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Obinutuzumab Effectively Depletes Key B-cell Subsets in Blood and Tissue in End-stage Renal Disease Patients

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Background. The THEORY study evaluated the effects of single and multiple doses of obinutuzumab, a type 2 anti-CD20 antibody that induces antibody-dependent cell-mediated cytotoxicity and direct cell death, in combination with standard of care in patients with end-stage renal disease. **Methods.** We measured B-cell subsets and protein biomarkers of B-cell activity in peripheral blood before and after obinutuzumab administration in THEORY patients, and B-cell subsets in lymph nodes in THEORY patients and an untreated comparator cohort. **Results.** Obinutuzumab treatment resulted in a rapid loss of B-cell subsets (including naïve B, memory B, double-negative, immunoglobulin D⁺ transitional cells, and plasmablasts/plasma cells) in peripheral blood and tissue. This loss of B cells was associated with increased B cell–activating factor and decreased CXCL13 levels in circulation. **Conclusions.** Our data further characterize the mechanistic profile of obinutuzumab and suggest that it may elicit greater efficacy in indications such as lupus where B-cell targeting therapeutics are limited by the resistance of pathogenic tissue B cells to depletion.

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Dysregulated B cells are considered pathogenic in multiple autoimmune indications because of their multifaceted role in immune response: presentation of antigen, activation of innate and adaptive immune cells via costimulation and cytokine secretion, and generation and secretion of antibodies.[1] Therefore, targeting B cells via the CD20 antigen has been of great interest. Since 1997, rituximab, an anti-CD20 monoclonal type 1 antibody highly dependent on complement-dependent cytotoxicity to mediate B-cell death, has been the established standard for B-cell depletion therapy.[2] Its effectiveness in B-cell malignancies has inspired evaluation in nononcologic indications, leading to approvals for rheumatoid arthritis and pemphigus vulgaris. Rituximab has been evaluated for other systemic autoimmune diseases;[3] however, randomized controlled trials of rituximab have failed to demonstrate superiority over standard of care in some indications, including lupus nephritis and multiple sclerosis.[4,5]

Rituximab administration results in peripheral B-cell depletion as measured by conventional flow cytometry methods. However, incomplete depletion of tissue-resident B cells (including key subsets such as memory B cells and plasma cells [PCs]) by rituximab has been observed in multiple indications.[6-9] In addition, suboptimal depletion of circulating memory B cells and plasmablasts (PBs) has been observed in a variety of autoimmune indications.[10-12] This persistence of B cells in the tissue and resistant subsets in blood has been hypothesized as a key reason for the observed suboptimal clinical efficacy in these indications.

To potentially enhance efficacy, next-generation B cell–targeted therapies have been engineered to induce greater B-cell depletion, particularly of these difficult-to-target tissue-resident B cells. Obinutuzumab is a glycoengineered type 2 antibody that mediates increased direct cell death and heightened antibody-dependent cellular cytotoxicity relative to rituximab. Redistribution of CD20 into lipid rafts and FcgRIIb-mediated internalization of CD20 is reduced compared with corresponding type 1 monoclonal antibodies.[13] Obinutuzumab has shown a greater ability than rituximab to deplete B cells both ex vivo in human blood and in vivo in tissue and peripheral blood of cynomolgus monkeys.[14,15] This increased depletion was associated with increased clinical benefit in chronic lymphocytic leukemia (CLL) over rituximab in a study where obinutuzumab demonstrated greater depletion of pathogenic B cells in bone marrow and blood.[16]

Although effective in CLL, it is not yet known if obinutuzumab will effectively reduce tissue-resident B cells, particularly recalcitrant memory B and PB subsets, in autoimmune indications. THEORY, a phase 1b study in sensitized end-stage renal disease (ESRD) patients, evaluated the safety, pharmacokinetics, and pharmacodynamics of obinutuzumab in combination with high-dose intravenous immunoglobulin (IVIG) in highly anti-HLA allosensitized patients with ESRD.[17]

Our results show that obinutuzumab led to a rapid loss of peripheral blood B-cell subsets, including memory B cells, double-negative (DN) cells, and PBs. Similarly, levels of B cells (including tissue-resident, CD20^{low} proliferating PBs) in retroperitoneal lymph nodes were profoundly repressed in the majority of obinutuzumab-treated patients relative to an untreated comparator cohort. In agreement, levels of BAFF (a survival factor consumed by B cells in blood and tissue)[18] were elevated and levels of CXCL13 (a key chemokine involved in the organization of B-cell follicles in tissue)[19-21] were reduced in patients treated with obinutuzumab, with T-cell levels in lymph nodes remaining comparable with the comparator cohort. These data suggest obinutuzumab mediates increased B-cell depletion compared with type 1 anti-CD20 antibodies, such as rituximab, in B cell–mediated diseases.

MATERIALS AND METHODS

Patients

THEORY was a phase 1b, open-label, sequential, 2-cohort study comparing single and repeated doses of obinutuzumab administered with IVIG in highly anti-HLA allosensitized candidates for renal transplant and has been previously described.[17] Briefly, the study included patients with ESRD who aged 18 to 65 y, had a history of sensitizing events such as pregnancy, blood transfusion, or a prior transplant, and a minimum calculated panel reactive antibody (cPRA) of 50%. Patients in cohort 1 (n = 5) received a single dose of 1000-mg IV obinutuzumab at week 0 (day 1). Patients in cohort 2 (n = 20) received 1000-mg IV obinutuzumab on days 1 and 15, with an optional dose at week 24. Both cohorts received 2 g/kg IVIG at weeks 3 and 6 and were monitored for 12 mo.

To assess the effect of obinutuzumab on tissue-resident B-cell subsets, we analyzed peritoneal lymph node tissue samples from ESRD patients undergoing kidney transplantation in both the THEORY study (both cohorts) and from a comparator cohort of ESRD patients who had not received any B cell–targeted therapy (including obinutuzumab) before receiving transplants. For the comparator cohort, lymph nodes were isolated from 9 ESRD subjects (**Table S1, SDC,** http://links.lww.com/TXD/A483) at time of kidney transplant (TP) at the UCSF Medical Center. Patients were not treated with a desensitizing regimen to enable transplant.

This study was conducted in accordance with local institutional review board ethical standards, good clinical practices, and the Declaration of Helsinki. All patients provided written informed consent before study participation.

Assessments

B-cell subsets in blood were measured centrally via a highly sensitive flow cytometry assay at baseline and at weeks 3, 24, and 52 (Table 1). If patients underwent transplant, blood was collected at TP and at weeks 24 and 52 after TP. Cell measurements in tissue (flow cytometry and immunofluorescence) were performed only in patients undergoing transplant on lymph nodes collected at TP (THEORY, n = 12; comparator cohort, n = 9).

