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BRIEF REPORT

Post Hoc Biomarker Analyses from a Phase 4, Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial of Repository Corticotropin Injection (Acthar® Gel) for Persistently Active Systemic Lupus Erythematosus

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ABSTRACT

Introduction: We conducted post hoc analyses of biomarker results from a multicenter, randomized, double-blind, placebo-controlled study of repository corticotropin injection (RCI; Acthar® Gel) in patients with persistently active systemic lupus erythematosus (SLE) despite treatment with moderate-dose glucocorticoids.
Methods: Adults with active SLE and moderate to severe rash and/or arthritis were enrolled in the primary study. Patients had active SLE despite treatment with stable glucocorticoids, antimalarials, and nonsteroidal anti-inflammatory drugs and/or immunosuppressants. Patients were randomly assigned to 80 U of RCI or placebo subcutaneously every other day for 4 weeks and then twice weekly through week 24. Blood samples were analyzed for serum cytokines and complement proteins using enzyme-linked immunosorbent or Luminex assays and for circulating leukocytes using flow cytometry. Biomarker levels were reported as percentages of the baseline and were further evaluated in subgroups stratified by baseline SLE Disease Activity Index-2000 (SLEDAI-2K) scores (< 10 vs. ≥ 10), baseline anti-double-stranded DNA levels (< 15 IU/mL vs. ≥ 15 IU/mL), and BILAG-based Combined Lupus Assessment (BICLA) responses at week 20 and 24.
Results: RCI treatment resulted in reduced levels of B cell-activating factor and interleukin-6 cytokines in all subgroups compared with placebo. RCI treatment also resulted in lower levels of $CD19^+$ B cells and $CD19^+IgD^-CD27^-CD95^+$ atypical activated memory B cells than did placebo in the higher baseline disease activity subgroups and in BICLA non-responders. Furthermore, RCI treatment led to greater increases in complement component (C)3 and C4 levels than did placebo in the higher baseline disease activity subgroups and in BICLA responders.
Conclusions: RCI may reduce inflammation through B cell immunomodulation in patients with persistently active SLE, particularly in those with higher disease activity.
Trial registration: ClinicalTrials.gov identifier NCT02953821.

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Key Summary Points

Why carry out this study?

In a 24-week, multicenter, randomized, double-blind, placebo-controlled study of the efficacy and safety of repository corticotropin injection (RCI; Acthar® Gel) in patients with persistently active systemic lupus erythematosus (SLE), treatment with RCI resulted in a larger decrease from baseline in B cell activating factor (BAFF) levels at week 8 than treatment with placebo, suggesting that RCI may have an immunomodulatory effect on B cells.

The goal of these post hoc analyses was to further characterize the immunomodulatory effects of RCI by assessing the cytokine, circulating leukocyte, and complement protein results from the primary study.

We also performed subgroup analyses to determine whether there were any differences in the immunomodulatory responses of patients with different baseline disease severities.

What was learned from this study?

In contrast with placebo, treatment with RCI led to reduced levels of BAFF and interleukin-6 in all patient subgroups (ie, baseline SLE Disease Activity Index-2000 [SLEDAI-2K] scores <10 or ≥10, baseline anti-double-stranded DNA [dsDNA] levels <15 IU/mL or ≥15 IU/mL, and British Isles Lupus Assessment Group-based Combined Lupus Assessment [BICLA] responders and non-responders). Treatment with RCI was also associated with lower levels of total B cells and atypical activated memory B cells than was placebo in patients with baseline SLEDAI-2K scores ≥10 and baseline anti-dsDNA levels ≥15 IU/mL and in BICLA non-responders. Additionally, RCI treatment led to increased levels of complement component (C)3 and C4 in the baseline SLEDAI-2K ≥10 and baseline anti-dsDNA ≥15 IU/mL subgroups and in BICLA responders.

These results suggest that RCI may reduce inflammation through B cell immunomodulation and provide insight into the potential mechanism of RCI for the treatment of persistently active SLE despite use of moderate-dose glucocorticoids.

INTRODUCTION

Systemic lupus erythematosus (SLE), a multisystem disease with heterogeneous manifestations, is characterized by periods of flare and remission [1]. Patients with SLE exhibit multiple immune cell abnormalities [2] and dysregulation of inflammatory cytokines [1]. Leukocyte and cytokine profiles have been evaluated as potential biomarkers of disease exacerbation [3]. Consequently, observed differences in these biomarker levels over time could be important for identifying changes in SLE disease activity and assessments of therapeutic response [1, 4–6].

Repository corticotropin injection (RCI; Acthar® Gel) is approved by the US Food and Drug Administration for use during an SLE exacerbation or as maintenance therapy in select cases of SLE [7]. RCI is a naturally sourced complex mixture of adrenocorticotropic hormone analogs and other pituitary peptides. RCI exhibits anti-inflammatory effects that are both steroid-dependent and -independent by binding to all five melanocortin receptors (MCRs) [7, 8]. Owing to the wide distribution of MCRs on multiple cell types throughout the body, RCI has the potential to affect several biologic pathways that contribute to inflammation in SLE [9–14].

RCI has demonstrated efficacy in patients with persistently active SLE that has not responded to standard-of-care treatments [15–19]. In a 24-week multicenter, randomized, double-blind, placebo-controlled study of RCI in patients with persistently active SLE despite use of moderate-dose glucocorticoids, we observed greater improvements in the 28 Swollen and Tender Joint Count and Cutaneous Lupus Erythematosus Disease Area and Severity Index-(CLASI) Activity scores at week 16 (predefined endpoint) for RCI vs. placebo [19]. In post hoc analyses, treatment with RCI also resulted in more British Isles Lupus Assessment Group British Isles Lupus Assessment Group–based Combined Lupus Assessment (BICLA) responders than placebo, and the effects of RCI were greater than placebo for the SLE Responder Index-4 (SRI-4) for patients with higher levels of baseline disease activity. Patients treated with RCI also showed a larger reduction from baseline in levels of B cell-activating factor (BAFF) at week 8 than patients who received placebo. These results suggested that RCI may have an immunomodulatory effect on B cells, as data indicate that BAFF is a vital survival factor for B cells that impacts B cell receptor activation and growth signals [20].

The objective of the current publication was to further analyze the biomarker results from the primary study and describe the post hoc analyses of the immunomodulatory effects of RCI in SLE.

