


# Safety and Biological Activity of Rozibafusp alfa, a Bispecific Inhibitor of Inducible Costimulator Ligand and B Cell Activating Factor, in Patients With Rheumatoid Arthritis: Results of a Phase 1b, Randomized, Double-Blind, Placebo-Controlled, Multiple Ascending Dose Study

Lubna Abuqayyas,<sup>1</sup>  Laurence E. Cheng,<sup>2</sup> Marcia Teixeira dos Santos,<sup>1</sup> Barbara A. Sullivan,<sup>2</sup> Norma Ruiz-Santiago,<sup>1</sup> Hui Wang,<sup>1</sup> Yanchen Zhou,<sup>2</sup> Vishala Chindalore,<sup>3</sup> Stanley Cohen,<sup>4</sup> Alan J. Kivitz,<sup>5</sup> Maximilian G. Posch,<sup>6</sup> and Jane R. Parnes<sup>1</sup>

**Objective.** To assess the safety and biological activity of rozibafusp alfa, a first-in-class bispecific antibody–peptide conjugate targeting inducible costimulator ligand (ICOSL) and B cell activating factor (BAFF), in patients with rheumatoid arthritis (RA).

**Methods.** This phase 1b, double-blind, placebo-controlled, multiple ascending dose study included 34 patients (18–75 years; 82.4% female) with active RA (Disease Activity Score of 28 joints–C-reactive protein [DAS28–CRP] >2.6, on stable methotrexate) randomized 3:1 to receive rozibafusp alfa (n = 26, in four ascending dose cohorts of 70, 140, 210, and 420 mg) or a placebo (n = 8) subcutaneously once every 2 weeks for 10 weeks (six total doses), with 24 weeks of follow-up. The primary end point was the incidence of treatment-emergent adverse events (TEAEs). Additional assessments included serum pharmacokinetics (PK), pharmacodynamics (PD), immunogenicity, and RA disease activity measures (DAS28–CRP, Patient Global Assessment of Disease, and Physician Global Assessment of Disease).

**Results.** TEAEs occurred in 96.2% and 87.5% of patients receiving rozibafusp alfa and the placebo, respectively; most were mild or moderate in severity. Two (7.7%) patients treated with rozibafusp alfa reported serious TEAEs; none were considered treatment related. Multiple doses of rozibafusp alfa showed nonlinear PK (mean  $t_{1/2}$  = 4.6–9.5 days) and dose-related, reversible PD (>90% ICOSL receptor occupancy in 210- and 420-mg cohorts; reduction in naïve B cells and increase in memory B cells in all cohorts). Five (20%) patients developed anti-rozibafusp alfa antibodies, with no apparent impact on safety. RA disease activity showed greater numerical improvement from baseline with rozibafusp alfa versus the placebo in the 210- and 420-mg cohorts.

**Conclusion.** Multiple ascending doses of rozibafusp alfa were well tolerated, with PK and PD reflecting dual ICOSL and BAFF blockade. Findings support further clinical evaluation of rozibafusp alfa in autoimmune disease.

## INTRODUCTION

Sustained dysregulated interactions between T and B cells disrupt the balance between immune activation and inhibition, resulting in the production of autoantibodies, proinflammatory

cytokines, and tissue damage associated with autoimmune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (1,2). Two key mediators implicated in altered T and B cell activity are inducible costimulator ligand (ICOSL) and B cell activating factor (BAFF) (3–6).

This phase 1b study was funded by Amgen Inc.

<sup>1</sup>Lubna Abuqayyas, PhD, Marcia Teixeira dos Santos, PhD, Norma Ruiz-Santiago, MD, Hui Wang, PhD (current address: Ultragenyx Pharmaceuticals, Novato, California), Jane R. Parnes, MD: Amgen Inc., Thousand Oaks, California; <sup>2</sup>Laurence E. Cheng, MD, PhD (current address: RAPT Therapeutics, South San Francisco, California), Barbara A. Sullivan, PhD (current address: Ultragenyx Pharmaceuticals, Novato, California), Yanchen Zhou, PhD: Amgen Inc., South San Francisco, California; <sup>3</sup>Vishala Chindalore, MD: Pinnacle Research Group, Anniston, Alabama; <sup>4</sup>Stanley Cohen, MD: Metroplex Clinical Research Center, Dallas, Texas; <sup>5</sup>Alan J. Kivitz, MD: Altoona Center for Clinical Research, Duncansville, Pennsylvania; <sup>6</sup>Maximilian G. Posch, MD: Charité Research Organisation GmbH, Berlin, Germany.

Qualified researchers may request data from Amgen clinical studies. Complete details are available at <https://wwwext.amgen.com/science/clinical-trials/clinical-data-transparency-practices/>.

Author disclosures are available at <https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Facr2.11487&file=acr211487-sup-0001-Disclosureform.pdf>.

Address correspondence to Lubna Abuqayyas, PhD, Amgen Inc., 360 Binney Street, Cambridge, MA 02141. Email: [lubnaa@amgen.com](mailto:lubnaa@amgen.com).

Submitted for publication March 16, 2022; accepted in revised form June 20, 2022.

BAFF is critical for B cell maturation and survival (7), and BAFF expression levels are associated with autoimmune manifestations in transgenic mice (8) and disease activity in patients with SLE (9). Several BAFF-targeted drugs have been developed and investigated in clinical studies, such as belimumab (10), blisibimod (11), tabalumab (12), and atacicept (13). Of these, belimumab is the only approved therapy and is indicated for the treatment of SLE and lupus nephritis (14), with clinical efficacy observed in RA in a phase 2 study (15,16).

The inducible costimulator (ICOS)/ICOSL costimulatory pathway is important for T cell activation, migration, differentiation, and antibody production via interactions between follicular helper T cells and B cells (17). T cells in patients with SLE and RA show elevated ICOS expression (18,19), and in SLE, the magnitude of ICOS expression correlates with anti-double-stranded DNA titers (20). Phase 1 studies of AMG 557, a human immunoglobulin G2 monoclonal antibody that binds to ICOSL and prevents functional interaction with ICOS on activated T cells, showed that multiple doses were safe and well tolerated in patients with SLE (5) and lupus arthritis (21). Furthermore, treatment with AMG 557 led to selective inhibition of the antigen-specific immunoglobulin G (IgG) response in patients with SLE and improvements in measures of disease activity, including tender and swollen joint counts (5,21).

