HHS Public Access

Author manuscript

Am J Transplant. Author manuscript; available in PMC 2023 June 12.

Published in final edited form as:

Am J Transplant. 2023 June ; 23(6): 759–775. doi:10.1016/j.ajt.2023.02.022.

Effects of *in vivo* CXCR4 Blockade and Proteasome Inhibition on Bone Marrow Plasma Cells in HLA-Sensitized Kidney Transplant Candidates

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Abstract

To date, plasma cell (PC)–targeted therapies have been limited by suboptimal PC depletion and antibody rebound. We hypothesized this is partly because of PC residence in protective bone marrow (BM) microenvironments. The purpose of this proof-of-concept study was to examine

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. E.S.W. reports associations with Novartis, Amgen, and Bristol Myers Squibb. The remaining authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2023.02.022.

the effects of the CXCR4 antagonist, plerixafor, on PC BM residence; its safety profile (alone and in combination with a proteasome inhibitor, bortezomib); and the transcriptional effect on BMPCs in HLA-sensitized kidney transplant candidates. Participants were enrolled into 3 groups: group A (n = 4), plerixafor monotherapy; and groups B (n = 4) and C (n = 4), plerixafor and bortezomib combinations. $CD34^+$ stem cell and PC levels increased in the blood after plerixafor treatment. PC recovery from BM aspirates varied depending on the dose of plerixafor and bortezomib. Single-cell RNA sequencing on BMPCs from 3 group C participants pretreatment and posttreatment revealed multiple populations of PCs, with a post-treatment enrichment of oxidative phosphorylation, proteasome assembly, cytoplasmic translation, and autophagy-related genes. Murine studies demonstrated dually inhibiting the proteasome and autophagy resulted in greater BMPC death than did monotherapies. In conclusion, this pilot study revealed anticipated effects of combined plerixafor and bortezomib on BMPCs, an acceptable safety profile, and suggests the potential for autophagy inhibitors in desensitization regimens.

Keywords

desensitization; plasma cells; plerixafor; bortezomib; proteasome inhibitors; antibody; single-cell genomics; kidney transplant; DSA; donor-specific antibodies; HLA antibodies

1. Introduction

Despite improvements in organ availability and allocation, obtaining a well-matched kidney transplant remains challenging for patients with high degrees of human leukocyte antigen (HLA) sensitization. HLA sensitization increases the waiting time on dialysis and the associated mortality risk.[1] Moreover, after transplantation, sensitized patients are at increased risk of antibody-mediated rejection (AMR), and concomitant allograft failure.[2–4] To date, desensitization therapies, such as intravenous immunoglobulin and plasmapheresis, have been associated with transient reductions in anti-HLA antibody (aHLA Ab) titers, which rebound upon treatment cessation.[5–7] To achieve sustained reduction in aHLA Ab, we have focused on developing plasma cell (PC)–targeting approaches using proteasome inhibitors (PIs) because PCs are dependent on proteasomes to degrade misfolded proteins resulting from their high rate of antibody synthesis.[8–19] Although PIs deplete PCs, toxicities and aHLA Ab rebound remain a challenge.[9,20] The mechanism(s) of rebound is unclear but may be owing to incomplete PC depletion, the resolution of unfolded protein responses, and/or repopulation of bone marrow (BM) plasma cells (BMPCs) by newly differentiated PCs generated after treatment.[21]

One approach for enhancing PC depletion is to target multiple survival pathways using multiagent regimens. During an immune response, a portion of newly generated PCs home to the BM through CXCL12 chemokine gradients. Although the mechanisms driving long-term PC survival remain incompletely understood, consensus holds that PCs reside in CXCL12-rich niches where proximity to stromal and immune cells provides prosurvival signals through cytokines and cell–cell contact.[22–24] CXCR4 signaling in PCs induces the expression of the adhesion factors VLA-4 and LFA-1, which are important for physically maintaining PCs in the BM.[25,26] Moreover, CXCR4 signaling promotes survival in other

cell types but has not been demonstrated in PCs.[27,28] Given the importance of the BM niche, we hypothesized that disrupting niche access would sensitize PCs to death by survival factor deprivation and/or PI treatment. We sought to mobilize BMPCs using the CXCR4 antagonist, plerixafor, to interrupt the CXCR4:CXCL12 axis that recruits and retains PCs in the BM.[29] We conducted a proof-of-concept and safety evaluation of plerixafor alone and with bortezomib in HLA-sensitized patients awaiting kidney transplantation.

2. Materials and methods

A detailed report of materials and methods is provided in the Supplementary Materials.

2.1. Study approval

The study (NCT02522572) was approved by the University of Cincinnati Institutional Review Board (#2014–4773) and conducted in accordance with the tenets of the Declaration of Helsinki. All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at Cincinnati Children's Hospital Medical Center.

3. Results

3.1. Demographics

Between 2015 and 2021, 11 participants were enrolled in the study (Fig. 1A). Group A participants 1 and 2 were re-enrolled in group B (as participants 7 [8 months after completing group A] and 5 [16.5 months after completing group A but was transplanted before starting group B]).

Baseline demographics are reported in Table 1. Characteristics do not differ significantly between groups. The median age was 58.8 years, 83.3% of participants were women, and 25% were of Black race. At enrollment, 75% of the participants were on dialysis for a median time of 5.3 years. The mechanism(s) of sensitization included pregnancy for 88.9% of the female participants, blood transfusion for 50% of participants, and previous transplantation for 41.7% of the participants. The median number of sensitizing events per participant was 3. The median calculated panel reactive antibody (cPRA) was 100% at mean fluorescence intensity $(MFI)_{1500}$; 100% at MFI_{4000}; and 88.6% at MFI_{8000}.

3.2. Toxicities

Treatment was well tolerated with the most common adverse events (AEs) being tinnitus (50%), anemia (50%), vivid dreams (50%), and insomnia (41.7%) (Table 2). AE frequency did not differ significantly between the groups. All but 1 AEs were mild and grade 1 or 2 on the Common Terminology Criteria for Adverse Events scale. No AEs resulted in dosing adjustments or study discontinuation, and no participant died during the study. A grade 3 neuropathic ulcer, assessed as unrelated to study treatment, occurred in participant 13 with a known peripheral vascular disease. Peripheral neuropathy occurred in a single participant (participant 13), lasted for a few minutes, and resolved without intervention.

Hematologic toxicities were monitored by complete blood count. No participants experienced neutropenia, lymphocytopenia, or thrombocytopenia. Two participants from each group (n = 6) developed grade 2 anemia during the study (Supplementary Fig. 1). Participant 13 had grade 2 anemia at the baseline, which transiently worsened, and the remaining 5 participants had grade 1 anemia at the baseline. No participants developed grade ≥3 anemia.

3.3. Plerixafor and bortezomib had minimal effects on immunodominant antibody levels, cPRA, and donors required to match

As an indirect measure of PC response to treatments, we measured aHLA Ab titers at defined time points (Fig. 1B–D). Before plasmapheresis, immunodominant antibody (iAb) levels were modestly decreased in participant 3 and unchanged in participants 1, 2, and 4 (Fig. 2A). After plasmapheresis, iAb levels decreased up to $\log_2$ fold change (FC) − 1.2 and returned to near-baseline in participants 1, 3, and 4; although iAb gradually increased up to $\log_2$FC 1.7 in Participant 2. All group B participants showed decreased iAb before plasmapheresis, which further declined to $\log_2$FC −1.5 to −2.83, and rebounded to near or above the baseline (Fig. 2B). Group C participants had less pronounced decreases in iAb before plasmapheresis, which decreased to $\log_2$FC − 0.77 to − 4.64 after plasmapheresis, and returned to near the baseline (Fig. 2C).

All participants had high degrees of HLA sensitization as measured by cPRA and donors required to match (Table 3). The cPRA was measured at the predefined MFI cutoffs: $cPRA_{1500}$, $cPRA_{4000}$, and $cPRA_{8000}$. At all cutoffs, variable and minimal changes were observed, consistent with the known insensitivity of cPRA to aHLA Ab-lowering treatments.[9,30] However, donors required to match demonstrated greater sensitivity as we have previously described, yet these effects were also variable.[9] Note that the short treatment period was not expected to provide substantial aHLA Ab reductions.

3.4. Plerixafor increases peripheral blood leukocytes

We used complete blood count data to track peripheral blood leukocyte dynamics (Fig. 3). All participants had at least a 2-fold increase in absolute neutrophil, monocyte, and lymphocyte counts after plerixafor treatment. Within group A, the degree of change in cell counts was generally similar across leukocyte subsets. In groups B and C, participants showed a greater variability in the degree of change across subsets, even before bortezomib dosing, although there was not a discernable pattern common among participants. Across all participants, the greatest increase in cell counts occurred in neutrophils in 50% of the participants (n = 6), lymphocytes in 33% of the participants (n = 4), and monocytes in 16.6% of the participants (n = 2).

3.5. Plerixafor increases peripheral blood hematopoietic stem cells and PCs

To assess the effects of plerixafor on PCs, we used flow cytometry to temporally monitor their appearance in peripheral blood (Fig. 4). As a positive control, we also monitored $CD34^+$ hematopoietic stem cells (HSCs) in peripheral blood as plerixafor induces their mobilization.[31] For group A, we initially examined day 4 and found high levels of HSCs, which subsequently tapered off, making it unclear if day 4 was the peak of mobilization.

So, we subsequently included earlier time points to determine the temporal effects of plerixafor. HSC mobilization was observed in all participants analyzed but with significant variability in the cell counts per milliliter blood and FC from the baseline (participant 6 was not analyzed owing to cytometry issues). For group B, HSC mobilization into peripheral blood peaked 48 hours after the fourth plerixafor dose and 24 hours after the bortezomib dose. In participant 10, HSCs peaked after each bortezomib dose. In participant 11, HSCs peaked after the first plerixafor dose, declined to the near-baseline after the first bortezomib dose and rebounded to approximately half of the peak after the last 2 plerixafor doses. In participants 12 and 13, HSCs peaked 24 hours after the sixth plerixafor dose.

Compared with HSC mobilization, PC mobilization was less robust and more variable across the participants. In group A, all participants showed increased PCs in peripheral blood after the plerixafor treatment, but to varying degrees. In groups B and C, PC mobilization dynamics mirrored that of HSCs for participants 7, 9, 10, and 11. For participant 6, PCs remained increased for 3 days after the first plerixafor dose. In participant 12, PC mobilization peaked 2 days after HSC mobilization. In participant 13, PC mobilization was unremarkable except on days 4 and 9, and HSC mobilization was similarly modest. Thus, plerixafor successfully mobilized PCs in all participants, which mostly mirrored the kinetics of HSC mobilization.

