

Experimental/VCA  
Monday January 26, 2015  
7:00am - 8:30am  
Grand Ballroom AB

**7:00 AM - 7:04 AM**

Lentivirally-delivered gene therapies protect against the late adverse effects of radiotherapy in free flaps

The Institute of Cancer Research & The Royal Marsden Hospital, London, , United Kingdom  
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**INTRODUCTION AND AIMS**

Adjuvant radiotherapy can be harmful to autologous, free flaps, leading to late adverse effects (LAEs) characterized by fat necrosis, volume loss and contracture that often require salvage reconstruction. This study aims to radioprotect free flaps using a virally-delivered gene therapy strategy, without compromising oncological efficacy.

**MATERIAL AND METHODS**

Using a rodent, superficial inferior epigastric artery (SIEA) free flap irradiation model, we characterized the LAE phenotype that developed after irradiation with 50 Gy/3 fractions. Flap outcomes were measured using clinical, imaging, histological and molecular end-points. LAE severity was scored using the Radiation Therapy Oncology Group (RTOG) adverse effects scoring system.

Separate lentiviral vectors encoding the superoxide dismutase 2 (LVSOD2) gene and a small hairpin RNA (shRNA) against connective tissue growth factor (CTGF) (LVshCTGF) were generated. Vectors were used to infect SIEA flaps *ex vivo* prior to irradiation (50 Gy/3 fx) 1 month post-operatively. Flap outcomes post-irradiation were quantified using the same endpoints described previously.

A tumor recurrence model was developed to investigate the oncological safety of free flap radioprotection. After flap infection with vector, syngeneic breast cancer cells were engrafted directly into the flap. Established tumors were irradiated (20 Gy/5 fractions) and tumor volume measurements performed.

## **RESULTS**

Irradiated free flaps developed a LAE phenotype characterized by significant skin paddle contracture ( $p < 0.05$ ), volume loss ( $p < 0.001$ ), fat necrosis ( $p < 0.05$ ), fibrosis ( $p < 0.05$ ), SOD2 depletion and CTGF over-expression compared to non-irradiated flaps.

LVSOD2-infected flaps irradiated with 50 Gy/3fx showed significantly less skin paddle contracture ( $p < 0.05$ ), volume loss ( $p < 0.05$ ) and acute/late toxicity severity ( $p < 0.05$ ) compared to control flaps irradiated with 50 Gy/3 fx. .

Flaps infected with LVshCTGF did not experience a reduction in acute toxicities but did exhibit a significant improvement in skin paddle surface area ( $p < 0.05$ ), (but not volume loss) compared to LVSOD2 (relative skin paddle surface area: LVSOD2 = 48% v. LVshCTGF = 71%).

Tumor recurrence experiments demonstrated that tumors growing in LVSOD2-infected flaps responded better to radiotherapy compared to those growing in PBS-sham flaps (median survival: no RT (PBS flap) = 10 days, 20 Gy/5 fx (PBS flap) = 27 days, 20 Gy/5 fx (LVSOD flap) = n/a;  $p = 0.0005$ ).

## **CONCLUSION(S)**

LVSOD2 and LVshCTGF appear to have different radioprotective qualities. LVSOD2 resulted in better retention of flap volume post-irradiation, whereas, LVshCTGF appeared to selectively protect against cutaneous LAEs. Flap radioprotection did not result in the radioprotection of tumor recurrences.

### **7:04 AM - 7:08 AM**

The Stromal Vascular Fraction of Autologous Fat Graft Induces Proliferation of Mammary Progenitor Cells in Healthy and Cancer-Containing Breast Tissue

Imran Ratanshi, Winnipeg, MB, Canada

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**PURPOSE:** Autologous fat grafting is widely used for managing contour asymmetries in breast reconstruction. Adipose tissue contains a rich population of both multipotent mesenchymal stem cells and vascular progenitor cells (collectively referred to as adipose-derived stem cells, or ASCs). In preparing lipoaspirate for grafting, centrifugation results in three distinct layers: (i) an oily supernate, (ii) a middle fat layer, and (iii) an infranate layer. This infranate layer of lipoaspirate is referred to as the stromal vascular fraction (SVF) and contains enriched

populations of ASCs. Despite widespread clinical use, interactions between the SVF of lipoaspirate and breast parenchyma remain poorly understood. Our study investigated the potential proliferative effect of SVF on (i) healthy breast tissue and (ii) cancer-adjacent breast tissue.

**MATERIALS AND METHODS:** Healthy breast tissue and abdominal lipoaspirate were obtained from patients undergoing elective reduction mammoplasty and liposuction. Cancer-adjacent breast tissue (defined as breast tissue greater than 3cm from the primary breast cancer) and lipoaspirate were obtained from patients undergoing mastectomy and abdominally based primary free flap reconstruction. Samples of SVF and breast parenchymal cells were then isolated from both sources using established cell digestion protocols. The presence of ASCs within the SVF was identified using established multi-lineage differentiation, colony-forming unit-fibroblast (CFU-f), and cell surface marker assays. Lastly, SVF cells were co-cultured with (i) healthy breast tissue cells and (ii) cancer-adjacent breast tissue cells using a three-dimensional matrix called Matrigel™. Control cultures consisted of breast cells from (i) healthy breast tissue and (ii) cancer-adjacent breast tissue in the absence of SVF. After 14 days, the total cell numbers and mammary progenitor cell populations from each culture group were quantified using colony forming cell (CFC) assays.

**RESULTS:** Differentiation, CFU-f, and surface marker assays demonstrated the presence of ASCs in all SVF samples. Co-cultures of cancer-adjacent breast cells with SVF showed a 9-fold expansion of breast progenitor cells (control = 3-fold) compared to a 5.5-fold expansion in co-cultures of healthy breast cells (control = 2-fold) with SVF based on CFC assays.

**CONCLUSIONS:** Our study demonstrates a marked proliferative effect of SVF on cancer-adjacent breast cells compared to cells derived from healthy breast tissue. This may mimic the clinical scenario of SVF-enriched fat grafting in patients who have undergone a lumpectomy for breast cancer treatment.

#### **7:08 AM - 7:12 AM**

Vascularized, Tissue-Engineered Skin Equivalents With and Without Dermal Appendages  
Weill Cornell Medical College, New York, NY, USA

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**Introduction:** Reconstruction of large areas of skin loss is challenging, especially with limited sites for autologous tissue harvest. Despite widespread use, contemporary xenografts, allografts and autografts are avascular and rely upon non-guided host cellular invasion for neovascularization and incorporation. This takes several weeks, especially in patients with significant co-morbidities. Furthermore, none of these products have dermal appendages. Here, we fabricate full-thickness skin equivalents with a hierarchical vascular supply consisting of an interweaving network of microvessels in circuit with macrovessels, also with and without dermal appendages.

