising inflamed GCA arteries, which have identified prominent Th1-type inflammation, B cell and PC gene signatures, and ECM remodelling as key features of the vascular immune landscape [13,15,16]. Extending these insights, our spatially resolved analysis uniquely links these molecular features to long-term treatment outcomes, revealing significant molecular and cellular divergences between patients with GCA who will achieve SR following a standard GC monotherapy regimen and those who will not (NR). Patients with SR exhibited an IgG PC-enriched profile, while patients with NR displayed a more complex inflammatory

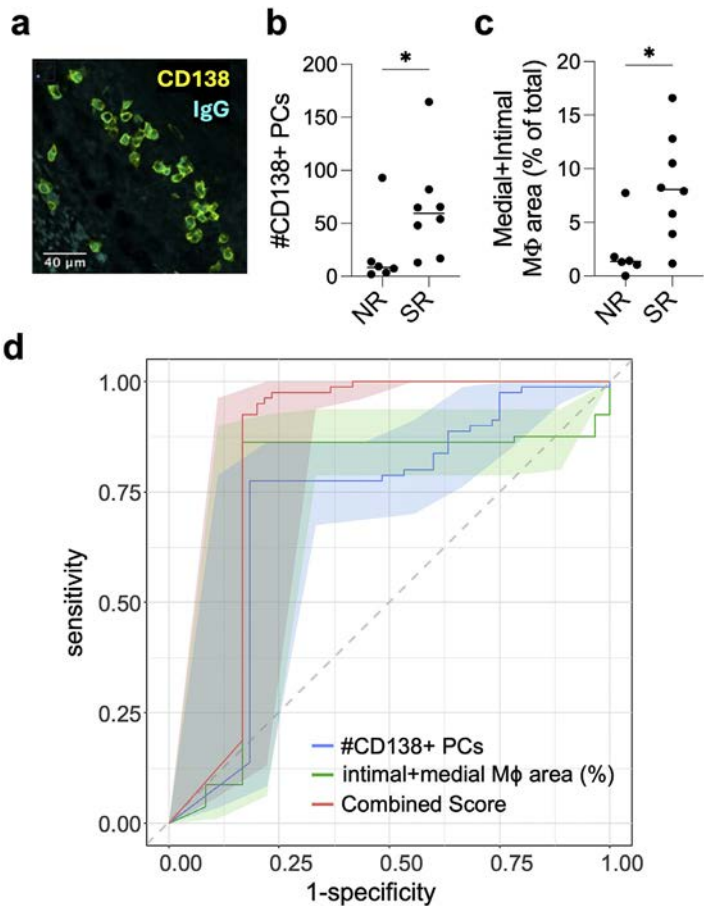


Figure 6. Plasma cells and intimal macrophages can predict response to steroids. (a) Representative immunofluorescent staining of adventitial plasma cells identified as CD138+ (yellow) and costained with IgG (cyan). Scale bar = 40 μ m. (b) Quantification of total numbers of CD138+ plasma cells in NR and SR. (c) Quantification of area covered by macrophages (M ϕ) within ROI defined for media + intima. (b, c) Each dot represents 1 individual case (n = 6 NR and n = 8 SR); line at the mean. Data analysed with 2-tailed Mann-Whitney test. **P* < .05. (d) Receiver operating characteristic curve (ROC) for prediction of sustained remission based on total numbers of CD138+ PCs (from b), intimal+medial M ϕ area (%) (from c), and a combined weighted score, obtained through a weighted sum of the normalised counts and percentages. Curves show sensitivity vs 1-specificity with 95% CIs (n = 14 combining NR and SR). IgG, immunoglobulin G; NR, non-remission; PC, plasma cell; ROI, regions of interest; SR, sustained remission.

landscape, including upregulation of ECM remodelling and T cell activation. By linking spatial signatures to treatment response, our study provides novel insights that may inform future biomarker-stratified studies and guide mechanistic investigation.

The identification of molecular pathways that distinguish SR from NR cases has both clinical and biological significance. Clinically, our results suggest that patients at risk of GC non-responsiveness could be identified early using tissue-based biomarkers. This supports a shift towards more personalised treatment approaches in GCA. Importantly, these findings also highlight a continued diagnostic and prognostic role for TABs. Although advanced imaging is increasingly adopted as a first-line diagnostic tool, TAB remains the gold standard for diagnostic specificity and is the only modality capable of providing molecular insights into vascular inflammation. As in other immune-mediated

diseases (such as oncology or nephrology) where tissue sampling remains essential, these findings suggest that moving away from biopsy in GCA may overlook key aspects of disease biology and prognosis.

Mechanistically, the NR group was characterised by persistent Th1-associated inflammation, marked by expression of IFN- γ -related and NF- κ B-driven transcripts (eg, *IFNGR2*, *IKBKB*), alongside fibroblast and myofibroblast-related genes (*ACTA2*, *TAGLN*, *PDGFRB*, *MYH11*). This profile was accompanied by an enriched transcriptional signature of CD4⁺ memory T cells, a subset recently shown to mediate vascular inflammation and contribute to disease persistence in an adoptive humanised model that captures key immune features relevant to large-vessel vasculitis [17]. Additionally, a recent study identified a positive feedback loop in which myofibroblasts (CD90+, *ACTA2*+, *MYH11*+) respond to IFN- γ and tumour necrosis factor- α by producing IL-12, thereby sustaining Th1 polarisation in GCA TABs [18]. This may contribute to the inflammation and tissue remodelling observed in NR cases [19,20]. Persistent fibroblast activity and ECM remodelling, leading to vascular fibrosis, have been previously reported in GCA rebiopsies, further corroborating these findings [14,19–21]. These insights raise the possibility that therapies targeting stromal or fibroblast-mediated remodelling could complement immunosuppressive regimens in selected patients. However, whether these remodelling pathways drive disease chronicity or serve protective roles (eg, by stabilising the arterial wall) remains to be clarified. Our results also showed upregulation of the IFNGR2/JAK1/STAT4 axis in NR cases, suggesting a potential role for JAK/STAT pathway inhibition in refractory GCA. While causality cannot be directly inferred, these findings provide a rationale for further studying the contribution of CD4⁺ memory T cells and stromal signalling

Table 2
Predictive performance of plasma cells and macrophages for sustained remission under GCs

	#CD138+ PCs	Intimal+medial M ϕ area (%)	Combined score
Sensitivity	90.0	90.0	90.0
Specificity	31.7 (16.7-48.3)	3.3 (0.0-88.3)	83.3 (71.7-91.7)
PPV	63.7 (59.0-69.9)	55.4 (54.5-91.1)	87.8 (80.9-93.5)
NPV	70.4 (55.6-78.4)	20.0 (0.0-86.9)	86.2 (84.3-87.3)

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for 3 parameters: #CD138+ PCs, intimal+medial macrophage (M ϕ) area (%), and the combined score. Sensitivity is fixed at 90% for each parameter. Specificity, PPV, and NPV are reported as median values with 95% CIs in brackets. All values are shown as percentages. GC, glucocorticoids; PC, plasma cells.

to relapse risk, as well as for evaluating GC-sparing agents in biomarker-stratified trials. For instance, JAK inhibitors like bari-citinib and upadacitinib have shown promise in early trials [22,23], and their use could be further refined through bio-marker-driven stratification.

A key novel finding of our study is the enrichment of IgG-producing PCs in the adventitia of SR. Although GCA is primarily viewed as a T cell and macrophage-driven disease, emerging evidence implicates B cells in disease modulation [24–27]. PCs have been observed in up to half of TABs in prior studies [28,29], but their association with treatment response has not been examined. Our results add to this growing body of evidence and demonstrate that PCs and their IgG transcripts are strongly enriched in SR cases and largely absent in NR cases. This finding emphasises the possibility that GCs exert their beneficial effects, in part, by targeting B cell activity and modulating PC function, as seen in other immune-mediated diseases like systemic lupus erythematosus [30]. This may act in concert with the well-established direct suppressive effects of GCs on macrophage activation and granuloma formation, aligning with prior reports of progressive granuloma resolution under effective GC treatment [21]. Alternatively, the presence of PCs may serve as a marker of a tissue environment more permissive to GC-mediated resolution. If further validated, their presence alongside macrophages in diagnostic TABs could provide a valuable tool for predicting GC responsiveness and guiding therapeutic decisions early in the disease course. However, whether PCs actively contribute to disease remission or represent an epiphenomenon remains unknown.

Despite the novelty and translational relevance of our findings, several limitations must be acknowledged. First, while the cohort size is the largest in spatial biology studies of GCA to date, it remains relatively small, limiting the generalisability of our findings. Future multicentre studies with larger cohorts will be essential to validate our findings and confirm the prognostic value of PCs ($\pm$ macrophages) as biomarkers of GC response. Second, patients were not steroid-naïve at the time of biopsy, which could have impacted the expression of certain GC-sensitive immune markers. However, all patients received similar initial GC doses for comparable durations, and exposure was well-matched between NR and SR groups. The persistence of distinct immune signatures despite this uniform exposure suggests that observed differences reflect intrinsic immune programmes rather than treatment effects. Moreover, this design reflects routine clinical practice, where GC initiation typically precedes biopsy to prevent ischaemic complications. As such, our findings retain real-world clinical relevance and applicability. Third, while clinicians generally adhered to recommended tapering schedules, individualised adjustments cannot be excluded and may have influenced relapse definition. Further prospective studies, ideally incorporating steroid-naïve samples and standardised treatment, will be necessary to clarify the full impact of GCs on tissue immunopathology.

In conclusion, our study identifies distinct immune transcriptional signatures at diagnosis that are associated with subsequent treatment responses in GCA, with PCs and macrophages emerging as potential biomarkers of sustained GC responsiveness. These findings support the rationale for a more precision-based approach to GCA management, where early identification of individuals at higher risk of relapse could inform more targeted and effective treatment strategies. Furthermore, this work lays the foundation for future mechanistic studies and provides a strong rationale to retain tissue-based profiling as part of the clinical and research toolkit in GCA, with the ultimate goal of

improving outcomes in this heterogeneous and challenging disease.

Competing interests

ESP, LZ, DCG, IHW, AN, and RJB reports financial support was provided by Eli Lilly and Company. ESP, LZ, DCG, IHW, AN, and RJB reports a relationship with Eli Lilly and Company that includes: employment and equity or stocks. CSG reports a relationship with Eli Lilly and Company that includes: funding grants. CSG reports a relationship with AstraZeneca Pharmaceuticals LP, Galvani, MedAnnex, and Bristol-Myers Squibb (BMS) that includes: consulting or advisory and funding grants. NB reports a relationship with Eli Lilly and Company that includes: consulting or advisory and funding grants. NB reports a relationship with Galapagos that includes: consulting or advisory. NB reports a relationship with AbbVie Inc that includes: speaking and lecture fees. NJ reports a relationship with NanoString Technologies Inc that includes: speaking and lecture fees. IM reports a relationship with AbbVie, AstraZeneca, Absci, Amgen, BMS, Boehringer, Causeway Therapeutics, Cabaletta, Compugen, Dextera, Eli Lilly, Gilead, Janssen, Montai, Moonlake, Novartis, Pfizer, Roche, Recludix, Sanofi, and UCB that includes: consulting or advisory, funding grants, and speaking and lecture fees. IM reports a relationship with Cabaletta, Compugen, Causeway Therapeutics, and Dextera that includes: equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contributors

CA: conceptualisation, methodology, investigation, formal analysis, visualisation, supervision, writing – original draft, writing – review and editing, and project administration. DM and VK: data curation. SW, PC, TB, and LH: resources. SM and LZ: data curation, software, and formal analysis. ATV: methodology. ESP: methodology, investigation, and formal analysis. EQ: data curation and investigation. AP, HL, YD, CKD, IG, and MLV: investigation. DCG and JC: software, formal analysis, and supervision. NBJ, IHW, AN, and RJB: supervision and resources. IBM: writing – review and editing. NB and CSG: conceptualisation, supervision, writing – review and editing, project administration, and funding acquisition.

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Patient consent for publication

Not applicable.

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Access to patient tissue biopsies was authorised by the NHS Greater Glasgow and Clyde Biorepository under NHS Research Ethics Committee approval (biorepository application #612; West of Scotland Ethics 22/WS/0020) and in accordance with the Declaration of Helsinki. Access to clinical records via the NHS Greater Glasgow and Clyde Clinical Portal was granted under Caldicott Guardian approval.

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Data availability statement

The spatial transcriptomic dataset generated using the NanoString GeoMx DSP platform has been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE299070. The submission includes raw digital counts, segment (ROI) metadata, clinical annotations, and DESeq2-normalised expression matrices to support reanalysis.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.09.009.

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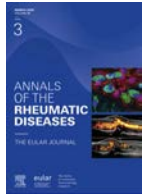
Cecilia Ansalone: <http://orcid.org/0000-0001-8683-1718>
 Ishita Gupta: <http://orcid.org/0000-0002-5101-6876>
 Evelyn Qian: <http://orcid.org/0000-0001-7709-2175>
 Maria Laura Vieri: <http://orcid.org/0000-0002-7309-0647>
 Claire Kennedy Dietrich: <http://orcid.org/0009-0006-4526-6402>

Lisa Hutton: <http://orcid.org/0000-0003-0597-9679>
 Nigel B. Jamieson: <http://orcid.org/0000-0002-9552-4725>
 Isabella H. Wulur: <http://orcid.org/0000-0001-7599-014X>
 Robert J. Benschop: <http://orcid.org/0000-0001-6313-5218>

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Autoinflammatory disorders

Insights from a novel monogenic autoinflammatory disease: overview of a multicentric European cohort of 38 patients with COPA syndrome

Clémence David^{1,2}, Nadia Nathan³, Eslam Al-Abadi^{4,†}, Peter D. Arkwright⁵, Brigitte Bader-Meunier⁶, Sebastian Becker^{7,8}, Alexandre Belot⁹, Mary Brennan¹⁰, Sylvain Breton¹¹, Vincent Bondet¹², Jacques Cadranel¹³, Aurore Coulomb l'Hermine¹⁴, Sébastien De Almeida¹⁵, Darragh Duffy¹², Teresa Clavaguera Poch¹⁶, Alix de Becdelièvre¹⁷, Siham El Khalifi-Boulisfane¹⁸, Marco Gattorno¹⁹, Montse Gispert-Saüch¹⁶, Florian Gothe^{8,20}, Yves Hatchuel²¹, Daniela Herdliczko²², Ayse Ayzit Kilinc^{8,23}, Vaclav Koucky^{8,24}, Géraldine Labouret²⁵, Raffaele Manna²⁶, Hélène Maillard²⁷, María Luisa Matoses Ruipérez²⁸, Caterina Matucci-Cerinic^{19,29}, Anna Mensa-Vilaro³⁰, Katarzyna Michel^{8,20}, Thierry Jo Molina³¹, Berta Lopez Montesinos³², William G. Newman^{5,33,34}, Julia Papenkort^{8,35}, Christina Rapp^{8,20}, Cinthia Rames³⁶, Gillian I. Rice³⁴, Markus A. Rose^{8,35}, Heloise Reumaux³⁷, Laurent Sailler¹⁵, Nicolaus Schwerk^{8,38}, Luis Seabra¹, Jérémie Sellam³⁹, Andrea Taddio^{40,41}, Caroline Thumerelle⁴², Alberto Tommasini^{40,41}, Maud Tusseau^{43,44}, Stefano Volpi^{19,29}, Martin Wetzke^{8,38}, Laurence Weiss⁴⁵, Anne Welfringer-Morin⁴⁶, Marie Wislez⁴⁷, Matthias Griesse^{8,20}, Yanick J. Crow^{1,48}, Marie-Louise Frémond^{1,6,*}

¹ Laboratory of Neurogenetics and Neuroinflammation, Imagine Institute, Paris, France

² Department of Internal Medicine, Hôpital Bichat-Claude Bernard, Assistance Publique-Hôpitaux de Paris, Université Paris Cité, Paris, France

³ Paediatric Pulmonology Department and Reference Centre for Rare Lung Diseases RespiRare, INSERM UMR_S933 Laboratory of Childhood Genetic Diseases, Armand Trousseau Hospital, Sorbonne University and APHP, Paris, France

⁴ Childhood Arthritis and Rheumatic Diseases Unit, Birmingham Women's and Children's Hospital NHSFT, Birmingham, UK

⁵ Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, UK

⁶ Department of Paediatric Haematology-Immunology and Rheumatology, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (APHP), and Reference Centre for Inflammatory Rheumatism, Autoimmune Diseases and Systemic Interferonopathies in Children (RAISE), Université Paris Cité, Paris, France

⁷ Pneumology Department, Darmstädter Kinderkliniken Prinzessin Margaret, Darmstadt, Germany

⁸ Member of the chILD-EU Register Group

⁹ National Reference Center for Inflammatory Rheumatism, Autoimmune Diseases and Systemic Interferonopathies (RAISE), Paediatric Nephrology, Rheumatology, Dermatology Unit, Hospital of Mother and Child, Hospices Civils of Lyon, Lyon, France

* Correspondence to Pr Marie-Louise Frémond, Paediatric Haematology-Immunology and Rheumatology Department, Hôpital Necker-Enfants Malades, Paris, France.

E-mail address: marie-louise.fremond@aphp.fr (M.-L. Frémond).

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- ¹⁰ Paediatric & Adolescent Rheumatology, Royal Hospital for Children & Young People, Edinburgh, UK
- ¹¹ Pediatric Radiology Department, Hôpital Necker-Enfants Malades, AP-HP Centre-Université de Paris, Paris, France
- ¹² Translational Immunology Unit, Institut Pasteur, Université Paris Cité, Paris, France
- ¹³ Department of Pneumology and Thoracic Oncology, Tenon Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP) Sorbonne Université, Paris, France
- ¹⁴ Pathology Department, Trousseau University Hospital, Assistance Publique-Hôpitaux de Paris, Sorbonne Université, Paris, France
- ¹⁵ Department of Internal Medicine, CHU de Toulouse, Toulouse, France
- ¹⁶ UF Paediatric Rheumatology Department, Hospital Doctor Trueta, Girona, Spain
- ¹⁷ Laboratory of Genetics, Henri Mondor University Hospital, APHP, University Paris Est Creteil, INSERM, IMRB, Creteil, France
- ¹⁸ Emergency, Infectious Disease and Paediatric Rheumatology Department, Centre Hospitalier Régional Universitaire Lille, University of Lille, Lille, France
- ¹⁹ UOC Reumatologia e Malattie Autoinfiammatorie, IRCCS Istituto Giannina Gaslini, Genova, Italy
- ²⁰ Pediatric Pneumology, Dr von Hauner Children's Hospital, University of Munich, German Center for Lung Research (DZL), Munich, Germany
- ²¹ Department of General Paediatrics, Competence Centre for Inflammatory Rheumatism, Autoimmune Diseases and Systemic Interferonopathies (RAISE) Antilles-Guyane, EpiCtiV Research Unit, University of the French West Indies, Martinique University Hospital, Fort-de France, France
- ²² Rheumatology, Universitäts - Kinderspital Zürich, Zurich, Switzerland
- ²³ Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Division of Pediatric Pulmonology, Istanbul, Turkey
- ²⁴ Department of Paediatrics, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic
- ²⁵ Paediatric Pulmonology Department, University Hospital for Children, Toulouse, France
- ²⁶ Periodic Fevers Research Center, Catholic University, Rome, Italy
- ²⁷ Department of Internal Medicine and Clinical Immunology, Referral Centre for Rare Systemic Autoimmune Diseases in the North of France, Northwest, Mediterranean and Guadeloupe (CeRAINOM), CHU de Lille, Lille, France
- ²⁸ Section of Pediatric Nephrology, Hospital Universitari i Politècnic (HUIP) La Fe, Valencia, Spain
- ²⁹ Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINO GMI), University of Genoa, Genoa, Italy
- ³⁰ Department of Immunology-CDB, Hospital Clínic-IDIBAPS, Barcelona, Spain
- ³¹ Pathology Department, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Centre-Université de Paris, Paris, France
- ³² Section of Pediatric Rheumatology, Hospital Universitari i Politècnic (HUIP) La Fe, Valencia, Spain
- ³³ Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust, Manchester, UK
- ³⁴ Division of Evolution Infection & Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK
- ³⁵ Stuttgart Children's Hospital, Center for Congenital Lung Diseases, Stuttgart, Germany
- ³⁶ Paediatric Pneumology and Allergy Unit, CHU of Amiens, Amiens, France
- ³⁷ Paediatric Rheumatology Unit, University of Lille, Jeanne de Flandre Hospital, Lille, France
- ³⁸ Department of Paediatrics, German Center for Lung Research, Medical University of Hannover, Hannover, Germany
- ³⁹ Department of Rheumatology, Saint-Antoine Hospital, APHP, Centre de Recherche Saint-Antoine (CRSA) Inserm UMR5308, Sorbonne Université, Paris, France
- ⁴⁰ Department of Pediatrics, Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy
- ⁴¹ Department of Medical, Surgical and Health Sciences University of Trieste, Trieste, Italy
- ⁴² Paediatric Pulmonology and Allergy Department, Hôpital Jeanne de Flandre, CHU Lille, Univ. Lille, Lille, France
- ⁴³ Centre International de Recherche en Infectiologie, Inserm, U1111, University Claude Bernard, Lyon 1, UMR5308, ENS de Lyon, Lyon, France
- ⁴⁴ Hospices Civils de Lyon, Department of Medical Genetics, University Hospital of Lyon, Lyon, France
- ⁴⁵ Pediatric Pulmonology, Strasbourg University Hospital, Strasbourg, France
- ⁴⁶ Department of Dermatology, Reference Centre for Genodermatoses and Rare Skin Disease (MAGEC), Necker-Enfants Malades Hospital, Assistance Publique-Hôpitaux de Paris, Université Paris Cité, Paris, France
- ⁴⁷ Service de Pneumologie, Hôpital Cochin, AP-HP, Inserm U1138, Université Paris Cité, Paris, France
- ⁴⁸ MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK

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ABSTRACT

Objectives: COPA (coatamer subunit alpha) syndrome is a rare monogenic autoinflammatory disease due to heterozygous mutations in *COPA*. It has phenotypic overlap with STING (Stimulator of interferon genes)-associated vasculopathy with onset in infancy (SAVI), although the spectrum of clinical manifestations is not yet fully defined. Our aim was to better delineate the clinical phenotype of this rare disorder in a European cohort.

Methods: Methods include assessment of clinical, imaging, and immunological data from 46 individuals (29 families) carrying a *COPA* mutation.

Results: Among the 46 individuals carrying a *COPA* mutation, 38 had at least 1 clinical manifestation likely related to their mutant state (clinical penetrance of 83%). Twenty-two (58%)

symptomatic patients were female, with a median age at disease onset of 3 years (range 0–50 years). Pulmonary involvement was observed in 34 patients, with interstitial lung disease in most cases ($n = 31$) and diffuse alveolar haemorrhage in 11 individuals. Twenty-six patients demonstrated joint involvement, and 7 had documented kidney disease. Previously undescribed features included skin ($n = 12$), cardiac ($n = 8$), gastrointestinal ($n = 7$), and hepatic involvement ($n = 5$). All but 1 patient tested positive for autoantibodies, and increased interferon signalling was noted in all those tested. Twenty-two patients were treated with Janus kinase inhibitors with promising efficacy.

Conclusions: We report a large European cohort of patients with COPA syndrome. While confirming the core organ features (lung, joint, and kidney) of the disease, our data expand the phenotype to include cardiac, skin, and gastrointestinal features, further demonstrating the clinical overlap with SAVI and other type I interferonopathies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- COPA syndrome is a rare monogenic type I interferonopathy.
- Known features of the disease are lung, joint, and kidney disease.

WHAT THIS STUDY ADDS

- Expansion of the known phenotype with skin, cardiac, digestive, and hepatic involvement.
- Possibility of clinical nonpenetrance, *de novo* or somatic mutations.
- Morbidity and mortality driven by pulmonary and renal disease.
- Promising efficacy of Janus kinase (JAK) inhibition.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Sequence the *COPA* gene in the context of a concordant phenotype, even in the absence of a family history of disease.
- Perform a chest computed tomography scan, pulmonary function tests, and a screen for proteinuria in any patient carrying a *COPA* mutation.
- JAK inhibition can be considered as a first-line treatment, and anifrolumab may be a promising therapy to be trialled in COPA syndrome.

INTRODUCTION

COPA syndrome is a monogenic autoinflammatory disease originally described in 2015 due to heterozygous mutations in the gene *COPA*, encoding the coatamer subunit α [1]. In the original paper, 5 families were reported, with symptomatic individuals variably manifesting interstitial lung disease (ILD), inflammatory arthritis, and high autoantibody titres [2]. The authors hypothesised that the disease resulted from defective retrograde transport between the Golgi and endoplasmic reticulum (ER) mediated by the coat protein complex I complex. A major advance in the understanding of COPA syndrome pathogenesis came from the observation of an enhanced expression of interferon (IFN)-stimulated genes (ISGs) in the whole blood of symptomatic individuals, a hallmark of the type I interferonopathies [3]. In addition, clinical overlap was noted with the well-defined type I interferonopathy due to gain-of-function mutations in *STING1* [4], termed STING-associated vasculopathy with onset in infancy (SAVI). STING activation was subsequently shown to be a key driver of the pathogenesis of COPA syndrome [5–8]. Indeed, dominant-negative mutations in *COPA* prevent STING retrograde transport back to the ER from the Golgi, with associated chronic activation of STING and the induction of the

expression of type 1 IFNs and nuclear factor kappa-B-related inflammatory cytokines [5–9].

Since COPA syndrome was first described, approximately 70 cases have been published [2,3,6,10–19]. The core clinical features are lung inflammation with either recurrent alveolar haemorrhage (AH) or ILD [20], joint disease that may mimic juvenile idiopathic arthritis [10–21], and renal involvement [12] with mainly glomerular disease. Given the pivotal role of type I IFN overproduction in the pathogenesis of COPA syndrome, Janus kinase (JAK) inhibition has been proposed as a targeted therapeutic strategy, with apparently promising effects in a number of case reports [13,21].

Here, we aimed to assemble a large European cohort of patients with molecularly confirmed COPA syndrome, thereby providing insights into the clinical and immunological spectrum of the disease, which has been undercharacterised due to the limited number of reported cases.

METHODS

Patients

Patients with genetically confirmed COPA syndrome were included in this retrospective study. Thirteen patients have been previously reported [3,6,10,12,15,21,22]. Patients were recruited from referent rare diseases centres in France, Germany, Italy, Spain, Switzerland, Turkey, Czech Republic, and the United Kingdom. Variants were annotated according to HGVS nomenclature using the MANE select transcript NM_004371.4. All patients had fulfilled the requirements of their local ethics committee for clinical data sharing.

Data collection

Data collected included sex, clinical manifestations at presentation and during follow-up, and age at genetic diagnosis of COPA syndrome. Results of chest computed tomography (CT), pulmonary function tests (PFTs), bronchoalveolar lavage (BAL) fluid analyses, and lung biopsies were recorded. ILD was defined according to CT scan findings, and AH either on CT scan and/or BAL and/or lung biopsy. The presence of honeycombing and/or traction bronchiectasis and/or inter- and intralobular septal thickening radiologically defined pulmonary fibrosis [23]. Biological parameters such as inflammatory markers, autoantibodies, and immunological status were collected, as well as treatment characteristics and the response to therapy.

