PURPOSE: Vascularized Composite Allograft (VCA) transplantation is a clinical reality but limited by toxicities of chronic immunosuppression and rejection. Current clinical tolerance protocols rely on recipient conditioning and donor cell mobilization that limits use to living donor transplants. We sought to design a clinically relevant protocol applicable to cadaveric organs. We have previously demonstrated stem cell engraftment using AMD3100 (Plerixafor) as a single dose agent for stem cell mobilization in a haploidentical canine model. We wanted to increase clinical relevance by testing our existing non-myeloablative stem cell canine VCA transplant model to full DLA-mismatched, unrelated canine donor-recipient pairs.

METHODS: 8 DLA haploidentical, related canine recipients (Group I) and 4 full DLA-mismatched, unrelated canine recipients (Group II) received conditioning with 350-450cGy TBI, AMD3100-mobilized donor stem cells + Bone Marrow (BM) infusion and simultaneous VCA transplantation with a short course of immunosuppression (MMF: 56 days/CSP: 70 days. Sirolimus 28 days added as third agent for Group II). 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 12 canines tolerated the conditioning regimen. Stem cell engraftment and donor chimerism was seen in all dogs. Mean COBE apheresis count was 3.98x10^8 cells/kg and mean BM aspirate count was 1.56x10^8 cells/kg across both groups (12 dogs). Outcomes were varied. In Group I there was evidence of an acute rejection episode (self-limited) in 1 dog. Evidence of GVHD (skin, liver) was seen in 3 dogs and pulmonary hemorrhage noted in 3 dogs. In Group II there was no evidence of acute rejection, however GVHD (skin, liver) was seen in 2 dogs. 2 dogs were lost post-transplant to complications of intussusception while still seemingly tolerant to the VCA.

CONCLUSION: This study demonstrates proof of principle for AMD3100 as a single-dose stem cell mobilizing agent for a clinically relevant tolerance protocol in both related haplotype and in mismatched, unrelated donor-recipient pairs. Use of AMD3100 led to stem cell engraftment in all animals transplanted with evidence of acute rejection in the VCA in only one canine. AMD3100 use limited by thrombocytopenia in our previous studies continue to appear be resolved with the addition of BM Aspirate in this model. Continued experiments should allow for longer-term follow up in future canine recipients that should optimistically not experience bowel complications or GVHD.

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Adipose-derived Stem Cell-Based Immunomodulatory Therapy in a Translational Porcine Limb Transplant Model

Deokyeol Kim, MD, Matthias Waldner, MD, Wensheng Zhang, MD, Mario Solari, MD, Kia Washington, MD, Kacey Marra, PhD, J. Peter Rubin, MD

Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, USA

PURPOSE: Mesenchymal stem cells deriving from bone marrow and adipose tissue have immunomodulatory effects and low immunogenicity. Cell therapy using donor-derived bone marrow cells is already being used clinically and has reduced the burden of immunosuppressive drugs in vascularized composite allotransplantation (VCA) patients. However, the quantity of donor-derived bone marrow cells that can be effectively harvested from an individual donor is limited. Adipose-derived stem cells (ASCs) represent another clinically useful source of cell therapy for allotransplantation. This study was designed to investigate whether ASCs treatment could prolong graft survival in a porcine large animal model.

METHODS: Full-major histocompatibility complex (MHC) mismatched heterotopic hind-limb transplantations were performed in MGH mini-swine. Animals receiving no therapy and animals treated with tacrolimus for the initial 30 days served as control and standard therapy group, respectively. Experimental animals were treated with a standard immunosuppressive protocol including tacrolimus for 30 days, followed by ASC therapy (donor-derived ASCs [1.0x10^6 cells/kg] administered intravenously in recipients at post-operative day 7). Allograft survival was compared across the different treatment protocols.

