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ORIGINAL RESEARCH

Induction of regulatory T cells and efficacy of low-dose interleukin-2 in systemic sclerosis: interventional open-label phase 1–phase 2a study

François Barde ,[1] Roberta Lorenzon,[2] Eric Vicaut,[3] Sébastien Rivière,[1] Patrice Cacoub,[4] Carlotta Cacciatore,[1] Michelle Rosenzwajg ,[5] Anne Daguenel-Nguyen,[6] Olivier Fain,[1] David Klatzmann,[5] Arsène Mekinian [1]

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FB and RL contributed equally.

DK and AM are joint senior authors.

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For numbered affiliations see end of article.

Correspondence to
Dr Arsène Mekinian;
arsene.mekinian@aphp.fr

ABSTRACT

Background Systemic sclerosis (SSc) is a chronic autoimmune disease, with impaired immune response, increased fibrosis and endothelial dysfunction. Regulatory T cells (Tregs), which are essential to control inflammation, tissue repair and autoimmunity, have a decreased frequency and impaired function in SSc patients. Low-dose interleukin-2 (IL-2_{LD}) can expand and activate Tregs and has, therefore, a therapeutic potential in SSc.

Objective We aimed to assess the safety and biological efficacy of IL-2_{LD} in patients with SSc.

Methods As part of the TRANSREG open-label phase IIa basket trial in multiple autoimmune diseases, we studied nine patients with SSc without severe organ involvement. Patients received 1 million international units (MIU)/day of IL-2 for 5 days, followed by fortnightly injections for 6 months. Laboratory and clinical evaluations were performed between baseline and month 6.

Results At day 8, the primary endpoint (Treg frequency) was reached with a 1.8±0.5-fold increase of Treg levels among $CD4^+$ T lymphocytes (p=0.0015). There were no significant changes in effector T cells nor in B cells. IL-2_{LD} was well tolerated, and no serious adverse events related to treatment occurred. There was a globally stable measurement in the modified Rodnan skin score and Valentini score at month 6. Disease activity and severity measures, the quality of life evaluated by EuroQL-5D-5L and pulmonary function test parameters remained stable during the study period.

Conclusion IL-2_{LD} at a dosage of 1 MIU/day safely and selectively activates and expands Tregs. Clinical signs remain stable during the study period. This opens the door to properly powered phase II efficacy trials investigating IL-2_{LD} therapeutic efficacy in SSc.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Most studies report decreased frequency and/or impaired function of circulating regulatory T cells in systemic sclerosis (SSc).

WHAT THIS STUDY ADDS

⇒ Regulatory T cells from SSc patients can be safely and efficiently activated and expanded by low-dose interleukin-2 (IL-2_{LD}) at a dosage of 1 million international units/day, without activating effector T cells.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our results open the door to phase II efficacy trials of IL-2_{LD} in SSc.

INTRODUCTION

Systemic sclerosis (SSc) is a chronic autoimmune systemic disease, with impaired immune response, increased fibrosis and endothelial dysfunction.[1 2]

Regulatory T cells (Tregs) are crucial components of immune system, which contribute to prevent autoimmunity and control inflammation.[3 4] Data about the regulatory T cells in SSc are scarce and controversial. Most studies reported decreased frequency and/or impaired function of circulating Tregs, but few other studies reported a lack of suppressive capacity despite increased Treg levels.[5–9]

Low-dose interleukin-2 (IL-2_{LD}) can expand and activate Tregs lymphocytes while blocking the differentiation of naive CD4 T cells into follicular helper or proinflammatory helper T cells (Th17).[10–12] This activation has been confirmed in several autoimmune diseases.[13–17]

TRANSREG trial recently reported the potential efficacy of IL-2_{LD} in 11 autoimmune diseases. Indeed, it shows promising efficacy and safety results concomitant to significant increase of Treg lymphocytes without activating effector T cells (Teffs), regardless of the underlying disease.[18]

However, IL-2_{LD} use in SSc is still debated. Indeed, (1) Tregs also produced TGF-ß, a key fibrotic cytokine in SSc pathophysiology and (2) a small open-label study investigating basiliximab, a monoclonal antibody targeting the IL-2 receptor alpha chain (highly expressed in Tregs) showed beneficial effects in patients with progressive SSc.[19–21]

Here, we report the safety and biological efficacy of IL-2_{LD} in patients with SSc in an open-label prospective non-randomised pilot trial from TRANSREG study.

