Daratumumab in Sensitized Kidney Transplantation: Potentials and Limitations of Experimental and Clinical Use


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ABSTRACT

Background Donor-specific antibodies are associated with increased risk of antibody-mediated rejection and decreased allograft survival. Therefore, reducing the risk of these antibodies remains a clinical need in transplantation. Plasma cells are a logical target of therapy given their critical role in antibody production.

Methods To target plasma cells, we treated sensitized rhesus macaques with daratumumab (anti-CD38 mAb). Before transplant, we sensitized eight macaques with two sequential skin grafts from MHC-mismatched donors; four of them were also desensitized with daratumumab and plerixafor (anti-CXCR4). We also treated two patients with daratumumab in the context of transplant.

Results The animals treated with daratumumab had significantly reduced donor-specific antibody levels compared with untreated controls (57.9% versus 13% reduction; \( P<0.05 \)) and prolonged renal graft survival (28.0 days versus 5.2 days; \( P<0.01 \)). However, the reduction in donor-specific antibodies was not maintained because all recipients demonstrated rapid rebound of antibodies, with profound T cell-mediated rejection. In the two clinical patients, a combined heart and kidney transplant recipient with refractory antibody-mediated rejection and a highly sensitized heart transplant candidate, we also observed a significant decrease in class 1 and 2 donor-specific antibodies that led to clinical improvement of antibody-mediated rejection and to heart graft access.

Conclusions Targeting CD38 with daratumumab significantly reduced anti-HLA antibodies and anti-HLA donor-specific antibodies in a nonhuman primate model and in two transplant clinical cases before and after transplant. This supports investigation of daratumumab as a potential therapeutic strategy; however, further research is needed regarding its use for both antibody-mediated rejection and desensitization.

Kidney transplantation is the preferred treatment for patients with ESRD because of improved patient survival, quality of life, and reduced cost compared with dialysis.1–3 However, donated organs are scarce, wait times are increasing, and many patients die awaiting transplantation. Highly sensitized patients have very low rates of eventual transplantation, and if they have donor-specific anti-HLA antibodies (DSAs), such patients have higher rates of antibody-mediated rejection (AMR) and early graft loss.4–7 Patients with prior organ transplantation, pregnancies, or blood transfusions have an increased risk of AMR.8–10 We present the results of daratumumab in a nonhuman primate model and in two transplant clinical cases before and after transplant.

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likelihood of becoming sensitized by developing preformed anti-HLA antibodies. Thus, since the late 1990s, therapeutic strategies to desensitize and safely transplant this challenging patient population have been pursued.

Currently, variations of two protocols, high-dose intravenous Ig (IVIG) and/or plasmapheresis, are predominantly used in an attempt to desensitize potential recipients and allow successful transplantation across this barrier. Although desensitization decreases wait times and increases the rate of transplantation, these approaches are resource intense, AMR rates are high,8–13 and graft and patient survival are well below national averages for compatible live-donor transplantation.14 These early data underscore the urgent need for continued research into novel desensitization therapies. These observations are most likely due to a B cell–mediated response in the post-transplant period, leading to the production of de novo DSAs (dnDSAs). dnDSAs are the leading cause of late transplant failure after kidney transplantation and up to 30% of kidney allograft recipients develop dnDSAs. Despite numerous treatment strategies to boost conventional immunosuppression or to suppress B cell activity by targeting plasma cells (PCs), antibodies, and/or complement, no therapy reliably or satisfactorily reverses the effects of DSAs once established in the graft. Taken together, these detrimental effects of the humoral immune response strongly support the need for improved immune therapy that reduces pretransplant sensitization and better controls DSA-mediated AMR.

Daratumumab is an IgG1k human mAb that binds to CD38 and inhibits the development of CD38-expressing cells via multiple mechanisms, including complement–dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and apoptosis.15 CD38 is a transmembrane glycoprotein expressed on the surface of many immune cells—including PCs, plasma blasts, transitional cells—and is involved in functions such as receptor-mediated adhesion and signaling.16 It has been conjectured that PCs, not directly targeted by current desensitization methods, contribute to rebound humoral responses.17,18 Rituximab therapy in desensitization protocols aims to deplete B cells, thereby reducing antibody production.19 However, B cells lose expression of CD20 upon terminal differentiation to PCs, and rituximab consequently conveys limited efficacy with respect to PC depletion. In this context, two teams have analyzed the potential benefit of daratumumab to control PC production of anti-HLA antibodies in an experimental non-human primate (NHP) model and in two separate human clinical conditions. Using a rigorous sensitized NHP model, we hypothesized that CD38 targeting with daratumumab would be an effective means of PC depletion that would facilitate desensitization. In combination with daratumumab, we used plerixafor, a CXCR4 chemokine inhibitor, to increase mobilization of PCs from the marrow compartment. In this model, we demonstrate that this novel dual immunotherapy reduces levels of PCs and DSA, and significantly prolongs kidney allograft survival compared with nondesensitized controls. Concomitantly, daratumumab was used to clinically treat refractory life-threatening heart and kidney AMR and desensitize a candidate for heart transplant. In both cases, we observed a significant reduction of DSAs and panel-reactive antibodies (PRAs), improved allograft function, and finally patient and graft survival.