METHODS

The methodology and procedures for the primary study were described in more detail previously [19, 21].

Ethics and Compliance

The study protocol was approved centrally by the Western Institutional Review Board and by ethics committees and institutional review boards at the individual study sites. Patients provided written informed consent. The study was conducted in agreement the ethical principles outlined in the Declaration of Helsinki and with the requirements for registered clinical trials (ClinicalTrials.gov identifier NCT02953 821).

Patients

The clinical trial enrolled adults aged $\geq$ 18 years with active SLE, defined as $\geq$ 4 of 11 American College of Rheumatology criteria [22] and SLE Disease Activity Index-2000 (SLEDAI-2K) score [23] $\geq$ 6 at screening with a clinical SLEDAI-2K (excluding laboratory results) score $\geq$ 4 at both screening and randomization. Patients were required to have moderate to severe rash and/or arthritis defined as BILAG-2004 scores [24] A or B in the mucocutaneous or musculoskeletal domains at both screening and randomization. Patients had active SLE despite receiving stable glucocorticoid doses (7.5–30 mg daily prednisone equivalent) and were permitted to enroll if they were on stable doses of antimalarials or nonsteroidal anti-inflammatory drugs (NSAIDs) for at least 4 weeks and/or immunosuppressants for at least 8 weeks prior to screening. Patients were excluded if they had severe active lupus nephritis (defined as serum creatinine > 2.5 mg/dl, proteinuria > 1.5 g/g, or required hemodialysis) or active central nervous system lupus within 3 months before screening.

Procedures

Patients were randomly assigned to receive 80 U RCI subcutaneously or placebo every other day for 4 weeks followed by twice-weekly dosing through week 24. Randomization was stratified by study site location (US or outside US) and glucocorticoid dose (prednisone or equivalent of $\leq$ 20 mg/day and $>$ 20 mg/day). Glucocorticoid doses remained stable until week 16; steroid taper was permitted between week 16 and week 24 if clinically indicated. Antimalarial, NSAID, and immunosuppressant doses remained stable throughout the study. Circulating leukocytes were analyzed using flow cytometry, and serum cytokines and complement proteins were analyzed using enzyme-linked immunosorbent or Luminex assays.

Exploratory endpoints included the changes from baseline through week 24 in levels of cytokines (interleukin [IL]-6, IL-10, IL-17, type I interferon-alpha, soluble vascular cell adhesion molecule-1, and BAFF), circulating lymphocytes (cluster of differentiation [CD]19^+ B cells, $CD3^+$ total T cells, and $CD4^+$ total regulatory T cells), and bone turnover markers (N-terminal propeptide of type I collagen and C-terminal crosslinking telopeptide of type I collagen). With the exception of BAFF, no differences were observed for these biomarkers in patients treated with RCI compared to placebo [19]. To further characterize the potential immunomodulatory effects of RCI, we assessed these cytokines, lymphocytes, and bone turnover markers in post hoc analyses of subgroups stratified by baseline disease severity or BICLA response [19].

Post Hoc Analyses

Serum cytokine, circulating leukocyte, and serum complement protein levels were measured at baseline and weeks 8, 16, and 24 and reported as percentages of the baseline level. Subgroup analyses stratified by disease activity and BICLA response were conducted for all biomarkers, and those that showed differential responses between RCI and placebo are reported here, namely BAFF, IL-6, $CD19^+$ B cells, $CD19^+$immunoglobulin (Ig)$D^-CD27^-CD95^+$ atypical activated memory B cells, and complement component (C)3 and C4. Disease activity was stratified on the basis of baseline SLEDAI-2K scores ($<$ 10 vs $\geq$ 10) and baseline anti-double-stranded DNA (dsDNA) levels ($<$ 15 IU/mL vs. $\geq$ 15 IU/mL). The use of SLEDAI-2K $\geq$ 10 and anti-dsDNA $\geq$ 15 IU/mL to indicate higher disease activity is consistent with thresholds used in previous studies [25–27]. BICLA responders were patients with persistent BICLA responses at both week 20 and week 24.

Statistical Analyses

Analyses were performed in the modified intention-to-treat (mITT) population, defined as patients who received $\geq$ 1 dose of study drug and contributed any postbaseline efficacy data. Endpoints were analyzed using analysis of covariance models with the change from baseline as the dependent variable, treatment as the factor, and baseline value of the corresponding endpoint as the covariate and were stratified for location (US and outside the US) and baseline glucocorticoid dose ($\leq$ 20 mg/day and $>$ 20 mg/day). Because the proportion of patients who achieved an SRI-4 response at week 16 (primary endpoint) was not significantly different ($p < 0.05$) between RCI and placebo, all p values presented here are nominal and are for informational purposes only.

RESULTS

Demographics and baseline disease characteristics for patients in each treatment group have been previously described [19, 21]. The mITT population (N = 169; RCI, n = 85; placebo, n = 84) had a mean age of 39.7 years. Patients were predominantly female (91.7%) and located outside of the US (66.9%), and most were of Hispanic or Latino ethnicity (80.5%). Most patients (95.3%) were receiving $\leq$ 20 mg of daily prednisone or equivalent glucocorticoid doses. Baseline mean SLEDAI-2 K scores and anti-dsDNA levels for each treatment group are presented in Table 1.

Table 1 Baseline disease characteristics, mITT[a] population

	RCI (n = 84)	Placebo (n = 85)
SLEDAI-2K total score [scale 0–105], mean (SD)	10.1 (3.1)	9.7 (3.0)
SLEDAI-2K < 10, no. (%)	37 (44.0)	41 (48.2)
SLEDAI-2K ≥ 10, no. (%)	47 (56.0)	44 (51.8)
Anti-dsDNA levels [normal range 0–6.3 IU/mL], mean (SD)	99.9 (215.3)	62.6 (165.2)
Anti-dsDNA < 15, no. (%)	49 (58.3)	55 (64.7)
Anti-dsDNA ≥ 15, no. (%)	35 (41.7)	30 (35.3)

[a] Patients who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data

Anti-dsDNA anti-double-stranded DNA, *mITT* modified intention-to-treat, *RCI* repository corticotropin injection, *SD* standard deviation, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

Biomarker Percentage of Baseline Levels

BAFF

RCI treatment resulted in a reduction in BAFF levels at all time points compared with placebo regardless of baseline disease activity or BICLA response, with the exception of week 24 in BICLA non-responders (Fig. 1). Differences between RCI and placebo were nominally significant at week 8 in the low-disease-activity subgroups (Figs. 1A, B) and at weeks 8, 16, and 24 in BICLA responders (Fig. 1C).

