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# Characterization and changes of lymphocyte subsets in baricitinib-treated patients with rheumatoid arthritis: an integrated analysis

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## ABSTRACT

**Objective:** Baricitinib is an oral selective inhibitor of Janus kinase (JAK) 1 and JAK2 that is efficacious in patients with rheumatoid arthritis (RA). This study analyzed changes in lymphocyte cell subsets during baricitinib treatment and correlated such changes with clinical outcomes of baricitinib treatment.

**Methods:** An integrated analysis was conducted by pooling data from three completed Phase 3 trials comparing placebo and baricitinib treatment (RA-BEAM, RA-BUILD, and RA-BEACON) and one ongoing long-term extension study (RA-BEYOND) in patients (N=2,186) with active RA.

**Results:** Baricitinib treatment was associated with an early transient increase in total lymphocyte count at week 4, which returned to baseline by week 12. Up to week 104, transient changes within normal reference ranges in T cells and subsets were observed with baricitinib treatment. B cells and relevant subpopulations increased after 4 weeks of baricitinib treatment with no further increases noted through 104 weeks of treatment. Natural killer (NK) cells transiently increased after 4 weeks of baricitinib treatment, before decreasing below baseline levels and then stabilizing over time. Few correlations were observed between changes in lymphocyte subsets and clinical endpoints with baricitinib treatment, and most were also observed within the placebo arm. A modest potential association was observed for baricitinib 4-mg between low NK cells and treatment emergent infections but not serious infections or herpes zoster.

**Conclusion:** Overall, changes in lymphocyte subsets were largely within normal reference ranges across the baricitinib Phase 3 RA clinical program, and were not associated with increased risk of serious infections.

## INTRODUCTION

Targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) are orally available low-molecular weight molecules being developed, or already available for the treatment of rheumatoid arthritis (RA). The most recent additions include Janus kinase (JAK) inhibitors which target cytokine signaling pathways implicated in RA pathogenesis (1-5).

Baricitinib is an oral selective inhibitor of JAK1 and JAK2 (6) approved for the treatment of moderately to severely active RA in adults in over 40 countries including European countries, Japan, and the United States. Phase 3 trials showed significant clinical efficacy for baricitinib in patients with active RA who were naïve to conventional synthetic DMARDs (csDMARDs) or had an inadequate response (IR) to csDMARDs or biologic DMARDs. Baricitinib was associated with significant clinical improvements compared not only to placebo, typically including stable background csDMARDs, but also to the TNF-inhibitor, adalimumab, in the RA-BEAM trial (7-10).

Baricitinib, along with other JAK inhibitors, has been reported to change circulating lymphocyte subset populations, largely within normal ranges (11-13). However, detailed analysis of effects on subsets are lacking, and their relevance to efficacy and safety is unclear (14). This study reports changes in lymphocyte numbers, including subpopulations, during treatment in patients (N=2,186) with active RA comprising an integrated analysis of three completed Phase 3 RA trials comparing placebo and baricitinib treatment (RA-BEAM, RA-BUILD, and RA-BEACON) and one ongoing Phase 3 long-term extension (LTE) study (RA-BEYOND). This study also tested whether changes in lymphocyte subsets were associated with efficacy or adverse events.

## PATIENTS AND METHODS

### Patients and study designs

This study includes data from three previously reported randomized, double-blind, multicenter Phase 3 studies and one ongoing Phase 3 LTE study with data up to January 1, 2016. Patients were ≥18 years of age with moderate to severe active RA. Descriptions of the patient cohorts for each of the trials are given in Table S1 and detailed methods were reported previously (7, 8, 10). The criteria for exclusion and permanent discontinuation included an abnormal lymphocyte count of <750 cells/μL and <200 cells/μL, respectively.

After completion of the randomized studies (RA-BEAM, NCT01710358; RA-BUILD, NCT01721057; and RA-BEACON, NCT01721044), patients could enter the LTE study, RA-BEYOND (NCT01885078), and receive baricitinib 2- or 4-mg once daily for up to 60 months. Patients entering RA-BEYOND after 52 weeks in RA-BEAM continued baricitinib 4-mg or switched to baricitinib 4-mg from adalimumab. Patients entering RA-BEYOND after 24 weeks of treatment in RA-BUILD and RA-BEACON continued baricitinib 2- or 4-mg, or switched to baricitinib 4-mg from placebo. In RA-BEYOND, patients who received baricitinib 4-mg for ≥15 months (including in the preceding originator study) without rescue and who achieved sustained low disease activity or remission were blindly re-randomized to continue baricitinib 4-mg or to step-down to baricitinib 2-mg. Studies were conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the

ethics committee or institutional review board of each center. All patients provided written informed consent.

**Analytical methodology**

Whole blood samples were collected at weeks 0 (Placebo N=856-892; Baricitinib 2-mg N=392-403; 4-mg N=869-891), 4 (Placebo N=747-825; 2-mg N=358-384; 4-mg N=765-846), 12 (Placebo N=706-790; 2-mg N=348-361; 4-mg N=755-835), 24-32 (Placebo N=481-530; 2-mg N=272-295; 4-mg N=637-706), 48-52 (2-mg N=182-196; 4-mg N=364-582), and 96-104 (2-mg N=79-91; 4-mg N=120-135). Total lymphocyte count was analyzed using a Beckman Coulter cell counter. Lymphocyte subsets were analyzed using a BD FACS Canto ll flow cytometer and a previously optimized panel (Table S2) (15). Data for Th1 and Th17 cells at the 96-104 week timepoint were not acquired, and data for these subsets for the baricitinib 2-mg group were not available for the 48-52 week timepoint due to an insufficient sample size. T cells, including CD4 and CD8 subpopulations, B cells, and NK cells were identified using the BD Multitest 6-color TBNK reagent (BD Biosciences). The Human FoxP3 Buffer Set (BD Biosciences) was used to identify regulatory T cells. Additional flow cytometry methods, and representative gating for cell subsets in the primary flow cytometry data (Figures S1-S4), are included in the Supplementary File.