**METHODS**

**Experimental Model**

**Animals**

We used eight male rhesus macaques (*Macaca mulatta*) from Alpha Genesis Inc. (Yemassee, SC). We paired donors and recipients for maximal mismatching of MHC class 1 and 2 loci. The Genetics Services Unit (Wisconsin National Primate Research Center; Madison, WI) performed the deep sequencing assays MHC genotyping. The Duke University Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines for the care and use of primates approved all medications, surgical procedures, and postoperative care of animals.

**Sensitization, Desensitization, and Kidney Transplantation**

For allosensitization, recipient animals received two serial skin grafts as previously reported.20,21 Donor cell flow crossmatch confirmed allosensitization. Briefly, we exchanged full-thickness skin grafts from the dorsum between donor-recipient pairs without immunosuppression. We performed the second skin graft 6 weeks after the first skin graft. Four animals received 16 mg/kg daratumumab (DARZALEX; Janssen Pharmaceutica NV, Beerse, Belgium) and 0.24 mg/kg plerixafor (Mozobil; Sanofi, Gentilly, France) approximately 8–12 weeks after the second skin transplantation once a week for 1 month while four animals (controls) received no desensitization. Lymph node and posterior-iliac-crest bone marrow biopsies were performed before (on the day of first infusion) and after desensitization therapy (a week after the final infusion). For induction, recipient animals received 50 mg/kg rhesus...
anti-CD4 mAb (CD4R1) intravenously a week before (day −7) kidney transplantation and 25 mg/kg rhesus anti-CD8 mAb (MT807R1) on the day of kidney transplantation (postoperative day 0) (both mAbs from National Institutes of Health [NIH] Nonhuman Primate Reagent Resource). We performed kidney transplantation approximately 2 weeks after the final daratumumab and plerixafor infusions (Figure 1A). All eight animals swapped kidneys with their paired skin graft donor and underwent simultaneous native bilateral nephrectomy such that kidney transplants were life supporting. Animals received maintenance immunosuppression with tacrolimus (Prograf; Astellas Pharma, Northbrook, IL), mycophenolate mofetil (Cellcept; Genentech, San Francisco, CA), and a methotrexate taper. The recipients underwent serial sectioning (5-μm thick) and staining for hematoxylin and eosin and periodic acid–Schiff for routine evaluation and grading for rejection. A trained transplant pathologist (A.B.F.) evaluated histology blindly and scored according to updated Banff criteria.23,24

Histology, Immunohistochemistry, and Quantitative Image Analysis
Allograft tissues were obtained at time of biopsy or necropsy, fixed, and embedded in paraffin. The embedded tissue blocks underwent immunohistochemistry and stained for hematoxylin and eosin and periodic acid–Schiff. We analyzed kidney allograft biopsies using the updated Banff criteria.23,24 For germinal center visualization, lymph node biopsies were fixed, embedded, and stained with anti-human Ki67 (clone MM1; Vector, Burlingame, CA) and anti-CD20 (Thermo scientific, Rockford, IL) antibodies. We analyzed heart allograft biopsies using the International Society for Heart and Lung Transplantation criteria.25

RESULTS

NHP Experimental Model
Desensitization with Daratumumab (anti-CD38 mAb) and Plerixafor (anti-CXCR4) Reduces Preformed DSA
For depletion of PC populations, we treated sensitized rhesus monkeys weekly with daratumumab and plerixafor for a month (Figure 1A). To maximize depletion, plerixafor was