3.6. Plerixafor and bortezomib induce dynamic changes in BMPC frequency

To assess the effect of plerixafor and bortezomib on BMPCs, we collected BM aspirates before and after treatment from group B participants 6, 8, and 9 and group C participants 11, 12, and 13 and used flow cytometry to quantitate PC abundance (Fig. 4). Participants 6, 8, and 9, showed an increase in the absolute number of PCs per milliliter of BM aspirate ($\log_2$ FC: 1.64, 1.44, and 1.93, respectively) with similar dynamics in total mononuclear cells (MNCs) per milliliter BM aspirate ($\log_2$ FC: 1.64, 0.93, and 1.67, respectively). BMPC data for participant 11 were not recorded; however, we observed an increase in the MNCs per milliliter of BM aspirate ($\log_2$ FC: 0.93) in the post sample drawn 24 hours after the first bortezomib dose, eg, participant 9. Conversely, we observed a decrease in PC abundance in BM from participants 12 and 13 ($\log_2$ FC: −1.43, −1.41) and similar decreases in MNCs ($\log_2$ FC: −1.03, −1.41). Thus, although the number of PCs was different between the 2 treatment groups, the frequency of PCs among BM MNCs did not change significantly for any of the participants.

3.6.1. Single-cell RNA sequencing analysis reveals BMPC heterogeneity, independent of treatment—

To better understand the responses of BMPCs to plerixafor and bortezomib, we performed single-cell RNA sequencing on flow-sorted PCs from pretreatment and posttreatment BM aspirates of participants 11, 12, and 13. After quality control measures, such as stringent removal of non-PCs and data set integration with pre samples as the reference, we retained transcriptomic data from 11 856 total BMPCs (6588 presamples and 5268 postsamples). Owing to differences in aspirate volumes, BMPC numbers varied across samples (Fig. 5A).

To investigate BMPC heterogeneity, we performed graph-based clustering and annotated 6 clusters of BMPCs (Fig. 5A). First, we verified that cells within these clusters did not express B cell transcription factors *BCL6*, *BACH2*, and *PAX5*, nor the CD20 gene, *MS4A1*; but did express the PC transcription factors *XBP1* and *PRDM1* (Blimp-1) and other PC-associated genes such as *TNFRSF17* (BCMA), *TNFRSF13B* (TACI), *SDC1* (CD138), and *MZB1* (Fig. 5B). All samples contained cells in each of the 6 clusters, with clusters 0, 1, and 2 capturing ~80% of cells and having similar baseline proportions across the participants (Fig. 5C).

To determine whether individual clusters correlated with the antibody isotype, we analyzed the isotype composition of each cluster (Fig. 5D). Importantly, isotype annotations were encoded in the metadata and individual heavy chain genes (eg, IgA1 and IgG4) were condensed into a single annotation in the gene expression matrix before integration and clustering to ensure that clustering was not directly influenced by isotype-specific genes. IgM^+ BMPCs were most abundant in clusters 2 and 4, whereas the proportion of IgA^+, IgG^+, and IgD^+ BMPCs did not differ substantially by cluster. In addition, clusters 2 and 4 had the highest expression of *JCHAIN*, which encodes the joining-chain protein required for multimeric IgM and IgA (Fig. 5E).

3.6.2. Differential gene expression informs biological interpretation of BMPC clusters—To ascertain if genes driving BMPC clustering, independent of treatment, were associated with unique biological processes, we analyzed the cluster marker genes conserved across time points. Figure 5E shows a subset of the top differentially expressed genes (DEGs) by cluster and putative annotations. Cluster 0 has relatively greater expression of genes relating to lysosomal structure and function (eg, *PSAP*, *GRN*, and *NPC2*), ER-associated redox (*PRDX4*), cell-matrix adhesion (*SDC1*, *ICAM3*, and *PECAM1*), and peptidyl-proline hydroxylation (*P4HA1*, *CRTAP*, and *P4HTM*). Cluster 1 has relatively increased expression of genes related to AU-rich element mediated decay (*ZFP36*), cellular response to stress (*DUSP1*, *FOS*, *JUN*, and *IER2*), and suppression of inflammation (*KLF2* and *KLF6*). The gene set enrichment analysis (GSEA) suggests cluster 1 expresses relatively fewer genes relating to protein folding, cytoplasmic translation, and ERAD, suggesting these cells have greater ER stress at baseline. Cluster 3 had the smallest list of DEGs, among which are genes involved in protein glycosylation (*TMEM59*) and the inhibition of proteolysis (*TIMP1*, *CST3*, and *ITM2B*). Clusters 2 and 4 show relatively increased the expression of genes relating to B-cell receptor signaling (*CD79A*), joining chain for multimeric IgM and IgA (*JCHAIN*), and the chemokine receptor CCR10, which is associated with IgA PCs.[32] In addition, cluster 4 signature genes are enriched for the oxidation of glucose (*GAPDH*, *LDHA*, *LDHB*, and *ATP5F1B*) and cytoskeletal rearrangement (*PFN1*, *TSMB10*, and *LSP1*). Finally, cluster 5 has increased expression of genes involved in glycosylation and protein folding (*OGT* and *TXNDC5*), regulation of gene expression (*MPHOSPH8*, *MBNL1*, *NEAT1*, *N4BP2L2*, and *DDX17*), and oxidative phosphorylation (*PDK1*), consistent with the PC antibody synthesis.

Notably, because all cells are PCs and ~40%−80% of their transcriptomes encode immunoglobulins, we acknowledge that the subtle differences in gene expression noted earlier, may be more reflective of variations in mRNA detection than truly different

biological states (Fig. 6B). An exception is cluster 4, which has a significantly lower median immunoglobulin transcript contribution to the overall transcriptome.

3.6.3. Plerixafor and bortezomib treatment drives transcriptional changes across BMPC subsets and reduces immunoglobulin transcripts—

To understand the BMPC responses to plerixafor and bortezomib treatment, first, we determined whether treatment altered the frequency of any of the subsets (Fig. 5C). Overall, there were no consistent changes in the frequency of BMPC clusters after treatment, the exception being participant 13 where the percentage of PCs in cluster 1 decreased from 22.9% to 7% and increased in cluster 0, from 37.5% to 49.9%. Although it is possible that we missed rare PC populations owing to our sorting strategy, we do not think that treatment preferentially affected any BMPC subpopulation.

Next, we identified genes that were differentially expressed between posttreatment and pretreatment cells, considering all PCs together and at the level of individual clusters (Fig. 6A).

Taking all PCs together, GSEA showed a significant posttreatment enrichment of cytoplasmic translation and oxidative phosphorylation (normalized enrichment score [NES]: 1.7 and 1.6, respectively). Cluster-level GSEA showed, in response to treatment, cells in cluster 0 decreased their expression of autophagy-suppressive genes and increased the expression of proteasomal protein catabolism genes (NES: −2.4 and 1.8); cluster 1 cells decreased the expression of mRNA decay and cytokine signaling genes and increased the expression of DNA damage response genes (NES: −2.3, −2.5, and 1.8); cluster 2 cells decreased the expression of lipid catabolism and increased the expression of chaperone-mediated protein refolding genes (NES: −2.5 and 1.9); cluster 3 cells increased the expression of protein catabolism genes (NES: 1.7); cluster 4 cells decreased expression of receptor tyrosine kinase signaling genes and increased expression of RNA splicing and proteasomal protein catabolism genes (NES: −2.3, 2.0, and 1.8); and cluster 5 cells decreased cytokine signaling genes (NES: −2.3) (Fig. 6A). Thus, there were both common and unique responses to plerixafor and bortezomib treatment among BMPCs.

Next, we quantitated the percentage of each cell's transcriptome comprised of immunoglobulin genes and found it decreased by ~10% across all clusters after the treatment (Fig. 6B). Because GSEA can miss subtle, but biologically relevant changes in gene expression, we also examined individual DEGs that were common to all participants (Fig. 6C). The DEG analysis was generally consistent with GSEA but uniquely revealed that *SQSTM1*, encoding the autophagy cargo-adaptor protein p62, was among the most upregulated genes. We subsequently analyzed several other autophagy-related genes (Supplementary Fig. 2) and found the expression of these genes was modestly increased after the treatment.[33–36]

3.7. Combined inhibition of autophagy and the proteasome enhances BMPC death

Previous work has shown the importance of autophagy in basal PC homeostasis and work in multiple myeloma has a shown a role for p62-dependent autophagy in PI resistance.[37–39]

Because our data showed enrichment of *SQSTM1*/p62 expression in BMPCs in response to PI treatment, we investigated the role of p62 in BMPC survival after PI treatment using a PC-specific, tamoxifen-inducible p62 knockout (p62 iKO) mouse model.[40,41] Using the BM from vehicle-treated and tamoxifen-treated p62 iKO mice, we cultured cells in the presence or absence of bortezomib or carfilzomib and measured PC death by flow cytometry (Fig. 7A). We included carfilzomib as an additional PI because it is more clinically relevant.[9] Across several independent experiments, we found that PC-specific loss of p62 significantly increased sensitivity to both bortezomib and carfilzomib (Fig. 7A).

To directly test the importance of autophagy, we performed additional experiments using an autophagy inhibitor, Lys05, which is a more potent version of the FDA-approved lysosomal inhibitor, hydroxychloroquine. [42] We cultured BMPCs in the presence or absence of bortezomib or carfilzomib and/or Lys05 and measured BMPC death (Fig. 7B). We determined synergy using the Bliss definition of drug independence as described.[43] Bortezomib and Lys05 combinations produced modest increases in BMPC death and showed statistically significant synergism at bortezomib 1.6 nM with 2.5 or 5 μM Lys05 (13% synergy, $P = .048$; 26% synergy, $P = .009$). Conversely, carfilzomib and Lys05 combinations produced substantial increases in BMPC death and demonstrated statistically significant synergism across all dose combinations (61%−326%, $P < 0.05$) except carfilzomib 100 nM with Lys05 10 μM (Fig. 7B). Importantly, we observed minimal non-BMPC death from bortezomib ≤ 5 nM, carfilzomib <100 nM, or Lys05 <10 μM alone or in combination (Supplementary Fig. 3). Thus, our data are consistent with a scenario in which PCs use autophagy to cope with stress induced by a failure of normal protein turnover downstream of proteasomal inhibition.