Rozibafusp alfa (formerly AMG 570) is a first-in-class bispecific antibody-peptide conjugate that binds both BAFF and ICOSL and is designed to simultaneously block B cell maturation, T cell activation, and aberrant T cell and B cell communication associated with autoimmune sequelae. The molecule contains two tandem copies of BAFF-binding peptides fused to the C-terminus

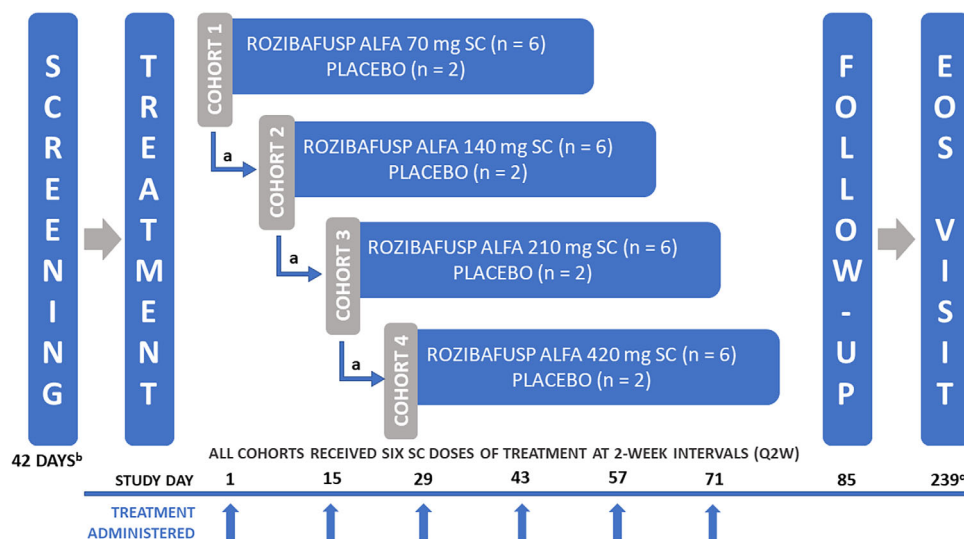
of the anti-ICOSL antibody AMG 557 (6). Dual-target engagement of BAFF and ICOSL with rozibafusp alfa demonstrated superior efficacy compared with that of single BAFF or ICOSL inhibition in murine models of arthritis and lupus, and also demonstrated acceptable safety and tolerability in healthy human participants in phase 1 studies (6,22,23). Rozibafusp alfa is currently in phase 2 clinical development for the treatment of SLE (NCT04058028).

This report presents the results of a phase 1b, multiple ascending dose clinical study investigating the effect of rozibafusp alfa on safety, pharmacokinetics (PK), pharmacodynamics (PD), immunogenicity, and disease control in patients with RA.

## PATIENTS AND METHODS

**Study design and participants.** This was a phase 1b, placebo-controlled, double-blind, multiple ascending dose study (NCT03156023) conducted at four treatment centers (three in the United States and one in Germany) between August 2017 and October 2019. The study comprised four ascending dose cohorts, each including approximately eight patients with RA, to evaluate rozibafusp alfa doses of 70 mg, 140 mg, 210 mg, and 420 mg. For each dose cohort, patients were randomized (3:1) to receive either rozibafusp alfa or a placebo subcutaneously (SC) once every 2 weeks (Q2W) for 10 weeks for a total of six doses (Figure 1). Escalation to a higher dose level was considered only if the previous dose regimen demonstrated acceptable tolerability in at least six participants who completed the day 29 visit.

Eligible patients were 18 to 75 years old (inclusive) with a diagnosis of active RA based on the 2010 American College of Rheumatology (ACR) or European League Against Rheumatism



**Figure 1.** Design of the phase 1b multiple ascending dose study. <sup>a</sup>Cohorts 2, 3, and 4 were initiated only after six participants in cohorts 1, 2, and 3, respectively, had completed their day 29 visit, demonstrating that the preceding dose level was safe and well tolerated. <sup>b</sup>Patients were maintained on 5 to 25 mg of methotrexate weekly through the duration of screening and treatment. <sup>c</sup>If patients had abnormal B cell counts at the EOS assessment on day 239, they were followed up every 3 months for up to 12 months after EOS. EOS, end of study; Q2W, once every 2 weeks; SC, subcutaneous.

(EULAR) classification criteria (disease duration  $\geq 6$  months); were on a stable methotrexate (MTX) dose of 5 to 25 mg weekly with folic acid supplementation ( $\geq 4$  weeks before treatment and throughout the study duration); had a Disease Activity Score of 28 joints–C-reactive protein (DAS28-CRP) greater than 2.6; and had a body mass index (BMI) of 18 to 35 (inclusive). Limited use of oral corticosteroids ( $\leq 10$  mg/day of prednisone or equivalent), nonsteroidal anti-inflammatory drugs, hydroxychloroquine ( $\leq 400$  mg/day), or sulfasalazine ( $\leq 3000$  mg/day) in combination with MTX was permitted if doses remained stable for 4 or more weeks before treatment and throughout the study duration. Participants were required to have their immunizations up to date based on recommendations for immunizations by the Centers for Disease Control and Prevention or by equivalent governmental agencies for sites outside the United States. Patients were excluded if they had been diagnosed with clinically significant disorders that could pose a risk to patient safety or interfere with the study or interpretation of study findings. Prohibited medications included prior use of rituximab; any commercial parenteral biologic or oral synthetic disease-modifying antirheumatic drugs (DMARDs) in the 3 months before enrollment; cyclophosphamide, cyclosporine, mycophenolic acid, or gold within 6 months before day  $-1$ ; and treatment with greater than or equal to 80 mg of a prednisolone equivalent for acute RA flare within 4 weeks before randomization. Patients who were on any other experimental or investigational biologic DMARD that impacts the immune system for less than or equal to 6 months prior to enrollment were excluded from the trial.

Written informed consent was obtained for each patient. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice guidelines and was formally approved by the appropriate institutional review board, ethical review committee, or equivalent at each study site.