**Statistical analysis**

An integrated data set was generated by pooling patient data from RA-BEAM, RA-BUILD, RA-BEACON, and RA-BEYOND. The placebo group came from RA-BEAM,

RA-BUILD and RA-BEACON; data were censored at rescue or the end of the 24-week placebo treatment period. The baricitinib 2-mg group came from RA-BUILD and RA-BEACON with associated data from RA-BEYOND; data were censored at rescue. The baricitinib 4-mg group came from RA-BEAM, RA-BUILD and RA-BEACON with associated data from RA-BEYOND; data were censored at rescue or dose step-down.

For changes in lymphocyte subsets, a restricted maximum likelihood-based mixed model for repeated measures (MMRM) was used which included treatment, visit, and treatment-by-visit-interaction as fixed categorical effects, and baseline as fixed continuous effects to estimate change from baseline after modeling 4 covariance structures (autoregressive [AR(1)], heterogeneous autoregressive [ARH(1)], compound symmetry [CS] and Toeplitz [TOEP]), selecting the variance-covariance structure with the smallest Akaike information criterion (AIC). The Fisher exact test was used for comparisons of incidence of treatment emergent (TE) infections between treatment groups by NK cell count category.

For rheumatoid factor (RF) and anti-citrullinated peptide antibody (ACPA), treatment comparisons in mean change from baseline were made using analysis of covariance (ANCOVA), with treatment and baseline values as covariates. Type III sums of squares were used for comparisons of each baricitinib dose versus placebo, and least square (LS) means, standard errors, and p-values were reported.

The weighted Pearson correlation coefficient and its associated p-value were used to test whether correlations between change from baseline in lymphocyte subset and Disease Activity Score for 28 joint-counts based on the high-sensitivity C-reactive protein level (DAS28-hsCRP) were significant by treatment group.

## RESULTS

### Changes in lymphocyte subsets

Total lymphocyte count increased transiently at week 4 for baricitinib 2- and 4-mg compared to placebo, returning to baseline by week 12 and remaining stable thereafter (Figure 1). At baseline, low lymphocyte counts were observed in 8.7%, 6.2%, and 6.6% of patients for placebo, baricitinib 2- and 4-mg, respectively, and high lymphocyte counts were observed in 4.3%, 4.7% and 4.0% of patients. Based on 24 weeks of data up to rescue, the proportions of patients with ≥1 TE low lymphocyte count were 10.7%, 8.2%, and 8.9% and the proportions of patients with ≥1 TE high lymphocyte count were 4.4%, 11.2%, and 11.9% for placebo, baricitinib 2- and 4-mg, respectively, (Figure 1).

CD3+, CD4+, and CD8+ T cell counts increased at week 4 for baricitinib 2- and 4-mg compared to placebo and returned to baseline by week 12. Inconsistent changes in these T cell subsets were observed at weeks 24-32 (Figures 2A-C), but were trending downwards compared to baseline. At baseline, low CD3+ T cell counts were observed in 11.3%, 9.3%, and 9.1% of patients for placebo, baricitinib 2- and 4-mg, respectively; low CD4+ T cell counts were observed in 14.2%, 13.4%, and 13.5% of patients; low CD8+ T cells counts were observed in 7.9%, 7.6%, and 6.4% of patients. Based on 24 weeks of data up to rescue, the proportions of patients with ≥1 TE low CD3+, CD4+, or CD8+ T cell count did not differ between baricitinib and placebo (Figures 2A-C). Th1 (CD4+CXCR3+CCR6-) counts increased at week 4 with baricitinib treatment and decreased below baseline through weeks 24-32 (Figure 2D). Th17 (CD4+CXCR3-CCR6+) counts increased at week 4 with baricitinib treatment and returned to baseline

by week 12 for baricitinib 4-mg and decreased at weeks 24-32 for baricitinib 2-mg (Figure 2E). Compared to placebo, which demonstrated a modest increase at week 12, regulatory T cells decreased at week 12 for both baricitinib groups and returned to near baseline by weeks 24-32, remaining stable thereafter (Figure 2F). For CD3+, CD4+, and CD8+ T cells, within-group analysis demonstrated a significant decrease from baseline by weeks 96-104 for baricitinib 2- and 4-mg. For Th1 cells, a significant decrease from baseline by weeks 48-52 was observed for baricitinib 4-mg (Figures 2A-D; markers of statistical significance not shown for within-group testing).

CD19+ B cells and B cell subpopulations, including switched memory, non-switched memory, mature naïve, and immature transitional B cells, significantly increased at weeks 4 through 24-32 for both baricitinib groups compared to placebo, and remained slightly above baseline or gradually returned to baseline over time (Figures 3A-E). At baseline, high CD19+ B cell counts were observed in 3.5%, 3.3%, and 3.6% of patients for placebo, baricitinib 2- and 4-mg, respectively. Based on 24 weeks of data up to rescue, the proportions of patients with ≥1 TE high CD19+ B cell count were 1.2%, 3.7% and 5.2% for placebo, baricitinib 2- and 4-mg, respectively (Figure 3A).

Finally, NK cell counts were increased significantly by week 4 for baricitinib compared to placebo; changes were transient with NK cell counts decreased by week 12 through weeks 24-32 for both baricitinib groups (Figure 4). In a change from baseline analysis, baricitinib 2- and 4-mg were below baseline at weeks 12 through 48-52 (p-values ≤0.05), and while baricitinib 4-mg was below baseline by weeks 96-104, the change from baseline was no longer significant for baricitinib 2-mg (p-value >0.05)

(Figure 4 and data not shown). At baseline, low NK cell counts were observed in 15.1%, 11.1%, and 14.9% of patients for placebo, baricitinib 2- and 4-mg, respectively. The proportions of patients with ≥1 TE low NK cell count were 9.9%, 14.8% and 20.4% for placebo, baricitinib 2- and 4-mg, respectively (Figure 4). Among patients with a TE low NK cell count at their last visit on baricitinib who had a follow-up value (N=16), 11/16 normalized during the post-treatment follow-up period.

