Kidney360

Ixazomib for Desensitization (IXADES) in Highly Sensitized Kidney Transplant Candidates: A Phase II Clinical Trial

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Key Points

- Ixazomib treatment resulted in decreases in B-cell subsets and bone marrow lymphocytes.
- Ixazomib treatment resulted in modest decreases in certain anti-HLA antibody specificities.
- Ixazomib treatment was tolerated, with modest adverse events.

Abstract

Background Ixazomib is a second-generation oral proteasome inhibitor approved for treatment of refractory multiple myeloma. We conducted an open-label phase II trial, IXAzomib for DESensitization (IXADES), testing the safety of ixazomib treatment as an approach to decreasing the level and diversity of specificities of anti-HLA antibodies in subjects awaiting kidney transplantation. The trial (NCT03213158) enrolled highly sensitized kidney transplant candidates, defined as subjects with calculated panel reactive antibodies (cPRA) >80%, awaiting kidney transplantation >24 months. The subjects were treated with 12 monthly cycles of ixazomib 3 mg+dexamethasone 20 mg. Efficacy was defined as a decrease of cPRA >20% or kidney transplantation. The safety end point was tolerability.

Methods In ten enrolled subjects, no grade IV, five grade III, 11 grade II, and 43 grade I adverse events were noted. The adverse events included infection, transient paresthesia, nausea, vomiting, and diarrhea. The IXADES regimen was not associated with significant change in levels or diversity of anti-HLA antibodies (cPRA).

Results Although the IXADES regimen did not exhibit a clear impact on levels and diversity of anti-HLA antibodies in this small cohort, the prolonged half-life of IgG could necessitate a longer duration of treatment for accurate evaluation of efficacy.

Conclusions In conclusion, treatment with ixazomib/dexamethasone engendered mild-to-moderate toxicity. The impact on anti-HLA was modest and paradoxical in the case of anti-HLA-DR. Clinical trials combining ixazomib with other immunosuppressive agents may be more effective in addressing antibody-mediated processes in kidney transplantation.

KIDNEY360 4: 796–808, 2023. doi: https://doi.org/10.34067/KID.0000000000000113

Introduction

Alloantibodies present a significant hurdle to kidney transplantation. Thirty-nine percent of those awaiting kidney transplant have detectable anti-HLA antibodies directed against a fraction of potential transplant donors (denoted by panel reactive antibody [PRA]) ≥1%.[1] Nearly 15,000 (15%) have antibodies against ≥80% of potential donors.[1] The time a patient must wait to

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undergo transplantation varies directly with the PRA. On average, those with PRA <1% undergo transplantation within a year, and those with PRA >98% wait longer, 6.9 years on average, to undergo transplantation.[1] The period of waiting for kidney transplantation is not merely an inconvenience because a significant percent of those waiting for kidney transplantation die every year.[1] The morbidity and mortality associated with the delay in kidney transplantation spark efforts to identify treatments that decrease the levels of anti-HLA antibodies and the PRA. Despite some success, current desensitization protocols are limited because of their complexity, toxicities, and reduced success to decrease donor-specific antibodies (DSAs). The lack of success is in part due to the absence of effective agents against plasma cells, which produce most HLA alloantibodies. A significant number of highly sensitized subjects die before receiving a transplant, outlining the critical importance of desensitization strategies.

Two approaches for helping highly sensitized subjects are to increase the chance of finding a crossmatch negative donor or to remove the preexisting antibodies using desensitization protocols.[2–6] Emerging evidence suggests that strategies to improve transplant rates in highly sensitized subjects enhance survival rates and the quality of life while reducing costs compared with chronic dialysis.[7,8] Current desensitization protocols combine rituximab (anti-CD20 monoclonal antibody) to deplete B cells, costimulation blockade and proteasome inhibition to inhibit antibody secretion,[9,10] and plasmapheresis plus intravenous immunoglobulins to block or remove preformed DSAs and replace loss of protective IgG.[2–6] Overall, current desensitization protocols are limited by their toxicity, logistical challenges, and failure in 30%–90% of subjects.[5,11,12]

First-generation proteasome inhibitors bortezomib and carfilzomib have been investigated therapies for desensitization[9,10] and for the treatment of antibody-mediated rejection. However, delivery requires injection, and the treatments are associated with gastrointestinal, neurological, and infectious complications.[12–16] Ixazomib is a second-generation oral proteasome inhibitor approved for the treatment of multiple myeloma.[17–22] This compound is a dipeptidilic boronic acid that is rapidly hydrolyzed in water and converts into the active form: ixazomib. The active form of ixazomib potently, reversibly, and selectively inhibits the proteasome.[18,20,22] It is more effective than earlier generation proteasome inhibitors with improved side effect profiles.[17,23–28] Having a more potent, less toxic proteasome inhibitor that does not need to be delivered intravenously and can be used in subjects with reduced kidney function will be clinically advantageous.[27] We have demonstrated that ixazomib is safe and effective for the treatment of antibody-mediated rejection in a robust preclinical model.[29] In this study, we investigated the safety and efficacy of ixazomib in a single-center phase II clinical trial for desensitization.

Materials and Methods

Study Population and Design

IXAzomib for DESensitization (IXADES) was a single-center prospective observational study (NCT03213158) funded by Takeda Pharmaceutical Company Limited and approved by the Institutional Review Board at University of Wisconsin (UW IRB: 2017-0429, NCT03213158). Subjects were treated with 12 monthly cycles of ixazomib 3 mg+dexamethasone 20 mg (Figure 1). The lower 3-mg dose (instead of 4 mg) of ixazomib was selected because previous clinical trials of ixazomib for the treatment of multiple myeloma had excluded patients with end-stage renal disease. The efficacy end point was a decline in calculated panel reactive antibodies (cPRA) >20% or kidney transplantation. The safety end point was based on tolerability. Adverse events were monitored and graded according to Common Terminology Criteria for Adverse Events v5.0 US Dept Health and Human Services. Grade 1 adverse events include conditions that are asymptomatic or mildly symptomatic, with no intervention needed. Grade 4 adverse events would have life-threatening consequences requiring urgent interventions. Ancillary studies addressed changes in HLA antibodies, bone marrow (BM) and circulating T-cell and B-cell phenotypes, and cytokines at two time points: T1 (3 months) and T2 (last measurement, see below). The main inclusion criteria included adult (age 18–70 years) kidney transplant candidates who were highly sensitized (cPRA>80%) and were active on the waitlist for more than 2 years. Main exclusion criteria included active or treated infection for HIV, hepatitis C virus, or hepatitis B virus, liver cirrhosis, elevated transaminases, hypersensitivity to ixazomib, platelet count of<30,000/ml, absolute neutrophil count <1000/ml, hemoglobin<6 g/dl, grade 2 or greater peripheral neuropathy, myocardial infarction within 6 months before enrollment or New York Heart Association class III or IV heart failure, uncontrolled angina, electrocardiogram evidence of acute ischemia or active conduction system abnormalities, active substance abuse, psychiatric disorder or a condition that in the opinion of the investigator invalidated communication with the investigator, female subject was pregnant or breast-feeding, or the subject was not taking any investigational drug in the year on study. Subjects were followed for 12 months or until 1 month after transplantation.

