

## ***Pittsburgh Healthcare System University of Pittsburgh, Pittsburgh, PA***

**PURPOSE:** Globally, 39 million people suffer from blindness. Whole eye transplantation (WET) offers the opportunity to provide a viable optical system to recipients with irreversible vision loss. We have previously established a viable orthotropic model for vascularized whole eye transplant in the rat. The purpose of our study is to evaluate gross morphology, structural integrity and aqueous humor dynamics in a long-term WET survivor, as well to evaluate for any adverse effects of WET in the unoperated, contralateral eye.

**METHODS:** Syngeneic whole eye transplants were performed with Lewis rats. The donor flaps included ocular tissues anterior to the optic chiasm, eyelid and periorbital tissue, and external ear. The recipient site was prepared by removing a similar region of skin and ocular tissue, with optic nerve division at its exit from the globe, vascular anastomoses, and optic nerve coaptation. Optical coherence tomography (OCT), gadolinium-enhanced magnetic resonance imaging (Gd-enhanced MRI) and electroretinography (ERG) were performed to evaluate the viability and structural integrity of the eyes of the long-term WET survivor and compared with a naive, age-matched control. In a subsequent series of syngeneic transplants, we evaluated the unoperated, contralateral eye with OCT, slit lamp exam, funduscopy, and histology.

**RESULTS:** The long-term WET survivor and corresponding control animal were >400 days old at time of evaluation. Corneal opacification prohibited OCT imaging of the retina of the transplanted eye. OCT of the cornea and retina of the contralateral eye corresponded with the naive eyes of the control rat. Gd-enhanced MRI imaging revealed existing aqueous humor dynamics in the contralateral unoperated eye in the transplanted eye had a normal electrical response with ERG, which was similar to the control. In the transplanted eye, aqueous humor dynamics was compromised, and there was no evidence of electrical response with ERG analysis. As for our follow-up series of syngeneic transplants (n=6, sacrifice at 3mos), we continued to find no abnormalities in the contralateral eye with respect to OCT, slit lamp exam, funduscopy, and histology.

**CONCLUSION:** In this study, we demonstrate findings in a long-term survivor. We did not identify any adverse changes in structural integrity and function in the contralateral eye >400 days after WET in comparison to the age-matched control eyes. This preservation of the structure and function

of the contralateral eye was recapitulated in a subsequent series of animals.

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## **Delayed Tolerance Induction Protocol for Vascularized Composite Allografts in Non-Human Primates: The Immunomodulatory Effect of Donor Bone Marrow Transplantation Does Not Prevent the Development of Chronic Rejection in the Absence of Durable Mixed Chimerism**

***Alexandre G. Lellouch, MD, Zhi Yang Ng, MD, Ivy A. Rosales, MD, Robert B. Colvin, MD, Amon-Ra Gama, BS, Ilse M. Schol, BS, Luke Geoghegan, BS, Josef M. Kurtz, PhD, Curtis L. Cetrulo, Jr., MD***

***Massachusetts General Hospital, Boston, MA***

**PURPOSE:** Our laboratory is currently developing a delayed tolerance induction protocol for vascularized composite allografts (VCAs) in a clinically relevant non-human primate (NHP) model using donor bone marrow transplantation (DBMT) after VCA to induce durable mixed chimerism and tolerance. DBMT has a demonstrated immunomodulatory effect in our previous experimental work and in clinical VCA, enabling some patients to be maintained on low-dose tacrolimus monotherapy. With longer-term follow-up of clinical VCA, recent reports of chronic rejection have emerged. We sought to investigate in further detail the immunomodulatory effect of DBMT on acute and chronic rejection of VCAs in NHPs.

**METHODS:** Following VCA transplantation and maintenance on standard triple immunosuppression (IS) for 2 months, donor bone marrow cells that were previously harvested from the vertebrae, minimally processed and cryopreserved were thawed and infused into MHC-mismatched recipient NHPs (n=6) conditioned with irradiation (total body and thymic), T cell depletion, co-stimulatory blockade and anti-inflammation (with anti-IL-6 receptor monoclonal antibody). A bridging course of calcineurin inhibition (CNI) was given for 4 weeks before IS withdrawal and assessment of the VCA for tolerance. Observed rejection episodes of the VCA were biopsied and treated with CNI and a tapering

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.

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

**Bruce J. Swearingen, MD<sup>1</sup>, Scott S. Graves, PhD<sup>2</sup>, Rainer Storb, MD<sup>2</sup>, David W. Mathes, MD<sup>1</sup>**

**<sup>1</sup>University of Colorado School of Medicine, Aurora, CO, <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA**

**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.