**Study end points.** The primary study end point was the patient incidence of treatment-emergent adverse events (TEAEs) and changes from baseline in vital signs, laboratory safety test results, and electrocardiograms (ECG). All TEAEs and serious TEAEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.0, and recorded events were graded for severity using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The secondary end point was characterization of the serum PK of roziabafusp alfa after SC administration of multiple doses. Serum drug concentrations were measured by an immunoassay on the Gyrolab xP Workstation equipped with Gyrolab Control version 7.2 software (Gyros Protein Technologies AB), and PK parameters were calculated using noncompartmental analysis on the Phoenix WinNonlin version 6.4 software (Certara). Assessed PK parameters included the maximum observed concentration ( $C_{max}$ ), the time taken to reach  $C_{max}$ , half-life, and the accumulation ratio.

Exploratory end points included PD, immunogenicity, and effects on RA disease activity. An immunophenotyping assay

was used to measure ICOSL mean fluorescence intensity (MFI). Baseline levels and postdose levels of ICOSL MFI on peripheral blood B cells were used to calculate ICOSL receptor occupancy (RO). Baseline levels, postdose levels, and changes from baseline in absolute count and percentage were calculated for peripheral blood lymphocytes and monocytes, such as total B cells and B cell subsets, including naïve B cells (CD19+IgD+CD27 $-$ ) and switched memory B cells (CD19+IgD $-$ CD27 $+$ ). Formation of anti-drug antibodies (ADAs) against roziabafusp alfa was assessed using a validated electrochemiluminescence-based bridging immunoassay. The incidence of ADAs was summarized, and the potential impact of ADAs on safety, PK, and PD was investigated. Scores and the changes in scores from baseline in DAS28-CRP, Patient Global Assessment of Disease (PtGA), and Physician Global Assessment of Disease (PhGA) were assessed using summary statistics at all evaluable time points.

**Statistical analysis.** No formal sample size calculation was performed because the primary objective of the study was to assess the preliminary safety and biological activity of roziabafusp alfa. Instead, the planned total sample size of  $\sim 32$  patients with active RA, with eight patients enrolled per cohort (six active, two placebo), was based on practical considerations typical for this type of study.

Descriptive statistics (means, medians, standard deviations [SDs], first quartile [Q1], third quartile [Q3], and ranges) were used to define continuous measurements (patient disposition, demographics, safety, PK, PD, and biomarker data). Categorical data were summarized using frequency counts and percentages. Data were summarized by treatment and by scheduled time point and were graphically presented, when appropriate. For comparison of the changes from baseline in continuous variables, only patients with both baseline and one or more postbaseline assessments were included. All assessments (including the unscheduled ones) were used to evaluate the minimum and maximum changes. All statistical analyses were conducted using sponsor-supported versions of the Statistical Analysis Software (SAS) system version 9.4 or later (SAS Institute Inc.).

Missing TEAE dates and concomitant medication dates were imputed on the basis of a previously established statistical analysis plan (available on request). Laboratory measurements and biomarker data below quantification limits were counted at the lower limit and at half of the lower limit, respectively. Concentrations that were below the quantification limits were set to zero before data analysis.

## RESULTS

**Patient disposition and baseline demographics.** A total of 34 patients were enrolled (82.4% female; mean [SD] age, 59.0 [9.2] years; mean [SD] BMI, 28.37 [3.94]), of whom 26 received roziabafusp alfa and eight received a placebo. Baseline characteristics, including use of concomitant medications, are summarized in Table 1. Six (17.6%) patients discontinued the study: five (14.7%) patients withdrew consent, and one (2.9%)

**Table 1.** Patient demographics at baseline and concomitant medication use

Characteristics	Placebo (N = 8)	Rozibafusp alfa (N = 26)
Age, mean (SD), years	55.1 (8.6)	60.2 (9.2)
Sex, female, n (%)	6 (75.0)	22 (84.6)
BMI, mean (SD)	27.2 (3.6)	28.8 (4.0)
DAS28-CRP, mean (SD)	4.6 (1.5)	4.9 (1.4)
PtGA, mean (SD)	43.9 (23.3)	40.7 (26.7)
PhGA, mean (SD)	36.4 (18.6)	37.6 (20.3)
Rheumatoid factor, mean (SD), IU/ml	168.9 (267.2)	48.3 (60.6)
Anticitrullinated protein, mean (SD), AU/ml	135.3 (137.0)	123.9 (113.9)
Race, n (%)		
Black	1 (12.5)	1 (3.8)
White	7 (87.5)	25 (96.2)
Ethnicity, n (%)		
Hispanic/Latino	0 (0.0)	2 (7.7)
Not Hispanic/Latino	8 (100.0)	24 (92.3)
Concomitant medication use, <sup>a</sup> n (%)	8 (100.0)	26 (100.0)
DMARDs		
Methotrexate	8 (100.0)	26 (100.0)
Sulfasalazine	2 (25.0)	2 (7.7)
Hydroxychloroquine	1 (12.5)	1 (3.8)
OCS		
Prednisone	5 (62.5)	6 (23.1)

Note: N = the number of participants allocated to either treatment group with on-study results; n = the number of participants with the characteristic of interest. All enrolled patients had a diagnosis of active rheumatoid arthritis with a disease duration of  $\geq 6$  months. Abbreviations: AU, arbitrary unit; BMI, body mass index; DAS28-CRP, Disease Activity Score of 28 joints-C-reactive protein; DMARD, disease-modifying antirheumatic drug; OCS, oral corticosteroids; PhGA, Physician Global Assessment of Disease; PtGA, Patient Global Assessment of Disease; SD, standard deviation.

<sup>a</sup>Medications included in the study inclusion criteria and used by  $\geq 10\%$  of patients at baseline and on-study in either treatment group.

patient was lost to follow-up. Of the five patients who withdrew consent, four were administered rozibafusp alfa (two in the 140-mg dose cohort and one each in the 210- and 420-mg dose cohorts), and one was administered a placebo.

