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ORIGINAL ARTICLE

Safety and Efficacy of B-Cell Depletion with Rituximab for the Treatment of Systemic Sclerosis–associated Pulmonary Arterial Hypertension

A Multicenter, Double-Blind, Randomized, Placebo-controlled Trial

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Abstract

Rationale: Systemic sclerosis (SSc)–pulmonary arterial hypertension (PAH) is one of the most prevalent and deadly forms of PAH. B cells may contribute to SSc pathogenesis.

Objectives: We investigated the safety and efficacy of B-cell depletion for SSc-PAH.

Methods: In an NIH-sponsored, multicenter, double-blinded, randomized, placebo-controlled, proof-of-concept trial, 57 patients with SSc-PAH on stable-dose standard medical therapy received two infusions of 1,000 mg rituximab or placebo administered 2 weeks apart. The primary outcome measure was the change in 6-minute-walk distance (6MWD) at 24 weeks. Secondary endpoints included safety and invasive hemodynamics. We applied a machine learning approach to predict drug responsiveness.

Measurements and Main Results: We randomized 57 subjects from 2010 to 2018. In the primary analysis, using data through Week 24, the adjusted mean change in 6MWD at 24 weeks favored the treatment arm but did not reach statistical significance (23.6 ± 11.1 m vs. 0.5 ± 9.7 m; $P = 0.12$). Although a negative study, when data through Week 48 were also considered, the estimated change in 6MWD at Week 24 was 25.5 ± 8.8 m for rituximab and 0.4 ± 7.4 m for placebo ($P = 0.03$). Rituximab treatment appeared to be safe and well tolerated. Low levels of RF (rheumatoid factor), IL-12, and IL-17 were sensitive and specific as favorable predictors of a rituximab response as measured by an improved 6MWD (receiver operating characteristic area under the curve, 0.88–0.95).

Conclusions: B-cell depletion therapy is a potentially effective and safe adjuvant treatment for SSc-PAH. Future studies in these patients can confirm whether the identified biomarkers predict rituximab responsiveness.

Clinical trial registered with www.clinicaltrails.gov (NCT 01086540).

Keywords: pulmonary hypertension; systemic sclerosis; treatment

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

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At a Glance Commentary

Scientific Knowledge on the Subject: Pulmonary arterial hypertension is one of the most serious complications of systemic sclerosis. B cells may be involved in disease pathogenesis. New studies can help define the role of adjunctive immune modulation for this frequently lethal condition.

What This Study Adds to the Field: This trial is the first to evaluate the safety and efficacy of B-cell depletion in pulmonary hypertension. Systemic sclerosis is a highly heterogeneous condition with many manifestations; we evaluated the subset of patients with evidence of significant group I pulmonary hypertension. In this proof-of-concept trial, we enlisted a selective cohort with rigorous enrollment criteria focused on World Health Organization group I pulmonary hypertension. In the primary analysis based on longitudinal data through Week 24, the adjusted mean change in the 6-minute-walk distance from baseline to 24 weeks did not differ significantly between arms ($P = 0.12$). However, in a secondary analysis using 6-minute-walk distance data through Week 48, the rituximab arm was superior to placebo ($P = 0.03$). Machine learning identified a subgroup based on biomarkers that appeared to gain the greatest benefit from rituximab. This study suggests a possible role for adjunctive immunotherapy for targeted patients with systemic sclerosis–associated pulmonary arterial hypertension.

Systemic sclerosis (SSc) is a rare systemic connective tissue disease characterized by fibrosis and atrophy of the skin and internal organs (1); its protean manifestations are due to autoimmune microvascular injury and fibrosis. Disease progression likely occurs when genetic, epigenetic, and environmental factors negatively affect immune and vascular systems, causing fibrotic remodeling (2). Abnormalities in B-cell function may contribute to pathogenic autoantibodies, immune dysregulation, and fibrosis, and, consequently, B-cell immunity is a rational therapeutic target for certain manifestations of this disease (3–6). Pulmonary hypertension is a serious condition complicating SSc, with preclinical (7, 8) and clinical studies demonstrating a putative role for B cells and autoantibodies in its development (9–14).

Pulmonary hypertension in patients with SSc has several etiologies, including progressive interstitial lung disease (ILD), pulmonary venoocclusive disease, chronic thromboembolic disease, pulmonary arterial hypertension (PAH), or a combination of these factors (15, 16). The limited cutaneous form of SSc has been more commonly associated with isolated PAH, otherwise known as SSc–associated PAH (SSc-PAH) (17, 18). SSc-PAH (the focus of the current study) develops in approximately 10% of individuals with SSc (19). Patients with SSc-PAH are more likely to have severe intrinsic right ventricular dysfunction, high BNP (B-type natriuretic factor), low D_{LCO}, and poor survival (16, 20–22). Lungs from patients with SSc-PAH exhibit a characteristic vascular pathology; the plexiform lesion is less common than in other forms of PAH, and vascular lesions display perivascular lymphocytes and intimal fibrosis (23, 24). The complex clinical presentation and distinct vascular pathology of SSc-PAH suggest a unique biology among group I pulmonary hypertension conditions (25). There have been increasing efforts to understand the relationship between autoimmunity, inflammation, and the evolution of PAH (9, 14, 26).

Because B-cell immunity is implicated in SSc pathogenesis, B-cell depletion was an attractive therapeutic candidate after standard-of-care vasodilator therapy. In other SSc manifestations, rituximab attenuates fibrosis, and so its benefit in attenuating the fibroproliferative aspects of SSc-PAH was plausible (5, 27, 28). Case reports suggest that rituximab might be therapeutic for several forms of PAH (29–33), most notably a patient with treatment-refractory SSc with mild ILD and severe PAH (34). Developmental B-cell anomalies temporarily reverse with rituximab treatment in patients with SSc-PAH (35). Given the strong evidence implicating autoimmune vascular injury and the poor survival of patients with SSc-PAH, the putative benefits of carefully proceeding with B-cell depletion appeared to be justified. Here, we sought to target the adaptive arm of immunity implicated in the microvascular injury of SSc (36, 37). We designed and implemented a proof-of-concept, multicenter, double-blind, placebo-controlled, randomized clinical trial to evaluate the safety, tolerability, and potential efficacy of rituximab in patients with SSc-PAH diagnosed within 5 years. This trial focused on patients with isolated group I pulmonary hypertension without severe ILD. We hypothesized that B-cell depletion with rituximab would improve exercise tolerance in patients with SSc-PAH. Some of the results of these studies have been previously reported in the form of abstracts (38, 39).

Methods

Study Design

We conducted a proof-of-concept, prospective, double-blind, multicenter, phase 2 randomized clinical trial of patients with SSc-PAH (NCT 01086540). The study was sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), and the study drug was provided by Genentech. Recruiting field centers included 26 academic pulmonary, rheumatology, and cardiology programs in the United States. The protocol development committee, in collaboration with the NIAID and the statistical and clinical coordinating center (Rho Federal Systems Division) designed the study protocol (*see* the online supplement), and institutional review boards at each center approved the protocol. Data were collected and analyzed according to a prespecified statistical analysis plan. An

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A complete list of ASC01 Study Group members may be found before the beginning of the References.

independent data monitoring committee supervised the study, and all authors had access to the source-verified data and attest to the accuracy and completeness of this report. The trial was conducted in accordance with the tenets of the Declaration of Helsinki.