PATIENTS AND METHODS

Study design and patients' selection

TRANSREG (NCT01988506) study is a multicentre, interventional open-label trial including fourteen autoimmune diseases. This study recruited nine SSc patients whose individual results have not been reported. Patients were eligible for inclusion if they met the 2013 American College of Rheumatology/European Alliance of Associations for Rheumatology diagnostic criteria for SSc, if they were 18 years of age or older and if their background therapy was stable during the 2 months prior to inclusion. Authorised background treatments included corticosteroids ≤15 mg/day, hydroxychloroquine 200–400 mg/day and immunosuppressants (except cyclosporine, rapamycin, tacrolimus, cyclophosphamide, rituximab and mycophenolate mofetil). We excluded patients with severe form of the disease, vital organ failure or active infections (online supplemental table 1). Patients were recruited in the Department of Internal Medicine of the Saint-Antoine Hospital and of the Pitié-Salpêtrière Hospital in Paris, France, from 1 June 2017 to 31 October 2019.

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

Treatment

The rationale for the dose and scheme of administration of IL-2 used in TRANSREG was previously described.[22] Each patient received 1 million international units (MIU)/day of IL-2 (ILT101, ILTOO Pharma, Paris, France) administered subcutaneously from day 1 to day 5 (induction period), and then every 2 weeks from day 15 to month 6 (maintenance period), as validated and used in previous studies.[14 17] There was a follow-up period without treatment lasting up to 12 months (online supplemental figure 1).

Immunomonitoring and flow cytometry

Blood samples were collected in lithium heparin according to the planned protocol: absolute numbers of lymphocyte subsets and Treg were monitored at each patient visit: day 1 (baseline), before treatment and during IL-2 administration: day 8; 15, 30, 90 and at 180 of the follow-up.

Flow cytometry analysis was performed according to previously published methods.[17 23] Blood subsets (CD3+, CD4+, CD8+T lymphocytes, CD19+B lymphocytes and CD3-CD56$^{bright/dim}$ NK cells) counts (cells/µL) were established from fresh blood samples using CYTO-STAT tetraCHROME kits with Flowcount fluorescents beads and tetra CXP software with a FC500 cytometer according to manufacturer's instructions. Treg cells were gated in CD4+T cells and identified as CD25hiCD127lo/-Foxp3+cells.

Cells acquisition and analysis were performed using a Navios Cytometer and data were analysed with Kaluza software (Beckman Coulter). Plasma samples were collected, aliquoted and stored at −80°C until analysed.

Endpoints

Primary endpoint was the change in Tregs, expressed as a percentage of total CD4 count, after the induction period, on day 8 compared with baseline.

Secondary biological endpoints were changes in Tregs at day 15, month 1, month 3 and month 6 compared with baseline and in other immunological cells (lymphocytes counts/mm^3, CD3$^+$T cells/ mm^3, CD4$^+$ T cells, CD8$^+$ T cells, CD19$^+$ B cells/ mm^3, CD3$^-$CD56 $^{bright/dim}$ NK cells mm^3 and ratio Tregs/Teffs in induction and maintenance period.

Exploratory secondary endpoints assessed during follow-up included:

- Specific clinical evaluation of tender and swollen joint, and of gastrointestinal involvement.
- Disease-specific clinical scores: modified Rodnan skin score (mRSS) and Valentini Disease Activity Index which is a good indicator of SSc activity assessment.[24]
- Pulmonary function test parameters (total lung capacity (TLC) and diffusing capacity for carbon monoxide (DLCO).
- Measure of quality of life with the EuroQL-5D-5L questionnaire.[25]
- Measure of symptom severity with the clinical global impression severity (CGI-Severity or CGI-S), a 7-point scale from 1 a normal situation to 7 among the most extremely ill patients and measure of treatment response with CGI-activity or CGI-A, a 4-scale from 0 a normal situation to 3, a severe activity of the disease.[26]

Adverse events were analysed by frequencies and percentage of administration, and classified by System Organ Class (SOC) according to Medical Dictionary for Regulatory Activities.