**Safety.** All 34 patients in the study were included in the safety analysis. Twenty-five (96.2%) patients in the rozibafusp alfa group and seven (87.5%) patients in the placebo group reported at least one TEAE (Table 2). Most TEAEs were mild or moderate

**Table 2.** Summary of TEAEs

TEAE classification	Placebo all (N = 8)	Rozibafusp alfa				Total (N = 26)
		70 mg (N = 7)	140 mg (N = 6)	210 mg (N = 6)	420 mg (N = 7)	
Any TEAEs, n (%)	7 (87.5)	6 (85.7)	6 (100.0)	6 (100.0)	7 (100.0)	25 (96.2)
Grade $\geq 2$ , n (%)	4 (50.0)	5 (71.4)	3 (50.0)	4 (66.7)	6 (85.7)	18 (69.2)
Grade $\geq 3$ , n (%)	0 (0.0)	1 (14.3)	1 (16.7)	0 (0.0)	0 (0.0)	2 (7.7)
TEAEs occurring in $\geq 3$ patients, n (%)						
Upper respiratory tract infection	0 (0.0)	1 (14.3)	2 (33.3)	2 (33.3)	1 (14.3)	6 (23.1)
Headache	1 (12.5)	1 (14.3)	0 (0.0)	2 (33.3)	2 (28.6)	5 (19.2)
Cough	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (28.6)	4 (15.4)
Arthralgia	0 (0.0)	1 (14.3)	1 (16.7)	1 (16.7)	0 (0.0)	3 (11.5)
Back pain	2 (25.0)	1 (14.3)	0 (0.0)	1 (16.7)	1 (14.3)	3 (11.5)
Bronchitis	0 (0.0)	1 (14.3)	0 (0.0)	2 (33.3)	0 (0.0)	3 (11.5)
Dermatitis contact	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	1 (14.3)	3 (11.5)
Nasopharyngitis	3 (37.5)	0 (0.0)	0 (0.0)	1 (16.7)	2 (28.6)	3 (11.5)
Seasonal allergy	0 (0.0)	2 (28.6)	1 (16.7)	0 (0.0)	0 (0.0)	3 (11.5)
Serious TEAEs, n (%)	0 (0.0)	1 (14.3)	1 (16.7)	0 (0.0)	0 (0.0)	2 (7.7)
Infections <sup>a</sup>	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	1 (3.8)
Neoplasms <sup>b</sup>	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)

Note: N = the number of participants allocated to either treatment group with on-study results; n = the number of participants with the characteristic of interest. Severity of each TEAE was graded using CTCAE version 4.0. All TEAEs and serious TEAEs were coded using MedDRA version 22.0 and sorted by system organ class and preferred term.

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event.

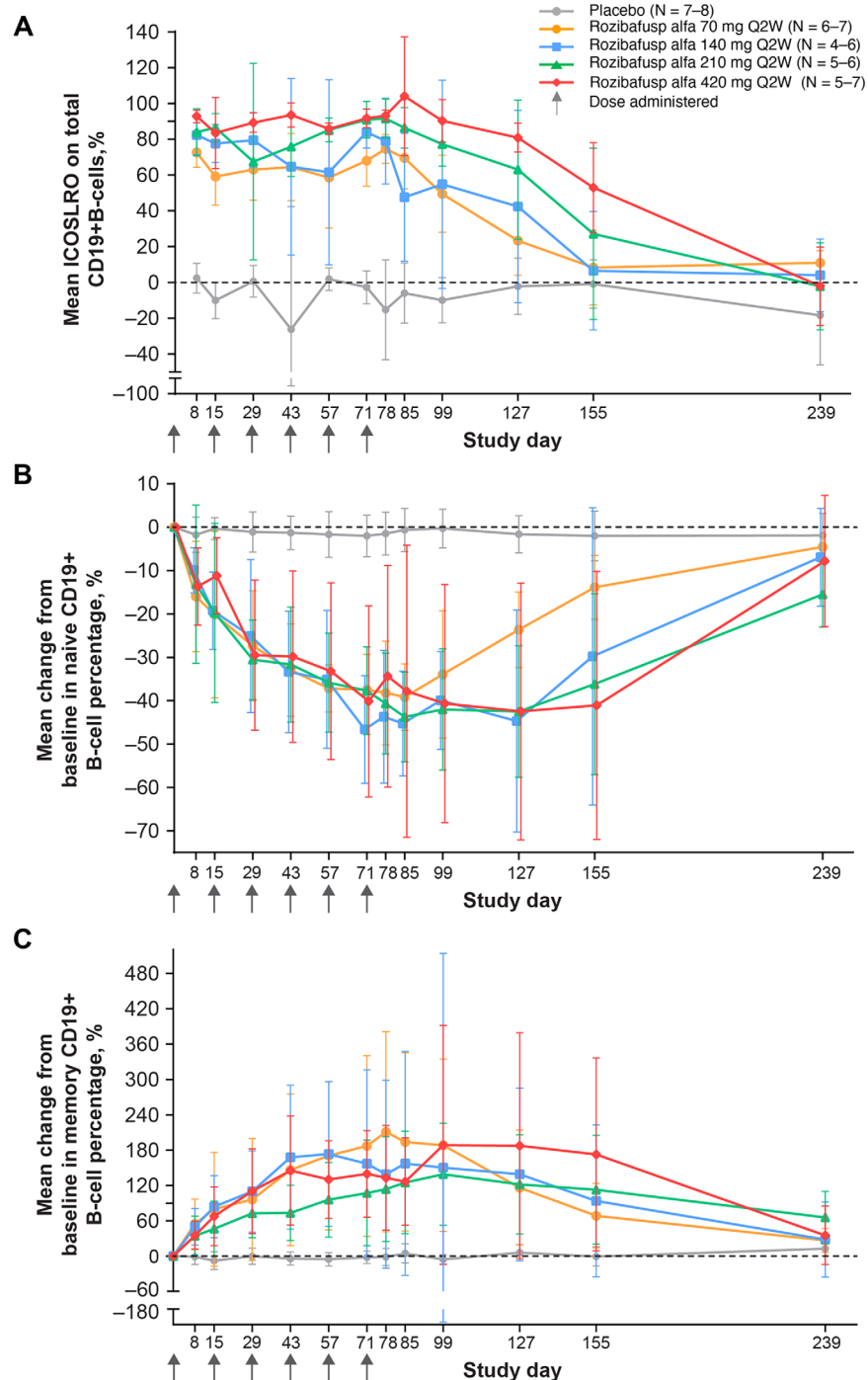
<sup>a</sup>A single patient presented with pneumonia and sepsis.

