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Lysophosphatidic Acid Receptor 1 Antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis

A Double-Blind, Randomized, Eight-Week Placebo-Controlled Study Followed by a Sixteen-Week Open-Label Extension Study

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Objective. **Preclinical studies suggest a role for lysophosphatidic acid (LPA) in the pathogenesis of systemic sclerosis (SSc). We undertook this study to assess SAR100842, a potent selective oral antagonist of the LPA_1 receptor, for safety, biomarkers, and clinical efficacy in patients with diffuse cutaneous SSc (dcSSc).**

Methods. **An 8-week double-blind, randomized, placebo-controlled study followed by a 16-week open-label extension with SAR100842 was performed in patients with early dcSSc who had a baseline modified Rodnan skin thickness score (MRSS) of at least 15. The primary end point was safety during the double-blind phase of the trial. Exploratory end points included the identification of an LPA-induced gene signature in patients' skin.**

Results. **Seventeen of 32 patients were randomly assigned to receive placebo and 15 to receive SAR100842; 30 patients participated in the open-label extension study. The most frequent adverse events reported for SAR 100842 during the blinded phase were headache, diarrhea, nausea, and falling, and the safety profile was acceptable during the open-label extension. At week 8, the reduction in MRSS was numerically greater in the SAR100842 group than in the placebo group (mean ± SD change −3.57 ± 4.18 versus −2.76 ± 4.85; treatment effect −1.2 [95% confidence interval −4.37, 2.02]; P = 0.46). A greater reduction of LPA-related genes was observed in skin samples from the SAR100842 group at week 8, indicating LPA_1 target engagement.**

Conclusion. **SAR100842, a selective orally available LPA_1 receptor antagonist, was well tolerated in patients with dcSSc. The MRSS improved during the study although the difference was not significant, and**

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additional gene signature analysis suggested target engagement. These results need to be confirmed in a larger controlled trial.

Systemic sclerosis (SSc) is characterized by fibrosis of the skin and internal organs, prominent alterations of the microvasculature, and frequent abnormalities of cellular and humoral immunity (1). SSc is an orphan disease with high morbidity, which strongly impairs the quality of life, and it has a high case-specific mortality (2). The high burden of severe skin and internal organ involvement in the early stages of diffuse cutaneous SSc (dcSSc) has been highlighted by many cohort studies. Safe and effective treatments for skin and other manifestations of dcSSc are lacking (3).

The pathogenesis of SSc is complex, and at present there is no unifying theory that may explain all its aspects. There is consensus that early vascular events associated with autoimmunity and inflammation lead to fibroblast activation and differentiation, promoting subsequent fibrosis. A broad range of biologic processes interact in SSc, and these include involvement of key profibrotic cytokines and growth factors, an imbalance in Th1/Th2/Th17/Treg cell systems promoting inflammation and fibrosis, and activation of B cells promoting production of autoantibodies (1).

Lysophosphatidic acid (LPA) is a lipid mediator that signals through specific G protein–coupled receptors, designated LPA_1 through LPA_6. It is generated at sites of inflammation or cell injury by the action of lysophospholipase D, also known as autotaxin, on lysophosphatidylcholine and other lysophospholipids (4). LPA exerts various physiologic effects on the receptors of parenchymal cells with some tissue specificities with regard to the various receptors (5–7). LPA mediates a variety of cell activities, including mitogenesis, cell differentiation, cell survival, cytoskeletal reorganization, cell migration, and extracellular matrix production. Recent studies of circulating markers, in vitro cell activation, or animal models have suggested that LPA is involved, and plays an important role, in the pathogenesis of SSc. The role of LPA has also been demonstrated in several animal models of organ fibrosis independently of SSc (8–11).

SAR100842 is a potent selective LPA_1 receptor antagonist (Sanofi R&D). In vivo, SAR100842 reversed dermal thickening and significantly inhibited myofibroblast differentiation and reduced collagen content in a mouse model of skin fibrosis. Similar antifibrotic properties were observed using the *Tsk1* mouse model (Illiano S, et al: unpublished observations). Mechanistic investigations showed that the antifibrotic effects of LPA_1 blockade could be mediated partly via inhibition of the Wnt signaling pathway. Taking into account the promise of LPA_1 receptor blockade in preclinical models of fibrosis and the unmet need of patients with early dcSSc, we performed a randomized proof of biologic activity study assessing the effects of SAR100842 in patients with early dcSSc.

PATIENTS AND METHODS

Study design. This was a double-blind, randomized, placebo-controlled, 8-week phase IIa study followed by an open-label extension study for 16 weeks (see Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract). The objective was to investigate the effects of orally administered SAR100842 in patients with dcSSc, characterizing safety as well as plasma pharmacokinetics and pharmacodynamics, with a focus on clinical efficacy and on SSc-related biomarkers. In the double-blind phase of the study, SAR100842 at 300 mg or matching placebo was administered orally twice a day (in the treatment arm, patients received 100 mg plus 200 mg tablets of SAR100842 twice daily for a total daily dose of 600 mg). Following a screening period of up to 14 days, eligible patients were randomized. Clinical and biologic parameters were assessed, and skin biopsy samples were obtained from a predefined area of the forearm at baseline and end of treatment (week 8).

