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A randomised study of rituximab and belimumab sequential therapy in PR3 ANCA-associated vasculitis

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Abstract

B-cell depletion with rituximab combined with glucocorticoids is routine therapy for ANCA-associated vasculitis (AAV), yet 10–30% of patients fail to achieve remission and relapse is common, particularly after rituximab withdrawal. We conducted a double-blind trial in 35 patients with active proteinase-3 (PR3)-AAV, comparing belimumab-rituximab (BEL-RTX) with placebo-rituximab (PBO-RTX) over 52 weeks, followed by 52 weeks of observation after treatment withdrawal. ANCA negativity occurred in 29.4% BEL-RTX and 13.3% PBO-RTX patients, with a trend toward faster PR3-ANCA clearance with BEL-RTX (adjusted HR 4.70, 95% CI 0.65–33.76, p=0.12). In patients with high baseline ANCA levels, BEL-RTX produced greater PR3-ANCA suppression. Faster remission and delayed total and naive B-cell reconstitution occurred with BEL-RTX. Relapses occurred in 35% BEL-RTX versus 60% PBO-RTX. PR3-ANCA re-emerged in 80% after belimumab withdrawal. Overall, BEL-RTX may enhance disease control but does not provide durable immunological reset.

Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV) is a group of multisystem autoimmune diseases characterised by necrotizing small-vessel inflammation and the presence of circulating ANCAs directed against the neutrophil cytoplasm proteases proteinase 3 (PR3) or myeloperoxidase (MPO)(1). Autoreactive B-cells and ANCA are central to pathogenesis, as ANCA-activated neutrophils drive endothelial injury and perivascular inflammation, and ANCA levels typically mirror disease activity at presentation and fall rapidly with effective immunosuppression(2).

B-cell depletion with the type 1 anti-CD20 monoclonal antibody rituximab (RTX) in combination with glucocorticoids is an effective remission induction strategy in AAV, yet 10–20% of patients fail to enter remission(3)(4). Peripheral B-cell reconstitution typically begins 4–6 months after RTX, and fixed-interval 6-monthly RTX re-dosing has become standard practice for maintenance therapy(5)(6). Despite this approach, even after two years of repeat RTX dosing, 50–60% of patients remain PR3-ANCA positive and relapse is common once RTX is withdrawn(6)(7). Prolonged RTX exposure can be complicated by hypogammaglobulinaemia and increases risk of serious infection(8). Mechanistically, RTX often fails to achieve complete B-cell depletion within lymphoid tissues, with persistence of CD20-negative B-cell populations and long-lived plasma cells(9), and CD20-positive B-cells can persist or re-emerge within granulomatous inflammatory lesions in refractory or relapsing PR3-AAV despite the absence of B-cells in blood(10)(11)(12)(13)(14).

A key goal for next generation B-cell-targeted therapies is to deepen tissue depletion of autoreactive B-cell populations and pathogenic autoantibody production; and to prevent the re-emergence of autoreactivity during B-cell repopulation, enabling an immunological “reset” and durable, treatment-free remission(15). BAFF (B-cell activating factor of the TNF receptor family), also known as BLyS (B-lymphocyte stimulator), is a survival cytokine for B-cells that is elevated in AAV and is thought to favour positive selection and persistence of autoreactive B-cells within tissue niches(16)(17)(18). Neutrophils and monocytes activated via C5a–C5aR1 signalling are key sources of BAFF/BLyS, providing a mechanistic link between complement-driven inflammation, B-cell activation and autoantibody production(19)(20)(21)(22), Belimumab (BEL), a monoclonal antibody that binds soluble BAFF/BLyS with high affinity, inhibits BAFF/BLyS-mediated signalling via three receptors on B-cells (particularly the BAFF receptor), resulting in a reduction in circulating B-cells through impaired maturation and survival(23). BEL is an approved adjunctive therapy for systemic lupus erythematosus (SLE) (24)(25) and reduces autoantibody levels and prolongs disease-free remission when used alongside rituximab for remission induction (26).

BAFF/BLyS inhibition before and during RTX therapy could enhance the depth of CD20-positive B-cell depletion achieved with RTX alone and broaden B-cell targeting in line with BAFF/BLyS-receptor expression across both CD20-positive and CD20-negative B-cell compartments. Class-switched, CD20-negative plasmablasts and plasma cells in germinal centres are key sources of IgG1 PR3-ANCA and may be particularly susceptible to BAFF/BLyS blockade(27). RTX is followed by a surge in circulating BAFF/BLyS that promotes B-cell reconstitution, and continued BEL during this phase imposes more stringent selection on emerging B-cell clones and limits the re-establishment of autoreactivity(28). On this basis, we designed COMBIVAS, a multicentre, randomised, double-blind, placebo-controlled experimental medicine trial (ClinicalTrials.gov NCT03967925) to define the mechanistic effects, potential clinical efficacy and capacity for immunological reset of sequential BEL plus RTX during both B-cell depletion and repopulation in patients with active PR3-AAV.

Results

Study participant characteristics

Between March 2019 and April 2022, 43 patients were screened and 35 were randomly assigned at five sites to BEL-RTX (n=17) or PBO-RTX (n=18). One participant allocated to PBO-RTX was withdrawn on day 6 due to incorrect AAV diagnosis. The modified intention-to-treat (mITT) population included 34 patients and the per protocol (PP) population included 32 patients. All patients received 2 g RTX with a standardised rapid glucocorticoid (GC) taper (complete withdrawal at 12 weeks) and no RTX maintenance immunosuppression, together with either weekly subcutaneous BEL 200 mg or matched placebo for 52 weeks, followed by 52 weeks of observation. Patient flow through screening, randomisation and follow-up is summarised in Figure 1. Baseline characteristics were well balanced between treatment groups (Table 1).

Primary outcome

Time to PR3-ANCA negativity was assessed in 32 of 34 patients in the the mITT population. Two patients from the RTX-PBO group were withdrawn from protocol early: one due to early major progressive disease requiring rescue therapy, and one due to a serious RTX reaction requiring unblinding. Of the 32 patients included in the primary PP analysis, PR3-ANCA negativity (<2 IU/L) was observed in 5 of 17 patients (29.4%) from the BEL-RTX group and in

2 of 15 patients (13.3%) from the RTX-PBO group [adjusted HR for time to PR3 ANCA negativity = 4.70, 95% confidence interval (CI) 0.65 to 33.76, p = 0.12] (Figure 2A). Cyclophosphamide use in the screening period did not affect time to ANCA negativity when adjusted for in the Cox model [adjusted HR = 1.73, 95% CI 0.31 to 9.47 p = 0.53].

Secondary outcomes

PR3 ANCA, lymphocyte subsets and BAFF/BLYS concentration

A rapid decline in PR3-ANCA occurred in both groups with comparable time to less stringent PR3-ANCA thresholds (<5 IU/L, <10 IU/L or ≥50% reduction from baseline, HRs 1.35–1.96, all p>0.18) and proportions of patients achieving PR3-ANCA negativity at 26, 52, 78 and 104 weeks between treatment arms (all p>0.05; Supplementary Table 1).

Among five BEL-RTX–treated patients who became PR3-ANCA negative, PR3-ANCA re-emergence occurred in all except one patient following BEL withdrawal (during weeks 52-104), indicating that 52 weeks of combination therapy induced PR3-ANCA suppression during active treatment, but did not achieve sustained response / immunological reset after belimumab withdrawal (Supplementary Figure 1A).

Peripheral blood B-cells were depleted in both groups by week 12 in the PP population but remained detectable at low levels by high-sensitivity flow cytometry, with no between-group differences in the residual $CD19^+$ cells [BEL-RTX: median 2128 cells/mL (IQR 1512–4309); RTX-PBO: median 1933 (IQR 1366–6750); p=0.93] or $CD19^+CD20^+$ cells [BEL-RTX: median 96 (IQR 54–247); RTX-PBO: median 111 cells/mL (IQR 71–1920); p=0.73]. B-cell reconstitution occurred earlier in the PBO-RTX group, which had higher absolute B-cell counts at week 52 (HLE for the median difference, –2.15; 95% CI –3.81 to –0.69; p=0.01) and week 78 (HLE –1.98; 95% CI –3.53 to –0.41; p=0.01), but not at week 104 (HLE –0.64; 95% CI –2.44 to 1.37; p=0.41). Between-group differences in total B-cell numbers were driven largely by naïve B-cells, with no differences at any time point in other B-cell subsets (Figure 2B-F). $CD4^+$ and $CD8^+$ T-cell and natural killer cell counts were comparable between groups throughout follow-up (Supplementary Figure 2).

In the PBO-RTX group, BAFF/BLyS concentrations rose after RTX and remained elevated throughout the trial, whereas in the BEL-RTX group BAFF/BLyS was neutralized during the 52-week treatment period and rebounded after BEL discontinuation. Peak BAFF/BLyS levels were observed at week 78 and coincided with accelerated B-cell repopulation in the BEL-RTX group (Figure 2G). These findings suggest that B-cell repopulation was delayed during BEL treatment, and was accelerated in a BAFF/BLyS-high environment following BEL withdrawal.

Clinical remission and progressive disease

In the mITT population, all patients allocated to BEL-RTX attained remission without minor or major disease progression, compared with 88% of those receiving PBO-RTX (median time to remission 42 vs 91 days; aHR 2.29, 95% CI 1.08–4.85; p=0.031) (Figure 3A). Early progressive disease occurred exclusively in the RTX-PBO arm (two with minor progressive disease managed with a temporary increase in glucocorticoid and one with major progressive disease requiring cyclophosphamide rescue and trial withdrawal) (Table 2).

Relapse

In the mITT cohort, relapses (minor or major) occurred in 6 of 17 (35.3%) of BEL-RTX treated patients and 9 of 15 (60%) of RTX-PBO-treated patients, with a trend towards delayed time to relapse in the BEL-RTX arm (HR 0.64, 95% CI 0.23–1.80) (Figure 3B). Cyclophosphamide use during screening did not affect time to remission or first relapse after adjustment in the Cox model (aHR for remission, 2.21; 95% CI, 1.01-4.83; p=0.083; aHR for first relapse, 0.64; 95% CI 0.22-1.84; p=0.41). No relapses occurred while patients remained PR3-ANCA-negative, and individuals who became ANCA-negative experienced fewer relapses after ANCA re-emergence during follow up than those who remained ANCA-positive throughout [1/7 (14%) vs 14/25 (56%); odds ratio 0.14, 95% CI 0.003–1.40] (Supplementary Figure 1B).

Safety

Safety was assessed in the intention-to-treat (ITT) population. Serious adverse events occurred in 6 of 17 patients (35.3%) in the BEL-RTX group and 6 of 17 (35.3%) in the RTX-PBO group. There were no differences in the incidence of infections, serious infections or withdrawal due to adverse events between groups (Table 3). During the treatment phase, serum IgG and IgM concentrations were lower in the BEL-RTX group at several timepoints (Supplementary Figure 3), and IgG hypogammaglobulinaemia was more common in the BEL-RTX group across all severity categories: 64.7% vs 41.1% for mild (IgG <7.0 g/L), 29.4% vs 5.8% for moderate (IgG <5.0 g/L), and 5.8% vs 0% for severe (IgG <3.0 g/L) hypogammaglobulinaemia. Differences in IgG and IgM levels diminished during the follow-up phase, suggesting a reversal effect of BEL on IgG and IgM levels.