<sup>b</sup>Invasive ductal breast carcinoma.

(CTCAE grade 1 or 2) in severity. There were no fatal or life-threatening TEAEs, and none led to treatment discontinuation in either treatment group. The most commonly reported TEAEs for rozibafusp alfa versus the placebo, respectively, were upper respiratory tract infection (23.1% vs 0.0%), headache (19.2% vs 12.5%), and cough (15.4% vs 0.0%). Two (7.7%) patients who received rozibafusp alfa reported serious TEAEs (pneumonia and sepsis in one patient in the 140-mg

dose cohort and invasive ductal breast carcinoma in one patient in the 70-mg dose cohort); neither case was considered related to the study treatment by the investigator(s). No significant changes were observed in clinical chemistry, hematology, urinalysis, vital signs, or ECG results (data not shown).

A total of 12 patients who received rozibafusp alfa had total CD19+ B cell counts of less than 107 cells/ $\mu$ L (the kit



**Figure 2.** Pharmacodynamic effects of rozibafusp alfa on B cells. Data show the mean and 95% confidence interval. ICOSL, inducible costimulator ligand; Q2W, once every 2 weeks; RO, receptor occupancy. **A**, change in percentage ICOSL RO on total B cells. **B**, change from baseline in naïve B cell percentage. **C**, change from baseline in memory B cell percentage.



manufacturer's lower limit of normal) on day 239 (end of study) and were followed up on a quarterly basis as part of safety monitoring after the end of the study. B cell counts normalized for seven patients and remained low for one patient, and four patients were lost to follow-up within the 1 year of designated follow-up.

**Pharmacokinetics.** Rozibafusp alfa demonstrated a greater than dose-proportional increase in exposure over the dose range of 70 to 420 mg. However, an approximately dose-proportional increase in area under the concentration–time curve extrapolated to infinity ( $AUC_{inf}$ ) over the dose range of 140 to 420 mg was observed. Median time to maximum serum concentration was 2.5 to 6.0 days after SC dosing. Mean terminal elimination half-life ranged from 4.6 to 9.5 days, with longer half-lives observed with higher doses. Functional half-life at steady state ranged from 20 to 43 days for the 210- to 420-mg Q2W doses. Upon Q2W dosing, the mean accumulation ratios ranged from 3.00 to 4.06 for  $C_{max}$  and from 2.94 to 4.41 for  $AUC_{inf}$ .

**Pharmacodynamics.** ICOSL RO on circulating B cells was dose related and reversible. Mean ICOSL RO values were greater than 70% for all rozibafusp alfa dosing groups on day 8. On multiple dosing, greater than or equal to 90% RO was observed in the 210- and 420-mg dose cohorts, which was maintained up to day 99 for the 420-mg cohort (Figure 2A). Similar trends were observed for ICOSL RO on memory B cells and monocytes (data not shown). Treatment with rozibafusp alfa reduced the percentage of naïve B cells (Figure 2B) and increased the percentage of memory B cells in all cohorts (Figure 2C); the effects were reversed after treatment cessation and returned to near-baseline levels by the end of the study in the majority of patients. Naïve and memory B cells remained steady at or near baseline levels in placebo-treated patients for the entire study duration.

**Immunogenicity.** Five (20%) patients developed anti-rozibafusp alfa binding antibodies after treatment with rozibafusp alfa (Table 3). In addition, one (3.8%) patient treated with rozibafusp alfa had pre-existing antibodies at baseline and maintained

low-level positive binding at the end of the study. No clear effects on PK or PD were observed in five of these six ADA-positive patients. One patient in the 70-mg dose cohort who developed binding ADAs during treatment had lower PK parameters ( $C_{max}$  and area under the concentration–time curve for the dosing interval) and reduced ICOSL RO on day 57 compared with other patients in the same dose cohort. The presence of ADAs was not associated with TEAEs (data not shown).

**Effect on RA disease activity.** Exploratory assessment of DAS28-CRP, PtGA, and PhGA scores showed greater numeric improvement from baseline with rozibafusp alfa versus a placebo in the 210- and 420-mg dose cohorts, and this effect on disease activity persisted through day 183 (Figure 3). Maximum change in DAS28-CRP was observed on day 57, with a mean change from baseline of  $-1.6$  in the 420-mg cohort (Figure 3A). The PtGA scores also showed the greatest reduction from baseline in the 420-mg cohort, with a mean change of  $-33.0$  on day 113 (Figure 3B). With a mean change of  $-28.0$ , PhGA scores showed the largest improvement on day 57 in the 210-mg cohort (Figure 3C).

## DISCUSSION

Rozibafusp alfa is a first-in-class antibody–peptide conjugate that combines a BAFF inhibitor with an anti-ICOSL antibody. This phase 1b, placebo-controlled, multiple ascending dose trial was designed to assess the safety, PK, and PD of rozibafusp alfa in patients with RA. The results indicate that multiple doses of rozibafusp alfa were well tolerated, with a greater magnitude of improvement in exploratory efficacy results observed in the highest dose cohorts. The PK/PD analysis demonstrated nonlinear target-mediated disposition consistent with cell surface target interaction and PD activity consistent with dual ICOSL and BAFF neutralization. These findings support and extend the safety, PK, and PD results previously reported from preclinical and first-in-human studies (6,22,23).