Immune Cell Monitoring
For monitoring immune cells, cells from blood, lymph nodes, bone marrow, spleen, and graft were stained with the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Life Technologies, Grand Island, NY) and then the following mAbs against human: CD3, CD4, CD8, CD14, CD20, CD25, CD27, CD28, CD56, CD95, CD159a, CD278 (ICOS), CD279 (PD-1), IgM, IgG, CXCR5, and—after fixation—Ki67 and FoxP3. We collected samples with a BD Fortessa flow cytometer and analyzed them using FlowJo software v9.6. We harvested, fixed, embedded, and stained grafts as previously described.20 All tissue samples were stained with hematoxylin and eosin and periodic acid–Schiff, evaluated blindly by an experienced transplant pathologist (A.B.F.), and scored according to updated Banff criteria.23,24

Statistical Analyses
Statistical analyses were performed using Prism software version 7.0 (GraphPad Software, San Diego, CA). Data are presented as mean ± SD (error bars). We calculated P values using the two-tailed t test in normally distributed data. For survival analysis, we used the Kaplan–Meier method and log-rank test. P values <0.05 were considered to be statistically significant. For original data related to NHP study, please contact stuart.knechtle@due.edu. For original data related to Créteil study, please contact philippe.grimbert@aphp.fr.

Patients
Two patients from Henri Mondor Hospital (Créteil, France) gave written consent to use daratumumab in this indication. High resolution Luminex assay technology (One Lambda) analyzed circulating anti-HLA antibodies and anti-HLA DSAs. A mean baseline normalized MFI >1000 was classified as a positive result. For each serum sample, we reported the number of antibody subclasses, the sum of MFIs, and the maximum MFI. For further analysis, we used the Kaplan–Meier method and log-rank test. P values <0.05 were considered to be statistically significant. For original data related to NHP study, please contact stuart.knechtle@due.edu. For original data related to Créteil study, please contact philippe.grimbert@aphp.fr.

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Desensitization with daratumumab and plerixafor reduces donor-specific alloantibody significantly in sensitized NHPs. (A) Schematic representation of sensitization and desensitization. Maximally MHC-mismatched NHP pairs received two serial skin transplants for sensitization and desensitization treatment or no treatment (control group) before kidney transplantation. (B) The absolute number of peripheral white blood cells and immune cell population. (C) Effect of DPT on peripheral B cells. B cell subpopulations, including CD20+ B cells, IgG+ B cells, and IgG+CD27+ B cells, were not changed. (D) The frequency of naive, central memory, effector memory CD4, and CD8 T cells were not changed with DPT. (E) Desensitization with daratumumab and plerixafor reduces DSA significantly compared with control. DSA levels were measured by T cell flow crossmatch and shown as MFI shift. *P<0.05. (F) Representative normalized MFI of single antigen bead testing before and after DPT. NHP serum samples were evaluated with HLA single antigen bead assay. BASO, basophil; EO, eosinophil; LN/ BM, lymph node/bone marrow; LYPH, lymphocyte; MONO, monocyte; NEUT, neutrophil; Tcm, central memory T cell; Tem, effector memory T cell; Tn, naive T cell; Tx, transplant; WBC, white blood cell.

given 11 hours before daratumumab. Because of plerixafor, circulating white blood cell counts fluctuated (Supplemental Figure 1). Whereas overall white blood cell counts normalized, lymphocytes showed a trend toward a proportional decrease (Figure 1B, P=0.06). Because CD38 is expressed in hematopoietic and nonhematopoietic cells, including activated B cell...
and T cell populations, we evaluated circulating B and T cell populations, including circulating B cells, IgG+ B cells, and memory B cells (IgG+CD27+CD20+), as well as naive (CD28+CD95-), central memory (CD28+CD95+), and effector memory (CD28-CD95dim) subsets of CD4 and CD8 T cells (Figure 1, C and D). We did not observe any significant changes in these populations. Surprisingly, DSA measured via donor T cell flow crossmatch showed significant reduction compared with pretreatment or date-matched untreated controls (Figure 1E). In addition, daratumumab and plerixafor treatment (DPT) profoundly reduced HLA antigen crossreactive antibodies (Figure 1F). A month of DPT diminished DSA levels with no changes in circulating B and T cell populations.