Patients who had completed the 8-week treatment and who did not meet any discontinuation criteria (see Amended Clinical Trial Protocol 5, http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract) were invited to participate in the open-label noncontrolled 16-week extension phase of the study with the same dosage of SAR100842 as in the initial part of the trial. Patients were evaluated at the end of the extension phase (week 24) for clinical and biologic assessments including 2 additional skin biopsies in those who consented.

The dose of 300 mg twice a day was selected for the study based on activity/efficacy data from in vitro pharmacology models and in vivo animal disease models as well as on the safety profile observed in healthy volunteers (Illiano S, et al: unpublished observations). The duration of 8 weeks was chosen based on expert opinion, which suggested that an 8-week treatment duration would be sufficient to demonstrate significant changes in SSc-related biomarkers. This design reduced the exposure of dcSSc patients to an experimental drug in this phase IIa study, while it provided the necessary data on safety and activity to support full development of the drug. A total of 12 active clinical sites located in Switzerland, France, the UK, Italy, and the US participated in this study.

Patients. Patients met the 1980 American College of Rheumatology (ACR) preliminary classification criteria for SSc (12), with diffuse cutaneous involvement according to the criteria of LeRoy et al (13), and had disease duration of less than 36 months since the onset of the first SSc manifestation other than Raynaud's phenomenon. Other key inclusion criteria were a baseline modified Rodnan skin thickness score (MRSS) $\geq$15 of 51 (14) together with an area of definite involvement of the mid-volar forearm allowing 4-mm skin biopsy samples.

Immunosuppressive therapies stable for 4 weeks prior to enrollment were permitted including prednisolone up to 10 mg/day, methotrexate up to 25 mg/week, azathioprine up to 100 mg/day, and mycophenolate mofetil up to 2 gm/day (for

exclusion criteria for medication dosages, see Amended Clinical Trial Protocol 5, http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract). We excluded patients experiencing orthostatic hypotension (postural reduction of systolic blood pressure by >20 mm Hg or reduction of diastolic blood pressure by >10 mm Hg), moderate-to-severe postural dizziness, or presyncope or syncope within the last 6 months of screening. These exclusion criteria were related to the current knowledge of the study drug obtained in phase I studies.

Study end points. The primary end point was safety and tolerability during the 8-week treatment period. Secondary end points were change from baseline to week 8 in skin and blood biomarkers, changes from baseline to week 8 in the MRSS and Scleroderma Health Assessment Questionnaire (SHAQ) (15), safety and tolerability during the extension treatment period, and pharmacokinetics. Skin biopsy samples were used for RNA extraction, and some messenger RNA (mRNA) biomarkers were assessed using quantitative reverse transcription–polymerase chain reaction (qRT-PCR), including cartilage oligomeric matrix protein (COMP), thrombospondin 1 (TSP-1), plasminogen activator inhibitor 1, Wnt-2, and secreted Frizzled-related protein 4 (sFRP-4). Other skin biopsy samples were dedicated to immunohistochemistry. Labeling for α-smooth muscle actin (α-SMA) was performed on serial slides, and skin thickness (histology) was evaluated. LPA markers were selected based on literature data and internal confirmation using dermal fibroblasts from SSc patients treated with LPA. The choice of other markers (COMP, TSP-1, type I collagen, and α-SMA) was based on literature data selecting genes or proteins that may play a key role in the evolution of fibrosis in SSc patients (16,17).

To explore the effect of SAR100842 on the LPA pathway, we used the results of a parallel study performed using cultured dermal fibroblasts from patients with SSc. LPA gene expression response was defined in the cultured dermal fibroblasts study. This LPA response was used in combination with the expression profile in patient skin biopsy samples for identifying an LPA signature, according to a guided clustering algorithm. The goal of using this data integration approach was to ensure that the identified gene cluster with high LPA treatment response was also consistently expressed and correlated in skin biopsy samples. The identified fibroblast LPA signature was subsequently reduced to a single composite biomarker called the pathway activation index (PAI), computed as the coefficient of a robust regression on the expression matrix of the LPA signature at each treatment visit (median polish algorithm) (18). The PAI was then used as a surrogate biomarker for investigating SAR100842 treatment response. Exploratory end points were change from baseline to week 24 on the MRSS and SHAQ, and also the change in pain or pruritus from baseline to week 8 and week 24.

Statistical analysis. *Sample size determination.* No formal sample size calculation was performed for this proof of biologic activity study, and the sample size for the study was based on empirical considerations.

Safety analyses. The safety analyses were based on the safety population of all randomized patients who actually received at least 1 dose of the investigational medicinal product, and they were performed according to the treatment actually received in the core or extension phases of the study. The safety analyses were descriptive.