IL-6

Lower levels of IL-6 were observed after treatment with RCI than after treatment with placebo at the following time points in each subgroup (Fig. 2): baseline SLEDAI-2 K score < 10 (weeks 16 and 24) and ≥ 10 (weeks 8, 16, and 24); baseline anti-dsDNA levels < 15 IU/mL (week 24) and ≥ 15 IU/mL (weeks 8 and 16); and BICLA non-responders (weeks 8, 16, and 24) and responders (weeks 16 and 24).

$CD19^+$ Total B Cell Count

Treatment with RCI resulted in an initial increase in $CD19^+$ B cells at week 8 in all subgroups, followed by a substantial decrease at weeks 16 and 24 in patients with baseline SLEDAI-2 K ≥ 10 and baseline anti-dsDNA ≥ 15 IU/mL and in BICLA non-responders (Fig. 3).

$CD19^+IgD^-CD27^-CD95^+$ Atypical Activated Memory B Cell Count

After treatment with RCI, fewer $CD19^+$ $IgD^-CD27^-CD95^+$ atypical activated memory B cells were observed in patients with baseline SLEDAI-2 K scores ≥ 10 (at week 24) and baseline anti-dsDNA levels ≥ 15 IU/mL (at weeks 8, 16, and 24) and in BICLA non-responders (at weeks 8, 16, and 24) than after treatment with placebo (Fig. 4).

Complement C3 and C4

Higher levels of complement C3 were observed after treatment with RCI than after treatment with placebo at the following time points in each subgroup (Fig. 5): baseline SLEDAI-2K score ≥ 10 (weeks 8, 16, and 24); baseline anti-dsDNA levels < 15 IU/mL (week 24) and ≥ 15 IU/mL (weeks 8 and 24); and BICLA non-responders (week 24) and responders (weeks 8, 16, and 24). Differences between RCI and placebo were nominally significant at week 8 in patients with baseline SLEDAI-2K < 10 and at week 24 in patients with baseline SLEDAI-2 K ≥ 10 (Fig. 5A).

RCI treatment resulted in an increase in complement C4 levels at all time points compared with placebo in the high baseline disease activity subgroups, apart from week 16 in the baseline anti-dsDNA ≥ 15 IU/mL subgroup (Fig. 6). Differences between RCI and placebo were nominally significant at the following time points in each subgroup: week 16 for baseline SLEDAI-2 K < 10 and weeks 8 and 24 for ≥ 10 (Fig. 6A); week 8 for baseline anti-dsDNA ≥ 15 IU/mL (Fig. 6B); and weeks 8 and 24 for BICLA responders (Fig. 6C).