4. Discussion

After the initial demonstration of PC targeting for AMR with bortezomib, our group focused on desensitization hypothesizing that targeting BMPCs would be key to enhancing therapeutic results.[8,9,12,14–16] Therefore, progression to mechanistic studies has been seminal for enhancing PC depletion and duration of therapeutic effects. Although promising, our results demonstrated the need for additional PC-targeting strategies, such as survival factor deprivation. In this study, we used the CXCR4 antagonist, plerixafor, to test the hypothesis that interrupting CXCR4:CXCL12 interactions would mobilize PCs from their niches. Our study examined the safety and tolerability of plerixafor with or without bortezomib and the effects of these drugs on leukocyte dynamics in peripheral blood, PC mobilization and homeostasis, and gene expression in BMPCs.

Plerixafor alone or with bortezomib was well tolerated in our population with end-stage renal disease (ESRD), with no grade ≥3 toxicities or unexpected AEs.[44] Next, given this tolerability, we explored its effectiveness on PC mobilization.

We hypothesized that disrupting CXCR4 signaling would result in what we refer to as follows: (1) micromobilization, where PCs remain in the BM but are released from their niche and, presumably, associated survival factors and/or (2) macromobilization, where PCs are mobilized to the peripheral blood and lose access to all BM factors.

Given the importance of the BM niche, we hypothesized that either micromobilization or macromobilization would cause a portion of PCs to die and that those remaining would have increased sensitivity to agents such as bortezomib, ultimately resulting in BMPC depletion.

Quantitation of PCs in peripheral blood demonstrated macromobilization after plerixafor treatment in all participants studied. Seven of the 10 participants with both PC and HSC data had PC kinetics closely mirroring HSC kinetics, adding to our confidence that the changes we observed were in response to plerixafor treatment. PC mobilization into the blood was an order of magnitude less than HSC mobilization for most participants, despite HSCs comprising an even smaller frequency of the BM than PCs.[45,46] There are several possible explanations: (1) PCs have additional mechanisms to maintain BM residence including integrins and chemokine receptors; (2) mobilization induces PC but not HSC death; (3) PCs migrate into other niches such as the intestines or spleen; (4) PCs favor micromobilization whereas HSCs favor macromobilization; and (5) CXCR4 antagonism induces HSC, but not PC, proliferation.[25,47–50]

Our analyses of BM aspirates showed increased MNCs per milliliter of aspirate and corresponding PC increases at early time points, whereas samples from later time points had the opposite trend. Unfortunately, our cytometry panels did not allow us to cleanly dissect the composition of the MNC compartment to determine whether a subset or all BM MNCs were equally affected. Nonetheless, one interpretation of these data is at early time points, plerixafor globally micromobilizes MNCs/PCs without significant killing, thereby increasing our ability to aspirate them. However, after additional doses of plerixafor and bortezomib, there is increased preference for MNC/PC macromobilization and/or death. The effects on non-PCs and non-HSCs that we observed in BM and peripheral blood are consistent with other plerixafor studies and attributable to the expression of CXCR4 on many BM cell subsets.[51–53]

Our data are in contrast to a previous study in mice evaluating plerixafor and bortezomib treatment on PCs.[54] The authors found plerixafor mobilized PCs into the blood with peak mobilization occurring within 1 hour of dosing and returning to baseline by 24 hours and the number of PCs in the BM and spleen were unaffected by the combined treatment. We suspect that differences in drug dosing/stability between species and distinct underlying biology between mice and humans likely explain the different data.

A striking observation from our study is that immunoglobulin transcripts were decreased by ~10% after the combination treatment. We suspect that the decreased immunoglobulin transcripts are part of a PI-induced unfolded protein response that aims to decrease the burden of protein synthesis on the ER. Indeed, previous work has shown flow-sorted PCs temporarily decrease antibody synthesis, presumably owing to the stress of being removed from BM and processed.[55] Notably, because immunoglobulin transcripts comprise such a significant portion of the PC transcriptome, this 10% change in transcriptome composition after the treatment is a caveat to the interpretation of gene expression changes after the treatment.

Another significant finding from our single-cell RNA sequencing analyses is that the autophagy-related gene, *SQSTM1*, was increased in all posttreatment samples. Our subsequent *in vitro* experiments suggest that PCs engage autophagy to compensate for proteasome dysfunction, partly through a p62-dependent mechanism. This is consistent with previous work in non-PCs and multiple myeloma models, showing that *SQSTM1*/p62 expression rapidly increases after proteasome inhibition.[39,56,57] In the study by Milan *et al*,[39] the authors found that bortezomib-resistant myeloma cells had a p62-dependent autophagic reserve that compensated for proteasome dysfunction whereas bortezomib-sensitive cells already relied on p62-dependent autophagy in steady state, thus lacked a protective reserve.

Because p62 has nonautophagy functions and is not required for autophagy, we also evaluated the effects of inhibiting autophagic flux by blocking autophagosome degradation. Although the absence of p62 modestly increased BMPC sensitivity to both bortezomib and carfilzomib, global autophagy inhibition showed a much greater effect on carfilzomib sensitivity. Thus, it is likely that factors in addition to p62 are contributing to autophagy initiation. Nonetheless, the potential for safely and effectively combining PIs and autophagy inhibitors *in vivo* was demonstrated in a phase I trial using hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma.[58] Thus, the potential to use more-potent and less-toxic drugs (eg, Lys05 and carfilzomib) in combination is a promising strategy to desensitize patients awaiting transplantation.

Furthermore, this study provides rationale that plerixafor may be an additional desensitization tool because it was well tolerated and mobilized PCs in our patient population with ESRD. Moreover, plerixafor may be uniquely well suited as a strategy to inhibit PC survival by preventing their establishment in the BM, which has applicability to both desensitization and AMR (eg, during insufficient immunosuppression). In a study combining plerixafor and bortezomib in a mouse lupus model, the authors found that although the addition of plerixafor to bortezomib did not drastically enhance BMPC depletion, extended plerixafor treatment after bortezomib-based depletion did prevent PC repopulation of the BM.[59] Thus, plerixafor treatment, through the prevention of PC homing to marrow, may significantly delay or prevent antibody rebound.

We acknowledge there are several limitations to this study. First, this was a pilot safety study on plerixafor and bortezomib in a patient population with ESRD that was not powered to determine efficacy. Moreover, we did not include a bortezomib-alone treatment group given the limited pool of patients to enroll and knowledge that 1 or 2 doses of bortezomib was likely inadequate to induce a longer-term effect on aHLA Ab as observed in our previous trials. Hence, we were unable to determine whether plerixafor enhanced bortezomib-based depletion of BMPCs. Nevertheless, this study provides the first human experience on the effects of combined CXCR4 antagonism and proteasome inhibition on primary BMPCs and suggests the potential for novel approaches to desensitization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to Dr Toru Yanagawa (University of Tsukuba, Japan) who generously approved the shipment of the $p62^{fl/fl}$ mice; Dr Jing Fang (University of South Carolina) who extensively characterized the $p62^{fl/fl}$ mice; and Dr Se-Jin Jeong (Washington University) who sent initial breeding pairs of the mice.

Funding

This work was supported in part by Sanofi and by a Public Health Service Grant R01AI154932 (to D.A., D.A.H., and E.S.W.). All flow cytometric data were acquired using equipment maintained by the Research Flow Cytometry Core in the Division of Rheumatology at Cincinnati Children's Hospital Medical Center, which is supported in part by NIH AR070549.

Data availability

The data that support the findings of this study are available from the corresponding authors (D.A.H., E.S.W.) on a reasonable request.

Abbreviations:

AE	adverse event
aHLA Ab	anti human leukocyte antigen antibody
AMR	antibody-mediated rejection
BM	bone marrow
BMPC	bone marrow plasma cell
cPRA	calculated panel reactive antibody
DEG	differentially expressed gene
DRTM	donors required to match
GSEA	gene set enrichment analysis
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
iAb	immunodominant antibody
iKO	inducible knockout
MNC	mononuclear cell
PC	plasma cell
PI	proteasome inhibitor

References

1. Annual Data Report. 2022.

2. Khalil AK, Slaven JE, Mujtaba MA, et al. Re-transplants compared to primary kidney transplants recipients: a mate kidney paired analysis of the OPTN/UNOS database. Clin Transpl. 2016;30(5):566–578. 10.1111/ctr.12722.

3. Clark S, Kadatz M, Gill J, Gill JS. Access to kidney transplantation after a failed first kidney transplant and associations with patient and allograft survival. Clin J Am Soc Nephrol. 2019;14(8):1228–1237. 10.2215/cjn.01530219. [PubMed: 31337621]

4. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol. 2010;21(8): 1398–1406. 10.1681/ASN.2009101065. [PubMed: 20634297]

5. Vo AA, Choi J, Kim I, et al. . A Phase I/II trial of the interleukin-6 receptor-specific humanized monoclonal (tocilizumab) intravenous immunoglobulin in difficult to desensitize patients. Transplantation. 2015;99(11):2356–2363. 10.1097/TP.0000000000000741. [PubMed: 26018350]

6. Marfo K, Lu A, Ling M, Akalin E. Desensitization protocols and their outcome. Clin J Am Soc Nephrol. 2011;6(4):922–936. 10.2215/CJN.08140910. [PubMed: 21441131]

7. Jordan SC. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 Trial. J Am Soc Nephrol. 2004;15(12): 3256–3262. 10.1097/01.asn.0000145878.92906.9f. [PubMed: 15579530]

8. Woodle ES, Tremblay S, Brailey P, et al. Proteasomal adaptations underlying carfilzomib-resistance in human bone marrow plasma cells. Am J Transplant. 2020; 20(2):399–410. 10.1111/ajt.15634. [PubMed: 31595669]

9. Tremblay S, Driscoll JJ, Rike-Shields A, et al. A prospective, iterative, adaptive trial of carfilzomib-based desensitization. Am J Transplant. 2020;20(2):411–421. 10.1111/ajt.15613. [PubMed: 31550069]

10. Woodle ES, Shields AR, Ejaz NS, et al. Prospective iterative trial of proteasome inhibitor-based desensitization. Am J Transplant. 2015;15(1):101–118. 10.1111/ajt.13050. [PubMed: 25534446]

11. Ejaz NS, Alloway RR, Halleck F, Dürr M, Budde K, Woodle ES. Review of bortezomib treatment of antibody-mediated rejection in renal transplantation. Antioxid Redox Signal. 2014;21(17):2401–2418. 10.1089/ars.2014.5892. [PubMed: 24635140]

12. Schmidt N, Alloway RR, Walsh RC, et al. Prospective evaluation of the toxicity profile of proteasome inhibitor-based therapy in renal transplant candidates and recipients. Transplantation. 2012;94(4):352–361. 10.1097/TP.0b013e318257acf6. [PubMed: 22836132]

13. Walsh RC, Alloway RR, Girnita AL, Woodle ES. Proteasome inhibitor-based therapy for antibody-mediated rejection. Kidney Int. 2012;81(11):1067–1074. 10.1038/ki.2011.502. [PubMed: 22336990]

14. Everly MJ, Everly JJ, Arend LJ, et al. Reducing de novo donor-specific antibody levels during acute rejection diminishes renal allograft loss. Am J Transpl. 2009;9(5): 1063–1071. 10.1111/j.1600-6143.2009.02577.x.