PBMC and BM Isolation

PBMCs and BM samples were isolated as previously described.[30] In brief, heparin anticoagulated blood is layered over Ficoll-Hypaque (Global Life Sciences, cat 17144002). Buffy coat was removed, red blood cells were lysed, and PBMCs were counted. BM was first filtered through a 40-mm tube top filter to remove bone fragments and other materials and then was layered over Ficoll and processed similarly to PBMCs. PBMCs were collected every 3 months, whereas BM was collected at baseline and the end of the study or at the time of transplantation if applicable.

Immunophenotyping by Flow Cytometry

Single-cell suspensions of PBMCs were prepared from thawed samples and stained for B-lymphocyte and T-lymphocyte subsets. See Supplemental Table 1 for markers used to define subsets. Antibodies used for immunophenotyping are listed in Supplemental Table 2. Fluorescently labeled cells were detected on a BD LSR Fortessa at the UW-Carbone Cancer Center Flow Cytometry core facility. Flow analyses were conducted using FlowJo v.10.5.3 (BD Biosciences Inc., Ashland, OR). Cells were gated for singlets and live cells and then CD45+ white blood cell lineages were gated. B cells were identified as $CD3^-$ cells, $CD19^+$, and/or $CD20^+$ and

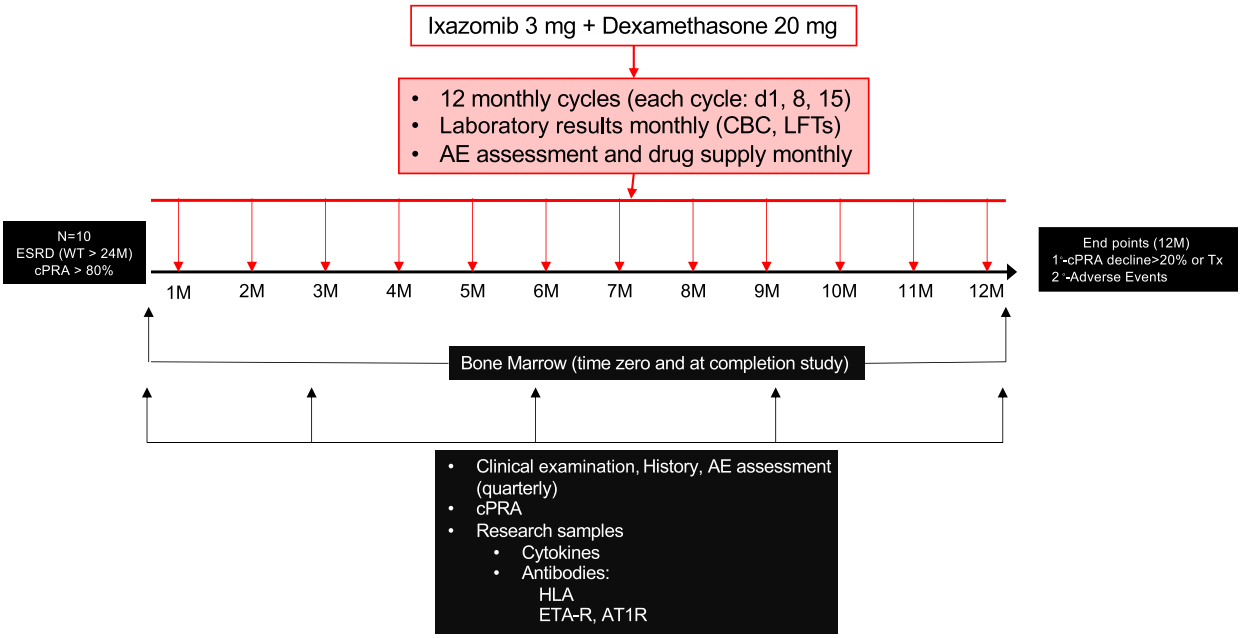

Figure 1. IXADES in kidney transplantation study design. Highly sensitized subjects (cPRA>80%) active on the waitlist for >2 years were recruited to this prospective study. Primary efficacy end point was a decline in cPRA of >20%. Secondary efficacy end point was successful transplantation within 12 months. Primary safety end point was incidence of infections, malignancies, hematological complications, changes in left ventricular ejection fraction, distal neuropathy, gastrointestinal symptoms, and immunosuppression-related adverse effects. Subjects were to receive 12 monthly cycles of 3 mg of ixazomib with 20 mg of dexamethasone. Clinical laboratory results were assessed monthly, and bone marrow samples were taken at the time of enrollment and at 12 months on study. Research blood was drawn at 3, 6, 9, and 12 months to assess for cytokines, antibodies to HLA and non-HLA antigens, and immunophenotyping. LFT, liver function test, AE, adverse event; AT-1R, angiotensin 1 receptor; ETA-R, endothelin II type A receptor.

then gated into subsets according to CD38 and CD27 labels. T cells in the lymphocyte gate were identified by their $CD3^+$, $CD4^+$ or $CD3^+$, and $CD8^+$ staining. Data were exported from FlowJo for statistical analyses in Prism (GraphPad, version 9).

Luminex Cytokine Analysis

Ten cytokines were simultaneously measured in the plasma using the Human Magnetic Luminex Assay kit from R&D (R&D LXSAHM). These cytokines included IFN-γ, IL-6, IL-10, IL-13, IL-15, IL-17A, programmed death ligand-1, TNF-related apoptosis-inducing ligand (TRAIL), and vascular endothelial growth factor C. All samples were run in duplicate. Plasma was incubated with antibody-coated MagPlex beads according to the manufacturer's instructions (R&D Biosystems LXSAHM-10). Antibody binding was detected using biotinylated primary antibody and streptavidin-PE and read on a Luminex 200 analyzer.

Single-Antigen Bead Analysis

Donor-specific HLA class I and class II antibodies were assessed at each time point using Luminex single-antigen (Ag) beads (Terasaki lab). The strength of the DSA was represented as the sum of the mean fluorescence intensity (MFI_{sum}). Data were acquired as reported in refs. 31–34. In brief, plasma samples were frozen at −80 and shipped to the Terasaki laboratory on dry ice. When thawed, plasma was incubated with multiplexed single-antigen beads (SABs) containing single alleles of HLA class I or class II alleles. The SAB assay includes built-in control beads, coated with human IgG (positive control) or albumin (human or bovine; negative control). After washing, the reaction was incubated with a PE-conjugated secondary antibody, washed, and resuspended in buffer for acquisition on the Luminex. Non-HLA antibodies to angiotensin II receptor type I and endothelin A receptor were performed as reported previously using a sandwich ELISA technique.[35]

For each subject, we examined the response of the top three immunodominant HLA antibodies to treatment. Specifically, the median of the highest three MFIs was selected. If multiple beads recognized the same antigen, the next highest MFI specificity was selected.