Selection of Subjects

We included adults between 18 and 75 years old with SSc-PAH diagnosed for no more than 5 years. Subjects were required to have a baseline mean pulmonary arterial pressure ⩾25 mm Hg, pulmonary capillary wedge pressure ⩽15 mm Hg, pulmonary vascular resistance (PVR) >240 dyn/cm/s^5 with TLC >60% predicted, and no evidence of significant valvular or left ventricular dysfunction. Subjects with a TLC within 60–70% of normal values were required to have computed tomography imaging demonstrating no more than mild fibrosis. We excluded subjects who received prior lymphocyte-depleting agents (at any time) or biologic immunotherapy within 4–8 weeks before screening. For full eligibility criteria, please refer to the online supplement.

Trial Procedures

Subjects were randomized in a 1:1 manner to receive two infusions of either rituximab at 1,000 mg each or placebo 14 days apart. We applied an adaptive randomization procedure to maintain balance between treatment groups overall, by study site, PVR at baseline, and time since diagnosis of SSc-PAH. Infusions were administered in a supervised telemetry unit contiguous with a hospital where subjects would have access to critical care ward in case of hypotension or anaphylaxis. Active blood pressure monitoring and hypotension treatment guidelines were provided (online supplement section E1). All subjects, including the placebo group, received prophylactic premedication with 40 mg of prednisone (day before), methylprednisolone (immediately before infusion), diphenhydramine, and acetaminophen. Subjects were followed over a 48-week period, with the primary endpoint measured at 24 weeks. Invasive hemodynamics and pulmonary function testing were completed at baseline and Week 24. Throughout the study, subjects were routinely evaluated for symptoms, 6-minute-walk test, NT-pro BNP (N-terminal pro B-type natriuretic peptide), and routine laboratory testing. We also collected blood for mechanistic biomarkers including CD19$^+$ B-cell count, autoantibodies, serum cytokines, and immunoglobulins.

Outcome Measures

The primary efficacy endpoint was the change from the time of enrollment to Week 24 in the 6-minute-walk distance (6MWD). Other important secondary efficacy endpoints included change from baseline to Week 24 in PVR, change from baseline to Week 48 in 6MWD, and time to clinical worsening. Values are reported as mean ± SE. Time to clinical worsening was defined as the first occurrence of one of the following events: death, hospitalization for SSc-PAH, lung transplantation, atrial septostomy, addition of other PAH therapies, or worsening of 6MWD by >20% with a decrease in function class. Other secondary efficacy endpoints included time to change or addition of PAH therapies, changes at Week 24 and 48 in the Short Form Health Survey quality of life questionnaire and the disability index of the Scleroderma Health Assessment Questionnaire, the number of new digital ulcers, and the severity of Raynaud phenomenon as measured by a visual analog scale of the Health Assessment Questionnaire–Disability Index (HAQ-DI), $D_{L_{CO}}$, and oxygen saturation at rest.

For evaluation of safety, adverse events (AEs) and laboratory safety data were graded for severity using the National Cancer Institute—Common Terminology Criteria for Adverse Events (version 4).

Statistical Analysis

The study was originally designed as an 80-subject study powered to detect a change in PVR at 24 weeks. After 3 years of recruitment, only 30 subjects had enrolled. Furthermore, the lower-than-expected baseline PVR made the originally anticipated percentage improvement in PVR unachievable. These factors prompted a redesign of the study, including a new primary endpoint of change in 6MWD at Week 24. No 6MWD data from the ongoing study were used in the development of the redesign. Based on prior studies, a difference between arms of 33 m was deemed to be the minimal clinically meaningful improvement in 6MWD, and an SD of 58 m was assumed (40). Under these assumptions and accounting for 20% dropouts, a study of 60 subjects had 50% power to detect a treatment group difference based on a two-sided *t* test with $\alpha = 0.05$ and 80% power if there was an underlying mean difference between arms of 50 m. This 60-person design was reasonable given the enrollment challenges and exploratory objectives for this study. Changes to the primary endpoint and sample size were reviewed and approved by the U.S. Food and Drug Administration, NIH Data and Safety Monitoring Board (DSMB) and the NIH/NIAID/Division of Allergy, Immunology, and Transplantation Autoimmunity Centers of Excellence Steering Committee. The primary outcome was changed in the protocol in 2014 (version date October 23, 2014) and updated in clinicaltrials.gov after the database lock in 2019 in preparation for posting study results.

Efficacy analyses were based on a modified intent-to-treat population including all randomized subjects who met entry criteria and initiated therapy. The primary analysis of the primary efficacy endpoint includes data collected through the Week 24 visit. A random regression model was fit to the 6MWD as a function of fixed effects for treatment, time (in weeks), and a treatment-by-time interaction. A quadratic term for time as an additional fixed effect was also included in the model. Random slopes for week and intercept were fit for each subject using a separate unstructured covariance matrix for each treatment group. This model assumes missing longitudinal data are missing at random; all available data contribute to the model, and missing data are not imputed. The *P* value is for the contrast, [(mean at Week 24 –mean at Week 0) rituximab – (mean at Week 24 – mean at Week 0) placebo]. The treatment group comparison is evaluated using a two-sided test with $\alpha = 0.05$. This hypothesis test was prespecified in the statistical analysis plan, with the knowledge that the design had only 50% power to detect the minimal clinical relevant difference (i.e., 33 m).

This was the only formal hypothesis test conducted for this proof-of-concept study. Prespecified secondary and exploratory analyses of efficacy endpoints were envisioned to explore relationships and better understand treatment group differences. Associated *P* values are not adjusted for multiple comparisons. *P* values ⩽0.05 are highlighted, as these suggest that the observed data would be unexpected if, in fact, treatment groups did not differ, and the underlying statistical model and assumptions are correct. These results should be interpreted with caution and considered with respect to the clinical relevance of associated parameter estimates. Estimates for these analyses are presented irrespective of the *P* values.

In a secondary analysis of the primary endpoint, a similar random regression model was fit using all available 6MWD data through Week 48 (online supplement section E1). A random regression model analogous with the primary analysis model was also fit to PVR, a

secondary efficacy endpoint. Kaplan-Meier survival curves for time to clinical worsening and time to change or addition of PAH medications were compared using the log-rank statistic; subjects were censored at their last study visit if an event did not occur.

Safety analyses were conducted on all subjects who initiated treatment. Descriptive statistics are presented for AEs.

As an exploratory approach, several circulating biomarkers were assessed in relation to the primary clinical outcome. In an agnostic untargeted analysis, we developed supervised machine learning (ML) models to identify baseline biomarkers that predicted a clinical response to rituximab at 24 weeks (6MWD increase ⩾33 m). We implemented a variety of ML algorithms in parallel to develop comparative models (random forests, extreme gradient boosted ensemble trees, and support vector machines with polynomial and radial kernel transformations). Training of each model involved 168 input variables (cytokines, chemokines, factors, immunoglobulin subclasses, autoantibodies, B-cell subsets, and clinical features), iterative algorithm resampling runs (Monte Carlo cross-validation), and recursive feature elimination to select the variable subset that best classified clinical responders. Areas under receiver operating characteristic and precision-recall curves were used to evaluate the classification accuracy of each ML model (online supplement section E3).