15. Everly MJ, Everly JJ, Susskind B, et al. Proteasome inhibition reduces donor-specific antibody levels. Transplant Proc. 2009;41(1):105–107. 10.1016/j.transproceed.2008.10.073. [PubMed: 19249489]

16. Everly MJ, Everly JJ, Susskind B, et al. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. Transplantation. 2008;86(12): 1754–1761. 10.1097/TP.0b013e318190af83. [PubMed: 19104417]

17. Cenci S.The proteasome in terminal plasma cell differentiation. Semin Hematol. 2012;49(3):215–222. 10.1053/j.seminhematol.2012.04.005. [PubMed: 22726544]

18. Cenci S, van Anken E, Sitia R. Proteostenosis and plasma cell pathophysiology. Curr Opin Cell Biol. 2011;23(2):216–222. 10.1016/j.ceb.2010.11.004. [PubMed: 21169004]

19. Cenci S, Mezghrani A, Cascio P, et al. Progressively impaired proteasomal capacity during terminal plasma cell differentiation. EMBO J. 2006;25(5):1104–1113. 10.1038/sj.emboj.7601009. [PubMed: 16498407]

20. Kwun J, Burghuber C, Manook M, et al. Humoral compensation after bortezomib treatment of allosensitized recipients. J Am Soc Nephrol. 2017;28(7):1991–1996. 10.1681/asn.2016070727. [PubMed: 28232617]

21. Kwun J, Knechtle S. Pharmacological approaches to antibody-mediated rejection—are we getting closer? Am J Transpl. 2020;20(10):2637–2638. 10.1111/ajt.16061.
22. Hauser AE, Debes GF, Arce S, et al. Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response. J Immunol. 2002;169(3):1277–1282. 10.4049/jimmunol.169.3.1277. [PubMed: 12133949]
23. Cassese G, Arce S, Hauser AE, et al. Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. J Immunol. 2003;171(4): 1684–1690. 10.4049/jimmunol.171.4.1684. [PubMed: 12902466]
24. Zehentmeier S, Roth K, Cseresnyes Z, et al. Static and dynamic components synergize to form a stable survival niche for bone marrow plasma cells. Eur J Immunol. 2014; 44(8):2306–2317. 10.1002/eji.201344313. [PubMed: 24777940]
25. Nakayama T, Hieshima K, Izawa D, Tatsumi Y, Kanamaru A, Yoshie O. Cutting edge: profile of chemokine receptor expression on human plasma cells accounts for their efficient recruitment to target tissues. J Immunol. 2003;170(3):1136–1140. 10.4049/jimmunol.170.3.1136. [PubMed: 12538668]
26. Roldán E, García-Pardo A, Brieva JA. VLA-4-fibronectin interaction is required for the terminal differentiation of human bone marrow cells capable of spontaneous and high rate immunoglobulin secretion. J Exp Med. 1992;175(6):1739–1747. 10.1084/jem.175.6.1739. [PubMed: 1588291]
27. Suzuki Y, Rahman M, Mitsuya H. Diverse transcriptional response of CD4 T cells to stromal cell-derived factor (SDF)-1: cell survival promotion and priming effects of SDF-1 on CD4 T cells. J Immunol. 2001;167(6):3064–3073. 10.4049/jimmunol.167.6.3064. [PubMed: 11544290]
28. Hernández-López C, Valencia J, Hidalgo L, et al. CXCL12/CXCR4 signaling promotes human thymic dendritic cell survival regulating the Bcl-2/Bax ratio. Immunol Lett. 2008;120(1–2):72–78. 10.1016/j.imlet.2008.07.006. [PubMed: 18692524]
29. De Clercq E.Mozobil® (Plerixafor, AMD3100), 10 years after its approval by the US Food and Drug Administration. Antivir Chem Chemother. 2019;27, 204020661982938. 10.1177/2040206619829382.
30. Kransdorf EP, Pando MJ. Calculated panel reactive antibody with decimals: a refined metric of access to transplantation for highly sensitized candidates. Hum Immunol. 2017;78(3):252–256. 10.1016/j.humimm.2016.12.009. [PubMed: 28069404]
31. Uy GL, Rettig MP, Cashen AF. Plerixafor, a CXCR4 antagonist for the mobilization of hematopoietic stem cells. Expert Opin Biol Ther. 2008;8(11):1797–1804. 10.1517/14712598.8.11.1797. [PubMed: 18847313]
32. Kunkel EJ, Kim CH, Lazarus NH, et al. CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA Ab-secreting cells. J Clin Invest. 2003; 111(7):1001–1010. 10.1172/JCI17244. [PubMed: 12671049]
33. Funakoshi M, Li X, Velichutina I, Hochstrasser M, Kobayashi H. Sem1, the yeast ortholog of a human BRCA2-binding protein, is a component of the proteasome regulatory particle that enhances proteasome stability. J Cell Sci. 2004;117(Pt 26): 6447–6454. 10.1242/jcs.01575. [PubMed: 15572408]
34. Cronin SJF, Woolf CJ, Weiss G, Penninger JM. The role of iron regulation in immunometabolism and immune-related disease. Front Mol Biosci. 2019;6:116. 10.3389/fmolb.2019.00116. [PubMed: 31824960]
35. Liang JR, Lingeman E, Luong T, et al. A genome-wide ER-phagy screen highlights key roles of mitochondrial metabolism and ER-resident UFMylation. Cell. 2020;180(6): 1160–1177.e20. 10.1016/j.cell.2020.02.017. [PubMed: 32160526]
36. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. Cell. 2019;176(1–2):11–42. 10.1016/j.cell.2018.09.048. [PubMed: 30633901]
37. Pengo N, Scolari M, Oliva L, et al. Plasma cells require autophagy for sustainable immunoglobulin production. Nat Immunol. 2013;14(3):298–305. 10.1038/ni.2524. [PubMed: 23354484]
38. Pengo N, Cenci S. The role of autophagy in plasma cell ontogenesis. Autophagy. 2013; 9(6):942–944. 10.4161/auto.24399. [PubMed: 23528926]

39. Milan E, Perini T, Resnati M, et al. A plastic SQSTM1/p62-dependent autophagic reserve maintains proteostasis and determines proteasome inhibitor susceptibility in multiple myeloma cells. Autophagy. 2015;11(7):1161–1178. 10.1080/15548627.2015.1052928. [PubMed: 26043024]
40. Xu AQ, Barbosa RR, Calado DP. Genetic timestamping of plasma cells in vivo reveals tissue-specific homeostatic population turnover. Elife. 2020;9, e59850. 10.7554/elife.59850. [PubMed: 33136000]
41. Harada H, Warabi E, Matsuki T, et al. Deficiency of p62/Sequestosome 1 causes hyperphagia due to leptin resistance in the brain. J Neurosci. 2013;33(37): 14767–14777. 10.1523/JNEUROSCI.2954-12.2013. [PubMed: 24027277]
42. McAfee Q, Zhang Z, Samanta A, et al. Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. Proc Natl Acad Sci U S A. 2012;109(21):8253–8258. 10.1073/pnas.1118193109. [PubMed: 22566612]
43. Demidenko E, Miller TW. Statistical determination of synergy based on Bliss definition of drugs independence. PLoS One. 2019;14(11), e0224137. 10.1371/journal.pone.0224137. [PubMed: 31765385]
44. MOZOBIL®(plerixafor) Product Monograph. Sanofi-Aventis Canada Inc; 2019. https://products.sanofi.ca/en/mozobil.pdf.
45. Terstappen L, Johnsen S, Segers-Nolten I, Loken M. Identification and characterization of plasma cells in normal human bone marrow by high-resolution flow cytometry. Blood. 1990;76(9):1739–1747. 10.1182/blood.v76.9.1739.1739. [PubMed: 2224123]
46. Pang WW, Price EA, Sahoo D, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci U S A. 2011;108(50):20012–200217. 10.1073/pnas.1116110108. [PubMed: 22123971]
47. Kang Y, Chen BJ, Deoliveira D, Mito J, Chao NJ. Selective enhancement of donor hematopoietic cell engraftment by the CXCR4 antagonist AMD3100 in a mouse transplantation model. PLoS One. 2010;5(6), e11316. 10.1371/journal.pone.0011316. [PubMed: 20596257]
48. Hoggatt J, Singh P, Tate TA, et al. Rapid mobilization reveals a highly engraftable hematopoietic stem cell. Cell. 2018;172(1–2):191–204.e10. 10.1016/j.cell.2017.11.003. [PubMed: 29224778]
49. Wilmore JR, Allman D. Here, there, and anywhere? Arguments for and against the physical plasma cell survival niche. J Immunol. 2017;199(3):839–845. 10.4049/jimmunol.1700461. [PubMed: 28739594]
50. Wilmore JR, Gaudette BT, Gómez Atria D, et al. IgA plasma cells are long-lived residents of gut and bone marrow that express isotype- and tissue-specific gene expression patterns. Front Immunol. 2021;12, 791095. 10.3389/fimmu.2021.791095. [PubMed: 35003110]
51. Schroeder MA, Rettig MP, Lopez S, et al. Mobilization of allogeneic peripheral blood stem cell donors with intravenous plerixafor mobilizes a unique graft. Blood. 2017; 129(19):2680–2692. 10.1182/blood-2016-09-739722. [PubMed: 28292947]
52. Pawig L, Klasen C, Weber C, Bernhagen J, Noels H. Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family: molecular perspectives. Front Immunol. 2015;6:429. 10.3389/fimmu.2015.00429. [PubMed: 26347749]
53. De Filippo K, Rankin SM. CXCR4, the master regulator of neutrophil trafficking in homeostasis and disease. Eur J Clin Invest. 2018;48(suppl 2), e12949. 10.1111/eci.12949. [PubMed: 29734477]
54. Moore N, Moreno Gonzales M, Bonner K, Smith B, Park W, Stegall M. Impact of CXCR4/CXCL12 blockade on normal plasma cells in vivo. Am J Transpl. 2017;17(6): 1663–1669. 10.1111/ajt.14236.
55. Nguyen DC, Garimalla S, Xiao H, et al. Factors of the bone marrow microniche that support human plasma cell survival and immunoglobulin secretion. Nat Commun. 2018;9(1):3698. 10.1038/s41467-018-05853-7. [PubMed: 30209264]
56. Demishtein A, Fraiberg M, Berko D, Tirosh B, Elazar Z, Navon A. SQSTM1/p62-mediated autophagy compensates for loss of proteasome polyubiquitin recruiting capacity. Autophagy. 2017;13(10):1697–1708. 10.1080/15548627.2017.1356549. [PubMed: 28792301]
57. Sha Z, Schnell HM, Ruoff K, Goldberg A. Rapid induction of p62 and GABARAPL1 upon proteasome inhibition promotes survival before autophagy activation. J Cell Biol. 2018;217(5):1757–1776. 10.1083/jcb.201708168. [PubMed: 29535191]