As BAFF is a survival factor consumed by B cells in the blood and in the tissues[18] and CXCL13 is a key cytokine involved in the formation of lymphoid follicles in both secondary lymphoid organs and ectopic tertiary lymphoid follicles,[19-21] we measured the levels of BAFF and CXCL13 in circulation in patients treated with obinutuzumab. Serum samples for BAFF and CXCL13 analysis were collected from all patients at baseline and weeks 2, 3, 6, 12, 24, 36, 52, and peritransplant if applicable. Additional details are included in the **Supplemental Methods** (**SDC,** http://links.lww.com/TXD/A483).

Statistics

Paired *t* tests were used to compare between baseline and week 52 per peritransplant levels of blood B-cell subsets. Patients without values at both time points were excluded

Flow cytometry markers used in blood and tissue

Population	Blood	Tissue
Total B cells	CD19$^+$ Dump^{neg}	CD19$^+$ Dump^{neg}
Memory B cells	CD19$^+$ Dump^{neg} CD27$^+$	CD19$^+$ Dump^{neg} CD27$^+$
Naive B cells	CD19$^+$ Dump^{neg} CD27^{neg} IgD$^+$	CD19$^+$ Dump^{neg} CD27^{neg} IgD$^+$
Plasmablasts/plasma cells	CD19$^+$ Dump^{neg} CD27$^+$ CD38^{high}	CD19$^+$ Dump^{neg} CD27$^+$ CD38^{high}
Plasmablasts		CD19$^+$ Dump^{neg} CD27$^+$ CD38^{high} Ki67$^+$
Plasma cells		CD19$^+$ Dump^{neg} CD27$^+$ CD38^{high} Ki67^{neg}
T cells		CD3$^+$
T$_{FH}$ cells		CD3$^+$ CD4$^+$ CD45RA^{neg} CXCR5$^+$ PD-1$^+$ ICOS$^+$

Dump = CD3, CD56, and CD14.
B-cell subsets are reported as a percentage of total lymphocytes, T follicular helper (T$_{FH}$) cells are reported as percentage of total T cells.
CD, cluster of differentiation.

from analysis (n = 3 in cohort 1, n = 9 in cohort 2). An unpaired 2-group *t* test was used to compare levels of B cells and subsets in trial nodes to comparator nodes. 95% confidence intervals are provided. All statistical tests were not prespecified and were run for exploratory purposes.

RESULTS

Obinutuzumab Administration Led to a Rapid Loss of Key Peripheral Blood B-cell Subsets

After treatment with a single dose of obinutuzumab, peripheral blood naive cells in cohort 1 patients (n = 5) were detectable but were reduced substantially at weeks 3 and 24 and repleted somewhat by week 52 in 2 of 3 patients (Figure 1A; **Table S2, SDC**, http://links.lww.com/TXD/A483). Switched memory B cells were below the limit of quantification (BLoQ) in 4 of 5 patients at week 3 and 2 of 5 patients at week 24, whereas unswitched memory B cells were BLoQ in all patients at week 3 and 2 of 5 patients at week 24 (Figure 1C and D). Immunoglobulin (Ig)D$^+$ transitional B cells were also BLoQ at week 3 in patients with available samples and in 3 of 5 patients at week 24 (Figure 1E). All aforementioned subsets remained generally below pre-obinutuzumab treatment levels through week 52. DN cells were BLoQ in all patients at week 3 (Figure 1F); by week 24, 3 patients had detectable DN cells but still below predose levels. At week 52, all 3 patients with measurements at that time point had DN cells lower than predose levels. PBs)/PCs were BLoQ at week 3; by week 24, 1 patient had a slight recovery of PB/PC and 1 patient had a spike in PB/PC, which decreased again by week 52 despite having no obinutuzumab infusions between these time points (Figure 1G).

In contrast to cohort 1 patients, cohort 2 patients (n = 20) receiving 2 doses had naive B cells, memory B (switched and unswitched) cells, DN B cells, IgD$^+$ transitional B cells, and PB/PC in peripheral blood BLoQ by week 3 (Figure 1H–N; **Table S2, SDC**, http://links.lww.com/TXD/A483). B-cell subset levels remained BLoQ through TP or week 52 in all patients except 1 patient (patient 120). This patient had rapid repopulation of total B cells; after full depletion by week 3, B cells began to reappear in blood by week 14, and at TP, had total B cells at near-baseline levels; this patient also had antidrug antibodies (ADAs) and reduced obinutuzumab exposure.[17] Consequentially, IgD$^+$ transitional B cells and, to a lesser extent, PB/PC reappeared initially in the circulation in this patient at week 24, followed by naive cells at TP, at which point IgD$^+$ transitional B cells and PB/PC decreased. Memory

B cells (switched and unswitched) and DN B cells repleted to a lesser extent versus naive cells. Also, in contrast to cohort 1, all subsets measured in cohort 2 were significantly reduced from baseline, whereas in cohort 1, only unswitched memory and IgD$^+$ transitional B cells were significantly reduced from baseline.

Obinutuzumab Administration Specifically Reduced Tissue B-cell Subsets

We characterized lymph node B cells in the 12 transplanted obinutuzumab-treated THEORY patients and 9 comparator patients (Figure 2). The etiology for ESRD in the comparator cohort was roughly comparable with THEORY patients (**Table S1, SDC**, http://links.lww.com/TXD/A483).[17] In the comparator cohort, total B cells varied from 20% to 55% of total lymphocytes (median 32.0%). In the obinutuzumab-treated patients, 9 of 12 patients (16 nodes) had B cells ≤2% of total lymphocytes (median 0.14%). Of the 3 obinutuzumab-treated patients with lymph node B cells >2% of total lymphocytes, 1 was the patient with ADAs, rapid drug clearance, and recovered B cells in the periphery at TP (16.7%). The other 2 (patient 110: 33.5%, and patient 117: 23.6%) had B-cell BLoQ in the periphery at time of transplant. Overall, obinutuzumab-treated patients had a significantly lower level of total lymph node B cells.

Memory B cells were significantly lower in obinutuzumab-treated (median 0.08%) versus comparator patients (median 15.90%; Figure 2B). Two obinutuzumab-treated patients had higher percentages of memory cells than other obinutuzumab-treated patients (patient 117: 17.6%, and patient 110: 32.7%). Patient 120, despite having recovered B cells in the periphery, still had very low (0.73%) tissue memory B cells.

Naive B cells were significantly lower in obinutuzumab-treated patient nodes (median 0%) versus comparator patients (median 10.60%; Figure 2C). Patient 120, the patient with ADAs and recovered total B cells in the periphery at TP, had naive cells at similar levels to comparator nodes (15.6%). Patient 117 had slightly higher percentages of naive cells than other obinutuzumab-treated patients (5.9%).