All tested doses of rozibafusp alfa were well tolerated by patients with RA in the current study. The majority of reported

**Table 3.** Patient incidence of anti-rozibafusp alfa binding antibodies

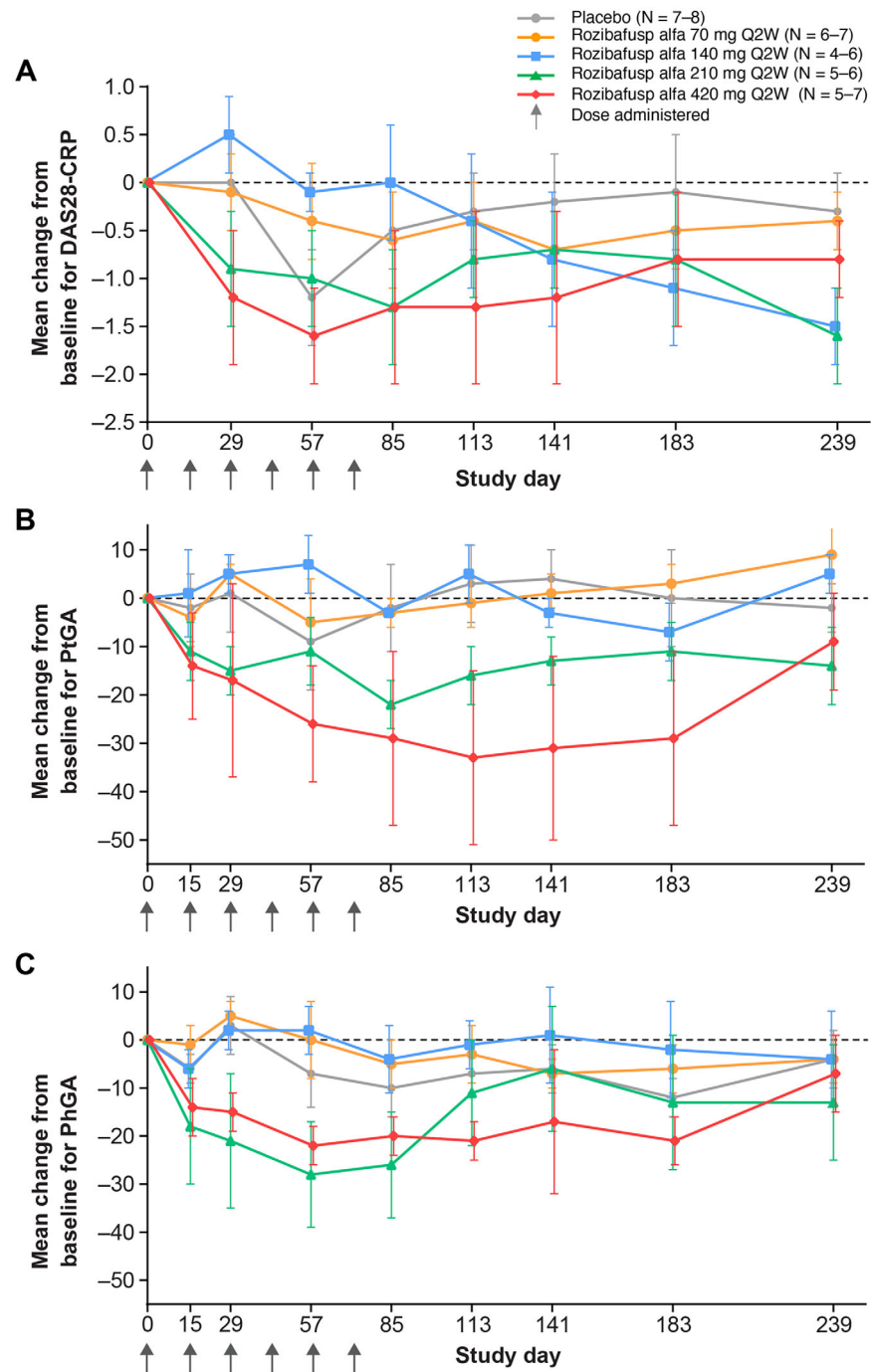
ADA response	Rozibafusp alfa				Total (N = 26)
	70 mg (N = 7)	140 mg (N = 6)	210 mg (N = 6)	420 mg (N = 7)	
Pre-existing ADA, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	1 (3.8)
Developing ADA, n (%)	2 (28.6)	0 (0.0)	3 (50.0)	0 (0.0) <sup>a</sup>	5 (20.0) <sup>b</sup>

*Note:* N = the number of participants allocated to each cohort with on-study results; n = the number of participants with the characteristic of interest.

Abbreviation: ADA, antidrug antibody.

<sup>a</sup>No postbaseline assessment data were available for one patient in the 420-mg dose cohort, so N = 6 was used as the denominator for calculating the ADA incidence.

<sup>b</sup>No postbaseline assessment data were available for one patient in the 420-mg dose cohort, so N = 25 was used as the denominator for calculating the ADA incidence.



**Figure 3.** Change from baseline in rheumatoid arthritis disease activity. Data show the mean and standard deviation. DAS28-CRP, Disease Activity Score of 28 joints–C-reactive protein; PhGA, Physician Global Assessment of Disease; PtGA, Patient Global Assessment of Disease; Q2W, once every 2 weeks. **A**, change from baseline in DAS28-CRP. **B**, change from baseline in PtGA. **C**, change from baseline in PhGA.

TEAEs were mild or moderate (CTCAE grade 1 or 2) in severity, with upper respiratory tract infection being the most commonly reported TEAE in patients who received rozibafusp alfa. There were no treatment-related serious TEAEs, fatalities, or treatment discontinuations in the study. These findings are consistent with the safety profile demonstrated by rozibafusp alfa in healthy study participants (Supplementary Table 1) (22,23), as well as by the

anti-ICOSL monoclonal antibody AMG 557 in patients with SLE (5) and lupus arthritis (21). Although ~35% of patients exhibited low B cell counts at the end of the study assessment on day 239, the effects were not long lasting and resolved within 1 year of treatment cessation in the majority of patients.