Post hoc analyses

Longitudinal analyses of PR3-ANCA concentration demonstrated greater and more durable suppression of PR3-ANCA with BEL-RTX among patients with high baseline PR3-ANCA concentrations. In the high-PR3-ANCA stratum (≥50 IU/L), BEL-RTX was associated with a continued decline in PR3-ANCA level out to week 78 indicating a persistent incremental effect of BEL that was still evident at its withdrawal at week 52 and maintained through week 78. In contrast, among patients with baseline PR3-ANCA <50 IU/L, PR3-ANCA trajectories were similar in both treatment arms at all time points (Figure 3D-F).

Baseline PR3-ANCA concentration was also associated with clinical response. In the low PR3-ANCA stratum (<50 IU/L), relapse rates were similar between arms (1/4 [25%] with BEL-RTX vs 1/5 [20%] with RTX-PBO), whereas in the high-PR3-ANCA stratum (≥50 IU/L), relapse occurred in 5/13 (38.5%) BEL-RTX recipients and 8/10 (80%) RTX-PBO recipients (Figure 3C), consistent with a more pronounced mechanistic effect of BEL-RTX in patients presenting with higher PR3-ANCA levels.

Exploratory outcomes

B-cell receptor repertoire dynamics

High-throughput B-cell receptor (BCR) sequencing was performed to define treatment-associated changes in B-cell composition and clonal structure beyond peripheral immunophenotyping. This was performed on a subset of PP samples meeting predefined quality control criteria (>1,000 high-confidence BCR sequences per sample) taken at baseline, week 52 and week 104 (n=13 BEL-RTX, n=14 RTX-PBO).

BEL did not alter immunoglobulin isotype usage at any time point, indicating preserved global class-switch recombination across switched and unswitched compartments (Supplementary Figure 4.1A). In contrast, some measures of clonality increased with BEL-RTX. At 52 weeks, D5 and D50 indices (proportion of sequences occupied by the top 5 and 50 clones) were higher in IGHG1 and total BCRs, respectively in BEL-RTX recipients (Supplementary Figure 4.1B-C), and clonal diversity index (measure of unevenness of clone diversity) was elevated in IGHG2 at 52 weeks (Supplementary Figure 4.2A). These findings are consistent with contraction of B-cell diversity following depletion(29) and indicate progressive narrowing of the circulating repertoire, most evident in class-switched compartments. BEL-RTX was associated with selective depletion of autoreactivity-associated features. The reduced IGHV4-34 usage containing the autoreactive NHS/AVY motif in IGHG2 in the BEL-RTX group and reduced signatures of secondary IGHV rearrangement in IGHA1 at week 104 are consistent with more stringent B-cell selection (Supplementary Figure 4.2B-C). Somatic hypermutation

(SMH) frequencies were unchanged between groups at all time points, indicating no global effect on SHM accumulation (Supplementary Figure 4.3).

Together, BEL in combination with RTX induced contraction of BCR diversity with increased clonality and preferential depletion of autoreactive class-switched B-cell populations. Clonal overlap between individuals was unchanged, indicating reshaping of repertoire composition rather than inter-individual clonal sharing.

Patient-reported outcomes

Between-group differences in change from baseline in patient-reported outcomes (assessed using an AAV-specific patient-reported outcomes tool(30)) were generally small across domains and time points. In this instrument, higher scores indicate better patient-reported status. Social and emotional impact scores were higher in the BEL-RTX group at week 12 (median difference 0.96, 95%CI 0.10-1.79; p=0.02), and physical function scores were higher at weeks 24 (0.81, 95%CI 0.00-1.98; p=0.03) and 78 (0.90, 95%CI 0.00-2.05; p=0.03), with borderline significance at week 52 (1.12, 95%CI −0.00-2.93; p=0.05), although these effects were not sustained. The systemic symptom score reflects the burden of general vasculitis-related symptoms, with higher scores indicating fewer or less severe symptoms; systemic symptom scores were higher in the BEL-RTX group at week 104 (0.75, 95%CI 0.01-1.54; p=0.04) (Supplementary Table 2). Overall, these data suggest modest, domain-specific improvements in selected patient-reported outcomes with BEL-RTX versus PBO-RTX over 104 weeks.

Discussion

The COMBIVAS trial was designed to assess whether BAFF/BlyS inhibition enhances the biological and clinical effects of RTX in PR3 AAV and whether effects persist after treatment withdrawal. Although the primary endpoint of PR3-ANCA negativity did not differ between groups, BEL-RTX was associated with faster remission, greater PR3-ANCA suppression in patients with high baseline PR3-ANCA, and delayed naïve and total B-cell reconstitution. These findings provide evidence for a meaningful treatment effect of BEL-RTX in AAV, consistent with deeper depletion of germinal centre, memory and plasma cell populations in nasal-associated lymphoid tissue (NALT) at 12 weeks with BEL-RTX (Nature Medicine companion paper, Tan et al).

Recognising the central role of ANCA in AAV pathogenesis, the strong association of PR3-ANCA with clinical outcomes after RTX, and the persistence of low-level PR3-ANCA positivity in the majority of patients reflecting a high relapse risk, we included only PR3-ANCA disease; the AAV subtype with greatest unmet need for enhanced B-cell depletion and disease control(31). Granulomatous inflammation of the upper and lower respiratory tract occurs frequently in PR3 AAV and incomplete B-cell depletion 12 weeks post RTX in PR3 AAV has previously been observed in NALT, a disease relevant lymphoid site (McClure thesis unpublished). Sequential sampling of NALT in a subset of COMBIVAS trial patients allowed detailed analyses of tissue responses before and 12 weeks after treatment initiation (Nature Medicine companion paper, Tan et al).

Mechanistic readouts across tissue and blood were consistent. As described in our companion paper, patients with highest baseline PR3-ANCA exhibited germinal centre B-cell expansion in NALT (Nature Medicine companion paper, Tan et al), suggesting local tissue autoantibody production, and defining a patient subgroup who may derive greatest benefit from enhanced B-cell depletion. In the PR3-ANCA high subgroup, post hoc analyses revealed that BEL-RTX was associated with greater incremental PR3-ANCA suppression throughout the 52 week treatment phase, analogous to the progressive reduction in anti-dsDNA antibodies observed with BEL in SLE(32). Failure to achieve a stable nadir in PR3-ANCA by week 52, suggests that belimumab treatment beyond 52 weeks may be beneficial. Conversely in patients with low baseline PR3-ANCA, irrespective of treatment group, PR3-ANCA suppression to low levels occurred, suggesting that the addition of BEL provided greatest benefit in those with highest baseline PR3-ANCA and germinal centre B-cell expansion.

After BEL withdrawal, initial contraction of the B-cell repertoire, increased clonality, and selective loss of autoreactivity-associated features was observed, particularly in class-switched compartments, supporting a role for BAFF/BLyS inhibition in refining peripheral tolerance during B-cell reconstitution rather than inducing a complete immunological reset. However, mechanistic effects observed during and early after BEL treatment withdrawal were not sustained. BAFF/BLyS levels rose sharply after the expected washout period (33), exceeding levels observed with RTX alone, in keeping with increased BAFF transcripts in NALT cells at 12 weeks (Nature Medicine companion paper, Tan et al). The high BAFF/BlyS environment coincided with accelerated naïve B cell repopulation and re-emergence of PR3-ANCA in almost all previously negative patients(34), Relapses occurred in both groups although there was a trend towards fewer or later relapse in the BEL-RTX group. No relapses occurred during periods of ANCA negativity, and the majority of relapses by 24 months occurred in those with high baseline ANCA, emphasising the association between

autoantibody and tissue B cell activity with disease recurrence. Overall, incomplete ANCA suppression at week 52 week and the high BAFF/BLyS environment after BEL withdrawal, favoured the re-emergence of autoreactivity and prevented immunological reset.

The relapse rate in the BEL-RTX group at 24 months (35%) was higher than rates reported in rituximab maintenance trials(5)(6), reflecting the high risk PR3-ANCA population, rapid glucocorticoid taper, absence of maintenance therapy and that durable immunological reset was not achieved. The pattern of incremental PR3-ANCA suppression and delayed B-cell repopulation observed, suggests that longer BAFF/BLyS inhibition maybe required to achieve sustained remission, analogous to observations in SLE(35). Achieving complete tissue B-cell depletion and autoantibody suppression is a particular challenge in SLE, where B-cell expansion, high BAFF/BlyS levels, and Fc gamma receptor polymorphisms frequently occur(36) and contribute to rituximab's failure to achieve complete peripheral B-cell depletion resulting in limited clinical effect(37). Even when combined with BAFF/BLyS inhibition, the varying clinical outcomes from BEL-RTX trials in SLE and Sjorgren's disease highlight the challenge in achieving complete B cell depletion and durable autoantibody suppression with a single RTX cycle(26)(38)(39). In AAV, where rituximab produces more effective peripheral B-cell depletion and greater clinical efficacy, the incremental benefit required from BEL is smaller and clinical priorities include faster remission, reduced glucocorticoid and cyclophosphamide exposure, and more sustained remission without prolonged immunodeficiency. Despite differences in treatment paradigms, experience from BEL-RTX trials in both SLE and AAV highlights the need for deeper initial tissue B-cell depletion and prolonged control of BAFF/BLyS-driven autoreactivity to deliver meaningful clinical benefit.

The frequency and pattern of adverse events observed in this study was consistent with a severe AAV population (40)(3). BEL-RTX was generally well tolerated, with similar rates of serious adverse events, infections and treatment discontinuation in both groups. Hypogammaglobulinaemia was more frequent in the BEL-RTX group, consistent with observations from SLE and Sjogren's disease(39). Unlike hypogammaglobulinaemia associated with repeated RTX dosing in AAV, which is typically associated with prolonged B-cell depletion and suggests an underlying defect in B-cell repopulation(41), the IgG decline with BEL-RTX was abrupt, plateaued during treatment, and improved after discontinuation. Long-term follow-up is required to determine the stability and clinical significance of this pattern particularly given the association between persistent hypogammaglobulinaemia and infection risk(42).

This trial has limitations. The small sample size and large effect size prediction, may have limited power for the primary endpoint. Permitted immunosuppression at screening may have influenced early mechanistic and clinic endpoints. Restriction to PR3-ANCA positive disease, limits generalisability of findings and further studies are needed in MPO-ANCA disease.