58. Vogl DT, Stadtmauer EA, Tan KS, et al. Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. Autophagy. 2014;10(8):1380–1390. 10.4161/auto.29264. [PubMed: 24991834]

59. Cheng Q, Khodadadi L, Taddeo A, et al. CXCR4–CXCL12 interaction is important for plasma cell homing and survival in NZB/W mice. Eur J Immunol. 2018;48(6): 1020–1029. 10.1002/eji.201747023. [PubMed: 29427452]

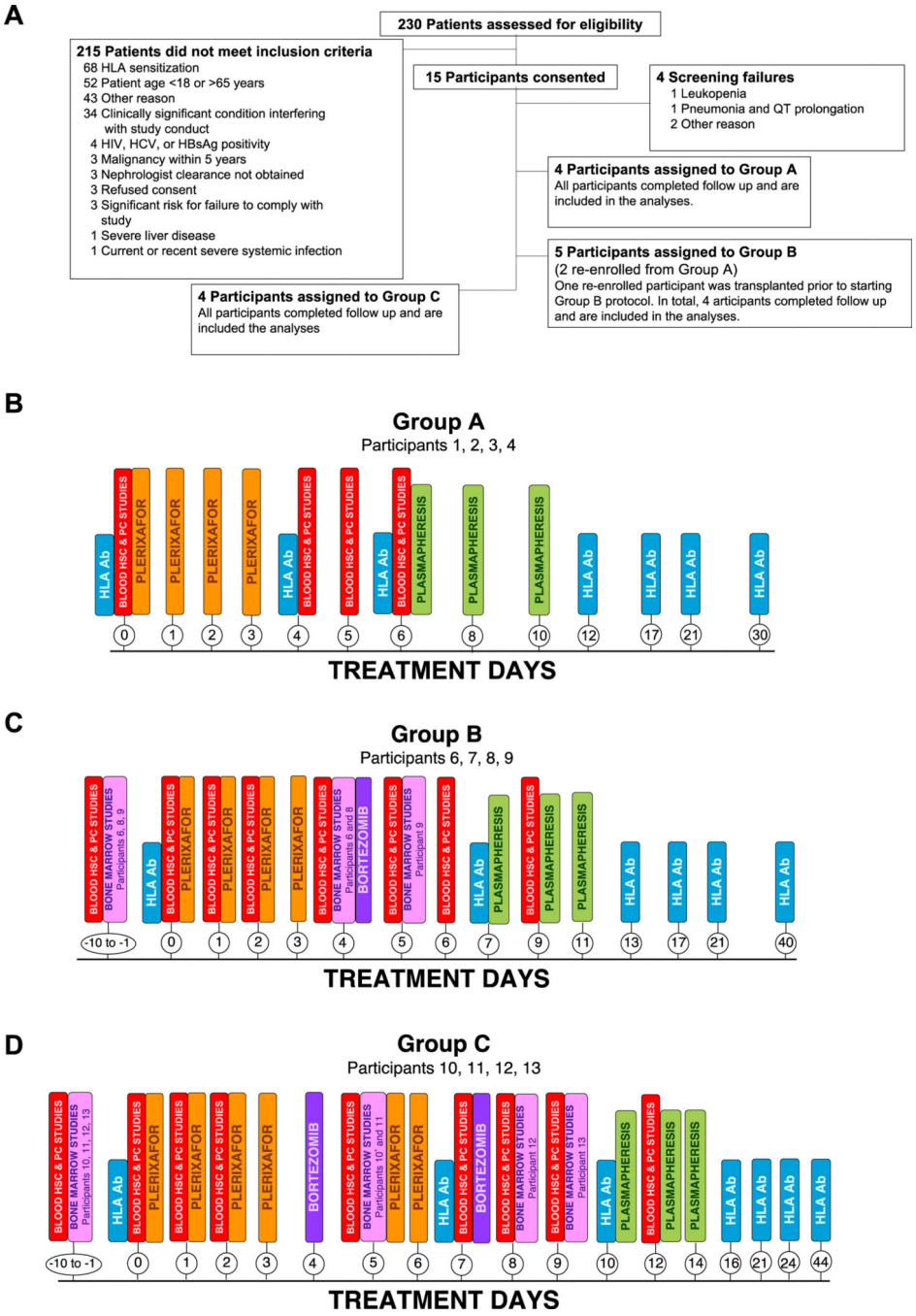

Figure 1.

Study recruitment and treatment regimens: (A) Patient disposition diagram. (B) Group A treatment and sample collection regimen. (C) Group B treatment and sample collection regimen. Bone marrow aspirates were collected before treatment and either 24 hours after the final plerixafor dose but before bortezomib for Participants 6 and 8 or 24 hours after the bortezomib dose for participant 9. (D) Group C treatment and sample collection regimen. Bone marrow aspirates were collected before treatment and 24 hours after the first bortezomib dose for Participant 11, 24 hours after the second bortezomib

dose for Participant 12, and 48 hours after the second bortezomib dose for participant 13. Participant 10 declined a posttreatment BM aspirate/biopsy. Plerixafor doses were 0.16 mg/kg subcutaneously and bortezomib doses were 1.3 mg/m^2 intravenous push, preceded by methylprednisolone 100 mg intravenous push.

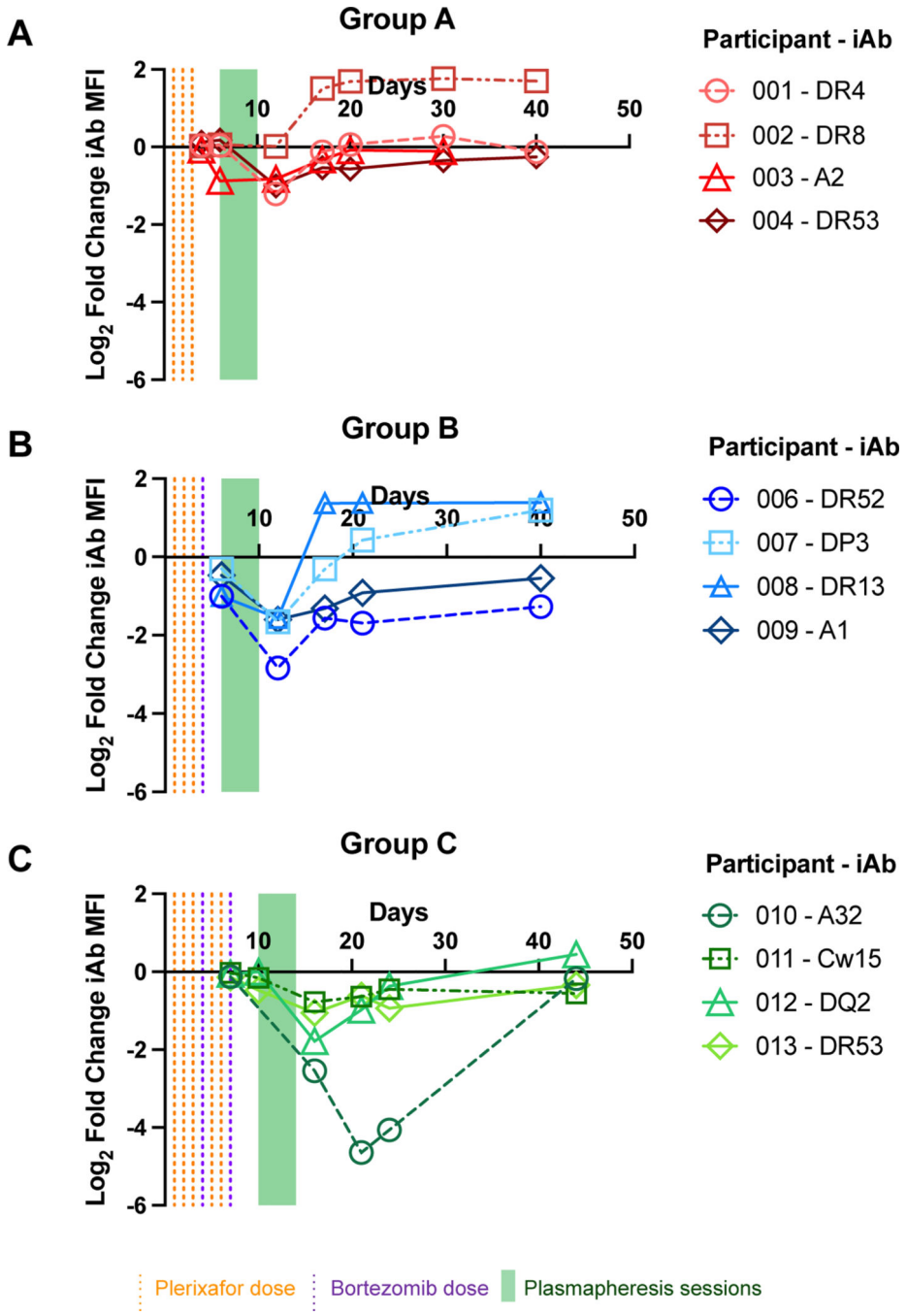

Figure 2.

Immunodominant antibody changes throughout the study. Data are presented as the $\log_2$ fold change in the median fluorescence intensity from the baseline. The iAb for each participant is annotated in the legends. (A) Group A iAb measurements. (B) Group B iAb measurements. (C) Group C iAb measurements. iAb, immunodominant antibody.

