course of steroids before IS withdrawal after 2 weeks. Systemic immune function was assessed by CFSE-based mixed lymphocyte reaction (MLR) proliferation assays and allo-sensitization was evaluated by the detection of serum allo-antibody formation. Evidence for mixed chimerism was assayed in peripheral blood.

RESULTS: Durable mixed chimerism (only detected transiently at 6 weeks post-DBMT) did not develop but recipients managed to come off all IS for 4–5 weeks before acute rejection (Banff II) developed between POD115-POD126. Rejection could be treated and reversed both clinically and histologically. However, following subsequent re-withdrawal of IS, Banff II rejection developed again within 2 weeks (on POD172), could not be reversed despite treatment (Banff II on POD195) and ultimately culminated in necrosis of the VCA (on POD224). Final histology showed severe acute cell-mediated and antibody-mediated rejection with C4d deposition in subcutaneous arteries and arterioles as well as chronic allograft vasculopathy. Despite these findings, corresponding serial MLR assays demonstrate unresponsiveness after DBMT and no allo-antibody formation was detected.

CONCLUSION: While DBMT demonstrated systemic immunomodulation based on MLR, in the absence of durable mixed chimerism, this effect was not enough to prevent acute rejection episodes and the development of chronic rejection in this cohort of animals. These rejection episodes may be related to the subsequent development of chronic rejection. The presence of vascular lesions (based on C4d deposition) throughout all time points following DBMT may predate clinical progression to eventual chronic allograft vasculopathy and rejection, and may represent a potential prognostic factor in clinical VCA.

A Clinically Relevant Protocol for Vascularized Composite Allotransplantation Using A Single Dose of AMD3100 for Stem Cell Mobilization

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PURPOSE: Vascularized Composite Allograft (VCA) transplantation is a clinical reality but its application is limited by the toxicities of chronic immunosuppression and rejection. Current clinical tolerance protocols require recipient conditioning that limits use to living donor transplants. We sought to design a clinically relevant protocol applicable to cadaveric organs. We modified our existing non-myeloablative stem cell canine VCA transplant model to use AMD3100 (Plerixafor) for stem cell mobilization.

METHODS: 4 DLA-haploidentical, related canine recipients [Group I] received conditioning with 350cGy TBI, AMD3100-mobilized donor stem cells and VCA transplantation with a short course of immunosuppression (MMF:56 days/CSP 70 days +/- taper). 5 DLA-haploidentical, related canine recipients [Group II] underwent identical conditioning plus an infusion of Bone Marrow (BM) harvested on the day of transplant. Aspirate in addition to AMD3100. CD34+ hematopoietic progenitor cells were quantified via flow cytometry. Peripheral blood chimerism was evaluated by PCR techniques weekly. VCA graft survival was followed clinically and histologically.

RESULTS: All dogs in the first group exhibited prolonged thrombocytopenia and one dog was euthanized secondary to this complication (POD 32). All 4 demonstrated initial engraftment of the stem cells. One dog had very poor initial engraftment and went on to reject the VCA on POD 146. The remaining 2 dogs remained tolerant to their VCA transplant (POD 79 and 101). The addition of marrow eliminated the problems with prolonged thrombocytopenia. In Group II, Two of the dogs were euthanized secondary to pneumonia (POD 12 and 95) and one for liver dysfunction (POD 45). Currently two dogs are doing well with no evidence of GVHD or loss of the VCA transplant (POD 186 and POD 67).

CONCLUSION: This study demonstrates that a clinically relevant protocol using a single dose of AMD3100 and addition of a bone marrow aspirate combined with our non-myeloablative protocol can lead to tolerance the VCA across a significant genetic barrier.