Statistical Analyses

Comparisons between time points were achieved using a paired *t* test, Mann-Whitney, or Wilcoxon signed-ranks test as appropriate. We used the Spearman correlation test to measure the strength and direction of monotonic association between two variables. *P* values of ≤0.05 were considered statistically significant. Analyses were performed using GraphPad Prism version 9.

Results

Baseline Characteristics

A total of ten potential kidney transplant recipients were enrolled in this trial. Subject characteristics are summarized in Table 1. The median age was 37 years; there were three female and seven male subjects and two subjects reported African American ethnicity. All subjects were previously transplanted: Two had received two previous kidney transplants (4 and 9),

Table 1. Baseline subject characteristics

Study ID	Age	Sex	Race	cPRA	Cause ESKD	Previous Tx	Tx-IXADES (yr)
IXA001	45	M	W	100	Congenital	K	14.0
IXA002	38	M	B	100	DM	KP	11.2
IXA003	30	F	W	100	GN	K	17.2
IXA004	36	M	W	100	Alport syndrome	KK	5.9
IXA005	28	M	B	100	Congenital	K	18.8
IXA006	51	F	W	83	PKD	K	5.0
IXA007	61	M	W	88	DM	K	10.3
IXA008	47	F	W	87	SLE	K	8.5
IXA009	28	M	W	96	Congenital	KK	9.7
IXA010	34	M	W	100	FSGS	K	10.1

cPRA, calculated panel reactive antibodies; IXADES, IXAzomib for DESensitization; DM, diabetes mellitus; KP, kidney pancreas; KK, then another kidney; PKD, polycystic kidney disease; SLE, systemic lupus erythematosus; FSGS, focal segmental glomerulosclerosis.

and one subject had received a kidney-pancreas transplant (2). The interval from initiation of dialysis to enrollment varied between 5 and 18.8 years.

Primary End Points

To determine whether our experimental treatment compromised subject safety, we examined white blood cells, hemoglobin, hematocrit, platelets, and left ventricular ejection fraction (LVEF) (Figure 2, A–E). No significant change in complete blood count or LVEF was noted. There were zero grade IV, three grade III, 11 grade II, and 43 grade I adverse events. These were primarily infections,[12] paresthesia,[3] and nausea/vomiting/diarrhea[5] (Table 2). Subjects 1 and 2 had hematomas at the site of BM aspirations, likely related to coagulopathy often seen in dialysis patients. Of the ten subjects, two were transplanted (subjects 3 and 10) (Figure 3); subject 3 received an HLA-matched kidney allograft while subject 10 was transplanted across the HLA barrier. Neither had a significant change in their cPRA or HLA DSA (Figure 2F). Two subjects were lost to follow-up (subjects 7 and 8), and two were removed from the study: subject 6 for progressive frailty because she was no longer eligible for transplantation and subject 9 because of substance abuse (Table 3).

Another primary efficacy end point was a reduction in cPRA of >20% (Figure 2F). Subjects included in this study started with a cPRA >80%. IXA006 had the lowest starting cPRA at 83% (blue triangle) at the start. No subjects experienced a significant decrease in cPRA >20% over time.

Ixazomib Treatment Was Associated with a Reduction in the Number of Circulating Mature B Cells and Memory B Cells

We next examined the effect of ixazomib treatment on circulating B-cell subsets. Although the decline in total B cells ($CD3^-CD19^+CD20^+$) and naïve B cells ($CD3^-CD19^+CD20^+CD27^-CD38^{lo}$) did not reach statistical significance, mature/activated B cells ($CD3^-CD19^+CD20^+CD27^+$

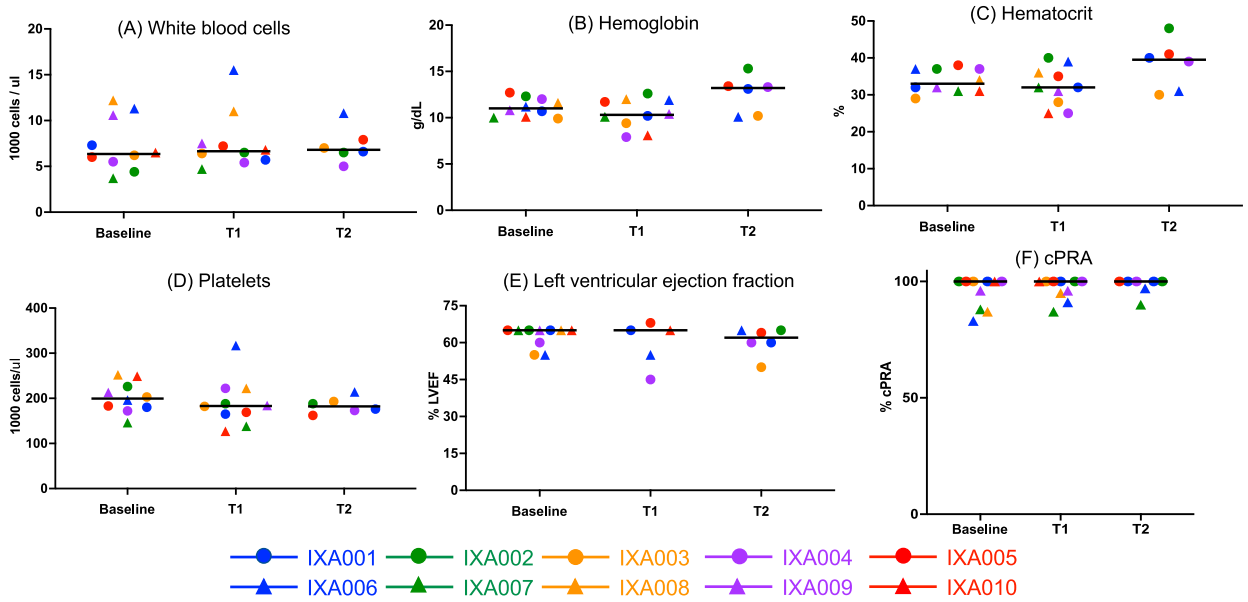

Figure 2. No significant changes were seen in complete blood count, LVEF, and cPRA. Blood was drawn at baseline, 3 months after enrollment (T1), and the time of last follow-up (T2). We analyzed white blood cells, hemoglobin, hematocrit, platelets, and left ventricular ejection fraction. As seen in the graphs, there were no significant changes at any time point. The black horizontal line is the median of values at that time point. Individual subjects are indicated by color and shape of symbols. LVEF, left ventricular ejection fraction.