IFN pathway assessment

Status of IFN biomarkers was determined by studying the expression of ISGs (by qPCR [24] or by NanoString [25]) in

peripheral blood and by measuring IFN α protein in serum using a Simoa ultrasensitive digital enzyme-linked immunosorbent assay [26].

Study approval

The study was approved by the Comité de Protection des Personnes (ID-RCB/EUDRACT: 2014-A01017-40; revalidated in 2022). Written informed consent was obtained for all patients.

Statistics

Data are expressed as median (range) or number (percentages). Analyses were performed using PRISM software (v10, GraphPad Inc.). A *P* value less than .05 was considered significant.

RESULTS

Demographics

Patients from 29 families with genetically confirmed *COPA* mutations were ascertained. Among these, 38 patients demonstrated at least 1 feature suggestive of *COPA* syndrome (Table 1 and Supplementary Table S1), while 8 individuals were clinically asymptomatic after a full clinical evaluation, pulmonary function testing, lung CT imaging, and assessment for proteinuria. These findings indicate a clinical penetrance of 83% where at least 1 individual in each family had been clinically ascertained. Twenty-two symptomatic patients were female (ratio F:M 1.4:1), while all asymptomatic *COPA* mutation carriers (*n* = 8), identified through family testing, were female. Among symptomatic patients, the median age at disease onset was 3 (neonatal period–50) years, with 12 patients manifesting disease before the age of 5 years. The median age at molecular diagnosis was 13 (0.3–57) years. At the last follow-up, 34 (89%) symptomatic patients were alive.

Molecular data

All mutations were located in the well-defined mutational hotspot in the WD40 domain of the *COPA* protein (Fig 1A). Most mutations (61%) identified in symptomatic patients were inherited in a dominant manner from a mutation-positive parent, while 5 occurred *de novo* and 2 patients were somatic mosaic [27], with a variant allele frequency (VAF) of 10% and 29%, respectively. We were unable to test the parents of 8 patients (Table 2). Among symptomatic patients, the p.(Arg233His) (R233H) and p.(Arg281Trp) (R281W) substitutions were the most prevalent, occurring in 13 patients (34%) from 9 pedigrees and 9 patients (24%) from 5 pedigrees, respectively. Additionally, 7 individuals carried a substitution of the amino acid p.(Trp240) (W240) (Table 2). Eight further substitutions were identified in single patients. Half of asymptomatic individuals (*n* = 4) carried the p.(Arg233His) (R233H) substitution (Table 2). No genotype-phenotype correlation was apparent, and the patients who were somatic mosaic for a *COPA* mutation did not present with a phenotype milder than those seen in the overall cohort (Supplementary Table S1).

Clinical features in symptomatic patients

Clinical characteristics of patients are summarised in Table 1 and detailed in Supplementary Table S1. Features at onset are

Table 1

Clinical characteristics of affected patients with *COPA* pathogenic variants at last report.

Demographics	n = 38 patients
Sex ratio (F:M)	1.4:1
Age at onset	3 (0 ^a –50)
Age at molecular diagnosis	13 (0.3–57)
Age at last report	15.5 (0.5–58)
Alive/dead at last report	34/4
Organ involvement	
Failure to thrive	14 (37%)
Recurrent fever	9 (24%)
Lung involvement	34 (89%)
ILD	31 (82%)
AH	3 (8%)
ILD and AH	8 (21%)
Fibrosis	14 (37%)
Joint disease	26 (68%)
Polyarthritis	17 (45%)
Arthralgias	6 (16%)
Isolated Boutonniere deformity	2 (5%)
Joint disease without specification	2 (5%)
Skin involvement	12 (32%)
Purpura	2 (6%)
Malar rash	2 (6%)
Acral ulcers/acrosyndrome	3 (8%)
Livedo	1 (3%)
Oral ulcers	1 (3%)
Nasal perforation	1 (3%)
Vitiligo	1 (3%)
Panniculitis	1 (3%)
Psoriasis	1 (3%)
Chilblains	1 (3%)
Not specified skin disease	1 (3%)
Kidney involvement	7 (18%)
ANCA-associated glomerulonephritis	3 (8%)
Not specified kidney insufficiency	2 (5%)
Lupus-like glomerulonephritis	1 (3%)
Membranous glomerulonephritis	1 (3%)
Liver involvement	5 (13%)
Cytolytic hepatitis/secondary to methotrexate	4 (11%)/2 (5%)
Hepatosplenomegaly	1 (3%)
Gut involvement	7 (18%)
Gastro-oesophageal reflux	4 (11%)
Chronic diarrhoea	2 (5%)
Digestive IgA vasculitis	1 (3%)
Stercoral peritonitis	1 (3%)
Cardiac involvement	8 (21%)
Pulmonary hypertension	4 (11%)
Myocarditis	1 (3%)
Mitral insufficiency	1 (3%)
Cardiac hypertrophy	1 (3%)
Not specified cardiac insufficiency	1 (3%)
Ear-nose-throat involvement	1 (3%)
Nasal perforation	1 (3%)
Necrotising sinusitis	1 (3%)
Other features	Retrocerebellar cyst (<i>n</i> = 1), thyroiditis (<i>n</i> = 1), dyslexia (<i>n</i> = 1)

AH, alveolar haemorrhage; ANCA, antineutrophil cytoplasmic antibody; IgA, immunoglobulin A; ILD, interstitial lung disease.

Data are presented as number (percentages) or median (range). Ages are given in years.

^a Neonatal presentation.

described in Supplementary Table S1, where recorded. While 10 (26%) symptomatic patients manifest single organ involvement, most individuals experienced at least 2, and up to 6, organs involvement during the course of the disease (Fig 1B). Among patients with single organ involvement, pulmonary disease was

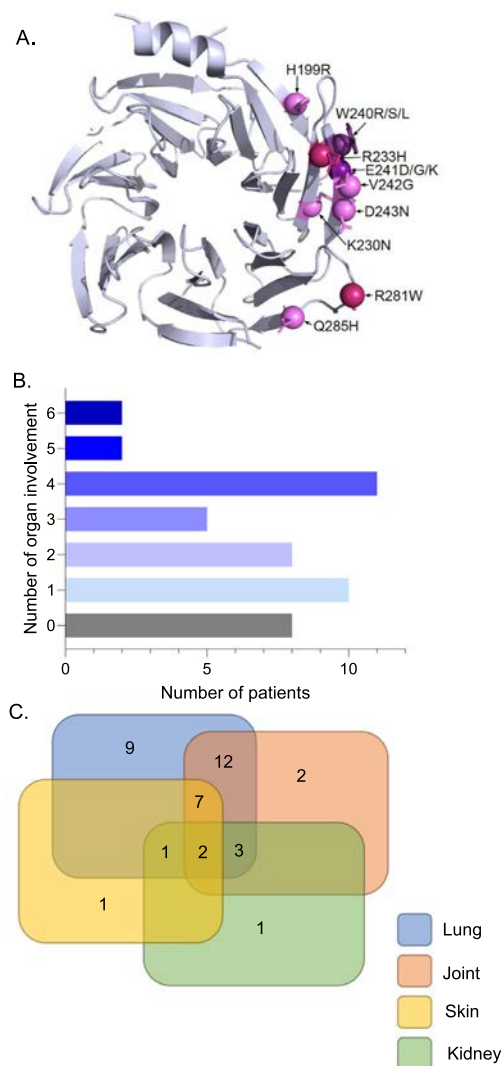


Figure 1. Clinical and molecular spectrum of *COPA* mutations. (A) Location of pathogenic missense mutations within the crystal structure of *COPA* WD-repeat domain (PDB ID: 6PBG). Substitutions affecting W240 and E241 amino acids are shown in purple. The 2 most frequent substitutions reported in the cohort are represented in darker pink, with other mutations shown in light pink. (B) Number of organs involved per patient in the cohort. (C) Specific and overlapping features present in patients of the cohort, including lung, skin, joint and kidney disease.

the most common (n = 9) (Fig 1C). Regarding general features, 14 (37%) patients manifested failure to thrive, and 9 (24%) recurrent fever.

Lung phenotype

The lung was the most commonly affected organ in the cohort, with 34 (89%) symptomatic patients manifesting pulmonary disease. The majority of patients (n = 21) complained of chronic cough and 14/23 (61%) of dyspnoea, while, at medical examination, 10 individuals had nail clubbing and tachypnoea, respectively. Only 2 patients reported episodes of haemoptysis (Supplementary Table S2). The majority of patients were diagnosed with isolated ILD (n = 23) (Supplementary Table S2 and Fig 2G). Three patients had AH, and 8 patients had both ILD and AH. Radiological (n = 12) and/or histological (n = 5) evidence of fibrosis was observed in 14 patients (41%), including patients aged less than 10 years. Of the 26 patients for whom a chest CT scan was available, ground-glass opacities (n = 22) and cysts

Table 2
Clinical penetrance status of the *COPA* mutations identified in the cohort.

Mutation of <i>COPA</i>	Symptomatic patients (n = 38)	Asymptomatic carriers (n = 8)
Amino acid substitution		
Het: c.698G>A/p. (Arg233His), R233H	13 (34%)	4 (50%)
Het: c.841C>T/p. (Arg281Trp), R281W	9 (24%)	1 (12%)
Het: c.718T>C/p. (Trp240Arg), W240R	4 (11%)	1 (12%)
Het: c.719G>C/p. (Trp240Ser), W240S	2 (5%)	0
Het: c.719G>T/p. (Trp240Leu), W240L	1 (3%)	0
Het: c.723G>C/p. (Glu241Asp), E241D	2 (5%)	0
Het: c.722A>G/p. (Glu241Gly), E241G	1 (3%)	0
Het: c.721G>A/p. (Glu241Lys), E241K	1 (3%)	0
Het: c.596A>G/p. (His199Arg), H199R	1 (3%)	1 (12%)
Het: c.690G>T/p. (Lys230Asn), K230N	1 (3%)	0
Het: c.725T>G/p. (Val242Gly), V242G	1 (3%)	0
Het: c.727G>A/p. (Asp243Asn), D243N	1 (3%)	1 (12%)
Het: c.855G>C/p. (Gln285His), Q285H	1 (3%)	0
Inheritance		
Autosomal dominant	23 (61%)	
De novo	5 (13%)	
Mosaic ^a	2 (5%)	
Unknown	8 (21%)	

Het, heterozygous.
Data are presented as number (percentages). Amino acid substitutions are annotated according to HGVS. *COPA* (ENST00000241704.8/NM_004371.4/NP_004362.2).
^a For F14.P1, the variant allele frequency (VAF) was 29% and 17% in blood and the kidney biopsy, respectively; for F15.P1, the VAF was 10% in blood, 9% in nails, 12% in hair bulbs, and 8% in buccal swab.

(n = 15) were the most frequently recorded anomalies, followed by honeycombing (n = 6), micronodules (n = 5), septal thickening (n = 10), and hilar lymphadenopathy (n = 5) (Fig 2A-C). Where PFTs were performed (n = 19), a restrictive pattern was seen in most patients (n = 13), with other patients displaying either an obstructive (n = 1) or mixed pattern (n = 4). BAL was performed in 16 patients, with fluid analysis revealing AH (n = 6), lymphocytic (n = 8), or neutrophilic alveolitis (n = 2). Lipid-laden macrophages were detected in 2 patients. Ten patients underwent lung biopsy, with follicular hyperplasia (ie, the presence of nonclonal lymphocytic aggregates) observed in all cases (Fig 2E,F). In addition, inflammatory lymphocytic infiltrates were recorded in 6 patients, and signs of fibrosis in 5 individuals. Other histopathological findings included cholesterol pneumonitis, focal organising pneumonitis, cellular nonspecific interstitial pneumonia, fibrosing pleuritis, and alveolar simplification. Six patients required long-term oxygen therapy, while disease in 3 patients necessitated noninvasive ventilation, and 1 individual underwent invasive ventilation with extracorporeal membrane oxygenation during severe AH episodes. Two patients underwent lung transplantation at the age of 23 and 28 years, respectively (See *treatment* section), and 1 other was considered as a candidate for lung transplant at the age of 30 years, but was not eligible due to other clinical disease features (Fig 2G).

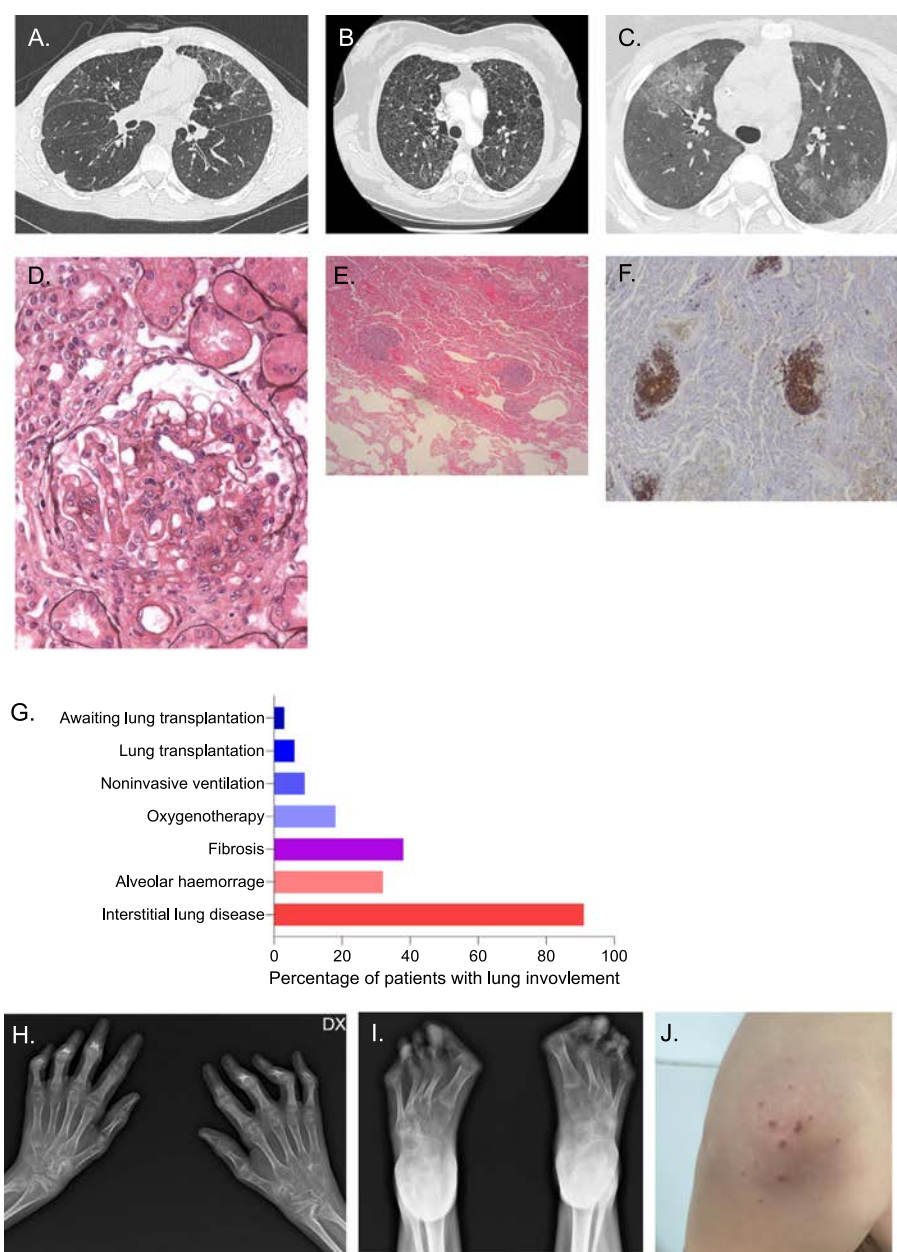


Figure 2. Representative lung imaging and joint disease in COPA syndrome. (A) Chest CT scan of F3.P1 aged 27 years showing ground-glass opacities, cysts and septal thickening. (B) Chest CT scan of F5.P1 aged 24 years demonstrating diffuse cystic lesions. (C) Chest CT scan of F1.P1 aged 12 years with recurrent AH. (D) Kidney biopsy of F2.P4 showing wire-loop thickening of the capillary walls without endocapillary proliferation, presence of a hemi-circumferential cellular crescent and hypertrophy and vacuolation of podocytes. (E) Haematoxylin and eosin (H&E) and Giemsa staining of the lung biopsy of F6.P1 showing subpleural emphysema with local interstitial thickening of the remaining interalveolar septa and lymphoid follicles. (F) CD20 staining of lymphoid follicles showing a predominance of CD20+ B cells. (G) Distribution of pulmonary disease, and requirement for oxygen therapy, noninvasive ventilation or lung transplantation. (H) Hand radiographs of F10.P1 aged 12 years showing diffuse demineralisation, bilateral carpal erosion and ankylosis, erosions with joint pinching of the right 2nd and 5th metacarpophalangeal spaces, and bilateral flaccid deformities of the proximal 2nd to 5th interphalangeal spaces. (I) Foot radiographs of F10.P1 aged 12 years showing diffuse demineralisation and joint misalignment with bilateral major hallux valgus and multifocal metatarsophalangeal dislocations. (J) Purpuric lesions of the elbow of F26.P1 aged 13 years. AH, alveolar haemorrhage; CT, computed tomography.

Joint and muscle involvement

Joint involvement was frequent in the cohort ($n = 26$ patients, 68%). In particular, 17 (45%) patients presented with polyarthritis, often initially diagnosed as juvenile idiopathic polyarthritis, affecting small, large, and axial joints. Of these, 4 patients suffered from severe disease with joint destruction (Fig 2H,I) and deforming arthritis, 1 of whom required bilateral knee replacement and now uses a wheelchair. Arthralgia without arthritis was seen in 6 patients. Of interest, 2 patients presented Jaccoud-like arthropathy as observed in other type I interferonopathies [28], and Boutonniere deformity of the fingers was documented in 2 further individuals.

Skin disease

Cutaneous manifestations were noted in 12 patients (32%), which were predominantly vascular. Notably, 4 patients presented with acral ulcers, chilblains, or acrosyndrom, 1 with *livedo* and 1 with nasal perforation. Other skin features included purpura ($n = 2$) (Fig 2J), malar rash ($n = 2$), panniculitis

($n = 1$), oral ulcers ($n = 1$), and psoriasis ($n = 1$). Skin biopsy of a patient with purpuric lesions of the elbows showed focal leukocytoclastic vasculitis.

Kidney disease

Seven (18%) patients had renal disease. The most common histological pattern was pauci-immune glomerulonephritis associated with antineutrophil cytoplasmic antibody (ANCA) positivity ($n = 3$ with a specificity for anti-myeloperoxidase (MPO)). One patient had lupus-like glomerulonephritis with proliferative lesions associated with focal and segmental glomerulosclerosis and endomembranous deposits of C1q, C3, immunoglobulin M, and immunoglobulin G on immunofluorescence (Fig 2D). Another patient presented a lupus-like membranous glomerulonephritis with a full-house pattern on immunofluorescence and negative antiphospholipase A2 receptor antibodies. In addition, 2 patients had renal insufficiency without further clinical and histological characterisation. Two patients required kidney transplantation (Supplementary Table S3).

Other features

Five (13%) patients presented with hepatic involvement, including 4 individuals with chronic transaminitis, which occurred during methotrexate treatment in 2 cases. Liver biopsy was performed in 1 patient and showed no abnormalities. Seven (18%) patients presented gastrointestinal dysfunction, ie, gastro-oesophageal reflux disease (GERD) ($n = 4$), chronic diarrhoea ($n = 2$), gastrointestinal IgA vasculitis ($n = 1$), and stercoral peritonitis ($n = 1$). Cardiac involvement was identified in 8 (21%) patients. Pulmonary hypertension was found in 4 (11%) individuals, all presenting with ILD. Right heart catheterisation data were available for 2 patients, showing a precapillary pulmonary hypertension. Three patients were treated with phosphodiesterase 5 inhibitors combined with an endothelin receptor inhibitor in 1 case. The fourth patient affected by pulmonary hypertension had no specific treatment. Further cardiac features included myocarditis, mitral insufficiency, cardiac hypertrophy, and unspecified heart failure (Table 1). Two patients had neurological involvement, ie, asymptomatic retrocerebellar cyst in 1 patient and dyslexia with no brain imaging available in the second one. A patient with ANCA presented with nasal perforation and necrotising sinusitis. Finally, autoimmune thyroiditis was documented in 1 patient.

Asymptomatic carriers were all clinically assessed at molecular diagnosis (between the ages of 24 and 48 years) with normal respiratory examination. Pulmonary investigations (chest CT scan and PFTs) were performed in 3 carriers and were normal.

Immunological features

Inflammatory markers

Inflammatory markers were available in less than half of the cohort. When tested, 6 (55%) patients had a dissociation of their inflammatory markers, ie, an elevated erythrocyte sedimentation rate (ESR) with normal levels of C-reactive protein (CRP) (Supplementary Table S4). Specifically, 9/11 (82%) patients had a high ESR, while 12/19 (63%) and 7/19 (37%) patients had, respectively, normal or only mildly elevated CRP.

Type I IFN assessment

All symptomatic patients evaluated ($n = 20$) had a positive-type I IFN gene signature (Supplementary Table S4 and S5). Notably, half of the asymptomatic carriers tested also had a positive IFN signature ($n = 2$), albeit at a lower level than the symptomatic patients (Fig 3A). Serum IFN α protein levels measured by Simoa were high in all symptomatic patients tested ($n = 9$) (Fig 3B), and the 2 asymptomatic carriers had intermediate levels.

Autoantibodies and immunological features

When tested ($n = 31$), all but 1 symptomatic patient (97%) had positive autoantibodies (Fig 3C and Supplementary Table S4). Twenty-four (77%) patients had positive antinuclear antibodies, almost all of which were nonspecific except for 5 patients (anti-DNA, $n = 1$, anti-RNP, $n = 2$, anticentromere, $n = 1$, anti-Scl70, $n = 1$). Fourteen (45%) patients had positive ANCA antibodies (anti-MPO [$n = 9$], anti-PR3 [$n = 2$], or both anti-PR3 and anti-MPO [$n = 2$]). Twelve (39%) patients were positive for rheumatoid factor, and 5 (16%) had positive anticyclic citrullinated peptide (CCP) antibodies (Supplementary Table S4). Six patients presented with hypergammaglobulinaemia.

Lymphocyte immunophenotyping was performed in 7 patients, showing variable modification of lymphocyte subpopulations.

Therapeutics and outcome

Outcome

After a median follow-up period of 48 (6–96) months since the diagnosis of COPA syndrome, 34 patients were alive at last assessment with a median age of 14 (0.5–58) years. Four patients had died. Causes of death included fibrotic progression of ILD and suspected systemic lupus erythematosus without specification at the age of 35 years, respiratory failure at the age of 23 years, and cardiac arrest at home at the age of 31 years, 4 years after lung transplantation. One patient died by suicide.

Treatment

The median number of different immunomodulatory treatments received by each patient was 4 (0–9). More than two-thirds of the patients received at least 2 immunomodulatory drugs (Supplementary Table S3). Seventeen (45%) patients were treated intensively, receiving more than 4 different immune modulators (Fig 3D). Half (55%) of the patients received a disease-modifying antirheumatic drug (DMARD) or a biotherapy (47%) (Fig 3E) during the course of their disease. Twenty-two patients received a JAK inhibitor, most commonly baricitinib ($n = 17$, 45%) (Supplementary Table S3), with a median age at initiation of 13 (0.5–57) years and a median follow-up under treatment of 24 (0–72) months. Of these 22 patients, 16 (73%) had previously received a DMARD and/or a biotherapy without sufficient efficacy, thereby justifying the introduction of a JAK inhibitor (Supplementary Table S6). For 16 patients, JAK inhibition was clinically assessed to have been associated with disease stabilisation or improvement: joint and lung disease improved in 7 patients each, and stabilised in 1 and 6 patients respectively. However, despite treatment, disease progression was noted in 5 patients, including uncontrolled joint disease in 3 cases and lung disease progression in 2 patients. One additional patient had just started treatment. No serious infectious adverse events were noted except in 1 patient on high-dose baricitinib who presented with meningococcal pneumonitis. Two patients received a lung transplantation, and 2 patients received a kidney transplantation. The first patient who underwent lung transplantation had a favourable pulmonary outcome, but subsequently died of cardiac failure 4 years post transplantation. The other one is stable 6 months post transplantation. Kidney function of the 2 patients who underwent kidney transplantation is stable 2 years post transplantation.

DISCUSSION

Previous case descriptions of COPA syndrome provided initial insights into the phenotypic pleiotropy of this rare autosomal dominant disorder [2,5–7,10,12,17,19,29]. The European cohort described here, comprising 38 symptomatic patients, confirms the known core features of COPA syndrome, ie, lung inflammation, arthritis, and kidney disease [2], and further expands the disease spectrum.