**Methods:** “U-shaped,” 1.5 mm diameter Pluronic F127 macrofibers, bridged by dense, 3D networks of 100-500  $\mu\text{m}$  Pluronic F127 microfibers were sacrificed in type I collagen with encapsulated human foreskin fibroblasts (HFF1) at a density of  $1 \times 10^6$  cells/mL. Twenty-four hours following fiber sacrifice,  $5 \times 10^6$  cells/mL of human aortic smooth muscle cells (HASMC) were seeded into the patent intraluminal space. The following day,  $5 \times 10^6$  cells/mL of human umbilical vein endothelial cells (HUVEC) were seeded. Lastly,  $1 \times 10^6$  cells/mL of human epidermal keratinocytes (HEK) were topically seeded onto scaffolds. Hair follicles from adult rats were implanted into the surface of a subset of scaffolds. Scaffolds underwent daily media changes and were fixed and processed for histology after 7 or 14 days of culture.

**Results:** Macrochannels were successfully lined with HUVEC and HASMC, generating anatomically appropriate neointimal and neomedial layers by day 7 and maintained by day 14. Proliferation of HFF1 was evident after 7 days and increased after 14 days. HEK proliferated and increased in thickness in a time-dependent manner, leading to the formation of a stratified “epidermal-like” layer along the construct surface. Immunohistochemical analysis revealed  $\text{CD31}^+$  HUVEC along the luminal surface of the macrochannel and the one cell layer thick microvessel linings, fibroblast specific-1-expressing fibroblasts within the collagen bulk, and involucrin-expressing keratinocytes along the scaffold surface after both 7 and 14 days. Scaffolds with implanted rat hair follicles demonstrated appendages with intact inner root sheaths and hair shafts, with surrounding viable cells by day 7.

**Conclusions:** We successfully fabricated vascularized tissue-engineered skin equivalents with and without hair follicles. We successfully incorporated an inherent vascular network that recapitulates the hierarchical organization of an arteriole, venule, and capillary bed that is suitable for microanastomosis and immediate perfusion. With a built-in vascular network, vital epidermal (HEK) and dermal (HFF, collagen) components, and dermal appendages, these constructs hold tremendous promise for the future of tissue-engineered, full-thickness skin equivalents.

**7:12 AM - 7:18 AM**

### **Discussion**

**7:18 AM - 7:22 AM**

Periosteal Prefabrication of Pre-vascularized Artificial Bone Scaffolds

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**Background:** Over the last decades, bone tissue engineering with tailor-made scaffolds fit to defect dimensions developed into a promising alternative to overcome the drawbacks of autologous bone grafts. Still, early neovascularization, crucial for successful implantation, presents the major challenge of these constructs.

**Purpose:** The aim of this study was to evaluate the neovascularization potential and bone forming capacity of an autologous artificial space (bioreactor) between the medial femur condyle and its periosteum that facilitates the design of a defined vascular pedicle flap (descending genicular vessels and superomedial genicular vessels) on bioresorbable scaffold structures.

**Material and Methods:** In four skeletally mature swiss alpine sheep the bioreactor, composed of a PEEK construct (side walls), a vascular pedicle flap (roof) and the deperiosteated compacta (floor), was locked into position by two 2.4 standard screws. The sheep were separated in two groups: In one group (n=2) the created rectangular space was filled with a bioresorbable  $\beta$ -tricalcium phosphate scaffold, whereas the bioreactor of the other group (n=2) was left empty (negative control).  $\mu$ CT scans were performed immediately and 12 weeks postoperatively. Fluorochrome labeling was performed four (Calcein Green) and eight (Xylenol Orange) weeks postoperatively to document new bone formation. Animals were euthanized 12 weeks postoperatively and surgery sites are examined histologically (Giemsa Eosin, pre-mortal intravascular injection of Indian Ink to visualize vessels).

**Results:** Fluorochrome Labelling displayed moderate early bone formation after 4 weeks with already in-growth into the bioreactor. After 8 weeks pronounced new bone formation into the bioreactor could be shown. After 12 weeks Eosin-Giemsa staining showed degradation and bony replacement of the scaffold material, bone in-growth into scaffold voids and extended neovascularization into the scaffold originating from the periosteal flap.  $\mu$ CT data showed that after 12 weeks of implantation 45% of the ROI contains new bone like tissue composed of new bone and ossified scaffold material. The overall volume increase of bone like tissue in the ROI is +9% and the calcification level increases by 30%.

**Conclusion:** Results indicate that the bioreactor concept strongly promotes new bone formation, ossification and extended vascularization of the scaffold material. The newly formed bone is highly calcified and thus of ideal quality for transplantation purposes.

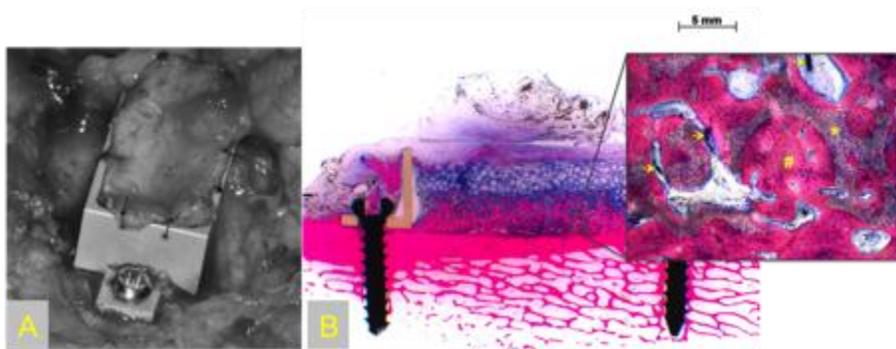


Figure 1: A) Bone box with periosteal flap and B) histological image after 12 weeks of implantation showing new bone formation (\*bone in-growth, #bony degradation & scaffold replacement) and neovascularization (arrows).

## 7:22 AM - 7:26 AM

A Novel, In-Vitro, 3D, vascularized Platform to Study the Role of Pericyte Function in Large Vessel Vasculogenesis

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**Introduction:** Mesenchymal in origin, pericytes are thought to have the ability to differentiate into smooth muscle cells. In previous work we demonstrated that human aortic smooth muscle cells (HASMC) co-cultured with human umbilical vein endothelial cells (HUVEC) within microchannels resulted in the formation of anatomically correct neointimal and neomedial layers. Here, we co-culture fibroblasts, pericytes and endothelial cells within microchannels to observe the spatial relationship among the cells as well the ability of endothelial cells to mediate pericyte to vascular smooth muscle cell transition, forming a medial layer in this “large vessel” model.

**Methods:** “U-shaped” Pluronic F127 fibers, 1.5 mm in diameter, were sacrificed in type I collagen. Twenty four hours following fiber sacrifice,  $5 \times 10^6$  cells/mL of human foreskin fibroblasts (HFF) and  $5 \times 10^5$  cells/mL of human placental pericytes (HPLP) were seeded into the microchannel. The following day,  $5 \times 10^6$  cells/mL of HUVEC were seeded. For comparison, a subset of microchannels was seeded with  $5 \times 10^6$  cells/mL of HASMC and  $5 \times 10^6$  cells/mL of HUVEC, 24 hours apart. All constructs underwent daily media changes and were fixed and processed for histology after 7 and 14 days of culture.

