concentrations of collagen was plated in the pre-designed well, surrounding the BCS and VC. Twenty-four hours after plating, fluorescently labeled endothelial cells (EC) and smooth muscle cells (SMC) were seeded within the channel at a concentration of 5 million cells/mL. Control constructs were made by generating vascular structures and BCS within a collagen-only matrix, and by creating full biomimetic platforms with vascular channels in the absence of cancer cells. Constructs were cultured for 7 days, formalin-fixed, counterstained with DAPI, and analyzed with confocal microscopy.

RESULTS After 7 days in culture, confocal microscopy revealed successful fabrication of biomimetic platforms with a type I collagen (different concentrations) extracellular matrix containing patient-derived adipocytes, SVF and breast duct organoids. Patent VC lined with fluorescently labeled SMC and EC were visualized within the platform, with vascular walls located within 1mm of the red fluorescent, triple-negative MDA-MB-231 cancer foci. Invasion of BC cells into the surrounding tissue was identified by the presence of red fluorescent cells within the biomimetic platform in constructs containing type I collagen at both 0.3% and 0.6% w/v. Decreased vascular integrity was observed in constructs containing BCS when compared to those without BC cells.

CONCLUSION With the aid of three-dimensional printing technology, we have successfully engineered an advanced, patient-specific, biomimetic platform of the breast cancer microenvironment that not only replicates patient tissue characteristics, but also includes vascular structures and cancer foci that closely resemble early tumors. Observed BC invasion into the surrounding microenvironment, and the platform’s ability to mimic patient specific tissue with extremely high fidelity, make this platform a highly versatile and powerful tool that holds significant promise for diagnostic and therapeutic applications in the study of breast cancer.

QS37

Induction Of Delayed Immune Tolerance After Reconstructive Transplantation By Combining Donor Bone Marrow Transplantation And High-dose Cyclophosphamide Treatment

YINAN GUO¹, Franka Messner, MD¹, Byoung Chol Oh, DVM, PhD¹, Georg J. Furtmüller, MD¹, W.P. Andrew Lee, MD¹, Damon S. Cooney, MD, PhD¹, Leo Luznik, MD², Gerald Brandacher, MD¹

¹Department of Plastic and Reconstructive Surgery, Vascularized Composite Allotransplantation Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, USA, ²Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA

PURPOSE: Developing novel treatment concepts to minimize/avoid immunosuppression by induction of immune tolerance represents a primary goal in the field of transplantation. Immunosuppression-free allograft survival has been achieved in several animal models as well as in humans in living-related combined kidney and donor bone marrow transplantation by inducing mixed hematopoietic chimerism. However, success of this concept relies on extensive pre-transplant recipient preconditioning which is not feasible in VCA. Many VCAs though inherently contain vascularized donor bone marrow and thus a vital bone marrow niche home to donor-derived hematopoietic progenitor cells facilitating chimerism induction. In this study we therefore explored a novel approach to induce delayed immune tolerance subsequent to conventional immunosuppressive treatment combining high-dose cyclophosphamide treatment and donor bone marrow transplantation.

METHODS: Orthotopic hind limb transplantation from Balb/c to C57BL6 mice is performed across a full MHC mismatch barrier. Recipient animals are assigned to a course of long-term treatment with conventional mTOR inhibitor-based immunosuppression. Induction treatment comprises non-myeloablative total body irradiation (TBI) and T-cell depletion and a single dose of cyclophosphamide (Cy) on POD 30 combined with donor bone marrow transplantation (dBMT) in selected groups. Animal survival, donor bone marrow engraftment, and frequency of memory T cells are assessed via flow cytometry on a weekly basis prior and after the application of the delayed tolerance regimen.