**Changes in lymphocytes and clinical effects of baricitinib treatment**

Negative correlations between changes from baseline in DAS28-hsCRP and counts of CD19+ B cell and several B cell subpopulations were observed for placebo and baricitinib treatment, indicating larger improvements in DAS28-hsCRP with larger increases in B cell counts (Table S3). A negative correlation was also observed between changes in DAS28-hsCRP and Th17 and regulatory T cell counts for baricitinib 4-mg (Table S3). The increase in CD19+ B cells observed with baricitinib (Figure 3A) occurred while RF titers significantly decreased from baseline at week 24 for baricitinib 2- and 4-mg compared to placebo, and ACPA titers significantly decreased from baseline at week 24 for baricitinib 4-mg only (Table S4).

Additionally, we tested whether changes in NK cells were associated with increased infection rates in baricitinib-treated RA patients, based on 24 weeks of data up to rescue. Compared to placebo in patients who never experienced a low NK cell count throughout treatment, increases in overall infections and herpes zoster were seen for both baricitinib groups and baricitinib 4-mg, respectively. However, the rate of serious infection was similar between groups (Table 1). Patients who experienced ≥1

low NK cell value throughout the treatment period also had a dose-related increase in overall infections with baricitinib versus placebo; herpes zoster and serious infection rates were similar between groups (Table 1). Similar associations were observed between changes in CD4+ T cell counts and infection rates (Table S5). Among patients who experienced a low NK cell count prior to their first TE infection, the majority experienced a low NK cell count less than 3 months before the infection across treatment groups (Table S6).

## DISCUSSION

This analysis characterized changes in lymphocyte subsets in patients with RA (N=2,186) from Phase 3 and LTE trials in the baricitinib clinical program, and assessed whether observed lymphocyte changes correlated with clinical effects of baricitinib treatment.

Changes in lymphocyte subsets were generally within the normal range and were largely consistent across the baricitinib Phase 3 RA clinical trials, which included patients with different responsiveness to prior DMARD therapies (11-13). In this integrated analysis, a transient increase in total lymphocyte count was observed after 4 weeks of treatment with baricitinib, returning to baseline by week 12. Overall changes in lymphocyte subsets from a Phase 3 RA study (RA-BEGIN) of baricitinib in the treatment of DMARD-naïve patients generally reflected similar patterns of change as those in the integrated analysis (13). Similarly, an early transient increase in lymphocyte count was also observed in patients with psoriasis treated with baricitinib (16). These findings may in part relate to the timing of baricitinib administration. In previous Phase 1 studies,

transient increases in total lymphocyte counts were observed within hours of baricitinib dosing, returning to baseline prior to the next daily dose (6). Transient changes in T cells and subsets were observed with baricitinib treatment, with cell counts remaining largely within normal reference ranges. While CD3+, CD4+ and CD8+ T cells decreased below baseline by weeks 96-104, longer term data will be required to inform whether the change continues or the patients establish a new baseline. B cells and subpopulations increased after 4 weeks of baricitinib treatment, and remained above baseline or stabilized over time. JAK inhibition modulates cytokine signaling, therefore, possible downstream alterations in adhesion molecule expression and chemokine production may affect B cell trafficking with baricitinib treatment, resulting in elevated peripheral B cells. Furthermore, differentiation of the B cell lineage may be induced in compensation for JAK1/2-mediated inhibition of production of cytokines such as type l and type ll IFNs. Importantly, baricitinib treatment did not result in increased autoantibody titers, suggesting that the increase in B cells may not be pathological. It is possible that some of the class-switched memory B cells, increased by baricitinib in a dose-dependent manner, may be regulatory B cells which cease disease activity. Further studies would be needed to clarify these possibilities.

NK cells transiently increased after 4 weeks of baricitinib treatment, before decreasing below baseline levels and then gradually stabilizing over time. Of note, baseline low NK cell counts were observed in up to 15.1% of patients in this analysis. The overall effect of JAK1 and JAK2 inhibition on peripheral blood mononuclear cell subsets may potentially reflect the summation of a number of different biological processes. These include demargination consequent upon endothelial deactivation and

altered marrow production of cellular subtypes. Which of these processes predominates in determining the observed change in peripheral blood may depend on the half-life of the circulating cell.

Changes in lymphocyte subsets have also been characterized for tofacitinib, a JAK inhibitor that preferentially inhibits JAK1 and JAK3 (17). Phase 2 and Phase 3 clinical trials involving patients with RA treated with tofacitinib showed a transient increase in total lymphocytes early in treatment, with a gradual decrease over time (18-20). In Phase 2 RA clinical trials, variable changes in T cells were observed with short-term tofacitinib treatment, while B cells and NK cells increased and decreased from baseline, respectively (18, 19). To our knowledge, robust data systemically following tofacitinib-treated RA patients from baseline through long-term treatment are unavailable.

Changes in lymphocyte subsets have been characterized in clinical trials with other JAK inhibitors. Phase 2 clinical trials involving patients with RA treated with the JAK1-selective inhibitor filgotinib showed no clear dose-dependent changes in the rates of TE low lymphocytes or NK cells during short-term treatment while similar studies with upadacitinib showed decreased lymphocyte and NK counts following administration of that agent (21-24). These data suggest the possibility that different JAK inhibitors may be associated with slightly different lymphocyte changes over time, and in vitro JAK selectivity is not an easy predictor of these changes. It is important to point out that in the current study, lymphocyte subpopulations were followed across 2,186 active patients with RA in the baricitinib Phase 3 program, including long-term treatment,

making it the most extensive analysis of lymphocyte subset changes with a JAK inhibitor described to date.