Proliferating PBs were significantly lower in obinutuzumab-treated (median 0%) versus comparator patients (median 0.06%; Figure 2D). Two obinutuzumab-treated patients, patients 120 and 117, had higher PBs than the other obinutuzumab-treated patients (0.05% and 0.03%).

The effect of obinutuzumab on B cells in tissue was supported by immunohistochemistry analysis (Figure 3). B cells
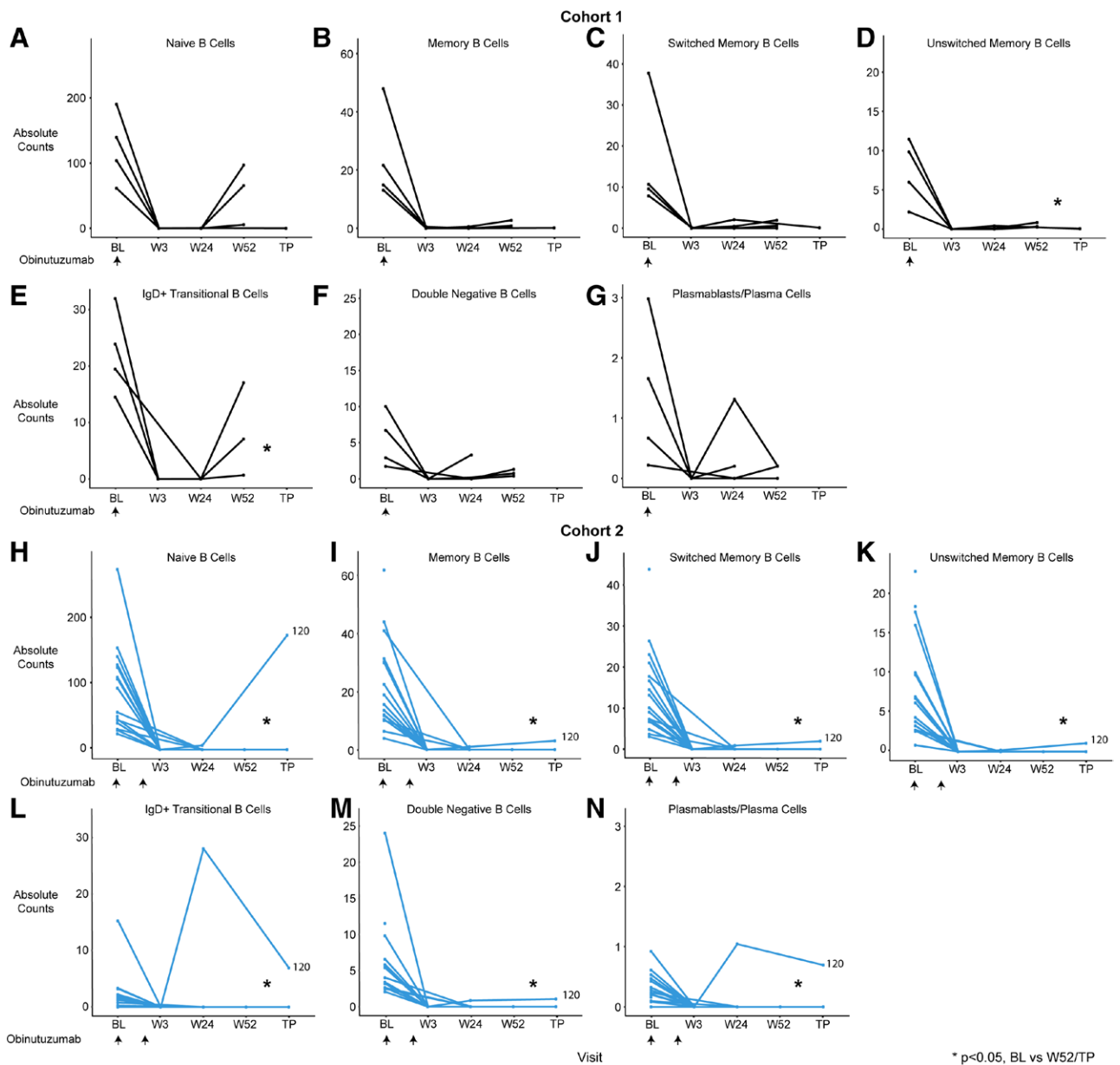
FIGURE 1. Multiple doses of obinutuzumab elicited a rapid and sustained depletion of B-cell subsets in peripheral blood in ESRD patients. B-cell subsets (black=cohort 1, blue=cohort 2) were measured in patients before and after doses of obinutuzumab (naive, memory, switched memory, unswitched memory, immunoglobulin D+ transitional, double negative, and plasmablasts/plasma cells are A–G for cohort 1 and H–N for cohort 2, respectively). One cohort 2 patient (noted here) had antidrug antibodies that reduced exposure and resulted in return of detectable B cells in circulation by wk 24. Baseline samples were drawn preinfusion. ESRD, end-stage renal disease.

were reduced in lymph nodes from obinutuzumab-treated patients (median 0.00035 cells/μM) relative to the comparator cohort (median 0.0037 cells/μM), an approximately 10-fold reduction. Interestingly, although by flow cytometry 3 of 12 patients had B cells at the level of comparator nodes, by immunohistochemistry only 2 of 12 patients had B cells at the level of comparator nodes (patient 110: 0.0022 cells/μM, and patient 120: 0.0024 cells/μM). This slight difference may be explained by sampling differences, as flow cytometry assesses homogenized tissue, whereas immunohistochemistry is constrained by the sections analyzed. This assessment also demonstrated that T cells did not differ in obinutuzumab-treated patients versus the comparator cohort. Consistent with this observation, flow cytometry analysis demonstrated that T follicular helper (T_{FH}) cells did

not differ in obinutuzumab-treated patients versus the comparator cohort (data not shown).

Obinutuzumab Increased Levels of BAFF in Circulation

Levels of BAFF in cohort 1 increased from a mean of 4877 pg/mL at day 1 predose to 10614 pg/mL by week 3 and was 11000 pg/mL at week 52. Levels of BAFF in cohort 2 increased from a mean of 4162 pg/mL at day 1 predose to 10812 pg/mL by week 3 and was 14917 pg/mL at week 52 (Figure 4A).