Table 2. Adverse events, serious adverse events, and study withdrawals

Patient ID	SAE	Comment	AE	Comment	Comment
IXA-1	3	Hematoma at fistula surgery site, readmitted for hematoma from fistula surgery, hematoma from end of study bone marrow aspiration	3	Common cold, latent TB, cellulitis	N/A
IXA-2	1	Hematoma at bone marrow aspiration site	3	Outpatient surgery, vomiting, cough from common cold	N/A
IXA-3	0	—	16	Paresthesia, hip pain, thrush, dermatitis, hypercholesterolemia, nausea, vomiting, headache	Transplanted
IXA-4	1	Mechanical aortic valve placed	2	Paresthesia, wisdom tooth infection	N/A
IXA-5	4	Bacteremia, blood clot associated with dialysis catheter, liver abscess	12	Diarrhea, chest pain, nausea, hemorrhoids, bacteremia, blood clot associated with dialysis catheter, stomach pain, liver abscess	N/A
IXA-6	0	—	28	Postmenopausal bleeding, nausea, paresthesia, foot fracture	Withdrawn from transplant waitlist and study because of frailty
IXA-7	1	Pneumonia	13	Hyperglycemia, common cold with cough and shortness of breath, insomnia, edema, shingles, hallucinations, pneumonia	Lost to follow-up
IXA-8	0	—	4	Diarrhea, acid reflux	Lost to follow-up
IXA-9	1	Alcohol intoxication	4	Irritability, alcohol intoxication	Withdrawn from study and waitlist for active alcohol abuse
IXA-10	0	—	0	N/A	Transplanted

SAE, serious adverse event; AE, adverse event TB, tuberculosis; N/A, not applicable.

CD38^{moderate}) declined significantly from 313 (267–392) cells/10^5 lymphocytes to 64 (28–166), $P = 0.008$, and memory B cells ($CD3^-CD19^+CD20^+CD27^+CD38^-$) decreased from 608 (380–842) cells/10^5 lymphocytes to 101 (36–200), $P = 0.004$ (Figure 4).

Ixazomib Was Associated with Downregulation of T Cells

To determine the effect of ixazomib on circulating T cells and natural killer cells, we measured $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^-CD56^+$ cells at baseline and 3 months (T1, Figure 5). We noted that $CD3^+$ declined

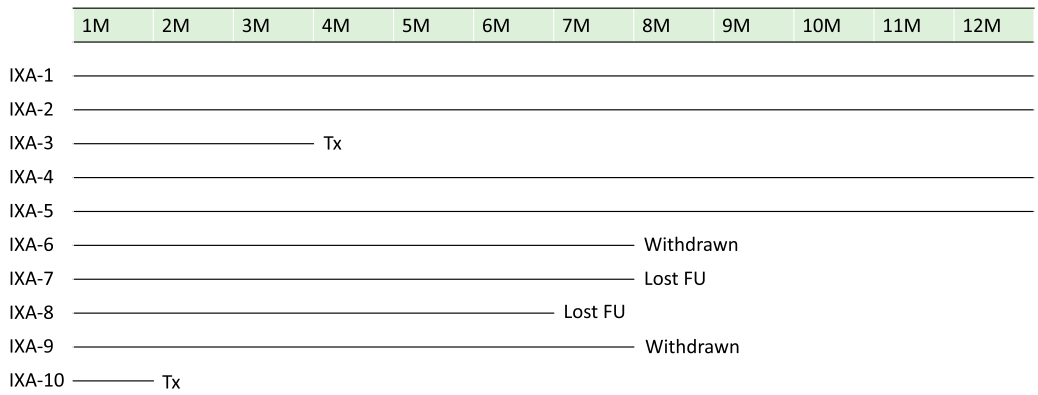

Figure 3. Clinical outcomes. Ten subjects were enrolled in the trial. Of them, two were transplanted (subjects 3 and 10); subject 3 received an HLA-matched kidney allograft while subject 10 was transplanted across the HLA barrier, without a significant change in their cPRA or HLA DSA (Figure 2F). Two subjects were lost to follow-up (subjects 7 and 8), and two were withdrawn: subject 6 for progressive frailty as she was no longer eligible for transplantation and subject 9 because of substance abuse. DSA, donor-specific antibody.

Table 3. Adverse events observed for all 10 subjects.

Adverse Event	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Drug Related?
	N	N	N	N	N	
Gastrointestinal disorders						
Diarrhea	0	4	0	0	0	Y
Nausea	0	17	0	0	0	Y
Hemorrhoids	0	0	1	0	0	N
Gastroesophageal reflux disease	0	2	0	0	0	Y
Infections and infestations						
Eye infection	0	0	1	0	0	Y
Gum infection	0	0	1	0	0	Y
Upper respiratory infection	0	1	0	0	0	Y
Hepatic infection	0	0	0	1	0	Y
Bacteremia	0	0	0	1	0	Y
Nervous system	0	0	0	0	0	
Paresthesia	0	10	0	0	0	Y
Respiratory, thoracic, and mediastinal						
Cough	0	0	1	0	0	Y
Dyspnea	0	1	0	0	0	N
Vascular						
Hematoma	0	2	0	1	0	N
Thromboembolic event	0	0	0	1	0	N
Reproductive system and breast disorders—other						
Postmenopausal bleeding						N
Psychiatric disorders	0	1	0	0	0	
Insomnia	0	1	0	0	0	Y
Irritability	0	3	0	0	0	N
General disorders and administration site conditions						
Edema limbs	0	1	0	0	0	Y
Metabolism and nutrition disorders						
Alcohol intolerance	0	0	0	1	0	N
Hyperglycemia	0	0	6	0	0	N
Cardiac disorders						
Chest pain	0	0	1	0	0	N
Total	0	43	11	5	0	
Percent of total		73%	19%	8%		

from 65,200 (54,950–74375) cells/10^5 lymphocytes to 37,750 (32,625–41,908) $P = 0.05$ and $CD3^+CD4^+$ from 30,878 (24,079–41,908) cells/10^5 lymphocytes to 18,147 (15,867–20,503), $P = 0.03$ (Figure 5). The decline in $CD3^+CD8^+$ cells did not reach statistical significance.

BM Lymphocytes Were Significantly Reduced After Ixazomib Therapy

To assess the effect of ixazomib treatment on BM cells, we collected BM samples at baseline and T2 in the five subjects who completed the studies (1, 2, 3, 4, and 10). Only the total number of lymphocytes decreased significantly from 31,228 cells per 100,000 live (16,300–33,700) to 7430 cells per 100,000 live (2660–13,900), $P = 0.05$ (Figure 6). Otherwise, no significant changes in T-cell or B-cell subpopulations were noted (data not shown).

Circulating TRAIL Decreased While BAFF Levels Increased

To determine the effect of ixazomib on circulating cytokines involved in T-cell and B-cell regulation, we measured chemokine (CXC motif) ligand 1, IFN-γ, IL-6, IL-10, IL-13, IL-15, IL17, programmed death ligand-1, vascular endothelial growth factor C, TRAIL, B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) levels at baseline and 3 months (T1, Figure 7). We determined that TRAIL (median 25%–75%) decreased from 38 (18–50) to 26[16–43] pg/ml ($P = 0.03$) while BAFF increased from 126 (113–146) to 160 (155–240) pg/ml ($P = 0.02$, Figure 7). There was no statistically significant change in the levels of other cytokines. The increase in BAFF is consistent with a decrease in the number of B cells, as a consequence of the desensitization treatment.