Statistical analysis

Data are expressed as mean or median with data range (minimum to maximum) for quantitative variables, and numbers and frequencies for qualitative variables.

Changes in Tregs and others immunological cells (lymphocytes counts, CD3$^+$, CD4$^+$, CD8$^+$ T cells, CD19$^+$ B cells and CD3$^-$CD56 $^{bright/dim}$ NK cells/ mm^3 and ratio Tregs/Teffs) during the induction period (between day 1 and day 8) were analysed using Wilcoxon signed-rank test (primary endpoint). Repeated measures analysis of variance (ANOVA) were used to analyse changes in

immunological cells during the maintenance period (secondary endpoints). Univariate descriptive analyses were performed on the clinical data (clinical scores and pulmonary function test parameters) due to the small number of patients.

RESULTS

Patients' characteristics

A total of nine patients were included from June 2017 to October 2019. One patient withdrew from the study before day 8. This patient was excluded from the analysis of the principal efficacy criteria but maintained in the analysis of the secondary efficacy criteria in accordance with the protocol (online supplemental figure 2).

At inclusion, the median age was 52.1 years (31–75) and eight patients were women (87.5%). Median duration of SSc was 15 years (0.8–25.5). According to disease duration: three patients had early and six had late SSc. Limited cutaneous SSc subgroup represented 89% of patients (8/9 cases). Anticentromere antibodies were found in seven cases (77.8% of patients). Two patients were affected by Reynold's syndrome (association of primary biliary cirrhosis and limited cutaneous SSc). Demographic data, disease features, clinical parameters, background treatments and clinical scores at baseline are summarised in online supplemental table 2.

Biological endpoints

At baseline, mean (±SD) relative Treg blood concentration among CD4+T cells was 6.0%±1.4%. At day 8, the primary efficacy endpoint was met, with an increase of Treg blood concentration to a mean of 10.7%±1.8%, corresponding to a 1.8±0.5-fold increase (p=0.0015). Regardless of their baseline Treg concentration, all patients responded to IL-2_{LD} by increasing their Tregs in peripheral blood by at least 35% after the induction period. The significant changes in percentages of Tregs among CD4+T lymphocytes were also observed for changes in Tregs/Teffs ratio at day 8 with a 1.8-fold increase (p=0.008) (figure 1, online supplemental figure 3). Importantly, during the maintenance period, Treg measurements were performed just before the IL-2 administration, and thus capture only the residual increase from the previous injection 14 days earlier. Despite this, Treg increase was still statistically significant at day 15 (p=0.0033) and, during maintenance period, the increase in Tregs was not significant (p=0.2195) but remained above the baseline value until month 6.