Efficacy analyses. The efficacy analyses were based on the modified intent-to-treat (ITT) population, which included all randomized patients who had actually received at least 1 dose of the investigational medicinal product and who had undergone at least 1 measurement after administration of the investigational medicinal product during the blinded period of the study (the double-blind phase). The modified ITT population for the open-label extension phase included all randomized patients who did actually receive at least 1 dose of the investigational medicinal product during the open-label extension phase and who had undergone at least 1 measurement after administration of the investigational medicinal product during the open-label extension phase.

An analysis of covariance (ANCOVA) was performed for the total MRSS and the Health Assessment Questionnaire disability index (HAQ DI) score (19) on the change from baseline to week 8 in the modified ITT population, with treatment group as the main factor and the baseline MRSS and HAQ DI scores centered on their means in the modified ITT population as a continuous covariate. Student's *t*-test was used to determine the superiority of 300 mg SAR100842 twice a day over placebo at week 8, with a nominal 2-sided Type I error rate of 5%. The analysis of other SHAQ variables was purely descriptive. All other secondary end points were described by treatment and analyzed within an ANCOVA.

Biomarker analyses. The biomarker analyses were based on the population of all randomized and treated patients who received at least 4 weeks of study drug with at least a baseline and a postbaseline assessment. Prior to all statistical analyses, mRNA data were normalized. Each biomarker was analyzed using descriptive statistics. For each of the skin biomarkers related to the disease (i.e., COMP, TSP-1, and type I collagen mRNAs and α-SMA [16]), the change from baseline to week 8 measurement was analyzed using a rank ANCOVA, with treatment group as fixed effect and baseline value as covariate.

Target engagement. SSc fibroblasts were prepared from forearm biopsy samples following established outgrowth conditions and were cultured in Ham's F-12 medium with 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.3 mg/ml L-glutamine. Dermal fibroblasts from 4 healthy volunteers and 10 SSc patients were seeded and treated with vehicle or LPA at 10 μ*M* for 24 hours. Supernatants were removed and cells were rinsed and stored, and total RNA was purified using an RNeasy Mini kit (Qiagen). The same methodology was used to extract RNA from skin biopsy samples from patients in Sanofi study no. ACT12339. Gene expression was measured by whole transcriptome profiling analysis using Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays. From each array result, a probe cell intensity data file was computed, which represented an individual gene expression profile. Samples were clustered based on Euclidean distance and correlation for evaluating the similarity of the quality of each array to the quality of the other arrays. We performed principal components analysis on expression data as well as on quality control metrics of the raw data provided by the Affymetrix platform (R-package simpleaffy; https://rdrr.io/bioc/simpleaffy/).

The guided clustering algorithm (18) was used for the identification of a set of genes that had high LPA perturbation in the cell culture study and that were consistently expressed in the skin biopsy samples from patients with SSc. A logistic regression model was computed for each probe set separately, with the LPA treatment label as the outcome variable (LPA = 1, placebo = 0) and the probe set as the independent variable. Each model was adjusted by fibroblast type (normal/SSc). The coefficient of the probe set in the model was used as the LPA activation strength for weighting the probe sets. The obtained weights were

used in conjunction with the expression profile in skin biopsy samples at baseline to extract the LPA signature. The LPA signature was condensed into 1 surrogate marker called the PAI. Descriptive statistics of change in the LPA PAI from baseline to the end of the 8-week treatment period were computed by treatment arm. The difference between SAR100842 and placebo was investigated using the following equation:

$$\Delta PAI_{\text{main part}} = \beta_0 + \beta_1 \times \text{treatment} + \beta_2 \times PAI_{\text{scaled baseline}} + \xi$$

where β_0 is the mean effect, β_1 is the treatment effect, β_2 is the PAI value effect at baseline, and ξ represents residuals from the model. Targeted gene expression analysis of selected LPA-related and fibrosis genes was carried out in the same skin biopsy samples using qRT-PCR.

Ethics approval. The protocol and its amendments were submitted to independent ethics committees and/or institutional review boards for review and written approval. All patients provided written informed consent prior to the conduct of any study-related procedures, and the optional skin biopsy informed consent form was obtained from patients who agreed to the collection of skin biopsy samples. In addition, dermal fibroblasts were grown from skin biopsy samples obtained from another cohort of SSc patients fulfilling the ACR/European League Against Rheumatism 2013 classification criteria (20). The procedure was approved by the local ethics committee (University of Naples), and patients signed informed consent forms.

RESULTS

Baseline characteristics of the patients, flow of enrollment into the study, discontinuations, and compliance. Of 48 patients screened, 16 (33.3%) were determined to be ineligible. Thirty-two patients were randomized into the study; for a period of 8 weeks, 15 received 300 mg SAR100842 twice a day and 17 received placebo twice a day. Patients in each group had comparable demographic characteristics at baseline, consistent with the overall population of dcSSc patients (Table 1).

One patient receiving SAR100842 discontinued treatment on personal request but was included in the modified ITT analysis. Of the 32 patients initially randomized to the double-blind phase, 30 were enrolled into the open-label extension phase (16 initially treated with placebo and 14 initially treated with SAR100842). One patient in the placebo/SAR100842 group and 1 patient in the SAR100842/SAR100842 group requested to discontinue treatment due to adverse events (AEs). The mean overall compliance was comparably high between treatment groups (99.6% in the placebo group versus 98.5% in the SAR100842 group).