Daratumumab-Plerixafor Combined Treatment Reduces Plasmablasts in Lymph Nodes but Not Follicular Helper T Cells in Sensitized NHP

Surprisingly, DPT did not affect circulating B and T cells although DSA declined profoundly. To address the effect of DPT on lymph node B and T cells, we performed lymph node biopsies before the first DPT infusion and a week after the final DPT infusion (Figure 1A). After desensitization, we observed a significant reduction in the frequency of plasmablasts (CD20+CD27+CD38+) (Figure 2A). Notably, the plasmablasts showed higher Ki67 staining after DPT, suggesting that these cells may have repopulated. We also measured the follicular helper T (Tfh) cell population in the lymph node to address whether the reduction of PCs and DSA correlated with Tfh cell reduction. However, DPT did not affect the frequency of Tfh cells (Figure 2B). In accordance with this, clonal B cell expansion (Ki-67+CD20+) which represents germinal center response remained unchanged and CD20+ B cell follicles were not affected after DPT (Figure 2C). Taken together, the data suggest that desensitization with DPT directly affects B cells and PCs independently of Tfh cells.

Delayed AMR after Desensitization with Daratumumab and Plerixafor in the Sensitized Kidney Transplant Model

DPT significantly reduced preformed DSA compared with untreated control animals, with more than 50% reduction compared with the pretreatment time point (Figure 1E). After desensitization, animals received kidney transplants from their maximally MAMU mismatched skin donors (Figure 3A). As shown in Figure 3B, sensitized animals without any desensitization showed rapid graft rejection within 10 days (mean survival time=5.0±3.3 days). Animals desensitized with daratumumab and plerixafor showed significantly prolonged graft survival (mean survival time=21.6±8.5 days); however, they uniformly developed rejection within 30 days of transplantation. Surprisingly, animals desensitized with daratumumab and plerixafor exhibited both increased AMR and cell-mediated rejection (Figure 3C). Increased AMR scores and acute rejection scores suggest rebound of both anti-donor T and B cell responses after kidney transplantation (Figure 3D). In parallel, circulating DSA rapidly increased after kidney transplantation, suggesting a short-lived PC/plasmablast depletion (Figure 3E).

Off-Target Effect of Daratumumab on Regulatory Cells

It is reported that daratumumab depletes CD38+ immune regulatory cells, including regulatory T cells (Tregs), regulatory B cells (Bregs), and myeloid-derived suppressor cells, and results in T cell expansion and skewing of the T cell repertoire in multiple myeloma patients. Therefore, we assessed CD8+/CD4+ and CD8+/Treg ratios. Overall, animals treated with daratumumab and plerixafor did not show significant increase of memory T cells (Figure 1D) or cytotoxic T cells in peripheral blood and lymph nodes (Figure 4A). However, it is notable that the CD8/Treg ratio showed a trend of increasing in lymph nodes (Figure 4B). Secondly, we evaluated conventional Treg populations pre- and post-treatment. Interestingly, the Treg (CD4+CD25+CD127-) population was not proportionally changed in the peripheral blood or lymph nodes after desensitization and we observed only a trend of reduction in the absolute number of Tregs in the lymph nodes (Supplemental Figure 2). We then assessed transitional B cells because they express CD38 on their surface. As shown in Figure 4C, transitional B cells showed a strong trend of reduction in the lymph nodes after DPT. Because rapid peripheral T cell immune deviation was not observed, we evaluated activated T cells after DPT as well as after kidney transplantation in the peripheral blood and lymph nodes. Interestingly, desensitization with daratumumab and plerixafor did not substantially affect T cell activation; however, CD69+ CD4 and CD8 T cells in the lymph nodes significantly increased after kidney transplantation (Figure 4D). This suggests that reduction of regulatory cells (Treg and B reg) by daratumumab and plerixafor did not affect T cells when they were immunologically inert but rather promoted a more vigorous response when challenged or activated. Taken together, targeting CD38 with daratumumab efficiently reduced memory B cell and PC populations but also depleted beneficial regulatory cell populations.

Clinical Results

DSA Outcome after Daratumumab in One Refractory Heart and Kidney AMR

A 32-year-old man, who had heart and kidney engrafted 17 months before due to systemic lupus, presented with severe acute kidney and heart mixed rejection after complete immunosuppressive drug discontinuation (complete clinical description is provided in Supplemental Material). In the kidney allograft biopsy, histologic analysis revealed T cell–mediated rejection (TCMR) and AMR according to the Banff updated classification, with PC-predominant infiltration, major interstitial edema, and interstitial hemorrhage (Figure 5, A–F); the heart biopsy revealed TCMR grade 2R, AMR pathologic AMR grade 3, and diffuse PC infiltration (Figure 5, I–K). Nine DSAs were found with four class 1 dnDSAs (two with MFI>6000, one with MFI 3000–6000, and one with MFI 1000–3000; MFI sum 26,554), four class 2 dnDSAs, and one preexisting class 2 DSA (all with MFI>6000; MFI sum 94,476) (Figure 6B). Despite conventional
therapy including high-dose steroid pulses, plasmapheresis, anti-thymocyte globulin, rituximab, and high-dose IVIG, the patient developed cardiogenic shock requiring intravenous dobutamine and ARF dependent to dialysis (Figure 6A). In this setting, we prescribed the compassionate use of eight weekly infusions of daratumumab (16 mg/kg each) combined with eculizumab. Outcome of anti-HLA antibodies MFI 3 months after the beginning of daratumumab revealed dramatic decline for eight of nine dnDSAs and MFI<1000 for seven of nine dnDSAs (Figure 6B). Heart allograft function returned to baseline without drugs and serum creatinine was 300 μmol/L (Figure 6A). Kidney allograft biopsy showed significant