In our cohort, most cases manifest early in life, although some cases demonstrated a later onset. Almost 90% of patients manifested pulmonary involvement. Some key features can be delineated to help clinicians diagnose COPA syndrome-related lung disease, which is of particular concern given the high associated morbidity due to progression to fibrosis and end-stage respiratory failure. Clinically, almost half of the patients had nail clubbing, as reported in other cases [14,17], which should be looked for in patients with unexplained autoinflammatory disease. However, lung disease can be strictly asymptomatic;

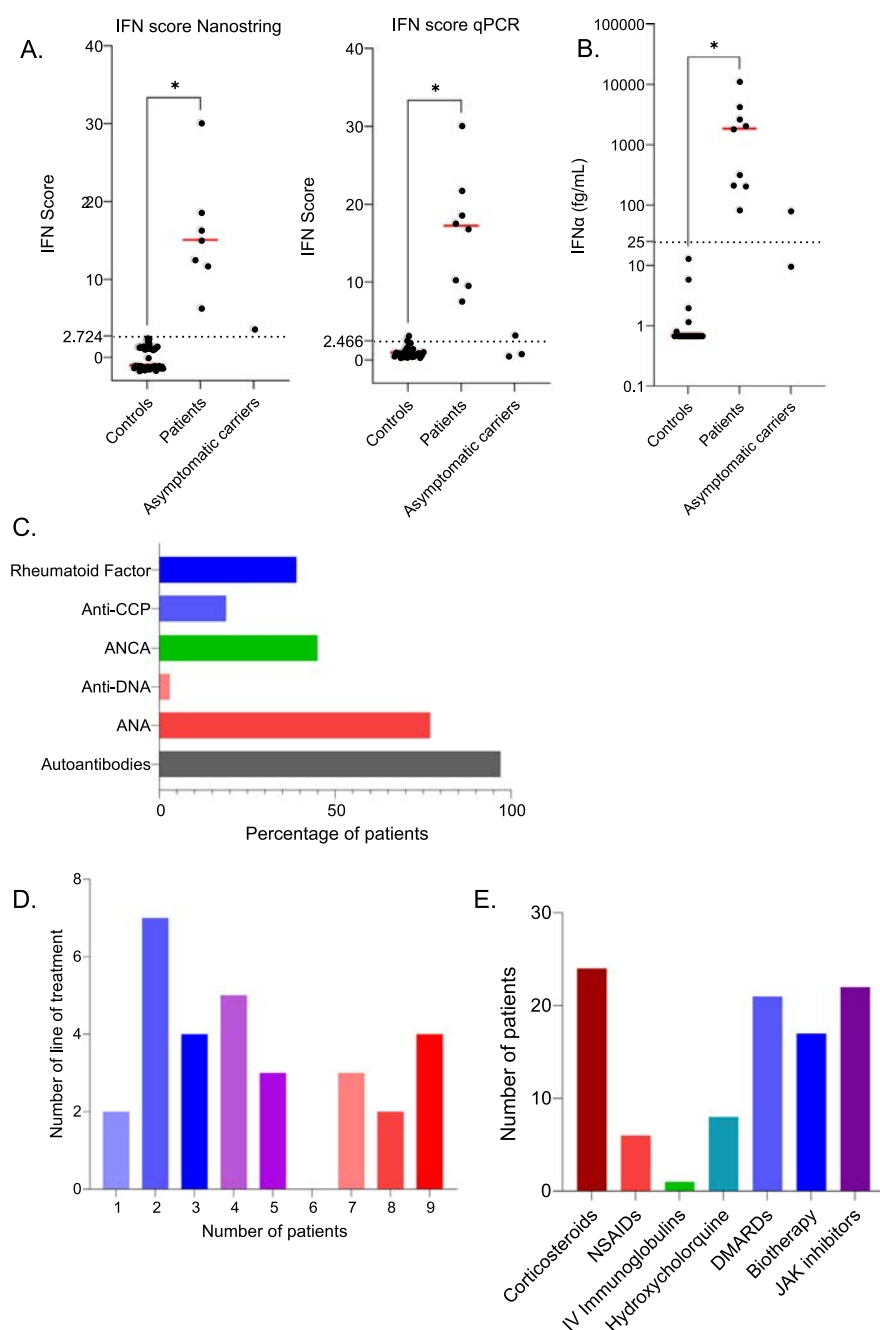


Figure 3. Immunological features and treatment exposures in symptomatic patients with COPA syndrome ascertained in this study. (A) Interferon-stimulated gene (ISG) expression measured in controls, patients and asymptomatic carriers, using either a 24 (left) or 6 (right) ISG panel (measured on a NanoString platform or using RT-qPCR, respectively) to calculate an IFN score. (B) Concentrations of IFN α protein assessed by ultrasensitive digital ELISA (Simoa) in serum from healthy controls ($n = 20$), patients and asymptomatic carriers. (C) Autoantibodies observed in the cohort: rheumatoid factor (RF), anticyclic citrullinated protein (CCP) antibodies, antineutrophil cytoplasm antibodies (ANCA), antidouble-stranded DNA (anti-DNA), antinuclear antibodies (ANAs). (D) Number of lines of treatment received by each patient. (E) Number of patients receiving corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), intravenous (IV) immunoglobulins, hydroxychloroquine, disease-modifying antirheumatic drugs (DMARDs), biotherapy or JAK inhibitors. **** $P < .0001$ using Mann-Whitney U test. ELISA, enzyme-linked immunosorbent assay; IFN, interferon; JAK, Janus kinase; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

therefore, a chest CT scan and PFTs should be performed. As previously described [14], AH can be insidious—with only 2 patients in our cohort presenting with episodes of obvious haemoptysis, and must be looked for on chest imaging (X-ray or CT scan) or in BAL. Regarding lung CT imaging, the most common pathological pattern was of ground-glass opacities. In more than half of the patients, a particular pattern consisting of diffuse cystic lesions was observed. Such cystic lung lesions have also been reported in other genetic type I interferonopathies, most particularly SAVI [30,31]. These cystic lesions might be the radiological consequence of the follicular bronchiolitis or lymphoid follicles found in all patients on biopsy [32]. The lung CT abnormalities seen in our cohort were consistent with other case series [33], except for ‘crazy paving’, which we did not record. BAL analysis was not specific, with the observation of diverse histopathological patterns.

The second major organ involved was the musculoskeletal system, as previously reported [2,11,13,21], with a wide spectrum and severity of manifestations ranging from arthralgia to

destructive polyarticular arthritis regardless of anti-CCP status. Although considered as a core feature of COPA syndrome [2,34], renal disease was recorded in less than a quarter of our cohort. The majority of patients presented with ANCA-like pauci-immune glomerulonephritis, highlighting the fact that COPA syndrome may mimic ANCA vasculitis, as also illustrated by the finding of nasal perforation and necrotising sinusitis in 1 patient. Given the high associated morbidity, renal disease should be searched for in all individuals carrying a pathogenic COPA mutation, with 2 patients in our cohort requiring kidney transplantation. Renal disease can be isolated, underscoring the need to enquire about family history and assess type I IFN signaling in patients with unexplained glomerulopathy [34].

We observed several clinical features not commonly or previously reported in COPA syndrome. Notably, almost one-third of our cohort manifests skin disease, reminiscent in some cases of the skin vasculopathy seen in SAVI and other type I interferonopathies [35]. Other rare organ involvement included the hepatic, gastrointestinal, and cardiac systems. While hepatic

disease has only been previously reported once [29], we recorded 5 patients with hepatic manifestations in our cohort, highlighting the possibility of an underestimation of hepatic disease in COPA syndrome. Of note, 2 patients developed transaminitis after methotrexate, raising concerns about the use of hepatotoxic drugs in the context of COPA syndrome and other type I interferonopathies, where severe liver damage has been observed in a few case reports [36,37]. Studies of metabolic dysfunction-associated steatotic liver disease and viral hepatitis suggest a deleterious effect of STING signalling on hepatocytes [38] that could account for a possible increased risk of hepatotoxicity in monogenic diseases driven by STING activation. Twenty per cent of patients experienced gastrointestinal symptoms, primarily GERD, for which the direct involvement of COPA dysfunction is not clear. Since GERD can worsen lung inflammation and has been reported in SAVI [30], it seems sensible to track and treat GERD in patients with COPA [39]. Finally, pulmonary hypertension has never been described in the context of COPA syndrome. However, 4 patients in our cohort had World Health Organization group 3 pulmonary hypertension. A direct effect of the type I IFN pathway in the development of pulmonary hypertension is also possible, as some evidence has been reported linking type I IFN and STING signalling to pulmonary hypertension [40,41], and pulmonary hypertension has been recorded in patients having type I interferonopathies without lung disease [42]. This study also highlighted another clinical overlap with SAVI: the lack of neurological involvement, which is a core feature of Aicardi-Goutières syndrome and other type I interferonopathies. The diversity of type I interferonopathy phenotypes is not fully understood but is likely to arise from differences in cellular or tissue sources of IFN. The number of patients with COPA syndrome reported in the literature is still small, and it is likely that the full extent of the associated clinical spectrum is yet to be defined. For these reasons, we have been comprehensive in our description of the clinical features observed in the patients described here. At the same time, we recognise that features such as vitiligo and GERD are seen at relatively high frequency in the general population, so that establishing a definitive relationship to COPA dysfunction is not possible at this time. Overall, the phenotypic spectrum of COPA syndrome is strikingly diverse and deserves careful clinical assessment, with some patients having isolated organ manifestation and others multiorgan involvement. As some patients develop novel organ involvement with age, physicians should continue to monitor patients over time.

COPA syndrome is an autosomal dominant disease due to heterozygous mutations inherited from a mutation-positive parent in most cases. In our cohort, the mutation occurred *de novo* in 4 patients. In addition, 2 patients presented with mosaicism, which has not been previously reported in COPA syndrome, but has been seen in other type I interferonopathies [43]. This observation indicates the requirement for a reduction of the VAF detection threshold in molecular diagnosis. In addition to the possibility of *de novo* mutation or mosaicism, another challenge in the diagnosis of COPA syndrome is the high degree of clinical nonpenetrance. Nearly 19% of the individuals in our cohort carried a pathogenic COPA mutation in the absence of clinical signs, a finding consistent with the literature [2]. All asymptomatic carriers in our cohort were women. However, several male asymptomatic carriers have been described by other teams [2]. Interestingly, half of the asymptomatic carriers presented a mildly elevated IFN score, suggesting that other factors—such as additional genetic, environmental, epigenetic influences [44] or monoallelic expression [45]—might play a protective or

aggravating role in determining phenotypic status. When assessing the pathogenic prediction scores of COPA variants *in silico*, the predicted scores were high for all mutations except for the Q285H variant [10]. Of possible note, the Q285H variant was seen in a patient with a relatively mild phenotype, ie, isolated joint disease. Except for this example, no genotype-phenotype correlation has been suggested or reported in COPA syndrome.

All of the mutations in our cohort were located in the same hotspot in the N-terminal part of COPA protein, encompassing exons 7, 8, 9, and 10. The location of these COPA mutations is directly linked to the pathogenesis of COPA syndrome as they affect the WD40 domain, which plays a key role in the recognition of STING for its retrograde transport. Recently, mutations in the C-terminal part of COPA have been published as causative of COPA syndrome [46]. These mutations do not seem to directly affect STING signalling and might be considered as causing a different disease with a more severe inflammatory phenotype. The precise pathogenesis of COPA syndrome is incompletely understood, as some features may be independent of IFN signalling [47], given that STING can mediate non-IFN-related effects [47]. Moreover, COPA mutations may affect protein trafficking beyond STING [1], having been shown to (also) cause ER stress and upregulated Th17 immunity [2]. The use of novel therapies, specifically targeting type I IFN signalling, such as the monoclonal anti-IFNAR1 antibody anifrolumab [48], will help in discriminating the IFN-dependent and independent pathogenesis of COPA syndrome.

Patients with symptomatic COPA experience a high disease burden, with the majority receiving multiple lines of immunosuppressive treatment. Although our study was not designed to assess the response to treatment, JAK inhibition targeting the type I IFN receptor downstream signalling was the most commonly used treatment approach, and was assessed to effectively control at least some aspect of the disease in more than two-thirds of the cases—as previously reported by us and others [13,18,21]. Except for 1 patient who developed meningococcal pneumonia, no other serious adverse events were recorded in the cohort. However, as severe infectious events have been reported with JAK inhibition, all patients should be screened for VZV (varicella-zoster virus) status and offered zoster vaccination when indicated, and BK, EBV (Epstein-Barr virus), and CMV (cytomegalovirus) viraemia should be monitored frequently during follow-up. Unfortunately, in some patients disease still progressed despite JAK inhibitor treatment, and end-stage organ failure occurred in several patients—as manifest by the requirement for lung and kidney transplantation in several patients in our cohort and in the literature [16,49]. Of note, lung transplantation in the context of COPA syndrome or SAVI is challenging, with poor outcomes in most patients [50,51]. Specifically, several patients experienced acute allograft dysfunction, with or without high levels of interleukin-6, highlighting the need to improve the immunosuppressant strategy in the context of constitutive activation of immune cells [16,49,50]. Understanding the specific and relative role of immune cells (monocytes, lymphocytes) and lung resident cells (alveolar epithelial cells, lung endothelial cells) in the pathogenesis of the lung inflammation seen in COPA syndrome and SAVI is crucial to optimise patient care [20].

Here, we describe, to our knowledge, the largest cohort of patients with COPA yet reported. While confirming the known core phenotype of COPA syndrome, we have broadened the clinical spectrum of this severe type I interferonopathy to include other features. Pulmonary and renal disease drive morbidity and mortality in COPA syndrome and should be a focus to prevent

progression to irreversible organ damage. The presence of *de novo* and mosaic mutations, in addition to clinical nonpenetrance, should prompt clinicians to assess type I IFN signalling status and/or sequence COPA in the presence of a concordant phenotype, even in the absence of a family history. JAK inhibition is currently the most common therapeutic strategy used. In the future, the availability of novel treatments that specifically target the type I IFN pathway, particularly anifrolumab [48], will be worth considering in the treatment of COPA syndrome. In the meanwhile, disease pathogenesis remains incompletely understood, particularly the mechanism of lung inflammation [20], an understanding of which will require further functional and therapeutic studies to improve patient management.

Competing interests

All authors declare they have no competing interests.

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Clémence David: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Nadia Nathan:** Writing – review & editing, Project administration, Investigation, Data curation. **Eslam Al-Abadi:** Writing – review & editing, Data curation. **Peter D. Arkwright:** Writing – review & editing, Validation, Investigation, Data curation. **Brigitte Bader-Meunier:** Writing – review & editing, Supervision, Data curation. **Sebastian Becker:** Writing – review & editing, Data curation. **Alexandre Belot:** Writing – review & editing, Data curation. **Mary Brennan:** Writing – review & editing, Data curation. **Sylvain Breton:** Writing – review & editing, Data curation. **Vincent Bondet:** Writing – review & editing, Data curation. **Jacques Cadranel:** Writing – review & editing, Data curation. **Aurore Coulomb l'Hermine:** Data curation. **Sébastien De Almeida:** Writing – review & editing, Data curation. **Darragh Duffy:** Writing – review & editing, Data curation. **Teresa Clavaguera Poch:** Writing – review & editing, Data curation. **Alix de Becdelièvre:** Writing – review & editing, Data curation. **Siham El Khalifi-Boulisfane:** Writing – review & editing, Data curation. **Marco Gattorno:** Writing – review & editing, Data curation. **Montse Gispert-Saüch:** Writing – review & editing, Data curation. **Florian Gothe:** Writing – review & editing, Data curation. **Yves Hatchuel:** Writing – review & editing, Data curation. **Daniela Herdliczko:** Writing – review & editing, Data curation. **Ayşe Ayzit Kilinc:** Writing – review & editing, Data curation. **Vaclav Koucky:** Writing – review & editing, Data curation. **Géraldine Labouret:** Writing – review & editing, Data curation. **Raffaele Manna:** Writing – review & editing, Data curation. **Hélène Maillard:** Writing – review & editing, Data curation. **María Luisa Matoses Ruipérez:** Writing – review & editing, Data curation. **Caterina Matucci-Cerinic:** Writing – review & editing, Data curation. **Anna Mensa-Vilaro:** Writing – review & editing, Data curation. **Katarzyna Michel:** Writing – review & editing, Data curation. **Thierry Jo Molina:** Data curation. **Berta Lopez Montesinos:** Writing – review & editing,

Data curation. **William G. Newman:** Writing – review & editing, Data curation. **Julia Papenkort:** Writing – original draft, Data curation. **Christina Rapp:** Writing – review & editing, Data curation. **Cinthia Rames:** Writing – original draft, Data curation. **Gillian I. Rice:** Writing – review & editing, Data curation. **Markus A. Rose:** Writing – review & editing, Data curation. **Héloise Reumaux:** Writing – review & editing, Data curation. **Laurent Sailer:** Writing – review & editing, Data curation. **Nicolaus Schwerk:** Writing – review & editing, Data curation. **Luis Seabra:** Writing – review & editing, Data curation. **Jérémy Sellam:** Writing – review & editing, Data curation. **Andrea Taddio:** Writing – review & editing, Data curation. **Caroline Thumerelle:** Writing – review & editing, Data curation. **Alberto Tommasini:** Writing – review & editing, Data curation. **Maud Tusseau:** Writing – review & editing, Data curation. **Stefano Volpi:** Writing – review & editing, Data curation. **Martin Wetzke:** Writing – review & editing, Data curation. **Laurence Weiss:** Writing – review & editing, Data curation. **Anne Welfringer-Morin:** Writing – review & editing, Data curation. **Marie Wislez:** Writing – review & editing, Data curation. **Matthias Giese:** Writing – review & editing, Data curation. **Yanick J. Crow:** Writing – review & editing, Writing – original draft, Supervision, Data curation. **Marie-Louise Frémond:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ard.2025.09.013.

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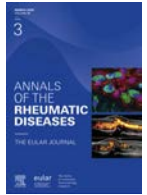
Clémence David: <http://orcid.org/0000-0001-6455-3251>

Marie-Louise Frémond: <http://orcid.org/0000-0002-2798-9141>

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Epidemiology

Tirzepatide and change in uric acid and its association with weight reduction: post hoc analyses of the SURMOUNT-1 randomised placebo-controlled trial

Naveed Sattar^{1,*}, Sabrina Scilletta^{1,2}, Adam Stefanski³, Hui Wang⁴, Jack W Daly³, Bruno Linetzky³

¹ School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, Scotland

² Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

³ Eli Lilly and Company, Indianapolis, Indiana, USA

⁴ TechData Services Company, King of Prussia, Pennsylvania, USA

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ABSTRACT

Objectives: This study aimed to test whether tirzepatide, a dual GLP-1RA/GIP agonist, approved for weight management, would be associated with lowered serum uric acid (SUA) levels via its weight reduction properties.

Methods: A post hoc analysis of the SURMOUNT-1 trial, a randomised placebo-controlled trial involving 2539 adults with obesity or overweight (body mass index [BMI] ≥ 30 kg/m² or ≥ 27 kg/m² and at least 1 weight-related complication), randomised to tirzepatide (5, 10, or 15 mg) or placebo for 72 weeks. Tirzepatide treatment over 72 weeks decreased weight by up to 20.9%. SUA was measured at baseline and multiple time points during the 72-week study across the 3 active trial arms, and changes were compared to the placebo arm. The association between weight changes and SUA changes was evaluated by mediation analysis.

Results: Treatment with all dose groups of tirzepatide was associated with significant reductions in SUA, compared to placebo. At week 72, the mean change in SUA was -0.69 mg/dL (SE: 0.04), -0.92 mg/dL (0.04), and -0.95 mg/dL (0.04) with 5, 10, and 15 mg of tirzepatide (all $P < .001$), respectively, and -0.18 mg/dL (0.04) with placebo. SUA levels reduced significantly over time compared to placebo, regardless of baseline uric acid quartiles ($P = .610$) and baseline BMI values ($P = .362$). Mediation analysis suggested that weight reduction explained 72.7% of SUA reduction.

Conclusions: In this post hoc analysis, in participants with obesity or overweight, tirzepatide was associated with meaningfully reduced SUA levels, regardless of participants' baseline BMI or SUA levels, and appeared to be so primarily via weight reduction. These findings warrant further investigation into the possible role that tirzepatide/intentional weight loss may play in the treatment of patients with gout living with obesity.

INTRODUCTION

Gout is the most common form of inflammatory arthritis that causes considerable pain in the acute phase, with a prevalence of

>5% in US adults [1]. It is also associated with an increased risk of a broad spectrum of cardiovascular diseases (CVDs) [2,3].

Gout has been traditionally associated with excess nutrition, with red meat and alcohol, 2 commonly associated lifestyle

*Correspondence to Dr Naveed Sattar, School of Cardiovascular and Metabolic Health, University of Glasgow, BHF Glasgow Cardiovascular Research Centre, Glasgow, Scotland.

E-mail address: Naveed.Sattar@glasgow.ac.uk (N. Sattar).

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Gout, the most common form of inflammatory arthritis, causes significant acute pain. Traditionally linked to excess nutrition like red meat and alcohol, gout and hyperuricaemia are also associated with obesity, increasing their incidence and prevalence. Weight reduction interventions, particularly bariatric surgery, have been shown to lower serum uric acid (SUA) levels and gout incidence. However, data on newer pharmaceutical therapies remain sparse, especially from large-scale randomised trials.
- Treatment with tirzepatide—a long-acting dual glucose-dependent insulinotropic polypeptide receptor and glucagon-like peptide-1 (GLP-1) receptor agonist—resulted in a clinically significant decrease in body weight of up to 20.9% in individuals living with obesity or overweight, with at least one weight-related comorbidity, in the SURMOUNT-1 trial.
- A recent meta-analysis showed no significant effect on SUA-lowering capacity for several GLP-1 agonists in placebo-controlled clinical trials. Notably, these GLP-1 agonists had a lesser effect on weight compared to tirzepatide. To our knowledge, limited data have been published on the effect of GLP-1 agonists with pronounced weight reduction capacity on SUA levels.

WHAT THIS STUDY ADDS

- This post hoc analysis investigated the SUA levels of all participants (2539 adults with a body mass index (BMI) of 30 or more, or 27 or more, and at least one weight-related complication, excluding diabetes) randomised to tirzepatide (5, 10, or 15 mg) or placebo for 72 weeks in the SURMOUNT-1 trial.
- Treatment with all dose groups of tirzepatide was associated with significant reductions of SUA at each time point, compared to placebo. Furthermore, SUA levels reduced significantly over time compared to placebo, regardless of baseline SUA levels and baseline BMI values.
- Finally, a mediation analysis suggested that weight reduction was responsible for 72.7% of SUA reduction.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Treatment with tirzepatide, primarily through meaningful weight reduction, might have an impact on gout development and aid in treating hard-to-treat gout in patients with obesity.

factors. However, obesity may also play a role, being associated with a greater incidence and prevalence of gout [4]. A 2018 meta-analysis reported that individuals with a body mass index (BMI) of ≥ 30 kg/m² had a >2-fold higher risk of gout vs a BMI of <30 kg/m² [5]. Higher genetically predicted BMI has also been associated with serum uric acid (SUA) levels and gout, consistent with causality [6]. More recently, Ferguson et al [2] demonstrated that individuals who developed gout were, on average, almost 3 BMI units heavier than those who did not. Additionally, in men, the younger a participant developed gout, the greater the BMI differential: men diagnosed with gout at a young age (aged 20–24 years at the index date) had a mean difference in BMI of 4.32 kg/m² vs matched individuals without gout. For women, the higher BMI in those with gout appeared more consistently elevated across the life course.

Weight reduction interventions, most notably bariatric surgery, have been shown to be associated with lower SUA levels and fewer incidents of gout. Over a follow-up of up to 26 years following bariatric surgery, the incidence of gout was 40% lower in the nonrandomised Swedish subjects with obesity intervention study (adjusted HR 0.60, 95% CI 0.48–0.75) [7]. However,

robust randomised trial data on potential changes in SUA with newer pharmaceutical therapies, now proven to cause weight reduction levels approaching those seen with bariatric surgery [8], are relatively sparse. These data are important, as approved weight reduction therapies are increasingly being used worldwide due to their potential to simultaneously improve multiple cardiometabolic outcomes [9].

Tirzepatide, a long-acting dual glucose-dependent insulinotropic polypeptide receptor and glucagon-like peptide-1 (GLP-1) receptor agonist, has been approved for the treatment of type 2 diabetes (T2D) and obesity. In the SURMOUNT-1 trial [8], tirzepatide treatment over 72 weeks resulted in a clinically significant decrease in body weight of up to 20.9% in individuals living with obesity or overweight, with at least 1 weight-related comorbidity. Such a degree of weight reduction in the context of a randomised trial therefore provided an excellent opportunity to test the hypothesis that tirzepatide, perhaps largely via weight reduction, would significantly lower SUA levels. If true, amongst its other proven benefits, tirzepatide may be predicted to lower incident gout and aid in the prevention of acute gout episodes through the reduction of SUA levels [10].

METHODS

This study represents a post hoc analysis of the SURMOUNT-1 trial. The trial design, inclusion and exclusion criteria, and procedures have been previously published [8]. Briefly, SURMOUNT-1 was a phase 3 randomised, double-blind, placebo-controlled trial in which 2539 adults with a BMI ≥ 30 kg/m² or ≥ 27 kg/m² and at least 1 weight-related complication (eg, hypertension, dyslipidaemia, obstructive sleep apnoea, or CVD), excluding diabetes, were assigned to once weekly, subcutaneous tirzepatide (5, 10, or 15 mg) or placebo for 72 weeks as an adjunct to lifestyle intervention. The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by an independent ethics committee or institutional review board at each trial site. All the participants provided written, informed consent before participation.

Outcomes

The main outcome of the current post hoc analysis was the change in SUA from baseline to week 72. SUA was measured at baseline and during follow-up at Labcorp (Labcorp Central Laboratory Services LP) as part of a chemistry panel. SUA was measured using an enzymatic colourimetric test performed by the Roche Cobas Analyser.

Statistical analyses

Statistical analyses were performed in the modified intent-to-treat population, defined as all randomised participants exposed to at least 1 dose of study drug. Postbaseline variables were assessed using the efficacy estimand, ie, excluding data obtained after discontinuation of study drug (last dose date + 7 days).

Change and percent change from baseline to week 72 in SUA and weight were assessed using a mixed model for repeated measurements (MMRM), where treatment, visits, treatment by visit interaction, pooled country, sex, prediabetes status at randomisation, and baseline values were modelled as fixed effects and subject as a random effect. Treatment effect and change in SUA from baseline were also evaluated within each subgroup of baseline SUA quartiles and BMI categories (<30, ≥ 30 , <35,

Table 1
Study population characteristics

Characteristic	Placebo (N = 643)	TZP 5 mg (N = 630)	TZP 10 mg (N = 636)	TZP 15 mg (N = 630)	Total (N = 2539)
Age (y)	44.4 ± 12.5	45.6 ± 12.7	44.7 ± 12.4	44.9 ± 12.3	44.9 ± 12.5
Female sex, no. (%)	436 (67.8)	426 (67.6)	427 (67.1)	425 (67.5)	1714 (67.5)
Race, no. (%)					
American Indian or Alaska native	58 (9.0)	56 (8.9)	58 (9.1)	59 (9.4)	231 (9.1)
Asian	71 (11.0)	68 (10.8)	71 (11.2)	66 (10.5)	276 (10.9)
Black or African American	55 (8.6)	48 (7.6)	47 (7.4)	51 (8.1)	201 (7.9)
White	450 (70.0)	447 (71.0)	452 (71.1)	443 (70.3)	1792 (70.6)
Native Hawaiian or other Pacific Islander	2 (0.3)	2 (0.3)	2 (0.3)	3 (0.5)	9 (0.4)
Multiple	7 (1.1)	9 (1.4)	6 (0.9)	8 (1.3)	30 (1.2)
Ethnic group, no. (%)					
Hispanic or Latino	310 (48.2)	308 (48.9)	297 (46.7)	299 (47.5)	1214 (47.8)
Duration of obesity (y)	14.0 ± 10.71	14.0 ± 10.81	14.7 ± 11.05	14.8 ± 10.75	14.4 ± 10.83
Height (cm)	165.6 ± 9.3	165.7 ± 9.0	166.1 ± 9.3	166.1 ± 9.7	165.9 ± 9.3
Body weight (kg)	104.8 ± 21.37	102.9 ± 20.71	105.8 ± 23.32	105.6 ± 22.92	104.8 ± 22.12
(1.3) Mean body mass index	38.2 ± 6.89	37.4 ± 6.63	38.2 ± 7.01	38.1 ± 6.69	38.0 ± 6.81
Waist circumference (cm)	114.0 ± 14.92	113.2 ± 14.25	114.8 ± 15.80	114.4 ± 15.59	114.1 ± 15.16
Baseline ORC, n (%)					
Hypertension	199 (30.9)	205 (32.5)	208 (32.7)	207 (32.9)	819 (32.3)
Dyslipidaemia	186 (28.9)	201 (31.9)	188 (29.6)	182 (28.9)	757 (29.8)
ASCVD	21 (3.3)	16 (2.5)	20 (3.1)	21 (3.3)	78 (3.1)
Gout	7 (1.1)	8 (1.3)	7 (1.1)	10 (1.6)	32 (1.3)
Estimated GFR, ml/min/1.73 m ²	98.1 ± 18.28	97.6 ± 17.87	98.3 ± 18.26	98.2 ± 17.67	98.1 ± 18.02
Prediabetes, no. (%)	270 (42.0)	247 (39.2)	262 (41.2)	253 (40.2)	1032 (40.6)
Uric acid levels (mg/dL)	5.61 (1.45)	5.50 (1.33)	5.65 (1.38)	5.62 (1.43)	5.59 (1.40)
Uric acid ≥ 6 mg/dL n (%)	233 (36.2)	214 (34.0)	243 (38.2)	229 (36.3)	919 (36.2)
Uric acid ≥ 7 mg/dL n (%)	95 (14.8)	86 (13.7)	102 (16.0)	107 (17.0)	390 (15.4)

ASCVD, atherosclerotic cardiovascular disease; eGFR, estimated glomerular filtration; N, total number of participants; ORC, obesity-related complications; TZP, tirzepatide.