Conclusion

In summary, BEL-RTX was associated with faster remission, greater PR3-ANCA suppression in patients with ANCA highest baseline levels, and delayed B-cell repopulation, supported by tissue findings of deeper B cell depletion, contraction of T follicular helper support, and reprogramming of BAFF/BLyS-dependent survival pathways with BEL-RTX (Nature Medicine companion paper, Tan et al). However, 52 weeks of BEL was insufficient to eliminate ANCA in most patients, and the high BAFF/BlyS environment following treatment withdrawal favoured re-emergence of PR3-ANCA and relapse. These findings support further study of RTX combined with longer term BAFF/BLyS inhibition, to determine whether more durable antibody suppression, sustained remission, and longer-term control of autoreactivity can be achieved in AAV.

Online methods

Trial design and oversight

This multicentre, randomised, double-blind, placebo-controlled phase 2b trial evaluated the mechanistic and clinical effects of sequential belimumab–rituximab therapy in adults with PR3 ANCA–positive AAV at five UK tertiary centres. The study design and protocol have been described previously(43). The trial complied with the Declaration of Helsinki, International Council for Harmonisation Good Clinical Practice and applicable regulatory requirements, and was approved by a UK research ethics committee and local institutional review boards; all participants provided written informed consent before any trial-specific procedures.

Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge acted as joint academic sponsors, with the Cambridge Clinical Trials Unit responsible for trial management, data management, monitoring and pharmacovigilance. An independent steering committee and an independent data and safety monitoring board (DSMB) oversaw the trial; the DSMB reviewed unblinded safety data at least every 6 months and could recommend protocol amendments or early termination for safety concerns. Remission and relapse events were adjudicated by a blinded, independent clinical end point committee using prespecified Birmingham Vasculitis Activity Score for Wegener's Granulomatosis (BVAS/WG)–based criteria (Supplementary Table 3). Statistical analyses were undertaken by an independent academic statistical centre at Newcastle University.

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Industry partnership and funding

This trial was funded by an academic grant from the U.K. Medical Research Council through the UKRI EMINENT industry-partnered collaboration with GlaxoSmithKline. GlaxoSmithKline provided belimumab, matching placebo, and an additional grant to support trial conduct, and contributed scientific input into trial design and data interpretation, but had no role in trial conduct, data collection, endpoint adjudication or the decision to submit the manuscript.

Patients

Eligible patients were aged 18 years or older, met Chapel Hill Consensus Conference criteria for AAV(44) and had PR3 ANCA–positive, active disease that was either newly diagnosed or relapsing. Active disease was defined as at least one major or three minor BVAS/WG(45) items together with a concurrent positive PR3 ANCA measured by enzyme-linked immunosorbent assay (ELISA). Key exclusion criteria were MPO-ANCA or anti–glomerular basement membrane antibody positivity, pulmonary haemorrhage with hypoxia, estimated glomerular filtration rate less than 15 ml min^{-1} 1.73 m^{-2}, previous biological B-cell–depleting therapy within 12 months, more than three intravenous cyclophosphamide doses within 6 months, undetectable peripheral blood B-cells, IgG less than 400 mg dl^{-1}, acute or chronic severe infection or malignancy within 5 years before screening. To allow safe recruitment of patients with severe presentations, up to 3 g intravenous methylprednisolone (or equivalent oral prednisolone) was permitted in the 30 days before enrolment and during screening. Full inclusion and exclusion criteria are provided in Supplementary Table 4.

Randomisation, masking and treatment

Participants were randomly assigned (1:1) to rituximab plus belimumab (BEL-RTX) or rituximab plus placebo (RTX-PBO) using a central, automated web-based system with permuted blocks and stratification by screening PR3 ANCA level (low versus high, threshold 24 IU/L, the median baseline concentration in a historical cohort(31)). Allocation was concealed using unique medication pack codes; participants, investigators, site staff, outcome

assessors and statisticians remained blinded to treatment assignment, with unblinded information restricted to DSMB members and a designated unblinded coordinator for safety oversight.

BEL 200 mg or matching placebo was administered subcutaneously once weekly from day 1 through week 52. RTX 1000 mg was given intravenously on days 8 and 22. All patients received oral prednisolone 20 mg per day starting on day 1, followed by a protocol-defined taper aiming for complete cessation by week 12. Concomitant azathioprine or rituximab remission-maintenance therapy and other B-cell–depleting or biological immunomodulatory agents were prohibited; cyclophosphamide and plasma exchange were not permitted after randomisation except as rescue, in which case patients were censored or withdrawn as defined below. Pneumocystis jirovecii prophylaxis and standard bone, gastric and vitamin D prophylaxis were recommended in line with local guidance. Patients were followed until week 104.

Endpoints

The primary endpoint was time from first trial dose to PR3 ANCA negativity, defined as PR3 ANCA less than 2.0 IU/L measured by a commercially available EliA fluoro enzyme immunoassay on the Phadia 2500/5000 platform in a central laboratory. Secondary biomarker endpoints included: change from baseline in PR3 ANCA; time to 50% reduction in PR3 ANCA; time to PR3 ANCA less than 5.0 IU/L and less than 10.0 IU/L; proportions of patients with PR3 ANCA negativity and sustained negativity at prespecified visits (weeks 12, 24, 52, 78 and 104); time to PR3 ANCA rise, defined as at least a 25% relative increase from nadir and an absolute increase of at least 10 IU/L; and changes in total B-cells, B-cell subsets (naïve, transitional, memory, activated, plasmablast and plasma cells), CD4 and CD8 T cells and natural killer cells in peripheral blood at weeks 12, 24, 52, 78 and 104.

Clinical secondary endpoints were time to remission, time to first relapse (major or minor), time to first major relapse, proportions of patients in remission at weeks 26, 52 and 104 and proportions with progressive disease before remission (major or minor, and major only). Remission, complete remission, minor and major progressive disease, relapse, minor relapse, major relapse and threatened major relapse were defined using BVAS/WG-based criteria and corticosteroid dose thresholds (Supplementary Table 3). Safety endpoints included the incidence of serious adverse events (SAEs) and adverse events of special interest (AESIs), including serious and opportunistic infections, infusion and injection reactions, hypogammaglobulinaemia, malignancy and other prespecified events (Supplementary Table 7), up to week 104.

Treatment of relapse and discontinuation

Patients developing major progressive disease (new or worsening major BVAS/WG items before remission) discontinued protocol therapy and received best local standard-of-care immunosuppression. After remission, a first minor relapse was treated within protocol using a standardised prednisolone regimen (no more than 30 mg per day for 1 week, then 5 mg decrements every 2 weeks to 5 mg per day). A second minor relapse, a first major relapse or a first threatened major relapse triggered withdrawal from trial treatment and management according to local practice. Additional discontinuation criteria included use of prohibited medications, unacceptable toxicity (including severe infection or severe hypogammaglobulinaemia), pregnancy or significant non-adherence.

Analysis populations and statistical analysis

Three prespecified analysis populations were used. The intention-to-treat (ITT) population comprised all randomised patients who received at least one dose of belimumab or placebo. The modified ITT (mITT) population included all ITT participants, with follow-up censored at initiation of cyclophosphamide for major progressive disease or rituximab retreatment for relapse. The per-protocol (PP) population comprised randomised patients who received at least 3 months of belimumab or placebo with at least 8 injections in the first 12 weeks, had no prohibited medications (with censoring at receipt of cyclophosphamide or rituximab for relapse), adhered to the corticosteroid taper (no more than one deviation above the taper in the first 12 weeks and, thereafter, mean prednisolone 0 ±10 mg per day with at most one temporary increase for minor flare) and had no major protocol deviations expected to affect mechanistic outcomes.

Baseline characteristics are summarised as medians (range) for continuous variables and counts (percentages) for categorical variables in the ITT population. The primary endpoint was analysed in the PP population with a Cox proportional hazards model using exact methods for ties and adjusting for treatment group (belimumab versus placebo) and baseline PR3 ANCA category (low versus high at 24 IU/L); results are reported as hazard ratios with 95% confidence intervals and p-values from likelihood ratio tests, with hazard ratios less than 1 indicating a longer time to PR3 ANCA negativity. Key secondary biomarker endpoints were evaluated in the PP population using Wilcoxon rank-sum tests, with Hodges–Lehmann estimates of median between-group differences and corresponding non-parametric 95% confidence intervals. Time-to-event clinical endpoints (time to remission, time to first relapse and time to first major relapse) were analysed in the mITT population with Cox models using the same covariates as for the primary analysis. Safety outcomes, including SAEs and AESIs,

were summarised descriptively in the ITT population; p-values and confidence interval widths were not adjusted for multiple comparisons and are not intended for confirmatory hypothesis testing.

Sample size

This exploratory phase 2b trial used a feasibility-based sample size, supported by assumptions about treatment effect. RTX is known to reduce PR3-ANCA levels and induce remission, and preliminary data from RTX-treated AAV cohorts indicated a median time to PR3 ANCA ELISA negativity of approximately 14 months. On this basis, a total of 30 patients (15 per arm) provided around 80% power at a two-sided 5% significance level to detect a reduction in median time to PR3 ANCA negativity to 3 months with rituximab–belimumab, using a log-rank test. Recruitment and protocol adherence were monitored to ensure that approximately 30 participants were evaluable in the per-protocol analysis.

Flow cytometric immunophenotyping

Flow cytometric immunophenotyping for secondary endpoints was performed in a central GlaxoSmithKline laboratory using a validated whole-blood panel. Major lymphocyte and B-cell subsets were defined according to pre-specified marker combinations, as detailed in Supplementary Table 5. Absolute counts were obtained using single-platform flow cytometry with TruCount tubes, and relative frequencies were derived from standardised gating strategies applied uniformly across all samples. In parallel, peripheral blood mononuclear cells (PBMCs) were isolated and analysed in university laboratories using an extended T-cell immunophenotyping panel, with marker combinations specified in Supplementary Figure 6, to provide higher-resolution characterisation of circulating T-cell subsets.

B-cell receptor repertoire analysis

BCR libraries were prepared from RNA samples extracted from PBMCs. BCR VHs were first amplified using a protocol we have previously described(29). Briefly, RNA was reverse transcribed to cDNA using a mixture of IgA/D/E/G/M isotype specific primers, incorporating 15 nucleotide UMIs. The resulting cDNA was used as template for PCR amplification using a set of 6 FR1-specific forward primers including sample-specific barcode sequences (7 nucleotides) along with a reverse primer specific to the RT primer. For three of the replicate libraries, a modified primer set was used where the sample-specific barcode was instead incorporated into the reverse transcription primers after the UMI. BCR VH amplicons (~450 bp) were quantified by TapeStation (Beckman Coulter) and gel-purified. Dual-indexed

sequencing adapters (KAPA) were ligated onto ≤500 ng of amplicon per sample using the HyperPrep library construction kit (KAPA). The adapter-ligated libraries were finally PCR-amplified (initial denaturation at 95°C for 1 min, for 2-83 cycles at (98°C for 15 s, 60°C for 30 s, 72°C for 30s, and final extension at 72°C for 1 min). The libraries were sequenced on an Illumina MiSeq using the 2 x 300 bp chemistry.