![Figure 2](image_url)

**Figure 2.** Desensitization with daratumumab and plerixafor reduces PC population, but not Tfh cells, in the lymph nodes. (A) Plasmablast population in the lymph nodes after desensitization. Representative flow plot shows the percentage of CD20+CD27+CD38+ cells within the CD3-IgD+ population of lymph nodes pre- (blue) and post- (orange) desensitization time points with DPT. (B) Tfh populations in the lymph nodes after desensitization. Representative flow cytometry plots show the percentage of PD1+ICOS+ Tfh cells within the CD4+ T cell population in lymph node pre- (blue) and post- (orange) desensitization with daratumumab and plerixafor. (C) Representative immunohistochemistry shows the Ki67-stained areas within the CD20+ B cell follicles in lymph nodes pre- and post-desensitization with daratumumab and plerixafor. Clonal B cell expansion (Ki67+CD20+) within B cell follicle was not significantly changed after desensitization. Images were adapted from whole slide scan; original magnification ×40 (inset, ×100). GC, Germinal Center.
Figure 3. Desensitization with daratumumab and plerixafor significantly prolongs but limits renal allograft survival in sensitized NHPs. (A) Dosing regimen of T cell depletional induction and maintenance immunosuppression for kidney transplantation after desensitization or without desensitization. (B) Percentage graft survival of sensitized NHPs with or without desensitization. Animals treated with DPT showed significantly prolonged graft survival compared with control group (P<0.05). (C) Representative hematoxylin and eosin (top panels) and C4d (bottom panels) staining of kidney allografts from control (left panels) and DPT (right panels) animals at the time of euthanasia. (D) Clustered Banff score related to AMR and TCMR. Animals treated with daratumumab and plerixafor showed elevated AMR score (g+ptc+C4d) and acute rejection (v+t+i) at euthanasia. (E) Post-transplant DSA kinetics. Animals with desensitization showed rapidly increased serum DSA (normalized to pre-renal transplant value=1). Post-kidney transplant peak DSA level showed strong trend of elevation compared with pretransplant time point. Original magnification ×200. ACR, acute cell-mediated rejection; g, glomerulitis; H&E, hematoxylin and eosin; i, interstitial inflammation; MMF, mycophenolate mofetil; ptc, peritubular capilaritis; t, tubulitis; TMA, thrombotic microangiopathy; Tx, transplant; v, intimal arteritis.
improvement in acute lesions and the PC infiltrate significantly decreased (Figure 5). Blood-circulating PCs were undetectable. Twenty weeks after the eighth infusion of daratumumab, the patient presented with AKI with recurrent acute PC-rich rejection on kidney biopsy and significant reascension of the MFI of two class 2 anti-HLA DSAs, which is associated with PC reappearance (Figure 6, A and B). Daratumumab was reinitiated weekly (four doses) and then bimonthly for 4 months (Figure 6A). Kidney allograft function improved to serum creatinine 350 µmol/L (Figure 6A). The class 2 anti-HLA DSA MFI decreased gradually and significantly (Figure 6B) and blood-circulating PCs were undetectable.

Anti-HLA Desensitization Using Daratumumab in a Candidate for Heart Transplant

The highly sensitized nature of a 62-year-old woman’s clinical situation confirmed the need for heart transplantation in December 2016 (complete clinical description is provided in Supplemental Material). Initial immunologic evaluation showed that up to 80% of heart allografts from deceased donors were incompatible (calculated PRA [cPRA]) with n=35 class 1 and n=5 class 2 alloantibody specificities. Desensitization included many plasmaphereses, multiple courses of high-dose IVIG, and rituximab. Because of no significant changes in sensitization (cPRA=98% and n=35 class 1 anti-HLA antibodies with MFI>3000) and deterioration of clinical condition, we used daratumumab as desensitization protocol (eight weekly injections of 16 mg/kg each). At the end of the treatment, we observed a significant decrease of cPRA to 62% with n=14 class 1 anti-HLA antibodies with MFI>3000 (Figure 6C) allowing heart transplantation in March 2018, with only two DSAs (one class 1 and one class 2). Heart allograft HLA included two prohibited antigens before daratumumab therapy.