Treatment had a Limited and Heterogeneous Effect on Circulating Antibodies

We determined short-term and long-term changes in HLA and non-HLA alloantibodies using SAB Luminex assays. For each antibody, changes in median MFIs for the top three immunodominant specificities were analyzed.

Ixazomib treatment did not decrease anti-HLA-DR antibodies in the long term. In fact, we observed a significant rise in the median HLA-DR antibodies' MFA at T2 from 11,595 (3745–15,652) at baseline to 14,012 (3617–16,988), $P = 0.01$ (Figure 8A). There was variability between subjects. For example, subjects IXA001, 002, and 004 showed a significant decline, whereas subject IXA005 demonstrated a significant increase in antibody levels (Figure 8A). Ixazomib treatment decreased anti-HLA class II DQ antibodies from

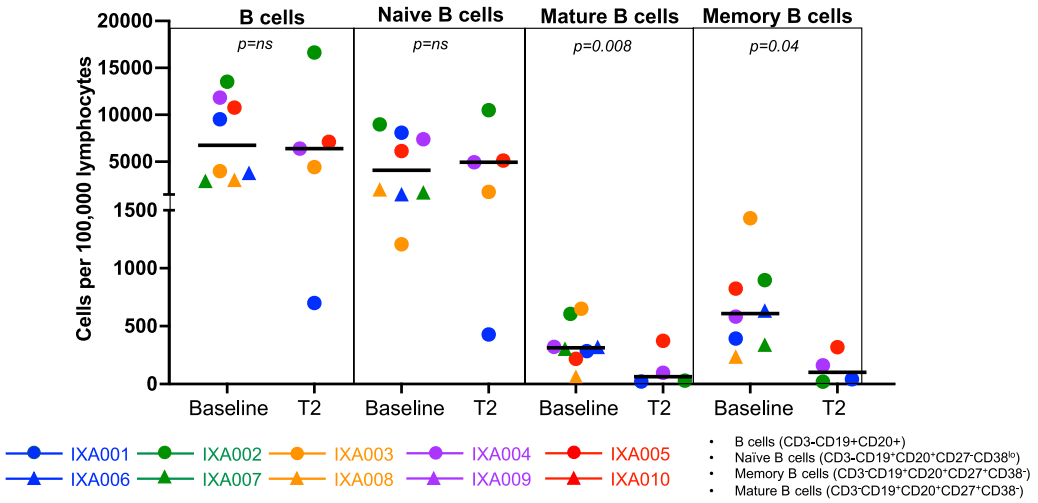

Figure 4. Ixazomib treatment reduces circulating mature B cells and memory B cells. We next examined the effect of ixazomib treatment on circulating B-cell subsets. Although the decline in total B cells ($CD3^-CD19^+CD20^+$) and naïve B cells ($CD3^-CD19^+CD20^+CD27^-CD38^{lo}$) did not reach statistical significance, mature B cells ($CD3^-CD19^+CD20^+CD27^+CD38^{moderate}$) declined significantly from 313 (267–392) cells/10^5 lymphocytes to 64 (28–166), $P = 0.008$, and memory B cells ($CD3^-CD19^+CD20^+CD27^+CD38^-$) decreased from 608 (380–842) cells/10^5 lymphocytes to 101 (36–200), $P = 0.004$.

baseline: 12,837 (7440–16,447) to T1: 11,364 (7564–16,543), P = ns and T2: 9261 (5693–13,798) $P = 0.008$ (Figure 8B). Anti-HLA class II DP antibodies also decreased with treatments, with baseline median MFI at 12,491 (10,210–17,857); T1: 2314 (0–14,337), $P = 0.001$; and T2: 2504 (950, 15,179), $P = 0.009$ (Figure 8C). The results indicate that ixazomib therapy was associated with a decline in anti-HLA class II DQ and anti-HLA class II DP but not anti-HLA class II DR antibodies.

Ixazomib treatment was not accompanied by a decrease in anti-HLA class I A antibodies. Instead, the anti-HLA class I A antibodies increased in subjects IXA002 and IXA008 and decreased in subjects IXA001 and IXA004 with time following ixazomib treatment (Figure 8D). Ixazomib treatment was also not associated with a decrease in anti-HLA class I B antibodies (Figure 8E). By contrast, anti-HLA class I C antibodies decreased in the short-term but not long-term after treatment (Figure 8F). We also did not find any association of ixazomib treatment and changes in non-HLA antibodies to angiotensin 1 receptor or endothelin II type A receptor at any time points (Figure 8, G and H).

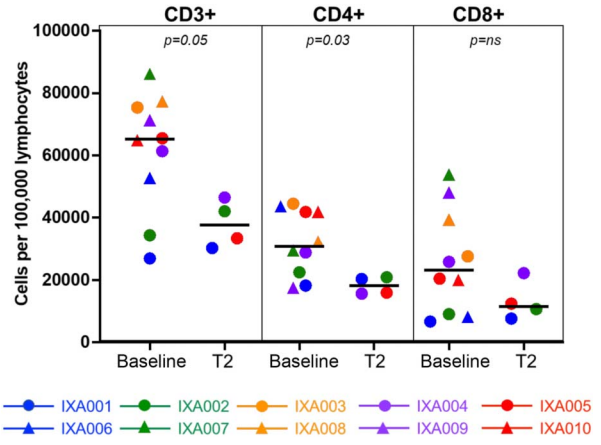

Figure 5. Ixazomib treatment reduces circulating $CD3^+$ and $CD4^+$ T cells. All values are expressed as cells per 100,000 lymphocytes. Circulating $CD3^+$ T cells ($CD3^+$, $P = 0.05$, baseline 65,200 [54,950, 74,375]), T2 (37,750 [32,625, 41,908]) and $CD4^+$ T cells ($CD3^+CD4^+$, $P = 0.03$, baseline 30,878 [24,079, 41,908]), T2 18,147 (15,867, 20,503) were significantly decreased after treatment with ixazomib. $CD8^+$ T cells ($CD3^+CD8^+$, P = ns, baseline 23,143 [11,810, 36,447], T2 11,559 [9936, 14,876]) were diminished, but the difference was not significant. The black horizontal line is the median of values at that time point. Individual subjects are indicated by color and shape of symbols.