Raw MiSeq reads were filtered for base quality (median Phred score >32) using QUASR (http://sourceforge.net/projects/quasr/). MiSeq forward and reverse reads were merged together if they contained identical overlapping regions of >50bp, or otherwise discarded. Universal barcoded regions were identified in reads and orientated to read from V-primer to constant region primer. The barcoded region within each primer was identified and checked for conserved bases (i.e. the T's in NNNNTNNNNTNNNNT). Primers and constant regions were trimmed from each sequence, and sequences were retained only if there was >80% sequence certainty between all sequences obtained with the same barcode, otherwise discarded. The constant region allele with highest sequence similarity was identified by 10-mer matching to the reference constant region genes from the IMGT database(46), and sequences were trimmed to give only the region of the sequence corresponding to the variable (V-D-J) regions. Isotype usage information for each IgH was retained throughout the analysis hereafter. Sequences without complete reading frames and non-immunoglobulin sequences were removed and only reads with significant similarity to reference IgHV and J genes from the IMGT database were retained using BLAST. IgH gene usages and sequence annotation was performed in IMGT V-QUEST, and sample-level repertoire statistics were computed as previously published(29).

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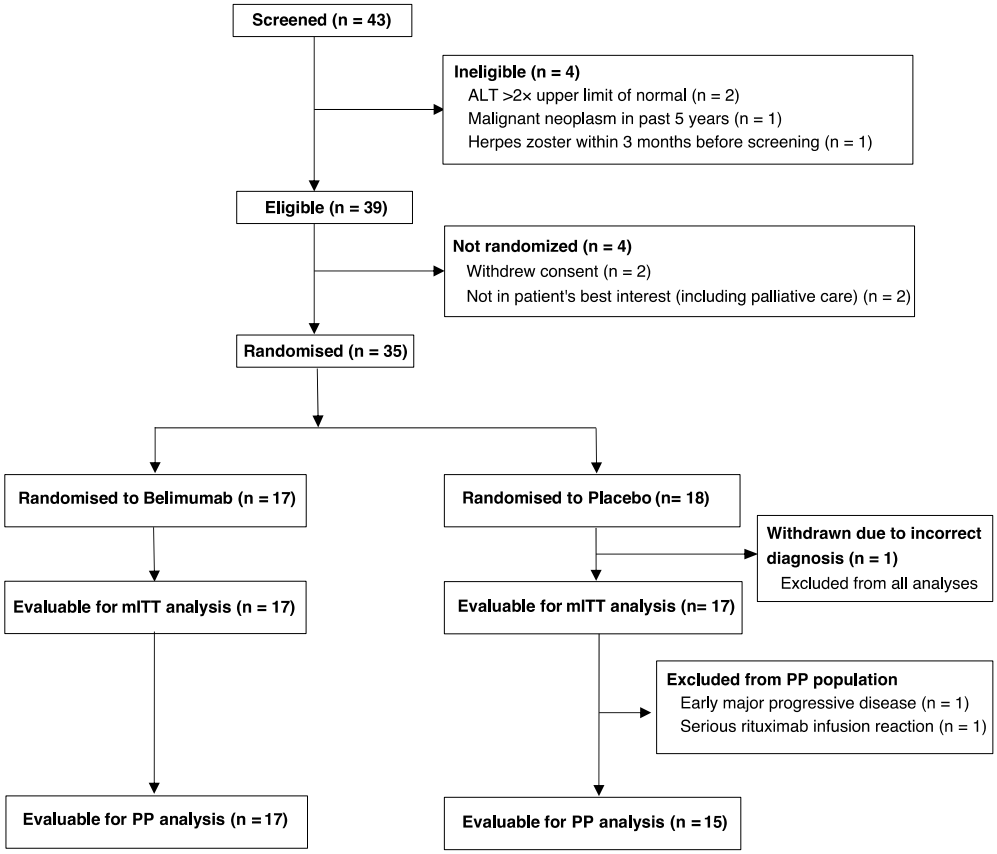
Figure 1. CONSORT: participant flow and analysis populations



mITT, modified intention-to-treat; PP, per protocol; ALT, alanine aminotransferase.

Table 1. Baseline demographics and clinical characteristics

Characteristic	BEL-RTX	PBO-RTX
mITT population, n	17	17*
PP population, n	17	15
Age, years, median (range)	59 (22–78)	52 (26–79)
Male sex, n (%)	13 (76)	10 (59)
Race, Asian / White, n (%)	0 (0) / 17 (100)	1 (5.9) / 16 (94.1)
Relapsing, n (%)	8 (47)	10 (58)
BVAS-GPA score at Day 1, median (range)	6 (3–19)	6 (3–11)
Prednisolone before Day 1, mg/day	20 (0–40)	20 (0–40)
Methylprednisolone during screening, n (%)	6 (35)	10 (58)
Cumulative dose, g, median (range)	1.5 (0.5–1.5)	1.0 (0.5–1.5)
CYC ever, n (%)	10 (59)	8 (47)
Cumulative dose, g, median (range)	3.4 (0.5–12.0)	6.0 (1.1–10.0)
CYC during screening, n (%)	5 (29)	2 (11.7)
Cumulative dose, g, median (range)	0.8 (0.5–1.2)	1.1 (1.1–1.2)
RTX ever, n (%)	7 (41)	4 (23.5)
Cumulative dose, g, median (range)	3.0 (2.0–8.0)	4.4 (2.0–7.8)
Creatinine, µmol/L, median (range)	85 (49–330)	69 (41–113)
CRP at Day 1, mg/dL, median (range)	11 (4–192)	16 (2–366)
PR3-ANCA at Day 1, IU/L, median (range)	65.0 (5.1–1580)	87.5 (10–3300)

Table 1. Baseline demographics and clinical characteristics. mITT, modified intention-to-treat; PP, per protocol; GPA, granulomatosis with polyangiitis; BVAS-GPA, Birmingham Vasculitis Activity Score for GPA; CRP, C-reactive protein; PR3, proteinase 3; RTX, rituximab; CYC, cyclophosphamide. *Thirty-five patients were randomised (17 to BEL-RTX and 18 to RTX–placebo); one PBO-RTX participant was diagnosed with acute myeloid leukaemia shortly after enrolment and was excluded from the mITT population.

Figure 2. PR3-ANCA negativity, B cell reconstitution and serum BAFF/BLyS

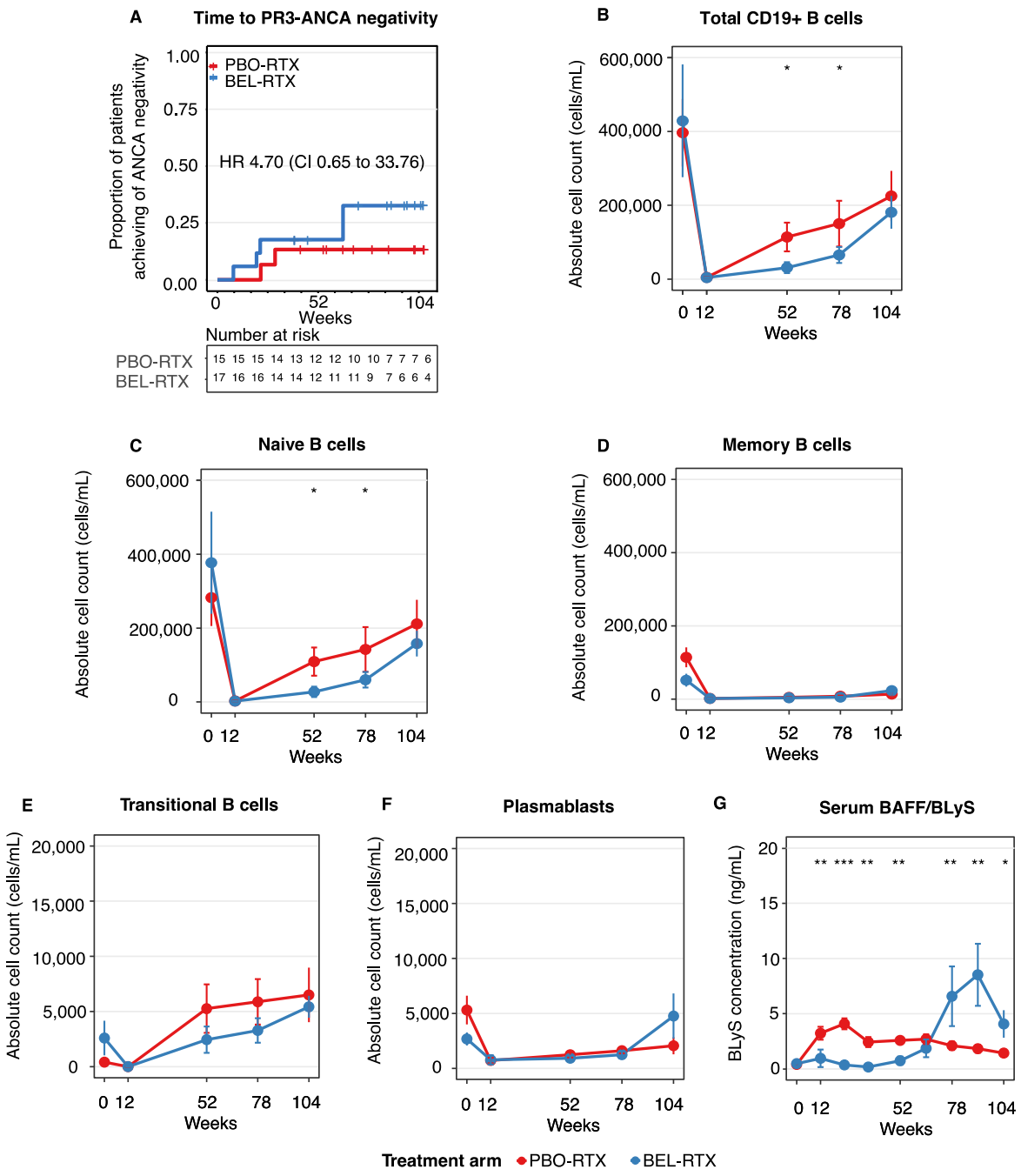


Figure 2. PR3-ANCA negativity, B cell reconstitution and serum BAFF/BLyS. A, time to PR3-ANCA negativity is shown as a Kaplan–Meier curve with hazard ratios (adjusted for baseline PR3-ANCA levels) comparing PBO-RTX and BEL-RTX, with numbers at risk displayed below the plot. B–F, absolute cell counts of total CD19⁺ B cells (B), naïve B cells (C), memory B cells (D), transitional B cells (E) and plasmablasts (F) over time, plotted as treatment-arm means with error bars denoting s.e.m.; between-arm differences at prespecified visits were assessed using Wilcoxon rank-sum tests with Hodges–Lehmann estimators and non-parametric 95% confidence intervals. G, serum BAFF/BLyS concentrations over time in weeks, plotted as mean ± s.e.m. by treatment arm; between-arm differences were assessed using mixed-effects models. Asterisks indicate between-arm P values with statistical

significance denoted by *, ** and *** for P<0.05, P<0.01 and P<0.001, respectively. All analyses in this figure are from the per-protocol population. PR3, proteinase 3; ANCA, anti-neutrophil cytoplasmic antibodies; BLyS, B-lymphocyte stimulator; RTX, rituximab; PBO, placebo; BEL, belimumab; CD19, cluster of differentiation 19; s.e.m., standard error of the mean.