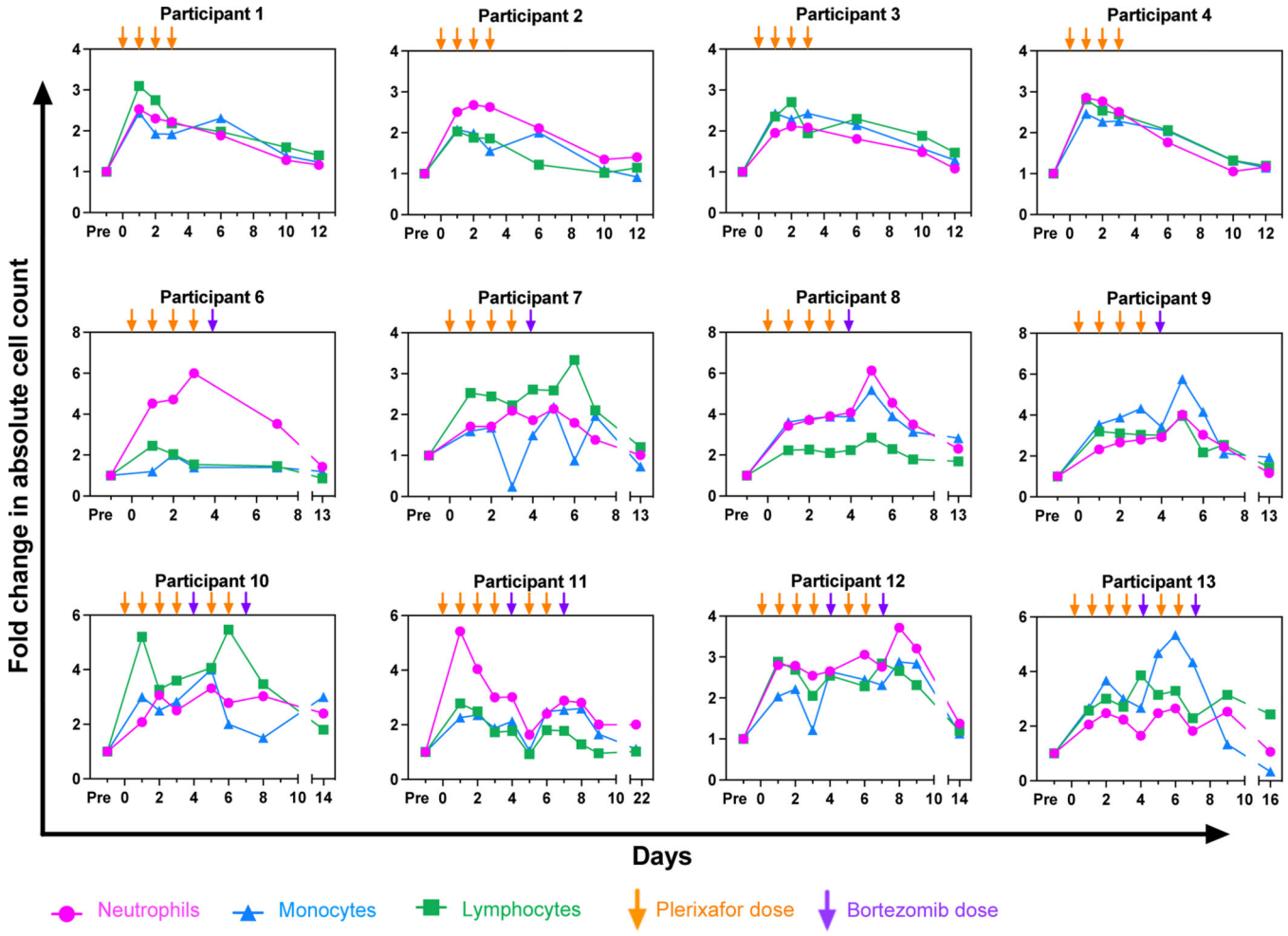

Figure 3.

Leukocyte dynamics in peripheral blood samples. Data are shown as the fold change in the absolute cell count relative to the baseline, as determined by complete blood count with differential. The timing of plerixafor and bortezomib dosing is indicated by vertical arrows. The “pre” time point was between 0 and 10 days before the first plerixafor dose. Group A, participants 1, 2, 3, and 4; group B, participants 6, 7, 8, and 9; and group C, participants 10, 11, 12, and 13.

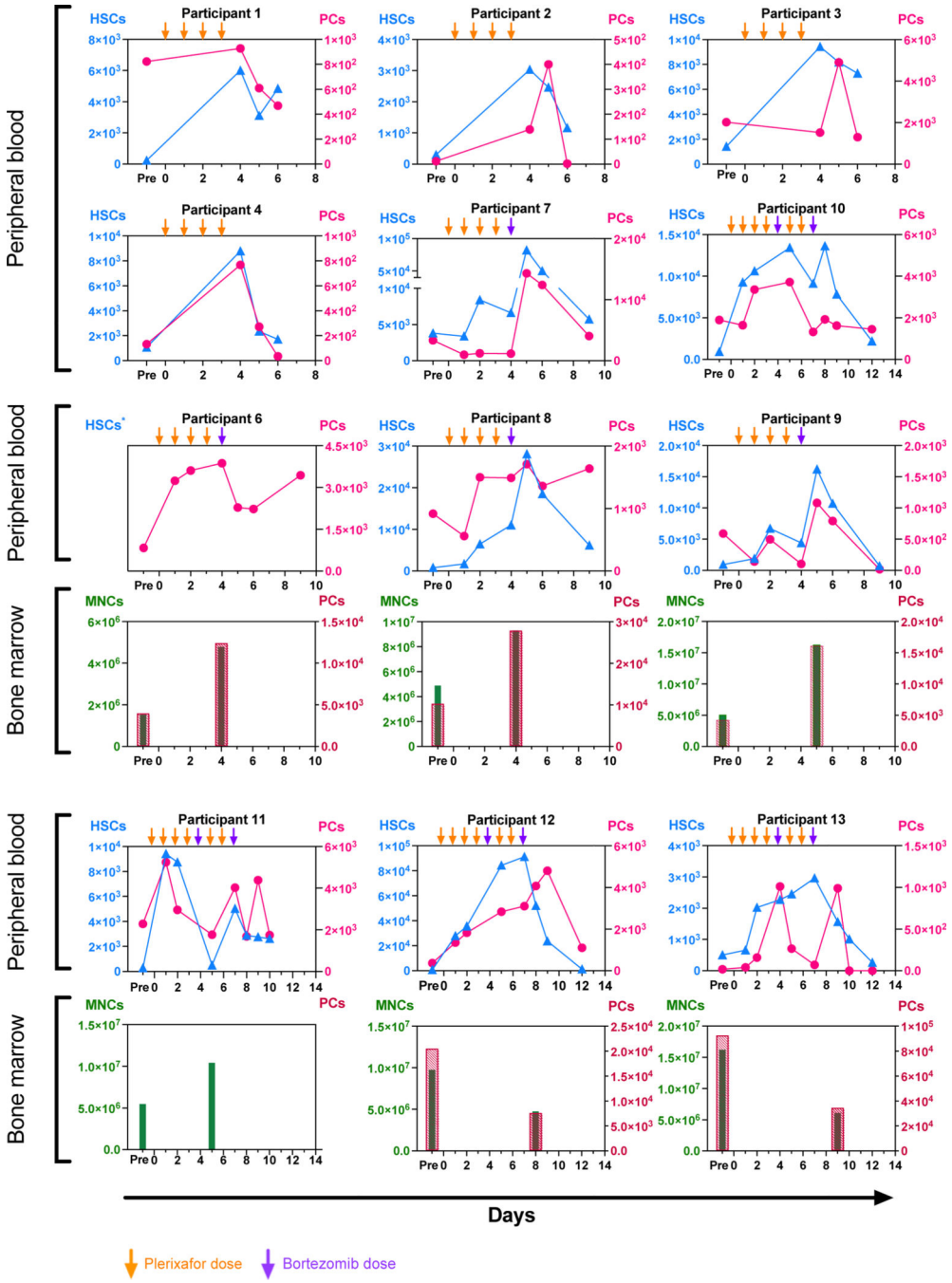

Figure 4.

Plerixafor mobilizes HSCs and PCs into peripheral blood samples and has varying effects on bone marrow MNCs and PCs. Data are shown as the number of cells per milliliter of either peripheral blood or bone marrow aspirate, as indicated. HSCs and PCs were quantitated by flow cytometry, and bone marrow MNCs were quantitated by hemocytometer after Ficoll enrichment of aspirates. The timing of plerixafor and bortezomib dosing is indicated by arrows. *HSC data unavailable owing to an issue with cytometry staining. [X]BMPC data

were not recorded. HSC, hematopoietic stem cell; PC, plasma cell; MNC, mononuclear cells.