The trial data are accessible from ImmPort at https://immport.niaid.nih.gov/research/study/studysearchmain#!/studysearch/viewStudyDetails/studySummarySDY1660.

Results

Between October 2010 and February 2018, 108 patients consented, and 57 subjects were randomized (Figure 1). Early withdrawals before Week 24 were imbalanced between arms (six in the rituximab group and one in the placebo group), but there was no apparent relationship between 6MWD and early withdrawal. The most common reasons for screen failure included SSc-PAH >5 years ($n = 11$), PVR <240 dyn/cm/s^5 ($n = 14$), and moderate to severe ILD ($n = 7$). Twenty-seven of the 29 subjects randomized to rituximab completed both infusions, whereas two subjects did not as a result of investigator decision.

The baseline characteristics were well balanced between study arms. Subjects were similar in age, predominantly female, and primarily New York Heart Association functional class II or III at enrollment (Table 1). The most common autoantibodies were anticentromere (43.9%) and anti-Th/To antibody (17.5%), as expected, and none of the subjects demonstrated positive SCL-70. Relative to normal values, the two cohorts had substantially reduced 6MWD (rituximab 338.7 ± 103.3 m and placebo 323.4 ± 97.1 m) and normal TLC but severely reduced $D_{L_{CO}}$ (rituximab 48.5 ± 16.2% predicted and placebo 42.0 ± 14.5% predicted). Baseline hemodynamics demonstrated elevated mean pulmonary arterial pressure (rituximab 40.6 ± 9.4 mm Hg and placebo 44.9 ± 9.4 mm Hg), normal pulmonary capillary wedge pressure, and modestly increased PVR (rituximab 531.6 ± 176.6 dyn/s/cm^5 and placebo 598.8 ± 199.7 dyn/s/cm^5).

Primary Efficacy Endpoint

For the primary analysis, the model-based mean increase in 6MWD at Week 24 was 23.6 ± 11.1 m in the rituximab group and 0.5 ± 9.7 m in the placebo group (least-squares mean difference, 23.1 ± 14.7 m) (Figure 2). The test for a treatment group difference based on the primary analysis model did not reach statistical significance ($P = 0.12$). The estimated treatment group difference at Week 24 based on the secondary analysis model that included 6MWD data to Week 48 was notable (25.1 ± 11.5 m; $P = 0.03$). This speculative improvement in rituximab-treated patients had diminished by Week 48 (16.4 ± 15.1 m; $P = 0.3$) (Figure 2, Table E2–1.0 in the online supplement). In addition, a higher proportion of the rituximab-treated group (for those who had a 24-wk 6MWD measured) achieved at least a 33-m (or more) improvement in the 6MWD than the placebo group (48% vs. 15%) (Figure 3). Similarly, although only 15% of placebo group demonstrated ⩾50 m change in 6MWD by Week 24, 38% of those treated with rituximab met that threshold.

Secondary and Exploratory Endpoints

The estimated change in PVR from baseline to Week 24 was −39.0 ± 28.9 dyn/s/cm^5 and 7.2 ± 48.5 dyn/s/cm^5 for the rituximab and placebo arms, respectively ($P = 0.42$). Time to clinical worsening and change in PAH-specific therapies were not notably different between the rituximab and placebo arms (Figure 4). There were four deaths (three in the rituximab arm and one in the placebo arm) in active subjects followed to Week 48. The three deaths in rituximab arm included adenocarcinoma, sudden death in a patient with a history of drug abuse, and cardiac arrest during elective surgery (the latter two diagnoses being attributed to SSc-PAH). The cause of death in the placebo arm was cardiac arrest related to underlying PAH. No notable differences between groups were observed at Weeks 24 or 48 for the Short Form Health Survey, disability index and visual analog scale of Scleroderma Health Assessment Questionnaire, number of new digital ulcers, $D_{L_{CO}}$, oxygen saturations, and NT-pro BNP between the arms (Section E2 and Table E2–14.0).

As anticipated, rituximab-treated subjects demonstrated a near complete depletion of B cells by Study Week 2 with a slow recovery after Week 24 (Figure 5). Although the rise in Δ6MWD at 24 weeks occurred during the time of B-cell depletion, an analysis of the data after 24 weeks could not confirm that the subsequent reduction in Δ6MWD was related to B-cell repletion. Using an ML approach, we identified baseline RF (rheumatoid factor), IL-12, and IL-17, when considered together, to be consistent markers of clinical response to rituximab (Figure 6 and Section E3: Table E3–1.0 and Figure E3–2.0). Low concentrations of RF, IL-12, and IL-17 were highly sensitive (80–100%) and specific (83.3–94.4%) as favorable predictors of rituximab response (receiver operating characteristic area under the curve, 0.88–0.95). These three factors were specific to subjects who received rituximab and did not identify "responder" (i.e., >33 m improvement at 24 wk) placebo subjects (Section E3: Table E3–2.0 and Figure E3–3.0).

Safety and Tolerability

Rituximab treatment appeared to be well tolerated (Table 2 and Section E2). B-cell depletion and reconstitution kinetics after rituximab treatment parallel those from patients with rheumatoid arthritis receiving the same dosing regimen (41, 42). There were no anaphylaxis or hypotensive events noted during infusion of rituximab. Differences in AE rates between arms were not remarkable or unexpected for the most common AEs (>5%). Higher number of AEs related to blood and lymphatic system disorders were reported in the rituximab arm (25 AEs in 20 subjects, rituximab; 17 AEs in 13 subjects, placebo). Infections were reported in 58.6% (17 subjects with 46 events) of rituximab and 82.1% (23 subjects with 43 events) of placebo subjects. There were 22 serious AEs (SAEs) in 14