**Results:** Microchannels seeded with HFF/HPLP/HUVEC formed an intact endoluminal lining with increasing thickness over time.  $\alpha$ -SMA- and desmin-expressing HPLP were interspersed throughout the endoluminal lining. CD31 and vWF expressing endothelial cells were noted along the luminal surface after 7 days and throughout the endoluminal lining after 14 days. Adherens junctions among the HUVEC were present along the luminal surface in close proximity to HPLP, contributing to vessel wall stability. Collagen IV deposition within the lining increased in a time-dependent manner.

**Conclusions:** Here, we demonstrate that the co-culture of pericytes and endothelial cells in a large vessel *in-vitro* model promotes the formation of an intact endoluminal lining, perhaps related to endothelial cell secretion of paracrine factors that promote a pericyte to vascular smooth muscle cell transition or perhaps through maintenance of the pericyte phenotype itself. As such, the role of the pericyte may be expanded from association with capillary-sized vessels to include association with arterioles and larger.

## 7:26 AM - 7:30 AM

### Discussion

### **7:30 AM - 7:34 AM**

Recipient autologous adipose-derived stem cells prolonged allotransplant survival in a miniature swine hind-limb model

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Purpose: Vascularized composite allo-transplantation (VCA) has tremendous potential application for reconstructive surgery. In this study, we investigated whether the recipient adipose-derived stem cells (ASCs) in addition to irradiation and short-term immunosuppressant (FK-506) as an immunosuppressant could prolong allotransplant survival by a swine hind-limb VCA model. We also trace the engraftment of recipient cells into allotransplant tissue.

Materials and Methods: The outbred miniature swine underwent heterotopic hind-limb transplant as a VCA model (female swine as donor and male swine as recipient). Group 1: VCA without treatment as a control group. Group 2: VCA followed by recipient autologous ASC infusions (given on days 0, +7, +14, +21). Group 3: VCA with Tacrolimus (FK-506) (day 0~+28 days). Group 4: VCA combined with irradiation, FK-506 (day 0~+28), and autologous ASC infusions (day 0, +1, +7, +14, +21). Tissue samples were biopsied, and flow cytometry was performed to quantify T cell populations. ELISAs were used to measure the levels of TGF- $\beta$ , IL-10, and IFN- $\gamma$ .

Results: Result revealed multiple injection of recipient ASCs combined with irradiation and FK506 could significantly prolong allo-transplanted hind-limb survival. Histological examination of the ASC-IR-FK506 group displayed the lowest degree of rejection in allo-skin and muscle tissues. The percentage of CD4+/CD25+/foxp3+ regulatory T-cells in the circulating blood revealed significant increase in the ASC-IR-FK506 group at 4 weeks post-transplant compared to the other groups. Analysis of recipient peripheral blood serum revealed that TGF- $\beta$ 1 was not apparently increased at 2 weeks in animals treated with ASCs-FK506-I/R group, as compared to that in controls. However, the TGF- $\beta$ 1 was significantly increased at 15 weeks post-transplant. The PCR analysis of donor tissues showed Y-chromosome positive expression cells (recipient cells) apparently existed in donor allo-skin and allo-muscular tissues. This indicated recipient cells could engraft and replace donor cells in allotransplant tissue.

Conclusion: Recipient ASCs in addition to irradiation and short-term FK-506 prolong allotransplant of swine hind-limb survival. The recipient cells could exist in allotransplant tissue.

### **7:34 AM - 7:38 AM**

Eyelid Transplantation: Lessons from a Total Face Transplant and the Importance of Blink  
Michael Sosin, Baltimore, MD, USA

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Trauma Center, (3)Johns Hopkins Hospital, (4)North Shore – Long Island Jewish Hospital System, (5)Johns Hopkins Bayview Medical Center

## **Purpose**

Despite inclusion of periorbital structures in facial transplants, critical assessment of post-transplant short and long-term periorbital function has not been reported. The purpose of this manuscript is to report recovery of ocular and periorbital functional with critical appraisal of post-transplant blink in the setting of revisional surgery.

## **Methodology/Design**

Prospective ocular and periorbital functional assessments were completed at multiple time points in a patient undergoing facial transplantation and subsequent revision surgeries. Function was evaluated using clinical ocular examinations, visual acuity assessments, photography, and video at various intervals from preoperative baseline to 13.5 months post-transplant. During this period, revisional surgeries involving periorbital structures were performed at 6 and 9-months post-transplant.

## **Results**

Pre-transplant, volitional blink was 100% in both eyes. Involuntary blink was 40% on the right and 90% on the left, with occasional full closure. Following face transplantation, voluntary blink was preserved (Figure 1), partial skin sensation was present, and involuntary blink improved to 70% in the right eye and 100% in the left eye. Following revision surgery, visual acuity, voluntary, and involuntary blink were impaired. By 7.5-months post-revision, improvement comparable to the pre-transplantation assessment was observed (Table 1 and Video 1).

## **Conclusion**

Adherence to principles of blink preservation is critical in periorbital transplantation. Involuntary blink is essential to preserving vision, and can be improved post-transplantation. Revisional surgery may temporarily impair advances made with initial allotransplantation. A comprehensive understanding of ocular biomechanics and function is invaluable to the reconstructive surgeon performing facial transplantation involving periorbital structures, and post-transplant revision surgeries.

Figure 1. Volitional blink (a) pre transplant, (b) 5-months posttransplant, and (c) 13.5-months following face transplant surgery (7.5-months following the first revision surgery).