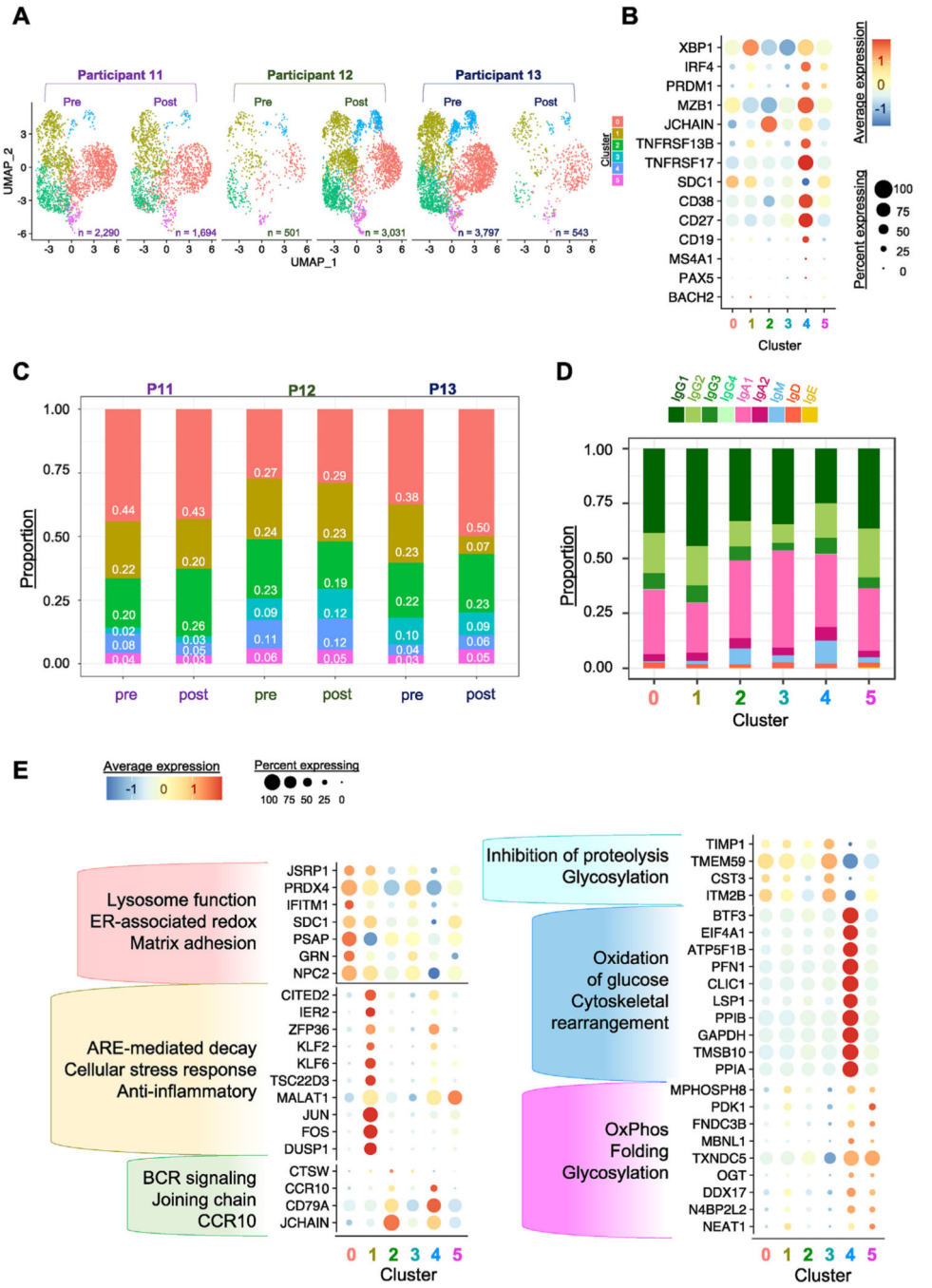

Figure 5.

Single-cell RNA sequencing results. (A) Dimensional reduction plots of each sample, with the number of plasma cells indicated for each. (B) Expression of key B cell and plasma cell genes to confirm plasma cell identity of each cluster. *BCL-6* expression not shown because it was not detected in the data set. The average gene expression is scaled to a mean of zero and a standard deviation of 1. The circle size corresponds to the percentage of cells within the cluster that express the gene and color corresponds to the scaled average expression. (C) Proportion of plasma cells in each of the 6 clusters for each sample, before and after,

from each participant, P11, P12, and P13. (D) Proportion of each isotype by cluster. (E) The selection of top genes defining each cluster and annotated with associated pathways. The average gene expression is scaled to a mean of zero and a standard deviation of 1. The circle size corresponds to the percentage of cells within the cluster that express the gene and color corresponds to the scaled average expression. Highlighting of genes/annotations corresponds to the cluster color that is the most enriched for those genes.

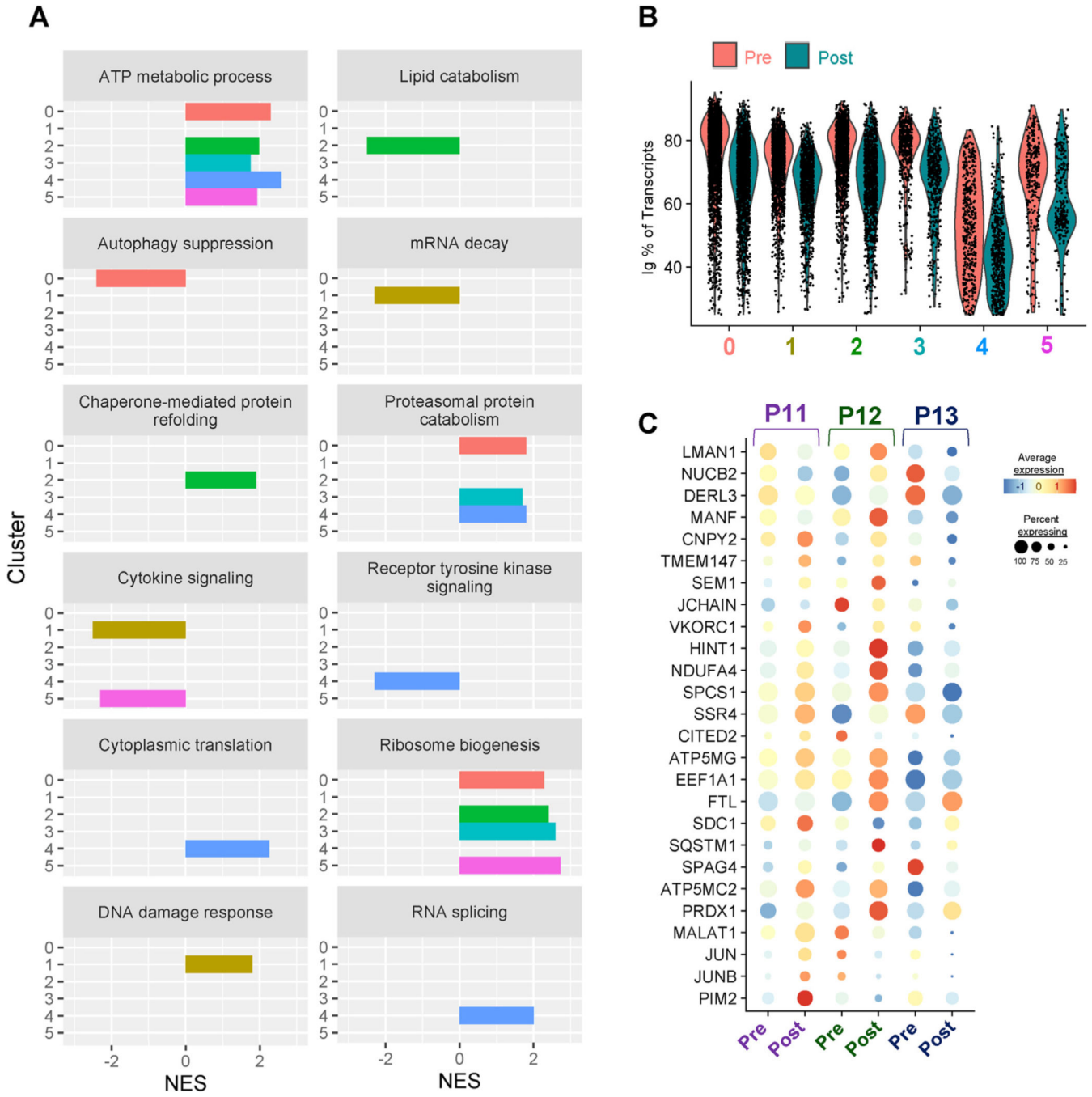

Figure 6.

Changes in gene expression after treatment with plerixafor and bortezomib. (A) The gene set enrichment analysis of the pathways most upregulated or downregulated in each cluster. Data are shown as the normalized enrichment score (NES) where a positive value indicates enrichment of the pathway in the post-sample relative to that of the presample. (B) The percentage of transcripts that code for immunoglobulin proteins. Each dot represents a single cell. (C) Differentially expressed genes (post vs pre) common to participants 11, 12, and 13. The average gene expression is scaled to a mean of zero and a standard deviation of 1. The

circle size corresponds to the percentage of cells within the cluster that express the gene and color corresponds to the scaled average expression.

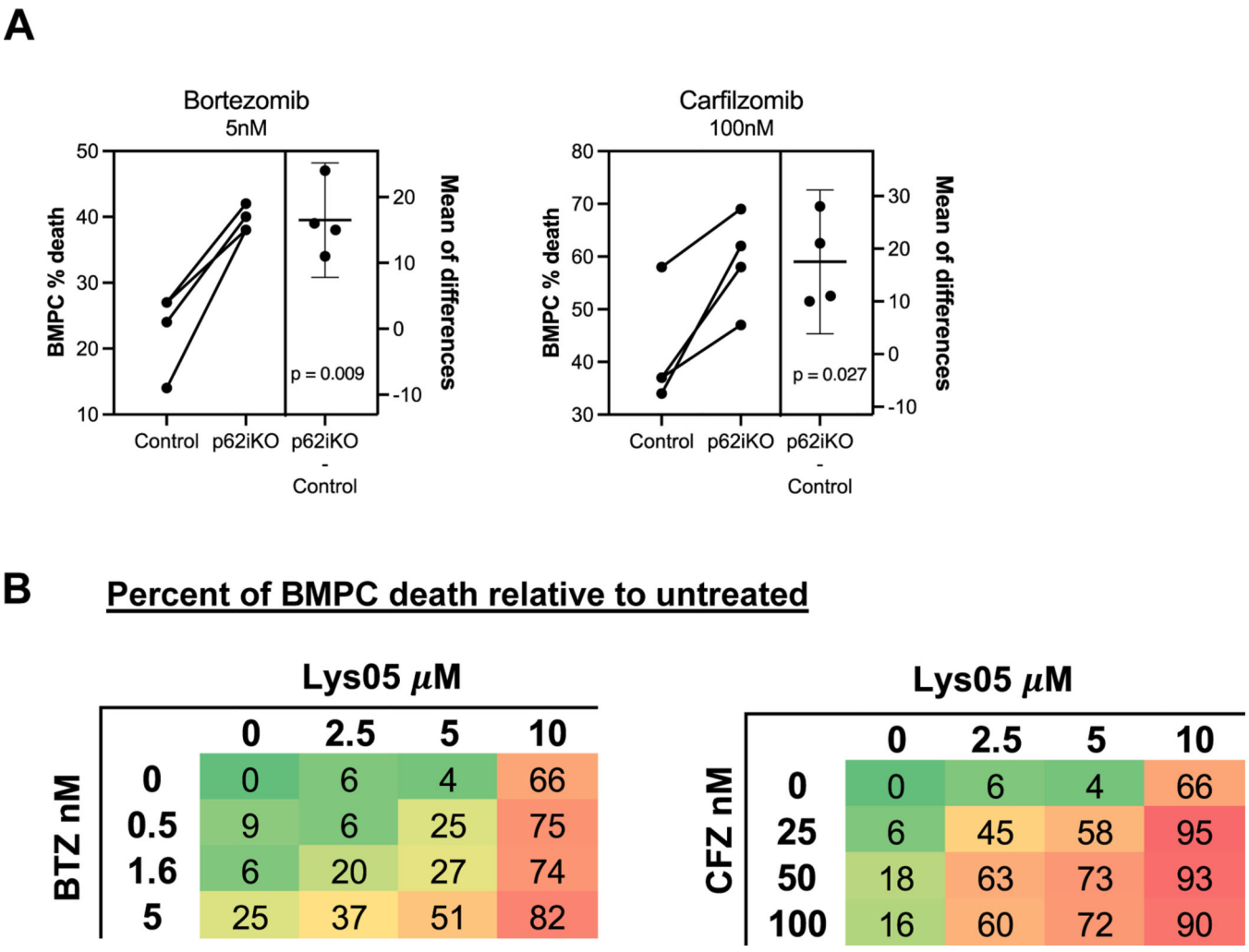

Figure 7.