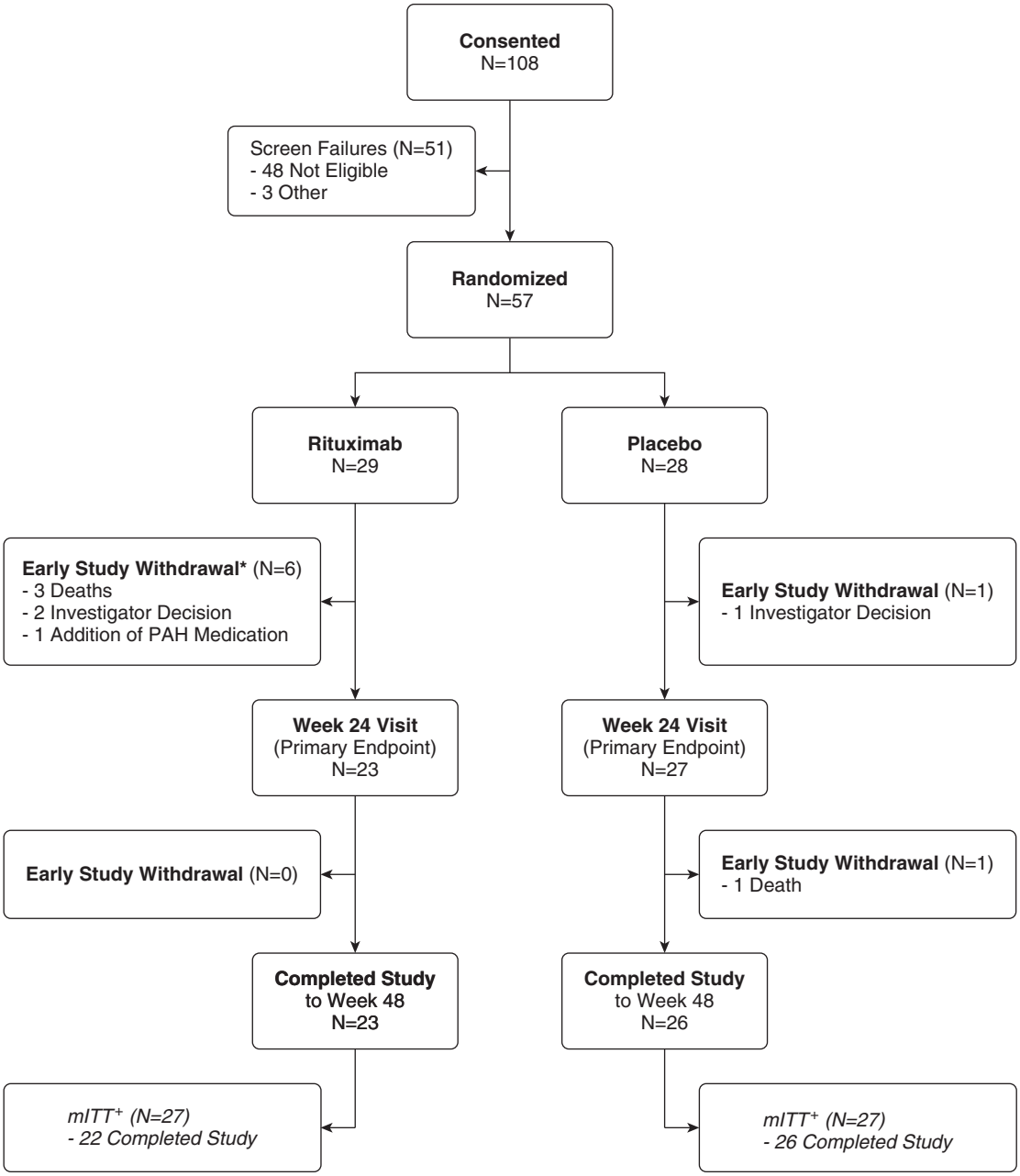

Figure 1. CONSORT diagram. *Two subjects who withdrew early from the study also discontinued study treatment early. Three subjects (two rituximab and one placebo) were excluded from the *mITT* population as they were ineligible. The reasons included *1*) abnormal neutrophil count $<1.5 \times 10^9$/L at screening (1.3 and 1.0×10^9/L at screening and baseline, respectively), *2*) not on stable dose of pulmonary arterial hypertension medication for 4 weeks before randomization (stopped bosentan 5 d before randomization), and *3*) not on stable dose of prednisone for 4 weeks before randomization (prednisone 40 mg/d on Days −8 to −4 and tapered over the first 100 d). The number at the "Week 24 Visit" box indicates the number of participants in the randomized population who completed the Week 24 visit. *mITT* = modified intent-to-treat; PAH = pulmonary arterial hypertension.

rituximab subjects compared with 14 SAEs in nine placebo subjects. Infections were the most common SAEs in rituximab-treated patients (10 SAEs in 7 [24.1%] subjects, rituximab; three SAEs in two [7.1%] subjects, placebo], but none were fatal (Table 2). The 10 infection SAEs in the rituximab arm included two catheter-related, two upper respiratory tract/pneumonia, one endocarditis, and three sepsis events. Sepsis was observed in the same individual over the short- and long-term course of observation. One event was due to an exophytic herpes simplex virus lesion found to be acyclovir resistant. Most importantly, 7 of these 10 events resolved without significant clinical sequelae (Section E2). The three events in the placebo arm were pneumonia, sialoadenitis, and parainfluenza virus infection (Section E2). As described above, there were four deaths in subjects followed to Week 48. None of these deaths were attributed to rituximab use. The 1-year mortality was 14% (95% confidence interval [CI], 5–33%) in the rituximab arm and 4% (95% CI: 1–23%) in

Table 1. Baseline Demographic and Clinical Characteristics

Patient Characteristics	Rituximab (*N* = 29)	Placebo (*N* = 28)	Total (*N* = 57)
Age, yr	57.3 (8.9)	59.1 (9.4)	58.2 (9.1)
Sex, *n* (%)			
M	4 (13.8)	1 (3.6)	5 (8.8)
F	25 (86.2)	27 (96.4)	52 (91.2)
Autoantibody profile,* mean (SD)			
Centromere positive	11 (37.9)	14 (50.0)	25 (43.9)
Th/To positive	5 (17.2)	5 (17.9)	10 (17.5)
Other autoantibody positive	8 (27.6)	5 (17.9)	13 (22.8)
Not performed	5 (17.2)	4 (14.3)	9 (15.8)
WHO class, *n* (%)			
II	12 (41.4)	12 (42.9)	24 (42.1)
III	17 (58.6)	15 (53.6)	32 (56.1)
IV	0	1 (3.6)	1 (1.8)
PAH medications, *n* (%)			
PDE-I	20 (68.9)	22 (78.6)	42 (73.7)
ERA	19 (65.5)	16 (57.1)	35 (61.4)
Prostacyclin	14 (48.3)	16 (57.1)	30 (52.6)
6MWD, mean (SD), m	338.7 (103.3)	323.4 (97.1)	331.2 (99.7)
NT-pro BNP,† mean (SD), pg/ml	988.6 (1,142.9)*	629.0 (838.7)†	820.8 (994.6)
BNP,‡ mean (SD), ng/L	230.7 (366.5)‡	280.5 (369.8)‡	255.6 (364.5)
Pulmonary function, mean (SD)			
TLC, % predicted	84.5 (15.4)	92.8 (11.8)	88.5 (14.3)
$D_{L_{CO}}$, % predicted	48.5 (16.5)	42.0 (14.5)	45.3 (15.7)
Hemodynamics, mean (SD)			
RA, mm Hg	6.6 (4.4)	8.7 (3.5)	7.6 (4.1)
mPAP, mm Hg	40.6 (9.4)	44.9 (9.4)	42.7 (9.6)
PCWP, mm Hg	9.6 (3.2)	10.9 (2.9)	10.3 (3.1)
CO, L/min	4.95 (1.1)	4.79 (1.0)	4.87 (1.1)
PVR, dyn/cm/s^5	531.6 (176.6)	598.8 (199.7)	564.6 (189.7)

Definition of abbreviations: 6MWD = 6-minute-walk distance; BNP = B-type natriuretic factor; CO = cardiac output; ERA = endothelin receptor antagonist; mPAP = mean pulmonary artery pressure; NT-pro BNP = N-terminal pro BNP; PAH = pulmonary arterial hypertension; PCWP = pulmonary capillary wedge pressure; PDE-I = phosphodiesterase inhibitor; RA = right atrial; PVR = pulmonary vascular resistance; WHO = World Health Organization.