In the transplanted patients of cohort 1 and 2, BAFF increased and stayed higher than baseline levels through TP, increasing from a mean of 4967 pg/mL at day 1 predose to 10850 pg/mL at TP (Figure 4B). Figure 4C presents the BAFF profiles of the 3 patients with persistent B cells in tissue. Levels
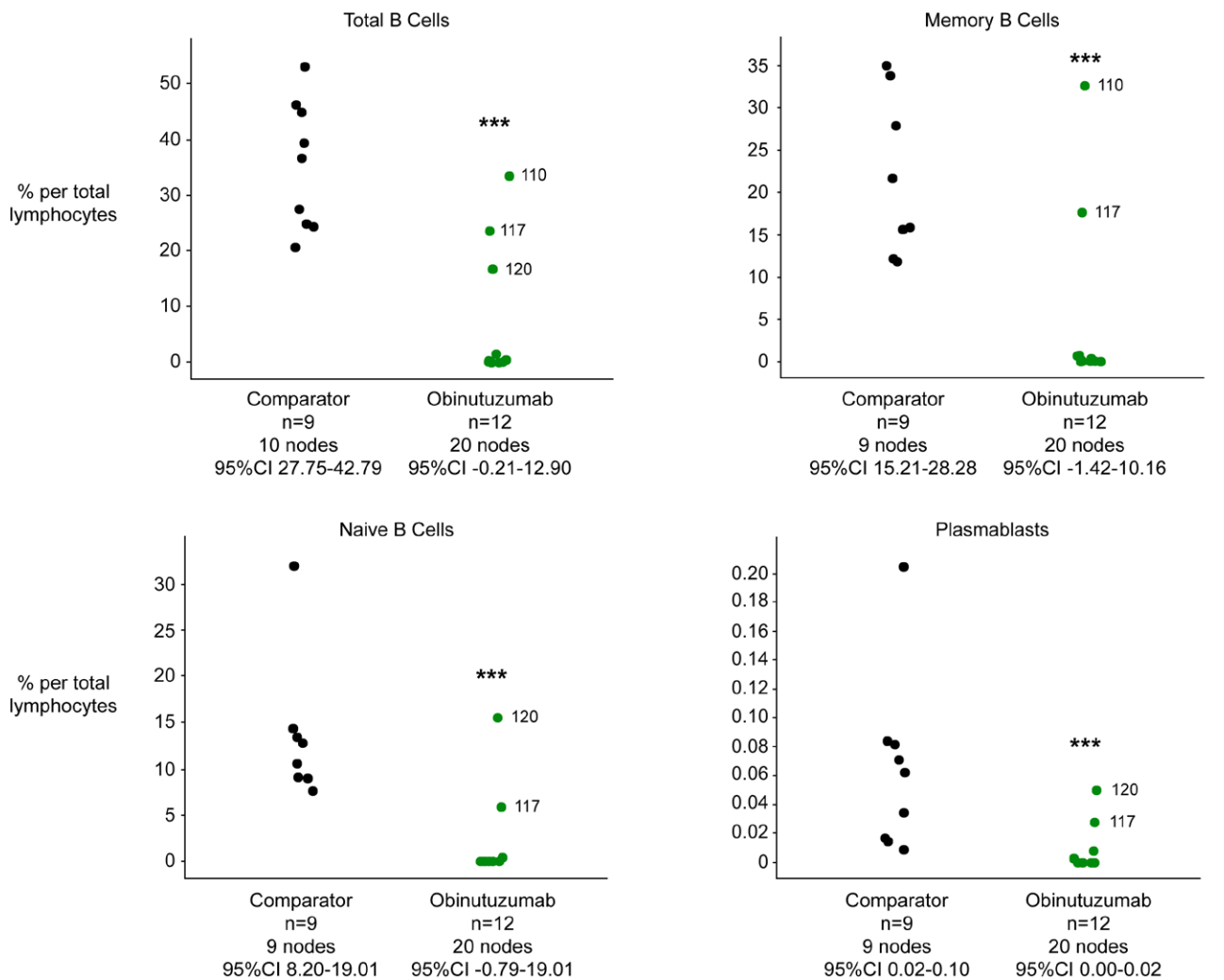
FIGURE 2. B cells in lymph nodes are lower in patients treated with obinutuzumab vs untreated comparators. Lymph nodes collected during kidney transplant, for both study and comparator nodes, were bisected; half was subjected to cell dissociation and stained for flow cytometry. CI, confidence interval.

in patient 110 increased from 5250 pg/mL at day 1 predose to 6520 pg/mL at week 3 and 7710 pg/mL at TP. Levels in patient 117 increased from 5120 pg/mL at day 1 predose to 12 700 pg/mL at week 3 and 20 600 pg/mL at TP. Notably, in patient 120, in whom ADAs developed and whose B cells in blood recovered up through TP and had tissue populated mostly by naive B cells, BAFF started to drop in the periphery after the nadir of B-cell levels at week 3 (from 8680 pg/mL at day 1 predose to 15 000 pg/mL at week 3, to 13 700 pg/mL by week 24), returning to baseline levels (7700 pg/mL) by TP.

Obinutuzumab Reduced Levels of CXCL13 in Circulation

In patients treated with obinutuzumab, CXCL13 levels in cohort 1 decreased from a mean of 187 pg/mL at day 1 predose to 99 pg/mL by week 3 and was 105 pg/mL at week 52 (Figure 5A). Levels in cohort 2 decreased from a mean of 127 pg/mL at day 1 predose to 70 pg/mL by week 3 and was 86 pg/mL at week 52.

In transplanted patients (Figure 5B), CXCL13 decreased from a mean of 123.0 pg/mL at day 1 predose to 100.2

pg/mL at TP. Figure 5C presents the CXCL13 profiles of 3 patients with persistent B cells in tissue. In patient 110, levels decreased from 96.1 pg/mL at day 1 predose to 56.4 pg/mL at week 3 and were 59.0 pg/mL at TP. In patient 117, levels decreased from 89.4 pg/mL at day 1 predose to 42.8 pg/mL at week 3 and were 43.8 pg/mL at TP. Notably, in patient 120, who developed ADAs and whose B cells in blood recovered through TP and had tissue populated mostly by naive B cells, CXCL13 changes again inversely mirrored BAFF changes, dropping from 364 pg/mL at day 1 predose to 111 pg/mL at week 3, to 190 by week 24, then returning to near-baseline levels (311 pg/mL) by TP.