Table 2. Clinical outcomes in the mITT population

Outcome	BEL-RTX	PBO-RTX
Remission, n/N (%)	17/17 (100)	15/17 (88)
Progressive disease before remission, n/N (%)	0/17 (0)	3/17 (17)
Relapse after remission, n/N (%)	6/17 (35)	9/15 (60)
Relapse or progressive disease, n/N (%)	6/17 (35)	10/17 (58)
RTX or CYC retreatment, n/N (%)	6/17 (35)	10/17 (58)

Table 2. Clinical outcomes in the mITT population. mITT, modified intention-to-treat; RTX, rituximab; BEL, belimumab; PBO, placebo; CYC, cyclophosphamide.

Figure 3. Clinical endpoints and effect of baseline PR3-ANCA

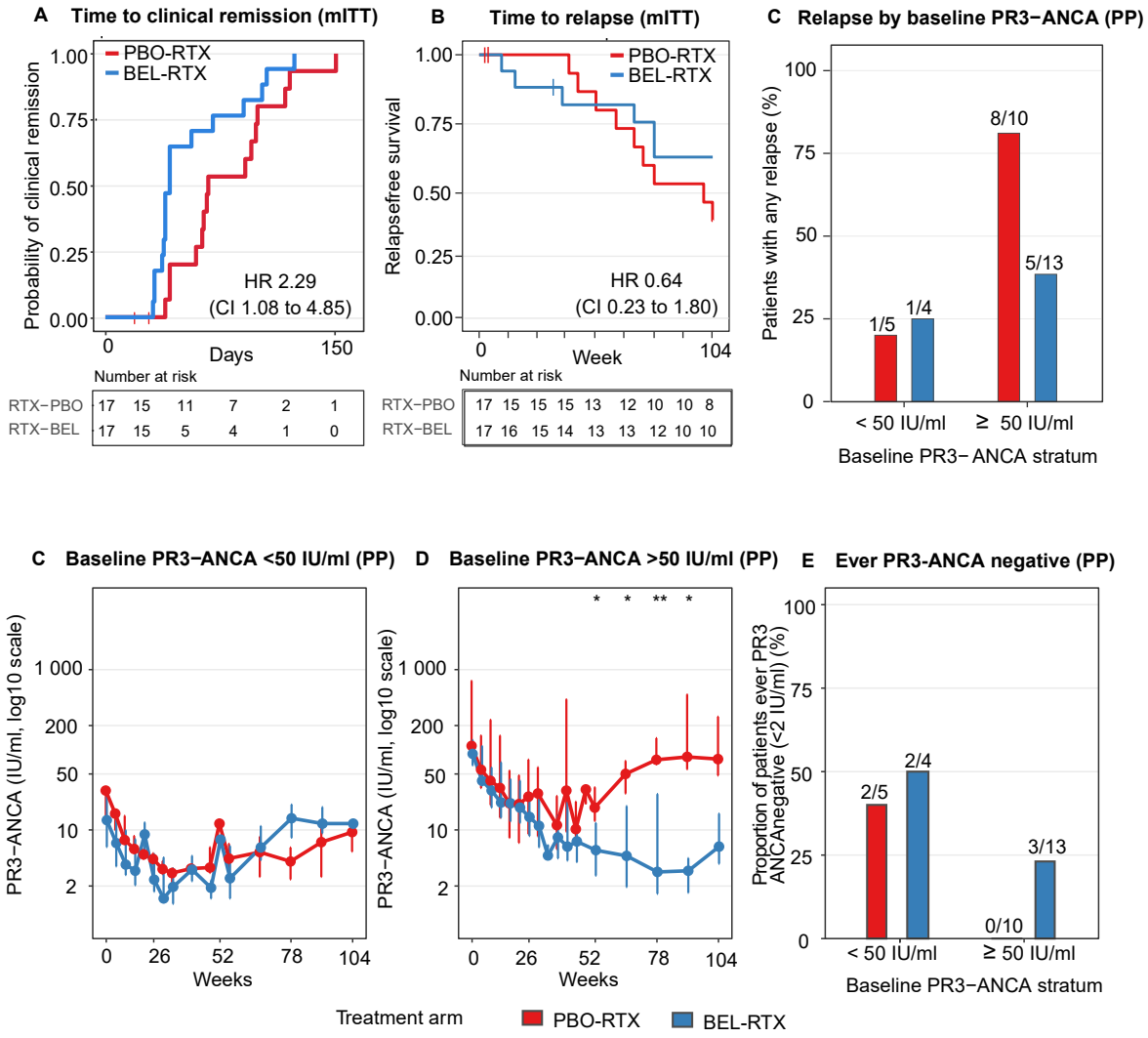


Figure 3. Clinical endpoints and effect of baseline PR3-ANCA. A, time to clinical remission and B, time to first relapse in the modified intention-to-treat (mITT) population are shown as Kaplan–Meier curves, with hazard ratios adjusted for baseline PR3-ANCA comparing PBO-RTX with BEL-RTX. C, proportions of patients in the per-protocol (PP) population with any relapse during follow-up are shown by baseline PR3-ANCA stratum (<50 or ≥50 IU/ml) and treatment arm, with bars labelled n/N. D, PR3-ANCA trajectories in the PP population, stratified by baseline PR3-ANCA (<50 or ≥50 IU/ml), are displayed as log10-scale line plots by treatment arm, censored at rituximab retreatment; thin lines show individual patients and coloured lines with IQR error bars show treatment-arm medians at each visit. Between-arm differences at each visit are estimated from a linear mixed-effects model of log10-PR3-ANCA over time, adjusted for baseline log10-PR3-ANCA, with statistical significance denoted by *, ** *and* ** for P<0.05, P<0.01 and P<0.001, respectively. E, proportions of PP patients who ever achieved PR3-ANCA <2 IU/ml during follow-up are shown by baseline PR3-ANCA stratum and treatment arm, with bars labelled n/N (percentage). ANCA, anti-neutrophil cytoplasmic antibody; PR3, proteinase 3; RTX, rituximab; PBO, placebo; BEL, belimumab; mITT, modified intention-to-treat; PP, per protocol.

Table 3. Adverse Events

Event	BEL-RTX	RTX-PBO
N (ITT population)	17	17
Deaths, n (%)	0 (0%)	0 (0%)
Patients with AE leading to discontinuation of intervention, n (%)	1 (6%)	1 (5.8%)
SAEs		
Patients, n (%)	6 (35.3%)	6 (35.3%)
Events, n	8	10
Patients with infection-related SAEs, n (%)	1 (5.8%)	3 (17.6%)
Patients with infection-related SAEs (events), n	1	4
Patients with non–infection-related SAEs, n (%)	6 (35.3%)	5 (29.4%)
Patients with non–infection-related SAEs (events), n	7	6
AESIs		
Patients with infection-related AESIs, n (%)	15 (88.2%)	16 (94.1%)
Patients with infection-related AESIs (events), n	41	33
Ear nose throat	10	5
Gastrointestinal	1	0
Genito-urinary	8	5
Oral	0	1
Respiratory	13	13
Skin	5	7
COVID-19	5	2
Patients with non–infection-related AESIs, n (%)	4 (23.5%)	2 (11.7%)
Patients with non–infection-related AESIs (events), n	6	2
Hypogammaglobinaemia		
Mild (IgG <7.0 g/L), n (%)	11 (64.7%)	7 (41.1%)
Moderate (IgG <5.0 g/L), n (%)	5 (29.4%)	1 (5.8%)
Severe (IgG <3.0 g/L), n (%)	1* (5.8%)	0 (0%)
Systemic infusion/injection reactions	2	1
Hypersensitivity reactions	1	1
Malignancy	1	1
Psychiatric events	2	0
Severe skin reactions (including TEN/SJS)	0	0
Cardiac disorders (including angina, myocardial infarction, arrhythmia, heart failure)	4	0
Thromboembolic event	0	1
PRES	0	0

Table 3. Adverse Events. ITT, intention to treat; AE, adverse event; SAE, serious adverse event; AESI, adverse event of special interest; TEN/SJS, Toxic Epidermal Necrolysis / Stevens-Johnson syndrome; PRES, posterior reversible encephalopathy syndrome. *This patient was diagnosed with myelodysplasia during the study.

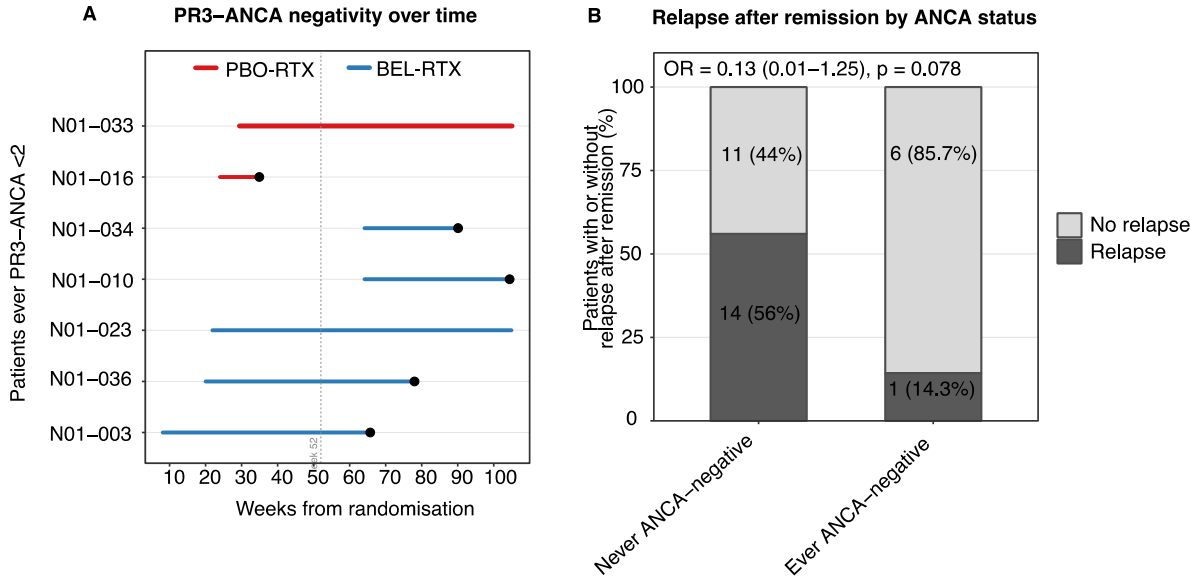
Supplementary appendix

Supplementary Table 1. PR3-ANCA–related outcomes

Endpoint	HR	CI	p value
Time to ANCA negativity *(primary endpoint)*	4.70	0.65 to 33.76	0.12
Time to ANCA <5.0 iU/L	1.96	0.72 to 5.36	0.19
Time to ANCA <10.0 iU/L	1.50	0.63 to 3.56	0.36
Time to 50% reduction from baseline ANCA	1.35	0.64 to 2.86	0.44
Endpoint	**OR**	**CI**	**p value**
Proportion of patients achieving ANCA negativity at 12 months	4.60	0.29 to 73.28	0.28
Proportion of patients achieving ANCA negativity at 18 months	6.07	0.49 to 75.55	0.16
Proportion of patients achieving ANCA negativity at 24 months	0.92	0.05 to 16.46	0.96
Proportion of patients with sustained ANCA negativity at 12 months	2.83	0.14 to 58.57	0.50
Endpoint	**Median differences**	**CI**	**p value**
Percentage change from baseline in ANCA at 3 months	-3.11	-16.87 to 6.72	0.62
Percentage change from baseline in ANCA at 6 months	-1.34	-11.43 to 6.32	0.83
Percentage change from baseline in ANCA at 12 months	-6.60	-34.29 to 1.71	0.08
Percentage change from baseline in ANCA at 18 months	-13.48	-62.92 to 20.34	0.31
Percentage change from baseline in ANCA at 24 months	-6.91	-66.55 to 69.50	0.71