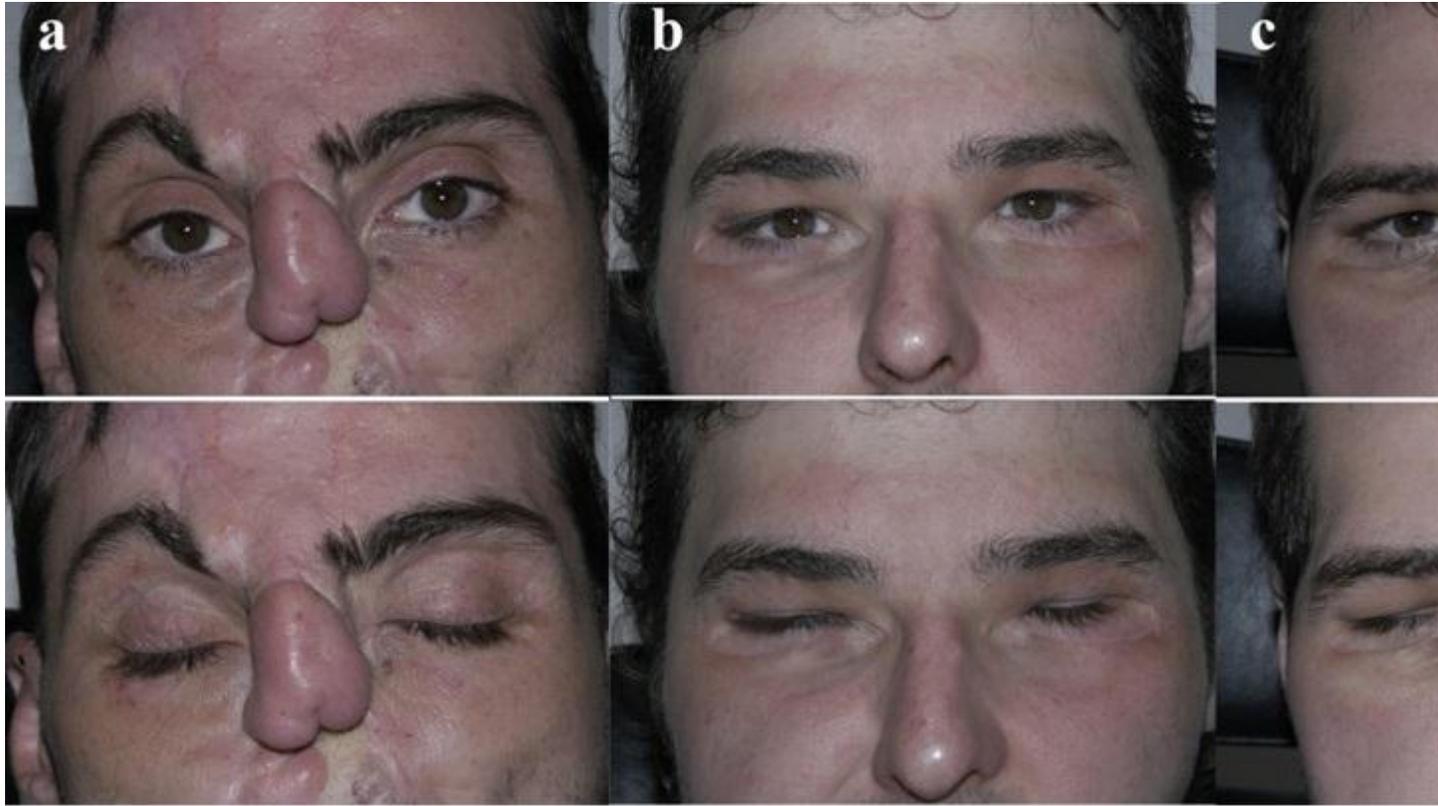


Table 1. Critical ophthalmologic and periorbital assessment at 4 distinct time points.

	Preoperative		6-Months Post Facial VCA		1-Week Post Initial Revision		7.5-Months Post Initial Revision/ 4.5 Months Post 2 <sup>nd</sup> Revision	
	R	L	R	L	R	L	R	L
<b>Visual Acuity</b>	20/80*	20/25	20/80*	20/25	20/200*	20/50	20/80*	20/25
<b>Pupillary Assessment</b>	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
<b>Cornea</b>	Normal	Normal	Normal	Normal	Surface Irregularity	Normal	Normal	Normal
<b>Extraocular Movements</b>	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact
<b>Globe Position</b>	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate
<b>Lateral Canthal Position</b>	Adequate	Adequate	Low	Low	Adequate	Adequate	Adequate	Adequate
<b>Periorbital Soft Tissue</b>	Normal	Normal	Excess Increased Laxity	Excess Increased Laxity	Edematous, Increased Tension	Edematous, Increased Tension	Normal	Normal
<b>Voluntary Blink (lid contact)</b>	Complete	Complete	Complete	Complete	2mm Lagophthalmos	Complete	Complete	Complete
<b>Involuntary Blink (lid contact)</b>	40%	90%	70%-90%	90-100%	10%	60%	40%	90-100%
<b>Periorbital Skin Sensation</b>	Present	Present	Present up to brow, 5mm below lower lid	Present up to brow, 5mm below lower lid	Mild sensory detection above brow and 10mm below lid	Mild sensory detection above brow and 10mm below lid	Sensation distribution near complete in V1, V2 distribution	Sensation distribution near complete in V1, V2 distribution
<b>Lacrimal System</b>	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

\*paracentral scotoma

Video: (available for presentation)

(Part 1) Blink reflex assessment, slowed to capture involuntary blink 6-months post transplant and 1-week prior to first revision surgery. Blink is approximately 90% in the patient's right eye and 100% in the left eye.

(Part 2) Blink reflex assessment, slowed to capture involuntary blink 1-week following initial revision surgery (Le Fort III advancement). Findings demonstrate impaired blink reflex bilaterally, approximately 10% in the right eye and 60% in the left eye.

(Part 3) Blink reflex assessment, slowed to capture involuntary blink 7.5-months following initial revision surgery (13.5-months following the face transplant). Marked improvement from the previous video, showing 40% of blink in the right eye and 90% in the left eye.

### 7:38 AM - 7:42 AM

Skin-specific Immunobiology of Vascularized Composite Allografts in Tolerance and Acute Rejection

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### Purpose

We have previously reported induction of vascularized composite allograft (VCA) tolerance across major histocompatibility (MHC) barriers by establishment of durable hematopoietic mixed chimerism in MGH Miniature Swine. Tissue-specific mechanisms appear to play a critical role in VCA outcome. The purpose of this study was to characterize the skin immune system in VCA tolerance and acute rejection.

## **Methods**

Two MGH miniature swine underwent VCA and chimerism-mediated tolerance induction. Control animals (n=2) received VCA alone. VCA and host skin biopsies were performed regularly from day 14 to 250 for chimeric recipients, and on alternate days from transplant until complete rejection in controls. Biopsies were incubated in Dispase II to separate epidermis from dermis, then further digested in trypsin and collagenase D respectively. For FACS, dermal and epidermal cell suspensions were analyzed for lineage (CD3, CD4, CD8, g/d T cells, MHC Class 2, Langerin) and donor/host hematopoietic origin. Langerhans cells were FACS sorted and plated as stimulators to peripheral blood leukocyte responders in mixed lymphocyte reaction (MLR).

## **Results**

In mixed chimeras, host-derived T cells were identified in VCA dermis two weeks post-transplant (CD4+ 20-30%, CD8+ 5-6%, g/d 20-60%) and host-derived Langerhans' cells in VCA epidermis (5-15%). No signs of rejection were observed. Infiltration of host dermis with donor T cells was also observed but donor-derived Langerhans' cells were not detected in host epidermis until day 150. Over time, skin chimerism levels equilibrated with peripheral blood. In controls, donor-derived T cells and Langerhans' cells in the VCA were rapidly replaced with recipient cells as rejection progressed. Donor-derived cells reached the limited of detection by day 8, and rejection was complete by day 8. In the absence of mixed chimerism, neither donor-derived T cells nor Langerhans' cells were identified in the host skin of control animals at any point.

In MLR, Langerhans cells were potent stimulators of naïve donor-type, host-type and 3<sup>rd</sup> party responders. However, no proliferation was observed in responders from the chimeric VCA recipient, demonstrating tolerance of both host- and donor-derived lymphocytes in these animals.