Data are mean ± SD unless stated otherwise.

≥35, and < 40, ≥40 kg/m²), respectively. The interaction effect between the subgroup variables was estimated using a similar MMRM model with additional fixed-effect terms for the baseline subgroup variable and interactions including subgroup variable, treatment by subgroup variable interaction, visit by subgroup variable interaction, and treatment by visit by subgroup variable interaction.

The Pearson correlation coefficients were calculated between the weight change and SUA change from baseline to week 72 by treatment group. Direct and indirect effect (mediated via weight change) of pooled tirzepatide (5/10/15 mg) vs placebo in SUA change from baseline to week 72 was estimated using a mediation analysis, where the weight change at week 72 served as the mediator. The model for mediation analysis included treatment, weight change at week 72, weight change at week 72 and treatment interaction, and baseline SUA level.

Other data, such as uric acid-lowering medications, were summarised descriptively by treatment group.

Role of the funding source

The funder of the study had a role in study design, data collection, data analysis, data interpretation, and writing of the report.

RESULTS

Study population characteristics

A total of 2539 participants from SURMOUNT-1 who had serum SUA measurements at baseline and at least 1 postbaseline measurement were included in the analyses. The characteristics

of the population, extracted from the SURMOUNT-1 trial, are presented in Table 1. The average age of the participants was 44.9 years; the majority were White (70.6%) and female (67.5%); the mean waist circumference was 114.1 cm; the mean body weight was 104.8 kg; the mean BMI was 38.0; and 94.5% of the individuals had a BMI of 30 or higher. The use of gout or uric acid-lowering medications was reported in the medical history of 1.7% of the participants. At baseline, hyperuricaemia with SUA higher than 7 mg/dL was observed in 390 participants (15.4%). At baseline, the mean SUA was 5.6 ± 1.4 mg/dL.

Change in body weight

The mean change in weight at week 72 was −15.0% (95% CI −15.9% to −14.2%) with a 5-mg weekly dose of tirzepatide, −19.5% (95% CI −20.4% to −18.5%) with a 10-mg dose, −20.9% (95% CI −21.8% to −19.9%) with a 15-mg dose, and −3.1% (95% CI −4.3% to −1.9%) with placebo, as reported in Figure 1.

Change in SUA levels

Treatment with all dose groups of tirzepatide was associated with significant reductions of SUA at each time point, compared to placebo, as shown in Figure 2A and B.

The mean change in SUA at week 72 was −0.69 mg/dL (SE: 0.04) with a 5-mg weekly dose of tirzepatide, −0.92 mg/dL (0.04) with a 10-mg dose, −0.95 mg/dL (0.04) with a 15-mg dose, and −0.18 mg/dL (0.04) with placebo. The pattern of change in SUA levels matched the pattern of weight change, with more pronounced changes observed with higher doses and with steeper declines initially, plateauing towards the 72 weeks' time point.

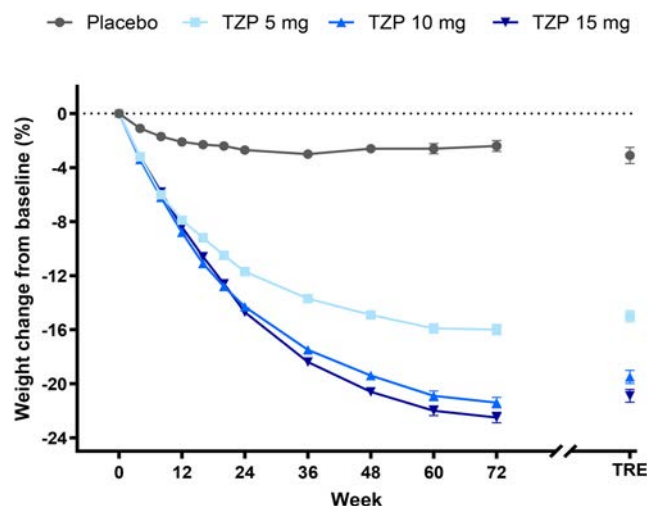


Figure 1. Changes in body weight with once weekly tirzepatide (TZP), compared with placebo. Data are least-squares means. Error bars indicate the SE. Percent change in body weight by week (from baseline to 72 wk), derived from a mixed model for repeated measures (MMRM) analysis for the efficacy estimand; week 72 estimates for the treatment regimen estimand are also shown.

Change in SUA levels by baseline BMI and SUA acid levels, and sex

SUA levels reduced significantly over time compared to placebo, regardless of baseline levels split into quartiles (<4.6 mg/dL, ≥ 4.6 and <5.4 mg/dL, ≥ 5.4 and <6.4 mg/dL, and ≥ 6.4 mg/dL), as reported in Figure 3. At week 72, no significant interaction was observed between baseline SUA and change in SUA ($P = .610$). Furthermore, the reduction of SUA levels did not appear to be dependent on the participants' baseline BMI values, as shown in Figure 4 ($P = .362$). Finally, significant reductions in SUA levels were observed in both sex subcategories, as shown in Figure 5.

Association of changes in SUA and body weight

In the mediation analysis, for the direct effect between tirzepatide and placebo at week 72, the estimated treatment difference in the mean SUA change from baseline was -0.185 (-0.304 , -0.064). The estimated indirect effect between tirzepatide and

placebo in the mean SUA was -0.492 (-0.567 , -0.421). Weight reduction was estimated to explain 72.7% of SUA reduction. Across all tirzepatide groups comparing change from baseline to week 72, there was a significant correlation between the change in body weight and change in SUA; placebo ($r = 0.187$, $P < .0001$), tirzepatide 5 mg ($r = 0.306$, $P < .0001$), tirzepatide 10 mg ($r = 0.263$, $P < .0001$), tirzepatide 15 mg ($r = 0.279$, $P < .0001$), shown in Table 2.

Other results

The incidence of gout was consistent among all treatment groups, but numbers were small; 3 participants in the placebo group (0.5%), 4 participants in the 5 mg tirzepatide group (0.6%), 2 participants in the 10 mg tirzepatide group (0.3%), and 3 participants in the 15 mg tirzepatide group (0.5%) (Supplementary Table S1). In the 5 mg tirzepatide group, 3 participants stopped the use of uric acid-lowering therapies postbaseline, while 2 participants in the 15 mg tirzepatide group stopped the use of these therapies (Supplementary Table S1).

DISCUSSION

Treatment with all dose groups of tirzepatide was associated with significant reductions of SUA at each timepoint, compared to placebo, with greater reductions observed in the higher dose groups. Moreover, SUA levels reduced significantly over time compared to placebo, regardless of baseline BMI and SUA. Finally, the patterns of change in SUA, weight, and mediation analysis suggested that weight reduction explained 72.7% of SUA reduction. However, the partial rebound of SUA levels observed in the safety follow-up may indicate a potential direct effect of tirzepatide on SUA levels, or it could possibly represent a reversion towards higher caloric intake after the cessation of treatment with tirzepatide, leading also to gradual weight regain. Collectively, these data suggest tirzepatide, by inducing meaningful weight and consequently SUA reductions, could assist with the regulation of uric acid levels. This could be further explored in both the treatment of hyperuricaemia and the prevention of gout in patients with obesity.

It is worth noting that tirzepatide treatment may also offer an effect on SUA that is nonweight mediated. This could be due to several factors related to GLP-1 agonism, such as an increase in

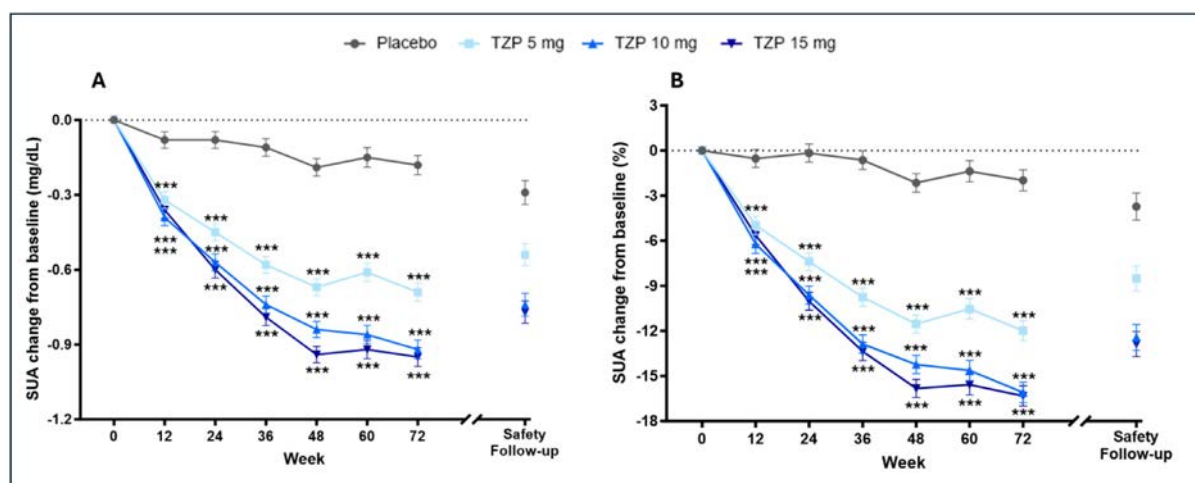


Figure 2. Changes in serum uric acid levels with once weekly tirzepatide, compared with placebo. Data are least-squares means. Error bars indicate the SE. Serum uric acid changes from baseline in (A) mg/dL and (B) %, by week (from baseline to 72 wk, and safety follow-up at 76 wk) across different treatment groups and placebo mg/dL. ** $P < .01$, *** $P < .001$. SUA, serum uric acid; TZP, tirzepatide.

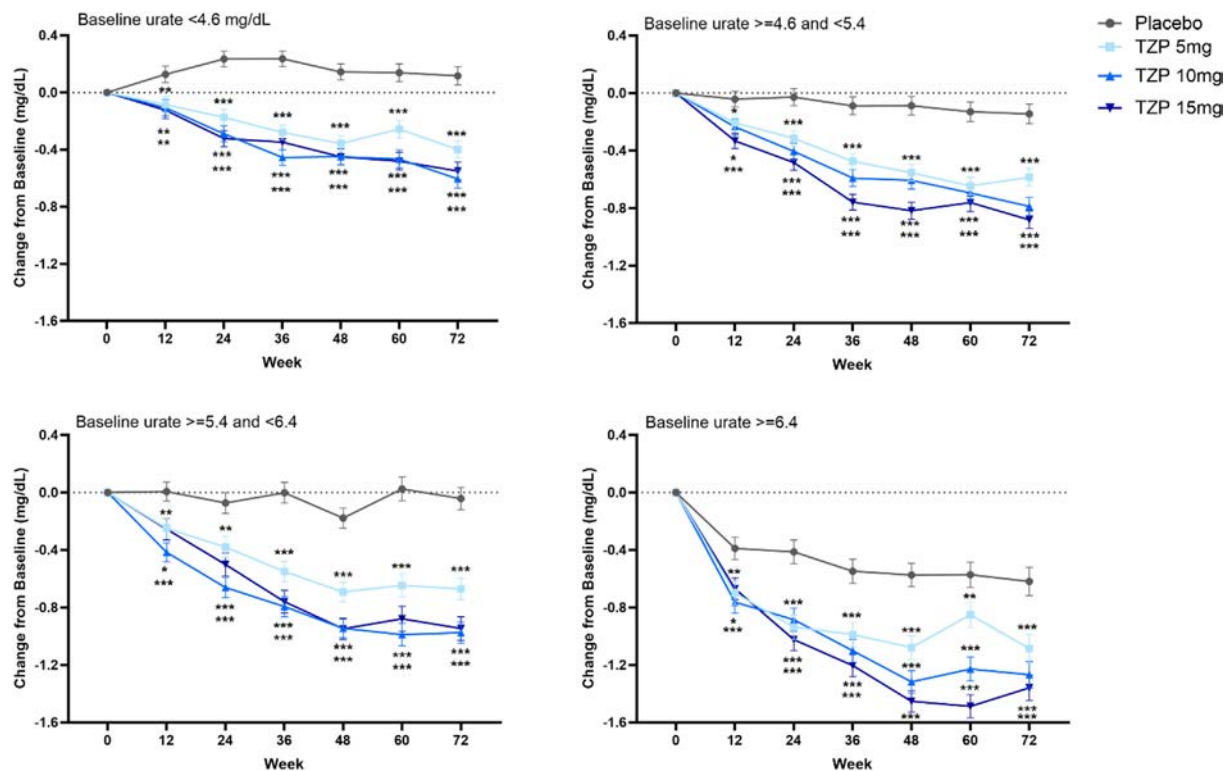


Figure 3. Serum uric acid levels by baseline serum acid levels observed in tirzepatide, compared with placebo. Data are least-squares means. Error bars indicate the SE. Change in serum uric acid (mg/dL) by week (from baseline to 72 wk) across groups stratified by quartiles of baseline serum uric acid, derived from a mixed model for repeated measures (MMRM) analysis. **P* < .05, ***P* < .01, ****P* < .001. TZP, tirzepatide.

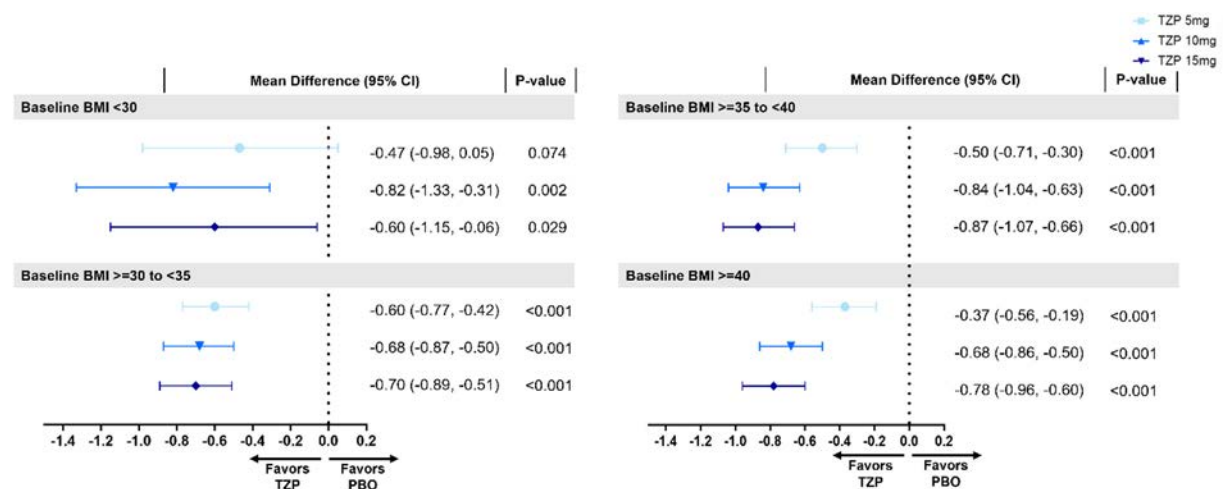


Figure 4. Serum uric acid levels by baseline BMI observed in tirzepatide, compared with placebo. Forest plot highlighting the mean serum uric acid changes associated with tirzepatide vs placebo in groups stratified by baseline BMI (<30, ≥30 to <35, ≥35 to <40, ≥40 kg/m²). BMI, body mass index; CI, confidence interval; PBO, placebo; TZP, tirzepatide.

the urinary excretion of uric acid, likely via inhibiting Na⁺/H⁺ -exchanger type-3 in the renal proximal tubule [11]. Also, the urinary clearance of uric acid could be affected by changes in insulin resistance levels [12]. Future research should explore these possibilities.

A meta-analysis of studies conducted primarily in patients with diabetes revealed a reduction in SUA from pre- to post-GLP-1 RA administration, despite agents with very modest effects on weight being used. However, placebo-controlled trials with those agents did not show significant reductions in SUA [13].

There are a limited number of pharmacological therapies available to lower SUA. Of these, allopurinol remains the most

used SUA-lowering therapy. In this post hoc analysis, treatment with higher doses of tirzepatide was associated with SUA reductions similar to those observed in participants treated with the lower dose ranges of allopurinol [14]. These findings do not suggest an equivalence but serve to conceptualise the potential of tirzepatide within the scope of contemporary SUA-lowering approaches, at least in patients with specific characteristics.

The pathophysiologic mechanism of hyperuricaemia in people living with obesity is not fully understood; overproduction of uric acid due to excess total caloric intake leading to obesity is likely [15], as well as reduced renal excretion [16]. The impaired renal excretion has been related to commonly observed risk factors associated with obesity, such as

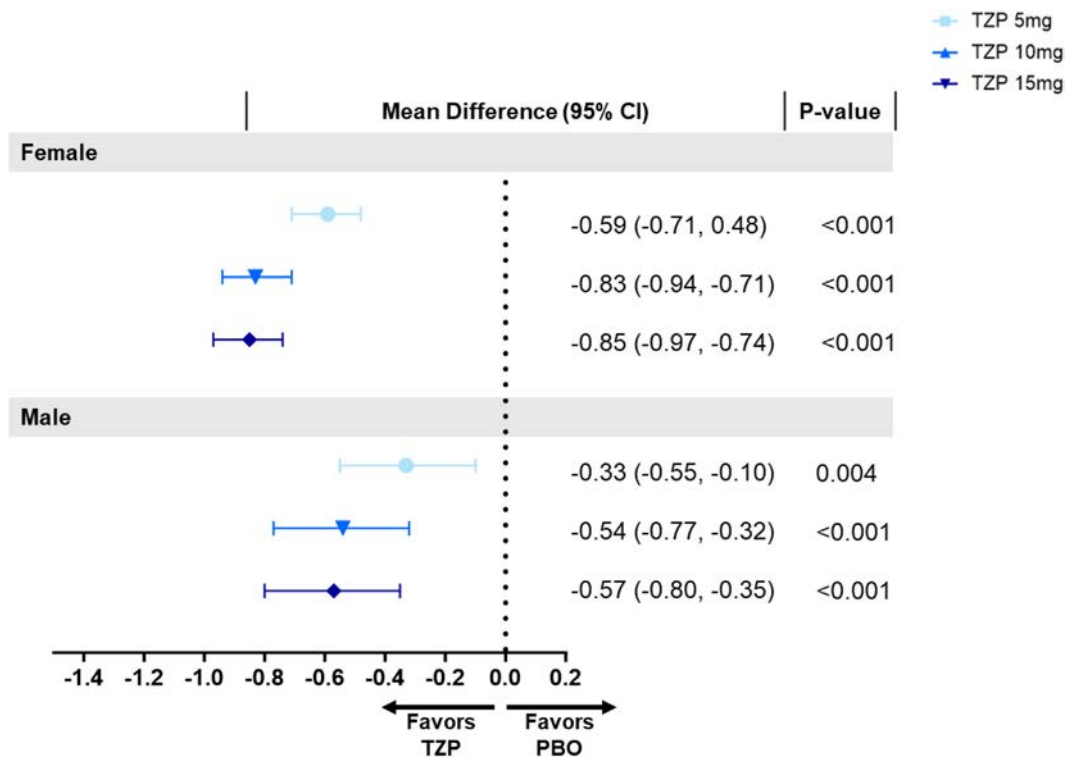


Figure 5. Serum uric acid levels by sex observed in tirzepatide, compared with placebo. Forest plot highlighting the mean serum uric acid changes associated with tirzepatide vs placebo in sex groups. BMI, body mass index; CI, confidence interval; PBO, placebo; TZP, tirzepatide.

Table 2
Correlations between weight change and SUA change in study groups at week 72

Treatment	r value	P value
Placebo	0.187	<.0001
Tirzepatide 5 mg	0.306	<.0001
Tirzepatide 10 mg	0.263	<.0001
Tirzepatide 15 mg	0.279	<.0001

P value from Pearson Correlation; r, Pearson correlation coefficient; SUA, serum uric acid.

hypertension- and diabetes-related circulatory and kidney complications [17].

Tirzepatide is most likely to be associated with rapid SUA reductions via rapid reductions in caloric intake. However, preliminary evidence also suggests that such drugs may reduce cravings towards alcohol intake, which could have added benefits beyond weight reduction [18]. Potential actions of tirzepatide on kidney function may also play a role [19]. In a post hoc analysis of SURPASS-4 trial of participants with T2D [20], tirzepatide was associated with a slower rate of estimated glomerular filtration decline and reduced albuminuria in clinically meaningful ways compared with insulin glargine. Tirzepatide was also associated with a greater decrease in albuminuria vs insulin, semaglutide, and placebo in a post hoc analysis of pooled SURPASS 1-5 trials [19]. Hence, multiple mechanisms may explain the impact of tirzepatide to reduce SUA, with weight reduction being the most dominant. These data are important, as clinicians managing rheumatological conditions are increasingly recognising the progressively widespread effects of obesity in the conditions they treat, such as gout [21]. Consequently, there is growing interest in incorporating weight reduction interventions into the therapeutic armoury.

The analysis has some limitations. First, it is a post hoc analysis, since the SURMOUNT-1 trial was not designed to assess

effects on SUA. Second, the study population differs somewhat from a truly representative gout population. Most of the study population was female (67.5%); however, gout is 3.26 times more prevalent in males in the general population [22]. Sex-specific analyses showed significant SUA reductions in both sex groups (Fig 5). Moreover, most participants were White (70.6%), highlighting the need for further studies in other ethnic groups to ensure broader generalisability. In addition, the mean BMI in SURMOUNT-1 was likely much higher than that of the average patient with gout. However, as previously demonstrated in a large cohort study, both men and women living with gout have higher BMI values across the life course as compared to people without gout [2]. In addition, there was no clear evidence for differences in uric acid reduction with tirzepatide by baseline BMI as demonstrated in Figure 4. Third, SURMOUNT-1 had a relatively young population with a mean age of 45 years. The incidence of gout is known to increase with age, with one study observing a mean age of 60 years for men and 68 years for women at the first diagnosis of gout [23]. Finally, while weight loss is commonly associated with a reduction in the incidence of gout flares, this was not observed in this study. However, this was likely due to the smaller sample size (and so limited power), the specific population included, and the short follow-up. Future trial work should investigate the impact of contemporary weight loss medications on the incidence of gout flares in a population living with obesity who have recently experienced gout flares.

In conclusion, all 3 doses of once-weekly tirzepatide were associated with significant reductions in SUA in adults with obesity or overweight in the present trial. Overall, the data suggest tirzepatide treatment, primarily by inducing meaningful weight reduction, may have an impact on SUA that could be further investigated for a possible role in preventing the development of gout and potentially in the treatment of patients with gout living with obesity. Such robust findings, in the context of a large-scale randomised trial, provide the basis for further appropriately

designed research to advance these clinically relevant concepts. These data also suggest that there may be a greater need to focus on weight reduction interventions per se (including lifestyle) in people who are living with overweight or obesity and present with gout.

CRedit authorship contribution statement

Naveed Sattar: Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Sabrina Scilletta:** Writing – review & editing, Writing – original draft, Conceptualization. **Adam Stefanski:** Writing – review & editing, Resources, Project administration, Methodology, Data curation. **Hui Wang:** Writing – review & editing, Formal analysis. **Jack W Daly:** Writing – review & editing. **Bruno Linetzky:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

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Competing interests

NS reports administrative support, article publishing charges, and writing assistance were provided by Eli Lilly and Company. NS reports a relationship with Abbott Laboratories Inc that includes: speaking and lecture fees. NS reports a relationship with AbbVie Ltd. that includes: consulting or advisory and speaking and lecture fees. NS reports a relationship with Amgen Inc that includes: consulting or advisory and speaking and lecture fees. NS reports a relationship with AstraZeneca that includes: consulting or advisory, funding grants, and speaking and lecture fees. NS reports a relationship with Boehringer Ingelheim GmbH that includes: consulting or advisory, funding grants, and speaking and lecture fees. NS reports a relationship with Carmot Therapeutics Inc that includes: consulting or advisory. NS reports a relationship with Eli Lilly and Company that includes: consulting or advisory and speaking and lecture fees. NS reports a relationship with GlaxoSmithKline that includes: consulting or advisory. NS reports a relationship with Hanmi Pharmaceuticals Co Ltd that includes: consulting or advisory. NS reports a relationship with Menarini-Ricerche that includes: consulting or advisory. NS reports a relationship with Metsera Inc that includes: consulting or advisory. NS reports a relationship with Novartis that includes: consulting or advisory and funding grants. NS reports a relationship with Novo Nordisk that includes: consulting or advisory and speaking and lecture fees. NS reports a relationship with Pfizer that includes: consulting or advisory. NS reports a relationship with Roche that includes: consulting or advisory, funding grants, and speaking and lecture fees. HW reports a relationship with Eli Lilly and Company that includes: consultant statistician, on behalf of TechData Services Company, employed by Eli Lilly and Co. for this analysis. AS reports a relationship with Eli Lilly and Company that includes: employment and equity or stocks. JWD reports a relationship with Eli Lilly and Company that includes: employment and equity or stocks. BL reports a relationship with Eli Lilly and Company that includes: employment and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Patient consent for publication

Not applicable.

Ethics approval

The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by an independent ethics committee or institutional review board at each trial site. All the participants provided written, informed consent before participation.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Eli Lilly and Company provides access to all individual participant data collected during the trial, after anonymisation, with the exception of pharmacokinetic or genetic data. Data are available to request 6 months after the indication studied has been approved in the USA and the EU and after primary publication acceptance, whichever is later. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, and blank or annotated case report forms, will be provided in a secure data sharing environment. For details on submitting a request, see the instructions provided at www.vivli.org.

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ard.2025.10.009](https://doi.org/10.1016/j.ard.2025.10.009).

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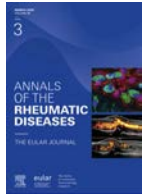
Naveed Sattar: <http://orcid.org/0000-0002-1604-2593>

Bruno Linetzky: <http://orcid.org/0009-0009-4015-7794>

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Pain disorders

Cannabidiol versus placebo in patients with fibromyalgia: a randomised, double-blind, placebo-controlled, parallel-group, single-centre trial

Marianne Uggen Rasmussen^{1,*}, Robin Christensen^{1,2},
Eva Ejlersen Wæhrens^{1,3}, Marius Henriksen¹, Pernille Hurup Duhn¹,
Henning Bliddal¹, Kirstine Amris¹

¹ Parker Institute, Bispebjerg and Frederiksberg Hospital, Frederiksberg, Denmark

² Research Unit of Rheumatology, Department of Clinical Research, University of Southern Denmark, Odense University Hospital, Odense, Denmark

³ User Perspectives and Community-based Interventions, Department of Public Health, University of Southern Denmark, Odense, Denmark

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ABSTRACT

Objectives: Cannabidiol (CBD) is used to alleviate fibromyalgia pain despite limited evidence for efficacy. This study assessed the efficacy and safety of CBD vs placebo in patients with fibromyalgia, hypothesising that CBD would be superior to placebo in reducing pain.