Discussion

IXADES was a pilot exploratory, proof-of-concept, open-label, single-center phase II clinical trial (NCT03213158) to test the efficacy of ixazomib+dexamethasone to desensitize highly sensitized kidney transplant candidates. Ten highly sensitized kidney transplant candidates were treated with 12 monthly cycles of ixazomib+dexamethasone. Of them, two were transplanted, two were lost to follow-up, and two withdrew from the study. There was no significant decline in cPRA; median immunodominant alloantibody MFIs declined for some subjects and HLA specificities but not consistently across the population. There was a significant decline in BM lymphocytes and in circulating $CD3^+$ T cells, $CD4^+$ T cells, and mature and memory B cells. Circulating TRAIL decreased, associating with a significant increase in BAFF. BAFF changes inversely with the number of B cells. The findings suggest that ixazomib alone has limited effectiveness on desensitization. Clinical trials combining ixazomib with other immunosuppressive agents may be more effective to induce desensitization in kidney transplantation.

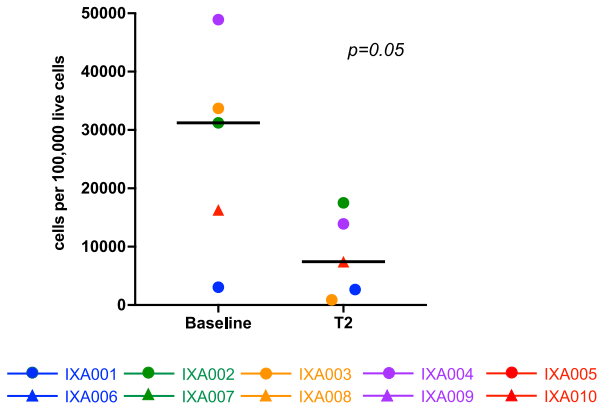

Figure 6. Lymphocyte population in the BM is significantly depleted after ixazomib therapy. To assess the effect of ixazomib treatment on BM cells, we collected BM samples at baseline and T2 in the five subjects who completed the studies (1, 2, 3, 4, and 10). Only the total number of lymphocytes decreased significantly from 31,228 cells per 100,000 live (16,300–33,700) to 7430 cells per 100,000 live (2660–13,900), $P = 0.05$. No significant changes in T-cell or B-cell subpopulations were noted (data not shown). Individual subjects are indicated by color and shape of symbols. BM, bone marrow.

Subjects in our study had cPRA >80% and were allosensitized because of previous transplantation. Except for imlifidase, most single-drug or dual-drug regimens have been unsuccessful in decreasing cPRA in highly sensitized kidney transplant candidates.[5,11,12] We designed the trial with this information in mind. We also hypothesized that IXADES would be a stepping stone for future clinical trials using combination therapies with oral proteasome inhibitors. For safety, ixazomib was overall well-tolerated. We used a lower dose (3 mg) instead of the standard 4 mg because there was no information on dosing in patients with end-stage renal disease. With this dose, we noted no grade IV, 5 grade III, 11 grade II, and 43 grade I adverse events (AEs), including infections, transient paresthesia, nausea, vomiting, and diarrhea. The AEs we observed were similar to those reported in the study by Eskandry, *et al.*[36] in which bortezomib was associated gastrointestinal and hematologic toxicity but little efficacy in halting late DSA-positive antibody-mediated rejection. We also observed unusual serious adverse events related to hematomas at the site of BM aspiration despite normal platelet counts, highlighting the risks of coagulopathy in patients with ESKD. Only in one case, ixazomib was held for shingles. Later, that subject was lost to follow-up. Other AEs were more consistent with known side effects of proteasome inhibitors (gastrointestinal distress, rash, and paresthesia), all temporary and resolving with minimal intervention. Importantly, there was no significant change in complete blood count and LVEF.

For efficacy, we noted no significant decline in cPRA, and although two subjects were transplanted, one received an HLA-matched kidney, and the other was transplanted across the HLA barrier with no significant decline in cPRA or immunodominant DSA. As noted by Schinstock *et al.* in their excellent review, of the various end points for desensitization, cPRA is advantageous because it can be applied to candidates with and without a living donor, it is easy to measure and directly related to a candidate's probability of receiving a kidney transplant, and can also be used in nonkidney solid organ transplant desensitization studies.[37] In our trial, ancillary studies demonstrated some interesting findings. Despite no change in the overall cPRA, we observed a significant decrease in median immunodominant MFIs for HLA-DP and HLA-DQ alloantibodies. This contrasts with Eskandry *et al.* who did not observe improvement in any disease features, including MFIs. Median MFIs for immunodominant HLA-A, HLA-B, and HLA-C alloantibodies showed no changes, but DR antibodies increased. These findings are consistent with previous observations highlighting heterogeneous antibody responses to proteasome inhibition.[38]

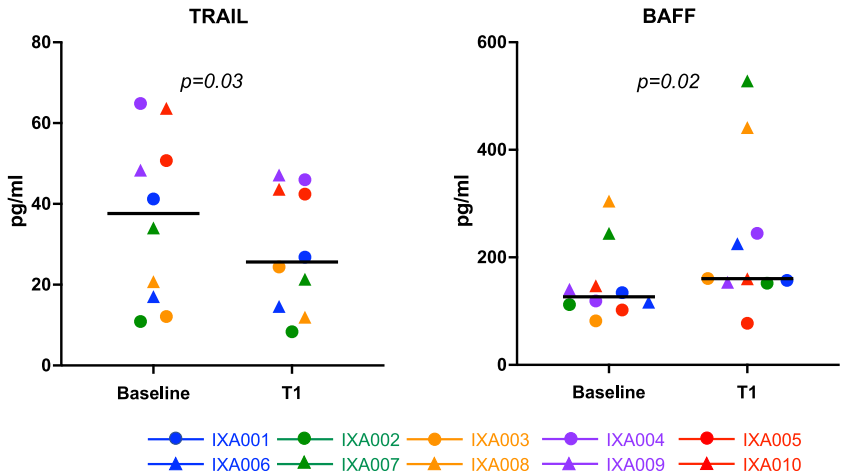

Figure 7. Effect of treatment on circulating cytokines. We analyzed 12 different cytokines, including BAFF and APRIL in plasma samples. CXCL1, IFN-γ, IL-6, IL-10, IL-13, IL-15, IL17, PD-L1, VEGF-C, and APRIL did not significantly change after treatment. However, expression of TRAIL was significantly down regulated by 3 months after treatment ($P = 0.03$, 25th percentile = 16, 75th percentile = 46). By contrast, BAFF was increased at 3 months after treatment ($P = 0.02$, 25th percentile =118, 75th percentile = 230). PD-L1, programmed death ligand-1; TRAIL, TNF-related apoptosis-inducing ligand; VEGF-C, vascular endothelial growth factor C.