RESULTS: Six allogenic hind limb transplantations in the mini-swine were performed in three groups. The control group reached Banff grade 4 acute rejection by an average of 7.5 days after transplantation. Allografts treated with ASCs demonstrated grade 4 rejection on day 119 and demonstrated rejection-free survival over 200 days.
postoperatively, which was longer than the standard therapy group. The long-term survivor of the ASCs therapy group showed donor-specific unresponsiveness and regulatory T cells upregulation.

CONCLUSION: Early results of ongoing in vivo experimentation show promising results of ASC therapy in prolonging acute rejection-free VCA survival across a full MHC mismatch. Achieved immune tolerance by cellular immunomodulation is considered to prolong allograft survival.

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Trends of Maintenance Immunosuppression in Hand and Facial Transplantation

Amit K. Manjunath, BS1, Rami S. Kantar, MD2, Michael J. Cammarata, BS2, Adam Jacoby, MD2, William J. Rifkin, BA2, Bruce E. Gelb, MD2, Rodrigo Diaz-Siso, MD2, Eduardo D. Rodriguez, MD, DDS2

1NYU School of Medicine, New York, NY, USA, 2Hansjörg Wyss Department of Plastic Surgery, NYU Langone Health, New York, NY, USA

PURPOSE: In select patients, vascularized composite allotransplantation (VCA) offers functional and aesthetic outcomes superior to autologous reconstruction. However, its role in the reconstructive armamentarium is limited by the need for lifelong immunosuppression. Furthermore, some studies have suggested that skin-containing VCA requires greater maintenance immunosuppression than solid organ transplants due to higher antigenicity. This study evaluates trends of maintenance immunosuppression in skin-containing VCA recipients and kidney recipients to determine differences in therapeutic risk.

METHODS: VCA immunosuppression data were collected through a review of hand and face transplant literature. Kidney transplant immunosuppression data were collected through an institutional review board-approved retrospective chart review of a randomly selected group of 100 patients whose transplants were performed between 1997 and 2016. Data from patients younger than 18, patients not receiving triple maintenance immunosuppression therapy, and patients with missing immunosuppression information were excluded from the analysis. Prednisone doses (mg/day) and mycophenolate mofetil (MMF) doses (gm/day) were compared between VCA and kidney recipients at predefined follow-up intervals (<1, 1–5, and >5 years). Tacrolimus target trough levels (TTTL) were stratified into our institution’s kidney transplant risk-based target ranges (low risk: 4–6 ng/ml, high risk: 6–8 ng/ml) or higher (>8 ng/ml). The distribution of VCA recipient and kidney recipient TTTL was calculated at follow-up intervals of 1–5 and >5 years within each cohort.

RESULTS: Immunosuppression data were available for 57 VCA recipients and 98 kidney recipients. There were no significant differences in prednisone doses between groups at all follow-up intervals. While the mean MMF dose of VCA recipients was significantly greater than that of kidney recipients at less than 1 year (1.71 ± 0.58 vs. 1.16 ± 0.55 gm/day; p=0.01), there was no significant difference at subsequent follow-up intervals. For VCA recipients, there was a significant difference (p=0.02) in TTTL distribution over the three predefined therapeutic ranges (4–6 ng/ml, 6–8 ng/ml, >8 ng/ml, respectively) between 1–5 years (24.0%, 20.0%, 56.0%, respectively) and greater than 5 years (28.6%, 42.9%, 28.6%). Kidney recipients showed no significant difference in TTTL distribution over the three defined therapeutic ranges (4–6 ng/ml, 6–8 ng/ml, and >8 ng/ml) between the follow-up intervals.

CONCLUSION: At longer follow-up, VCA and kidney recipients receive comparable MMF and prednisone doses. In addition, the majority of VCA recipients are treated with TTTL similar to kidney recipients after 5 years. These findings suggest that VCA recipients may not be subject to a higher level of dose-related immunosuppressive therapeutic risk than kidney recipients at longer follow-up. While the benefits of VCA have proven difficult to quantify, a detailed understanding of therapeutic risk may serve to enhance the informed consent process. Transparent outcome reports are warranted to determine safe and effective strategies for minimization of immunosuppression in VCA.

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