DISCUSSION

This study demonstrates that targeting CD38 with daratumumab significantly reduced anti-HLA antibodies and production of anti-HLA DSAs in both an experimental model and two transplant clinical cases before and after transplant.

Anti-HLA antibodies limit graft access and DSAs, preformed or dnDSAs, are increasingly recognized as the leading cause of early and late kidney allograft failure. Binding DSAs to antigens expressed on allograft endothelial cells can activate a classic complement pathway, a key pathologic process of AMR phenotypes. Even without complement activation, some DSAs can induce allograft damage through antibody-dependent cellular cytotoxicity. This pathogenesis may contribute to transplant glomerulopathy and vasculopathy which feature vascular intimal thickening with smooth muscle cell invasion. DSA-mediated rejection increases allograft dysfunction and is generally recalcitrant to the standard therapeutic approaches used for TCMR. To increase transplant access, immunosuppressive therapy combinations have been proposed for incompatible transplants but benefits are unclear, with acute rejection rates after transplant up to 40% and no difference in patient survival compared with dialysis. Such treatments allow elimination of circulating anti-HLA antibodies and effectively destroy B lymphocytes expressing CD20 markers; however, they have no effect on antibody-producing PCs.

Daratumumab is a first-in-class human IgG1 mAb that binds CD38-expressing cells with high affinity. Presently, daratumumab is used in cancer studies to promote tumor cell death through diverse mechanisms of action, including complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis, and apoptosis induction. In a phase 2 trial, daratumumab monotherapy showed encouraging efficacy in heavily pretreated and refractory patients with multiple myeloma with a favorable safety profile.

Although CD38-targeting antibodies were initially developed to kill malignant PCs, these mAbs may also abrogate the production of autoantibodies in autoimmune disorders or anti-HLA antibodies in transplant recipients before or after transplant, thereby reducing antibody-dependent effector mechanisms. In transplantation, CD38-targeting antibodies provide a therapeutic option for sensitized transplant candidates and refractory AMR. Notably, the ability of daratumumab to induce nonmalignant CD38-PC depletion in the field of auto- or alloimmune-mediated disorders has not yet been evaluated. Daratumumab specifically targets CD38+ cells on the basis of cell surface expression. Furthermore, plerixafor may mobilize PC populations from the bone marrow niche. Unlike our previous approaches targeting PCs and germinal centers with costimulation blockade and proteasome inhibitor, we targeted PCs and activated/memory B cells. We hypothesized that we could achieve a more selective and complete desensitization by mobilizing PC populations from the bone marrow and directly target CD38-expressing PCs (as well as memory B cells). As expected, serum DSA levels as well as serum antibodies that crossreact with HLA Ag dropped significantly after DPT (more than 50%; Figure 1). The cross-reactivity of anti-MAMU antibodies to HLA Ags is presumably due to the same Bw6 motif present in the Mamu-A1 haplotypes and the Mamu B alleles to HLA. The single antigen bead assay provided a better way to compare the rhesus result to a human DSA clinical situation. However, the HLA antigen beads with Bw6 motif (most >10,000 MFI) may be saturated with bound antibody, potentially underestimating the degree of antibody reduction. Diluted serum samples (1:8 dilution) showed a greater diminution compared with neat samples. The circulating B cell population was not significantly affected by the combination of daratumumab and plerixafor over time. However, serial biopsies during desensitization showed a reduction of plasmablasts in the lymph nodes without reducing the Tfh population, suggesting Tfh-independent mechanisms. The level of DSA reduction was comparable, or even superior, to that achieved by combined costimulation
Figure 4. A possible immune deviation after daratumumab and plerixafor treatment. (A) Ratio of CD8 to CD4 was not changed after desensitization with daratumumab and plerixafor in blood and lymph nodes. (B) Ratio of CD8 cells to Treg cells show a strong increasing trend \( (P=0.06) \) in the lymph nodes but not in the blood. (C) Transitional B cell population \( (CD20^+CD24^+CD38^+) \) showed a strong trend of reduction \( (P=0.08) \). (D) Increased frequencies of activated T \( (CD69^+) \) cells after kidney transplantation. The results are representative of four monkeys tested individually. LN, lymph node.
blockade–bortezomib. However, even with profound reduction of DSA before kidney transplantation, all treated animals showed limited durability in regulating the humoral response. Surprisingly, all animals rejected kidney transplants with enhanced acute rejection grade and AMR score, even though they showed pristine graft tissue on early biopsy (Supplemental Figure 3). It is unclear why treating sensitized NHP with daratumumab and plerixafor induces TCMR while effectively lowering DSA levels. We cannot attribute this to low animal numbers because we saw homogeneous outcomes. It is possible that the proximity to the sensitization events and MHC-mismatching status in the NHP model could favor a