Methods: In this single-centre, double-blind, randomised, placebo-controlled trial, patients diagnosed with fibromyalgia were recruited from a specialised outpatient clinic in Denmark. Eligible participants were randomised 1:1 and stratified by sex, defined as biological sex assigned at birth based on physical anatomy. Age (<45 vs ≥45), and pain level (<7 vs ≥7) on a 0 to 10 numeric rating scale (NRS) to receive 50 mg plant-derived CBD or placebo tablets. The primary outcome was change in pain intensity at week 24, assessed on the NRS pain subitem in the revised Fibromyalgia Impact Questionnaire in the intention-to-treat population. Adverse events were monitored throughout the study in the safety population.

Results: Of 273 participants screened for eligibility, 200 were included and randomised to receive CBD (n = 100) or placebo (n = 100). At week 24, mean change in pain intensity was −0.4 points (95% CI: −0.82 to 0.08) in the CBD group and −1.1 points (95% CI: −1.53 to −0.63) in the placebo group, corresponding to a between-group difference of −0.7 points (95% CI: −1.2 to −0.25; *P* = .0028) favouring placebo. Adverse events were generally mild and evenly distributed between groups.

Conclusions: The findings do not support CBD 50 mg daily as an analgesic supplement for patients with fibromyalgia. ClinicalTrials.gov number: NCT04729179.

*Correspondence to Dr. Marianne Uggen Rasmussen, The Parker Institute, Bispebjerg and Frederiksberg Hospital, Nordre Fasanvej 57, DK-2000 Frederiksberg, Denmark.

E-mail address: marianne.uggen.rasmussen@regionh.dk (M.U. Rasmussen).

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What is already known on this topic

- Cannabidiol (CBD) is commonly used by patients with fibromyalgia to manage pain.
- Evidence supporting the efficacy of CBD for fibromyalgia pain relief is limited and inconclusive.
- There is growing interest in exploring non-opioid alternatives for chronic pain conditions like fibromyalgia.

What this study adds

- This randomized, placebo-controlled trial found that 50 mg of plant-derived CBD daily was **not effective** in reducing fibromyalgia pain compared to placebo.
- Adverse events were generally mild and occurred at similar rates in both groups, suggesting CBD at this dose is safe but not efficacious.

How this study might affect research, practice or policy

- These findings challenge the assumption that CBD is a beneficial analgesic for fibromyalgia and highlight the need for more rigorous evidence before recommending its use.
- Clinicians may reconsider prescribing or endorsing CBD for fibromyalgia pain management based on this evidence.
- This study does not form basis for national clinical guidelines or recommendations for the treatment of Cannabidiol 50 mg in the treatment of fibromyalgia.
- Future research may focus on different dosages, formulations, or patient subgroups to better understand CBD's role in chronic pain treatment.

INTRODUCTION

Fibromyalgia is a chronic pain syndrome affecting 2% to 5% of the general population, with a higher prevalence in women [1]. Core clinical features include widespread pain and tenderness, and nonpain symptoms such as fatigue, nonrefreshing sleep, cognitive dysfunction, and emotional distress [2]. The condition leads to significant disability, including work disability, increased healthcare utilisation, and lower quality of life compared to other chronic diseases [3]. The pathophysiology of fibromyalgia is not completely understood but involves changes in pain processing brain networks and imbalance in anti- and pronociceptive pathways [4]. Current management strategies aim to alleviate symptoms and maintain functioning through both nonpharmacological and pharmacological approaches [5]. Recommended pharmacological treatments include antidepressants and anticonvulsants [6], but these adjuvant analgesics offer limited benefits and are often discontinued due to adverse effects [6]. Therefore, there is an urgent need for more effective and safer therapeutic options.

Cannabis-based medicine is used for fibromyalgia [7] despite the existing knowledge gap on the efficacy and safety of these treatments, as identified by The International Association for the Study of Pain, based on systematic reviews of the existing literature of randomised controlled trials (RCTs) [8,9]. Medical cannabis, which refers to medications derived from the top shoots of cannabis plants, are categorised into 2 subgroups: delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) [10]. THC is known for its psychoactive effects, appetite stimulation, and nausea reduction, whereas CBD is believed to have anti-inflammatory, anticonvulsive, and immune-modulating properties [10]. Both are suggested to have pain-relieving effects, with CBD having a more favourable safety profile [11]. However, there is a lack of evidence from high-quality dose-response and well-powered

pharmacokinetic studies to inform clinical practice on the appropriate dosing and administration of CBD for achieving clinical effectiveness. This gap necessitates expert guidance and treatment protocols based on expert consensus for its clinical use in the treatment of chronic pain, including fibromyalgia [12]. Given the limited evidence [13], this treatment option remains inaccessible to patients, leading some to self-administer unlicensed medical cannabis at their own risk and expense [14]. Thus, well-designed randomised trials are warranted. Consequently, we designed and conducted a study to evaluate the efficacy and safety of CBD in patients with fibromyalgia. The aim of the study was to evaluate the efficacy and safety of CBD for patients with fibromyalgia. We hypothesised that CBD would be superior to placebo in reducing pain intensity and improving key secondary outcomes.

METHODS

Study design

This investigator-initiated, single-centre, randomised, double-blind trial, was conducted in a specialised fibromyalgia outpatient clinic at Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark. The trial adhered to the principles of the Declaration of Helsinki and was approved by the health research ethics committee for the Capital Region of Denmark (Journal no. H-20047715) and the Danish Data Protection Agency (P-2020-229). The trial protocol is available in [Supplementary Appendix, File S1](#).

Patient research partners diagnosed with fibromyalgia contributed to aligning the research questions and outcomes in accordance with the patients' perspectives and endorsed the research protocol. The authors take responsibility for the accuracy and completeness of the data and analyses, the integrity of the trial and of this report.

Participants

Participants with an established clinical diagnosis of fibromyalgia were recruited from a multidisciplinary patient education and rehabilitation program at the fibromyalgia outpatient clinic at Bispebjerg and Frederiksberg Hospital, Denmark. Patients enrolled in this program that had previously given their consent to be contacted for the purpose of participation in research, were invited to participate through a secure digital mailbox. Those responding to the invitation and showing an interest in participation were subsequently prescreened for eligibility by telephone interview. Eligible participants were aged 18 to 75 years, fulfilling the American College of Rheumatology criteria for fibromyalgia [15], had an average pain intensity of ≥ 4 on a numeric rating scale (NRS) according to the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) recommendations [16], and had not used cannabis within the last 6 months.

Exclusion criteria included a primary diagnosis of systemic inflammatory rheumatic disorder, impaired liver/kidney function, epilepsy, cancer, major depression, other severe psychiatric disorders, substance abuse, and current use of opioids, opioid antagonists, or similar strong analgesics. Participants who were pregnant/planning pregnancy or breastfeeding were also excluded. A complete list of the inclusion and exclusion criteria is provided in the protocol. All included patients provided written informed consent prior to study procedures.

Randomisation and masking

After signing informed consent, all participants were assessed for eligibility by KA and PHD. If eligible, participants proceeded

to baseline assessment and were subsequently randomly assigned 1:1 to receive plant-derived CBD 50 mg daily or placebo for 24 weeks. Stratified randomisation was performed with a computer-generated randomisation sequence based on permuted blocks of up to 8 individuals according to sex (male vs female), age group (<45 vs ≥45 years), and pain intensity level (<7 vs ≥7 on the NRS). An independent biostatistician (RC) generated the randomisation sequence using a random number generator (SAS Proc Plan) that was entered into the electronic case report form (e-CRF) by an independent data manager (CCH) who was not involved in the rest of the trial. Participants were blinded to their treatment allocation until the final visit of the last participant. Outcome assessors and data analysts remained blinded to group assignments until the study database was locked, analysis of the primary outcome was completed, and a blinded abstract was drafted and saved (see [Supplementary Appendix, File S2](#), Abstract Blinded Interpretation).

Procedures

The investigational product was manufactured and supplied by Glostrup Pharmacy in Denmark, a facility specialising in the production of indistinguishable pharmaceutical formulations for research purposes, including magistral preparation of plant-derived medical cannabis. Both the active compound and the placebo were delivered to the clinical site in 4 separate batches over the course of the 2-year study. Prior to distribution, each batch underwent comprehensive quality assessment by the Danish Medicines Agency in accordance with national regulatory standards for magistral medicines. The analytical evaluations confirmed the accuracy and consistency of the active compound and verified that the placebo tablets were identical in appearance, taste, and smell. The pharmacy labelled the tablet packages with unique Dispensing Unit Numbers (DUNs). The database manager ensured correct administration of the study medication according to group allocation, by matching DUNs with participant numbers in the e-CRF system.

CBD and placebo were administered orally in tablet form. Based on expert guidance for CBD treatment of patients with chronic pain in routine clinical care [17], participants started with 10 mg daily and titrated up with 10 mg every third day until reaching 50 mg or the highest tolerable dose, which was maintained until the end of the 24-week treatment period. Study visits were conducted weekly during the titration period and subsequently every 4 weeks, alternating between site visit and telephone contacts. A postintervention follow-up visit occurred at week 36. Throughout the study, monitoring of vital signs and clinical and laboratory evidence of adverse events (AEs) was conducted.

Outcomes

The primary outcome was change in pain intensity from baseline to week 24, measured on the 0 to 10 NRS pain subscale of the revised Fibromyalgia Impact Questionnaire (FIQ-R) [18], where 0 indicates no pain and 10 indicates worst pain. Reporting also included the proportion of participants achieving 1 NRS unit, 30% and 50% pain reduction. Key secondary outcomes were changes in sleep quality and duration, measured with the Pittsburgh Sleep Quality Index [19], observed nightly rest defined as time spent lying down, and observed time out of bed during the night, measured with a triaxial accelerometer [20]. Activities of daily living were assessed using both the observation-based Assessment of Motor and Process Skills test [21] and the Activities of Daily Living Questionnaire [22]. Health-related

quality of life was measured with the EuroQol EQ-5D Questionnaire (EQ-5D) [23], and energy level was measured with the energy level 0 to 10 NRS subscale of the FIQ-R. Safety was monitored throughout the study period through AEs reporting and clinical monitoring of vital signs and laboratory tests.

Statistical analysis

The study was powered to detect a between-group difference of 1 NRS unit in mean pain intensity, based on a total sample of 200 participants. The sample size was calculated based on the assumption that a 2-sample pooled t-test of a normal mean difference with a 2-sided significance level of 0.05 ($P < .05$), with a common SD of 2 on a 0 to 10 pain NRS, a total sample size of 200 with a balanced design (1:1), would have the statistical power of 0.94 (94%) to detect a difference between the means of 6 and 7, (see Statistical Analyses Plan in [Supplementary Appendix, File S3](#)). The primary analysis was based on the intention-to-treat (ITT) population, while AEs were analysed in the safety population (all participants who received ≥1 dose of the study medication).

For continuous outcomes, we used repeated measures, mixed-effects models with restricted maximum likelihood estimation, with the patient identification number as a random effect and with the baseline score and stratifying baseline characteristics (sex, age group, pain severity group), treatment group, time (baseline, 4, 12, 20, and 24 weeks), and the interaction between treatment group and time. Missing data were handled with the use of the mixed-effects linear-models approach with an assumption that data were missing at random, in accordance with the working assumption underlying these models

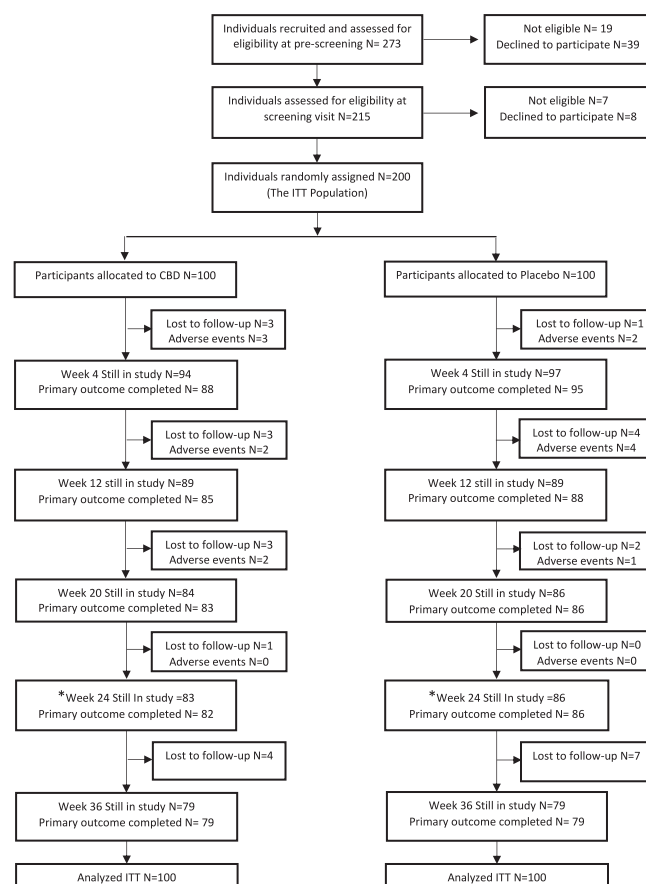


Figure 1. Trial profile: participant flow throughout the trial. CBD, cannabidiol; ITT, intention-to-treat.

Table 1
Baseline characteristics of patients in the intention-to-treat population

Variables	CBD (N = 100)	Placebo (N = 100)	Total (N = 200)
Age, y, mean (SD)	49.7 (10.5)	50.1 (10.9)	49.9 (10.7)
Female, n (%)	95 (95.0)	94 (94.0)	189 (94.5)
Body mass index, kg/m ² , mean (SD)	30.1 (7.7)	28.6 (6.1)	29.3 (7.0)
Duration of chronic pain: n (%)			
- <1 y	6 (6.0)	7 (7.0)	13 (6.5)
- 1–5 y	32 (32.0)	32 (32.0)	64 (32.0)
- >5 y	62 (62.0)	61 (61.0)	123 (61.5)
Tender point count, mean (SD)	15.9 (2.5)	15.6 (2.7)	15.7 (2.6)
Pain medication: n (%)			
- Weak analgesics (paracetamol/NSAIDs)	93 (93.0)	92 (92.0)	185 (92.5)
- Secondary analgesics (antidepressants/anticonvulsants)	24 (24)	19 (19)	43 (21.5)
Expecting a positive effect of the study drug, n (%)	89 (89.0)	88 (88.0)	177 (88.5)
Marital status: n (%)			
- Married/cohabiting	61 (61.0)	64 (64.0)	125 (62.5)
Education: n (%)			
- Secondary school (9th or 10th grade)	26 (26.0)	21 (21.0)	47 (23.5)
- High school	18 (18.0)	15 (15.0)	33 (16.5)
- Bachelor's/master's degree	56 (56.0)	64 (64.0)	120 (60.0)
Occupation: n (%)			
- Working full time	17 (17.0)	26 (26.0)	43 (21.5)
- Working part time with social benefit (flex job)	15 (15.0)	14 (14.0)	29 (14.5)
- Other social transfer income	10 (10.0)	6 (6.0)	16 (8.0)
- Disability pension	12 (12.0)	16 (16.0)	28 (14.0)
- On sick leave	7 (7.0)	7 (7.0)	14 (7.0)
- Pending social welfare application	29 (29.0)	22 (22.0)	51 (25.5)
Primary and key secondary outcomes			
Pain intensity, average last 7 d, FIQ NRS 0–10, mean (SD)	6.9 (1.6)	6.9 (1.6)	6.9 (1.6)
Sleep quality, PSQI 0–3, mean (SD)	2.2 (0.7)	2.1 (0.7)	2.2 (0.7)
Sleep duration, PSQI, h, mean (SD)	5.6 (1.2)	5.8 (1.3)	5.7 (1.3)
Observed nightly rest, h, mean (SD)	6.6 (1.1)	6.8 (1.1)	6.7 (1.1)
Observed time out of bed during the night, s, median (Q1, Q3)	256 (170, 363)	233 (155, 325)	240 (161, 338)
AMPS motor,* Logits, mean (SD)	1.2 (0.4)	1.2 (0.5)	1.2 (0.42)
AMPS process,* Logits, mean (SD)	1.01 (0.29)	0.95 (0.29)	0.98 (0.29)
Self-reported activities of daily living, ADLQ, Logits, median (Q1, Q3)	1.57 (0.68, 2.70)	1.83 (0.91, 2.78)	1.70 (0.74, 2.78)
Quality of life, EQ5D global VAS 0–100, mean (SD)	36.3 (20.5)	36.4 (21.0)	36.3 (20.7)
Energy, FIQ NRS 0–10, mean (SD)	7.5 (1.9)	7.0 (2.1)	7.2 (2)

ADLQ, Activities of Daily Living Questionnaire; AMPS, Assessment of Motor and Process Skills; CBD, cannabidiol; EQ-5D, EuroQol EQ-5D Questionnaire; FIQ, Fibromyalgia Impact Questionnaire; NRS, numerical rating scale; NSAID, nonsteroidal anti-inflammatory drug; PSQI, Pittsburgh Sleep Quality Index; VAS, visual analogue scale.

* Performance-based activities of daily living.

[24]. Changes from baseline are reported as least-squares means, and between-group differences are presented with 95% CIs. To account for multiplicity and preserve the overall type I error for the secondary outcomes, a hierarchical (gatekeeping) strategy was used. 95% CIs were not adjusted for multiplicity and should not be used in place of hypothesis testing. Responders, defined as participants with a >1-point improvement on the NRS or at least a 30% or 50% reduction in pain at 24 weeks, were analysed using risk ratios (RRs) with 95% CIs, with missing data addressed through nonresponder imputation.

The robustness of the main findings was confirmed through sensitivity analyses, including nonresponder imputation with baseline observations carried forward, and per protocol analyses, including participants with ≥80% adherence to the prescribed treatment and compliant with the scheduled activities. Analyses were performed using SAS software, version 9.4 M5 64 bit advanced (SAS Institute). The statistical analysis plan was finalised on January 3, 2024 and made publicly available before enrolment closed.

The study was registered with the Danish Medical Agency (EUDRACT: 2019-002394-59) on August 25, 2020, and the protocol was registered with www.clinicaltrials.gov on January 18, 2021 (NCT04729179). The study was overseen by an independent monitoring committee according to Good Clinical Practice.

RESULTS

Between April 12, 2021, and April 17, 2023, 215 individuals were screened for enrolment. Prior to screening, 273 individuals were prescreened for eligibility by telephone, of whom 19 were

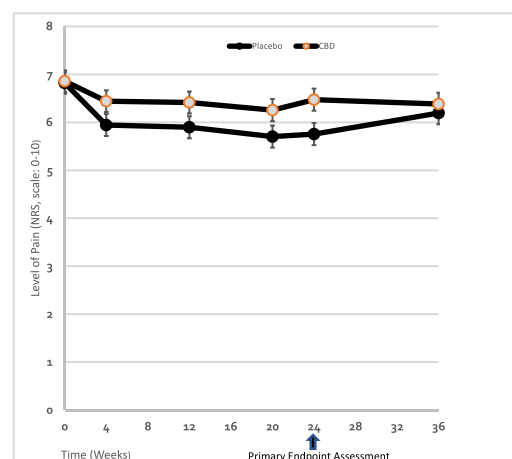


Figure 2. Trajectories of the least-squares means (with SE) of the average pain intensities from baseline to 36 weeks in the intention-to-treat population. CBD, cannabidiol; NRS, numerical rating scale.

Table 2
Change in primary and key secondary outcomes at 24 weeks from baseline based on the ITT population (repeated measures mixed-effects models)

Primary outcome	Change from baseline after 24 wk of treatment (SE)		Difference between groups (95% CI)	P
	CBD	Placebo		
Pain intensity, average last 7 d, FIQ NRS 0-10	−0.40 (0.20)	−1.10 (0.20)	−0.70 (−1.20 to −0.20)	.0028
Key secondary outcomes				
Sleep quality, PSQI 0-3	−0.20 (0.10)	−0.40 (0.10)	−0.19 (−0.30 to 0.04)	.12
Sleep quality, PSQI, h	−0.02 (0.14)	0.18 (0.14)	0.19 (−0.10 to 0.49)	.19
Observed nightly rest, h	0.17 (0.10)	−0.20 (0.10)	0.37 (0.16 to 0.58)	.0006
Observed time out of bed during the night, s	7.84 (13.58)	23.20 (13.10)	15.36 (−12.51 to 43.23)	.28
Performance-based activities of daily living, AMPS ADL motor ability, Logits	0.04 (0.03)	0.05 (0.03)	0.02 (−0.05 to 0.08)	.61
Performance-based activities of daily living, AMPS ADL process ability, Logits	0.03 (0.03)	0.08 (0.03)	0.05 (−0.01 to 0.10)	.088
Self-reported activities of daily living, ADLQ, Logits	−0.12 (0.19)	−0.10 (0.20)	0.02 (−0.37 to 0.41)	.91
Quality of life, EQ5D global VAS 0-100	0.20 (2.30)	6.50 (2.30)	6.30 (1.40 to 11.20)	.012
Energy, FIQ NRS 0-100	−0.50 (0.30)	−0.90 (0.30)	−0.40 (−1.00 to 0.20)	.19
Responder indices				
		CBD	Placebo	Risk ratio (95% CI)
>1 unit improvement in FIQ-R pain, n (%)		17 (17)	31 (31)	0.55 (0.33 to 0.92)
≥30% improvement in FIQ-R pain, n (%)		6 (6)	25 (25)	0.24 (0.10 to 0.56)
≥50% improvement in FIQ-R pain, n (%)		2 (2)	11 (11)	0.18 (0.04 to 0.80)

ADL, activities of daily living; ADLQ, Activities of Daily Living Questionnaire; AMPS, Assessment of Motor and Process Skills; CBD, cannabidiol; EQ5D, EuroQol EQ-5D Questionnaire; FIQ-R, Fibromyalgia Impact Questionnaire, revised; ITT, intention-to-treat; NRS, numerical rating scale; PSQI, Pittsburgh Sleep Quality Index; VAS, visual analogue scale. Estimates are least-squares means with standard errors unless otherwise indicated. The 95% CIs were not adjusted for multiplicity and should not be used in place of hypothesis testing.

ineligible and 39 declined to participate. Of the 215 individuals screened for enrolment, 7 were ineligible and 8 declined to participate, resulting in 200 participants enrolled and randomly assigned to receive CBD or placebo, with 100 participants in each group (Fig 1). All randomised participants were included in the ITT population. Participants' mean age was 49.9 (SD 10.7) years and 189 (94.5%) were female (Table 1). In the CBD group, 83 participants completed week 24, 10 were lost to follow-up, and 7 dropped out due to AEs. In the placebo group, 86 participants completed week 24, 7 were lost to follow-up, and 7 dropped out due to AEs. In the CBD group, 67 reached the maximum maintenance dose of 50 mg daily, 18 completed at 40 mg daily, and 1 at 30 mg. In the placebo group, 74 achieved the maximum dose, 3 completed at 40 mg daily, 3 at 30 mg, 2 at 20 mg, and 1 at 10 mg daily. Protocol deviations occurred in both groups; 5 participants in the CBD group and 2 in the placebo group required opioid treatment due to nonrelated AEs.

Follow-up data for all outcomes were available as of February 14, 2024. The trajectories of pain intensity in the 2 groups over the study period are shown in Figure 2. Primary and key secondary outcomes are reported in Table 2. The outcomes are reported for each group, along with between-group differences after 24 weeks of treatment, with 95% CIs and P values. The mean change in pain intensity was −0.4 (95% CI: −0.82 to 0.08) in the CBD group and −1.1 (95% CI: −1.53 to −0.63) in the placebo group, with a between-group difference of −0.7 favouring the placebo group (95% CI: −1.2 to −0.25; P = .0028). This difference, however, was not clinically meaningful. Additionally, a higher number of responders was observed in the placebo group, with significantly more participants achieving an improvement of >1 NRS unit (CBD n = 17 vs placebo n = 31, RR = 0.55, 95% CI: 0.33-0.92), 30% improvement defined as a clinically

meaningful change (CBD n = 6 vs placebo n = 25, RR = 0.24, 95% CI: 0.10-0.56), and 50% improvement defined as a substantial change (CBD n = 2 vs placebo n = 11, RR = 0.18, 95% CI: 0.04-0.80).

No differences between groups were observed in secondary patient-reported or observed outcomes, except for change from baseline in EQ-5D scores, which improved more in the placebo group than in the CBD group, with a between-group difference of 6.3 (95% CI: 1.4-11.2). The only objectively assessed secondary outcome with a difference between groups was change in observed nightly rest, which was greater in the CBD group than in the placebo group, with a between-group difference of 22 minutes (0.37 hours, 95% CI: 0.16-0.58), favouring the CBD group. The overall patterns of the results remained consistent in the sensitivity analyses (see Supplementary Appendix File S4; Table 2a and 2b, Sensitivity Analyses).

AEs were evenly distributed in the CBD and placebo groups regarding the number of events per participants (median = 4), number of mild, moderate, and severe AEs, and withdrawals due to AEs (Table 3). More participants experienced serious AEs (SAEs) in the placebo group than in the CBD group (8 vs 2). However, all SAEs reported were hospitalisations due to other and unrelated reasons and not related to the study medication. The most frequent AEs that were considered probably related to the study medication [25] were headaches/migraines, worsening in fatigue, nausea/vomiting, diarrhoea, and dryness of the mouth/throat. Other frequent AEs were worsening in pain, COVID-19 infections and common colds, which were evenly distributed in the 2 groups, followed by influenza, which was more frequent in the CBD group.

Table 3

Harms: adverse events in total and observed in $\geq 5\%$ of the safety population

Patients with ≥ 1 adverse events	Total N = 200	CBD N = 100	Placebo N = 100
Patients with ≥ 1 AE	192	97	95
AE events per patient (median/range)	4 (0-16)	4 (0-16)	4 (0-12)
Patients with ≥ 1 mild AE	168	82	86
Patients with ≥ 1 moderate AE	128	69	59
Patient with ≥ 1 severe AE	25	11	14
Patients with ≥ 1 serious adverse events	10	2	8
Withdrawals due to AE	14	7	7
Deaths	0	0	0
Observed AEs in $\geq 5\%$ of the ITT population			
Central nervous system			
Dizziness	33	16	17
Headaches/migraines	51	28	23
Sensory disturbances	18	8	10
Psychological/cognitive			
Depressed mood	21	10	11
Anxiety/distress	10	3	7
Cardiac system			
Palpitations	11	6	5
Hot flashes	11	3	8
Gastrointestinal system			
Nausea/vomiting	41	17	24
Diarrhoea	30	18	12
Constipation	20	8	12
Stomach pain	27	10	17
Increased appetite	14	7	7
Decreased appetite	15	6	9
Skin and mucus membranes			
Dryness of mouth/throat	28	16	12
Itchy skin	11	5	6
Rash	10	8	2
Infections			
Covid-19 infection	49	23	26
Common cold	46	21	25
Influenza	30	23	7
Gastroenteritis	10	5	5
Musculoskeletal system			
Worsening in pain	38	20	18
General events			
Worsening in fatigue	45	26	19
Worsening in sleep disturbances	16	7	9

AE, adverse event; ITT, intention-to-treat.

Data are number unless otherwise specified.