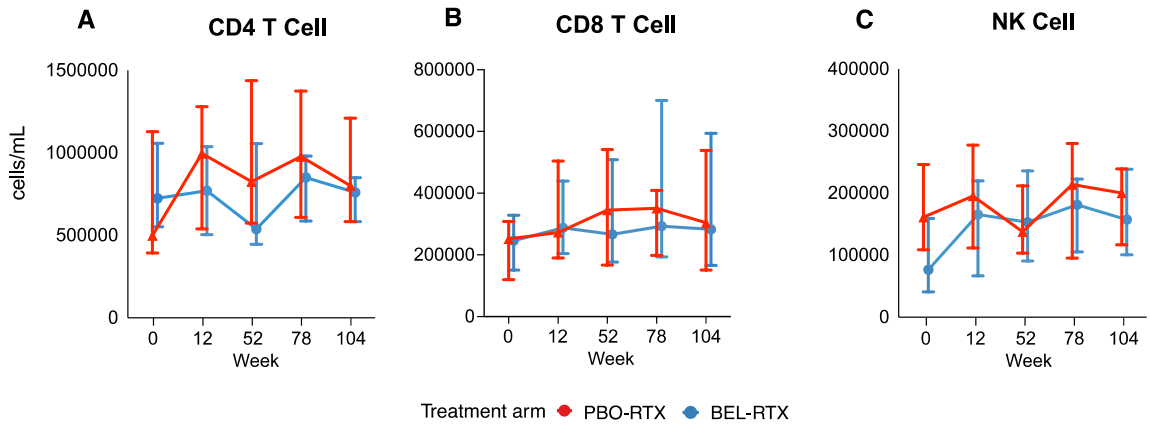
Supplementary Table 1. PR3-ANCA–related outcomes. PR3-ANCA–related secondary outcomes in the PP population, adjusted for baseline PR3-ANCA levels (low vs. high; <24 vs. ≥24 IU/mL), with Placebo as the Reference Group. HR, hazard ratio; OR, odds ration; CI, confidence interval

Supplementary Figure 1. Relapse after remission and PR3-ANCA dynamics.



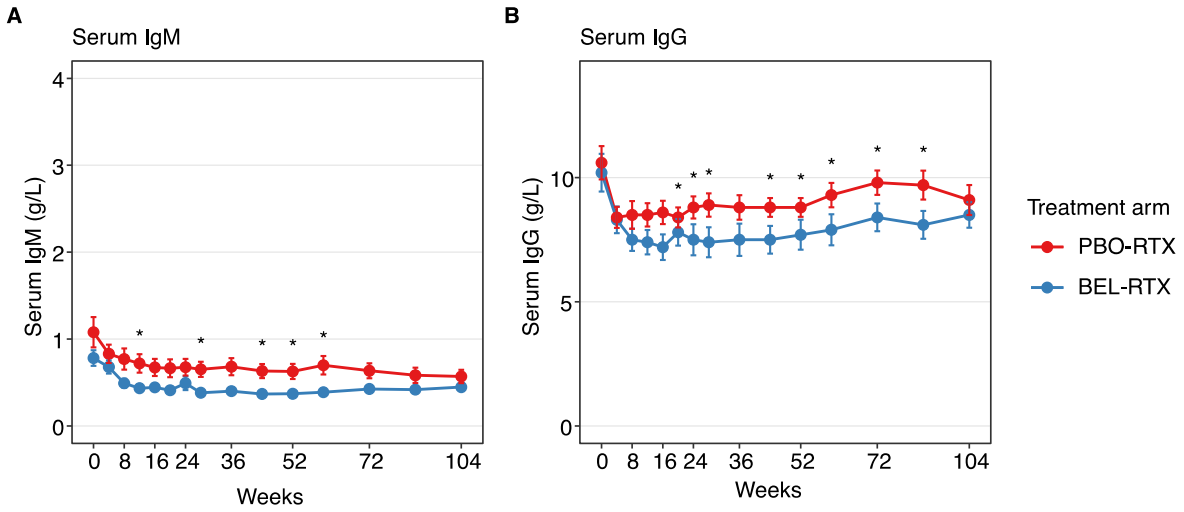
Supplementary Figure 1. PR3-ANCA dynamics and relapse after remission. A, per-protocol timelines display episodes of PR3-ANCA negativity and subsequent serorecurrence for individual patients, with episodes coloured by treatment arm and the first PR3-ANCA re-emergence marked by symbols. B, proportions of patients in all patients that achieved remission (treatment groups combined) with or without relapse after remission are shown according to ever versus never achieving PR3-ANCA negativity, with stacked bars labelled n/N (percentage). OR, odds ratio; PR3, proteinase 3; RTX, rituximab; PBO, placebo; BEL, belimumab.

Supplementary Figure 2. T cell and NK cell dynamics



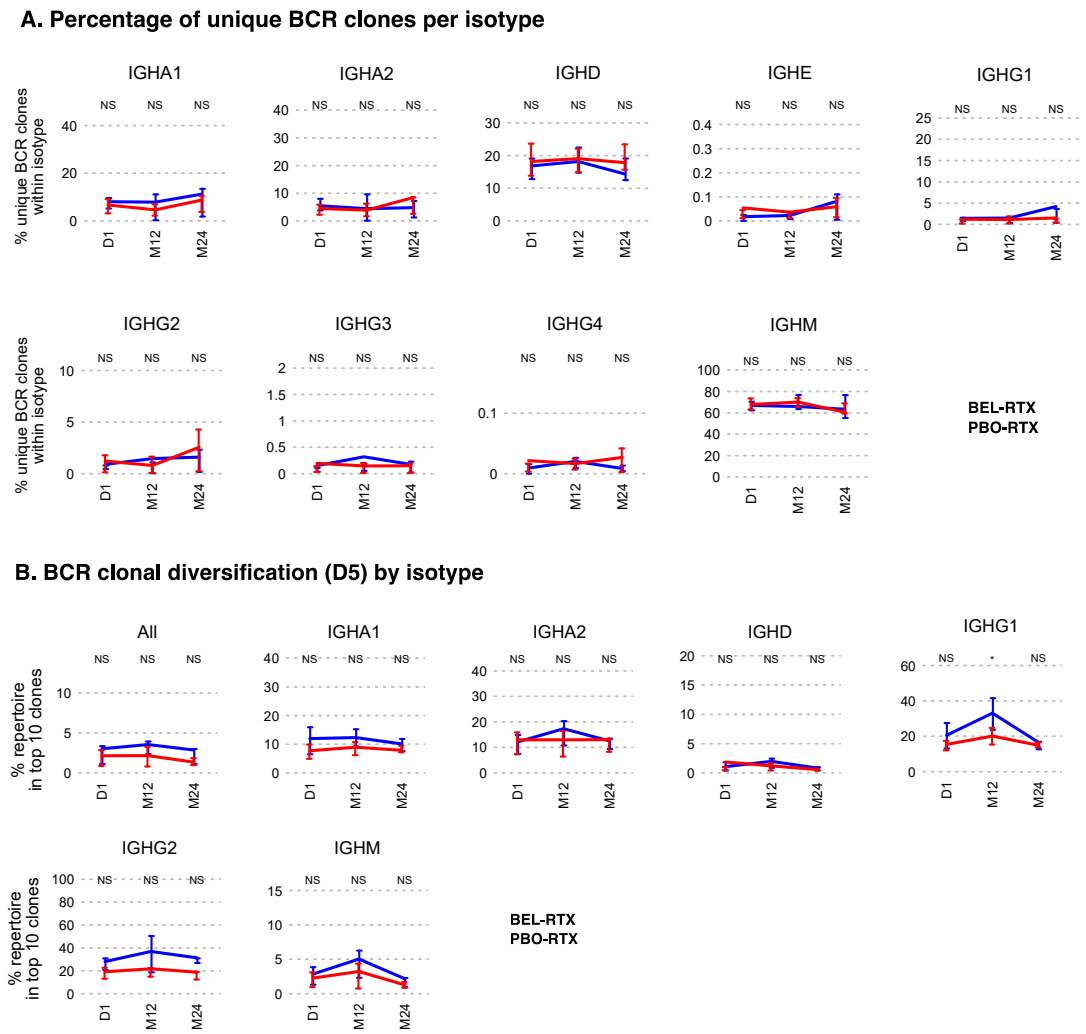
Supplementary Figure 2. T cell and NK cell dynamics. A–C, Absolute counts of CD4 T cells (A), CD8 T cells (B) and NK cells (C) over time in weeks, plotted as treatment-arm means with error bars denoting s.e.m., in the PP population and censored at time of rituximab; between-arm differences at prespecified visits were assessed using Wilcoxon rank-sum tests. Visits without asterisks are not statistically significant. RTX, rituximab; PBO, placebo; BEL, belimumab; NK, natural killer; PP, per protocol; s.e.m., standard error of the mean.