Table 1. Baseline characteristics of the patients*

	Placebo (n = 17)	SAR100842 (n = 15)	All (n = 32)
Age, mean ± SD years	50.6 ± 11.3	48.8 ± 10.3	49.8 ± 10.7
Female	12 (71)	9 (60)	21 (66)
Caucasian/white	13 (76)	13 (87)	26 (81)
Weight, mean ± SD kg	70.6 ± 16.8	75.1 ± 19.3	72.7 ± 17.9
Current smoker	3 (18)	2 (13)	5 (16)
Disease duration, mean ± SD months	19.6 ± 7.4	20.4 ± 8.9	20.0 ± 8.0
Raynaud's phenomenon	17 (100)	14 (93)	31 (97)
Digital ulcers (past or current)	6 (35)	4 (27)	10 (31)
Joint synovitis	5 (29)	4 (27)	9 (28)
Tendon friction rubs	6 (35)	7 (47)	13 (41)
Renal crisis	1 (6)	0	1 (3)
Dyspnea (significant)	7 (41)	2 (13)	9 (28)
Fibrosis on plain radiograph	3 (18)	1 (7)	4 (13)
ACA positive	1 (6)	0	1 (3)
Anti–Scl-70 positive	5 (29)	4 (27)	9 (28)
Anti–RNA polymerase III positive	4 (24)	8 (53)	12 (38)
MRSS			
Mean ± SD	24.8 ± 7.8	22.7 ± 8.2	23.8 ± 7.9
Median (range)	23 (15–38)	21 (15–44)	22 (15–44)
HAQ DI score			
Mean ± SD	1.27 ± 0.75	1.23 ± 0.77	1.25 ± 0.75
Median (range)	1.25 (0.0–2.5)	1.38 (0.0–2.4)	1.37 (0.0–2.5)
Previous immunosuppressive or steroid medications	14 (82)	10 (67)	24 (75)
Mycophenolate mofetil	5 (29)	7 (47)	12 (38)
Methotrexate	9 (53)	1 (7)	10 (31)
Systemic steroids	8 (47)	6 (40)	14 (44)
Topical steroids	1 (6)	1 (7)	2 (6)

* Except where indicated otherwise, values are the number (%). ACA = anticentromere antibody; MRSS = modified Rodnan skin thickness score; HAQ DI = Health Assessment Questionnaire disability index.

Good safety and tolerability of SAR100842. Overall, SAR100842 was well tolerated. AEs are described in Supplementary Table 1, http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract. Eighty percent of patients in the SAR100842 group versus 71% of patients in the placebo group reported at least 1 treatment-emergent AE. However, most treatment-emergent AEs were mild to moderate in intensity. There was 1 treatment-emergent serious AE (SAE) in the SAR100842 group (syncope) in a patient with a medical history of syncope in childhood. In the open-label extension phase, 2 patients reported a treatment-emergent SAE, 1 in each group. Dyspnea was reported in 1 patient 6 days after switching from placebo to SAR100842, and this was considered to be related to the investigational medicinal product, while an infected digital ulcer in another patient was not considered to be drug-related. Two patients discontinued prematurely due to treatment-emergent AEs, 1 for moderate arthritis in the SAR100842/SAR100842 group and 1 for pruritus, skin discoloration, and facial swelling in the placebo/SAR100842 group. With regard to the laboratory safety assessments, no safety concern emerged from the various laboratory parameters (see Supplementary Table 2, http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract).

Efficacy as determined by change in MRSS during the controlled and extension phases. A primary analysis was conducted in the modified ITT population on patients who were treated until week 8. There was a numerically greater decrease from baseline in the total MRSS in the SAR100842 group compared to the placebo group, although the difference did not reach statistical significance (mean ± SD change −3.57 ± 4.18 versus −2.76 ± 4.85; treatment effect −1.2 [95% confidence interval {95% CI} −4.37, 2.02]; $P = 0.46$) (median change −4.00 [interquartile range {IQR} −5, −1] versus −1.00 [IQR −5, 0], respectively) (Figure 1).

After 24 weeks of treatment, patients in the SAR 100842/SAR100842 group experienced a clinically meaningful decrease in total MRSS versus baseline (mean ± SD change −7.36 ± 4.24; median change −7.50), and a high percentage (78.6%) of patients improved by at least 5 points (the definition of responders). Patients initially receiving 8 weeks of placebo also demonstrated an improvement in MRSS after 24 weeks (mean ± SD change −7.31 ± 4.59; median change from baseline −7.00), with a responder rate of 69.2%.