DISCUSSION

Our data demonstrate that obinutuzumab/IVIG administration led to the loss of B-cell subsets in the peripheral blood of sensitized ESRD patients. Additionally, obinutuzumab treatment led to the loss of B cells in tissue, including key B-cell subsets thought to be resistant to conventional B cell–targeted agents, particularly memory and proliferating
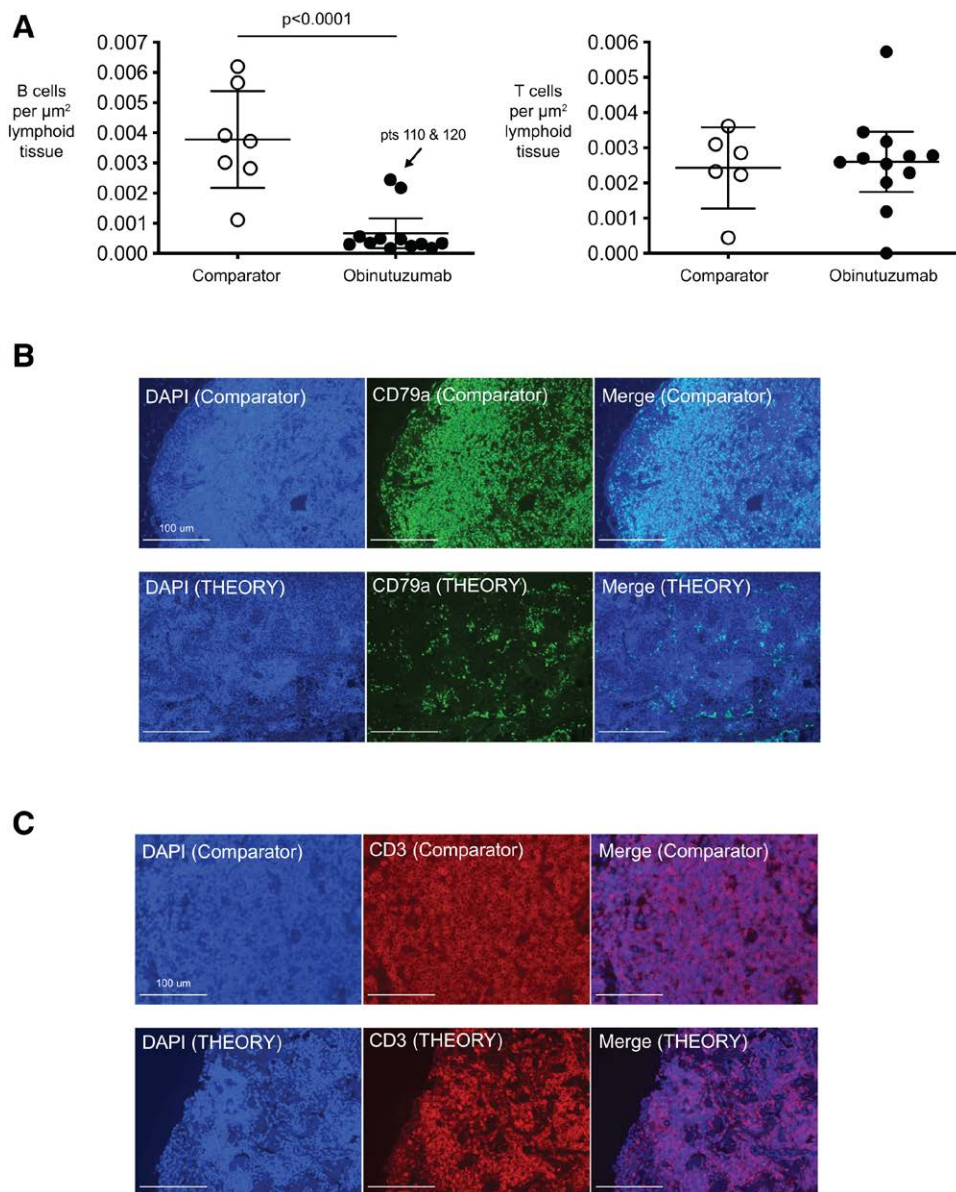
FIGURE 3. Immunohistochemistry confirms that B cells, not T cells, are decreased in patients treated with obinutuzumab. Lymph nodes collected during kidney transplant, for both study and comparator nodes, were bisected; half was subjected to cell dissociation, formalin-fixed and paraffin-embedded, and then stained via immunohistochemistry for the B-cell marker CD79a and the T-cell marker CD3. A, plot of B cells and T cells per analyzed tissue area (patient averages shown). Bars represent mean and 95% confidence interval. B, Representative B-cell staining in obinutuzumab-treated and comparator patients' nodes. C, Representative T-cell staining in obinutuzumab-treated and comparator patients' nodes. CD, cluster of differentiation; DAPI, 4′,6-diamidino-2-phenylindole.

tissue PBs—subsets hypothesized to drive disease pathogenesis in various autoimmune indications. This loss of tissue B cells was accompanied by elevated BAFF and reduced levels of CXCL13, a key chemokine for lymphoid follicle formation. Although 2 patients had higher levels of tissue B cells at TP than other obinutuzumab-treated patients, it is difficult to determine if the degree of depletion in the tissues was actually less than in others, given that we had no predose samples. It is possible that relative reductions in tissue B cells were equivalent to the relative reductions in the other patients or that the profound and sustained loss of peripheral B cells alone was enough to result in the observed BAFF elevation and CXCL13 reduction.

