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⊕ A Phase-2 NIH-sponsored Randomized Clinical Trial of Rituximab in Scleroderma-associated Pulmonary Arterial Hypertension Did Not Reach Significance for Its Endpoints End of Story? Not So Fast!

In this issue of the *Journal*, Zamanian and colleagues (pp. 209–221) report the results of a randomized placebo-controlled clinical trial (RCT) of rituximab in scleroderma-associated pulmonary arterial hypertension (SSC-PAH), a common form of PAH (1). The protocol excluded patients with significant interstitial lung disease, and all patients were on at least two PAH therapies, including epoprostenol. This NIH-sponsored trial showed acceptable drug safety and tolerability but failed to reach significance for the primary (6-min-walk distance [6MWD] change from baseline at 24 weeks) and secondary endpoints, which included a change in pulmonary vascular resistance. However, a closer look suggests that rituximab may be a promising adjuvant therapy for patients with SSC-PAH, opening important questions for the future.

Most clinicians do not understand how long and complicated an RCT can be. More than 75% of the RCT funding comes from industry (2), where large teams of employees and consultants design the protocol, which goes through several regulatory stages before it even reaches clinicians at participating centers, a process that can take years. In investigator-driven trials, all the work is completed by the principal investigator and his or her small team. The study received funding from the NIH/National Institute of Allergy and Infectious Diseases and the NIH Autoimmunity Centers of Excellence and, after years of planning, started enrolling in 2010 and closed in 2019. Three years into the trial, only 30 patients had been recruited, forcing the steering committee to change the protocol and decrease the original target sample size from 80 to 60, decreasing the power to only 50% (to detect a change in 6MWD > 33 m), contributing to the failure to detect significance in the endpoints.

There are at least two obvious reasons for this difficulty in recruiting: 1) as a publicly funded trial, it had to compete with industry trials, which typically compensate more, both at the physician (3) and

occasionally the patient level (4), creating a bias against enrolling in trials with less direct (trial cost) and indirect (consulting fees or institutional grants) funding (5); and 2) the trial included two right heart catheterizations that decrease enthusiasm on the patient side, although this is critical and necessary to assess treatment effects on the pulmonary circulation.

All the patients were already on approved PAH therapies compared with the original RCTs that led to the approval of prostacyclin (6), bosentan (7), and sildenafil (8), in which the drug was compared with placebo. In those trials, the placebo-corrected 6MWD was 47, 44, and 45 m, respectively. In this trial, this was 25.1 m. There was, however, variability in the group responses, with 38% of patients on rituximab having a >50-m increase in 6MWD compared with 15% on placebo.

In a *post hoc* analysis, the authors used machine learning and found that of the many serum biomarkers they tested, a panel of cytokines (low levels of RF [rheumatoid factor], IL2, and IL17) predicted response to rituximab. Because this is a publicly funded trial, the raw data are deposited in www.import.org and open to anyone for analysis, in contrast to industry trials, for which data are typically not publicly available. We asked the authors to provide us the endpoints for the subsets of patients that did and did not have this biomarker profile, now shown in Figure 1A. In patients that had the biomarker, the placebo-corrected 6MWD (24 wk) was +101 m, whereas the placebo-corrected Δ pulmonary vascular resistance was -2.56 Wood units, suggesting a significant improvement. Through a precision medicine (PM) lens, one would conclude that, in fact, rituximab may be a very effective therapy for a subset of patients with SSC-PAH. The trial design, however, prohibits such an analysis because these subgroups were not prospectively defined and thus are suffering from potential bias. There is one exception to that rule: when the biomarker is genetically driven (i.e., allocated in the drug–placebo group patients at the time of conception by Mendelian distribution) because this is immune to selection biases. In fact, some cancer drugs have been approved for patient subgroups based on *post hoc* analysis of genetic biomarkers, even though they were not prospectively assigned as “potential drug-response” predictors (9, 10).