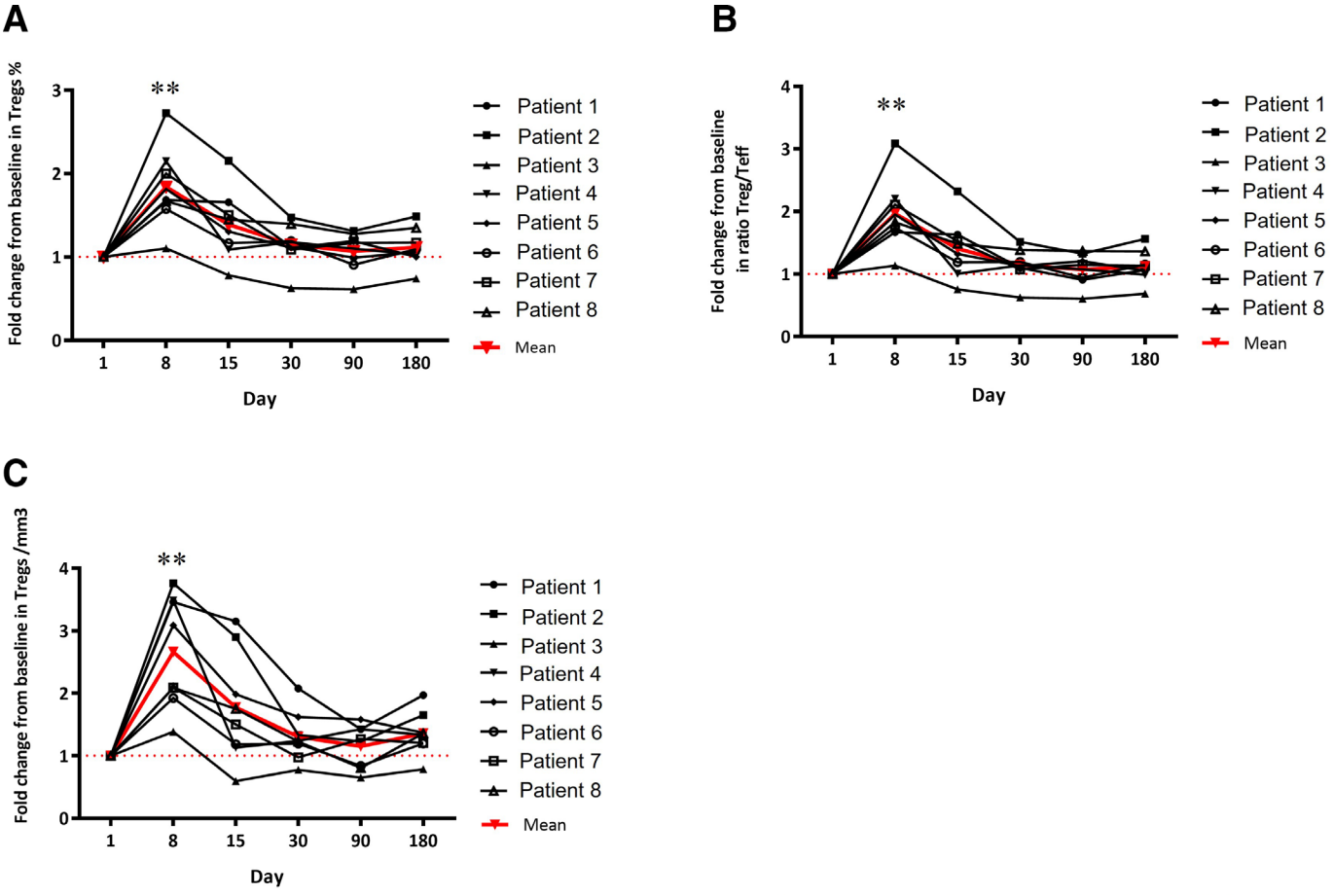

Figure 1 Treg lymphocytes (%) among CD4+T lymphocytes changes during TRANSREG trial data represent fold changes in Tregs (A) as percentages among CD4+T cells in mean from day 1 to month 6; (B) as cells/mm3 in mean from day 1 to month 6; (C) as ratio Tregs/Teff in mean from day 1 to month 6. Statistics were made on raw data (*p<0.05, **p<0.01, ***p<0.001).

Table 1 Lymphocyte subpopulations analysis during induction and maintenance period

	Day 1	Day 8	Day 15	Month 1	Month 3	Month 6
Treg cells cells/mm^3	46.1±15.5	121±7.4**	81.1±0.9**	59.9±7.4	51.9±9.4	62.2±24.5
% among CD4$^+$	6.3±1.4	10.7±0.8**	8.1±2.1**	7.1±1.6	6.2±0.8	6.5±1.1
Lymphocytes cells/mm3	1307±458	1819±14*	1636±25	1521±310	1437±502	1603±394
Treg cells/Teff cells % Among CD4$^+$	4.6±1.2	8.1±1.9**	5.9±1.8**	5.2±1.2	4.6±0.7	4.9±1.1
CD3$^+$T cells cells/mm^3	1039±443	1463±407*	1307±492	1184±270	1153±467	1287±395
CD4$^+$ T cells cells/mm^3	782±353	1108±369*	964±432	853±196	841±320	946±312
CD8$^+$ T cells cells/mm^3	281±156	358±150	352±151	317±124	311±187	346±178
CD19$^+$ B cells cells/mm^3	159±89	140±71	154±66	173±97	145±72	175±113
CD3$^-$CD56 $^\pm$ NK cells cells/mm^3	84.8±36.7	178±66.5***	145±55**	138±61.6*	112±59	110±44.8

Data are represented as mean±SD. Changes between baseline and day 8 were analysed values using by ANOVA (Analysis of Variance) using ANOVA for ranked data considering factor time.
*p<0.05, **p<0.01, ***p<0.001.