## **Conclusions**

We have demonstrated the establishment of T cell and Langerhans' cell chimerism in tolerant VCAs. The infiltration of host-type T cells without evidence of rejection suggests that tolerance is induced rapidly in this model. The establishment of durable cutaneous chimerism in tolerant VCAs is consistent with functional integration of donor and recipient derived T cells and Langerhans' cells and maintenance of homeostasis in the skin immune system.

**7:42 AM - 7:48 AM**

## **Discussion**

**7:48 AM - 7:52 AM**

Effects of thymus on donor-specific chimerism: Comparison of osseomyocutaneous sternum composite tissue allotransplantation model with and without thymus

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Donor-specific chimerism is believed to provide donor specific tolerance of allograft, so many studies aim to increase chimerism levels following allotransplantation. Thymus plays a major role in development of self-tolerance and have a crucial role for induction of acquired tolerance to exogenous antigens. However, the role of thymus on donor-specific chimerism following vascularized bone marrow transplantation is still unclear.

The aim of this study is to compare the effects of thymus on donor-specific chimerism by the application of osseomyocutaneous sternum composite tissue allotransplantation models with and without thymus transplantation.

## **Materials and Methods**

Five composite osseomyocutaneous (sternum, pectoralis muscles and skin) (group 1) and five osseomyothymocutaneous (sternum, thymus, pectoralis muscles and skin) (group 2) heterotopic allotransplantations were performed between 10 Lewis-Brown Norway (LBN, RT1<sup>l+n</sup>) donors and 10 Lewis rats (RT1<sup>l</sup>) recipients. Immunosuppressive treatment with cyclosporine A was used during the study.

Standard two-color flow cytometry was used to evaluate the presence of donor-specific chimerism for MHC class I (RT1<sup>n</sup>) antigen in the peripheral blood of Lewis rat recipients during follow-up period at day 7, 21, 63, 100, and the end point of the experiment. PCR analyses of donor-specific chimerism in lymphoid organs (lymph nodes, skin, spleen and thymus) were performed at the end of the study.

## **Results**

During follow-up period significant increase in CD4 positive T cell population of was observed in Group 1 and Group 2, however chimerism level in Group 2 (2.52% for RT1<sup>n</sup>/CD4 and 1.18% for RT1<sup>n</sup>/CD8) was almost two times higher than Group 1 (0.9% for RT1<sup>n</sup>/CD4 and 0.65% for RT1<sup>n</sup>/CD8).

Mean chimerism level of B lymphocyte population and monocyte/granulocyte/ dendritic cells subsets were detected at the level below 1% in both Group 1 and Group 2.

Chimerism was also confirmed with PCR in liver and spleen in all animals in Group 2. In Group 1 only one animal had LBN-derived DNA in the liver, two in the spleen. Only one animal in each of the groups showed chimerism in the recipient rat thymus.

## Conclusions

Our results showed that inclusion of thymus causes a significant increase in chimerism levels following vascularized composite allotransplantation. This study indicates a potential use of thymus transplantation for induction of stable and high level mixed hematopoietic chimerism and subsequent donor specific tolerance.

## 7:52 AM - 7:56 AM

### Dynamic Skeletal Changes of an Osteomyocutaneous Facial Allograft Five Years Following Transplantation

Cleveland Clinic, Cleveland, OH, USA

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**Background-**More than 30 face transplantations have been performed worldwide, most including part of the facial skeletal framework. The aim of this study was to evaluate if the skeletal component of a facial allograft undergoes changes following transplantation under the modified circulatory pattern and effects of the immunosuppressive regimen.

**Materials and Methods-**Pre and postoperative CT scans of the facial bones, CT angiogram (CTA) of the neck vessels and bone mineral densitometry (BMD) were evaluated. The pre and postoperative CT images were overlapped to assess skeletal changes and the changes were expressed both in a numeric and color-coded scale (Medical Modeling 3D Systems). The values of the serum calcium, phosphate, vitamin D, alkaline Phosphatase, thyroid and parathyroid hormones, TSH, FSH, LH, estradiol, total protein and albumin, serum creatinine and creatine clearance were reviewed.

**Results-**At 5 years follow up the patient was 51 year-old, clinically asymptomatic and presented good stability of the Le Fort III skeletal component of the facial allograft. Five years CT images revealed fibrous union of all of skeletal fixation sites except the right zygomatic arch. There was increased bone resorption at the osteotomy sites, left infraorbital rim and left maxillary buttress and anterior maxilla. Patchy areas of bone deposition were detected at the level of septum and alveolar bones. CTA showed segmental absence at the origin of the left external carotid artery, good opacification of the rest of the external carotid arteries and its branches likely due to retrograde flow and attenuated origin of the left lingual artery with good distal opacification. BMD evidenced osteopenia of the spine. The patient presented mild hypoalbuminemia (3.4 g/dL) and perimenopausal hormonal levels. All of the remaining laboratory values were within normal limits.

**Conclusions-**This is the longest follow-up reported for a facial allograft with an important bony component. Despite the patient presented multiple risk factors for bone resorption, facial allograft osteopenia was only discovered at the level of the left infraorbital rim and anterior maxilla. These findings could be explained with the occlusion of the left external carotid system and retrograde revascularization. Bilateral arterial repair is recommended in the event of full-face allotransplantation in order to maximize the normal physiology of the skeletal component of the allograft.

### **7:56 AM - 8:00 AM**

Presensitization of the VCA Donor with Bone Marrow Transplant of the Recipient Origin Facilitates Chimerism Induction and Extends Vascularized Composite Allograft Survival  
Cleveland Clinic, Cleveland, IL, USA

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**Introduction:** One of the successfully tested approaches is conditioning of the recipient with donor bone marrow cells (BMC) at the time of vascularized composite allotransplantation (VCA). Rodent models confirmed that infusion of donor-derived BMC at the time of allotransplantation induces donor-specific tolerance via establishment of mixed hematopoietic chimerism. We propose a new approach for induction of tolerance via conditioning of the VCA donor with BMC transplant of the recipient origin.

**Methods:** The ACI donors of the VCA (groin flaps) were conditioned with  $80 \times 10^6$  of PKH-26 pre-stained Lewis (recipient) BMC at 24 and 72hours prior to VCA transplantation. A total of 50 VCA were performed between ACI donors and Lewis recipients. Animals were divided into: groups I and II- donors were preconditioned with the recipient BMC at 24 or 72hours and was followed by VCA under 7 day protocol of anti- $\alpha\beta$ -TCR/Cyclosporine A. In groups III and IV, following donor presensitization at 24 or 72hours, the VCA was transplanted to the Lewis recipients without immunosuppression. In group V, VCA was performed without donor conditioning. In group VI, VCA was performed without donor preconditioning and without immunosuppression of the recipient. Engraftment of recipient-origin cells into different tissue compartments of the donor (Flow cytometry, PCR, immunofluorescence), induction of donor-specific chimerism and tolerance and VCA survival were assessed. .