The multiple-dose regimen (cohort 2) caused sustained loss of all peripheral blood B-cell subsets to BLoQ in all patients except for one through week 52 per TP. The reconstitution profile (predominantly naive B cells) of this outlier patient, who had detectable ADAs, is consistent with a deep loss of memory B cells, as seen in the tissue samples from this and the majority of patients analyzed. The initial return of blood B cells at week 24 in this patient was driven mainly by IgD[+] transitional B cells, suggesting "fresh" B cells emerging from the bone marrow were the main drivers of reconstitution (with a smaller contribution from PB/PC, which recovered at week 24 and remained recovered at week 52). Consistent with this hypothesis, at TP (the next time point analyzed for this patient), naive B cells were the majority of cells in the periphery, and IgD^{+} transitional cells had decreased; the B cells in the tissue at TP were almost entirely naive B cells (**Figure S1, SDC,** http://links.lww.com/TXD/A483). BAFF levels had returned
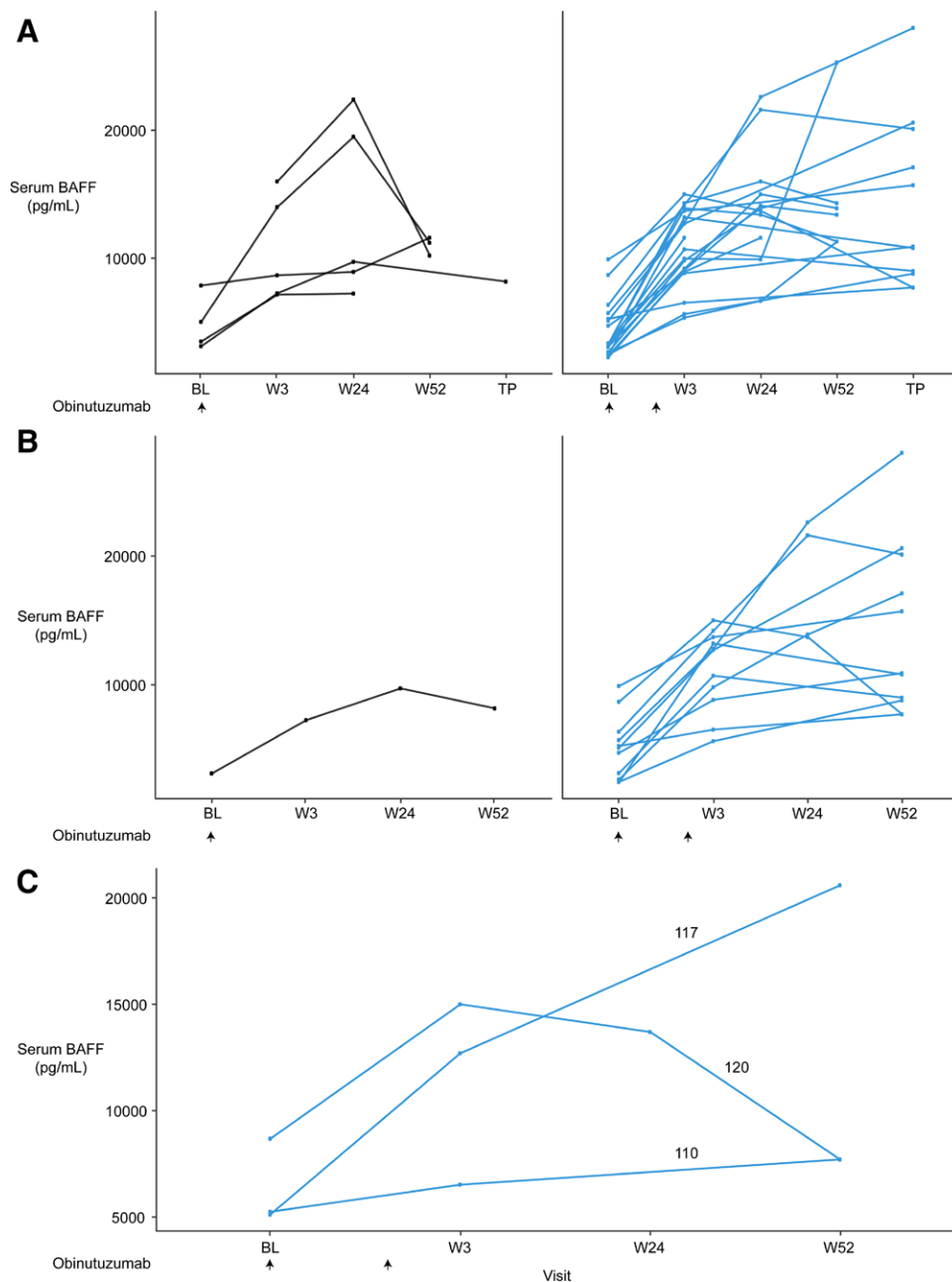
FIGURE 4. BAFF increased in patients treated with obinutuzumab. Serum samples were collected from patients before and after dosing with obinutuzumab and BAFF measured by ELISA ([A] all patients, [B] patients who went to transplant, [C] patients with detectable B cells in tissue; black = cohort 1, blue = cohort 2). Samples collected on days of obinutuzumab administration were collected predose. BAFF, B cell–activating factor; BL, baseline; ELISA, enzyme-linked immunosorbent assay; TP, time of kidney transplant; W, week.

to baseline levels by TP, possibly reflecting the return of B cells to tissue, and CXCL13 levels had increased to roughly baseline levels, potentially reflecting the expansion of naive cells in the lymph nodes.

It is particularly interesting to note that obinutuzumab/IVIG treatment reduced memory B cells, DN B cells, and CD20^{low} PBs, cells considered key pathogenic subsets in B cell–driven diseases including systemic lupus erythematosus,[22-25] while theoretically sparing CD20^{neg} PCs. Although we did not evaluate the effects of obinutuzumab on bone marrow PCs directly, no changes in vaccination titers were observed in treated patients,[17] suggesting that obinutuzumab had no or limited effect—as expected—on these cells. In agreement,

levels of total serum immunoglobulin G did not decrease and remained above the lower limit of normal (5.65 g/L) in all patients measured during the 52-wk treatment period.[17] It is theoretically possible that treatment with IVIG may confound the immunoglobulin measurements; however, total immunoglobulin G levels were assessed at weeks 12 and 24, which is 6 and 18 wk after the last administration of IVIG, respectively, at which point the contribution of externally administered IVIG should be limited.[26] PB/PCs not residing in long-term survival niches in the bone marrow appear to be CD20^{low} (**Figure S2, SDC**, http://links.lww.com/TXD/A483); however, it is not yet known what density of CD20 on the cell surface is required to mediate direct depletion by obinutuzumab.
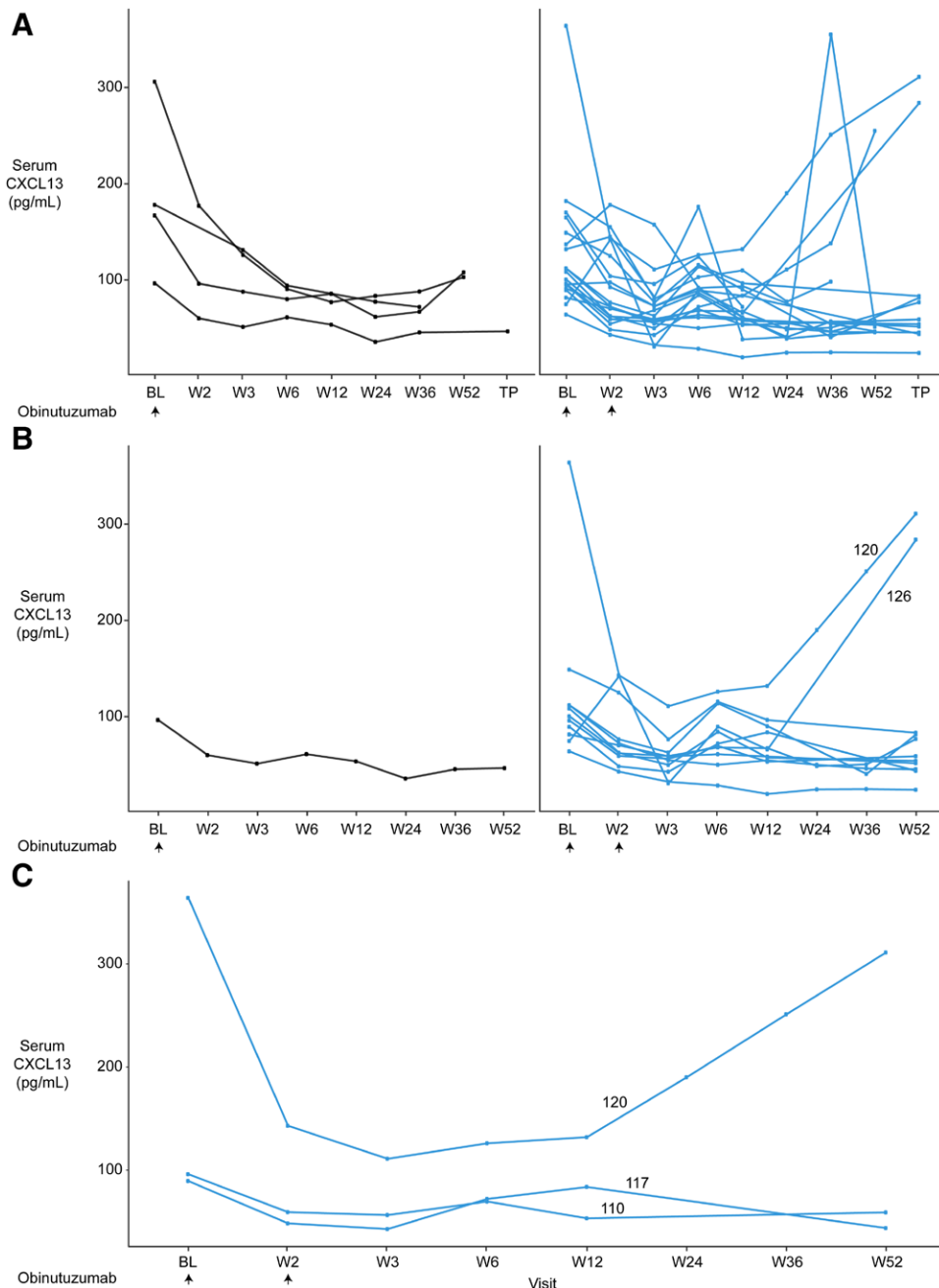
FIGURE 5. CXCL13 decreased in patients treated with obinutuzumab. Serum samples were collected from patients before and after dosing with obinutuzumab and CXCL13 measured by ELISA ([A] all patients, [B] patients who went to transplant, [C] patients with detectable B cells in tissue; black=cohort 1, blue=cohort 2). Samples collected on days of obinutuzumab administration were collected predose. BL, baseline; CXCL13, C-X-C motif chemokine ligand 13; ELISA, enzyme-linked immunosorbent assay; TP, time of kidney transplant; W, week.