DISCUSSION

In this double-blind, randomised trial, administration of plant-derived CBD at a dose of 50 mg daily for 24 weeks did not demonstrate superiority over placebo in reducing average pain intensity. In fact, the study findings indicated more favourable outcomes for pain and health-related quality of life in the placebo group, although the observed between-group differences were not clinically meaningful. Nevertheless, a significantly higher proportion of participants in the placebo group achieved a clinically relevant improvement of $\geq 30\%$ in the pain score compared to the CBD group. Among other key secondary outcomes, a significant difference was observed only in observed nightly rest, assessed by accelerometer, indicating slightly increased nightly rest in the CBD group. However, this finding was not corroborated by self-reported measures of sleep quality or sleep duration, nor by measures of energy level, and the magnitude of increased nightly rest is of questionable relevance. The study identified no safety concerns, with reported side effects being generally mild and evenly distributed across groups. Therefore, the more favourable outcomes in the placebo group could not be attributed to a higher occurrence of AEs in the CBD group.

Our findings of no benefits of CBD over placebo on pain and other clinical core outcomes align with the results of several RCTs, systematic reviews and meta-analyses investigating the efficacy of cannabis-based medicines for different types of pain [26–28]. Common to these systematic reviews is the fact that the quality of the included RCTs is generally low. Additionally, the lack of standardised treatment regimens among the heterogeneous patient groups makes it difficult to draw definitive conclusions. Thus, it is challenging to determine the efficacy of any type of cannabis-based medicine in patients with chronic pain [26–28]. Our study overcomes many of these limitations and robustly concludes no benefits of CBD for patients with fibromyalgia.

In this study, we used medicinal cannabis, natural CBD, extracted from the top shoot of the Cannabis sativa plant, rather than cannabis-based medicine containing synthetically produced CBD. This was partly due to the initiation of a 4-year medicinal cannabis pilot scheme on January 1, 2018 [29], which allowed physicians to prescribe plant-derived cannabis products approved by the Danish Medicines Agency for the treatment of various conditions, including neuropathic pain. Additionally, patient preference played a significant role, as many expressed a desire for a natural remedy over synthetic

alternatives. Although synthetic CBD is known for its superior dosage precision and purity [30], our study medication underwent a rigorous quality assessment by the Danish Medicines Agency, which ensured the accuracy and consistency of the active compound.

Few studies have evaluated the benefits and harms of CBD or low CBD-THC products for chronic pain [26]. Evidence from RCTs on cannabis products in fibromyalgia is also limited [28]. Consistent with our findings, one study reported no significant effect of CBD as an add-on analgesic for joint disease, with 12 weeks of CBD showing no significant clinical effects on pain, sleep, distress, or functioning in patients with hand osteoarthritis and psoriatic arthritis compared to placebo [31]. The study may have been limited by low CBD doses (20–30 mg daily), although another study found high-dose CBD (600 mg daily) added to paracetamol for painful knee osteoarthritis to have no additional effect compared to placebo [32]. CBD dose and route of administration are reported to be major determinants of CBD pharmacokinetics. Similar to other cannabinoids, CBD generally has poor bioavailability when consumed orally [12]. This has led some to advocate for future studies to examine higher doses (>200 mg) than the 50 mg used in our study based on expert consensus [12]. The importance of sex for drug bioavailability and clearance could also have significant implications, including a probably lower required dose of CBD in women to reach a certain blood level and clinical effect. There are noted sex differences in the metabolism of CBD [33], and the literature suggests slower clearance in women compared with men [34,35], but more studies are required to delineate the role of biological sex [12]. Unfortunately, the sex distribution in our study did not allow for a robust analysis stratified by sex.

The favourable outcomes in the placebo group in our study may be attributed to the placebo effect. A meta-research study suggests that at least half of the overall treatment effect in clinical trials could be due to contextual effects rather than the experimental intervention [36]. Placebo is recognised as an effective and ethical tool for relieving chronic pain [37]. In our study, the close follow-up and care provided by healthcare professionals may have contributed to the placebo effect [38]. Additionally, the participants' expectations and beliefs might have influenced the placebo response. It could be speculated that the difference between groups favouring placebo might be explained by the pharmacodynamics of CBD, specifically how CBD binds to and interacts with dopamine receptors in the central nervous system, potentially influencing and inhibiting the reward system responsible for the placebo effect [11]. Further research is needed to explore the CBD–dopamine interaction.

The main strength of our study is its methodological rigour. We used computerised random sequence generation to ensure blinding of participants, outcome assessors, investigators, and the statistician. The long follow-up period allowed for extended monitoring of pain intensity. The study had a large sample size, reducing the likelihood of type 2 errors, and treatment groups were comparable at baseline with high compliance and minimal loss to follow-up. The primary analysis was conducted on the ITT population, and sensitivity analysis returned similar results, indicating robustness. Patient research partners were involved in the study, ensuring relevance of outcomes and study aim.

Our study had limitations, including being a single-centre study recruiting from a specialised outpatient clinic, which may not represent the broader fibromyalgia patient population, especially patients with a low disease burden managed in primary care. The constrained sampling frame also resulted in a predominantly female study population, limiting robust sex-based

analysis. Given emerging differences in sex pain biology, extrapolation to male fibromyalgia patients should be done with caution. The use of the 1990 classification criteria to verify the fibromyalgia diagnosis at inclusion could have contributed to sex-based selection bias. However, our study population was recruited from a specialised care unit where men constitute only 5% of referrals, and no participants were excluded due to not meeting the 1990 criteria. The trial participants were either cannabis-naïve or had not used cannabis within the last 6 months. However, we did not collect data on the proportion of participants with prior experience using cannabis-related products (before the 6-month period) in either group. This information might have been relevant for interpreting the study results if we had been able to perform ancillary analyses exploring potential subgroup effects related to prior cannabis use.

In conclusion, our study did not support analgesic efficacy of treatment with CBD 50 mg daily compared to placebo in patients with fibromyalgia. Furthermore, the study did not show beneficial effects of CBD on other core outcomes such as functional ability, quality of sleep, and quality of life in this patient population. The study results do not support the use of CBD as an analgesic supplement in fibromyalgia.

Competing interests

All authors declare no competing interests.

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Contributors

The study was conceived and designed by MUR, KA and RC, who drafted the clinical trial protocol, which was subsequently approved by MUR, KA, RC, EEW, and HB. Data collection and data interpretation were conducted by MUR, KA, PHD, and EEW. KA was the principal investigator. MUR, RC and KA drafted the statistical analysis plan, which was reviewed and approved by MUR, RC, EEW, MH, HB, and KA. Blinded data analysis was carried out by RC and MUR. RC, EEW, MH, HB, and KA interpreted the results of the blinded analyses and reached consensus on the interpretation of the results before unblinding. MUR and KA drafted the manuscript, with critical revisions and final approval provided by all authors to ensure its intellectual quality. Funding for the clinical trial was secured by MUR and KA. All authors had full access to all the data in the study and MUR, RC and KA verified the data reported in this manuscript. All shared the final responsibility for the decision to submit for publication.

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Patient consent for publication

Not applicable.

Ethics approval

The trial was approved by the health research ethics committee for the Capital Region of Denmark (Journal no. H-20047715) and the Danish Data Protection Agency (P-2020-229).

Provenance and peer review

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Data availability statement

The complete and deidentified data set that was making basis for this trial can be requested and will be available after proper communication with MUR and KA and under the extent possible according to Danish national legislation. The study protocol and statistical analysis plan are part of the manuscript. The informed consent form is available upon request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.07.008.

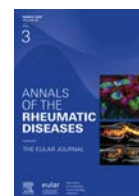
Orcid

Marianne Uggen Rasmussen: <http://orcid.org/0000-0001-9257-3073>

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Images in rheumatology

Multimodality imaging in primary central nervous system vasculitis

Pranjal Rai^{1,*}, James P. Klaas², Kenneth J. Warrington³, Waleed Brinjikji¹, Girish Bathla¹

¹ Department of Radiology, Mayo Clinic, Rochester, MN, USA

² Department of Neurology, Mayo Clinic, Rochester, MN, USA

³ Department of Rheumatology, Mayo Clinic, Rochester, MN, USA

A 75-year-old woman with a history of multiple sclerosis presented with a 1-week history of subacute decline in speech and cognition. Magnetic resonance imaging (MRI) of the brain demonstrated multifocal left hemispheric infarcts. Photon-counting computed tomography (PCCT) angiography revealed multifocal intracranial vessel stenoses, predominantly involving the medium and distal vessels in the anterior circulation bilaterally (M2/M3 segments of middle cerebral arteries and A2/A3 segments of anterior cerebral arteries) (Fig. A and B). Cerebrospinal fluid was notable only for elevated protein (83 mg/dL) and kappa free light chains (0.4010 mg/dL). Vessel wall imaging demonstrated irregular mural thickening and enhancement affecting arterial vessels bilaterally, suggestive of vasculitis (Fig. C). Digital subtraction angiography (DSA) confirmed multifocal irregular narrowing bilaterally (Fig. D). Extensive evaluations for underlying infectious or autoimmune aetiology, malignancy, or systemic vasculitis were negative, leading to a final diagnosis of primary central nervous system (CNS) vasculitis (PCNSV).

PCNSV is a rare idiopathic inflammatory vasculitis, with an estimated annual incidence of 2.4 cases per million person-years. Clinical manifestations are often nonspecific, most com-

monly presenting with headache or focal neurological deficits [1]. Existing literature highlights that multiple sclerosis and vasculitis may occasionally coexist, a phenomenon attributed to the underlying polyautoimmunity [2]. Ultrahigh resolution imaging, as offered by PCCT, can improve diagnostic confidence in the evaluation of medium and smaller CNS vessels and better evaluate the extent of PCNSV in a noninvasive manner [3]. Complementary imaging findings (PCCT, MRI, and DSA in this case) and a concordant clinical picture can help avoid invasive CNS biopsy in select cases, especially with the systematic exclusion of other potential aetiologies. Early initiation of aggressive immunosuppressive therapy is critical, as untreated disease is frequently fatal [1].

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Competing interests

All authors declare they have no competing interests.

*Correspondence to Dr. Pranjal Rai, Department of Radiology, Mayo Clinic, 200, 1st St SW, Rochester, MN 55905, USA.

E-mail address: raipranjal2@gmail.com (P. Rai).

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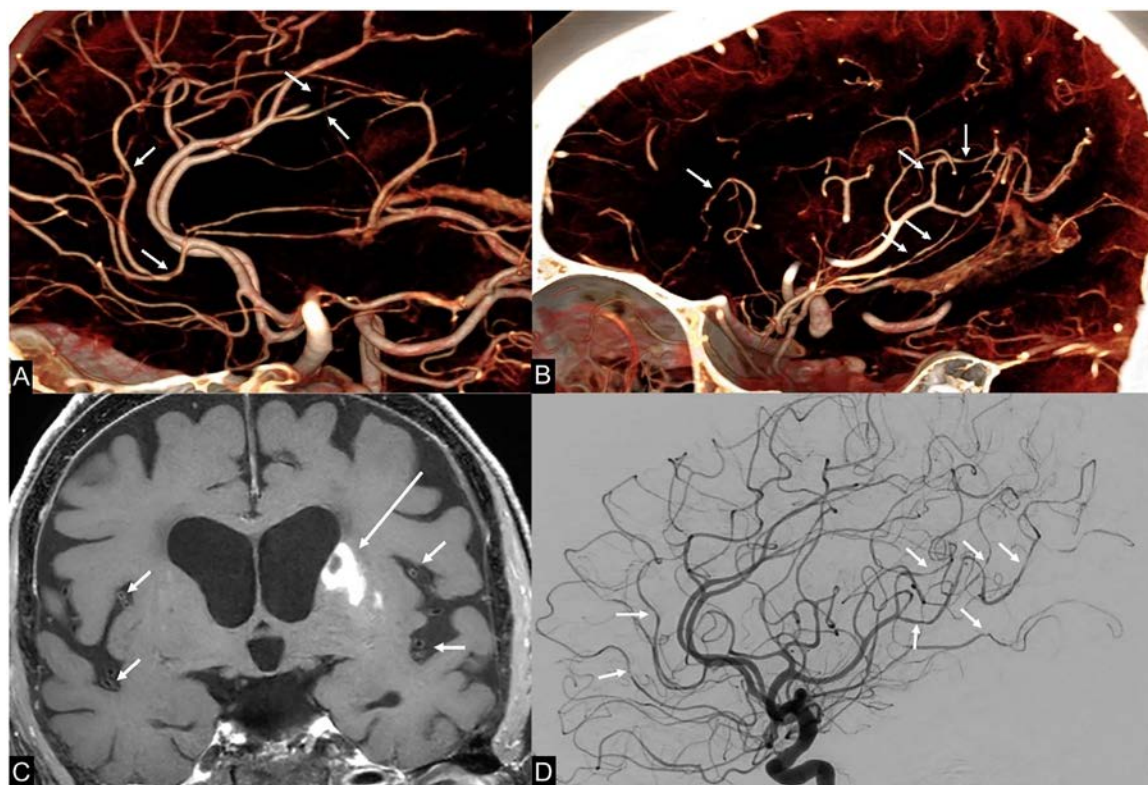


Figure. Volume rendered images (A and B) derived from the photon-counting computed tomography (CT) showing multiple areas of focal vessel narrowing involving the left Anterior Cerebral Artery (ACA; A) and Middle Cerebral Artery (MCA; B) branches distally. Post-Gadolinium Coronal T1-Weighted vessel wall imaging showing involvement of multiple distal MCA vessels bilaterally (short arrows), along with a subacute left MCA infarct (long arrow). Digital subtraction angiography (D) with selective left Internal Carotid Artery injection demonstrating multifocal luminal narrowing of the ACA and MCA branches.

CRedit authorship contribution statement

Pranjal Rai: Writing – original draft, Conceptualization. **James P. Klaas:** Writing – review & editing, Supervision. **Kenneth J. Warrington:** Writing – review & editing, Supervision. **Waleed Brinjikji:** Writing – review & editing, Supervision. **Girish Bathla:** Writing – review & editing, Supervision, Conceptualization.

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Provenance and peer review

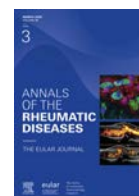
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Orcid

Pranjal Rai: <http://orcid.org/0009-0008-3796-8273>

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Letter

Efficacy of obinutuzumab in two rituximab refractory cases of idiopathic inflammatory myopathies with severe interstitial lung disease

Driven by the success of CD19-targeting chimeric antigen receptor T-cells (CAR-T) and bispecific T-cell engagers (BiTE), there is growing interest in innovative and deep B-cell depleting strategies [1–3]. However, due to associated risks and high costs, few patients are suitable for these treatments, highlighting the need for alternatives. Here, we report on the successful rescue therapy with obinutuzumab in two cases of rituximab (RTX)-refractory idiopathic inflammatory myopathies (IIM) with severe interstitial lung disease (ILD), including its first reported use in MDA5+ IIM. Obinutuzumab is a humanised anti-CD20 monoclonal antibody with deeper B-cell depleting capacity compared to RTX [4]. With a successful phase 3 study in lupus nephritis, obinutuzumab has now made its mark in rheumatology [5].

CASE 1

This 37-year-old female with anti-MDA5 positive dermatomyositis presented with skin ulcers, tenosynovitis and arthritis, deterioration in general condition with severe cachexia (minimum body mass index 13 kg/m²), and severe ILD requiring 2 to 5 L/min oxygen at rest (Fig. A–E). She was bedridden and hospitalised for months. Immunosuppressants showed limited efficacy (methotrexate, tocilizumab) or were discontinued due to leukopenia (azathioprine). Prednisolone (100 mg/day) showed positive responses but was associated with pneumonias and septicæmia. Subsequent RTX (2 × 1g) showed no improvement and failed to achieve B-cell depletion. Rapidly progressive ILD prompted addition of tofacitinib (10 mg daily), which improved cutaneous and musculoskeletal manifestations. Yet, respiratory insufficiency progressed, and a new cardiac involvement developed (indicated by troponinemia and cardiac magnetic resonance imaging). Diffusion capacity for carbon monoxide (DLCO) was not measurable at this time. As a second RTX cycle (2 × 1 g) again failed to achieve complete B-cell depletion, we applied obinutuzumab (1 g). Obinutuzumab led to prompt and complete B-cell depletion accompanied by reduced oxygen requirements (2.5 L/min to 0.2 L/min), C-reactive protein level normalisation, improved ground glass on computed tomography

(CT) scans and remission of tenosynovitis within days. The 4-month follow-up revealed constantly improved general condition and nutritional status. B-cells remained depleted and no relevant infections have occurred. The walking distance improved from 25 m with oxygen to >250 m without rest or oxygen. Prednisolone was successfully tapered and tofacitinib was reduced (5 mg daily). As the 4-month CT revealed renewed subclinical inflammation, 1 g obinutuzumab was repeated. One year after obinutuzumab initiation, her body weight had normalised, and she did not display any inflammatory symptoms and almost normal endurance without any oxygen. Forced vital capacity (FVC) was significantly improved by +100% of baseline and DLCO by at least +140% of baseline.

CASE 2

We previously presented a case series on obinutuzumab in RTX-refractory connective tissue diseases [6], including a 48-year-old female with multirefractory anti-Jo1 syndrome with disabling myositis (creatinine kinase [CK] 8946 U/L) and progressive ILD (Fig. F–I). Obinutuzumab induced remission of myositis and improvement of ILD. Here, we report a follow-up after more than 4 years. An asymptomatic CK increase (to 1824 U/L) at month 6 was successfully treated with another 1 g obinutuzumab cycle. After 12 months, azathioprine and prednisone were tapered and discontinued. Subsequently, with obinutuzumab as monotherapy 1 g every 6 months a stable remission of muscle involvement was achieved. Lung function slightly improved (FVC: 39% to 48%), and the CT-based pulmonary fibrosis index decreased. Obinutuzumab was well tolerated without hypogammaglobulinemia. Importantly, none of the severe infections (tuberculosis, cytomegalovirus pneumonitis, hepatitis B) that occurred during prior therapies reappeared. The patient now leads a normal life without activity restrictions.

Our cases demonstrate the potential of obinutuzumab as treatment for RTX-refractory IIM with musculoskeletal and lung involvement in both induction therapy and long-term disease control. Obinutuzumab was not associated with severe adverse events, both as monotherapy and in combination with azathioprine or tofacitinib, even in multimorbid patients with a history of infectious complications. Following prior treatment with rituximab, no cytokine release syndrome was observed after obinutuzumab. Given the lower costs compared to CAR-T and BiTE, obinutuzumab may offer potential as a novel disease-modifying drug for the management of refractory IIM, which should be further evaluated in future randomised controlled trials.

Competing interests

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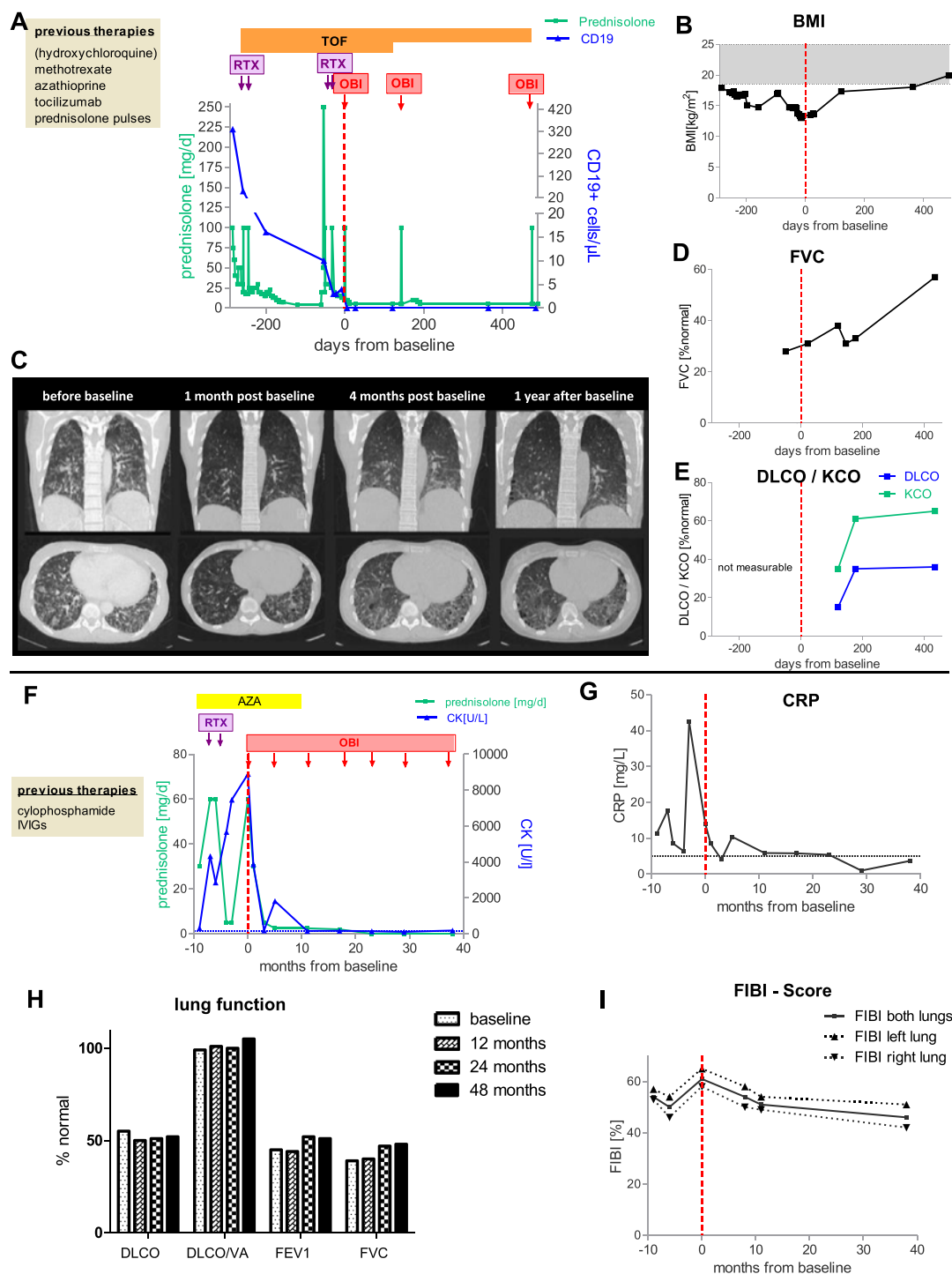


Figure. Clinical outcomes of 2 multirefractory patients with inflammatory idiopathic myopathy treated by obinutuzumab. A-E, Patient 1 suffering from an MDA5 syndrome with pulmonary involvement. F-I, Patient 2 suffering from multirefractory JO1-positive antisynthetase-syndrome. A, Schematic treatment overview and CD19 cell count in patient 1; prior to obinutuzumab initiation, in total 4 doses of RTX (each 1 g) were applied (days –257, –244, –50, and –32). B, BMI, grey area indicates normal BMI (>18.5). C, High-resolution computed tomography (CT) scans of interstitial lung disease over time. D-E, Lung function parameters (DLCO/KCO values before baseline were not measurable). F, Treatment overview and CK (upper limit of normal [ULN] 170 U/L). G, CRP, ULN 5 mg/L. H, Lung function parameters. I, FIBI score, the areas of fibrosis in lung CTs was assessed using a scoring algorithm as previously described [2]. AZA, azathioprine; BMI, body mass index; CK, creatine kinase; CRP, C-reactive protein; DLCO, diffusion capacity for carbon monoxide; DLCO/VA, ratio of DLCO to the alveolar volume; FEV1, forced expiratory volume in 1 second; FIBI score, radiologic pulmonary fibrosis index; FVC, forced vital capacity; IVIG, intravenous immunoglobulin; KCO, carbon monoxide transfer coefficient; OBI, obinutuzumab; RTX, rituximab; TOF, tofacitinib.

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CRediT authorship contribution statement

Ayla Nadja Stütz: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Peter Kvacskey:** Writing – original draft, Validation, Investigation. **Claus Peter Heußel:** Software, Formal analysis. **Nikolas Teichert:** Visualization, Formal analysis. **Hanns-Martin Lorenz:** Writing – review & editing, Resources. **Jörg H W Distler:** Writing – review & editing, Resources. **Johanna Mucke:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Wolfgang Merkt:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Conceptualization.

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The patients in this case report provided informed consent for the publication of their cases.

Ethics approval

Ethical approval was not required, as this report describes individual cases of off-label rescue treatment and does not involve a pharmaceutical study or experimental intervention.

Provenance and peer review

Not commissioned; externally peer reviewed.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used GPT-4o in order to improve the readability and language of the

manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Orcid

Wolfgang Merkt: <http://orcid.org/0000-0002-3108-154X>

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Ayla Nadja Stütz^{1,2,†}, Peter Kvacskey^{3,†}, Claus Peter Heußel⁴, Nikolas Teichert⁵, Hanns-Martin Lorenz³, Jörg H W Distler^{1,2,6}, Johanna Mucke^{1,7,‡}, Wolfgang Merkt^{1,2,3,‡,*}

¹ Department of Rheumatology, University Hospital Düsseldorf, Medical Faculty of Heinrich-Heine University, Düsseldorf, Germany

² Hiller Research Center, University Hospital Düsseldorf, Medical Faculty of Heinrich-Heine University, Düsseldorf, Germany

³ Division of Rheumatology, Department of Hematology, Oncology and Rheumatology, Internal Medicine V, University Hospital Heidelberg, Heidelberg, Germany

⁴ Thoraxklinik, Diagnostic and Interventional Radiology with Nuclear Medicine, University Hospital Heidelberg, Heidelberg, Germany

⁵ Department of Diagnostic and Interventional Radiology, University Hospital Düsseldorf, Düsseldorf, Germany

⁶ Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, and Fraunhofer Cluster of Excellence for Immune Mediated Diseases CIMD, Frankfurt am Main, Germany

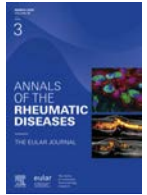
⁷ Rheumazentrum Ruhrgebiet Herne, Ruhr-University Bochum, Herne, Germany

*Correspondence to Dr. Wolfgang Merkt.

E-mail address: Wolfgang.Merkt@med.uni-duesseldorf.de (W. Merkt).

[†]Ayla Nadja Stütz and Peter Kvacskey contributed equally as cofirst authors.

[‡]Johanna Mucke and Wolfgang Merkt contributed equally as colast authors.



Letter

Confounding by indication in observational studies investigating glucocorticoid-associated adverse events in patients with rheumatoid arthritis: a systematic literature review including an assessment of *E*-values

Glucocorticoids (GCs) are commonly used to treat rheumatoid arthritis (RA) due to their rapid onset of action and disease-modifying properties. Despite these benefits [1], their safety profile has created widespread controversy regarding the overall benefit-to-risk ratio. Results from randomised trials are reassuring, but these are often short-term and underpowered. Observational studies correct for this and suggest greater harm, but have a high risk of bias. Observational studies on GC-related adverse events (AEs) in RA are particularly vulnerable to confounding by indication: patients with more severe disease are more likely to receive GCs, and they also have a higher likelihood of developing AEs (Fig 1) [2,3]. To address this issue, disease severity needs to be taken into account and adjusted for in statistical analyses; otherwise, the risk of GC-induced harm will be overestimated. As an example, studies of cardiovascular events and COVID-19-related death in patients with RA found that statistically significant associations between GCs and certain AEs were no longer present after adjustment for RA disease severity [4,5].

We conducted a systematic literature review to determine the extent to which observational studies of GC-associated toxicity in RA adjust for disease activity or systemic inflammation.