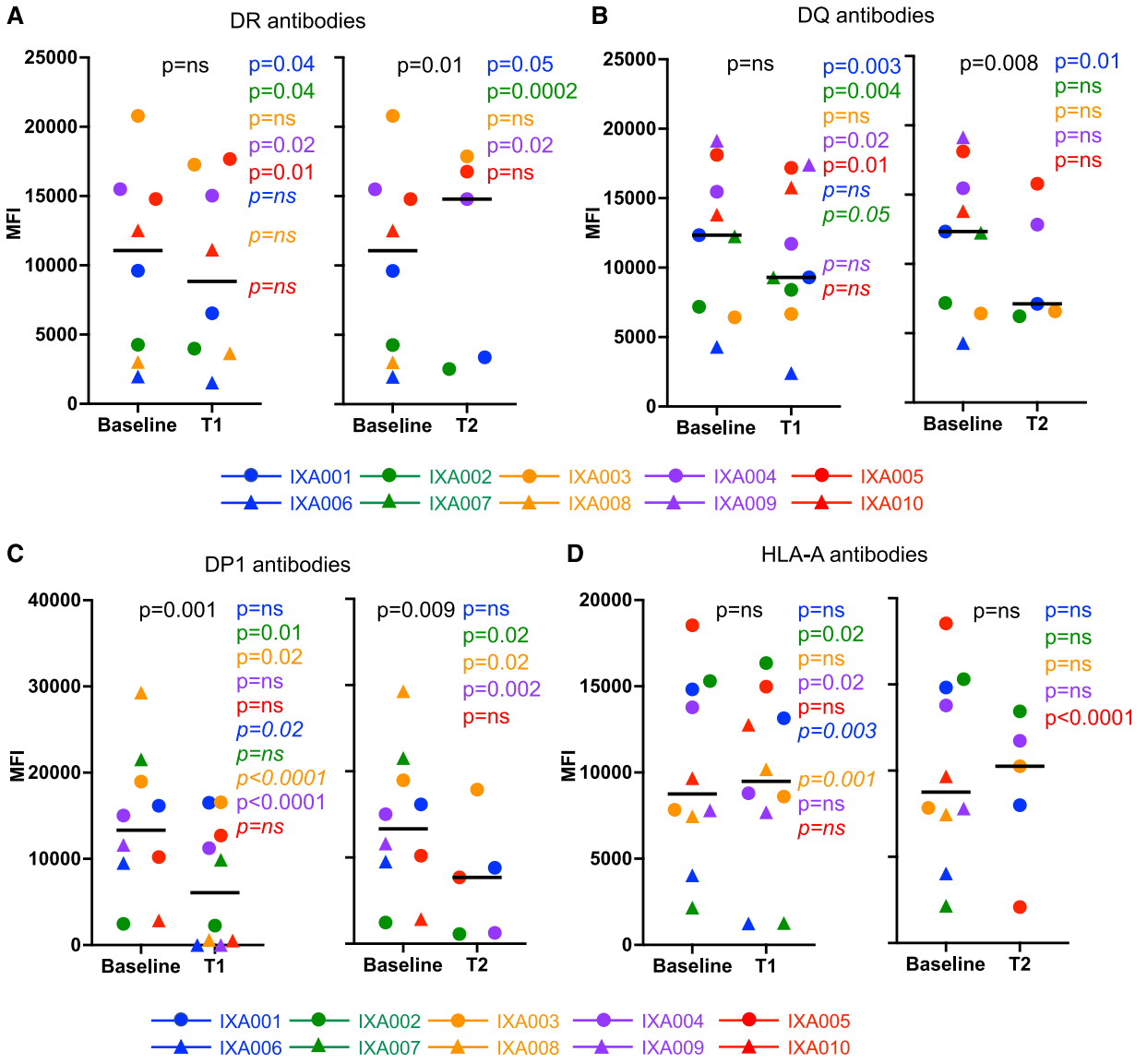

Figure 8. Treatment had a limited and heterogeneous effect on alloantibodies. We analyzed short-term and long-term changes in HLA and non-HLA alloantibodies using SAB Luminex assays. For each antibody, changes in median MFIs for the top three immunodominant specificities were analyzed. (A) For anti-HLA class II DR antibodies, the short-term decline in median (25%–75%) MFIs was not statistically significant: 11,595 (3745–15,652) to 9711 (3692–16,363), P = ns. However, treatment was associated with a significant rise in median MFI between baseline and T2 from 11,595 (3745–15,652) to 14,012 (3617–16,988), $P = 0.01$. There was variability between subjects. For example, subjects IXA001, 002, and 004 showed a significant decline, whereas subject IXA005 demonstrated a significant increase in their antibody levels. (B) Anti-HLA class II DQ antibody response was more consistent as median MFI declined from baseline: 12,837 (7440–16,447) to T1: 11,364 (7564–16,543), P = ns and T2: 9261 (5693–13,798) $P = 0.008$. (C) Similar trends were noted for anti-HLA class II DP antibodies with baseline median MFI at 12,491 (10,210–17,857); T1: 2314 (0–14,337), $P = 0.001$; and T2: 2504 (950, 15,179), $P = 0.009$. Overall, despite some variability per HLA specificity and subject, ixazomib therapy was associated with a decline in anti-HLA class II antibodies. (D) Changes in anti-HLA class I A antibody levels were not statistically different at baseline: 8756 (6502–14,817), T1: 9491 (6026–13,427), and T2: 10,250 (4943–12,487). We again noted variability among subjects. For example, IXA001 and 004 showed a decline, whereas IXA002 and IXA008 demonstrated significant increases in their antibody levels. (E) Changes in anti-HLA class I B antibodies were not statistically significant: baseline: 12,960 (4659–15,606), T1: 11,786 (3776–13,750), and T2: 11,530 (7185–13,611), P = ns. (F) The decline in anti-HLA class I C antibodies was significant short-term but not long-term: baseline: 10,055 (3980–13,395), T1: 1355 (0–8952) $P = 0.006$, and T2: 1832 (100–19,390) P = ns. No significant differences were found for non-HLA antibodies to (G) anti-AT1-R (angiotensin 1 receptor) or ***H. anti***-ETA-R (endothelin II type A receptor) at any time points. Overall, ixazomib therapy was associated with a more modest response in HLA class I antibodies despite persistent variability within subjects and HLA specificity. AT1-R, angiotensin 1 receptor; ETA-R, endothelin II type A receptor; MFI, mean fluorescence intensity.