Changes in lymphocyte levels have also been reported in patients with RA treated with compounds that target TNF- or IL-6-signaling. Our Phase 3 RA-BEAM trial, as well as other studies, showed that the TNF inhibitor, adalimumab, produced a sustained increase in total lymphocyte counts over time in patients with RA (12, 20, 25). Consistent with an increase in total lymphocyte levels over time, we showed that adalimumab treatment also resulted in increased T cells, B cells, and NK cells in the RA-BEAM trial (12). Several studies with small patient populations have analyzed changes in lymphocyte subsets in patients with RA treated with tocilizumab, a monoclonal antibody that inhibits the IL-6 receptor. Two studies showed that tocilizumab treatment resulted in increased regulatory T cells, one of which also showed decreased Th17 cells with treatment (26, 27). Tocilizumab treatment in patients with RA has shown conflicting changes in B cells; one study reported increased total B cells, with no significant changes in B cell subsets, whereas another reported decreased levels of memory B cell subsets (27, 28). Overall, these findings demonstrate that therapeutics in RA that target different biologic pathways are associated with distinct changes in lymphocyte subsets.

In view of these changes in lymphocyte subsets, this study attempted to determine whether there were clinical correlations between changes in lymphocyte subsets with DAS28-hsCRP in the integrated analysis. Negative correlations were observed between changes in DAS28-hsCRP and B cell subpopulations for placebo and baricitinib treatment, in addition to Th17 and regulatory T cells for baricitinib 4-mg.

Though statistically significant, the strengths of these associations were however modest. The increase in B cells with baricitinib treatment was not associated with an increase in autoantibody titers. Finally, patients who never experienced low NK cell counts as well as patients who experienced ≥1 low NK cell value on treatment with baricitinib 4-mg had a small increase in the rate of overall infection though not serious infection. Low NK cell counts did not relate to the rate of herpes zoster infection, a known adverse reaction for baricitinib, suggesting that NK modulation is not a dominant mechanism underlying this association.

The present study does not directly explain mechanistically the changes in lymphocyte subsets observed. Several mechanisms could contribute to the changes observed, operating either in isolation or in combination. Thus, cell number may have reflected transiently increased synthesis, perhaps reflecting an immediate effect of drug on marrow homeostasis, effects on cellular mobilization from lymphoid or inflamed tissues, effects on cellular egress from the circulation or perhaps effects on cellular survival. Moreover, the impact of JAK inhibition is distinct in different subsets, leading most notably in the B cell compartment to a new homeostatic circulatory level. Whether these effects are mediated directly on lymphocyte subsets via JAK sensitive receptors or via altered chemokine or chemokine receptor expression that is dependent on JAK driven pathways is unclear, and warrants further investigation.

A significant limitation of this study is that the follow-up period, although considerable, is not yet over many years, yet RA is typically lifelong and may potentially require lifelong treatment. Longer term lymphocyte subset data continue to be collected during the ongoing LTE study RA-BEYOND, which currently provides up to 5 years of

treatment with baricitinib after patients complete an originating study; evaluation of these longer term data, when available, will assist in addressing this limitation.

In conclusion, this integrated analysis of lymphocyte subsets during the course of treatment with baricitinib in a large Phase 3 RA dataset has shown distinct changes in different lymphocyte populations, including B cell increases, variable changes in T cell counts, and transient reduction in NK cells, without strong association with efficacy or safety endpoints. The later kinetics of these analytes during longer term baricitinib treatment, and any clinical correlates, continues to be evaluated.

## REFERENCES

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205-19.

2. Tanaka Y. Current concepts in the management of rheumatoid arthritis. Korean J Intern Med. 2016;31:210-8.

3. Furst DE, Emery P. Rheumatoid arthritis pathophysiology: update on emerging cytokine and cytokine-associated cell targets. Rheumatology (Oxford). 2014;53:1560-9.

4. Schwartz DM, Bonelli M, Gadina M, O'Shea JJ. Type I/II cytokines, JAKs, and new strategies for treating autoimmune diseases. Nat Rev Rheumatol. 2016;12:25-36.

5. Smolen JS, Landewe R, Bijlsma J, Burmester G, Chatzidionysiou K, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann Rheum Dis. 2017;76:960-77.

6. Shi JG, Chen X, Lee F, Emm T, Scherle PA, Lo Y, et al. The pharmacokinetics, pharmacodynamics, and safety of baricitinib, an oral JAK 1/2 inhibitor, in healthy volunteers. J Clin Pharmacol. 2014;54:1354-61.

7. Genovese MC, Kremer J, Zamani O, Ludivico C, Krogulec M, Xie L, et al. Baricitinib in patients with refractory rheumatoid arthritis. N Engl J Med. 2016;374:1243-52.

8. Dougados M, van der Heijde D, Chen YC, Greenwald M, Drescher E, Liu J, et al. Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: results from the RA-BUILD study. Ann Rheum Dis. 2017;76:88-95.

9. Fleischmann R, Schiff M, van der Heijde D, Ramos-Remus C, Spindler A, Stanislav M, et al. Baricitinib, methotrexate, or combination in patients with rheumatoid arthritis and no or limited prior disease-modifying antirheumatic drug treatment. Arthritis Rheumatol. 2017;69:506-17.

10. Taylor PC, Keystone EC, van der Heijde D, Weinblatt ME, Del Carmen Morales L, Reyes Gonzaga J, et al. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. N Engl J Med. 2017;376:652-62.

11. Emery P, McInnes I, Genovese M, Smolen J, Kremer J, Dougados M, et al. Characterization of changes in lymphocyte subsets in baricitinib-treated patients with rheumatoid arthritis in two phase 3 studies [abstract]. Arthritis Rheumatol. 2015;67 (suppl 10).

12. Tanaka Y, Fleischmann R, Schiff M, Takeuchi T, Keystone E, Weinblatt M, et al. Characterization of changes in lymphocyte subsets in baricitinib-treated patients with

rheumatoid arthritis in a phase 3 study (RA-BEAM) [abstract]. Annals of the Rheumatic Diseases. 2016;75 (suppl 2).

13. Takeuchi T, Fleischmann R, Schiff M, Issa M, Macias W, Rooney T, et al. Characterization of changes in lymphocyte subsets in baricitinib-treated patients with early, DMARD naïve, rheumatoid arthritis in a phase 3 study [abstract]. Annals of the Rheumatic Diseases. 2017;76 (suppl 2).