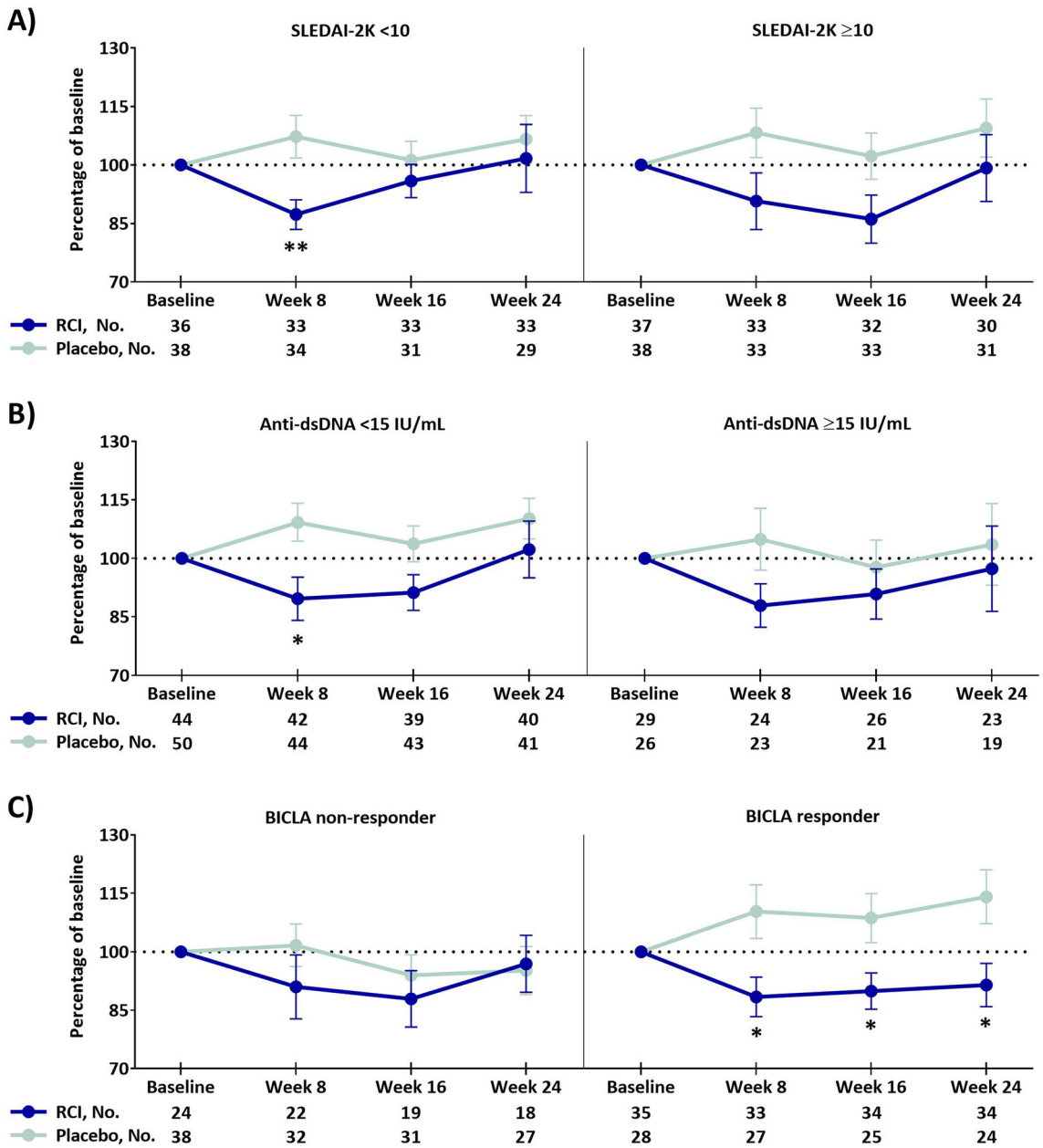

Fig. 1 Mean (SEM) percentage of baseline BAFF levels reported by baseline SLEDAI-2 K score (**A**), baseline anti-dsDNA levels (**B**), and BICLA response (**C**), mITT population[a]. [a]Patients who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data. The dotted line represents baseline. $^{*}p < 0.05$ (nominal), $^{**}p < 0.01$ (nominal) for the LS mean difference using ANCOVA models with the change from baseline as the dependent variable, treatments as the factor, and baseline values of corresponding endpoints as the covariate, with stratification for location (US and outside the US) and baseline prednisone or equivalent glucocorticoid dose (≤ 20 mg/day and > 20 mg/day). *ANCOVA* analysis of covariance; anti-dsDNA, anti-double-stranded DNA, *BAFF* B cell-activating factor, *BICLA* British Isles Lupus Assessment Group-based Combined Lupus Assessment, *LS* least squares, *mITT* modified intention to treat, *RCI* repository corticotropin injection, *SEM* standard error of the mean, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

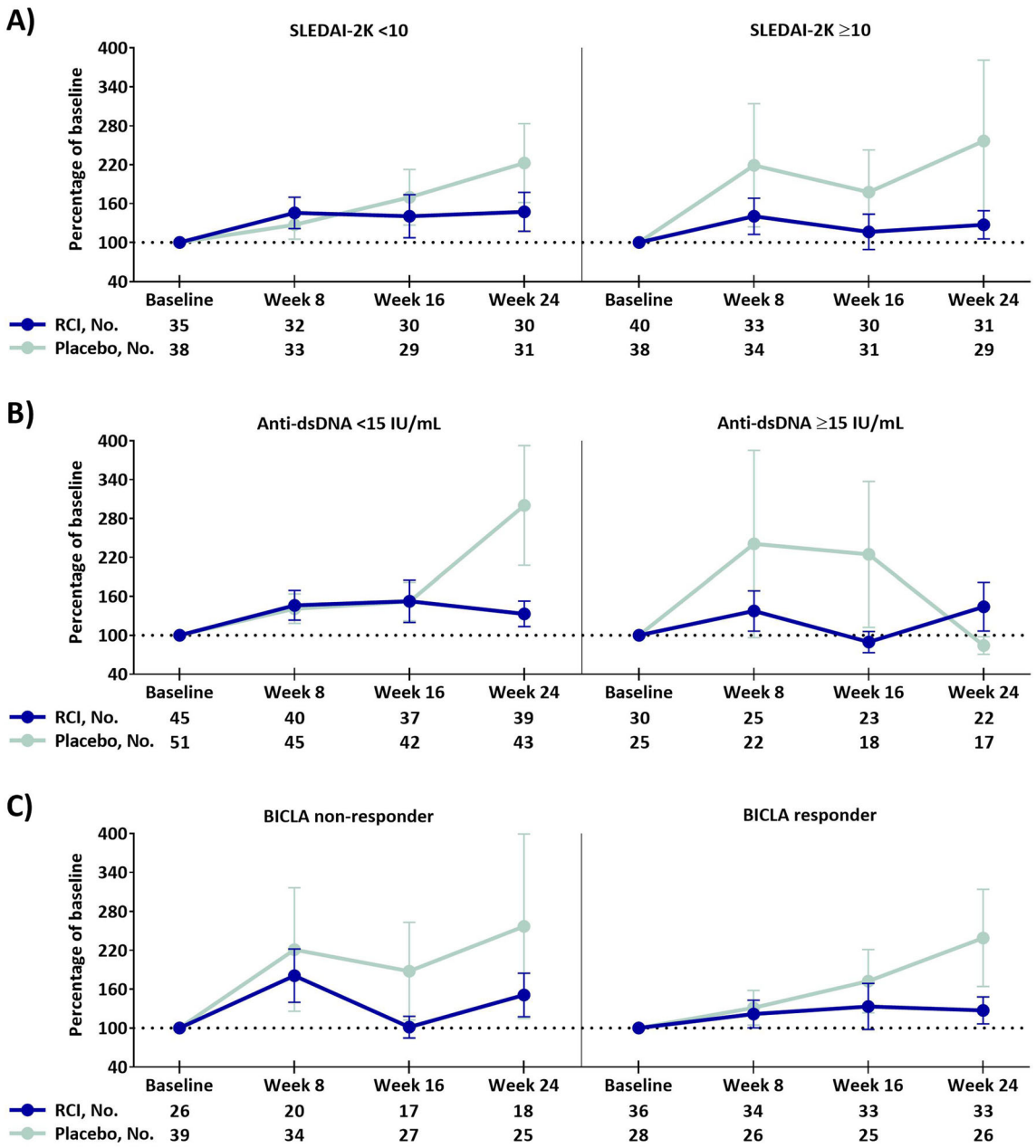

Fig. 2 Mean (SEM) percentage of baseline IL-6 levels reported by baseline SLEDAI-2K score (**A**), baseline anti-dsDNA levels (**B**), and BICLA response (**C**), mITT population[a]. [a]Patients who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data. The dotted line represents baseline. *anti-dsDNA* anti-double-stranded DNA, *BICLA* British Isles Lupus Assessment Group-based Combined Lupus Assessment, *IL-6* interleukin 6, *mITT* modified intention to treat, *RCI* repository corticotropin injection, *SEM* standard error of the mean, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