*Data were available for 24 rituximab + 24 placebo subjects.

†Data were available for eight rituximab + seven placebo subjects.

‡Data were available for 21 rituximab + 21 placebo subjects.

placebo arm; 2-year mortality was 28% (95% CI: 15–48%) in the rituximab arm and 18% (95% CI, 8–39%) in the placebo arm (Section E2: Figure E2–13.0).

Discussion

In this proof-of-concept, randomized, placebo-controlled study of B-cell depletion, we demonstrate an acceptable safety and tolerability profile for rituximab in patients with stable SSc-PAH. In the primary analysis that includes data through Week 24, the observed improvement in 6MWD favored rituximab-treated patients, but this difference did not reach statistical significance; for this reason, this trial is considered a negative study. When data through Week 48 were included in a secondary analysis, the estimated mean difference between arms for the change in 6MWD from baseline to Week 24 was slightly larger and more precise, with an associated *P* value of 0.03, suggesting the potential for benefit. In addressing the ASC01 Study hypothesis that posed a positive role for selective lymphocyte ablation in PAH, we found that this potential performance improvement occurred when B-cell counts nadired at 24 weeks. However, an evaluation of patients during the B-cell recovery period could not confirm that the converse was true (i.e., that worsening performance was related to rising B-cell counts). In this study, the estimated mean change in PVR from baseline to Week 12 again favored rituximab ($P = 0.42$). PVR, however, is an unreliable indicator of prognosis in SSc-PAH (20). The proportion of patients requiring additional PAH therapies after 24 weeks was lower in the rituximab group than in the placebo group. In short, although results of this proof-of-concept study are not definitive and *P* values that are not adjusted for multiple comparisons should be interpreted with caution, we observed internally consistent signals suggesting benefit of rituximab for patients with SSc-PAH.

The mechanisms by which rituximab exerts its potentially therapeutic effect are most likely related to altering B-cell immunity, and, as noted, the improvement ascribed to treatment in this study did correlate with a reduction in B-cell counts (43). Treatment with this drug may be effective because it removes cells that give rise to plasma cells that secrete autoantibodies and because it deletes lymphocytes that promote autoimmunity through antigen presentation (44). A separate mechanistic study of B cells from subjects in this trial demonstrated that SSc-PAH–specific

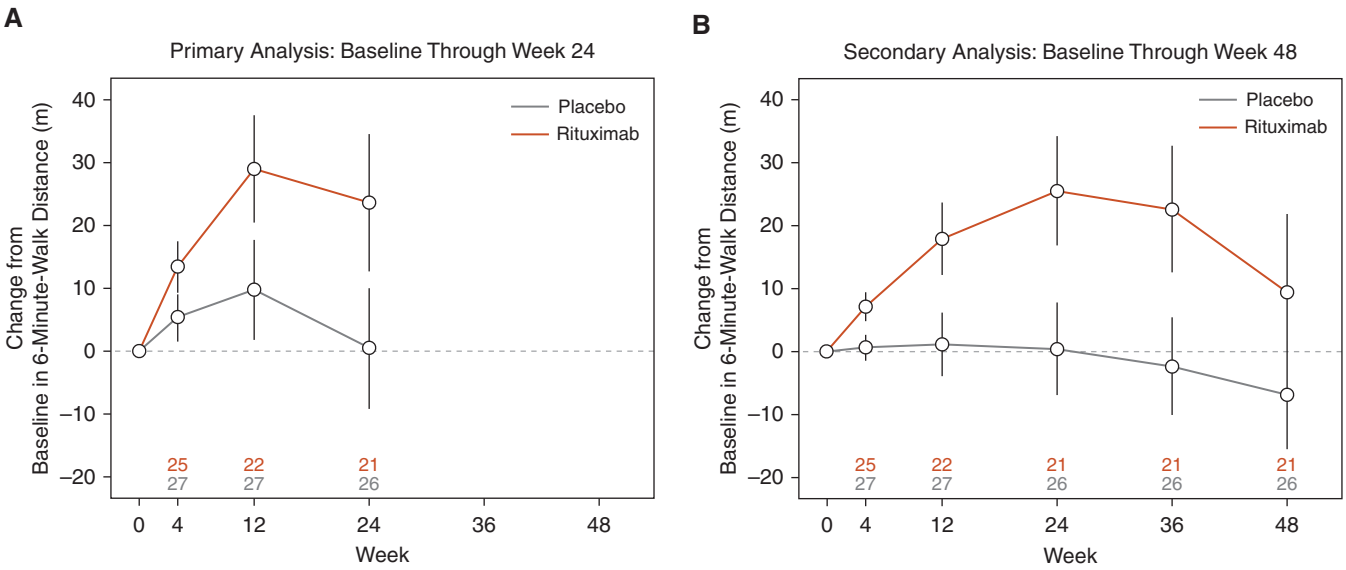

Figure 2. Model estimated mean (±SE) changes in 6-minute-walk distance (6MWD) from baseline are shown in the modified intent-to-treat (mITT) cohort. (*A*) The primary endpoint model, including 6MWD data up to Week 24, demonstrates a least-squares mean difference in the distances at Week 24 of 23.1 m ($P = 0.12$). (*B*) Secondary analysis of primary endpoint model, including all 6MWD data to Week 48, shows an estimated mean change walk distance from baseline to 24 weeks of 25.5 ± 8.79 m for rituximab and 0.4 ± 7.43 m for placebo ($P = 0.03$), a finding that diminished by Week 48. The numbers indicate the number of mITT participants with available 6MWD data at each time point and are shown at the bottom of the figure.

of B-cell anomalies were temporarily reversed after rituximab treatment (35). With a drug targeting B-cell immunity, even downstream pathologies such as fibrotic remodeling can be attenuated (45), and it will behoove future trials to further explore the biological basis of any discovered clinical improvements.