The effect of IL-2$_{LD}$ on other immune cells showed that changes in CD8+T cells, Teffs and CD19+B cells were not statistically significant throughout the study (table 1).

The overall CD3-CD56+NK cells were significantly increased at day 8 (p=0.0007), day 15 (p=0.009) and month 1 (p=0.04) and then not significant at month 3 and month 6 (table 1). This initial increase concerned the specific regulatory CD56^{bright} NK cell subset with at day 8, a 2.16±1.1-fold increase compared with baseline (p<0,01). At the same time, the CD56^{dim} NK cell subset showed a significant reduction at day 8 for all participants (p<0,01) (online supplemental figure 3).

No significant change was observed in eosinophils count during treatment. Only one patient shown abnormal values at day 8 (730/mm^3) and at day 15 (960/mm^3); but eosinophils count was normalised at month 1, month 3 and month 6.

Specific autoantibodies (anti-Scl70, anticentromere and antinucleolar antibodies) remained stable throughout the study period (data not shown).

With regard to other biological markers, liver function tests (AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase)) remained stable during treatment, particularly in the two patients with primary biliary cirrhosis (mean AST from 41 IU/L at day 1 to 80 IU/L at month 6 and mean ALT from 242 IU/L at day 1 to 123 IU/L at month 6).

Clinical endpoints

We observed a globally stable measurement in mRSS and Valentini scores. CGI-A and CGI-S measures and the quality of life evaluated by EuroQL-5D-5L remained also stable during the study period (table 2).

Tender and swollen joint counts were available for all patients at baseline. Tender joints were present in five patients at baseline, four of them showed a decrease in tender joint count during treatment. Swollen joints were present in two patients at baseline and have disappeared at month 3 in both, and one had a relapse at month 6. One patient showed a dramatic joint response at month 1 (swollen and painful joints decreased from 12 to 0) with efficacy maintained until 2 months after discontinuation.

No change in Raynaud's phenomenon and gastrointestinal involvement were observed during the treatment period (data not shown). One patient with gastro-oesophageal reflux disease refractory to proton

Table 2 Evolution of clinical scores during induction and maintenance period

	Day 1 N=8	Month 3 N=7	Month 6 N=7
Rodnan skin score	12.9 (4.1)	11.9 (5.5)	10.3 (4.8)
Valentini Activity Index	2.6 (1.21)	1.9 (0.7)	2.4 (1.5)
CGI act	2 (0.5)	1.6 (0.5)	1.4 (0.8)
CGI sev	3.3 (0.7)	3 (0.5)	2.3 (0.8)
EuroQOL 5D-5L	8.8 (3.8)	NA	7.7 (1.8)
Total lung capacity %	99.3 (8.4)	98.7 (8.3)	102 (16)
Diffusing capacity of the lungs for carbon monoxide %	71.3 (17)	73.7 (19.7)	68.9 (19.5)

Data are represented as mean (standard deviation). EuroQOL 5D-5L is a questionnaire assessing quality of life.
CGI, clinical global impression; NA, not available.

pump inhibitor treatment had a complete disappearance of symptoms at the end of the induction phase and throughout the study. Symptoms reappeared at the end of follow-up and IL-2_{LD} was restarted off-protocol 6 months after the end of the study with renewed efficacy.

Finally, pulmonary function test parameters remained stable throughout the study period with mean TLC rising from 99% at baseline to 102% at 6 months and mean DLCO from 71.3% at baseline to 68.9% at 6 months.

Safety

IL-2_{LD} was well tolerated. Four serious adverse events (SAE) were reported during the study, none related to trial treatment. One was a cholecystectomy for vesicular lithiasis in patient affected by associated primary biliary cirrhosis that occurred during the maintenance period.

Three SAE were reported in the follow-up period after IL-2_{LD} discontinuation for the same patient. Traumatic sacrum fracture and short episode of lack of words were evaluated as not related to the study treatment. Worsening of gastro-oesophageal reflux disease (occurred 3 months after IL-2_{LD} discontinuation) was also not related to the study treatment, as explained above, by the improvement of digestive disorders when treatment was restarted off-protocol. Most common non-SAEs (NSAEs) related to treatment were injection site reactions (n=4) most frequently in the induction period, fatigue (n=2), influenza-like syndrome (n=1), digestive troubles (n=5) and headache (n=4). NSAEs not related to treatment were classified according to SOC. All adverse events are listed in online supplemental table 3.