For the review, MEDLINE was searched for observational studies investigating the association between GCs and AEs in patients with RA. We chose AEs most feared by patients with RA and rheumatologists [6], published in English between 2008 and August 10, 2023 (date of database search). Search strings can be found in Supplementary Table S1, and further details are provided in the protocol ([dx.doi.org/10.17504/protocols.io.bp2l6x955lqe/v1](https://doi.org/10.17504/protocols.io.bp2l6x955lqe/v1)). Patients and the public were not involved in this study.

The primary outcome of this systematic review was the presence of statistical adjustment for *either* disease activity *or* systemic inflammation in each study's main analysis (yes vs no). We required disease activity to be measured by a validated composite score, and systemic inflammation by C-reactive protein (CRP) or erythrocyte sedimentation rate.

Joanna Briggs Institute's critical appraisal checklists judged study quality (<https://jbi.global/critical-appraisal-tools>). To correct for study type, we divided each study's score by the maximum achievable score and multiplied by 100 to get the percentage of the maximum attainable score. For interpretation: while there are no recommended thresholds for defining anything along the lines of 'high quality' or 'low quality', a prior study using the tools we applied defined >75% as 'low risk of bias' (25%-75%: 'moderate risk'; <25%: 'high risk') [7].

Chi-squared tests explored the effect of the following factors (without correction for multiple testing): year of publication (cutoff: median), study type, type of AE, critical appraisal score percentage (cutoff: median), and 2022 Journal Impact Factor (Clarivate, Philadelphia, PA, USA).

In an unprotocolled ancillary analysis, we computed so-called *E*-values for all studies that did not adjust for RA disease activity or systemic inflammation. *E* is defined as 'the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the treatment and outcome... to fully explain away a treatment-outcome association' [8].

Additionally, *E* for 95% CIs (*ECIs*) were calculated, ie, the minimum strength of association to make confidence intervals cross the line of no difference between groups (and make the comparisons statistically insignificant). To illustrate, if $E = 3$, then the unmeasured confounder (in our case, disease activity or systemic inflammation) would need to triple the risk of GC-related AEs *and* triple the risk of receiving GCs in order to null the association between GCs and AEs (eg, change the risk ratio to 1 or the regression coefficient to 0). If $ECI = 3$, then these associations would make the observed associations statistically insignificant. One *E*- and one *ECI* value were calculated for each study (for the primary analysis of each study, if it was defined and GC-related). Further information is presented in Supplementary Table S2.

Of the 609 search results, 47 articles were eligible for inclusion (Supplementary Figure S1 [search flow chart] and Supplementary Table S3 [referenced included studies]). Most were cohorts from North America or Europe, assessing only oral GCs. Bone-related AEs (eg, bone mineral density or fractures) were most commonly investigated, followed by cardiovascular and infectious AEs. Most publications had a 2022 Journal Impact Factor of <5 ($k = 26$ [55%]). More information on study characteristics is presented in Supplementary Table S4. Critical appraisal score percentages ranged from 30% to 100% (median, 89%; inner quartiles, 73%-100%; Supplementary Table S5).

Over half of the studies (27 [57%]) adjusted for *neither* disease activity *nor* systemic inflammation. Study characteristics had no effect on whether adjustment was performed (Fig 2).

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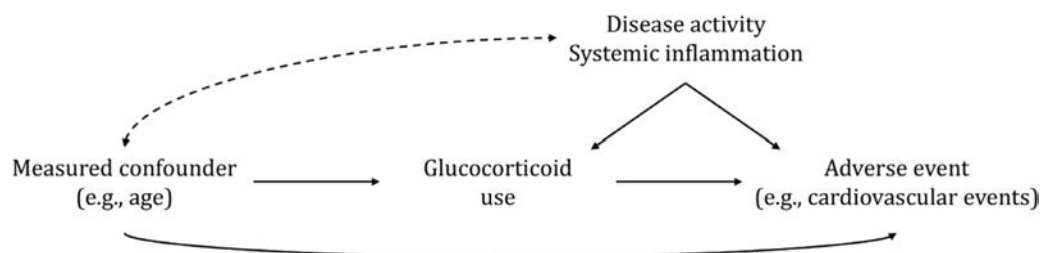


Figure 1. Directed acyclic graph showing the interplay between disease activity and systemic inflammation, glucocorticoid use, and adverse events.

The mean $E \pm SD$ (median; minimum–maximum) of these studies was 2.17 ± 1.12 (1.90; 1.04–5.43), and the mean ECI (for studies that had statistically significant results) was 1.93 ± 0.85 (1.57; 1.16–4.07). In more detail, 60% of studies had an E -value of <2 and 75% an ECI -value of <2 . Supplementary Table S6 shows the distribution of E - and ECI -values in more detail.

These E - and ECI values suggest that the association between GC and AE variables in the majority of studies could be nullified, or at least made statistically insignificant, if an increase in disease activity or systemic inflammation approximately doubles the risk of using GCs and experiencing AEs in patients with RA. This is well conceivable based on prior research findings. For example, for the association between disease activity or inflammation and AEs, including cardiovascular events, serious infections, and fractures, we found hazard ratios in the range of 1.06 to 3.56, with the caveat that various analysis methods and definitions of high disease activity were used in these studies [4,9–14]. For the association between disease activity and GCs, the odds ratio was 1.4 for each point increase in DAS28 in a German early arthritis cohort [15]. In another study, the mean CRP at baseline was 20 mg/L in those not using GCs vs 29 mg/L in

those using GCs. This difference increased when comparing patients who did not use GCs over 5 years (mean CRP at baseline = 19 mg/L) with those using ≥ 1 g of prednisone equivalent over 5 years (mean CRP at baseline = 34 mg/L) [16]. It is possible that the link between disease activity and GCs may become even stronger since the American College of Rheumatology began recommending the avoidance of GCs as a bridging therapy in patients with RA [17].

In summary, our findings suggest that most observational results on GC-associated harm in RA are biased by confounding by indication, including recent studies published in high-ranking journals. In the past, a study investigating bias in breast cancer treatments even found that no adjustment method fully controlled for confounding by indication [18]. Likewise, in RA, the proper adjustment technique is open for discussion. Patients with similar disease activity who are taking GCs differ from those who are not, as those on GCs would likely have higher disease activity if they were taken off GCs. These results emphasise the continued need for cautious interpretation of observational studies of drug-related harm in RA, even when disease severity is taken into account.

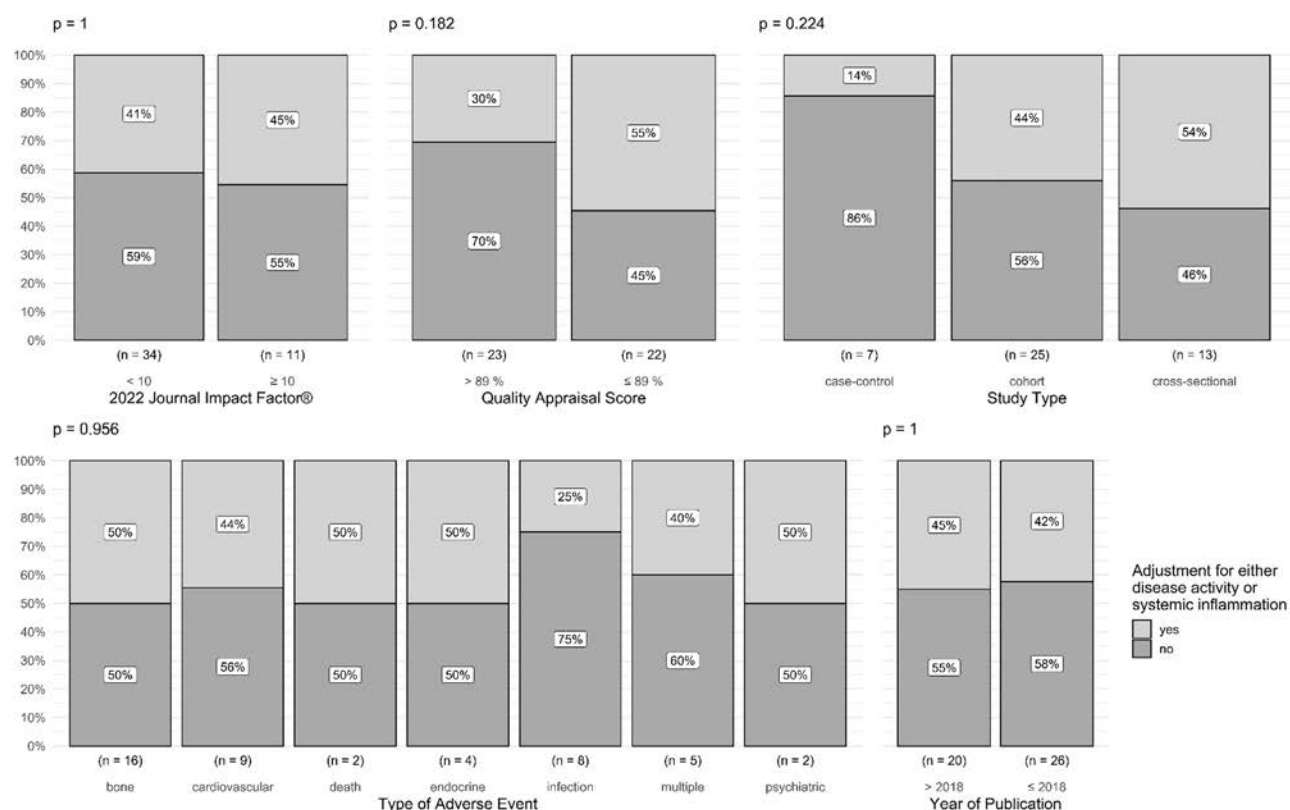


Figure 2. No effect of study characteristics on the proportions of studies adjusting for either disease activity or systemic inflammation. Studies in which it remained unclear whether adjustment took place were excluded from the analyses ($n = 1$). Articles with multiple study types were excluded from the analysis of study type ($n = 1$) and quality appraisal score ($n = 1$). Studies published in a journal without a 2022 Journal Impact Factor were excluded from the analysis of the 2022 Journal Impact Factor ($n = 1$).

Competing interests

FB has received consultancy fees, honoraria, and travel expenses from AbbVie, Pfizer, Gruenthal, and Horizon Therapeutics, all of which are unrelated to this manuscript. A Palmowski reports honoraria (Novartis and argenx) and travel support (Sanofi). TW reports travel cost (AbbVie, Johnson & Johnson, and UCB Pharma) and participation in an educational program (Johnson & Johnson). HK has received honoraria and travel expenses from Johnson & Johnson and AbbVie. DS has received consultancy fees, honoraria, and travel expenses from AbbVie, Johnson & Johnson, Novartis, Bristol Myers Squibb, UCB, Elli Lilly, Kyverna Therapeutics, Alfasigma, and Medac, all of which are unrelated to this manuscript. ELM has received consultancy fees for Data Safety Monitoring Boards related to Amgen and Horizon Therapeutics. The other authors report that they have no relevant competing interests to disclose.

CRediT authorship contribution statement

Andriko Palmowski: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anne Elisabeth Beenken:** Writing – review & editing, Methodology, Data curation. **Anne Pankow:** Writing – review & editing, Methodology, Data curation. **Judith Oldenkott:** Writing – review & editing, Methodology, Data curation. **Henriette Käding:** Writing – review & editing, Data curation. **Edgar Wiebe:** Writing – review & editing. **Zhivana Boyadzhieva:** Writing – review & editing, Data curation. **Eric L. Matteson:** Writing – review & editing. **Ioanna Minopoulou:** Writing – review & editing, Data curation. **Thorben Witte:** Writing – review & editing, Data curation. **David Simon:** Writing – review & editing. **Maarten Boers:** Writing – review & editing, Visualization. **Arnd Kleyer:** Writing – review & editing. **Frank Buttgerit:** Writing – review & editing, Supervision, Resources, Project administration.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ard.2025.10.008](https://doi.org/10.1016/j.ard.2025.10.008).

Orcid

Andriko Palmowski: <http://orcid.org/0000-0002-3456-0597>

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Andriko Palmowski ^{a,b,c,d,*}, Anne Elisabeth Beenken^a, Anne Pankow^a, Judith Oldenkott^a, Henriette Käding^e, Edgar Wiebe^a, Zhivana Boyadzhieva^a, Eric L. Matteson^f, Ioanna Minopoulou^a, Thorben Witte^a, David Simon^{a,g,h,i,k}, Maarten Boers^j, Arnd Kleyer^a, Frank Buttgereit^{a,k}

^a Department of Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany

^b Section for Biostatistics and Evidence-Based Research, Parker Institute, Bispebjerg and Frederiksberg Hospital, University of Copenhagen, Copenhagen, Denmark

^c Epidemiology and Health Services Research, German Rheumatism Research Center (DRFZ), A Leibniz Institute, Berlin, Germany

^d Berlin Institute of Health (BIH), Charité – Universitätsmedizin Berlin, Berlin, Germany

^e Department of Gastroenterology, Infectiology and Rheumatology, Charité – Universitätsmedizin Berlin, Berlin, Germany

^f Division of Rheumatology, Mayo Clinic College of Medicine and Science, Rochester, MN, USA

^g Fraunhofer Institute for Translational Medicine and Pharmacology, Allergy and Immunology, Berlin, Germany

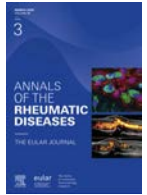
^h Department of Internal Medicine 3, Friedrich-Alexander-University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany

ⁱ Deutsches Zentrum Immuntherapie (DZI), Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Universitätsklinikum Erlangen, Erlangen, Germany

^j Department of Epidemiology and Data Science, Amsterdam UMC, Amsterdam, The Netherlands

^k Translational Rheumatology, German Rheumatism Research Center (DRFZ), A Leibniz Institute, Berlin, Germany

*Correspondence to Dr. Andriko Palmowski, Charité – Universitätsmedizin Berlin, Berlin, Germany.
E-mail address: andriko.palmowski@charite.de (A. Palmowski).



Correspondence

Correspondence on 'Expert consensus statement on the treatment of immune-mediated inflammatory diseases with Janus kinase inhibitors: 2024 update' by Nash et al

Dear Editor,

We have reviewed the recent publication entitled 'Expert consensus statement on the treatment of immune-mediated inflammatory diseases with Janus kinase inhibitors: 2024 update' [1]. We respectfully submit a request for an erratum related to Tables 2 and 3 of the paper by Nash et al [1]. In this publication, abrocitinib is listed as a Janus kinase (JAK) 1 and JAK2 inhibitor. However, abrocitinib is a highly selective JAK1 inhibitor, with minimal activity against JAK2, JAK3, and tyrosine kinase 2 (TYK2), as demonstrated in multiple preclinical and clinical studies [2–6]. Biochemical assays have demonstrated abrocitinib's selectivity for JAK1 (half-maximal inhibitory concentration [IC_{50}] = 0.029 μ M) over JAK2 (28-fold), JAK3 (>340-fold), and TYK2 (43-fold) [2]. Importantly, the IC_{50} values of abrocitinib in human primary immune cells treated with various cytokines and growth factors demonstrated the selectivity of inhibition of abrocitinib for cytokine signalling through JAK1 heterodimer pairs, including interferon (IFN) α (0.189 μ M), IFN γ (0.163 μ M), interleukin (IL)-21 (0.511 μ M), and IL-27 (0.271 μ M), over JAK2/TYK2 signalling (IL-12 [13.673 μ M] and IL-23 [>16.452 μ M]) [2]. Both the Food and Drug Administration- and European Medicines Agency-approved labels, as well as those in other regions, including Australia, Canada, and the UK, describe abrocitinib as a JAK1-selective inhibitor [7–11].

A concern with JAK2 inhibitors is thrombocytopenia, which is driven by inhibition of the JAK2-dependent haematopoietic growth factors erythropoietin (EPO) and thrombopoietin (TPO). Therefore, a JAK1-selective inhibitor would be expected to inhibit multiple cytokines involved in immune function but not EPO and TPO signalling. Abrocitinib's selective inhibition of JAK1 affects signalling through cytokines involved in the pathogenesis of atopic dermatitis while avoiding JAK2 inhibition and minimising the risk of JAK2-driven neutropenia and

thrombocytopenia. Notably, in the first publication characterising the pharmacology of abrocitinib, we showed that at the clinical dose of 200 mg once daily, the projected average inhibition of EPO signalling is less than 8% [2]. Similar data were obtained with TPO at the clinical dose [2]. Clinical occurrences of thrombocytopenia with abrocitinib 200 mg once daily would thus, most likely, not be driven by JAK2 inhibition.

To ensure scientific accuracy, we respectfully request that the entry for abrocitinib be amended to reflect its JAK1 selectivity.

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Competing interests

J-BT, AS, MW, and GAE are employees and shareholders of Pfizer.

CRedit authorship contribution statement

Jean-Baptiste Telliez: Conceptualization, Writing – review & editing. **Andrew Selfridge:** Conceptualization, Writing – review & editing. **Melissa Watkins:** Conceptualization, Writing – review & editing. **Gerardo A. Encinas:** Conceptualization, Writing – review & editing.

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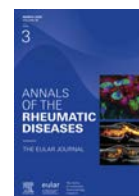
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Jean-Baptiste Telliez¹, Andrew Selfridge², Melissa Watkins²,
Gerardo A. Encinas^{3,*}
¹ *Pfizer Inc., Cambridge, MA, USA*
² *Pfizer Inc., New York, NY, USA*
³ *Pfizer SA DE CV, Mexico City, Mexico*

*Correspondence to Dr Gerardo A Encinas.

E-mail address: GerardoAbraham.Encinas@pfizer.com (G.A. Encinas).



Response

Response to ‘Correspondence on ‘Expert consensus statement on the treatment of immune-mediated inflammatory diseases with Janus kinase inhibitors: 2024 update’ by Nash et al.’ by Telliez et al

We thank Telliez et al [1] for their interest in our consensus statement [2] and appreciate their discussion of various publications and regulators’ information on abrocitinib. We are pleased to respond to their suggestion for a correction of the target specificity of this compound.

The task force has made great efforts to obtain an objective view of the *clinical* selectivity of Janus kinase inhibitors (JAKis). To this end, systematic literature reviews on the efficacy and safety of the drugs were conducted to provide the task force with the most recent insights [3,4], and expert discussions based on the large amount of data available for the many JAKis have subsequently led to the decisions made. The task force’s approach regarding Janus kinase (JAK) 1 vs JAK2 selectivity was quite simple: assess whether haematologic changes occur that suggest JAK2 inhibition, since various haematopoietic growth factors, such as erythropoietin and thrombopoietin (TPO), signal via JAK2 homodimers [5]. We were particularly interested in potential dose-dependent effects.

With respect to abrocitinib, we would like to refer Telliez et al [1] to recent publications showing an interaction between abrocitinib and JAK2, along with an image of the respective X-ray structure. This image was derived from the original study on abrocitinib by Vazquez et al [6,7]. Thus, abrocitinib appears to interact with JAK2. While the selectivity of JAK1 binding over JAK3 in biochemical assays is 340-fold, it is 28-fold over JAK2, a log₁-fold difference [6]. A 28-fold difference is certainly sufficient to claim selectivity in a biochemical assay, but, as mentioned above, the task force focused on *clinical*, not biochemical, selectivity.

Anaemia of chronic disease is one of the many abnormalities seen in most immune-mediated inflammatory diseases. Therefore, haemoglobin values were of particular interest to the task force, since a drug that inhibits the inflammatory response will usually also rapidly improve anaemia [8], unless it inhibits JAK2 [9]. Platelet values were also of interest due to the role of TPO. According to the integrated safety analysis of abrocitinib

in patients with atopic dermatitis who usually do not experience much anaemia of chronic disease, there was a dose-dependent decrease in platelet counts through week 12 and a decrease in haemoglobin levels at the higher dose (200 mg); these changes were not seen with placebo [10]. In the Assessment Report of the European Medicines Agency (EMA) on abrocitinib [11], haemoglobin levels <9 g/dL or decreases ≥2 g/dL were seen in 2.8% of patients on placebo, 3.1% on 100 mg abrocitinib and 6.8% on the 200 mg dose; about 13% of patients on the 200 mg dose compared with 0% on placebo experienced haemoglobin levels <11 g/dL [11]. Thus, we conclude that reductions in haemoglobin levels occur during treatment with the higher abrocitinib dose in about 7% to 13% of patients, depending on the cut point used.

The US Food and Drug Administration (FDA) also provided relevant wording [12]. They explicitly make the following statements: ‘Treatment with CIBINQO was associated with an increased incidence of thrombocytopenia and lymphopenia’ and ‘In the placebo-controlled trials, for up to 16 weeks, treatment with CIBINQO was associated with a dose-related decrease in platelet count’.

The authors are certainly aware of the fact that the following is stated in EMA’s summary of product characteristics for abrocitinib [13]: ‘In biochemical assays, abrocitinib has selectivity for JAK1 over the other 3 JAK isoforms JAK2 (28-fold), JAK3 (>340-fold) and tyrosine kinase 2 (TYK2, 43-fold),’ as we have just mentioned above. The EMA then continues, ‘In cellular settings, it preferentially inhibits cytokine-induced STAT phosphorylation by signalling pairs involving JAK1, and spares signalling by JAK2/JAK2, or JAK2/TYK2 pairs’. In other words, here the EMA is not using the term ‘selective’ but ‘preferential’. The EMA concludes by stating, ‘The relevance of selective enzymatic inhibition of specific JAK enzymes to clinical effect is not currently known’. This is what we looked at: *clinical* effects.

Further, in contrast to what Telliez et al [1] suggest, the FDA did *not* describe ‘abrocitinib as a JAK1 selective inhibitor’; they merely stated the biochemical findings we mentioned above, and concluded, just as the EMA did: ‘The relevance of inhibition of specific JAK enzymes to therapeutic effectiveness is not currently known’ [12]. We repeat: one of the task force’s main objectives was to investigate the *clinical* effects of different JAKis.

Telliez et al [1] can also be referred to a recent publication by Roskoski [14], who stated that abrocitinib ‘is a potent JAK1 antagonist with moderate activity against JAK2’ and also interpreted the findings of Vazquez et al [6,7] by mentioning that ‘the X-ray structure of abrocitinib bound to JAK2... mirrored its binding to JAK1’. Of note, in their tables of JAKi targets, both Shawky et al [7] and Roskoski [14] classified abrocitinib as a JAK1 *and* JAK2 inhibitor.

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Thus, based on all these data, we could not conclude that abrocitinib was free of an effect on JAK2, at least in some patients. However, if the authors can explain the mechanisms, other than JAK2 inhibition, by which the haematologic abnormalities mentioned above are mediated, we will be pleased to reconsider our decision.

CRedit authorship contribution statement

Peter Nash: Investigation, Supervision, Validation, Writing – review & editing. **Andreas Kerschbaumer:** Data curation, Validation, Writing – review & editing. **Victoria Konzett:** Formal analysis, Validation, Writing – review & editing. **Kevin Winthrop:** Methodology, Validation, Writing – review & editing. **Josef S. Smolen:** Conceptualization, Formal analysis, Writing – original draft.

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Peter Nash¹, Andreas Kerschbaumer², Victoria Konzett², Kevin Winthrop³, Josef S. Smolen^{2,*}

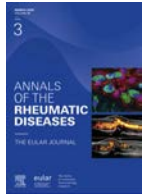
¹ Griffith University School of Medicine, Nathan, Brisbane, QLD, Australia

² Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria

³ Division of Infectious Diseases, School of Public Health, Oregon Health and Science University, Portland, OR, USA

*Correspondence to Prof Josef S. Smolen, Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria.

E-mail address: josef.smolen@meduniwien.ac.at (J.S. Smolen).



Correspondence

Correspondence on ‘EULAR/PRoS endorsed recommendations for the management of familial Mediterranean fever (FMF): 2024 update’ by Ozen et al

Dear Editor,

We read with great interest the 2024 EULAR/PRoS recommendations on familial Mediterranean fever (FMF) management by Ozen et al [1]. While we found the document both timely and valuable, we would like to respectfully raise several points for discussion.

The recommendations rightly highlight the importance of experienced clinicians. However, the growing potential of translational research also deserves explicit recognition. Functional assays assessing (pyrin) inflammasome dysregulation are increasingly available and may soon help refine the interpretation of genetic variants of uncertain significance [2]. Encouraging collaboration between clinicians and laboratories would facilitate diagnostic precision in ambiguous cases.

In addition, although the text promotes ‘holistic’ care, it does not explicitly address emotional or social stressors as genuine drivers of FMF flares. Given their documented impact [3], proactive screening and targeted interventions could improve disease control in many patients.

Concerning colchicine dosing, we question the rationale of titrating up to 3 mg/d. Evidence of added efficacy above 2 mg/d is limited, while toxicity rises significantly. The phrase ‘should not exceed 3 mg/day or the maximum tolerated dose’ might be misunderstood as permitting higher doses under certain conditions. Furthermore, it should be made clearer that dosing is not adjusted by body weight.

The algorithm for treatment escalation is focused solely on colchicine resistance (as an initial step) without acknowledging intolerance (far more common) as a legitimate indication for switching therapy. Patients with early and persistent gastrointestinal side effects may struggle to adhere to treatment. Recognising primary intolerance as a legitimate criterion for treatment escalation would improve the clinical applicability and flexibility of the recommendations.

There is also an inconsistency between the textual and graphical definitions of colchicine resistance. The text refers to >1 attack/mo for 3 months, while the figure uses >3 attacks in 6 months. We would go further: even 3 attacks over 6 months should prompt therapeutic reconsideration. Given the available options, this level of disease activity should no longer be considered acceptable.

We believe there is also an opportunity to address colchicine tapering and discontinuation in selected patients [4]. We think that for patients who have remained attack-free for several months (or even years), show no residual inflammation, and do not carry biallelic exon-10 mutations, a cautious dose reduction could be attempted. Such a strategy is already outlined in the French guidelines and would benefit from prospective evaluation.

Although briefly mentioned, the recommendations omit on-demand short half-life anti-interleukin (IL)-1 use in anticipation of predictable flares or stressful life events. Though off-label, this strategy is supported by emerging literature [5], and patients often feel reassured knowing a fast-acting option is available. Reduced anticipatory stress may itself prevent attacks. Yet, its evaluation in rigorously designed medico-economic trials (including healthcare use and absenteeism) would be highly valuable.

We also advocate for broader research into innovative therapies with less toxicity than colchicine. Inhibitors of the pyrin inflammasome may offer alternatives to patients with intolerance or contraindications.

Finally, we wish to clarify a potential misunderstanding regarding the Auto-Inflammatory Diseases Activity Index. While the document notes its limitations in reflecting disease activity, it is in fact a validated tool with a well-defined threshold and has the advantage of being a patient-reported outcome, an essential feature for conditions that fluctuate outside a physician’s view. Its specificity is high when interpreted alongside clinical context, especially when chronic pain syndromes are suspected.

We hope these reflections will enrich the current dialogue and support the ongoing refinement of FMF care.

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Competing interests

YJ received consulting fees from Sobi, Novartis, and Amgen. IK-P received consulting fees from Sobi, Novartis, Pfizer, and Amgen, and is a coshareholder of Patent Valor, a company exclusively licensing the Auto-Inflammatory Diseases Activity Index score patent owned by AP-HP.