RESULTS: Untreated animals rejected their grafts acutely within 8±1 days. In treated animals, allograft survival was maintained over 30 days with conventional immunosuppression (Rapamycin) followed by Cy +/- dBMT which prolonged graft survival to 76.5d (±25.89d) without dBMT
and 83.6d (±15.89d) with dBMT. Mixed chimerism levels in animals without dBMT were 7.17% (±4.22%) on POD7 and 2.15% (±1.09%) POD30. Comparatively, chimerism levels were at 1.70% (±1.08%) and 3.77% (±2.17%) on POD 7 and 30, respectively, for animals with dBMT on POD 30. Multilineage mixed chimerism persisted after PT/Cy with declining levels at the time of allograft rejection. CD8+ effector memory T cell (Tmem) frequency after transplantation in animals with and without dBMT on POD 7 was (dBMT-: 4.65% (±8.38%); dBMT+: 1.7% (±0.19%)) and POD 30 (dBMT-: 7.22% (±2.65%); dBMT+: 2.31% (±0.94%)). Of note, after induction treatment, the proportion of CD8+Tmem cells in groups with or without dBMT increased to 28.08%±20.90% and 41.08%±21.85%, respectively, representing a trend which negatively correlated with graft survival.

CONCLUSION: Delayed application of combined high dose cyclophosphamide and donor bone marrow transplantation following a long-term course of conventional immunosuppression leads to extended yet not indefinite allograft survival despite the presence of multilineage mixed chimerism. Further studies will focus on studying memory T cell barriers to achieve delayed immune tolerance in this murine model of VCA.

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Effect of Compound 21, a Selective Angiotensin II Type 2 Receptor Agonist, in an Abdominal Adhesion Murine Model

Colton Boudreau, MSc1, Courtney Jones, BSc1, Alison Gareau, PhD2, Terry Levatte, BSc1, Stephanie Legere, MSc1, Michael Bezuhly, MD, MSc, SM, FRCSC, FAAP1

1Dalhousie University, Halifax, NS, Canada, 2Calgary Lab Services, Calgary, AB, Canada

PURPOSE: Abdominal adhesions are fibrous bands that form in response to surgical trauma, and connect visceral and/or peritoneal surfaces. Adhesions occur in over 90% of patients post-laparotomy and can lead to serious long-term complications, including infertility, small bowel obstruction and perforation, and chronic pain. This study uses a murine model of induced abdominal adhesions to study the anti-fibrotic effect of a novel selective angiotensin II type 2 receptor agonist, compound 21 (C21), in reducing abdominal adhesion formation.

METHODS: The effects of C21 in vivo were assessed using a cecal abrasion model in female BALB/c mice. A laparotomy was performed and the cecum and overlying peritoneum was abraded with fine grit sandpaper. Mice were divided into systemic (oral gavage) or local (intraperitoneal injection) treatment groups and were treated with C21 (10 μg/kg) or saline (vehicle control) daily for 7 days. Mice were sacrificed 8 days post-surgery and adhesions were graded by a blinded observer. Peritoneal fluid was obtained at time of sacrifice and ELISA used to quantify TGFβ levels. Laparotomy incisions were excised and CD31, CD68, and αSMA immunostaining, and picrosirius red staining were performed to assess surgical laparotomy incisional wound properties. To study the in vitro effects of C21, parietal peritoneal fibroblasts and visceral mesothelial cells were isolated and scratch wound assays performed using C21 (10 μM), angiotensin II (AngII, 1 μM), or both.

RESULTS: Post-operative C21 administration, both systemically and locally, greatly reduced the formation of abdominal adhesions in vivo. Additionally, TGFβ in peritoneal fluid was reduced in mice treated with C21. Hydrological analysis of surgical incisions revealed no statistical difference in the number of CD31+ vessels or CD68+ cells. Expression of αSMA was reduced in C21-treated animals. Picrosirius red staining revealed no difference in collagen I and collagen III distribution in laparotomy scars between control and C21-treated animals. Laparotomy scar collagen density and surrounding dermis thickness was not significantly different between treatment groups. Cell migration of isolated parietal peritoneal fibroblasts and visceral mesothelial cells in vitro was markedly reduced in the presence of C21 compared to control or AngII.

CONCLUSIONS: C21 markedly reduced or completely prevented adhesion formation both with local and systemic administration. These findings may be attributed to decreased levels of pro-fibrotic TGFβ in vivo and decreased cell migration of parietal peritoneal fibroblasts and visceral mesothelial cell migration in the presence of C21. Importantly, C21 did not appear to have histologically quantifiable effects on laparotomy wounds compared to controls. This study suggests that C21 could reduce abdominal adhesions without impeding laparotomy wound healing.

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Fibroblasts Show Attenuated Wound Healing in the Setting of Dermal Melanoma