**Results:** Migratory pathways of Lewis BM PKH-26+ cells into different ACI lymphoid and non-lymphoid organs were confirmed by PCR and immunofluorescent analysis. Lewis-derived BMC were detected in the peripheral blood, lymph nodes, spleen, thymus, and liver of the ACI donor at 24 and 72 hours following BMC transplantation. Lewis BMC were detected in ACI thymus at 72 hours after BMC infusion. Skin and lungs were negative for presence of BMC of Lewis origin at both time points.

Groups III, IV and VI rejected VCA transplants, at an average of 8, 14 and 10 days. In groups I, II and V, the mean survival time was 80, 64 and 30 days respectively. In groups I and II, donor-specific chimerism assessed in the peripheral blood, decreased from 8.8% and 11.4%, on day 7, to 3.7% and 4.7% when the VCA flaps manifested grade-3 rejection.

**Conclusions:** Donor preconditioning with BMC of recipient origin is a novel approach, which results in development of both the recipient and donor-specific chimerism, leading to extension of the VCA survival without side effects of immunosuppression or myeloablation and without risk of GVHD development.

**8:00 AM - 8:06 AM**

**Discussion**

**8:06 AM - 8:10 AM**

The Vascularized Bone Marrow Is Temporarily Important for Achieving Donor-Specific Tolerance via Engraftment in Immediate VCA

Chang Gung Memorial Hospital & Chang Gung University, Taipei, , Taiwan

Nidal F. AL Deek, MSc, MD; Hui-Yun Cheng, PhD; Chih-Jen Wen, PhD; Cheng-Hung Lin, MD; Ling-Yi Shih, MSc; Fu-Chan Wei, MD, FACS; Chang Gung Memorial Hospital & Chang Gung Medical College & University

**Introduction:** Induction and maintenance of donor-specific tolerance without myeloablative conditioning and long-term immunosuppression was a challenge to vascularized composite allotransplantation (VCA). Few animal trials have ever investigated the success of immediate VCA without cytoreductive and myeloablative therapies.

We hypothesized that vascularized bone marrow (VBM) of VCA may induce donor-specific tolerance under short-duration immunosuppressant treatment.

**Materials and Methods:** Wild type and luciferase transgenic Lewis rats were used as VCA donors while Brown-Norway (BN) rats were used as VCA recipients. A novel model comprised of combined groin flap with whole femur osteomyocutaneous flap was transplanted under the following regiment: Antilymphocyte serum (ALS) at day -3, +1; cyclosporine (CsA 16mg/kg) at day 0-7; and rapamycin at day 8-28. *The femur bone in VCA was removed from tolerant group at day 90 for some recipients with survived VCA.* Different hematopoietic cell lineages in recipients' peripheral blood were assessed by flow cytometry. IVIS bioluminescence imaging system was used to non-invasively assess engraftment and trafficking of donor cells with luciferase expression. Secondary Lewis and F344 antigen challenge in the form of skin grafting was performed on BN recipients with long-term survived VCA .

**Results:** Overall graft survival rate, with rejection-free, was 71.4 %. No fibrosis or rejection in the bony component of the flap in all tolerant animals was detected at day 90. All tolerant animals demonstrated donor-specific tolerance with acceptance of Lewis skin graft and rejection of F344 skin graft at day 100. The femur bone was successfully removed without compromising flap survival and without inducing rejection up to day 200. Donor skin tolerance was maintained after bone removal, as confirmed by skin graft challenge. Peripheral lymphocyte panel of the tolerant recipients showed higher level of CD4 T cells and lower level of B cells compared to the

rejection counterparts. Donor cells engraftment was detected via IVIS bioluminescence imaging system.

**Conclusion:** The VBM is capable under short-term immunosuppressant treatment to achieve tolerance via donor cells engraftment. Once engrafted donor cells are well-established, the vascularized bone marrow may become expendable. Non-invasive imaging systems are promising and may have an increasing role in the future.

### **8:10 AM - 8:14 AM**

Optimizing Reconstruction with Periorbital Transplantation: Clinical Indications and Anatomic Considerations

Michael Sosin, Baltimore, MD, USA

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### **Purpose**

Reconstruction of composite tissue defects of the periorbital subunit is challenging since the goals of effective reconstruction may vary from one individual to another. Multiple facial transplants involving the transfer of periorbital contents as part of a larger (total) facial allograft have yielded encouraging results. The development of technical and anatomic feasibility toward transplanting isolated periorbital tissue is gaining momentum. However, it remains unclear which type of injury is suitable for application of periorbital subunit allotransplantation. The purpose of this manuscript is to explore the indications and anatomic feasibility of periorbital transplantation, emphasizing the importance of evaluating patient's preoperative vision and mechanical eyelid function by reviewing our institutions repository of facial injury.

### **Methodology/Design**

Institutional review board approval was obtained at the R Adams Cowley Shock Trauma Center for a retrospective chart review conducted on patients with periorbital defects, including candidates for facial allotransplantation. Patient history and images were critically evaluated to assess potential candidacy for an isolated periorbital VCA, or total face (incorporating the periorbital subunit) VCA. Patient's facial defects, visual acuity, and periorbital function were critically reviewed to identify indications for periorbital transplantation and to design an anatomically feasible periorbital allograft tailored for specific patient defects (Figure 1a, 1b). Allograft harvest dissections were performed at the Maryland State Anatomy Board (Baltimore, MD).