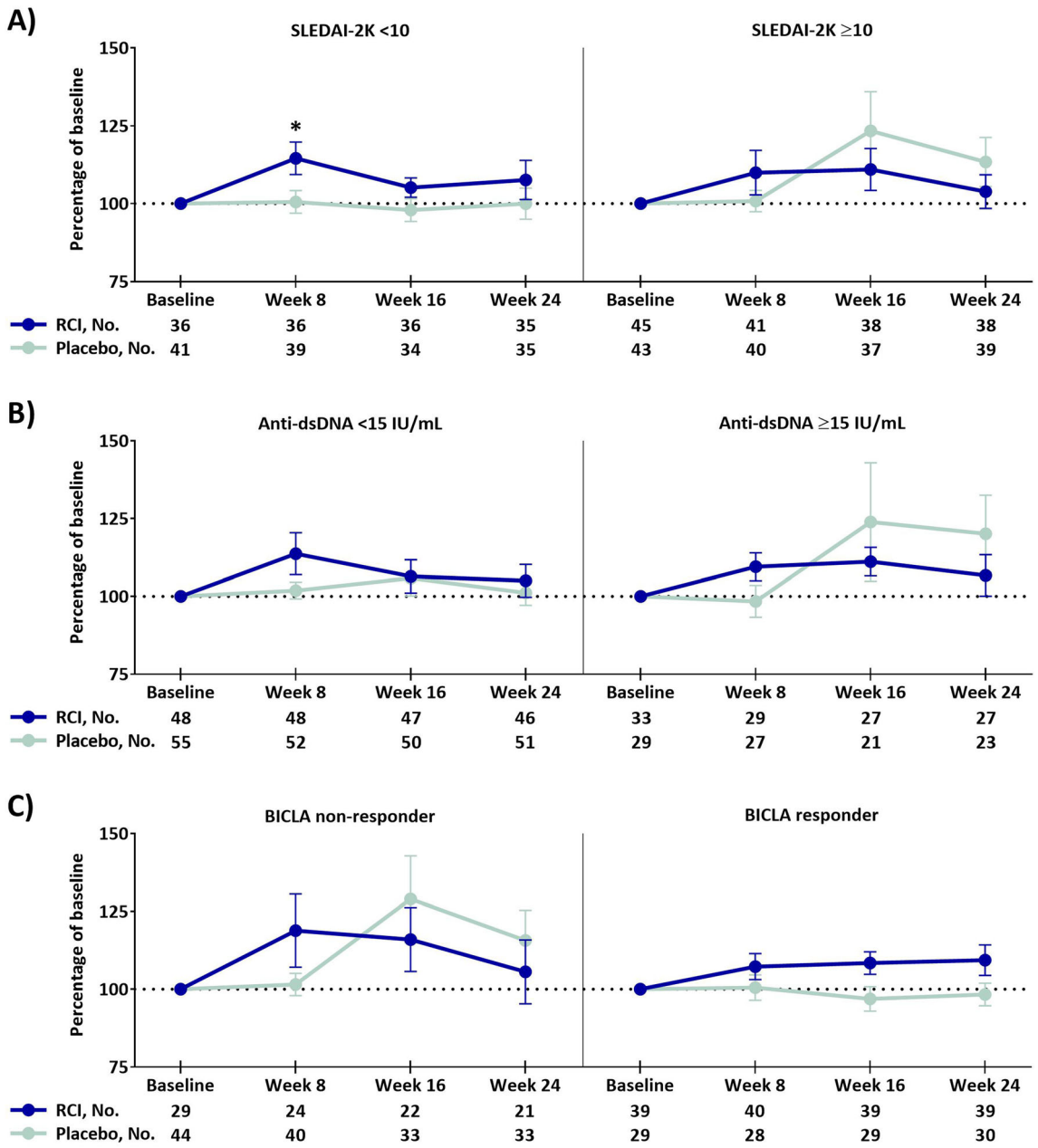

Fig. 3 Mean (SEM) percentage of baseline $CD19^+$ B cells reported by baseline SLEDAI-2K score (**A**), baseline anti-dsDNA levels (**B**), and BICLA response (**C**), mITT population[a]. [a]Participants who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data. The dotted line represents baseline. *$p < 0.05$ (nominal) for the LS mean difference using ANCOVA models with the change from baseline as the dependent variable, treatments as the factor, and baseline values of corresponding endpoints as the covariate, with stratification for location (US and outside the US) and baseline prednisone or equivalent glucocorticoid dose (≤ 20 mg/day and > 20 mg/day). *ANCOVA* analysis of covariance; anti-dsDNA, anti-double-stranded DNA, *BICLA* British Isles Lupus Assessment Group-based Combined Lupus Assessment, *CD* cluster of differentiation, *LS* least squares, *mITT* modified intention to treat, *RCI* repository corticotropin injection, *SEM* standard error of the mean, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