Figure 5. Histological improvement of heart and kidney acute rejection after daratumumab treatment. (A–F) Kidney allograft biopsy at the time of diagnosis showed (A) interstitial edema and diffuse infiltrate (periodic acid–Schiff stain), made of (B) >10% of PCs (immunohistochemical staining with CD138), (C) with severe tubulitis (methenamine silver-periodic Jones stain Marinozzi), (D) capillaritis (periodic acid–Schiff), and (E) interstitial hemorrhage (Masson trichrome). (F) Immunohistochemical stain with C4d is diffusely positive on peri-tubular capillaries. (G–H) Kidney allograft biopsy after daratumumab showed significant reduction of the interstitial infiltrate with (G) only mild persistent tubulitis (periodic acid–Schiff); (H) only scattered PCs are stained with CD138 antibody compared with the first kidney biopsy. (I–K) Heart allograft biopsy at the time of cardiogenic shock showed (I) diffuse edema (hematoxylin eosin saffron [HES] stain), (J) interstitial infiltrate with myocyte damage (HES stain), and (K) diffuse C4d positivity. (L) Heart allograft biopsy after daratumumab showed no significant anomaly (HES stain). Original magnification, ×200 in A; ×200 in B; ×400 in C; ×400 in D; ×400 in E; ×200 in F; ×100 in G; ×200 in H; ×200 in I; ×400 in J; ×100 in K; ×200 in L.
response. Perhaps if the sensitization had occurred years or months earlier and a well matched transplantation were available, this would be pursued in a human patient. With recent sensitization the cellular rejection may not be as difficult to suppress with conventional immunosuppression, but this is unclear. However, it is clear that, although both agents target PCs, they may also elicit broader immune modulation. Daratumumab could successfully target CD38-expressing memory B cells, plasmablasts, and PCs; however, these are not the only immune cells expressing CD38. In particular, B cells with regulatory function (Breg or B10 effector cells) are well known to express CD38. We observed reduction of Bregs and Tregs after DPT treatment with more rapid emergence of activated T cells after kidney transplantation. Krejcik et al. made a similar observation with daratumumab treatment in patients with multiple myeloma. They showed

Figure 6. Significant improvement of AMR and significant decrease of DSA after daratumumab treatment. (A) Heart and kidney function evolution after daratumumab as acute rejection treatment. After the first eight injections of daratumumab, heart and kidney allograft functions improved significantly. Inotropic positive drugs and hemodialysis could be stopped. Twenty weeks after the eighth daratumumab infusion, serum creatinine level increased significantly, and heart allograft function remained stable. Daratumumab was reinitiated and serum creatinine level decreased. (B) DSA evolution after daratumumab as acute rejection treatment. After the first eight injections of daratumumab, DSAs class 1 and class 2 MFI decreased significantly besides DQ7 anti-HLA antibody (class 2). Two DSAs class 2 (DR12 and DR52) remained detectable with MFI>1000. At the time of the AKI episode, three class 2 DSAs were detected with MFI>1000 (DQ7, DR12, and DR52). Of those, two of them (DR12 and DR52) decreased significantly after daratumumab reinfusion. (C) Anti-HLA antibodies evolution after daratumumab as desensitization treatment. After plasmapheresis, anti-HLA antibodies MFI and cPRA increased significantly, both class 1 and class 2. After eight weekly infusions of daratumumab, anti-HLA MFI and cPRA decreased significantly. Maintenance infusion, bimonthly, permitted to decrease MFI and number of significant anti-HLA antibodies.
depletion of CD38+MM cells, as well as immunosuppressive cells including Treg (CD4+CD25+CD127dimCD38+), Breg (CD19+CD38−CD24+), and G-MDSC (CD11b+CD14−HLA− DR−CD15+CD33+), along with T cells skewing toward a phenotype of memory T cells. More recently, it has been shown that CD38 inhibition promoted superior effector function via metabolic reprogramming of T cells. Furthermore, our observations are similar to the adverse events that occur with general B cell depletion at induction in transplantation. Clatworthy et al.43 showed increased TCMR with rituximab induction compared with noncytolytic induction. B cells with regulatory function may be targeted by both rituximab and daratumumab and contribute to graft rejection in organ transplantation. Whether the daratumumab-induced TCMR can be readily controlled with current or additional immunosuppressive regimens remains to be established.

