

required to prevent allograft loss, and recent studies suggest that repeated AR episodes can lead to VCA dysfunction and loss<sup>2</sup>. The mechanisms underlying variability in AR presentation remain poorly defined however.

**MATERIALS AND METHODS:** 8 cynomolgus monkeys received either an orthotopic hand (n=2) or heterotopic face VCA (n=6) from MHC-mismatched donors following induction with anti-thymocyte globulin. Post-operatively, triple immunosuppression – tacrolimus, mycophenolate mofetil, methylprednisolone – was maintained for up to 120 days before bone marrow transplantation (BMT) was performed. Protocol biopsies of VCA skin were performed at 30-day intervals for histopathology and flow cytometric analysis of resident skin leukocyte populations; VCA-resident cells were differentiated by H38 status (mouse antihuman HLA class I monoclonal antibody that cross reacts with cynomolgus monkeys) for donor or recipient derivation. Clinical AR was treated with steroids and further biopsies were taken for histologic confirmation; corresponding anti-donor responses were evaluated by mixed lymphocyte reaction (MLR) and allo-antibody formation.

**RESULTS:** Up to three episodes of AR (from POD 14, Banff I to II) developed while recipient animals were maintained on triple immunosuppression. Corresponding flow cytometric analyses demonstrate > 80% of skin-resident T lymphocytes (CD4+, CD8+) within VCA dermis were of recipient origin, suggesting rapid immigration of various lineages into the VCA. These observations coincided with the first episode of AR in fully mismatched recipients but haplomatched animals remained rejection-free. All but one episode of AR were successfully treated. No allo-antibodies were detected and anti-donor responses by MLR were comparable to that against third-party. Following BMT, mixed chimerism was detected and enabled immunosuppression withdrawal. However, this was transient and once lost, clinical AR developed and nearly 100% of both dermal and epidermal lymphocytes were recipient-derived.

**CONCLUSION:** We report a clinically-relevant model for studying AR in VCA. Our results suggest that further understanding of the relative importance of MHC differences in transplant pairs may lead to differences in outcomes for VCA recipients maintained under standard immunosuppression. Immunosuppression-free tolerance of non-hematopoietic antigens in composite tissues can be achieved, but require additional strategies to achieve stable, rather than transient mixed chimerism following BMT.

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### Immunomodulation in Vascularized Composite Allotransplantation – Preliminary Results in a Non-human Primate Model with Tocilizumab

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**INTRODUCTION:** Tolerance in vascularized composite allotransplantation (VCA) remains elusive and patients are faced with a lifetime of immunosuppression and associated risks. Tocilizumab (anti-IL-6 receptor monoclonal antibody) is currently FDA approved for use in rheumatoid and idiopathic arthritis. It mitigates inflammation, reduces the incidence of GvHD, and is potentially protolerogenic. We investigated the utility of a short course of tocilizumab in a non-human primate model (NHP) of facial VCA to achieve prolonged survival and/or tolerance.

**MATERIALS AND METHODS:** VCAs were transplanted into MHC-mismatched NHPs (n=4) after induction with anti-thymocyte globulin. Post-operative maintenance consisted of triple immunosuppression (FK506, methylprednisolone, MMF) before further conditioning (irradiation, lymphocyte depletion) in preparation for costimulatory blockade-based donor bone marrow transplantation (DBMT) on POD 60. Tocilizumab was administered on the day of DBMT, and at weekly intervals thereafter for

a total of 5 doses. Post-DBMT, the recipient was maintained on a tapering course of cyclosporine before complete withdrawal 28 days later. VCAs were assessed by serial clinical assessment and histopathology. Mixed chimerism in peripheral blood was monitored by flow cytometry and *in vitro* immunologic responses were assessed through mixed lymphocyte reaction (MLR) assays.

**RESULTS:** Two recipients were euthanized within 2 weeks of DBMT due to neutropenic sepsis and post-transplant lymphoproliferative disorder but both VCAs remained viable up to experimental endpoint. M4515 (full MHC-mismatched recipient) has been off of all immunosuppression for 3 weeks without any evidence of rejection. M3815 (haplomatched) developed mixed chimerism transiently at 6 weeks after DBMT and corresponding MLR assays demonstrated decreased anti-donor responses; immunosuppression was then successfully withdrawn for a total of 5 weeks before rejection developed. Although the rejection episode could be reversed with steroid bolus and a tapering course of FK506, recurrence occurred after another 2 weeks off immunosuppression.

**CONCLUSION:** As with the clinical experience with tocilizumab, vigilant monitoring is required following drug administration due to increased susceptibility to neutropenia and infections<sup>1</sup>. Tocilizumab appears to promote engraftment after DBMT to allow short-medium term immunosuppression-free VCA survival across haplomatched barriers in this NHP model. Continued follow-up is required to determine if similar results can be achieved across a full MHC mismatch. Further studies in our laboratory are focused on optimizing the current protocol to achieve stable engraftment and durable mixed chimerism for tolerance of VCA.

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## Ex-vivo Subnormothermic Oxygenated Machine Perfusion of Swine Forelimbs Enables Prolonged Graft Preservation Prior to Transplantation

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**BACKGROUND:** The current gold standard in tissue preservation is static cold storage (SCS) on ice-cold (0–4°C) UW solution. While SCS slows down graft deterioration, it does not have restorative capabilities. We previously developed an ex-vivo perfusion system for subnormothermic oxygenated machine perfusion (SNMP) to resuscitate cadaveric organs. Recovered livers were perfused for 3 hours and transplanted successfully into recipient rats in 5/6 cases<sup>1</sup>; when scaled up to DCD human livers, SNMP demonstrated sustained and enhanced viability of liver grafts. To expand the donor pool in VCA, we investigated the utility of SNMP on preservation time and resuscitation of ischemic limbs in a swine model.

**METHODS:** 2 porcine forelimbs were procured and flushed with ice-cold UW on the back table through the cannulated axillary artery and veins. Warm ischemia was 45 mins and SCS was 2 hours. Before starting SNMP, the forelimbs were flushed with 1500mL of cold Lactated Ringers. During SNMP (3 hours), the amputated forelimbs were perfused by a pressure-controlled system through the axillary artery. The perfusion solution consisted of William's E medium, which was enriched with dexamethasone, insulin and heparin. A venous outflow was prepared for sample collection. Hemodynamics of the limbs was monitored by evaluation of arterial flow and vascular resistance. Perfusion samples were collected at 30 min intervals for biochemical analysis. Lactate clearance was monitored as a marker of muscle injury. Muscle biopsies were collected at 60 min intervals for measurement of ATP production.

**RESULTS:** Arterial outflow and vascular resistance remained stable throughout the perfusion, between 270 and 320 mL/min and 0.23 and 0.26 mmHg/mL/min, respectively. Despite the initial increase in lactate levels from 0.2 mmol/L to > 6 mmol/L, this value remained stable during the final hour of perfusion. The increase in ATP production reflects successful resuscitation of the forelimb, increasing from a baseline of 5500 before perfusion to 7500 nmol/g protein during SNMP.