Although the loss of subsets expressing higher levels of CD20 is likely because of direct depletion, the loss of PB/PCs (ie, precursors of these subsets) may be because of direct or indirect effects of obinutuzumab.

Although CXCL13 is produced by other immune cells as well as B cells,[20,27-29] some trends in an obinutuzumab treatment effect were observed. Levels of serum CXCL13 decreased from predose levels in all obinutuzumab-treated patients—an effect seen after a single dose and continuing after the second—potentially because of loss of B cells in lymphoid follicles in secondary lymphoid tissue. In patients with detectable tissue B cells post-obinutuzumab, a recovery of CXCL13 levels from a week 3 nadir was observed. However,

the patterns of effects on CXCL13 were variable and did not always associate with loss of tissue B cells, possibly reflecting myriad cellular sources (including T_{FH} cells, which were not noticeably affected by obinutuzumab).

The pharmacodynamic effects of B cell–depleting agents have traditionally been measured in blood, as serial samples are easily drawn and evaluated. However, it is critical to evaluate B-cell depletion in the tissue when the indication allows for tissue sampling because peripheral changes are not always reflective of tissue effects[6-9,11,30] and tissues are often more relevant to pathogenic processes than blood.[31,32] Our results demonstrated that the changes effected by obinutuzumab in the blood are reflected in substantial effects on tissue B cells,

including pathogenic subsets of interest in inflammatory diseases, effects that obinutuzumab was specifically engineered to elicit.[14] IVIG was administered with obinutuzumab in the THEORY study. Although IVIG has been shown to have some effects on B cells,[33] it has not been shown to substantially affect B-cell levels in the periphery or spleen.[9,34]

One consideration in this study was the unavailability of predose tissue. However, although it is possible that the comparator nodes were not ideally representative of the THEORY patient population (eg, different cPRA levels), the causes of their ESRD broadly resembled THEORY patients (**Table S1, SDC,** http://links.lww.com/TXD/A483). Therefore, it is reasonable to use this group as a relevant comparator for obinutuzumab effects.

To minimize variables that can impact tissue staining performance, tissue handling and processing were standardized for the lymph nodes collected in THEORY and comparator cohorts. Immunofluorescence analysis of these intact tissue halves showed similar reductions in B cells in the obinutuzumab-treated group, supporting the conclusion that observations were pharmacodynamic effects of obinutuzumab.

Whether this profound reduction in B cells after treatment with obinutuzumab will occur in other nononcology indications is unknown. Although other indications do not allow the same degree of tissue sampling as in transplantation, in a recent study of obinutuzumab in lupus nephritis (NOBILITY), efficacy correlated with sustained peripheral B-cell depletion (via the highly sensitive flow cytometry assay used in this study), and an elevation of BAFF was similarly observed.[35,36]

Although the present study provides evidence of enhanced B-cell depletion with obinutuzumab/IVIG, changes in anti-HLA antibodies, numbers of unacceptable antigens, and cPRA scores were limited and not clinically meaningful.[17] It is not known whether this B-cell depletion in the periphery and lymph nodes with obinutuzumab will result in clinical benefits over rituximab in management of autoimmune diseases and chronic humoral sensitization conditions. Of note, 1 limitation was the lack of assessment of long-lived bone marrow PC populations. The relative contributions of the small circulating peripheral blood PC subset versus the bone marrow PC population in antibody-mediated diseases remain to be defined.

Previous publications have noted that rituximab treatment can reduce peripheral B cells while sparing tissue B cells.[6-9,11,30] Compared with rituximab, obinutuzumab, a type 2 antibody, is less reliant on complement-dependent cytotoxicity for the majority of its depletion potential and can mediate direct cell death via binding to CD20.[37] This difference may provide obinutuzumab a mechanistic advantage in depleting B cells in the tissue, which has been demonstrated in animal models and in CLL. In this study, we supported this proposed mechanism of action of obinutuzumab and added to existing preclinical and clinical data supporting the superiority of B-cell depletion via obinutuzumab compared with rituximab.[14,16] We demonstrated that obinutuzumab/IVIG effectively reduces B-cell subsets of interest in the tissue in addition to the blood in a nononcology indication, a difference that may mediate better therapeutic outcomes in B cell–mediated diseases. Consistent with this hypothesis, a phase 2 study of obinutuzumab in lupus nephritis has met its primary endpoint where the phase 3 study of rituximab did not.[4,36]