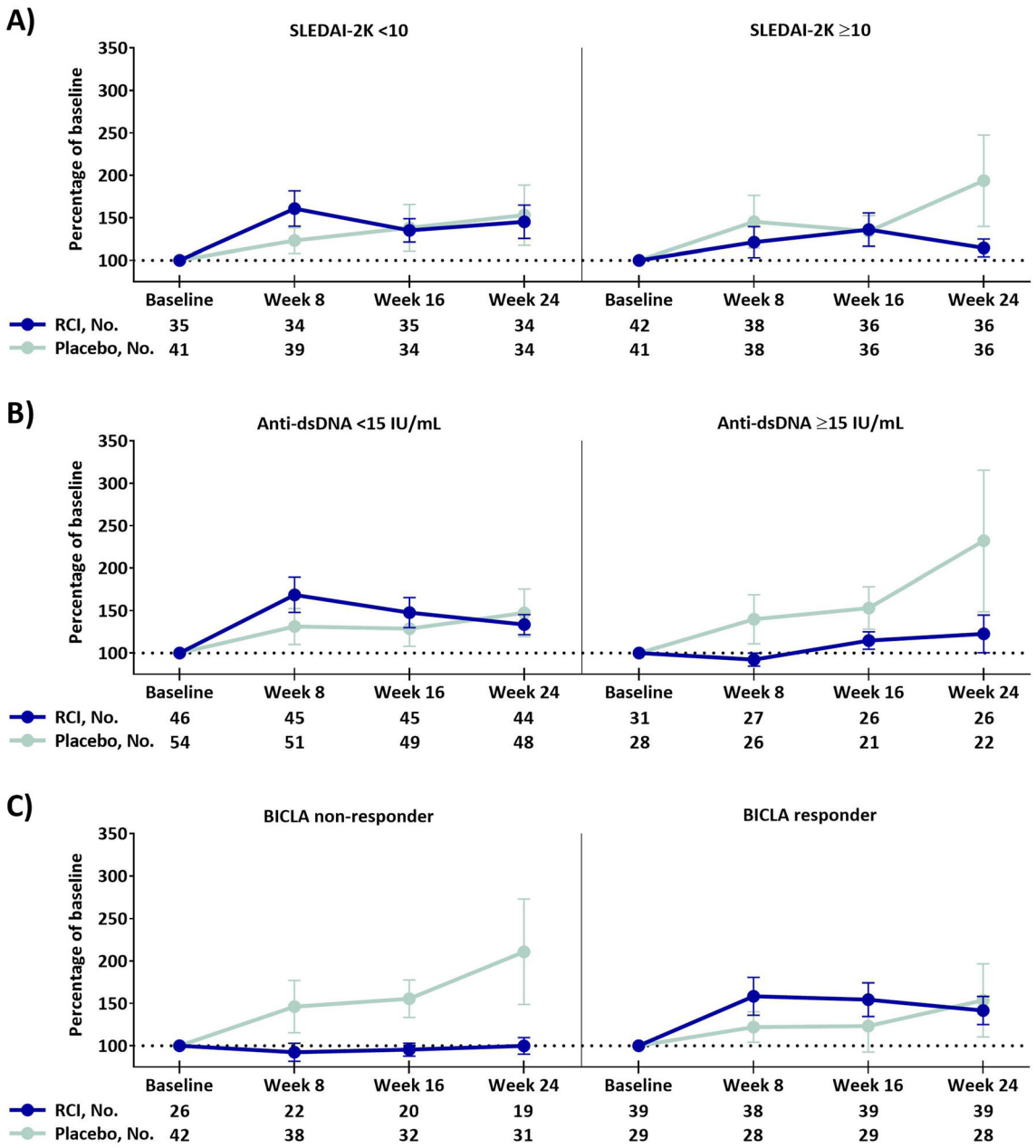

Fig. 4 Mean (SEM) percentage of baseline $CD19^{+}$ $IgD^{-}CD27^{-}CD95^{+}$ B cells reported by baseline SLEDAI-2K scores (**A**), baseline anti-dsDNA levels (**B**), and BICLA response (**C**), mITT population[a]. [a]Participants who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data. The dotted line represents baseline. *anti-dsDNA* anti-double-stranded DNA, *BICLA* British Isles Lupus Assessment Group-based Combined Lupus Assessment, *CD* cluster of differentiation, *IgD* immunoglobulin D, *mITT* modified intention to treat, *RCI* repository corticotropin injection, *SEM* standard error of the mean, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

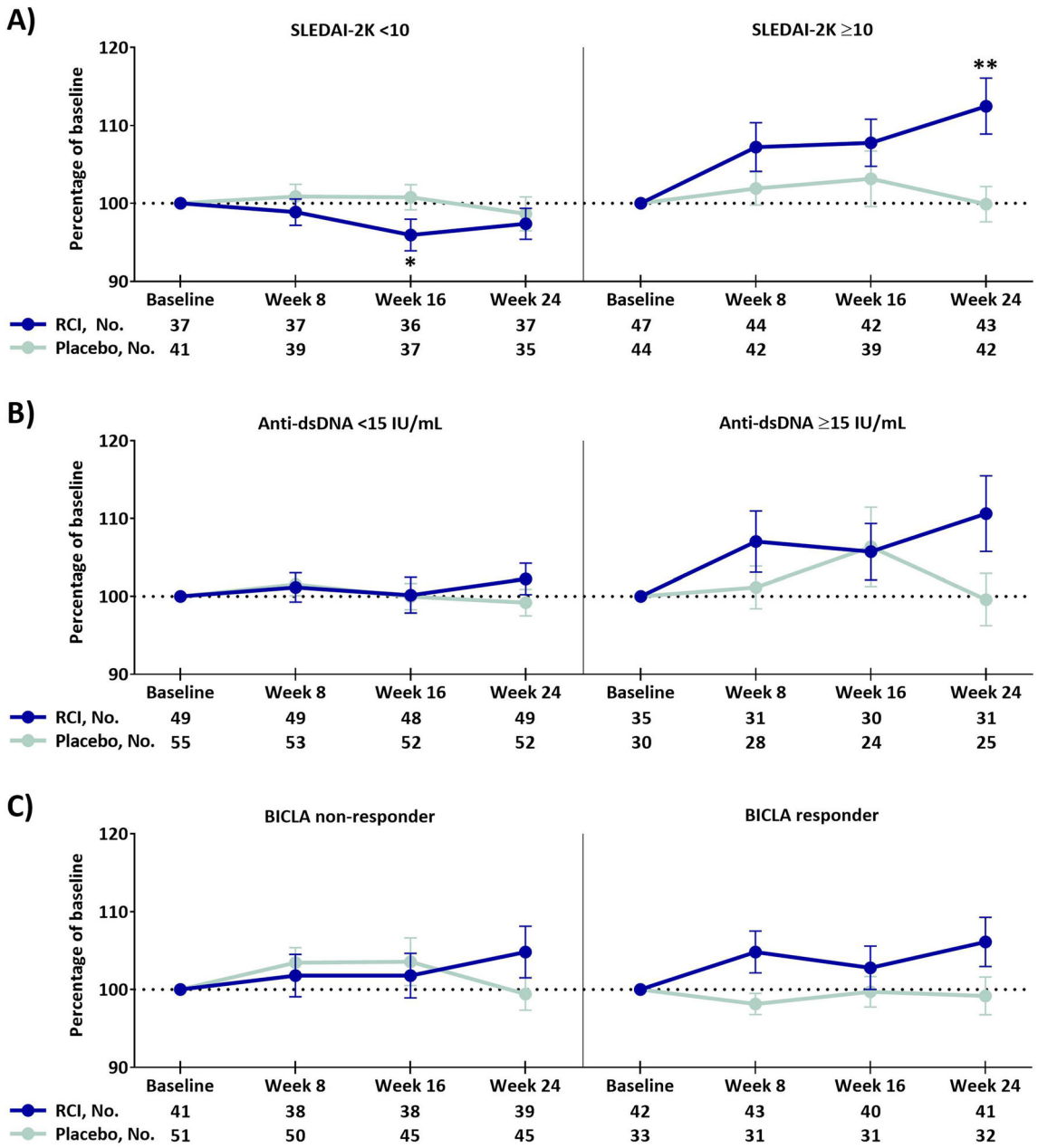

Fig. 5 Mean (SEM) percentage of baseline complement C3 levels reported by baseline SLEDAI-2K scores (**A**), baseline Anti-dsDNA levels (**B**), and BICLA response (**C**), mITT population[a]. [a]Participants who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data. The dotted line represents baseline. $^{*}p < 0.05$ (nominal), $^{**}p < 0.01$ (nominal) for the LS mean difference using ANCOVA models with the change from baseline as the dependent variable, treatments as the factor, and baseline values of corresponding endpoints as the covariate, with stratification for location (US and outside the US) and baseline prednisone or equivalent glucocorticoid dose (≤ 20 mg/day and > 20 mg/day). *ANCOVA* analysis of covariance, *anti-dsDNA* anti-double-stranded DNA, *BICLA* British Isles Lupus Assessment Group-based Combined Lupus Assessment, *C* component, *LS* least squares, *mITT* modified intention to treat, *RCI* repository corticotropin injection, *SEM* standard error of the mean, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