14. Winthrop KL. The emerging safety profile of JAK inhibitors in rheumatic disease. Nat Rev Rheumatol. 2017;13:320.

15. Mujtaba MA, Komocsar WJ, Nantz E, Samaniego MD, Henson SL, Hague JA, et al. Effect of treatment with tabalumab, a B cell-activating factor inhibitor, on highly sensitized patients with end-stage renal disease awaiting transplantation. Am J Transplant. 2016;16:1266-75.

16. Papp KA, Menter MA, Raman M, Disch D, Schlichting DE, Gaich C, et al. A randomized phase 2b trial of baricitinib, an oral Janus kinase (JAK) 1/JAK2 inhibitor, in patients with moderate-to-severe psoriasis. Br J Dermatol. 2016;174:1266-76.

17. Clark JD, Flanagan ME, Telliez JB. Discovery and development of Janus kinase (JAK) inhibitors for inflammatory diseases. J Med Chem. 2014;57:5023-38.

18. Hodge JA, Kawabata TT, Krishnaswami S, Clark JD, Telliez JB, Dowty ME, et al. The mechanism of action of tofacitinib - an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis. Clin Exp Rheumatol. 2016;34:318-28.

19. van Vollenhoven R, Choy E, Lee EB, Hazra A, Anisfeld A, Lazariciu I, et al. Tofacitinib, an oral Janus kinase inhibitor, in the treatment of rheumatoid arthritis: changes in lymphocytes and lymphocyte subset counts and reversibility after up to 8

years of tofacitinib treatment [abstract]. Annals of the Rheumatic Diseases. 2016;75 (suppl 2).

20. Schulze-Koops H, Strand V, Nduaka C, DeMasi R, Wallenstein G, Kwok K, et al. Analysis of haematological changes in tofacitinib-treated patients with rheumatoid arthritis across phase 3 and long-term extension studies. Rheumatology (Oxford). 2017;56:46-57.

21. Westhovens R, Taylor PC, Alten R, Pavlova D, Enriquez-Sosa F, Mazur M, et al. Filgotinib (GLPG0634/GS-6034), an oral JAK1 selective inhibitor, is effective in combination with methotrexate (MTX) in patients with active rheumatoid arthritis and insufficient response to MTX: results from a randomised, dose-finding study (DARWIN 1). Ann Rheum Dis. 2017;76:998-1008.

22. Kavanaugh A, Kremer J, Ponce L, Cseuz R, Reshetko OV, Stanislavchuk M, et al. Filgotinib (GLPG0634/GS-6034), an oral selective JAK1 inhibitor, is effective as monotherapy in patients with active rheumatoid arthritis: results from a randomised, dose-finding study (DARWIN 2). Ann Rheum Dis. 2017;76:1009-19.

23. Kremer JM, Emery P, Camp HS, Friedman A, Wang L, Othman AA, et al. A phase IIb study of ABT-494, a selective JAK-1 inhibitor, in patients with rheumatoid arthritis and an inadequate response to anti-tumor necrosis factor therapy. Arthritis Rheumatol. 2016;68:2867-77.

24. Genovese MC, Smolen JS, Weinblatt ME, Burmester GR, Meerwein S, Camp HS, et al. Efficacy and safety of ABT-494, a selective JAK-1 inhibitor, in a phase IIb study in patients with rheumatoid arthritis and an inadequate response to methotrexate. Arthritis Rheumatol. 2016;68:2857-66.

25. Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS, et al. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. Arthritis Rheum. 2004;50:1400-11.

26. Samson M, Audia S, Janikashvili N, Ciudad M, Trad M, Fraszczak J, et al. Brief report: inhibition of interleukin-6 function corrects Th17/Treg cell imbalance in patients with rheumatoid arthritis. Arthritis Rheum. 2012;64:2499-503.

27. Daien CI, Gailhac S, Audo R, Mura T, Hahne M, Combe B, et al. High levels of natural killer cells are associated with response to tocilizumab in patients with severe rheumatoid arthritis. Rheumatology (Oxford). 2015;54:601-8.

28. Roll P, Muhammad K, Schumann M, Kleinert S, Einsele H, Dorner T, et al. In vivo effects of the anti-interleukin-6 receptor inhibitor tocilizumab on the B cell compartment. Arthritis Rheum. 2011;63:1255-64.

## FIGURE LEGENDS

### Figure 1. Total lymphocyte counts throughout treatment

The median, 25$^{th}$ and 75$^{th}$ percentiles, in total lymphocyte counts through week 24 (placebo) and weeks 96-104 (baricitinib 2- or 4-mg) are shown, including the number (%) of patients with the specified treatment emergent abnormality at any time post-baseline up to the time of rescue (0-24 weeks), based on the following LLN and ULN reference ranges (cells/µL): LLN=800, ULN=4280. Percentages are based on the number of patients at risk for the specified abnormality in each treatment group (NAR).

LLN, lower limit of normal; N, the number of patients in the analysis population; ULN, upper limit of normal. *p ≤0.05, **p ≤0.01, ***p ≤0.001 compared to placebo based on the least square mean difference change from baseline.

**Figure 2. T cell counts throughout treatment**

The median, 25th and 75th percentiles, cell counts through week 24 (placebo) and weeks 96-104 (baricitinib 2- or 4-mg) are shown for CD3+ (A), CD4+ (B), CD8+ (C), Th1 (D), Th17 (E), and regulatory T cells (F). A-C include the number (%) of patients with the specified treatment emergent abnormality at any time post-baseline up to the time of rescue (0-24 weeks), based on the following LLN and ULN reference ranges (cells/µL): CD3+, LLN=603, ULN=2990; CD4+, LLN=441, ULN=2156; CD8+, LLN=125, ULN=1312. Percentages are based on the number of patients at risk for the specified abnormality in each treatment group (NAR). LLN, lower limit of normal; N, the number of patients in the analysis population; ULN, upper limit of normal. *p ≤0.05, **p ≤0.01, ***p ≤0.001 compared to placebo based on the least square mean difference change from baseline.