SSc-PAH is a unique form of group I PH; it is the only group I condition associated with ILD and exhibits a heterogenous response to PAH therapies (20). A disease treated by rheumatologists, pulmonologists, and cardiologists, the terminology associated with this condition has not always been unified. SSc-PAH was previously referred to as "isolated pulmonary hypertension in SSc" (17) that is commonly observed in the limited

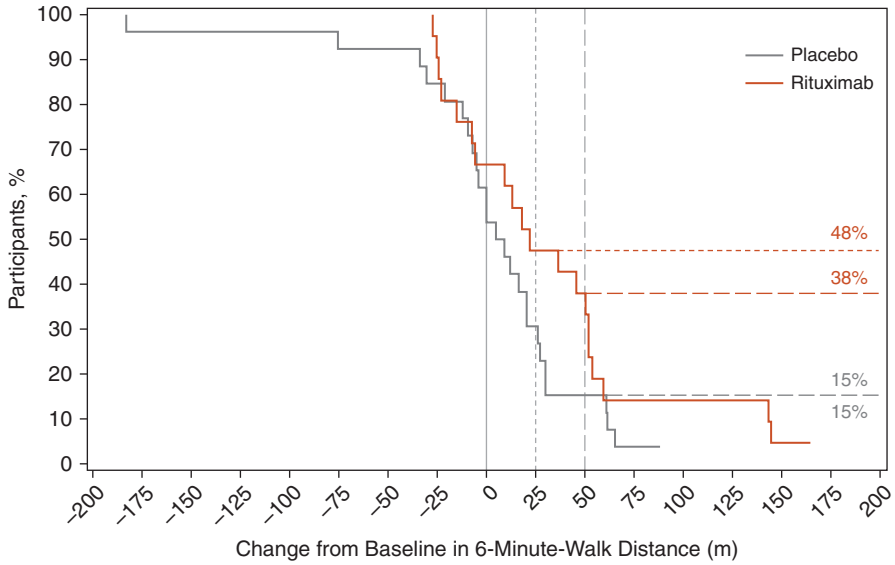

Figure 3. Cumulative distribution plot of change in 6-minute-walk distance (6MWD) from baseline to Week 24. Although 48% of the rituximab-treated subjects demonstrated ⩾33 m in 6MWD, only 15% of placebo-treated subjects achieved this threshold. Similarly, a higher proportion of rituximab subjects (38% vs. 15%) achieved an improvement of ⩾50 m. In this figure, the short-dashed vertical gray line represents the ⩾33 m threshold, and the long-dashed vertical gray line represents the ⩾50 m threshold.

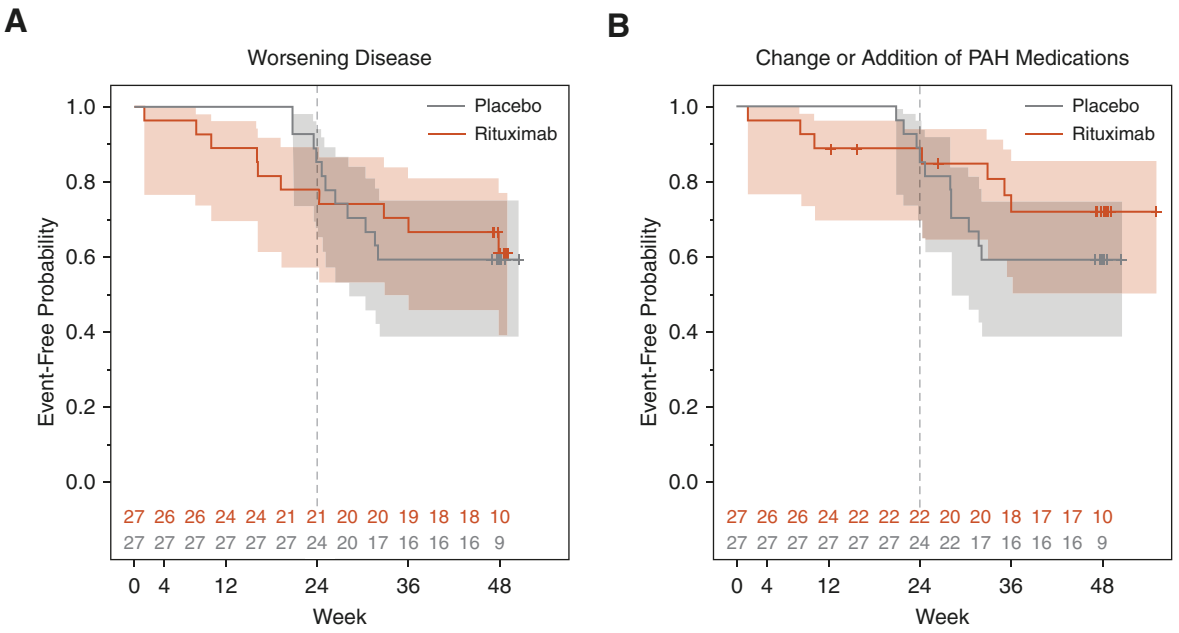

Figure 4. Kaplan-Meier curves of (*A*) time to clinical worsening and (*B*) time to addition or modification of pulmonary arterial hypertension–specific therapies across treatment arms. Subjects were censored at their last study visit if an event did not occur or if they terminated early from the study. Although overall rates of deterioration did not differ substantially across rituximab and placebo arms, the majority of worsening in the placebo arm appears to be just before or after the 24 weeks' time. Shaded areas represent confidence intervals. PAH = pulmonary arterial hypertension.

cutaneous form of SSc. Now, there is widespread acceptance of World Symposium on Pulmonary Hypertension terminology (i.e., "SSc-PAH") (46). This disorder is distinguished from other SSc pulmonary hypertension conditions by the presence of precapillary pulmonary vascular disease (16, 18). On the basis of this updated definition of SSc-PAH, the study sought subjects with no more than mild parenchymal disease (21). Although initial (or early) combination vasodilator therapy benefits patients with SSc-PAH (47, 48), this group has historically exhibited poor survival rates compared with patients with other forms of group I pulmonary hypertension (20, 49, 50). Characterization of connective tissue disease–associated PAH in the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL Registry) showed that patients with SSc-PAH demonstrate a unique phenotype, with the highest BNP concentrations, lowest D_{LCO}, and poorest survival of all connective tissue disease–associated PAH subgroups (20). Risk factors for poor outcome in SSc-PAH are male sex, a D_{LCO} <50%, exercised-induced oxygen

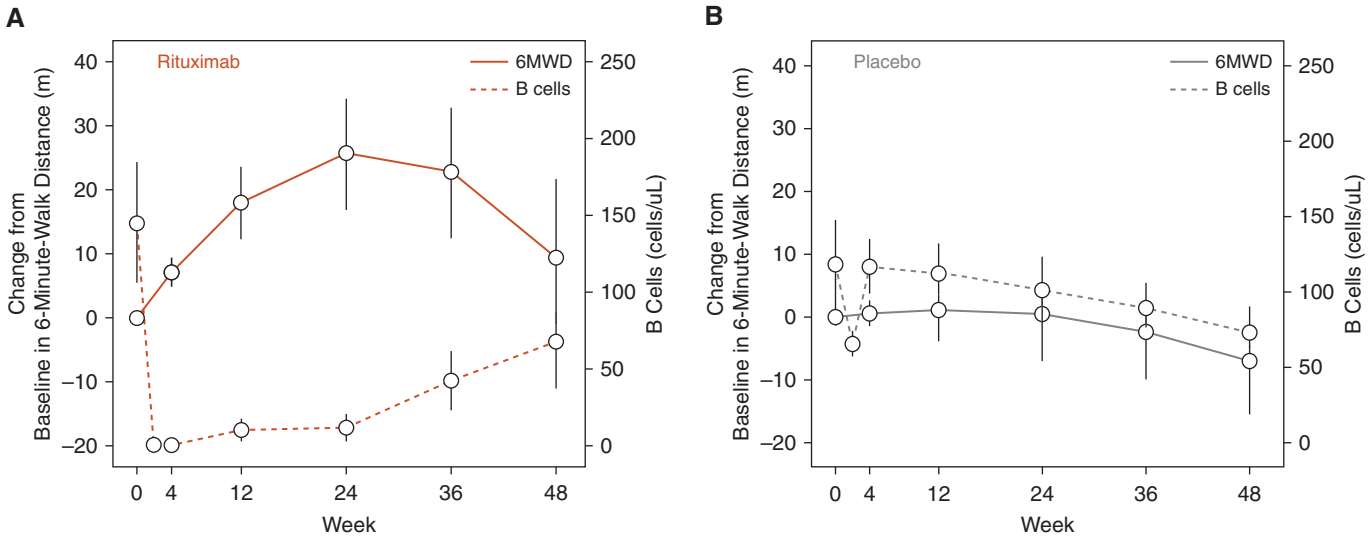

Figure 5. Relationship between B-cell depletion and clinical response in the (*A*) rituximab and (*B*) placebo treatment arms. Change in 6MWD (solid line) and raw B-cell count (dashed line) from baseline to Week 48. 6MWD = 6-minute-walk distance.