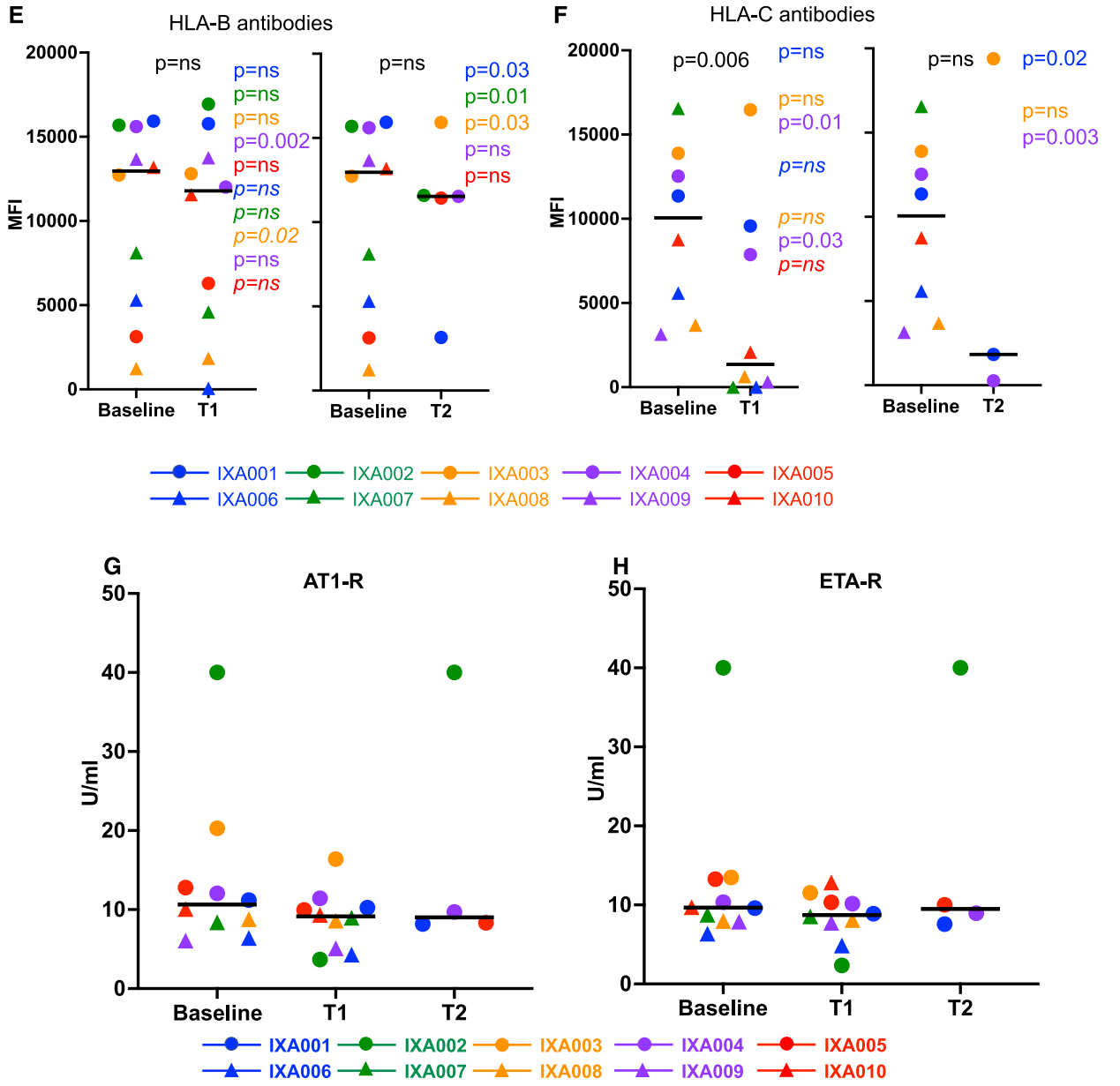

Figure 8. Continued.

We saw a significant decline in total BM lymphocytes and circulating $CD3^+$ T cells, $CD4^+$ T cells, mature B cells, memory B cells, and TRAIL associated with a significant increase in BAFF. Although these observations are novel in kidney transplant recipients, similar changes have been noticed in experimental and nontransplant studies. For example, ixazomib inhibited dendritic cell function and T-cell proliferation in a preclinical graft-versus-host disease model (GVHD).[39] Similarly, ixazomib was associated with an increase of regulatory T cells both in peripheral blood and in the GVHD target organs and a decrease of effector donor T cells.[40] Mice receiving ixazomib had a lower number of neutrophils in the GVHD target organs than in the vehicle group. Furthermore, ixazomib may sensitize TRAIL/death receptor signaling pathway–targeted colorectal cancer cells,[41] suggesting that proteasome inhibition and TRAIL may also have a synergistic immunomodulatory effect. Finally, downregulation of plasma cell activity by proteasome inhibition is associated with upregulation of BAFF, presumably due to negative feedback.[42,43] These observations indicate a broader immunomodulatory role for ixazomib than plasma cell inhibition.

Ixazomib is a second-generation proteasome inhibitor that has been used to target plasma cells in multiple myeloma.[17,23–28] Ixazomib may have higher potency and less toxicity compared with bortezomib.[9,10] It is also an oral formulation. Our proof-of-concept study had several limitations, including the small sample size. Calculated PRA can be an insensitive measure of desensitization if antibodies are not decreased enough. Furthermore, cPRA is often based on the MFI from undiluted serum samples, and MFI results are affected by inherent assay

limitations and intralaboratory variability. Limited enrollment, follow-up, and duration of the trial preclude obtaining rigorous conclusions about the safety and efficacy, especially regarding the impact on antibody levels. Considering these limitations, we showed that median immunodominant alloantibody MFIs declined for some subjects and HLA specificities but not consistently across the population. There was a significant decline in BM lymphocytes and in circulating $CD3^+$ T cells, $CD4^+$ T cells, and mature and memory B cells. Circulating TRAIL decrease associated with a significant increase in BAFF. Together, these findings suggest that ixazomib alone has a limited and heterogeneous effect on desensitization strategies. More studies are needed to determine if ixazomib alone or in combination with costimulation blockade[9,44–47] or proteasome inhibitor-free regimens are safe and effective desensitization strategies.

Disclosures

S. Denham reports the following: Consultancy: Omeat Inc. A. Djamali reports the following: Consultancy: CareDx, CSL; Research Funding: CareDx, Takeda; Honoraria: CareDx, CSL; and Advisory or Leadership Role: CSL; CareDx; Associate Editor of *Kidney360*. P. Hematti reports the following: Consultancy: scientific co-founder and chair of scientific board of Cellular Logistics, Inc; and Ownership Interest: scientific co-founder of Cellular Logistics, Inc. S. Parajuli reports the following: Research Funding: Veloxis; and Advisory or Leadership Role: CareDx; Eurofin; Horizon Pharmaceuticals. J. Platt reports the following: Advisory or Leadership Role: Editor in Chief: International Journal of Molecular Sciences; Section Chief Editor: Frontiers in Transplantation. All remaining authors have nothing to disclose.

Funding

Funding for the study was provided by Millennium Pharmaceuticals/Takeda for this work. UW award MSN203641, Takeda protocol X16,101 (Arjang Djamali).

Acknowledgments

Flow cytometry: We would like to thank UW Carbone Cancer Center Flow Cytometry Shared Instrumentation Facility and core staff.

Clinical Trial Protocol: 2014-100831 was approved by SMPH IRB at UW Madison.

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Data Sharing Statement

Data cannot be shared: Since the corresponding author has left UW, we do not have a persistent repository. Interested parties can contact the corresponding author. Anonymized data can be provided on request.

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Received: July 22, 2022 **Accepted:** January 30, 2023
Published Online Ahead of Print: March 23, 2023