**Figure 3. B cell counts throughout treatment**

The median, 25th and 75th percentiles, cell counts through week 24 (placebo) and weeks 96-104 (baricitinib 2- or 4-mg) are shown for CD19+ (A), switched memory (B), non-switched memory (C), mature naive (D), and immature transitional B cells (E). A includes the number (%) of patients with the specified treatment emergent abnormality at any time post-baseline up to the time of rescue (0-24 weeks), based on the following

LLN and ULN reference ranges (cells/µL): LLN=107, ULN=698. Percentages are based on the number of patients at risk for the specified abnormality in each treatment group (NAR). LLN, lower limit of normal; N, the number of patients in the analysis population; ULN, upper limit of normal. *p ≤0.05, **p ≤0.01, ***p ≤0.001 compared to placebo based on the least square mean difference change from baseline.

**Figure 4. NK cell count throughout treatment**

The median, 25$^{th}$ and 75$^{th}$ percentiles, NK cell counts through week 24 (placebo) and weeks 96-104 (baricitinib 2- or 4-mg) are shown, including the number (%) of patients with the specified treatment emergent abnormality at any time post-baseline up to the time of rescue (0-24 weeks), based on the following LLN and ULN reference ranges (cells/µL): LLN=95, ULN=640. Percentages are based on the number of patients at risk for the specified abnormality in each treatment group (NAR). LLN, lower limit of normal; N, the number of patients in the analysis population; ULN, upper limit of normal. *p ≤0.05, **p ≤0.01, ***p ≤0.001 compared to placebo based on the least square mean difference change from baseline.

**TABLES**

**Table 1. Incidence of TE infection by NK cell count, weeks 0-24 with data up to rescue**

| | Placebo (N=860) | Baricitinib 2-mg (N=393) | Baricitinib 4-mg (N=868) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Never low NK cell count, Ns (%)[a] | 718 (83.5) | 316 (80.4) | 618 (71.2)*** ††† |
| Patients with ≥1 TE infection, n (%)[b] | 213 (29.7) | 114 (36.1)* | 217 (35.1)* |
| Patients with ≥1 TE herpes zoster, n (%)[b] | 3 (0.4) | 3 (0.9) | 13 (2.1)** |
| Patients with ≥1 serious infection, n (%)[b] | 13 (1.8) | 4 (1.3) | 10 (1.6) |
| Low NK cell count, Ns (%)[c] | 142 (16.5) | 77 (19.6) | 250 (28.8)*** ††† |
| Patients with ≥1 TE infection, n (%)[d] | 47 (33.1) | 29 (37.7) | 112 (44.8)* |
| Patients with ≥1 TE herpes zoster, n (%)[d] | 0 | 1 (1.3) | 2 (0.8) |
| Patients with ≥1 serious infection, n (%)[d] | 1 (0.7) | 1 (1.3) | 3 (1.2) |

[a]Patients who never experienced low NK cell values at all times post-baseline up to the time of rescue, based on the LLN reference range (≥95 cells/μL). Percentages are based on N.

[b]Patients who never experienced low NK cell values and the specified abnormality. Percentages are based on Ns.

[c]Patients who experienced ≥1 low NK cell value at any time post-baseline up to the time of rescue, based on the LLN reference range (<95 cells/μL). Percentages are based on N.

[d]Patients who experienced ≥1 low NK cell value and the specified abnormality. Percentages are based on Ns.

*p ≤0.05, **p ≤0.01, ***p ≤0.001 for baricitinib 2- or 4-mg compared to placebo using Fisher exact test

[†††]p ≤0.001 for baricitinib 4-mg compared to baricitinib 2-mg using Fisher exact test

LLN, lower limit of normal; N, number of patients in the analysis set with ≥1 post-baseline NK cell measure in the corresponding analysis period; Ns, number of patients by subset in each treatment group; n, number of patients with the specified abnormality; TE, treatment emergent

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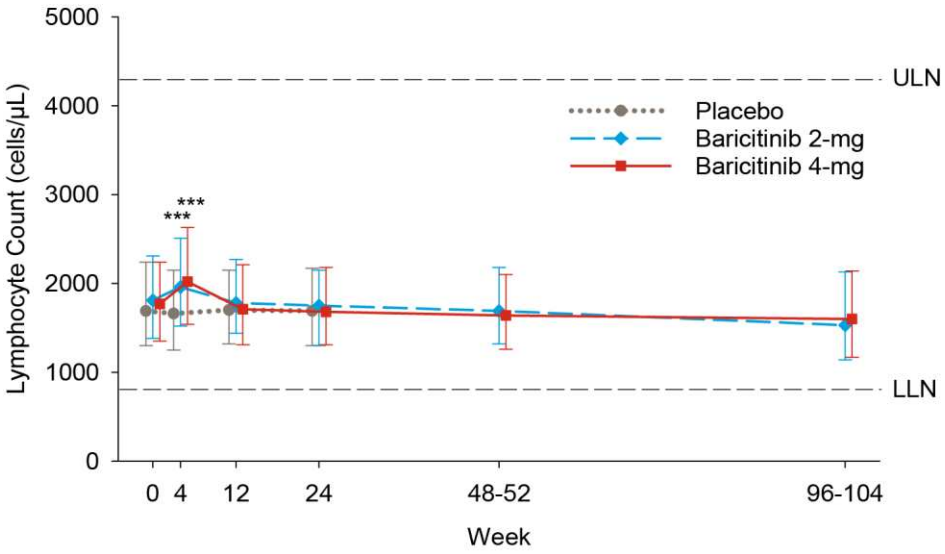
**Contributors**

All authors participated in the analyses and interpretation of data, wrote or critically reviewed the manuscript, and reviewed and approved the final version.