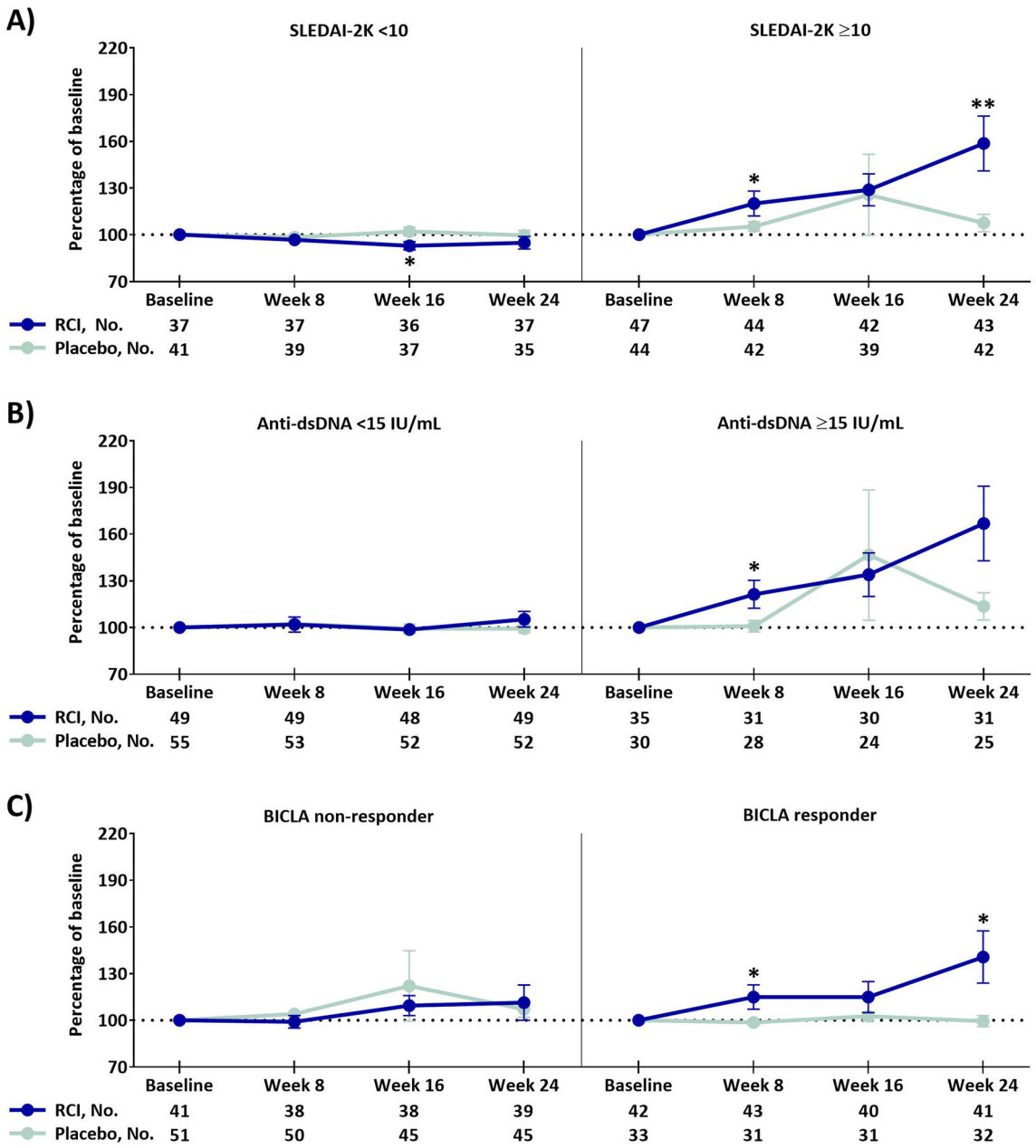

Fig. 6 Mean (SEM) percentage of baseline complement C4 levels reported by baseline SLEDAI-2K scores (**A**), baseline Anti-dsDNA levels (**B**), and BICLA response (**C**), mITT population[a]. [a]Participants who received ≥ 1 dose of study drug and contributed any postbaseline efficacy data. The dotted line represents baseline. *$p < 0.05$ (nominal), **$p < 0.01$ (nominal) for the LS mean difference using ANCOVA models with the change from baseline as the dependent variable, treatments as the factor, and baseline values of corresponding endpoints as the covariate, with stratification for location (US and outside the US) and baseline prednisone or equivalent glucocorticoid dose (≤ 20 mg/day and > 20 mg/day). *ANCOVA* analysis of covariance, *anti-dsDNA* anti-double-stranded DNA, *BICLA* British Isles Lupus Assessment Group-based Combined Lupus Assessment, *C* component, *LS* least squares, *mITT* modified intention to treat, *RCI* repository corticotropin injection, *SEM* standard error of the mean, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index-2000

DISCUSSION

These post hoc analyses of a 24-week randomized, double-blind, placebo-controlled study suggest that treatment with RCI reduces inflammatory cytokines (BAFF and IL-6) and circulating B lymphocyte profiles ($CD19^+$ and $CD19^+IgD^-CD27^-CD95^+$) and increases complement proteins in patients who have persistently active SLE despite receiving standard-of-care treatments. The observed differences from placebo propose that the immunomodulatory effects of RCI may be more pronounced in patients with higher disease activity as determined by baseline SLEDAI-2K scores and anti-dsDNA levels, while results for BICLA responders vs. non-responders were variable. These data are particularly intriguing because randomized controlled trials in SLE have shown inconsistent effects on circulating cytokines and leukocyte subgroups [28].