In contrast to the NHP data, we also observed the potential benefit on daratumumab on anti-HLA antibodies and DSA in two patients, including refractory AMR in a recipient of a combined heart and kidney transplant and a candidate for heart transplantation who was highly refractory and sensitized. We observed in both cases a significant decrease in DSA and anti-HLA antibody MFI after eight injections of daratumumab, associated with a high depletion in peripheral CD38+ PCs, strongly supporting the effectiveness of the molecule to reduce allosensitization intensity. In both cases, plasmapheresis, high doses of IVIG, and rituximab failed to control DSA or PRA levels. MFI decrease after plasmapheresis and high-dose IVIG has already been analyzed in the context of acute AMR and dnDSA.46,47 Mean MFI decrease after plasmapheresis procedure was 25%, but long-term analysis is not currently available.46 High-dose IVIG has no effect on dnDSA MFI.47 Furthermore, DSA MFI rebound has been reported after rituximab induction therapy in recipients of kidney allograft with preformed DSA.48 The efficacy of daratumumab could be enhanced by better control of memory responses, for example by increasing the amount of calcineurin inhibitors immunosuppression. In the AMR-resistant case, both control heart and kidney allografts biopsies (performed 12 weeks postrejection) showed significant intragraft PC depletion. PC-rich rejection has been identified as a morphologically distinct lesion49 that occurs late post-transplantation and can be an independent predictor of poor allograft survival.50 Previous studies have also associated PC-rich rejection with vascular rejection, transplant glomerulopathy, and inadequate immunosuppression due to noncompliance.51 Whether PC infiltrates participate in humoral rejection through local secretion of antibodies is still a challenging question. Our data suggest that daratumumab induces intragraft deletion of CD38+ PCs and could represent a therapeutic option for the management of PC-rich rejection in the context of humoral rejection.

In conclusion, both experimental and preliminary clinical results trends to suggest that daratumumab is a potentially therapeutic strategy to limit DSA production in the setting of allotransplantation. The effect of targeting the PC population with daratumumab could be dependent on contexts such as the level of sensitization, timing of immunologic response, and degree of pathogenesis. However, the potential risk and benefit of daratumumab for treatment of AMR and desensitization should be investigated further to determine the benefit/risk ratio related to concomitant T cell activation.

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Dr. Kwun, Dr. Matignon, Dr. Knechtle, and Dr. Grimbert designed the study. Dr. Kwun, Dr. Manook, Dr. Guendouz, Dr. Kheav, Dr. Poullot, Dr. Gautreau, Dr. Ezekian, Dr. Bodez, Dr. Damy, Dr. Faire, Mr. Yoon, and Dr. Belhadj carried out experiments. Dr. Kwun, Dr. Matignon, Dr. Chen, Dr. Bilewski, Dr. Yi, Dr. Farris, Dr. Knechtle, Dr. Park, and Dr. Grimbert analyzed the data. Dr. Poullot and Dr. Matignon made the figures. Dr. Kwun, Dr. Matignon, Dr. Knechtle, and Dr. Grimbert drafted and revised the paper. All authors approved the final version of the manuscript.

**DISCLOSURES**

Dr. Knechtle reports personal fees from Sanofi, outside of the submitted work. Dr. Belhadj reports personal fees from Celgene, personal fees from Takeda, personal fees from Amgen, and personal fees from Janssen, outside of the submitted work. All remaining authors have nothing to disclose.

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**SUPPLEMENTAL MATERIAL**

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2018121254/-/DCSupplemental.

Supplemental Figure 1. Longitudinal and transient changes of circulating leukocyte populations during daratumumab and plerixafor treatment.
Supplemental Figure 2. Effect of daratumumab and plerixafor on lymph node Treg cells.

Supplemental Figure 3. No profound prozone but some saturation effect were shown in serial diluted samples.

Supplemental Figure 4. Early renal biopsy after daratumumab and plerixafor treatment.

REFERENCES


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