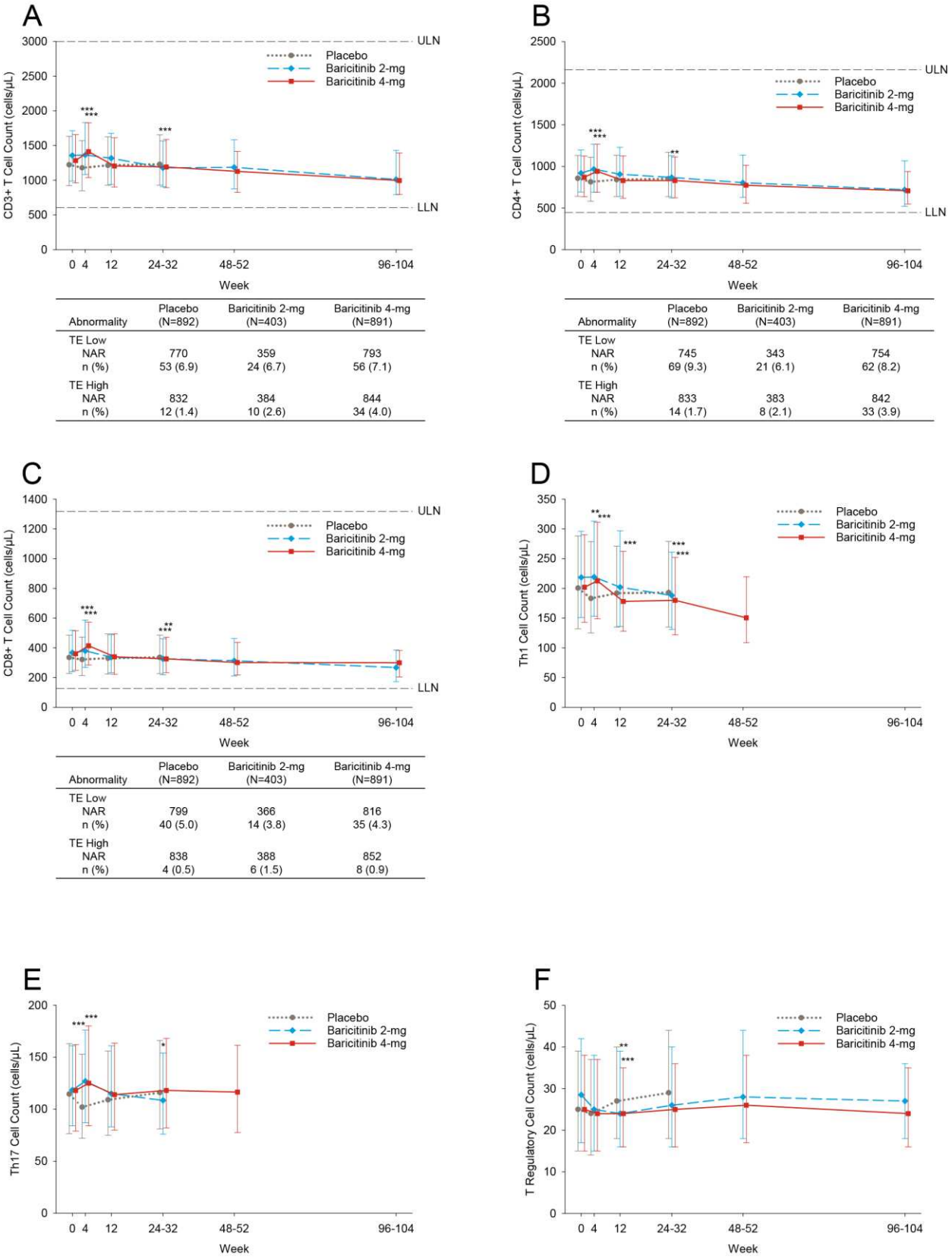
Lymphocyte subset changes in baricitinib-treated RA patients




| Abnormality | Placebo (N=892) | Baricitinib 2-mg (N=403) | Baricitinib 4-mg (N=891) |
|---|---|---|---|
| TE Low NAR n (%) | 810 87 (10.7) | 378 31 (8.2) | 827 74 (8.9) |
| TE High NAR n (%) | 849 37 (4.4) | 384 43 (11.2) | 850 101 (11.9) |