Aberrant T and B cell activity resulting in increased autoantibody and cytokine production is a key underlying mechanism for

autoimmune disease pathogenesis (3,24). Despite the strong evidence suggesting the efficacy of therapies targeting T cells and B cells in autoimmune diseases, there are a limited number of approved options (25–27). Abatacept, a selective T cell costimulator inhibitor, and rituximab, a chimeric mouse/human anti-CD20 monoclonal antibody, are the only T cell and B cell–targeted therapies, respectively, approved for the treatment of RA (28–31). With their overexpression in the peripheral blood of patients with autoimmune diseases, both ICOSL and BAFF represent promising therapeutic targets for the treatment of such diseases (4,32). Indeed, the anti-BAFF antibody belimumab has been approved for the treatment of SLE and lupus nephritis since 2011 (10), and until the recent approval of anifrolumab (33), belimumab was the only available biologic agent for the treatment of SLE. In a phase 1b study of patients with SLE and active lupus arthritis, the anti-ICOSL antibody AMG 557 showed safety and potential efficacy, with greater improvements in both Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and British Isles Lupus Assessment Group (BILAG) scores and in tender and swollen joint counts compared with a placebo (21). Thus, clinical findings support the utility of both ICOSL and BAFF as therapeutic targets for autoimmune diseases. As a bispecific antibody–peptide conjugate, rozibafusp alfa is designed to uniquely disrupt aberrant T and B cell activity by simultaneously inhibiting ICOS/ICOSL binding and BAFF-mediated B cell proliferation, respectively, and preclinical studies in murine models of lupus and arthritis showed that dual ICOSL and BAFF inhibition was more efficacious than targeting either ICOSL or BAFF alone (6).

BAFF neutralization with belimumab has been shown to decrease naïve B cell counts and increase memory B cell counts (15,34,35). The reduction in naïve B cell counts and increase in memory B cell counts observed with rozibafusp alfa in this study are consistent with the anti-BAFF functionality of the molecule. All tested doses of rozibafusp alfa in the current study achieved greater than or equal to 40% reduction from baseline in naïve B cell counts; this reduction is approximately consistent with the reduction observed with belimumab in a phase 2 study among patients with RA within 12 weeks of treatment duration at doses of 4 and 10 mg/kg (15).

Assessment of ICOSL inhibition with rozibafusp alfa was evaluated by measuring RO. ICOSL RO was used as a surrogate PD end point on the basis of findings for AMG 557 (an anti-ICOSL antibody) that showed selective inhibition of anti-keyhole limpet hemocyanin IgG responses (5) and a trend for clinical efficacy in lupus arthritis (21) when ICOSL RO saturation was achieved. The current results showed that multiple doses of rozibafusp alfa achieved greater than 90% mean ICOSL RO in the 210- and 420-mg dose cohorts. The higher doses also showed the highest numerical improvement in measures of RA disease activity (DAS28-CRP, PtGA, and PhGA). Together, these findings support further evaluation of the immunologic and clinical impact of dual ICOSL and BAFF blockade with rozibafusp alfa.

Certain limitations of this study should be noted. Similar to other phase 1 clinical trials, this study was primarily aimed at assessing

safety and PK in a relatively small cohort of patients with active RA to help inform the design of dose selection for future clinical investigations. As such, there was a relatively short study duration and small sample population overall and in each treatment group. PD assessments of ICOSL inhibition were limited to measurement of ICOSL RO, and additional biomarker data will be collected in future studies to provide a more functional readout of ICOSL inhibition. In addition, inhibition of BAFF in the context of a bispecific molecule will need to be investigated in the future to evaluate effects on B cell products (eg, immunoglobulin, rheumatoid factor, C-reactive protein, and erythrocyte sedimentation rate). Moreover, analyses were confined to peripheral blood samples instead of relevant central immunologic tissues, such as the spleen and lymph nodes, primarily because of the difficulty associated with the highly invasive nature of tissue collection procedures. Efficacy end points in this study were evaluated on an exploratory basis, and the study was not powered to provide statistics on changes in measures of RA disease activity.

In conclusion, the current findings demonstrate that multiple ascending doses of rozibafusp alfa were well tolerated by patients with active RA, showing nonlinear PK and dose-related and reversible PD indicative of dual-target engagement of ICOSL and BAFF. Moreover, exploratory disease activity findings suggest potential clinical improvement in RA disease control, especially at higher doses of rozibafusp alfa. Together, these results informed the design and dose selection of a phase 2 randomized, placebo-controlled study assessing the safety and efficacy of rozibafusp alfa in patients with active SLE and inadequate responses to standard-of-care therapy.

## ACKNOWLEDGMENTS

The authors acknowledge Cassandra E. Milmont, PhD, for providing biostatistics support and Sandra Cole for providing technical assistance in developing flow cytometry assays.

## 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. All authors had full access to all of the data in the study. Dr. Abuqayyas takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Abuqayyas, Cheng, Teixeira dos Santos, Sullivan, Wang, Zhou, Parnes.

**Acquisition of data.** Chindalore, Cohen, Kivitz, Posch.

**Analysis and interpretation of data.** Abuqayyas, Cheng, Teixeira dos Santos, Sullivan, Ruiz-Santiago, Wang, Zhou, Chindalore, Cohen, Kivitz, Posch, Parnes.

## ROLE OF THE STUDY SPONSOR

Kate Smigiel, PhD (Amgen Inc.), and Meenakshi Mukherjee, PhD (Cactus Life Sciences on behalf of Amgen Inc.), provided medical writing support. Amgen Inc. was involved in the study design and in the collection, analysis, or interpretation of the data. Publication of this article was not contingent upon approval by Amgen Inc.