The response to rituximab in patients with the biomarker is too robust to ignore. Can the responder–nonresponder separation have a

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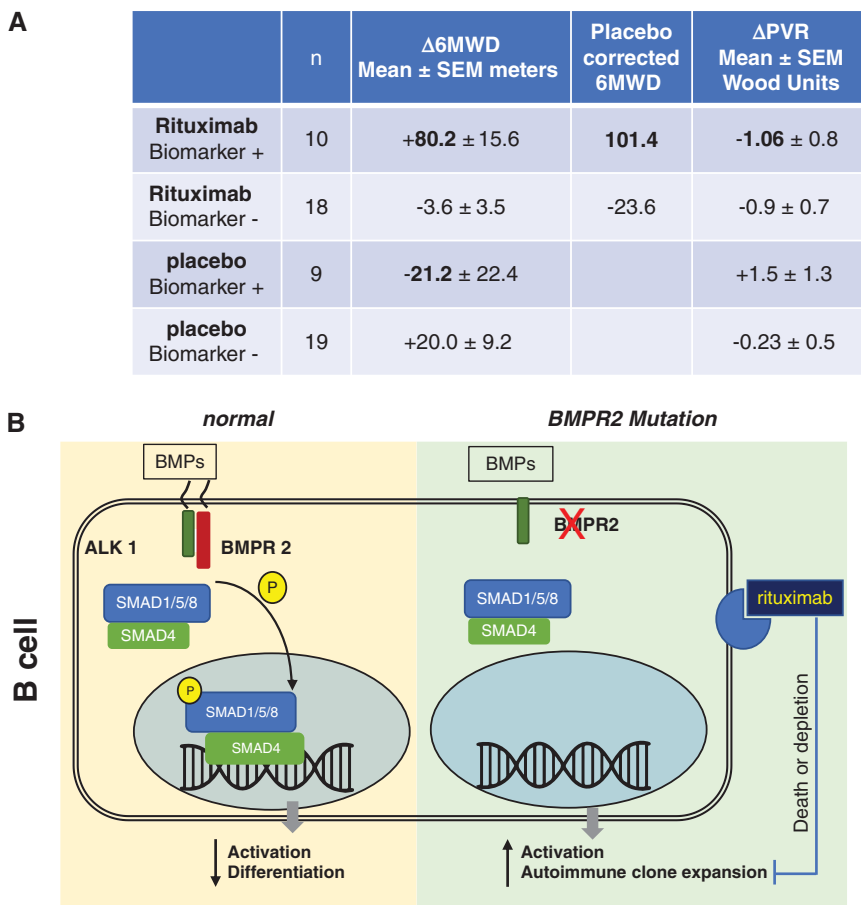


Figure 1. (A) Data sent to us by the authors show that patients with the proposed biomarker (low levels of RF, IL2, and IL17) have a remarkable improvement in both the 6MWD and PVR at 24 weeks in response to rituximab, whereas patients with the biomarker on placebo had a significant worsening in both the 6MWD and PVR. (B) A hypothetical schematic on how some patients with SSC-PAH can have an increased proportion of activated autoimmune B-cell lineages because of genetic mutations in BMP signaling (or perhaps other pathways) and how these lineages can be depleted by rituximab. 6MWD = 6-minute-walk distance; BMP = bone morphogenetic protein; PVR = pulmonary vascular resistance; RF = rheumatoid factor; SSC-PAH = scleroderma-associated pulmonary arterial hypertension.

genetic basis? The authors could explore the patient genomic profiles to test this. Several genes have been associated with scleroderma, some of them being important for B-cell activation, such as BANK1 (B cell scaffold protein with ankyrin repeats 1) or BLK (B-lymphoid tyrosine kinase), and several specifically linked to SSC-PAH (IL23R [IL23 receptor], KCNA5 (potassium voltage-gated channel subfamily A member 5), TLR2 [Toll-like receptor 2], TNFAIP3 [TNF α -induced protein 3], and UPAR [urokinase-type plasminogen activator receptor]) (11).