Changes in quality of life during controlled and extension phases. There was no statistically significant difference between the SAR100842 and placebo groups in change in HAQ DI total score from baseline to week 8 (mean ± SD change 0.00 ± 0.33 in the placebo group versus −0.14 ± 0.30 in the SAR100842 group; treatment effect −0.1 [95% CI −0.38, 0.09]). However, it should be pointed out that the mean absolute difference from baseline observed in the SAR100842 group (−0.14) was clinically meaningful; indeed, a HAQ DI score improvement of ≥0.14 is considered to be the minimum clinically important difference in patients with SSc. In contrast to the improvement between baseline and week 8, the improvement seen in the mean HAQ DI total score was clinically meaningful from baseline to week 24 in both the placebo/SAR100842 group and the SAR100842/SAR100842 group (mean ± SD change −0.23 ± 0.30 versus −0.15 ± 0.33, respectively), and the percentages of patients whose HAQ DI total scores decreased by ≥ −0.14 were comparable in the 2 groups.

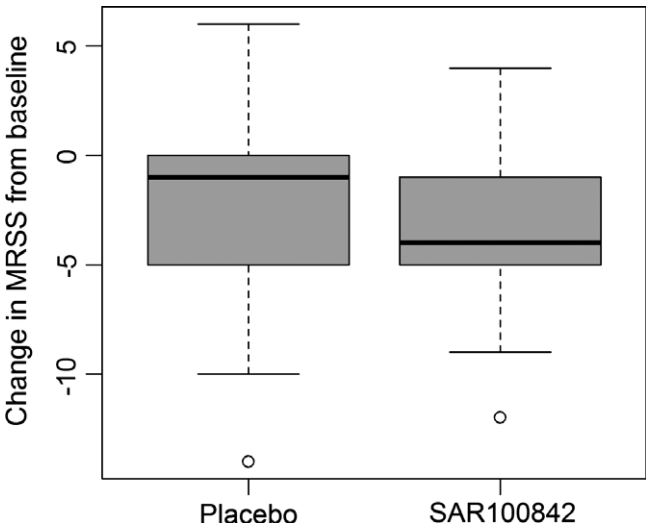

Figure 1. Change in modified Rodnan skin thickness score (MRSS) from baseline to week 8 in the modified intent-to-treat population. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.

Effects on pruritus and pain during controlled and extension phases. Based on the preclinical rationale, LPA receptor antagonists may be effective against pruritus. Interestingly, despite a low baseline value, there was numerical improvement in the SAR100842 group and worsening in the placebo group (mean ± SD change −0.37 ± 3.92 versus 0.25 ± 1.79) in the severity of pruritus assessed by patients from baseline to week 8 using a 0–10-cm visual analog scale. Similarly, reduced pruritus severity was observed in SAR100842/SAR100842-treated patients compared to placebo/SAR100842-treated patients (mean ± SD change −1.38 ± 2.85 versus −0.84 ± 1.67). Compared to week 8, the severity of pruritus was further decreased at week 24 in patients initially treated with SAR100842 or placebo. The severity of pain assessed by

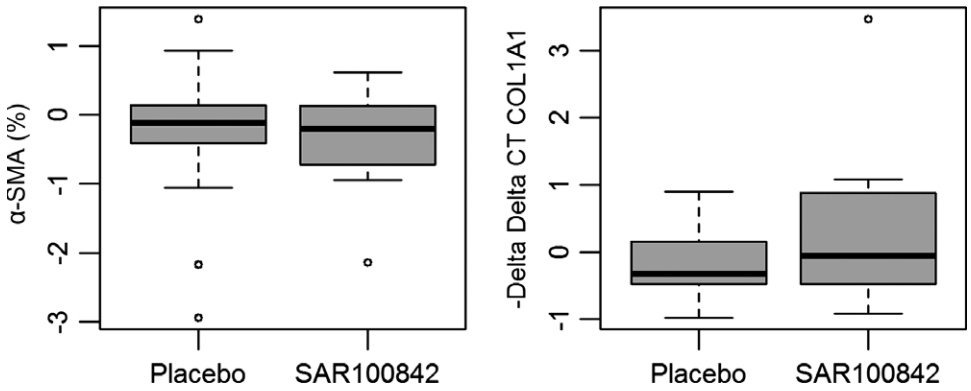

Figure 2. Changes in the skin fibrosis markers α-smooth muscle actin (α-SMA) and type I collagen from baseline to week 8. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.

patients using a numerical pain scale was low at baseline in the study population, and no conclusion could be drawn.

No significant differences between groups in skin and blood biomarker end points. There was no statistically significant differential expression of any skin mRNA and protein biomarkers or blood protein biomarkers between placebo- and SAR100842-treated patients. Type I collagen and α-SMA were used as fibrosis markers and were not modulated by SAR100842 treatment (Figure 2). Disease signature was evaluated using either the 4-gene biomarker as described by Farina et al (16) or a combination of TSP-1 and COMP. No changes in these genes were correlated with change in MRSS (Table 2). However, after 8 weeks there was a trend toward a reduction in TSP-1 with SAR100842 compared to placebo, although this did not reach statistical significance (Figure 3). We also evaluated the gene for membrane-spanning 4 domains, subfamily A, member 4A (a marker of M2-type macrophages). The expression of this marker, such as in the 2-gene signature (17), was not modulated by SAR 100842 and was not correlated with the change in MRSS (not shown).