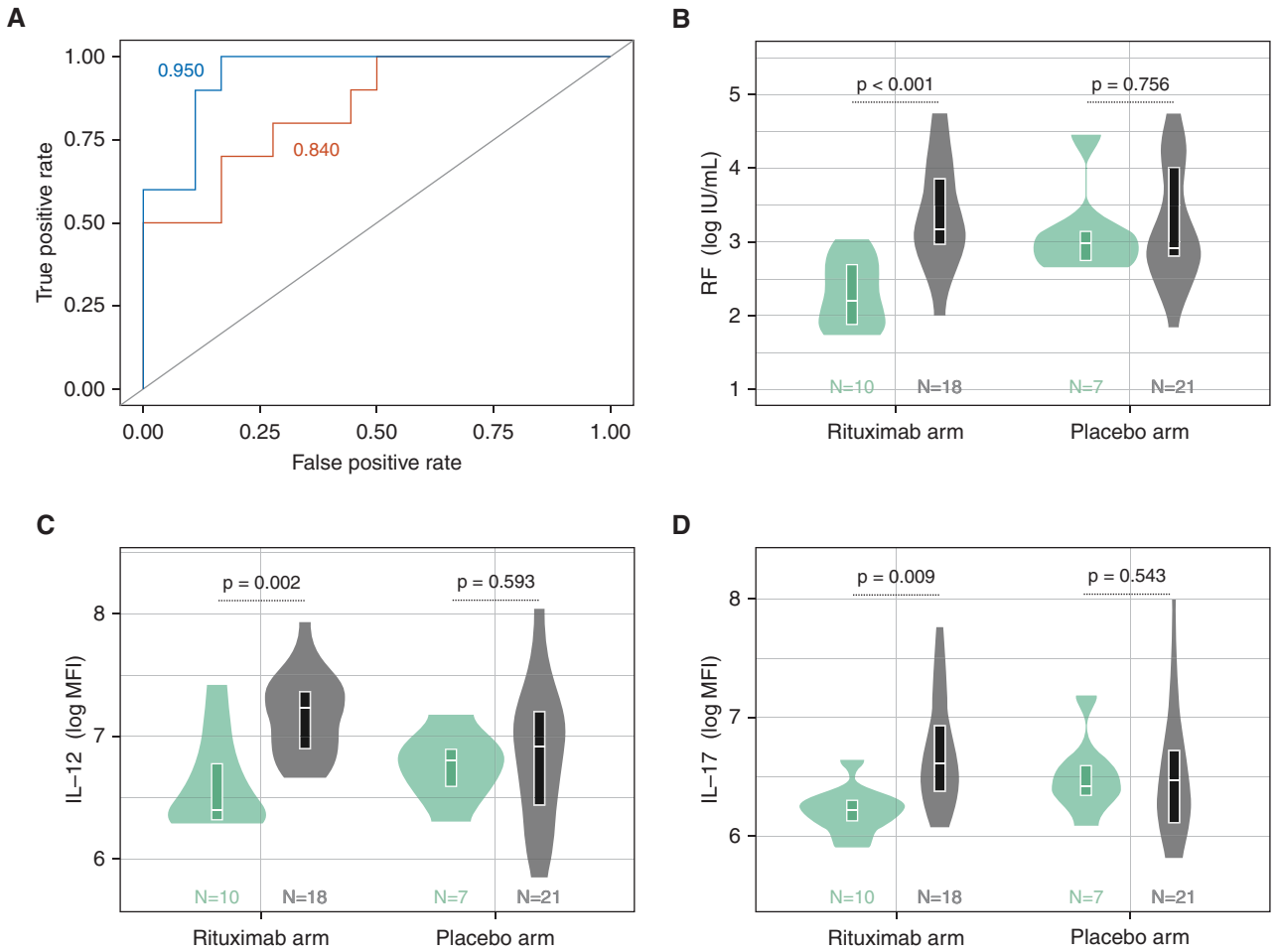

Figure 6. Machine learning discovery of baseline predictors of rituximab clinical response. (*A*) Receiver operating characteristic curves show performance of the extreme gradient boosted decision trees (XGBOOST) algorithm for predicting rituximab response (6-minute-walk distance [6MWD] improvement ⩾33 m). Cross-validated performance (across dataset subsampling iterations) is displayed for two XGBOOST models, including one model that included all measured input variables (red curve; area under the curve [AUC] = 0.84) and another model that was trained with recursive feature elimination and selected RF (rheumatoid factor), IL-12, and IL-17 as the most informative predictors (blue curve; AUC = 0.95). The XGBOOST algorithm outperformed three alternative machine learning algorithms (Table E1). These alternative algorithms selected a few additional candidate predictors of rituximab response (*see* Figure E4), although RF, IL-12, and IL-17 were consistently identified as the top biomarkers. Baseline plasma concentrations of (*B*) RF, (*C*) IL-12, and (*D*) IL-17 are compared between clinical responders (green) and nonresponders (black) stratified across treatment arms. Rituximab-treated patients with a 6MWD increase ⩾33 m at Study Week 24 had lower baseline concentrations of RF ($P < 0.001$), IL-12 ($P = 0.002$), and IL-17 ($P = 0.009$). Figures are box plots superimposed on shaded violin plots, which show the density of data points across the distribution. MFI = mean fluorescence intensity.

desaturation, and pericardial effusions (16); autoantibodies have less prognostic significance (51). In a study comparing patients with limited SSc with or without PAH, peripheral blood mononuclear cell profiles from patients with PAH suggested greater monocyte activation, vascular injury, and inflammation (52). As the ASC01 Study was being designed, the heightened immunity and poor outlook of patients with SSc-PAH were deemed consequential enough to warrant the risks associated with administering a lymphocyte-depleting antibody.