The immunomodulatory effects of RCI were evaluated by assessing cytokines and leukocytes that are associated with local inflammation, organ damage, tissue damage, and disruption of immune function in patients with SLE [1]. BAFF plays an important role in B cell survival and differentiation and has been shown to regulate antibody production [20]. Patients who received RCI in this study had reduced BAFF levels compared to patients who received placebo in both the primary [19] and post hoc analyses, including all disease severity and BICLA subgroups analyzed. The exact underlying mechanism for the effect of RCI on BAFF levels is not fully understood, and further study is warranted.

Additionally, increased production and elevated serum levels of IL-6 in patients with SLE result in local and systemic inflammatory effects [1, 4]. IL-6 induces B cell differentiation, T cell differentiation and proliferation, and macrophage proliferation [1]. Consequently, IL-6 levels have been shown to correlate with SLE disease activity, autoantibody production, and cardiac, pulmonary, renal, and skeletal manifestations [1, 4]. Preclinical studies have demonstrated reduced production of IL-6 from macrophages treated with RCI in vitro [29]. Our study further demonstrated inhibition of IL-6 production after RCI treatment in patients with SLE, with lower levels of IL-6 observed after RCI treatment than after placebo treatment.

Higher levels of $CD19^+$ B cells are associated with higher SLE disease activity [30]. $CD19^+IgD^-CD27^-CD95^+$ memory B cells are also increased in patients with SLE and are associated with active lupus nephritis, autoantibodies, and disease flares [5, 6]. In our study, total B cells and atypical activated memory B cells were lower for RCI-treated patients than for placebo-treated patients with higher baseline disease activity and in BICLA non-responders.

The trends observed in these post hoc analyses showed that BAFF and IL-6 (cytokines associated with B cell development, proliferation, and function [3]) decreased in all subgroups, yet only certain subgroups showed a reduction in B cells as a result of RCI therapy. The differences in B cell reductions in the high-disease vs. low-disease-activity subgroups suggest that factors other than BAFF and IL-6 may be involved in RCI-mediated B cell immunomodulation in patients with more severe disease. Since the BILAG does not incorporate measurements of autoantibodies and complement as does the SLEDAI-2K, distinction between B cell responses for RCI and placebo may be more difficult with BICLA. However, further studies that elucidate the molecular mechanisms involved in these immunomodulatory processes are warranted.

The initial increase in B cells after treatment with RCI was unexpected and differs from the results of murine models of SLE and in vitro studies that have shown that RCI decreases B cells [11] and inhibits B cell proliferation [12]. Consistent with these previously published results, a decrease in B cells was observed at later time points in the higher disease activity subgroups, while an increase in B cells was observed after RCI administration at week 8. However, given that the previous animal study evaluated leukocyte phenotypes in a cross-section of the spleen [11], one may speculate that the dynamics of B cell changes observed in blood may be different from changes observed in the spleen. Additional studies will be required to investigate this and other potential explanations.

Differences between RCI and placebo in levels of other circulating lymphocytes assessed in the primary study ($CD3^+$ total T cells and $CD4^+$ total regulatory T cells) were less pronounced [19]. In contrast to these results, studies of RCI in murine models suggest that RCI has an immunomodulatory effect on T cells [10, 11, 14]. Glucocorticoids and immunosuppressants have been shown to rapidly deplete circulating T cells [31–33]; thus, the continued use of stable background glucocorticoids and immunosuppressants in this study could have affected the ability to detect a differential response on T cells in patients treated with RCI compared to placebo. Other potential explanations for the differences in T cell response observed in murine models and clinical studies of SLE will require further investigation.

The complement system is a central component in the pathogenesis of SLE [34, 35]. Patients with active SLE often have reduced complement levels owing to a hyperactivation of the complement system that results in the degradation of complement proteins [36]. Routine measurement of complement protein levels over time may serve as a method for assessing changes in SLE disease activity, where increases in complement levels suggest a diminished inflammatory response [34–36]. In our study, complement C3 and C4 levels increased in the higher disease activity subgroups and in BICLA responders after treatment with RCI, suggesting that treatment with RCI reduced the inflammatory response in these patients. In the low disease activity subgroups and in BICLA nonresponders, C3 and C4 levels remained relatively stable through week 24 in the RCI and placebo groups. However, this was not unexpected given that patients with low disease activity have complement levels that are normal or only slightly reduced and are less likely to demonstrate large fluctuations.

Limitations of the primary study have been discussed previously [19, 21]. Inclusion criteria allowed for documented historical antinuclear antibody/anti-dsDNA/extractable nuclear antigen antibody positivity rather than requiring confirmation of these antibodies through testing at study entry. Additionally, continued use of stable background SLE therapy (i.e., NSAIDs, glucocorticoids, antimalarials, and immunosuppressants) was permitted during the study period and could have affected the observed response to RCI therapy. As with all post hoc analyses, the potential exists for subgroup selection bias. Thus, these results warrant further investigation in a randomized clinical trial in which disease activity subgroups have been determined a priori.

CONCLUSIONS

Results of these post hoc analyses provide insight into the mechanistic effects of RCI for reducing SLE disease activity via B cell immunomodulation in patients who have persistently active SLE despite treatment with glucocorticoids and other standard-of-care therapies, particularly patients with high disease activity. Preclinical evidence in vitro, in murine models of SLE, and in studies with healthy human participants has demonstrated immunomodulatory effects of RCI on B cells [10–13]. Additional clinical studies are needed to determine the direct immunomodulatory effects of RCI in patients with SLE.

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Compliance with Ethics Guidelines. The study protocol was approved centrally by the Western Institutional Review Board and by the ethics committees and institutional review boards at the individual study sites. Patients provided written informed consent. The study was conducted in agreement the ethical principles outlined in the Declaration of Helsinki and with the requirements for registered clinical trials (ClinicalTrials.gov identifier NCT02953 821).

Data Availability. The data sets generated during and/or analyzed during the current study are not publicly available. Patient data may be requested if allowed per informed consent and appropriately anonymized. Requests should be sent to Mallinckrodt Pharmaceuticals' department for Clinical Trial Disclosure and Transparency at clinicaltrials@mnk.com.

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