One of the most studied genetic factors in PAH is loss-of-function germline mutations in BMPR2 (bone morphogenetic protein receptor 2), promoting the characteristic PAH proliferative vascular remodeling (12). Although this specific mutation was not detected in a small study of 24 patients with SSC-PAH (13), it is not known whether other mutations affecting the whole BMP axis are present in SSC-PAH. BMPR2 signaling activated SMAD1/5/8 translocase into the nucleus to regulate gene transcription. Loss of that signal in vascular cells promotes proliferative vascular remodeling. However, BMPR2 signaling is also important in immune cells (12). In B cells, it inhibits immunoglobulin production, differentiation, and

proliferation. Therefore, BMPR2 deficiency may facilitate the emergence of activated autoimmune B-cell clones in SSC-PAH (Figure 1B). B cells not only respond to but also produce many cytokines, and they can both promote and regulate or suppress inflammation (14). Immune cells play a critical role in PAH, and although a lot is known about T cells, little is known about B cells (15). Rituximab, initially approved for B-cell lymphoma (1997) and rheumatoid arthritis (2006) but also used off-label in other autoimmune diseases, is a monoclonal antibody that binds the B cell CD20 surface marker, promoting complement and antibody-dependent cytotoxicity and apoptosis (16).

Can autoimmune clones be suppressed by B-cell depletion therapy? In a different study, B cells from patients in this trial were compared with healthy control subjects using immunoglobulin heavy chain sequencing (17). SSC-PAH B cells had expanded lineages of high antibody-secreting cells and altered variability, diversity, and joining rearrangement frequencies and somatic mutation-fixation probability, suggesting expanded autoimmune clonal selection; they also had increased proportions of immunoglobulin D-positive and high antibody-secreting cells. These anomalies in B-cell repertoire homeostasis were reversed by

rituximab, an effect diminished within a few months after injection. Thus, rituximab may (reversibly) remove autoimmune clones of B cells, attacking the disease-causing inflammation at its core (Figure 1B). That study also showed significant variability in B-cell homeostasis among patients. Is it possible that the proportion of autoimmune B-cell clones varies among patients with SSC-PAH, perhaps driven by germline mutations affecting the BMP2 or other signaling pathways? Could this also apply to other autoimmune diseases? For known mutations, this is easily tested by a simple PCR in any biological sample because germline mutations are present in all cells.

This study, one of the biggest cohorts of well-phenotyped patients with SSC-PAH, describes the presence of immunology biomarker-defined drug-response subgroups that need to be studied beyond SSC-PAH in idiopathic PAH or other autoimmune diseases (14).

The lessons learned are as follows: 1) Future publicly funded trials should consider adaptive design protocols to address challenges with enrollment, which allow a predetermined sample size recalculation well into the trial without compromising power (18). 2) Machine learning should be used more frequently in the analysis of biomarkers, facilitating the discovery of unexpected or counterintuitive profiles of response predictors. The biomarkers should include a genomics component to determine whether they have a genetic basis. 3) Publicly funded trials should pursue more PM designs that may lead to an approval of a drug for a biomarker-defined subgroup (18). The industry may be more reluctant to pursue such trials because approving a drug for a subgroup of a relatively uncommon disease significantly narrows the profit margin. 4) Physicians need to support enrollment in such trials, and journal editors need to support their publication, even if negative. The bias created by the tendency to not publish negative trials by the industry is well known (2, 5).

In summary, this “negative trial” offers many important lessons and opens intriguing questions for future research. The persistence of the authors and the support from the NIH is inspiring at a time when more publicly funded trials are needed to advance PM. ■

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Yongneng Zhang, M.D.
Evangelos D. Michelakis, M.D.
Department of Medicine
University of Alberta
Edmonton, Alberta, Canada

ORCID IDs: 0000-0002-0791-9381 (Y.Z.);
0000-0003-0787-7721 (E.D.M.).

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