Inhibiting autophagic flux improves plasma cell depletion with proteasome inhibitors. (A) Data are presented as the percentage of plasma cells that died during culture with either BTZ 5 nm or CFZ 100 nM relative to untreated, derived as the mean of technical replicates. Lines connect the paired samples corresponding to an individual experiment. *P* values are from the paired Student *T* test. The mean difference and 95% CI comparing plasma cell death in p62iKO and control cells are displayed. Control cells were from corn-oil vehicle-treated J-chain$^{ERT2Cre/WT}$ p62$^{fl/fl}$ mice and p62iKO cells were from tamoxifen-treated J-chain$^{ERT2Cre/WT}$ p62$^{fl/fl}$ mice. (B) Data are presented as the percentage of plasma cells that died during culture with BTZ or CFZ and/or Lys05, relative to that of the untreated cells, at indicated concentrations. Data are means from 3 to 5 independent experiments, each with 2–3 technical replicates. BTZ, bortezomib; CFZ, carfilzomib.

Table 1

Demographics of the study groups.

Demographics	A (n = 4)	B (n = 4)	C (n = 4)	Total (n = 12)	*P*
Age (y)	58.0 (46.2, 63.9)	63.3 (61.0, 64.9)	52.5 (44.7, 55.7)	58.8 (52.1, 63.7)	.146
Female	3 (75.0)	4 (100.0)	3 (75.0)	10 (83.3)	1.000
Black race	1 (25.0)	0 (0.0)	2 (50.0)	3 (25.0)	.709
Body mass index (kg/m^2)	27.9 (27.3, 29.9)	26.9 (22.5, 32.2)	31.9 (29.5, 33.4)	29.7 (25.3, 32.7)	.667
End-stage renal disease etiology					.564
Hypertension	0 (0.0)	0 (0.0)	1 (25.0)	1 (8.3)	
Polycystic kidney disease	0 (0.0)	2 (50.0)	0 (0.0)	2 (16.7)	
Systemic lupus erythematosus	0 (0.0)	1 (25.0)	0 (0.0)	1 (8.3)	
Type 1 diabetes mellitus	0 (0.0)	0 (0.0)	1 (25.0)	1 (8.3)	
Type 2 diabetes mellitus	2 (50.0)	1 (25.0)	1 (25.0)	4 (33.3)	
Congenital solitary kidney	0 (0.0)	0 (0.0)	1 (25.0)	1 (8.3)	
Congential obstructive uropathy	1 (25.0)	0 (0.0)	0 (0.0)	1 (8.3)	
Fabry disease	1 (25.0)	0 (0.0)	0 (0.0)	1 (8.3)	
Coronary artery disease	1 (25.0)	0 (0.0)	0 (0.0)	1 (8.3)	1.000
Hypertension	4 (100.0)	4 (100.0)	4 (100.0)	12 (100.0)	
Hyperlipidemia	2 (50.0)	2 (50.0)	1 (25.0)	5 (41.7)	1.000
Diabetes	2 (50.0)	1 (25.0)	2 (50.0)	5 (41.7)	1.000
Receiving immune-modulating drug(s)	0 (0.0)	0 (0.0)	2 (50.0)	2 (16.7)	.273
Receiving dialysis	4 (100.0)	2 (50.0)	3 (75.0)	9 (75.0)	.709
Peritoneal dialysis	0 (0.0)	1 (50.0)	2 (66.7)	3 (33.3)	.214
Time on dialysis (y)	7.9 (6.2, 9.8)	5.2 (5.2, 5.2)	3.3 (1.9, 4.9)	5.3 (4.5, 6.8)	.192
Previous transplant	2 (50.0)	1 (25.0)	2 (50.0)	5 (41.7)	1.000
No. of previous transplants	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	2.0 (1.5, 2.5)	1.0 (1.0, 1.0)	.472
Previous transplant type					1.000
Deceased donor kidney transplant	1 (50.0)	1 (100.0)	1 (50.0)	3 (60.0)	
Living related-donor kidney transplant	1 (50.0)	0 (0.0)	0 (0.0)	1 (20.0)	
Living unrelated-donor kidney transplant	0 (0.0)	0 (0.0)	1 (50.0)	1 (20.0)	
Cause of rejection					1.000

Demographics	A (n = 4)	B (n = 4)	C (n = 4)	Total (n = 12)	*P*
Acute cellular rejection	1 (50.0)	0 (0.0)	0 (0.0)	1 (20.0)	
Antibody-mediated rejection	0 (0.0)	1 (100.0)	1 (50.0)	2 (40.0)	
CMV nephropathy	1 (50.0)	0 (0.0)	0 (0.0)	1 (20.0)	
BK nephropathy	0 (0.0)	0 (0.0)	1 (50.0)	1 (20.0)	
Nephrectomy	2 (100.0)	1 (100.0)	1 (50.0)	4 (80.0)	1.000
Previous pregnancy	2 (100.0)	4 (100.0)	2 (66.7)	8 (88.9)	.556
No. of previous pregnancies	5.5 (3.8, 7.2)	2.5 (1.8, 3.0)	1.5 (0.0, 3.2)	2.5 (1.2, 3.0)	.62
Previous transfusion	4 (100.0)	1 (25.0)	1 (25.0)	6 (50.0)	.143
Total sensitizing events	3.5 (2.8, 5.5)	3.0 (2.8, 3.2)	3.5 (2.5, 4.0)	3.0 (2.8, 4.0)	.800
$cPRA_{1500}$	98.3 (94.9, 99.6)	100.0 (100.0, 100.0)	83.2 (62.8, 100.0)	100.0 (94.9, 100.0)	.368
$cPRA_{4000}$	94.5 (89.4, 95.8)	100.0 (100.0, 100.0)	100.0 (75.9, 100.0)	100.0 (94.5, 100.0)	.103
$cPRA_{8000}$	74.9 (65.9, 85.3)	96.9 (87.1, 100.0)	88.6 (69.3, 93.4)	88.6 (67.0, 96.0)	.255

Values are given as median (Q1, Q3) or n (%).

Table 2

Adverse events reported in at least 2 participants.

Adverse events	A (n = 4)	B (n = 4)	C (n = 4)	Total (n = 12)	*P*
Fatigue	1 (25.0)	1 (25.0)	2 (50.0)	4 (33.3)	1
Anemia	2 (50.0)	2 (50.0)	2 (50.0)	6 (50.0)	1
Tinnitus	1 (25.0)	2 (50.0)	3 (75.0)	6 (50.0)	.766
Insomnia	0 (0.0)	2 (50.0)	3 (75.0)	5 (41.7)	.212
Vivid dreams	2 (50.0)	3 (75.0)	1 (25.0)	6 (50.0)	.766
Nausea	1 (25.0)	1 (25.0)	2 (50.0)	4 (33.3)	1
Diarrhea	2 (50.0)	0 (0.0)	2 (50.0)	4 (33.3)	.418
Arthralgias/myalgias	1 (25.0)	2 (50.0)	1 (25.0)	4 (33.3)	1
Sleep hyperhidrosis	2 (50.0)	0 (0.0)	0 (0.0)	2 (16.7)	.273

Values are given as n (%).

Table 3

The cPRA and donors required to match.

Participant	Pre-cPRA	Post-cPRA	AcPRA	Pre-DRTM	Post-DRTM	ADRTM
cPRA $_{1500}$						
Group A						
P1	99.4587	97.5525	−1.9062	185	41	−144
P2	99.9994	99.9973	−0.0021	155514	36951	−118563
P3	88.4055	84.6414	−3.7641	9	7	−2
P4	97.1294	96.2582	−0.8712	35	27	−8
Group B						
P6	99.9848	99.9963	0.0115	6558	26728	20170
P7	99.9973	99.9974	0	37669	37809	140
P8	99.989	99.9926	0.0036	9050	13510	4459
P9	99.9947	99.9975	0.0027	18883	39247	20364
Group C						
P10	66.3979	73.9703	7.5724	3	4	1
P11	99.9944	99.9934	−0.001	17823	15178	−2645
P12	99.9905	99.9916	0.0011	10476	11894	1418
P13	51.901	50.9717	−0.9293	2	2	0
cPRA $_{4000}$						
Group A						
P1	94.1098	75.2452	−18.8646	17	4	−13
P2	98.2574	98.6902	0.4328	57	76	19
P3	75.2621	74.9943	−0.2677	4	4	0
P4	94.9762	92.5078	−2.4683	20	13	−7
Group B						
P6	99.9839	99.9152	−0.0687	6221	1179	−5041
P7	99.9989	99.9995	5.00E-04	94838	184861	90024
P8	99.9883	99.191	−0.7972	8517	124	−8393
P9	99.9975	99.9982	8.00E-04	39247	56656	17409
Group C						

Participant	Pre-cPRA	Post-cPRA	AcPRA	Pre-DRTM	Post-DRTM	ADRTM
P11	99.9934	98.1118	−1.8816	15178	53	−15125
P12	99.9916	99.9915	−1.00E-04	11911	11795	−116
P13	51.901	50.0129	−1.8882	2	2	0
cPRA $_{8000}$						
Group A						
P1	66.9871	56.0022	−10.9849	3	2	−1
P2	82.8699	95.3706	12.5007	6	22	16
P3	62.831	61.0655	−1.7655	3	3	0
P4	92.5078	91.7364	−0.7714	13	12	−1
Group B						
P6	99.985	99.2496	−0.7353	6653	133	−6519
P7	66.9871	96.5636	29.5766	3	29	26
P8	93.8027	93.9381	0.1354	16	16	0
P9	99.9982	99.9885	−0.0097	56656	8691	−47965
Group C						
P11	98.1118	95.6006	−2.5112	53	23	−30
P12	88.6173	89.7491	1.1318	9	10	1
P13	50.0129	50.0129	0	2	2	0

Participant 10 did not record an antihuman leukocyte antigen antibody of >4000 MFI.