Global change in gene expression in skin samples at 8 weeks between vehicle- and SAR100842-treated patients was evaluated using a stringent cutoff for false discovery rate ($P = 0.05$) or less stringent criteria ($P = 0.1$). No significant difference was observed under any conditions. Data obtained using a cutoff for a false discovery rate of <0.1 are presented in Supplementary Table 3, http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract.

Table 2. Absence of correlation of change in 4 gene biomarkers with change in MRSS*

Gene	Correlation of change in gene with change in MRSS	*P*
COMP	0.01	0.96
TSP1	0.0074	0.97
SIGLEC1	0.043	0.82
IFF44	−0.031	0.87

* MRSS = modified Rodnan skin thickness score.

SAR100842 induces target engagement in the LPA pathway. There was a nonsignificant numerical reduction from baseline in some LPA pathway biomarkers (plasminogen activator inhibitor 1, Wnt-2, and sFRP-4) in SAR 100842-treated patients compared to placebo-treated patients (Figure 4). Although the decrease in these biomarkers was not significant, it is of interest since they have been shown to be regulated by LPA and SAR100842 in dermal fibroblasts from SSc patients. Thus, a post hoc analysis was performed to identify a more global LPA signature in SSc dermal fibroblasts and skin biopsy samples and to evaluate the impact of SAR100842 on this signature in patient skin to assess target engagement.

The signature was identified using both microarray data obtained in SSc dermal fibroblasts treated for 24 hours with LPA and microarray data from skin biopsy samples from SSc patients at baseline. A guided clustering method was performed to give weight to genes that were expressed at a significant level following LPA treatment but that were also expressed at a significant level in skin biopsy samples. This led to a list of 47 genes identified as an LPA signature (see Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.40547/abstract). This signature reflects pathways such as those for proliferation and epidermal growth factor signaling, which are known to be mechanistically part of LPA responses in other cell types. These genes were reduced to a unique surrogate biomarker in 1 dimension called the PAI, using the median polish algorithm (21). The PAI was extracted as a row effect as it represents the summary expression in each patient. A significant decrease in the

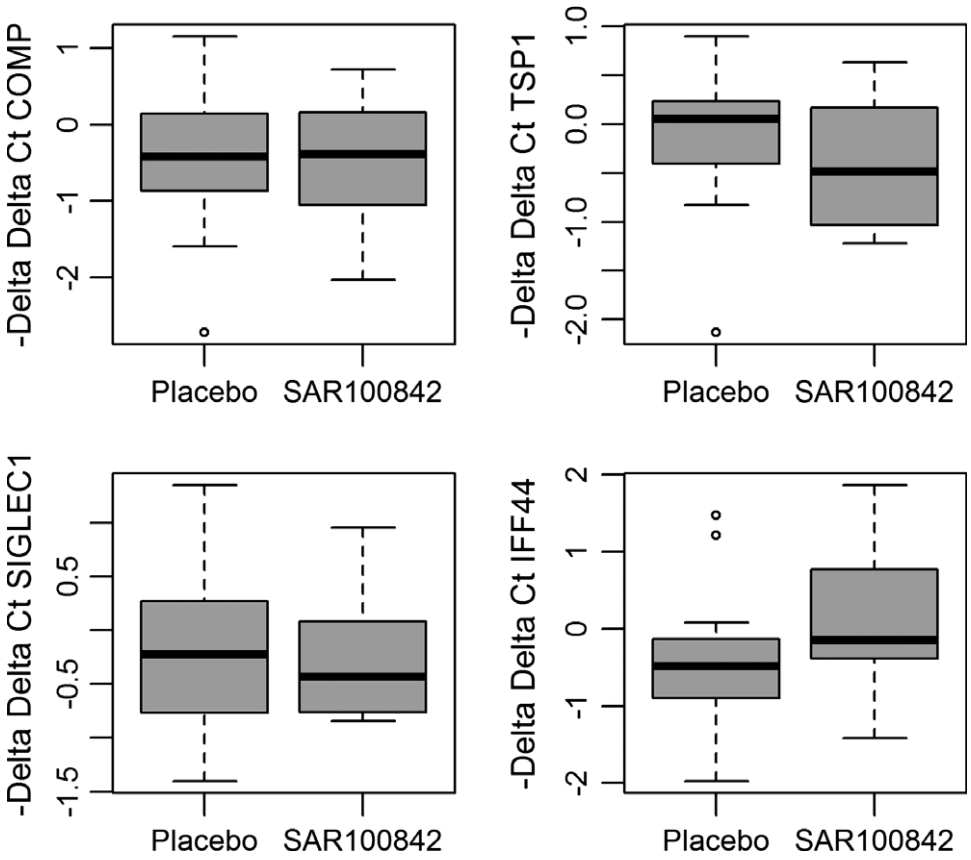

Figure 3. Changes from baseline to week 8 in 4 gene biomarkers of skin fibrosis. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.

PAI was observed in the SAR100842 group ($P = 0.0089$) (Figure 5).