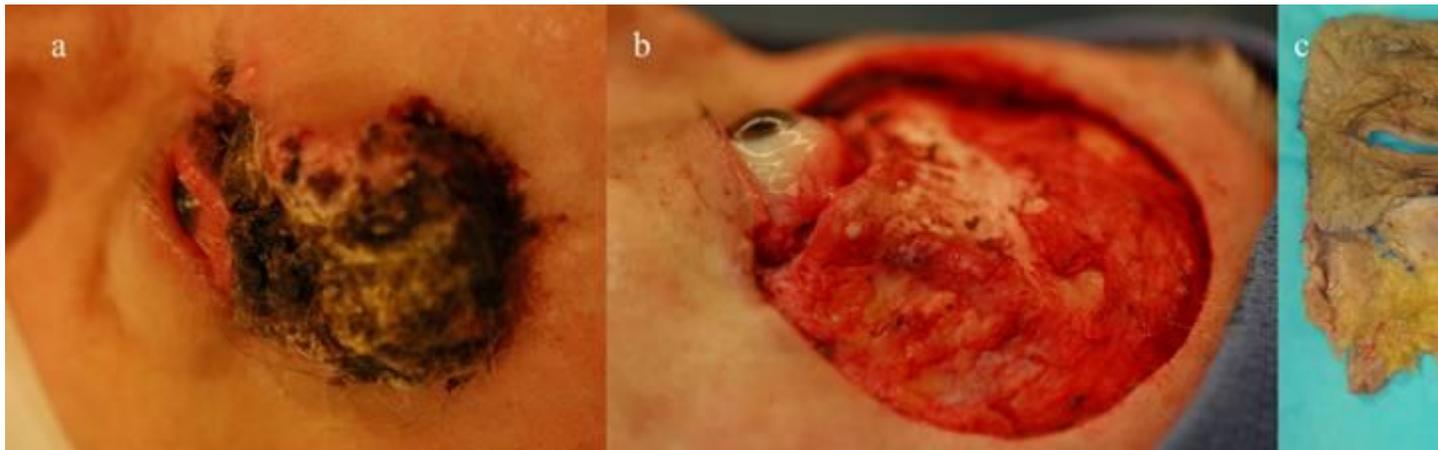
### **Results**

A total of 7 facial or periorbital transplant candidates representing 6 different etiological disease conditions were selected as suitable indications for periorbital transplantation. Other disease conditions not captured by our patient population warranting consideration were reviewed. Autoimmune disorders, warranted consideration but was ultimately excluded from the indications for periorbital transplantation. Cadaveric allograft harvest was successfully completed in 4 hemifaces and 1 full face. Isolated periorbital subunits were harvested based on a bipedicle vascular supply via the facial vessels and superficial temporal vessels (Figure 1c). The zygomatic and buccal branches of the facial nerve were preserved in 100% of hemifaces.

## Conclusion

Transplantation of isolated periorbital structures or full face transplantation including periorbital structures is technically feasible. The goal of periorbital transplantation is to re-establish the protective mechanisms of the eye, to prevent deterioration of visual acuity, and optimize aesthetic outcomes. Indications for periorbital transplantations include: ballistic trauma, animal attack, thermal burn, chemical (acid or alkali) burn, and neoplasm.

Figure 1. (a) Preoperative basal cell carcinoma of the left supraorbital region. (b) Candidate for isolated periorbital allotransplantation following resection. (c) Allograft for isolated periorbital transplantation.



## 8:14 AM - 8:18 AM

Pushing the Boundary of Solid Organ and Vascularized Composite Allotransplantation: A Clinical Chest wall, Thymus, and Heart Transplant Simulation

Johns Hopkins Hospital, Baltimore, MD, USA

Michael Sosin, MD<sup>1</sup>; Gianluca Torregrossa, MD<sup>2</sup>; Amir H. Dorafshar, MBChB<sup>1</sup>; (1)Johns Hopkins Hospital, (2)Mount Sinai Hospital

## Purpose

The integration of vascularized composite allotransplantation (VCA) into the field of clinical cardiac transplantation may serve as a potential solution or at the very least may improve multiple challenges to cardiac surgery by expanding the donor pool, prolonging allograft survival, reconstructing chest wall deformity, and decreasing immunosuppressive requirements. Recently, our group has described a proof-of-concept cadaveric model of harvesting the chest wall, thymus and heart as an en bloc donor allograft.<sup>1</sup> The purpose of this study is to design a proof-of-concept cadaveric model to describe technical aspects of allograft harvest and recipient inset of two different techniques termed, 'en bloc' and 'two stage.' Moreover, each technique is critically evaluated based upon advantages, disadvantages, potential risks, and ability to be integrated into a clinical practice.

## **Methodology/Design**

Following institutional review board approval, 4 cadavers (2 male and 2 female) were obtained and identified as donor or recipient. Two different techniques of combined solid organ and VCA entitled: 'en bloc' and 'two stage' were designed to assess for technical feasibility, degree of difficulty, and timing. The 'en bloc' combined solid organ and VCA technique incorporates the skin, sternum, costae, intercostal muscles, thymus, and heart as a single transferrable allograft. (Figure 1) The 'two stage' combined solid organ and VCA technique incorporates two transferrable allografts: (1) heart and (2) skin, sternum, costae, intercostal muscles, and thymus as two distinct entities that are anastomosed into one allograft during the recipient inset.

## **Results**

A total of 2 mock chest wall, thymus, and heart transplant procedures were performed in cadaveric donor and recipients. The 'en bloc' and 'two stage' procedures were both successfully performed. The 'en bloc' and 'two stage' procedures were both successfully performed in the presence of a multidisciplinary team of cardiac surgeons and plastic and reconstructive surgeons present for both procedures (Video 1). Table 1 and 2 summarizes the differences of both techniques.

## **Conclusion**

Our cadaveric surgical simulation demonstrates both, the 'en bloc' and the 'two stage' chest wall, thymus, and heart VCA are technically feasible models for clinical application that are associated with their respective advantages and disadvantages.

Figure 1. (a) En-bloc allograft, (b) recipient, and (c) inset.

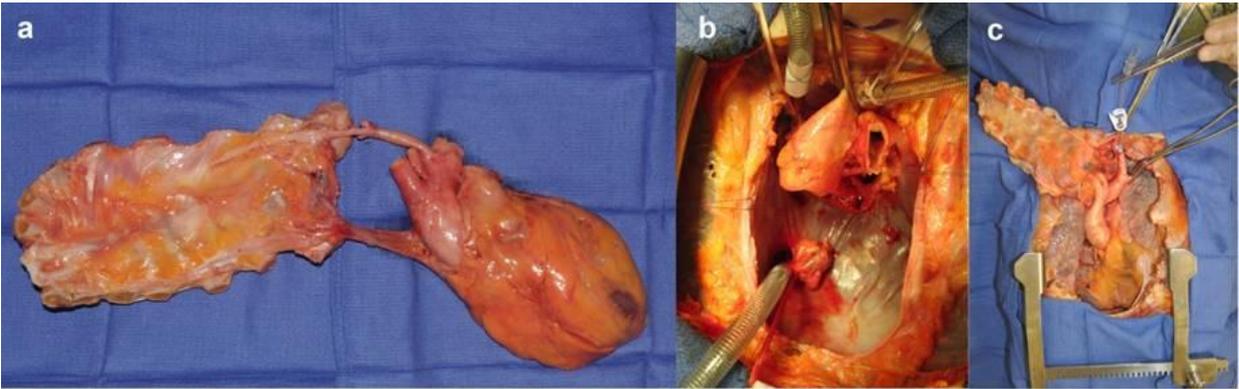


Table 1. Structures anastomosed for the ‘en bloc’ and ‘two stage’ chest wall, thymus, and heart transplant.

En Bloc		Two Stage	
Donor	Recipient	Donor	Recipient
Left atrial cuff	Left atrial cuff	Left atrial cuff	Left atrial cuff
IVC	IVC	IVC	IVC
PA	PA	PA	PA
Proximal aortic arch	Proximal aortic arch	Ascending aortic arch	Proximal aortic arch
SVC (end)	SVC (side)	SVC (end)	SVC (end)
		Right internal mammary vein (end)	Left innominate vein (side)
		Left internal mammary vein (end)	Left innominate vein (side)
		Right internal mammary artery (end)	Ascending aorta (side)
		Left internal mammary artery (end)	Ascending aorta (side)

IVC – inferior vena cava, PA – pulmonary artery, SVC – superior vena cava, \*microvascular anastomosis

Table 2. Advantages and disadvantages to the ‘en bloc’ and ‘two stage’ chest wall, thymus, and heart transplant.