REFERENCES

1. Chaplin DD. Overview of the immune response. *J Allergy Clin Immunol.* 2010;125(Suppl 2):S3–23.
2. Golay J, Zaffaroni L, Vaccari T, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. *Blood.* 2000;95:3900–3908.
3. Kaegi C, Wuest B, Schreiner J, et al. Systematic review of safety and efficacy of rituximab in treating immune-mediated disorders. *Front Immunol.* 2019;10:1990.
4. Rovin BH, Furie R, Latinis K, et al; LUNAR Investigator Group. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum.* 2012;64:1215–1226.
5. Hawker K, O'Connor P, Freedman MS, et al; OLYMPUS trial group. Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. *Ann Neurol.* 2009;66:460–471.
6. Audia S, Samson M, Guy J, et al. Immunologic effects of rituximab on the human spleen in immune thrombocytopenia. *Blood.* 2011;118:4394–4400.
7. Genberg H, Hansson A, Wernerson A, et al. Pharmacodynamics of rituximab in kidney allotransplantation. *Am J Transplant.* 2006;6:2418–2428.
8. Kamburova EG, Koenen HJ, Borgman KJ, et al. A single dose of rituximab does not deplete B cells in secondary lymphoid organs but alters phenotype and function. *Am J Transplant.* 2013;13:1503–1511.
9. Ramos EJ, Pollinger HS, Stegall MD, et al. The effect of desensitization protocols on human splenic B-cell populations in vivo. *Am J Transplant.* 2007;7:402–407.
10. Anolik JH, Barnard J, Cappione A, et al. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum.* 2004;50:3580–3590.
11. Thurlings RM, Vos K, Wijbrandts CA, et al. Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response. *Ann Rheum Dis.* 2008;67:917–925.
12. Vital EM, Dass S, Buch MH, et al. B cell biomarkers of rituximab responses in systemic lupus erythematosus. *Arthritis Rheum.* 2011;63:3038–3047.
13. Klein C, Lammens A, Schafer W, et al. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. *MAbs.* 2013;5:22–33.
14. Mossner E, Brunker P, Moser S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood.* 2010;115:4393–4402.
15. Reddy V, Klein C, Isenberg DA, et al. Obinutuzumab induces superior B-cell cytotoxicity to rituximab in rheumatoid arthritis and systemic lupus erythematosus patient samples. *Rheumatology (Oxford).* 2017;56:1227–1237.
16. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N Engl J Med.* 2014;370:1101–1110.
17. Redfield RR, Jordan SC, Busque S, et al. Safety, pharmacokinetics, and pharmacodynamic activity of obinutuzumab, a type 2 anti-CD20 monoclonal antibody for the desensitization of candidates for renal transplant. *Am J Transplant.* 2019;19:3035–3045.
18. Smulski CR, Eibel H. BAFF and BAFF-receptor in B cell selection and survival. *Front Immunol.* 2018;9:2285.
19. Havenar-Daughton C, Lindqvist M, Heit A, et al; IAVI Protocol C Principal Investigators. CXCL13 is a plasma biomarker of germinal center activity. *Proc Natl Acad Sci USA.* 2016;113:2702–2707.
20. Litsiou E, Semitekolou M, Galani IE, et al. CXCL13 production in B cells via toll-like receptor/lymphotoxin receptor signaling is involved in lymphoid neogenesis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2013;187:1194–1202.

21. Londono AC, Mora CA. Role of CXCL13 in the formation of the meningeal tertiary lymphoid organ in multiple sclerosis. *F1000Res.* 2018;7:514.

22. Jacobi AM, Mei H, Hoyer BF, et al. HLA-DRhigh/CD27high plasmablasts indicate active disease in patients with systemic lupus erythematosus. *Ann Rheum Dis.* 2010;69:305–308.

23. Wei C, Anolik J, Cappione A, et al. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J Immunol.* 2007;178:6624–6633.

24. You X, Zhang R, Shao M, et al. Double negative B cell is associated with renal impairment in systemic lupus erythematosus and acts as a marker for nephritis remission. *Front Med (Lausanne).* 2020;7:85.

25. Zhu L, Yin Z, Ju B, et al. Altered frequencies of memory B cells in new-onset systemic lupus erythematosus patients. *Clin Rheumatol.* 2018;37:205–212.

26. Bonilla FA. Pharmacokinetics of immunoglobulin administered via intravenous or subcutaneous routes. *Immunol Allergy Clin North Am.* 2008;28:803–819.

27. Ferretti E, Ponzoni M, Doglioni C, et al. IL-17 superfamily cytokines modulate normal germinal center B cell migration. *J Leukoc Biol.* 2016;100:913–918.

28. Manzo A, Paoletti S, Carulli M, et al. Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis. *Eur J Immunol.* 2005;35:1347–1359.

29. Rao DA. T cells that help B cells in chronically inflamed tissues. *Front Immunol.* 2018;9:1924.

30. Nakou M, Katsikas G, Sidiropoulos P, et al. Rituximab therapy reduces activated B cells in both the peripheral blood and bone marrow of patients with rheumatoid arthritis: depletion of memory B cells correlates with clinical response. *Arthritis Res Ther.* 2009;11:R131.

31. Arazi A, Rao DA, Berthier CC, et al; Accelerating Medicines Partnership in SLE network. The immune cell landscape in kidneys of patients with lupus nephritis. *Nat Immunol.* 2019;20:902–914.

32. Pollastro S, Klarenbeek PL, Doorenspleet ME, et al. Non-response to rituximab therapy in rheumatoid arthritis is associated with incomplete disruption of the B cell receptor repertoire. *Ann Rheum Dis.* 2019;78:1339–1345.

33. Mitrevski M, Marrapodi R, Camponeschi A, et al. Intravenous immunoglobulin and immunomodulation of B-cell – in vitro and in vivo effects. *Front Immunol.* 2015;6:4.

34. Mitrevski M, Marrapodi R, Camponeschi A, et al. Intravenous immunoglobulin replacement therapy in common variable immunodeficiency induces B cell depletion through differentiation into apoptosis-prone CD21(low) B cells. *Immunol Res.* 2014;60:330–338.

35. Vital E, Rémy P, Porras LQ, et al. SAT0166 biomarkers of b-cell depletion and response in a randomized, controlled trial of obinutuzumab for proliferative lupus nephritis. *Ann Rheum Dis.* 2020;79:1023–1024.

36. Furie RA, Aroca G, Cascino MD, et al. B-cell depletion with obinutuzumab for the treatment of proliferative lupus nephritis: a randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis.* 2022;81:100–107.

37. Herter S, Herting F, Mundigl O, et al. Preclinical activity of the type II CD20 antibody GA101 (obinutuzumab) compared with rituximab and ofatumumab in vitro and in xenograft models. *Mol Cancer Ther.* 2013;12:2031–2042.