DISCUSSION

LPA is a phospholipid growth factor that targets cells through a number of cell surface receptors, and it has been implicated in the pathogenesis of SSc. Of greatest interest, it appears that it may contribute to excessive tissue fibrosis, mainly through LPA_1 receptor activation (5), as observed in SSc. Recent findings further emphasize the key roles of autotaxin and the LPA axis in SSc (22). SAR100842 is a low molecular weight, selective inhibitor of the LPA_1 receptor that is being developed as a potential novel therapy for SSc with the aim of reducing or even reversing the progression of fibrosis. This phase IIa study is the first to assess oral administration of SAR100842 in patients with early dcSSc. The safety and tolerability of SAR100842 was the primary outcome measure, and SAR100842 was shown to be well tolerated in patients with dcSSc.

In preclinical studies, the administration of SAR 100842 to rats at doses up to 2,000 mg/kg/day caused no toxicologically relevant effects. Findings related to the compound were limited to a slightly higher incidence of regurgitation in females at a high dose, and the present study did not show any specific gastrointestinal AEs in SSc patients. In previous phase I studies, the safety profile was very good, and overall the most frequently reported SAR100842-related AEs were headache, symptomatic orthostatic hypotension or postural dizziness, and flatulence. Those AEs were not severe or serious. In the present study, both in the short-term double-blind phase and in the longer term open-label extension phase, no safety signals emerged regarding vital signs, orthostatic hypotension, electrocardiography, or laboratory parameters. A common toxicologic concern with antifibrotic agents is whether patients may exhibit a delay in normal wound healing. Studies with LPA receptor antagonists using incisional and excisional wounding in rats have been reassuring (22), but it is noteworthy that in the present study, although one-third of patients had digital ulcerations at

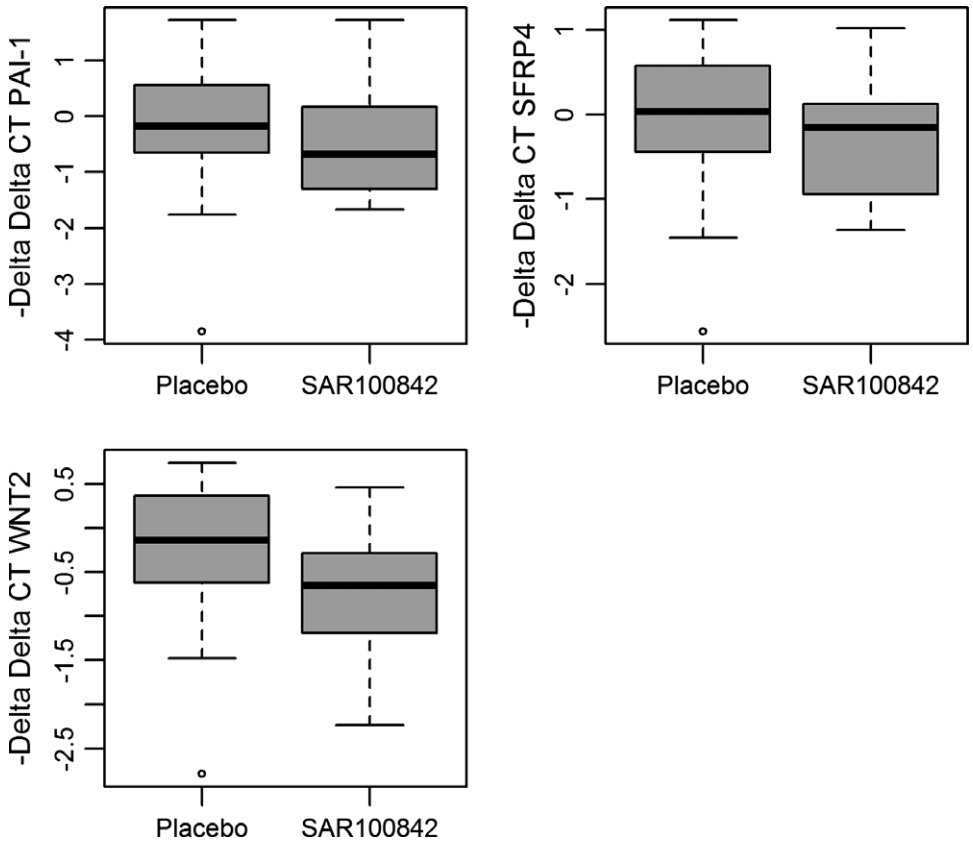

Figure 4. Changes from baseline to week 8 in lysophosphatidic acid pathway markers. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.