## REFERENCES

1. Catrina AI, Joshua V, Klareskog L, Malmström V. Mechanisms involved in triggering rheumatoid arthritis [review]. *Immunol Rev* 2016;269:162–74.
2. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet* 2016;388:2023–38.
3. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 2005;435:452–58.
4. Her M, Kim D, Oh M, Jeong H, Choi I. Increased expression of soluble inducible costimulator ligand (ICOSL) in patients with systemic lupus erythematosus. *Lupus* 2009;18:501–7.
5. Sullivan BA, Tsuji W, Kivitz A, Peng J, Arnold GE, Boedigheimer MJ, et al. Inducible T-cell co-stimulator ligand (ICOSL) blockade leads to selective inhibition of anti-KLH IgG responses in subjects with systemic lupus erythematosus. *Lupus Sci Med* 2016;3:e000146.
6. Zhang M, Lee F, Knize A, Jacobsen F, Yu S, Ishida K, et al. Development of an ICOSL and BAFF bispecific inhibitor AMG 570 for systemic lupus erythematosus treatment. *Clin Exp Rheumatol* 2019;37:906–14.
7. Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells [review]. *Nat Rev Immunol* 2002;2:465–75.
8. Mackay F, Woodcock SA, Lawton P, Ambrose C, Baetscher M, Schneider P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 1999;190:1697–710.
9. Petri M, Stohl W, Chatham W, McCune WJ, Chevrier M, Ryel J, et al. Association of plasma B lymphocyte stimulator levels and disease activity in systemic lupus erythematosus. *Arthritis Rheum* 2008;58:2453–59.
10. Navarra SV, Guzmán RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 2011;377:721–31.
11. Merrill JT, Shanahan WR, Scheinberg M, Kalunian KC, Wofsy D, Martin RS. Phase III trial results with blisibimod, a selective inhibitor of B-cell activating factor, in subjects with systemic lupus erythematosus (SLE): results from a randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis* 2018;77:883–89.
12. Smolen JS, Weinblatt ME, van der Heijde D, Rigby WFC, van Vollenhoven R, Bingham CO III, et al. Efficacy and safety of tabalumab, an anti-B-cell-activating factor monoclonal antibody, in patients with rheumatoid arthritis who had an inadequate response to methotrexate therapy: results from a phase III multicentre, randomised, double-blind study. *Ann Rheum Dis* 2015;74:1567–70.
13. van Vollenhoven RF, Kinnman N, Vincent E, Wax S, Bathon J. Atacicept in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase II, randomised, placebo-controlled trial. *Arthritis Rheum* 2011;63:1782–92.
14. Belimumab (BENLYSTA®) prescribing information. Philadelphia: GlaxoSmithKline LLC; 2021.
15. Stohl W, Merrill JT, McKay JD, Lisse JR, Zhong ZJ, Freimuth WW, et al. Efficacy and safety of belimumab in patients with rheumatoid arthritis: a phase II, randomised, double-blind, placebo-controlled, dose-ranging study. *J Rheumatol* 2013;40:579–89.
16. Kaegi C, Steiner UC, Wuest B, Crowley C, Boyman O. Systematic review of safety and efficacy of belimumab in treating immune-mediated disorders [review]. *Allergy* 2021;76:2673–83.
17. Mak TW, Shahinian A, Yoshinaga SK, Wakeham A, Boucher LM, Pintilie M, et al. Costimulation through the inducible costimulator ligand is essential for both T helper and B cell functions in T cell-dependent B cell responses. *Nat Immunol* 2003;4:765–72.
18. Hutloff A, Büchner K, Reiter K, Baelde HJ, Odendahl M, Jacobi A, et al. Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3211–20.
19. Ma J, Zhu C, Ma B, Tian J, Baidoo SE, Mao C, et al. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clin Dev Immunol* 2012;2012:827480.
20. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum* 2010;62:234–44.
21. Cheng LE, Amoura Z, Cheah B, Hiepe F, Sullivan BA, Zhou L, et al. Brief report: a randomized, double-blind, parallel-group, placebo-controlled, multiple-dose study to evaluate AMG 557 in patients with systemic lupus erythematosus and active lupus arthritis. *Arthritis Rheumatol* 2018;70:1071–76.
22. Cheng LE, Hsu H, Kankam M, Siebers N, Stoltz R, Abuqayyas L, et al. Development and first-in-human characterization of an ICOSL and BAFF bispecific inhibitor AMG 570 for SLE treatment [abstract]. *Arthritis Rheumatol* 2018;70 Suppl 10. URL: <https://acrabstracts.org/abstract/development-and-first-in-human-characterization-of-an-icosl-and-baff-bispecific-inhibitor-amg-570-for-sle-treatment/>.
23. Abuqayyas L, Cheng LE, Simiens MA, Dutta S, Houk BE. Phase 1, open-label, randomized, single-dose study to assess safety and pharmacokinetics of subcutaneous rozibafusp alfa in healthy Japanese subjects [abstract]. *Clin Pharmacol Ther* 2021;109 Suppl 1:S53.
24. Iwata S, Tanaka Y. B-cell subsets, signaling and their roles in secretion of autoantibodies. *Lupus* 2016;25:850–56.
25. Dörner T, Giesecke C, Lipsky PE. Mechanisms of B cell autoimmunity in SLE [review]. *Arthritis Res Ther* 2011;13:243.
26. Spicer P, Runkel L. Costimulatory pathway targets for autoimmune and inflammatory conditions: clinical successes, failures, and hope for the future [editorial]. *Expert Opin Investig Drugs* 2019;28:99–106.
27. Wallace DJ, Ginzler EM, Merrill JT, Furie RA, Stohl W, Chatham WW, et al. Safety and efficacy of belimumab plus standard therapy for up to thirteen years in patients with systemic lupus erythematosus. *Arthritis Rheumatol* 2019;71:1125–34.
28. Abatacept (ORENCIA®) prescribing information. New York: Bristol-Myers Squibb Company; 2013.
29. Korhonen R, Moilanen E. Abatacept, a novel CD80/86–CD28 T cell co-stimulation modulator, in the treatment of rheumatoid arthritis. *Basic Clin Pharmacol Toxicol* 2009;104:276–84.
30. Rituximab (RITUXAN®) prescribing information. San Francisco: Genentech; 2021.
31. Korhonen R, Moilanen E. Anti-CD20 antibody rituximab in the treatment of rheumatoid arthritis. *Basic Clin Pharmacol Toxicol* 2010;106:13–21.
32. Stohl W, Metyas S, Tan SM, Cheema GS, Oamar B, Xu D, et al. B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum* 2003;48:3475–86.
33. AstraZeneca. Saphnelo (anifrolumab) approved in the US for moderate to severe systemic lupus erythematosus. 2021. URL: [www.astrazeneca.com/media-centre/press-releases/2021/saphnelo-approved-in-the-us-for-sle.html](http://www.astrazeneca.com/media-centre/press-releases/2021/saphnelo-approved-in-the-us-for-sle.html).
34. Ramsköld D, Parodis I, Lakshmikanth T, Sippl N, Khademi M, Chen Y, et al. B cell alterations during BAFF inhibition with belimumab in SLE. *EBioMedicine* 2019;40:517–27.
35. Jacobi AM, Huang W, Wang T, Freimuth W, Sanz I, Furie R, et al. Effect of long-term belimumab treatment on B cells in systemic lupus erythematosus: extension of a phase II, double-blind, placebo-controlled, dose-ranging study. *Arthritis Rheum* 2010;62:201–10.