DISCUSSION

In this trial, we showed that treatment with IL-2_{LD} at a dose of 1 MIU / day during a 5-day induction phase increases effectively the level of Treg cells in patients with SSc. This increase is selective with no concomitant rise of Teffs. These results are consistent with recent studies showing that IL-2_{LD} stimulate Treg in various autoimmune diseases.[14 16–18]

After a major increase of Treg during the induction period, we observe a rapid decrease with a stabilisation close to the baseline rate. This profile has already been observed in previous studies using the same administration scheme.[17 18] Administration scheme using weekly injections of IL-2_{LD} during maintenance period could enhance or sustain higher Treg concentrations.

A slight increase in NK lymphocytes and especially in the non-cytotoxic $CD56^{bright}$ NK cell subset, also called regulatory NK cells, was also observed in our patients, similar to what was observed in patients with over autoimmune diseases from the TRANSREG study.[18]

There was a stability of the global evaluation studied by the CGI-act, CGI-sev and the EUROQol 5D-5L scales. Improvement in rheumatic symptoms was observed in four of five patients with joint pain at baseline. There was no worsening of respiratory or digestive problems, no change in Raynaud's phenomenon and the mRSS was stable. Comparable results have already been reported in two patients with off-protocol IL-2_{LD} treatments.[27] These data at least ensure the absence of disease aggravation under IL-2_{LD}, especially in fibrosis which could have been a justified fear due to a risk of elevation in TGF-β, a fibrotic cytokine. All these clinical data are, however, collected from a small number of people and other studies are needed to confirm the clinical efficacy.

Consistent evidence of a specific effect of IL-2_{LD} on Treg explains the number of past and ongoing therapeutic efficacy studies in autoimmune diseases. In a recent multicentre, double-blinded, randomised, placebo-controlled phase II proof-of-concept trial of IL-2_{LD} in active systemic lupus erythematosus showed an absolute and relative reductions in SELENA-SLEDAI (Safety of Estrogens in Lupus Erythematosus National Assessment - Systemic Lupus Erythematosus Disease Activity Index) score from baseline and a persistent increase of the numbers and proportions of Treg cells.[28]

CONCLUSION

The use of IL-2_{LD} at a dosage of 1 MIU/day for five consecutive days selectively activates and expands Tregs without activating Teffs in SSc. Phase II efficacy trials are now needed to validate the therapeutic potential of IL-2_{LD} in SSc.

Author affiliations
[1]Service de médecine interne, AP-HP, Hôpital Saint Antoine, Sorbonne Universite, Paris, France
[2]Clinical Investigation Center for Biotherapies and Inflammation-Immunopathology-Biotherapy Department (i2B), AP-HP, Hôpital Pitié-Salpêtrière, Sorbonne Universite, Paris, France
[3]Unité de recherche clinique, AP-HP, Hôpital Lariboisière, Paris Cité University, Paris, France
[4]Service de médecine interne et immunologie clinique, AP-HP, Hôpital Pitié-Salpêtrière, Sorbonne Universite, Paris, France
[5]Centre d'Investigation Clinique intégré en Biothérapies et immunologie (i2B), Sorbonne Universite, Paris, France
[6]Service de pharmacie, AP-HP, Hôpital Saint-Antoine, Sorbonne Universite, Paris, France

X François Barde @francoisbarde

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Contributors DK and AM conceptualisation, data curation and supervision. FB, RL, SR, DK and AM participated in the formal analysis, methodology, validation and writing the original draft. AM is responsible for the finished work and the conduct of the study, had access to the data, and controlled the decision to publish.

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Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval The TRANSREG study (NCT01988506) and all its amendments were approved by the institutional review board and conducted in accordance with the Declaration of Helsinki and good clinical practice. Written informed consent was obtained from all participants before enrolment in the study.

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ORCID iDs
François Barde http://orcid.org/0000-0002-5652-225X
Michelle Rosenzwajg http://orcid.org/0000-0001-5312-6203
Arsène Mekinian http://orcid.org/0000-0003-2849-3049

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