Supplementary Figure 3. Serum immunoglobulins over time



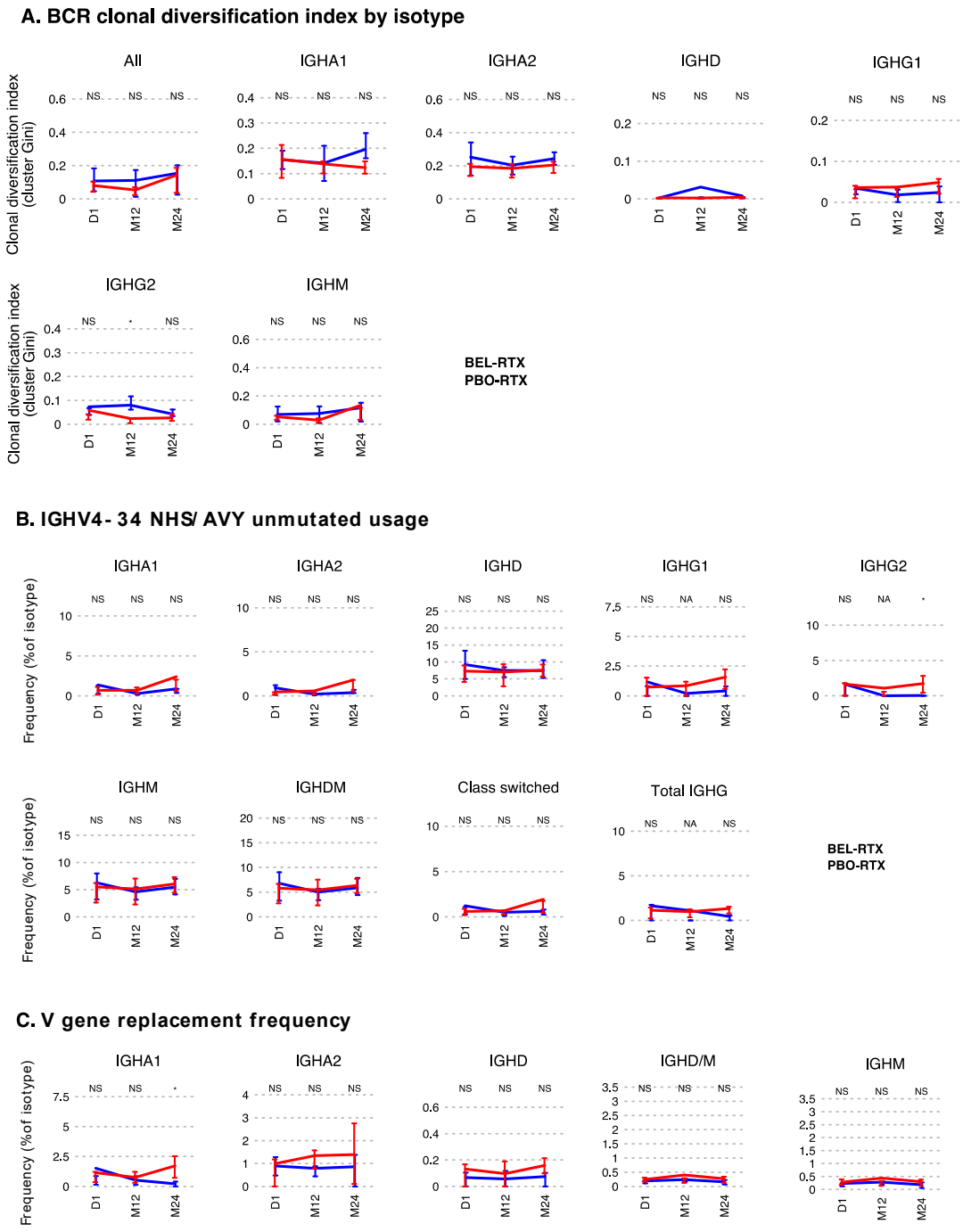
Supplementary Figure 3. Serum IgM and IgG over time. Serum immunoglobulin concentrations are shown over 104 weeks as mean values (± s.e.m.) by treatment arm, comparing PBO-RTX and BEL-RTX. Between-arm differences at each visit are estimated from a linear mixed effects model, with statistical significance denoted by *, ** and *** for P<0.05, P<0.01 and P<0.001, respectively. RTX, rituximab; PBO, placebo; BEL, belimumab; IgM, immunoglobulin M; IgG, immunoglobulin G

Supplementary Figure 4.1. Dynamics of B cell receptor repertoire features over time (1)



Supplementary Figure 4.1. Dynamics of BCR repertoire features over time (1). Longitudinal changes over 24 months in the percentage of unique BCR clones within each isotype (A), the proportion of the repertoire represented by the top 5 clones (D5; B), and the proportion of the repertoire represented by the top 50 clones (D50; C), shown by treatment arm (PBO-RTX and BEL-RTX). Data are presented at day 1 (D1), month 12 (M12) and month 24 (M24) as mean values with 25th–75th percentile intervals. Between-arm differences at each visit were estimated by MANOVA adjusted for age, sex and prior cyclophosphamide exposure. *P<0.05; NS, not significant. RTX, rituximab; PBO, placebo; BEL, belimumab; BCR, B cell receptor; Ig, immunoglobulin; D10, proportion of repertoire represented by the top 10 clones; D50, proportion of repertoire represented by the top 50 clones.

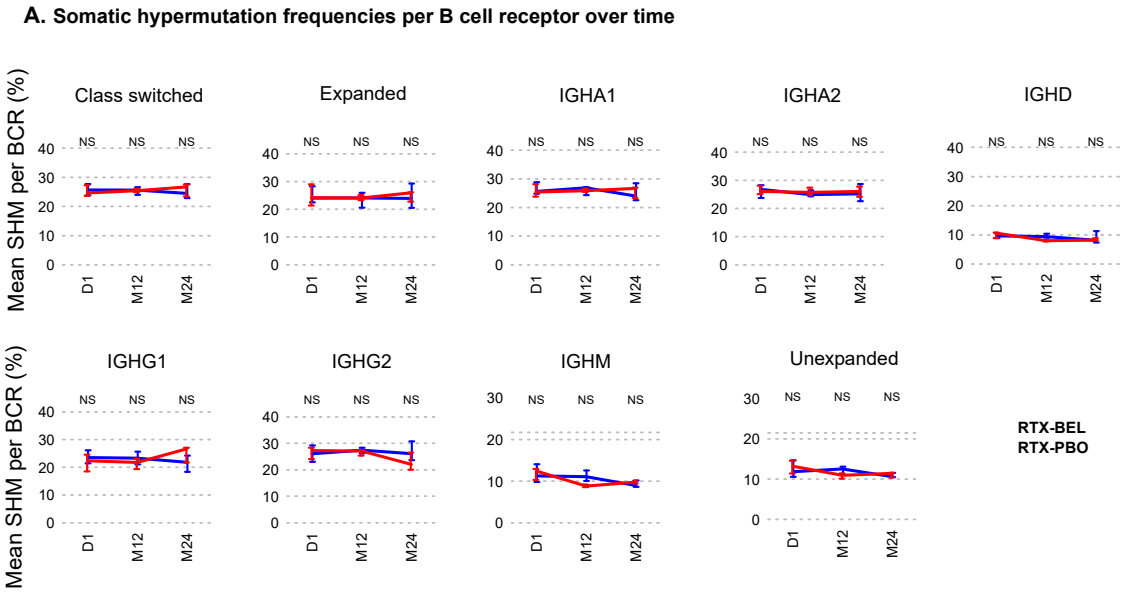
Supplementary Figure 4.2. Dynamics of B cell receptor repertoire features over time (2)



Supplementary Figure 4.2. Dynamics of BCR repertoire features over time (2). Longitudinal changes over 24 months in BCR clonal diversification (cluster Gini index; A), the frequency of unmutated IGHV4-34 NHS/AVY-expressing BCRs (B), and the frequency of V gene replacement events (C) are shown by isotype and treatment arm (PBO-RTX and BEL-RTX). Data are presented at day 1 (D1), month 12 (M12) and month 24 (M24) as mean values with 25th–75th percentile intervals. Between-arm differences at each visit were estimated using multivariate analysis of variance (MANOVA) adjusted for age, sex and prior cyclophosphamide exposure. Statistical significance is denoted by * for P<0.05; NS indicates no significant difference and NA indicates that a between-arm comparison was not available. RTX, rituximab; PBO, placebo; BEL, belimumab; BCR, B cell receptor;

Ig, immunoglobulin; IGHV4-34, immunoglobulin heavy-chain variable region 4-34; NHS, asparagine–histidine–serine motif; AVY, alanine–valine–tyrosine motif.

Supplementary Figure 4.3. Dynamics of B cell receptor repertoire features over time (3)



Supplementary Figure 4.3. Dynamics o B cell receptor repertoire features over time (3). Mean somatic hypermutation (SHM) per B cell receptor (BCR) is shown at day 1 (D1), month 12 (M12) and month 24 (M24) by treatment arm, comparing PBO-RTX and BEL-RTX. Data are plotted as mean values with 25th–75th percentile intervals for each treatment group. Between-arm differences at each visit are estimated from a multivariate analysis of variance (MANOVA) model adjusted for age, sex and prior cyclophosphamide exposure. Statistical significance is denoted by * for P<0.05, and “NS” indicates non-significant differences. BEL, belimumab; PBO, placebo; BCR, B cell receptor; SHM, somatic hypermutation; Ig, immunoglobulin; IgA, immunoglobulin A; IgD, immunoglobulin D; IgG, immunoglobulin G; IgM, immunoglobulin M;

Supplementary Table 2. Between-group differences in patient-reported outcome domain scores

Domain	Visit (Week)	Between-group difference in median (95% CI)	p value
Organ specific symptoms	12	−0.07 (−0.66 to 0.49)	0.88
	24	0.12 (−0.58 to 0.93)	0.85
	52	0.23 (−0.76 to 1.44)	0.65
	78	0.67 (−0.42 to 1.58)	0.17
	104	0.18 (−0.86 to 1.12)	0.78
Systemic symptoms	12	−0.36 (−1.48 to 0.12)	0.08
	24	0.19 (−0.90 to 0.92)	0.87
	52	0.29 (−0.96 to 1.07)	0.48
	78	−0.07 (−1.80 to 0.96)	0.86
	104	0.75 (0.01 to 1.54)	0.04
Belimumab side effects	12	0.01 (−0.67 to 0.67)	0.85
	24	−0.09 (−1.10 to 0.78)	0.80
	52	0.32 (−0.72 to 1.31)	0.63
	78	0.49 (−0.66 to 1.47)	0.37
	104	−0.10 (−1.06 to 0.77)	0.77
Social and emotional impact	12	0.96 (0.10 to 1.79)	0.02
	24	0.44 (−0.59 to 1.44)	0.45
	52	0.58 (−0.55 to 1.96)	0.24
	78	0.58 (−0.53 to 1.62)	0.31
	104	0.62 (−0.40 to 1.67)	0.25
Concerns about the future	12	0.49 (−0.59 to 1.22)	0.20
	24	0.26 (−0.94 to 1.06)	0.41
	52	0.46 (−1.07 to 1.83)	0.65
	78	0.44 (−0.91 to 1.71)	0.50
	104	0.73 (−0.36 to 1.96)	0.23
Physical function	12	0.95 (−0.11 to 2.17)	0.08
	24	0.81 (0.00 to 1.98)	0.03
	52	1.12 (−0.00 to 2.93)	0.05
	78	0.90 (0.00 to 2.05)	0.03
	104	0.50 (−0.91 to 1.45)	0.42

Supplementary Table 2. Between-group differences in change from baseline in patient-reported outcome domain scores. Differences are defined as BEL-RTX minus PBO-RTX; positive values indicate higher scores in the BEL-RTX group. Estimates are median differences with 95% confidence intervals. PRO, patient-reported outcome; CI, confidence interval.