Patient consent for publication

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT-4o in order to edit English grammar and increase readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Yvan Jamilloux^{1,2,*}, Isabelle Kone-Paut^{2,3}

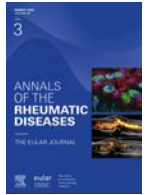
¹ Internal Medicine, CEREMAIA, University Hospital Croix-Rousse (HCL-Hospices Civils de Lyon), Inserm U1111 (CIRI), CNRS UMR5308, ENS de Lyon, University of Lyon-1, Lyon, France

² Reference Center for Auto-Inflammatory Diseases and Amyloidosis (CEREMAIA), France

³ Paediatric Rheumatology Department, Bicêtre University Hospital (APHP-Assistance Publique-Hopitaux de Paris), University of Paris-Saclay, Le Kremlin Bicêtre, Paris, France

*Correspondence to Dr Yvan Jamilloux

E-mail address: yvan.jamilloux@chu-lyon.fr (Y. Jamilloux).



Response

Reply to “Correspondence on: EULAR/PRES endorsed recommendations for the management of FMF: 2024 update”

Dear Editor,

We would like to thank Drs Jamilloux and Kone-Paut [1] for their interest in our paper and their comments. We have replied to their concerns in a point-to-point manner.

For Familial Mediterranean Fever (FMF), functional assays for the pyrin inflammasome dysregulation are indeed in the pipeline; however, we believe that it is too early to be mentioned in recommendations because we lack evidence-based data. These tests are not standardised nor validated for clinical decision-making, and adding them to the recommendations at this stage would create more ambiguity rather than clarity; they are still under development and have not yet been widely adopted as diagnostic tools in centres where patients with FMF are followed. Even once established, functional assays will be expensive and represent an additional layer of complexity in understanding genotype–phenotype interactions. For these reasons, they were included in the research agenda [2]. Indeed, in countries where it is feasible, encouraging collaboration between clinicians and laboratories will facilitate diagnostic precision in ambiguous cases.

Although psychosocial stress is something our patients frequently report, the current literature is very limited and mostly observational. Although clinicians frequently encounter such observations, they remain largely anecdotal, and there is insufficient systematic evidence to form the basis of formal recommendations. More robust data collection is needed before their impact can be meaningfully evaluated, and issues on better assessment have been included in the research agenda.

Regarding the colchicine dosage, the upper limit of 3 mg/d reflects the maximum tolerated dose reported in the literature. This should not be interpreted as a requirement for all patients to reach this dose. For some patients, the maximum tolerated dose may be as low as 0.5 mg/d due to gastrointestinal or laboratory abnormalities, whereas others may tolerate 2 to 3 mg/d. Intolerance is defined clinically and biochemically, and dose adjustments should follow individual tolerance. Thus, the argument that doses >2 mg/d offer no added benefit is not supported by robust evidence, and loosening this boundary would risk confusion in clinical practice. We would like to highlight that we have also specifically included a recommendation for colchicine toxicity [2].

The consensus group was concerned about appearing to endorse moving onto biologicals without robust evidence of colchicine resistance or severe intolerance. Concerning colchicine intolerance, patients who remain attack-free and maintain normal acute phase reactants on their maximum tolerated dose do not require additional therapy. True colchicine intolerance (complete inability to tolerate the drug or persistent attacks despite the highest tolerated dose) is extremely rare. The current recommendations already encompass these scenarios. We do not find it appropriate to broaden the indication for biologics to include early or mild gastrointestinal intolerance. These issues are usually manageable with slow titration and divided dosing; escalating straight to biologics would be an overreach.

For the definition of colchicine resistance, in our text, we noted that “an expert group (not the group that prepared the recommendations) had defined colchicine resistance as recurrent clinical attacks (average ≥ 1 per month over a 3-month period) or persistent laboratory inflammation between attacks.” Historically, other definitions have used ≥ 3 attacks in 6 months. Because practices vary between countries and expert groups, both definitions were included in the treatment algorithm to reflect the diversity of clinical approaches [2]. We would like to add that recent real-life data suggest that the percentage of patients with FMF requiring IL1 blockers is probably lower than anticipated.

With respect to colchicine tapering or discontinuation in heterozygous “patients,” long-term data on amyloidosis risk are lacking. For the time being, a limited attack-free period cannot be considered sufficient evidence to support tapering recommendations. Tapering colchicine in selected patients with long-term remission and low genetic risk should be further explored in prospective studies. On the contrary, in the recommendations, we mention on-demand anti-IL1 use; however, we have not included it as a concrete recommendation. This is because evidence-based data are as yet limited; we were concerned about recommending on-demand treatment outside highly experienced centres at present; thus, studies on dosing alternatives were suggested in the research agenda.

The evidence base for the long-term safety of colchicine is actually very reassuring across a wide age range and in reproductive outcomes. Regarding pyrin inflammasome–specific inhibitors, no meaningful data are currently available to support their inclusion in recommendations. Moreover, colchicine has an unmatched safety record spanning >3000 years and remains widely accessible, inexpensive, and cost-effective. No emerging therapy has yet demonstrated superiority in terms of safety, long-term experience, or affordability. Thus, new therapies will need to be addressed in future research.

As for the activity index, the authors acknowledge the current relevance and utility of Autoinflammatory Diseases Activity

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Index as the only evidence-based and validated tool for inherited recurrent fevers. Our statement was intended to highlight the possible overestimation of the score in certain conditions, such as in patients with comorbid chronic pain syndromes, particularly in adult patients. We believe that in the recommendation paper, our wording already acknowledges its strengths while being realistic about its limitations [2].

Finally, in the scope of holistic care, as physicians, we have concerns regarding sustainability and feasibility, especially in less developed healthcare settings. As experts, we have a duty to find the right balance between patient well-being and the long-term sustainability of treatment. EULAR recommendations follow defined methodological standards and are grounded primarily in the available evidence. Expert opinion does contribute, but its main role is to help interpret existing data and highlight areas where future research is needed. We hope that our recommendations for FMF will serve its purpose.

Competing interests

AG has received speaker fees and consultancy fees from SOBI, Novartis, and R-Pharm. JKD received speaker fees and research grants from Novartis and SOBI. MG received speaker fees and research grants from Novartis and SOBI, speaker fees from Fresenius Kabi, and consultancy from Kiniksa and Boehringer. SGL received speaker fees from SOBI and Novartis. HL received speaker fees from SOBI and Novartis. SO received speaker fees and consultancy from SOBI and Novartis. TO has no conflicts of interest with the topic and does not prescribe; nevertheless, she works for an institute that delivers services for many stakeholders, including pharmaceutical companies that produce drugs listed here, such as Roche or Novartis. OK received speaker fees and consultancy from Novartis, AbbVie, Pfizer, and UCB. ES received consultancy from Novartis. TK received speaker fees from SOBI and Novartis paid to institutional accounts. VH received consultancy and speaker fees from Novartis and SOBI. All other authors declare no competing interests.

CRediT authorship contribution statement

Seza Ozen: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Ahmet Gül:** Investigation, Writing – review & editing. **Cristiana Sieiro Santos:** Investigation, Writing – review & editing. **Erdal Sağ:** Investigation, Writing – review & editing. **Helen J Lachmann:** Investigation, Writing – review & editing. **Jasmin Kuemmerle Deschner:** Investigation, Writing – review & editing. **Marco Gattorno:** Investigation, Writing – review & editing. **Nuray Aktay-Ayaz:** Investigation, Writing – review & editing. **Ömer Karadağ:** Investigation, Writing – review & editing. **Shaye Kivity:** Investigation, Writing – review & editing. **Sophie Georgin-Lavialle:** Investigation, Writing – review & editing. **Tamara Sarkisian:** Investigation, Writing – review & editing. **Teresa Oton:** Investigation, Writing – review & editing. **Tilman Kallinich:** Investigation, Writing – review & editing. **Véronique Hentgen:** Investigation, Writing – review & editing. **Yosef Uziel:** Investigation, Writing – review & editing.

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Orcid

Seza Ozen: <http://orcid.org/0000-0003-2883-7868>

Ahmet Gül: <http://orcid.org/0000-0001-8219-3720>

Cristiana Sieiro Santos: <http://orcid.org/0000-0003-0889-9877>

Erdal Sağ: <http://orcid.org/0000-0002-6542-2656>

Helen J Lachmann: <http://orcid.org/0000-0001-8378-2498>

Jasmin Kuemmerle Deschner: <http://orcid.org/0000-0002-6365-6598>

Marco Gattorno: <http://orcid.org/0000-0003-0704-1916>

Nuray Aktay-Ayaz: <http://orcid.org/0000-0003-3594-7387>

Ömer Karadağ: <http://orcid.org/0000-0002-3443-3117>

Shaye Kivity: <http://orcid.org/0000-0002-8391-5446>

Sophie Georgin-Lavialle: <http://orcid.org/0000-0001-6668-8854>

Tamara Sarkisian: <http://orcid.org/0000-0002-4767-7567>

Teresa Oton: <http://orcid.org/0000-0003-3622-7146>

Tilman Kallinich: <http://orcid.org/0000-0003-2404-9397>

Véronique Hentgen: <http://orcid.org/0000-0003-1788-1898>

Yosef Uziel: <http://orcid.org/0000-0003-4655-1652>

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Seza Ozen 

Ahmet Gül 

Department of Internal Medicine, Division of Rheumatology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Türkiye

Cristiana Sieiro Santos 

Rheumatology Department, Complejo Asistencial Universitario de León, León, Spain

Erdal Sağ 

Department of Paediatric Rheumatology, Hacettepe University, Ankara, Türkiye

Helen J Lachmann 

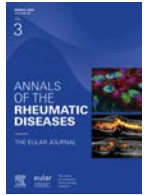
Royal Free Hospital London NHS Foundation Trust & University College London, London, United Kingdom

Jasmin Kuemmerle Deschner 

Division of Pediatric Rheumatology and Autoinflammation Reference Center Tübingen (arcT), Department of Pediatrics, University Hospital Tübingen, Tübingen, Germany

Marco Gattorno *IRCCS Istituto Giannina Gaslini, Reumatologia e Malattie Autoinfiammatorie, Genoa, Italy*Nuray Aktay-Ayaz *Department of Pediatric Rheumatology, Faculty of Medicine, Istanbul University, Istanbul, Turkey*Ömer Karadağ *Hacettepe University Faculty of Medicine, Department of Rheumatology, Ankara, Türkiye*Shaye Kivity *Department of Rheumatology, Meir Medical Center, Kfar Saba, Israel, Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*Sophie Georgin-Lavialle *Sorbonne University, Tenon Hospital, DMU 3ID, AP-HP, Paris, France**ERN RITA & CEREMAIA (National Reference Center for Autoinflammatory Diseases and AA Amyloidosis), Paris, France*Tamara Sarkisian *Center of Medical Genetics and Primary Health Care, Department of Medical Genetics, Yerevan State Medical University, Yerevan, Armenia*Teresa Oton *Instituto de Salud Musculoesquelética, Madrid, Spain*Tilman Kallinich *Department of Paediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité Universitätsmedizin, Berlin, Germany
German Center for Child and Adolescent Health (DZKJ), partner site Berlin, Berlin, Germany
Deutsches Rheuma-Forschungszentrum (DRFZ), an Institute of the Leibniz Association, Berlin, Germany*Véronique Hentgen *National French Reference Centre for Auto-inflammatory Diseases and Inflammatory Amyloidosis, Montpellier, France
Department of General Pediatrics, Versailles Hospital, Versailles, France*Yosef Uziel *Paediatric Rheumatology Unit, Department of Paediatrics, Meir Medical Center, Kfar Saba, Israel
Tel Aviv University School of Medicine, Tel Aviv, Israel*

***Correspondence to** Dr Seza Ozen, Department of Paediatric Rheumatology, Hacettepe University, Ankara, Türkiye
E-mail addresses: sezaozen@gmail.com (S. Ozen), dr.agul001@gmail.com (A. Gül), crstysieirosantos@gmail.com (C.S. Santos), sag.erdal@gmail.com (E. Sağ), h.lachmann@ucl.ac.uk (H.J. Lachmann), kuemmerle.deschner@uni-tuebingen.de (J.K. Deschner), marcogattorno@gaslini.org (M. Gattorno), nurayaktay@gmail.com (N. Aktay-Ayaz), omerkaradag@gmail.com (Ö. Karadağ), shaye.kivity@clalit.org.il (S. Kivity), sophie.georgin-lavialle@aphp.fr (S. Georgin-Lavialle), tamarasarkisian@gmail.com (T. Sarkisian), teresa.oton@inmusc.eu (T. Oton), tilmann.kallinich@charite.de (T. Kallinich), vhentgen@ght78sud.fr (V. Hentgen), uziely@zahav.net.il (Y. Uziel).



Correspondence

Correspondence on ‘ERS/EULAR clinical practice guidelines for connective tissue disease-associated interstitial lung disease’ by Antoniou et al.

The recent European Respiratory Society (ERS) and European Alliance of Associations for Rheumatology (EULAR) guidelines for managing interstitial lung disease in patients with connective tissue diseases suggest screening for rheumatoid arthritis-associated interstitial lung disease (RA-ILD) in patients with rheumatoid arthritis (RA) who exhibit specific risk factors [1]. These risk factors include older age, smoking history, elevated rheumatoid factor, anticitrullinated protein (anti-CCP) antibodies, increased inflammatory markers, male sex, and high articular disease activity. According to the guidelines, RA-ILD screening should include high-resolution computed tomography (HRCT). While we appreciate the considerable effort that went into developing these guidelines, it is important to consider that RA is a common disease affecting 0.5% to 1% of the population. Thus, understanding the proportion of RA patients with risk factors is important for effective resource planning. To quantify the potential implications of this screening suggestion, we analysed a national quality registry with relevant clinical data to estimate the proportion of RA patients who could be affected by the implementation of the ERS/EULAR guidelines.

The Norwegian Arthritis Registry (NorArthritis), established in 2014, is a national quality registry that includes all rheumatology departments in Norway and records patient and inflammatory joint disease characteristics for over 39,000 individuals. As of 2024, the registry covers almost three-quarters of all RA patients in Norway [2]. A patient representative was consulted for this study.

We defined RA-ILD risk factors as follows: (1) age of RA disease onset >60 years, (2) current or previous smoking history, (3) elevated rheumatoid factor and/or anti-CCP, (4) increased inflammatory markers, defined as C-reactive protein (CRP) and/or erythrocyte sedimentation rate above the reference range in the last available blood test, (5) male sex, and (6) high disease activity, characterised by a disease

activity score 28 with CRP (DAS28-CRP) greater than 4.6 in the last available evaluation [3].

From NorArthritis, we extracted data for 12,647 patients diagnosed with RA from 2013 to 2024 to assess the prevalence of the 6 risk factors. Due to missing data, 826 patients (6.5%) were excluded, leaving 11,821 patients for analysis. Of these, 68% were female, with a median (IQR) age and disease duration of 58 (47–68) and 5 (3–8) years, respectively. Our findings revealed that 94.9% of patients had at least 1 risk factor, and 75.9% had at least 2 risk factors that would warrant RA-ILD screening (Fig).

In a multinational survey of rheumatologists regarding RA-ILD screening practices, only 25% reported they would order an HRCT for a seropositive RA patient with a smoking history without respiratory symptoms [4]. Given the new guidelines and the prevalence of risk factors among RA patients in our registry, there is reason to believe that rheumatologists may substantially increase HRCT orders and subsequent investigations. Although RA-ILD screening in the presence of risk factors is conditionally suggested rather than strongly recommended, clinicians may struggle with this distinction and resort to a more comprehensive screening approach to avoid missing cases. Studies indicate that HRCT images for diagnosing RA-ILD should be interpreted by thoracic radiologists to enhance sensitivity and specificity, potentially leading to increased demand for these specialists and requiring substantially more healthcare resources than currently allocated [5]. Even in Norway, known for its strong fiscal capacity, the projected rise in healthcare expenditures has been highlighted as a concern, prompting calls for stricter resource prioritisation [6].

While the existing guidelines provide a valuable foundation, the absence of specific guidance on the number and nature of risk factors could lead to varied interpretations. This highlights the opportunity for further research and collaboration to develop effective screening strategies that optimise healthcare resource allocation.

CONTRIBUTORS

All authors contributed to the planning, conduct, and reporting of the work described in the article. EI conceived the study. CLA performed the majority of the statistical analyses. SAZ is responsible for the overall content.

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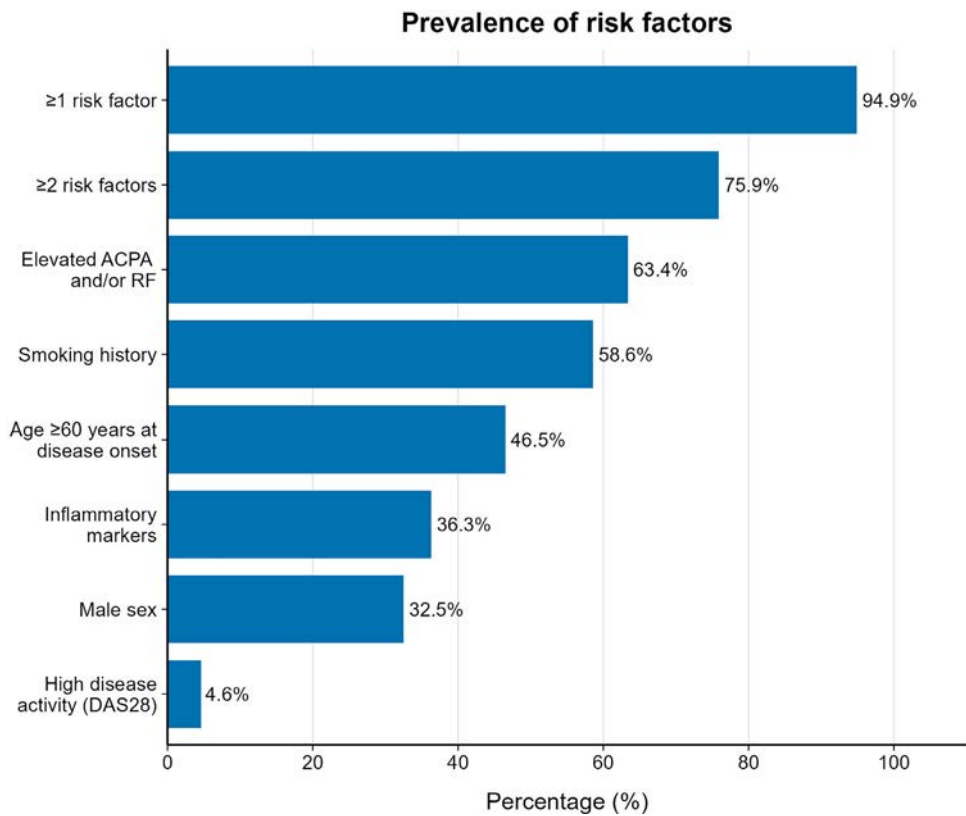


Figure. Prevalence of risk factors for rheumatoid arthritis-associated interstitial lung disease (RA-ILD). ACPA: anti-citrullinated protein antibody; DAS28: disease activity score 28; RF: rheumatoid factor.

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Competing interests

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CRedit authorship contribution statement

Salman Afaq Zarar: Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Bjørge-Tilde Svanes Fevang:** Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Christian Lillebø Alsing:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. **Eirik Ikdahl:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

Patient consent for publication

Not applicable.

Ethics approval

The project was approved by the Regional Committees for Medical and Health Research Ethics, Norway (approval number 824201).

Provenance and peer review

Not commissioned; not externally peer reviewed.

Patient and public involvement

A patient representative was consulted during the planning of the study and provided input from the patient’s perspective.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Claude Sonnet 4 in order to improve language clarity and readability, and to facilitate coding in R, mainly by generating functions. Datasets were never supplied to the AI. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Orcid

Salman Afaq Zarar: <http://orcid.org/0009-0009-0759-663X>

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Salman Afaq Zarar ^{a,*}, Bjørg-Tilde Svanes Fevang^{a,b},
Christian Lillebø Alsing^{b,c}, Eirik Ikdhahl^d

^a *Department of Rheumatology, Haukeland University Hospital, Bergen, Norway*

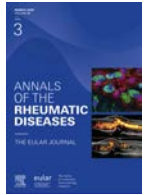
^b *Department of Clinical Science, University of Bergen, Bergen, Norway*

^c *Department of Internal Medicine, Haraldrsplass Deaconess Hospital, Bergen, Norway*

^d *REMEDY Center for Treatment of Rheumatic and Musculoskeletal Diseases, Diakonhjemmet Hospital, Oslo, Norway*

*Correspondence to Dr Salman Afaq Zarar.

E-mail address: salman.afaq.zarar@helse-bergen.no (S.A. Zarar).



Correspondence

Correspondence on the ‘ERS/EULAR 2025 CTD-ILD Guideline: Queries from an Indian Perspective’ and on the ‘ERS/EULAR clinical practice guidelines for connective tissue disease-associated interstitial lung disease’ by Antoniou et al.’

We appreciate the interest and engagement by Dr Mehta and colleagues as well as Dr Zarar and colleagues regarding our recently published European Respiratory Society (ERS)/European Alliance of Associations for Rheumatology (EULAR) 2025 Connective Tissue Disease–Associated Interstitial Lung Disease (CTD-ILD) Guideline [1,2].

We wish to clarify several points raised in the correspondence by Dr Mehta and colleagues. First, we recognised that differences in access to treatment between countries can be substantial, and the guideline clearly stated that reimbursement, availability, and labelling in the respective countries need to be considered when choosing treatment for CTD-ILD. We agree that treatment decisions should not follow a uniform or sequential scheme. The central goal is to ensure that treatment choices are highly individualised and guided by risk assessment. The guideline proposes early and/or aggressive treatment for patients identified as being at risk of progressive or severe ILD. In addition, treatment selection should consider patient-specific risk factors, extrapulmonary manifestations, potential side effects, and the inherent risk of progressive ILD. Regarding antifibrotics, nintedanib is suggested for any CTD-ILD patient (whether usual interstitial pneumonia [UIP] or other pattern) with progressive pulmonary fibrosis (PPF), which is based on evidence from the Interstitial Lung Disease Building Up Knowledge (INBUILD) trial with presence of PPF as an inclusion criterion [3]. For systemic sclerosis-associated ILD, the guideline does not require PPF, as it was not required for the supporting Safety and Efficacy of Nintedanib in Systemic Sclerosis-associated Interstitial Lung Disease trial as for the INBUILD trial [3,4]. Pirfenidone is recommended for rheumatoid arthritis-associated interstitial lung disease (RA-ILD) based on a specific high-risk phenotype (presence of a UIP pattern), regardless of explicit documented PPF status based on the Trial of Pirfenidone in RA-ILD (TRAIL) study [5]. In the absence of key factors to guide

treatment hierarchy, the guideline suggests using the inclusion criteria of randomised clinical trials to guide treatment decisions for CTD-ILD. This approach ensures that treatments are used in patient populations demonstrated to benefit in higher evidence clinical studies. The task force acknowledged the need for further evidence regarding the use of antifibrotics beyond the clinical scenarios mentioned above. A future research priority identified is to assess the efficacy of pirfenidone in patients with different high-resolution computed tomography (HRCT) patterns (UIP versus nonspecific interstitial pneumonia).

Second, the guideline directly addresses the concern about radiation exposure associated with repeated HRCTs during longitudinal follow-up, especially in younger patients undergoing long-term surveillance. The guidelines remark on mitigating radiation exposure, noting that for repeat HRCT used as regular follow-up, low-dose radiation protocols may be considered. The need for future research is stated to establish evidence-based monitoring algorithms, determining whether HRCT, pulmonary function tests, or a combination should be used for long-term follow-up.

Moreover, the guideline includes a future research agenda including the mentioned points. It emphasises the need for safety and efficacy studies of combination treatment (antifibrotic and immunosuppressive treatments) as well as novel agents in combination, both upfront combination and sequential to prevent ILD progression and lung damage. Research priorities emphasise also the need to evaluate the safety and efficacy of treatments in other disease groups such as undifferentiated or overlap CTD-ILD.

The correspondence by Dr Zarar and colleagues addresses the recent guidelines regarding the screening of RA-ILD, emphasising the necessary balance between proactive case identification and judicious resource allocation, particularly considering existing deficits in screening practices and ongoing research efforts. The analysis of the Norwegian registry is an interesting contribution to the literature on the identification of at-risk populations who could benefit from screening.

Although resource constraints mandate targeted screening, current practice suggests that even high-risk, asymptomatic RA patients are frequently overlooked in clinical practice. An international survey of rheumatologists found that 44% did not consider screening for RA-ILD necessary in patients with risk factors but no respiratory symptoms [6]. This lack of screening is concerning because substantial lung damage and loss of lung function may occur before patients report respiratory symptoms such as dyspnoea, and respiratory symptoms themselves may be underestimated in patients whose joint disease impairs exertion. Once ILD in RA patients is established and progressive, health care resource utilisation and related costs are extensive [7]. This

is likely reduced if screening for ILD in patients with RA is implemented and ILD is earlier diagnosed and well managed. Another important point to consider is that ILD is more prevalent than previously believed and is currently diagnosed at a severe stage. This was recently shown in a screening study, where the prevalence of ILD in new onset RA was 11% including 10.5% of patients patient having >30% lung involvement on HRCT and 35.3% having a forced vital capacity <80% [8]. Compared to these numbers, a current Norwegian study using the same registry as mentioned by the authors showed that only a minority of Norwegian RA patients were screened for ILD using an HRCT resulting in a very low prevalence of ILD of 1.9% [9]. It is worth noting that ILD screening could be better identified as ‘lung involvement screening’ because non-ILD lung involvement, including bronchiectasis and emphysema, is also common in RA patients [8]. Finally, many of these patients meet the criteria for lung cancer screening given their smoking history, which poses specific problems in this population due to the frequency of nodules [10].

On the other hand, this urgent need for the early detection of RA-ILD patients before severe damage has occurred has to be balanced against an optimal use of resources as mentioned by the authors. The guideline acknowledges the associated burden on health care infrastructure based on screening RA patients using HRCT. However, very little higher-level evidence has been published addressing this balance, and the optimal use and combination of risk factors is currently debated. The data presented in the letter are adding important information to this debate from a real-life registry. In addition, ongoing studies such as the Autoantibodies, Non-articular Manifestations, Cigarettes, He/him, Older Age at RA Onset, RA High Disease Activity-RA study will provide further information on how to quantify and weight the risk factors [6]. These and other studies will shed light on the optimal set of risk factors and allow for more precise targeting of HRCT resources. It is likely that with these upcoming new data a more precise guidance on when to screen RA patients will be possible.

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PATIENT CONSENT FOR PUBLICATION

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ETHICS APPROVAL

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PROVENANCE AND PEER REVIEW

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Declaration of competing interest

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Anna-Maria Hoffmann-Vold^{1,2,*}, Katerina Antoniou³,
Oliver Distler², Bruno Crestani⁴

¹ Department of Rheumatology, Oslo University Hospital, Oslo, Norway

² Department of Rheumatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

³ Department of Respiratory Medicine, Laboratory of Molecular and Cellular Pneumology, Medical School, University of Crete, Heraklion, Greece

⁴ Université Paris Cité, AP-HP, Hôpital Bichat-Claude Bernard, Respiratory Medicine, Paris, France

*Corresponding author. Dr Anna-Maria Hoffmann-Vold, Department of Rheumatology, Oslo University Hospital, Oslo, Norway.
E-mail address: a.m.hoffmann-vold@medisin.uio.no
(A.-M. Hoffmann-Vold).