**Figure 1. Total lymphocyte counts throughout treatment**

Lymphocyte subset changes in baricitinib-treated RA patients



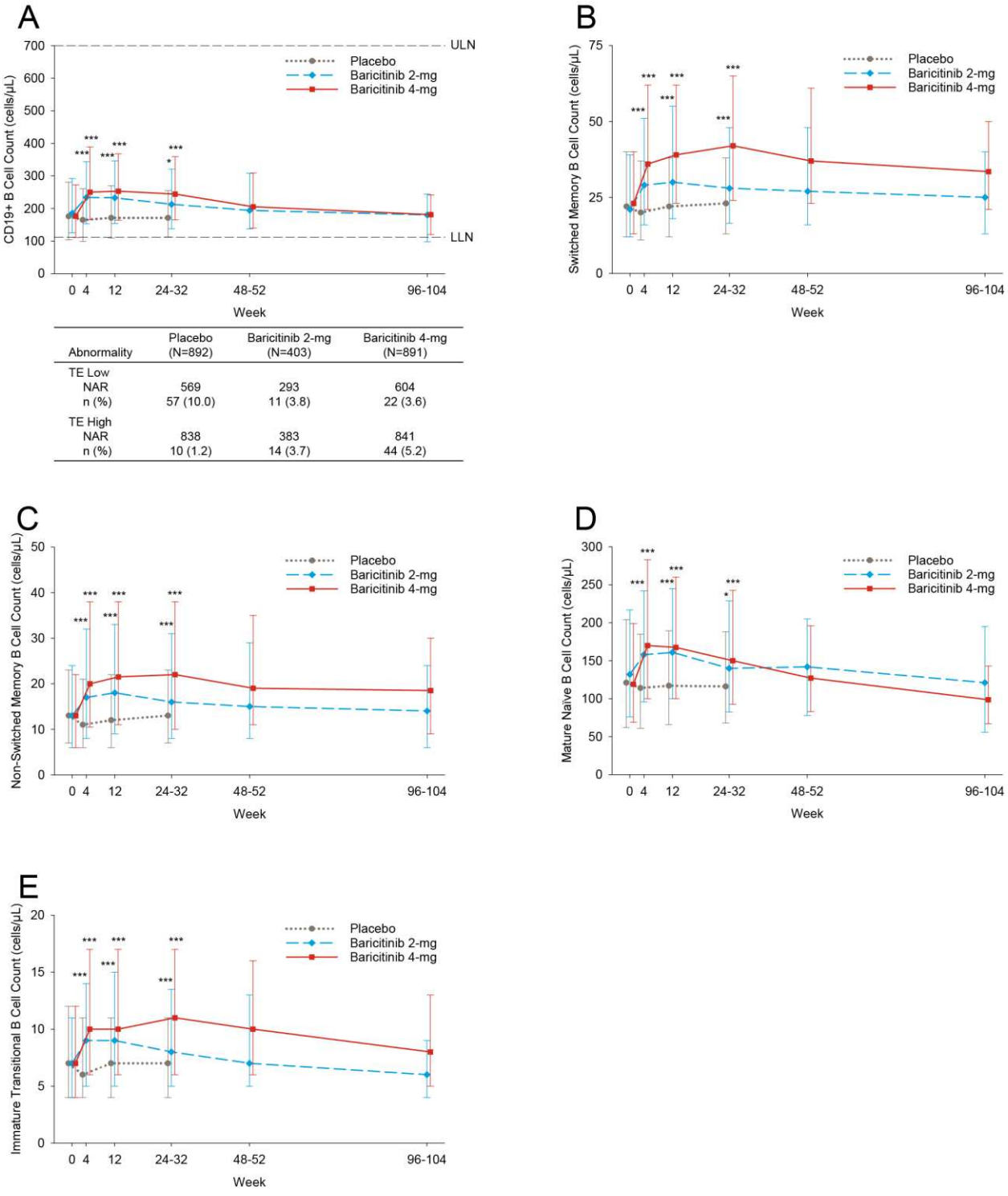

| Abnormality | Placebo (N=892) | Baricitinib 2-mg (N=403) | Baricitinib 4-mg (N=891) |
|---|---|---|---|
| TE Low NAR n (%) | 770 53 (6.9) | 359 24 (6.7) | 793 56 (7.1) |
| TE High NAR n (%) | 832 12 (1.4) | 384 10 (2.6) | 844 34 (4.0) |

| Abnormality | Placebo (N=892) | Baricitinib 2-mg (N=403) | Baricitinib 4-mg (N=891) |
|---|---|---|---|
| TE Low NAR n (%) | 745 69 (9.3) | 343 21 (6.1) | 754 62 (8.2) |
| TE High NAR n (%) | 833 14 (1.7) | 383 8 (2.1) | 842 33 (3.9) |

| Abnormality | Placebo (N=892) | Baricitinib 2-mg (N=403) | Baricitinib 4-mg (N=891) |
|---|---|---|---|
| TE Low NAR n (%) | 799 40 (5.0) | 366 14 (3.8) | 816 35 (4.3) |
| TE High NAR n (%) | 838 4 (0.5) | 388 6 (1.5) | 852 8 (0.9) |

**Figure 2. T cell counts throughout treatment**

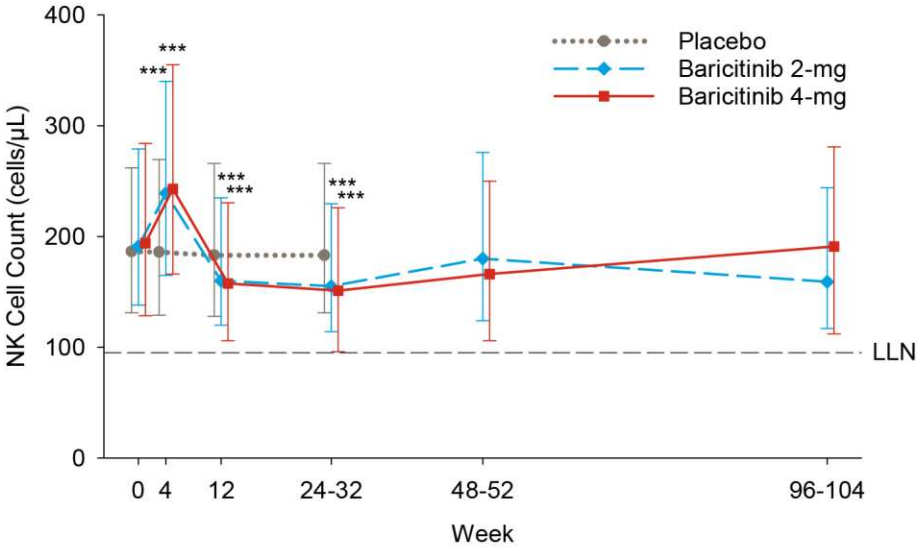
Lymphocyte subset changes in baricitinib-treated RA patients




| Abnormality | Placebo (N=892) | Baricitinib 2-mg (N=403) | Baricitinib 4-mg (N=891) |
|---|---|---|---|
| TE Low NAR n (%) | 569 57 (10.0) | 293 11 (3.8) | 604 22 (3.6) |
| TE High NAR n (%) | 838 10 (1.2) | 383 14 (3.7) | 841 44 (5.2) |

**Figure 3. B cell counts throughout treatment**

Lymphocyte subset changes in baricitinib-treated RA patients




| Abnormality | Placebo (N=892) | Baricitinib 2-mg (N=403) | Baricitinib 4-mg (N=891) |
|---|---|---|---|
| TE Low | | | |
| NAR | 737 | 352 | 742 |
| n (%) | 73 (9.9) | 52 (14.8) | 151 (20.4) |
| TE High | | | |
| NAR | 843 | 380 | 832 |
| n (%) | 13 (1.5) | 7 (1.8) | 37 (4.4) |

**Figure 4. NK cell count throughout treatment**