Supplementary Table 3. Disease state definitions

Minor progressive disease	Worsening disease activity with new/worse minor BVAS item(s) before remission is achieved.
Major progressive disease	Worsening disease activity with new/worse major BVAS item(s) before remission is achieved.
Remission	BVAS/WG≤1 (one minor persistent BVAS/WG item) confirmed by a consecutive assessment at least 30 days apart, and corticosteroid dose <10mg/day. Remission starts from the first date of BVAS/WG≤1
Complete Remission	BVAS/WG = 0 confirmed by a consecutive assessment at least 30 days apart, and corticosteroid dose <10mg/day. Complete remission starts from the first date of BVAS/WG=0
Relapse	The occurrence of any new BVAS/WG item after remission or being classified as a threatened major relapse
Minor Relapse	Any increase in disease activity after remission that does not meet the definition of Major Relapse
Major Relapse	The development of a new or recurrent major disease activity item after remission using the BVAS/WG assessment tool or being classified as a threatened major relapse
Threatened Major Relapse	Clinician defined rise in PR3 ANCA treated with additional immunosuppression in the absence of any new BVAS items

Supplementary Table 4: Full inclusion and exclusion criteria

Inclusion Criteria

Participants are eligible to be included in the trial only if all of the following criteria apply:

1. Participant must be ≥18 of age at the time of signing the informed consent form.
2. Have a diagnosis of AAV (granulomatosis with polyangiitis or microscopic polyangiitis), according to the definitions of the Chapel Hill Consensus Conference
3. PR3 ANCA positivity by ELISA at screening
4. Have active disease defined by one major or three minor disease activity items on the Birmingham Vasculitis Activity Score for Wegener's (BVAS/WG)
5. Women of child-bearing potential (WOCBP) must agree to use effective contraception methods and agree to follow these methods during the treatment period and for at least 16 weeks after the last dose of belimumab-placebo, or at least 12 months after the last dose of rituximab, whichever is greater.
6. Capable of giving signed informed consent

Exclusion Criteria

Participants are excluded from the trial if any of the following criteria apply:

1. Women who are pregnant, plan to become pregnant or are lactating during the trial period
2. Participation in any other investigational treatment trials unless observational in nature
3. MPO ANCA or anti–GBM antibody positivity by ELISA
4. Presence of pulmonary haemorrhage with hypoxia
5. Estimated glomerular filtration rate (eGFR) <15 ml/min/1.73m2
6. Symptomatic herpes zoster within 3 months prior to screening
7. Evidence of active or latent tuberculosis (TB) as documented by medical history and examination, chest X-rays (if required as part of SoC), and TB testing: a positive (not indeterminate) QuantiFERON®-TB Gold test (or equivalent)
8. Significant allergies to humanised monoclonal antibodies
9. Clinically significant multiple or severe drug allergies and/or history of hypersensitivity to belimumab and/or rituximab or known to have titres of human anti-mouse antibody or history of hypersensitivity reactions when treated with other diagnostic or therapeutic monoclonal antibodies
10. Malignant neoplasm within the past 5 years (from screening) with the exception of basal cell or squamous epithelial carcinoma of the skin treated with local resection only or carcinoma in situ of the uterine cervix treated locally and with no evidence of metastatic disease for 3 years.
11. Have a history of a primary immunodeficiency
12. IgA deficiency (IgA level < 10 mg/dL)
13. IgG < 400 mg/dL
14. Neutrophils < 1.5 x 109 cells/L
15. Pre-existing B cell lymphopenia (total CD19+ count < lower limit of detection)
16. Alanine transferase (ALT) >2x upper limit of normal (ULN)
17. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)
18. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
19. Active bleeding disorders, and/or inability to support interruption to anticoagulant (including but not limited to warfarin, rivaroxaban) or anti-platelet therapies (including but not limited to aspirin, clopidogrel, etc.), as assessed jointly by trial physician and physician responsible for managing anticoagulant / anti-platelet therapies
20. Severe heart failure (New York Heart Association Class IV) or other severe, uncontrolled cardiac disease

21. QT >450 msec or QTc >480 msec in participants with bundle branch block
22. Have a history of a major organ transplant (e.g., heart, lung, kidney, liver) or hematopoietic stem cell/marrow transplant
23. Have an acute or chronic infection requiring management as follows:

- Currently on any suppressive therapy for a chronic infection such as pneumocystis, cytomegalovirus, herpes simplex virus, herpes zoster, or atypical mycobacteria)
- Hospitalisation solely for treatment of proven infection within 60 days of Day 1
- Have had proven severe infection requiring treatment with parenteral (IV or IM) antibiotics (antibacterials, antivirals, antifungals, or anti-parasitic agents) within 60 days of Day 1. Prophylactic anti-infective treatment is allowed. Precautionary PO/IV antibiotics in a patient with active vasculitis will be permitted.

24. Have evidence of serious suicide risk, including any history of suicidal behaviour in the last 6 months and/or any suicidal ideation of type 4 or 5 on the C-SSRS in the last 2 months or who, in the investigator's opinion, pose a significant suicide risk
25. Have clinical evidence of significant unstable or uncontrolled acute or chronic diseases not due to vasculitis (i.e., cardiovascular, pulmonary, haematological, gastrointestinal, hepatic, renal, neurological, malignancy, or infectious diseases) which, in the opinion of the principal investigator, could confound the results of the trial or put the participant at undue risk
26. Have a planned surgical procedure, laboratory abnormality, or condition (e.g., poor venous access) that, in the opinion of the principal investigator, makes the participant unsuitable for the trial.

Prior/Concomitant Therapy
27. Live vaccine(s) within 30 days prior to Day 1, or plans to receive such vaccines during the treatment phase
28. Have received anti-CD52 [alemtuzumab] within 5 years of Day 1 (or total CD3 count <0.7 x109/L) at screening following previous anti-CD52 [alemtuzumab] exposure.
29. Have received any of the following within 180 days of Day 1:
Belimumab
Rituximab

- Any B cell targeted therapy (anti-CD20 agents other than rituximab, anti-CD22 [epratuzumab], BLyS-receptor fusion protein [BR3], TACI Fc, anti-BAFF (LY2127399), anti-Interferon alpha agents or anti-BLyS other than belimumab)
- A biologic investigational agent other than B cell targeted therapy or abatacept (e.g., abetimus sodium, anti CD40L antibody [BG9588/ IDEC 1311]). (Investigational agent applies to any drug not approved for sale in the country in which it is being used.)

30. Have received any of the following within 90 days of Day 1:

- More than 3 infusions of cyclophosphamide
- Anti-TNF or anti-IL-6 therapy (e.g., adalimumab, etanercept, infliximab, tocilizumab)
- Abatacept
- Interleukin-1 receptor antagonist (e.g., anakinra)
- Intravenous immunoglobulin (IVIG)
- Plasmapheresis, leukapheresis.

31. Have received a non-biologic investigational agent (investigational agent applies to any drug not approved for sale in the country in which it is being used) within 60 days of Day 1.
32. Have received any steroid injection (e.g., intramuscular [IM], intraarticular, or IV) within 60 days of Day 1 (unless given during or 30 days before screening period)
33. Have received emergency steroid >3g methylprednisolone (IV) or equivalent dose of oral prednisolone between 30 days prior to Screening Visit and Day 1 (including Day 1) - (please see section 10.2.2 for further information).
34. Positive human immunodeficiency virus (HIV) antibody test

35. Positive serology for Hepatitis B (HB), defined as: (i) HB surface antigen positive (HBsAg+) OR (ii) HB core antibody positive (HBcAb+)

36. Positive Hepatitis C (HCV) antibody test

37. Participants with current drug/alcohol dependence or drug/alcohol dependence within the last 6 months prior to Day 1

38. Compliance: is unlikely to comply with scheduled trial visits based on investigator judgment or has a history of substance abuse, psychiatric disorder or condition that may compromise communication with the investigator

39. Sensitivity to any of the trial treatments, or components thereof, or drug or other allergy that, in the opinion of the investigator, contraindicates participation in the trial

40. Unable to administer trial treatment (belimumab/ belimumab-placebo) by SC injection and has no other reliable resource to administer the injection

41. Unable to safely and reliably store trial treatment (belimumab/ belimumab-placebo). E.g., no permanent residence, no fridge

Supplementary Table 5: Flow cytometric surface markers used to define cell types (secondary analyses)

Cell type	Definition
CD4 T Cells	CD4+
CD8 T Cells	CD8+
B cells	CD19+
B cells (CD20+)	CD19+CD20+
NK cells	CD3-CD56+
Naïve B cells	CD19+IgD+CD27-
Transitional B cells	CD19+CD24+CD38+
Memory B cells	CD19+CD27+
Activated memory B cells CD95+	CD19+CD27+CD95+
Switched memory B cells	CD19+IgD-CD27+
Activated switched memory B cells CD95+	CD19+IgD-CD27+CD95+
Non-switched memory B cells	CD19+IgD+CD27+
Activated non-switched memory B cells CD95+	CD19+IgD+CD27+CD95+
Double negative B cells	CD19+IgD-CD27-
Activated double negative B cells CD95+	CD19+IgD-CD27-CD95+
Plasmablasts	CD19+CD27+CD38+
Activated plasmablasts CD95+	CD19+CD27+CD38+CD95+

Marker combinations in this table define secondary immunophenotyping endpoints measured by flow cytometry on whole blood; these analyses were performed in a central GlaxoSmithKline laboratory using a validated panel to quantify major lymphocyte and B-cell subsets.

Supplementary Table 6: Flow cytometric surface markers used to define cell types (exploratory analyses)

Cell type / marker	Definition
T cells	$CD3^+$
$CD4^+$ T cells	$CD4^+$
Naïve T cells	$CD4^+CD45RA^+CCR7^+$
Effector memory T cells	$CD4^+CD45RA^-CCR7^-$
Central memory T cells	$CD4^+CD45RA^-CCR7^+$
TEMRA cells	$CD4^+CD45RA^+CCR7^-$
Th1 cells	$CD4^+CXCR3^+CCR6^-$
Th17 cells	$CD4^+CXCR3^-CCR6^+$
Th2 cells	$CD4^+CXCR3^-CCR6^-$
T regulatory cells	$CD4^+CD127^{lo}CD25^{hi}$
T follicular helper (Tfh) cells	$CD4^+CXCR5^+PD1^{hi}$
Additional activation markers	HLA-DR, CD38, CD69, CD25

Marker combinations in this table define exploratory T-cell subsets and activation markers measured by flow cytometry on peripheral blood mononuclear cells (PBMCs); these exploratory immunophenotyping endpoints were performed in university laboratories using an extended T-cell panel.

Supplementary Table 7. Adverse Events of Special Interest

Infections requiring antimicrobial, antiviral or antifungal treatment (including opportunistic, progressive multifocal leukoencephalopathy)
Hypogammaglobinaemia
Systemic infusion/injection reactions
Hypersensitivity reactions
Malignancy
Psychiatric events (including suicidality)
Severe skin reactions (including Toxic Epidermal Necrolysis and Stevens-Johnson syndrome)
Cardiac disorders (including angina, myocardial infarction, arrhythmia, heart failure)
Thromboembolic event
Posterior reversible encephalopathy syndrome
Pregnancy