	Pros	Cons
<b>En Bloc</b>	<ul style="list-style-type: none"> <li>Large venous drainage outlet.</li> <li>Decreased number of anastomoses.</li> <li>No microvascular anastomosis necessary.</li> </ul>	<ul style="list-style-type: none"> <li>Single vein draining chest wall.</li> <li>Increased donor harvest operative time.</li> <li>Increased implantation/inset duration.</li> <li>Complexity of determining SVC length for end-to-side anastomosis (risk of redundancy and kinking).</li> <li>Complexity of aortic anastomosis (ascending arch to the proximal arch of the recipient with the cross clamp in place).</li> </ul>
<b>Two Stage</b>	<ul style="list-style-type: none"> <li>Decreased operative time during harvest and transplant (lower ischemia time).</li> <li>Standardized techniques are unaltered and does not impact cardiac surgeon familiarity with technique.</li> <li>Extra length of SVC and ascending aorta not necessary.</li> <li>Two stages allows for ‘bailout’ from second stage of chest wall transplant in the event of a comprised heart function or intraoperative complications.</li> </ul>	<ul style="list-style-type: none"> <li>Increased anastomotic sites.</li> <li>Complexity of optimizing mammary artery distance to the ascending aorta with C-clamp in place.</li> <li>Arterial anastomosis adjacent to aorto-aortic anastomosis.</li> <li>Complexity of venous end-to-side anastomosis.</li> </ul>

SVC – superior vena cava.

**8:18 AM - 8:22 AM**

Prevalence and Distribution of Potential Vascularized Composite Allotransplant Donors, Implications for Optimizing the Donor-Recipient Match in a UNOS-Based Allocation System  
 Institute for Plastic Surgery, Southern Illinois University, Springfield, IL, USA  
 Shaun D. Mendenhall, MD; Mauricio De la Garza; Tim Daugherty; Jennifer Koechle; Emma Hoffmann; Steven Verhulst; Michael Neumeister; Bradford West; Southern Illinois University School of Medicine

BACKGROUND: Vascularized composite allotransplantation (VCA) is the transplantation of multiple tissue types as a functional unit such as a hand, face, or abdominal wall. There have been close to 100 hand and 29 face transplants to date. The 5-year allograft survival rate for

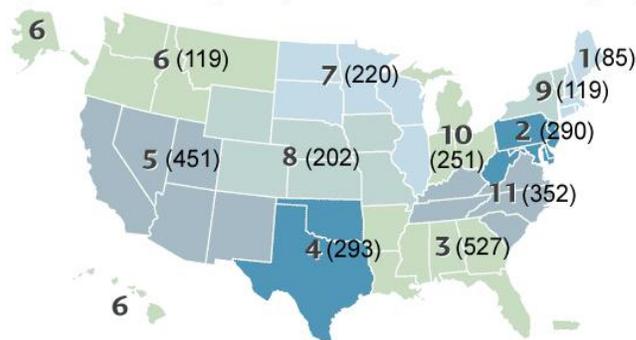
hand transplants is > 90% compared to 75% for kidney transplants. UNOS recently elected to classify VCAs as "organs" and began oversight of VCA allocation on July 3<sup>rd</sup>, 2014. Little is known about the prevalence and distribution of organ donors who could also be candidates for VCA donation in this new allocation system.

**METHODS:** A custom dataset of donor characteristics was obtained from UNOS of all brain-dead donors from 2008-2013. To identify the prevalence of potential VCA donors, inclusion and exclusion criteria used for VCA were applied to the dataset. Frequency analyses were then performed of characteristics important for VCA matching.

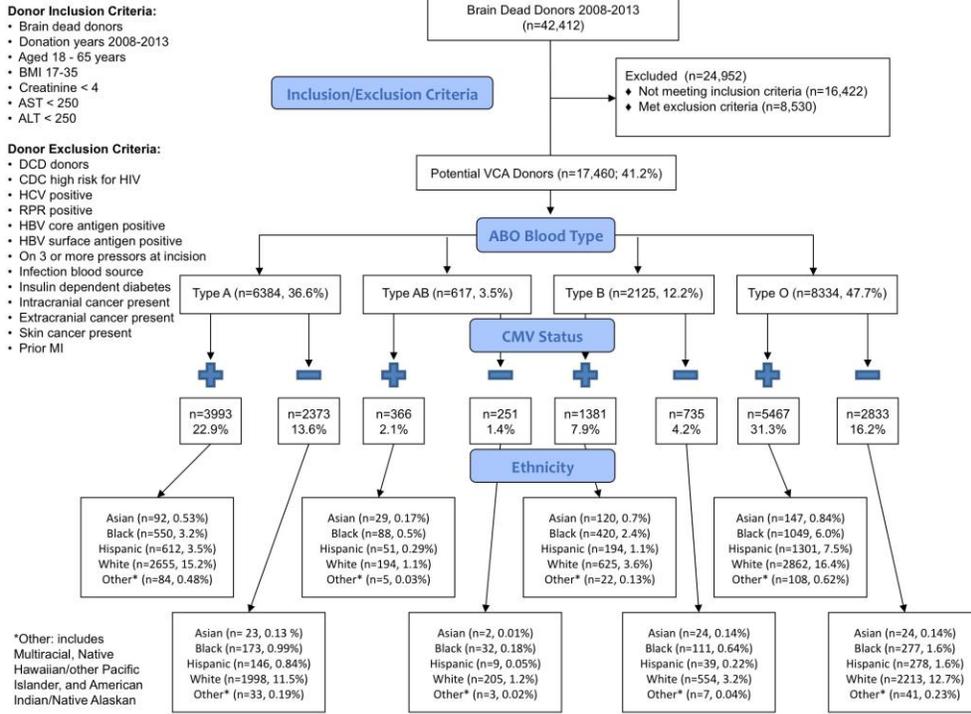
**RESULTS:** The dataset began with 42,414 brain-dead donors and after applying the inclusion and exclusion criteria, decreased to 17,460 (41.2%). The number of potential VCA donors per UNOS region ranged from 85-527/yr (Fig. 1). The majority of potential VCA donors were blood type O, CMV+, Whites, with the least common profile being blood type AB, CMV-, Asians (Fig. 2).

**CONCLUSIONS:** VCA is in the early stages of standard of care as evidenced by UNOS oversight and increasing acceptance by the medical community. Analysis of the UNOS donor database reveals a large potential donor pool. Understanding the characteristics of previous organ donors can guide VCA teams in optimizing the donor-recipient match in this new field of transplantation and thus maximize patient outcomes following transplantation.

**Figure 1. UNOS Regions (# of Potential VCA Donors/year)**



**Fig 2. Prevalence of Potential VCA Donors from UNOS Database 2008-2013**



8:22 AM - 8:28 AM  
Discussion