The ASC01 Study Group placed considerable importance on avoiding rituximab-induced hypersensitivity reactions during infusion so that any hemodynamic lability was minimized. With appropriate premedications, no anaphylaxis or significant hypotensive events occurred throughout the study. Rituximab, as an adjuvant approach, was well tolerated by these patients with SSc-PAH who were already being treated with standard-of-care vasodilator therapies. As anticipated, serious infections were more common in the rituximab-treated subjects (Table 2), but overall infection rates were similar in both arms and not beyond what was expected from prior reported data (Section E4). Infections were not associated with an increase in mortality. Three deaths occurred in the rituximab group in the 48-month period of observation but were not judged to be directly related to this therapy. The study team, the NIH, and the DSMB approached this trial with a very critical eye toward safety given the fragile population being studied and the potential toxicities of the study drug. The protocol had carefully specified rules for DSMB monitoring, which were strictly adhered to. There were at least three *ad hoc*

Table 2. Adverse Events

System Organ Class	Rituximab (*N = 29*)		Placebo (*N = 28*)	
	Subject [*n* (%)]	Events [*n*]	Subject [*n* (%)]	Events [*n*]
Adverse events (>5%) by class				
Infections	17 (58.6)	46	23 (82.1)	43
Blood and lymphatic system	20 (69.0)	25	13 (46.4)	17
Musculoskeletal and connective tissue	7 (24.1)	15	10 (35.7)	12
Gastrointestinal	8 (27.6)	12	7 (25.0)	10
Respiratory, thoracic, and mediastinal	6 (20.7)	9	9 (32.1)	13
Metabolism and nutrition	5 (17.2)	7	7 (25.0)	8
Nervous system	5 (17.2)	8	3 (10.7)	5
Investigations	6 (20.7)	6	9 (32.1)	10
Serious adverse events by class				
Any serious adverse event	14 (48.3)	22	9 (32.1)	14
Infections and infestations	7 (24.1)	10	2 (7.1)	3
Cardiac	4 (13.8)	4	3 (10.7)	4
Respiratory, thoracic, and mediastinal	1 (3.4)	2	2 (7.1)	2
Endocrine	0 (0)	0	1 (3.6)	1
Gastrointestinal	0 (0)	0	1 (3.6)	1
Neoplasms benign, malignant, and unspecified	1 (3.4)	1	0 (0)	0
Nervous system	1 (3.4)	1	0 (0)	0
Psychiatric	1 (3.4)	1	0 (0)	0
Renal and urinary	0 (0)	0	1 (3.6)	1
General and administration-site conditions	2 (6.9)	2	0 (0)	0
Grading of infections*				
Grade 2	15 (51.7)	36	22 (78.6)	39
Grade 3	6 (20.7)	6	3 (10.7)	4
Grade 4	3 (10.3)	4	0 (0)	0
Grade 5 (deaths)	0 (0)	0	0 (0)	0

*Adverse event severity was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.0, system. Only grades 2 and higher were collected during the study.

DSMB safety reviews over the life of the trial in addition to the regularly scheduled twice yearly reviews. In each case, the DSMB believed it was appropriate to continue the trial, especially balancing risk to potential benefit in a fatal disease such as SSc-PAH. A larger and more definitive trial can continue to assess the putative risks and benefits of B-cell targeting for SSc-PAH.

The application of unsupervised molecular phenotyping in PAH has significant potential for not only classifying disease (53) but also identifying signatures of response. Perhaps, counterintuitively, the biomarkers of interest were low rather than high in the rituximab-responsive group. Through the application of ML, we found that reduced baseline concentrations of RF, IL-12, and IL-17 identified the patients who subsequently had the best response to treatment, as reflected by an improvement in 6MWD of 33 m (a distance considered to be a minimally important difference) (40). First identified in rheumatoid arthritis, RFs are autoantibodies defined by their specificity to the Fc portion of IgG (54). Some studies have shown that up to 70% of patients with SSc are RF+ (55), and it is possible that the absence of RF may identify a subset of patients with SSc who have more aggressive pulmonary vascular disease, similar to how the absence of the SCL-70 antibody is associated with an increased risk for PAH in this group (18). Interestingly, RFs reduce complement-dependent cytotoxicity by binding to the Fc portion of rituximab (56); in this way, high RF concentrations directly inhibit rituximab function. Similarly, increased concentrations of IL-17 promote rituximab resistance in the treatment of B-cell lymphomas, possibly through the cytokine's suppression of p53 (57). Considered together, low RF and IL-17 concentrations may identify rituximab responsiveness because of the limitations imposed on rituximab action by high RF and IL-17 concentrations. Of potential interest, IL-12 is involved with B-cell and plasmablast differentiation (58), but the ramifications of low cytokine concentrations are less clear. Although this study illustrates the potential of agnostic ML approaches for defining drug-responsive cohorts, future trials are needed to validate the usefulness of this newly identified biomarker panel.

This study posed significant operational challenges and has limitations. Within 3 years of initiating the study, low enrollment mandated downsizing the planned enrollment from 80 to 60 patients. Furthermore, we changed the original primary outcome measure (ΔPVR) to 6MWD when it became clear that these patients with SSc-PAH treated in the contemporary era had unexpectedly low baseline PVR values. Ultimately, the study underenrolled and was insufficiently powered to detect smaller differences in the primary endpoint. Despite this limitation, the trend for our primary endpoint was in a therapeutic direction (a mean placebo-corrected change in 6MWD of 23.1 m favoring the rituximab arm), and a secondary analysis that included all 6MWD data up to Week 48 suggested a potential benefit of rituximab at Week 24. Most study subjects were taking background combination therapy that may also have impacted the magnitude of response seen in the 6MWD. It is possible that the increase in 6MWD does not reflect an improvement in cardiopulmonary status, especially given that there was no demonstrable improvement in resting PVR. Moreover, the results may not be entirely generalizable, as we excluded patients with a PAH diagnosis made more than 5 years previously. One of the corollary lessons of this

trial was that it can be challenging to conduct a study limited to a single subpopulation within group I pulmonary hypertension. Enrolling this trial was difficult for several reasons, including strict inclusion/exclusion criteria, competing industry-driven studies, and a lengthy consent process. However, this stringent accrual approach also yielded a highly circumscribed cohort of patients with PAH with valuable demographic and phenotype information. This study also included relatively fewer sites than industry-sponsored trials, and an argument can be made for the creation of a more effective clinical trial consortium for PAH. Finally, the discovery of putative biomarkers predicting responsiveness to rituximab requires validation in future clinical studies before being considered as a discriminator for trial enrollment (59).

Conclusions

In the PAH field, there is broad recognition that new therapies are needed that can help reverse cardiopulmonary disease. We used a therapy that was potentially effective against the adaptive arm of immunity implicated in the microvascular injury of SSc (36, 37). Although underpowered to definitively conclude benefit from B-cell depletion, an improvement in exercise tolerance was suggested. Rituximab treatment has also benefited the pulmonary function of patients with SSc-associated ILD (27, 60–64), but, similar to the current study, these findings in patients with ILD require validation in a larger randomized trial (28). Given a possible benefit to both SSc-PAH and SSc-associated ILD, rituximab treatment may be a rational option for patients with combined disease, just as it is being broadly considered as a treatment consideration for other SSc conditions (65). Finally, molecular signatures that describe SSc (66) and PAH phenotypes (53) hold great promise for revealing pathogenic mechanisms and guiding therapeutic decisions (67). Future trials should increasingly consider these new biomarkers as an enrichment strategy for optimum clinical trial candidate selection. ■

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