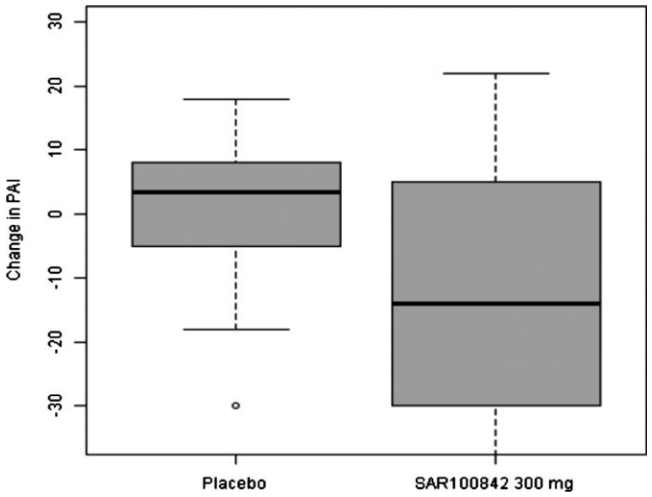

Figure 5. Change in the pathway activation index (PAI) from baseline to week 8. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.

baseline, no overt safety concerns emerged for them, confirming the good safety profile in SSc patients.

The clinical efficacy of SAR100842 was part of the secondary end points, but no effect was expected on the MRSS after 8 weeks of treatment because the MRSS is slow in changing. Nevertheless, at the end of the double-blind period, a numerically greater decrease in total MRSS score from baseline in the SAR100842 group compared to the placebo group was detected without reaching statistical significance (treatment effect −1.2 [95% CI −4.37, 2.02]; median change −4.00 versus −1.00, respectively). Also, there was a numerically greater reduction without reaching statistical significance in the HAQ DI score in the SAR100842 group (treatment effect −0.1 [95% CI −0.38, 0.09]). These findings are promising; they might be due to the mechanism of action of SAR100842 and/or they might also be explained by a large proportion of subjects receiving background immunosuppressive medications. These findings were supportive of the effect observed after 24 weeks of treatment, when patients experienced a clinically meaningful decrease in total MRSS

(median change −7.50) and a high percentage of them (78.6%) improved by at least 5 points from baseline (responders) (23); similar benefit was observed in the HAQ DI score. Although these were secondary end points and had weak statistical power, the size of the decreases must be noted and is larger than that observed in other trials targeting the same SSc population. Furthermore, the similar trend observed for skin changes and quality of life is encouraging and promising for future trials. Nevertheless, and despite being encouraging, the open-label data should be interpreted with caution.

This study must be interpreted while taking its limitations into account. The sample size was not large but was consistent with the design of a proof of biologic activity study looking primarily at safety. The duration may be considered short, and most trials are expected to last more than 6 months, but the observed changes in MRSS in this population are promising. SSc is a systemic disease and organ involvement defines the prognosis. No data could be provided on organ involvement from the present study, and this will have to be addressed in the future.

Pharmacodynamic and biomarker assessments were part of the secondary end points. There was no statistically significant differential expression of any biomarker between the 2 groups of patients.

Using a new unbiased statistical analysis, a guided clustering algorithm allowed the identification of a set of genes that had high LPA perturbation in the cell culture study and that were consistently expressed and correlated with similar expression in skin samples from patients. This LPA signature was then reduced to 1 dimension, and change in the resulting PAI was computed in skin biopsy samples from patients treated with placebo versus patients treated with SAR100842. A significant effect of SAR100842 on change from baseline in the PAI was indicative of an effect of SAR100842 on the LPA signature (15 patients per treatment arm), demonstrating target engagement upon SAR100842 treatment for 8 weeks.

The optimal clinical trial duration for patients with SSc is still unknown. Some observations regarding collagen metabolism suggest that a clinical trial duration of 24 weeks or longer might be recommended. Indeed, in the phase II tocilizumab data (24), the 2-gene biomarker was able to differentiate tocilizumab from placebo at 24 weeks. The biomarkers in clinical trials of SSc have been shown to correlate with skin fibrosis (as seen here) rather than to predict skin progression. In addition, the collagen turnover (which is a product of collagen production and collagen degradation) may require several weeks to be modulated, and this also depends on whether the pharmacologic agent directly (e.g., knockout) or indirectly (the current inhibitor) targets collagen products. A longer version of this trial with clinical and biologic outcome measures at 4–6 months might have shown statistically significant differences.

This study demonstrates that LPA_1 blockade by SAR100842 is well tolerated in patients with early dcSSc. The results show target engagement with SAR100842 as well as some promising clinical and biologic changes. Nevertheless, treatment effect cannot be inferred from skin fibrosis biomarkers, but these biomarkers may be informative as shown in other recent trials (24–26). Taken together, these results suggest the potential clinical benefit of SAR100842 in dcSSc patients with unmet needs (27), and SAR100842 deserves evaluation in confirmatory trials.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Allanore and Khanna had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Allanore, Distler, Jagerschmidt, Illiano, Ledein, Boitier, Agueusop, Denton, Khanna.

Acquisition of data. Allanore, Distler, Denton, Khanna.

Analysis and interpretation of data. Allanore, Distler, Jagerschmidt, Illiano, Denton, Khanna.

ROLE OF THE STUDY SPONSOR

Sanofi was involved in the design and conduct of the study, oversaw the collection, management, and statistical analysis of the data, and contributed to the interpretation of the data and the preparation, review, and approval of the manuscript. The final decision on manuscript submission was made by the authors. Publication of this article was not contingent upon approval by Sanofi.

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