2020 Pediatric Research and Career Development Symposium

Tuesday, August 4, 2020
9:00 am – 4:15 pm

Abstracts
BASIC SCIENCE POSTERS ONLY

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B5. A Patient-Specific 3D Bioprinted Platform for *In Vitro* Disease Modeling and Treatment Planning in Pulmonary Vein Stenosis

**Authors:** Serpooshan, Vahid; Tomov, Martin L.; Jing, Bowen; Kumar, Akaash; Panoskaltsis, Nicki; Mantalaris, Athanasios; Slesnick, Timothy C.; Lindsey, Brooks; and Bauser-Heaton, Holly

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Pulmonary vein stenosis (PVS) is an acute pediatric cardiovascular disease that is always lethal if not treated early. While current clinical interventions (stenting and angioplasties) have shown promising results in treating PVS, they require multiple re-interventions that can lead to re-stenosis and diminished long-term efficacy. Thus, there is an unmet need to develop functional *in vitro* models of PVS that can serve as a platform to study clinical interventions. Patient-inspired 3D bioprinted tissue models provide a unique model to recapitulate and analyze the complex tissue microenvironment impacted by PVS.

Here, we developed perfusable *in vitro* models of healthy and stenotic pulmonary vein by 3D reconstruction and bioprinting of patient CT data (Figure 1). Models were seeded with human endothelial (ECs) and smooth muscle cells (SMCs) to form a bilayer structure and perfused using a bioreactor to study cell response to stenotic geometry, and to the stent-based treatment. Flow hemodynamics through printed veins were quantified via CFD modeling, 4D MRI and 3D ultrasound imaging. Cell growth and endothelialization were analyzed. Our work demonstrates the feasibility of bioprinting various cardiovascular cells, to create perfusable, patient-specific vascular constructs that mimic complex *in vivo* geometries. Deeper understanding of EC-SMC crosstalk mechanisms in *in vitro* biomimetic models that incorporate tissue-like geometrical, chemical, and biomechanical ques could offer substantial insights for prevention and treatment of PVS, as well as other cardiovascular disease.
B6. Epigenetic and Transcriptional Dynamics in Acute and Chronic Human Myeloid Inflammation

Authors: Cammarata-Mouchtouris, Alexandre; Moncada, Diego; Giacalone, Vincent; Dobosh, Brian; Prahalad, Sampath; and Tirouvanziam, Rabindra

Presenting Author: Alexandre Cammarata-Mouchtouris, PhD; acammar@emory.edu

Type: Basic - Poster

Poster Session Zoom Room Link: Visit Cammarata-Mouchtouris Zoom Room

RATIONALE: Beyond receptors and signaling proteins, genetic, epigenetic and transcriptional regulators are important in the unfolding of immuno-inflammatory responses. Epigenetic and transcriptional regulators in particular allow for rapid adaptation of responses in different types of cells and pathophysiological contexts. For example, our laboratory demonstrated in patients with Cystic Fibrosis (CF) that neutrophils recruited from blood into the airway lumen undergo reprogramming that causes them to promote a complex reorganization of the local tissue, in coordination with macrophages and epithelial cells. Such reprogramming of human neutrophils runs counter to the conventional assumption that the fate of myeloid cells is pre-programmed and establishes these fast-acting subsets as adaptable coordinators of tissue responses.

APPROACH: In ongoing studies, we are expanding on our past investigations in CF to characterize epigenetic and transcriptional dynamics in other neutrophil-dominated diseases of peripheral tissues including Juvenile Idiopathic Arthritis, and late stage COVID-19. Our pipeline combines the Cut & Run epigenetic assay method with shotgun Illumina sequencing and long-read Oxford Nanopore MinION sequencing to understand the regulatory landscape of neutrophils and monocytes recruited to diseased airways and joints.

RESULTS: Output from Cut & Run assays conducted in tissue neutrophils and macrophages in CF and JIA will be compared to illustrate the scope and specificity of epigenetic and transcriptional adaptations occurring over time in these fast-acting subsets. Rapid epigenetic changes can sometimes remain in effect even after the initial stimulus has waned, leading to transcriptional memory (a component of trained innate immunity). Therefore, the potential impact on long-term immune polarization / dysregulation will also be outlined.

CONCLUSIONS: A better understanding of dynamic adaptations in myeloid subsets associated with human peripheral inflammation using this and other approaches will help identify novel epigenetic and transcriptional targets for therapeutic modulation of various intractable diseases.

ACKNOWLEDGMENTS: CTID Pilot and Cystic Fibrosis Foundation.

Authors: Chonat, Satheesh; Patel, Seema; Jeffers, Lauren; Cisneros de la rosa, Eduardo; Fields, Earl; Archer, David; Koval, Michael; Joiner, Clinton, and Stowell, Sean

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Sickle cell disease (SCD) is a common and life-threatening autosomal recessive hematological disorder that affects millions worldwide. Amongst acute complications in SCD, acute chest syndrome (ACS) is a leading cause of hospitalization and the most common cause of death due to SCD. Unfortunately, supportive care remains the primary approach to alleviate these complications. This in part reflects an incomplete understanding of the pathophysiology and accompanying pharmacological targets that could specifically mitigate acute disease complications. Retrospective analysis of stored plasma samples from our SCD patients with ACS revealed acute hemolysis, and significantly increased levels of anaphylatoxins (C3a and C5a) and markers of the alternative complement pathway (Ba, Bb) during episodes of ACS compared to their baseline values. To examine the underlying mechanism of the role of complement in ACS, we developed a pre-clinical model of acute lung injury (ALI) in humanized sickle cell (SS) mice. Injection of cobra venom factor (CVF) to SS mice, a commonly used approach to induce complement activation, resulted in rapid deoxygenation, hypopnea and bradycardia (all hallmarks of ALI in mice), followed by death. In contrast, CVF treated littermate control (AA) mice did not develop detectable hemolysis, pulmonary compromise or increase in mortality. The SS mice had markedly increased levels of plasma anaphylatoxin C5a and increased complement component (C3) deposition in kidneys and lungs by immunofluorescence when compared to their controls and those treated with vehicle. While erythrocytes in these SS mice had elevated levels of C3b/iC3b/C3c deposition when compared to AA mice, no difference was noted in the total plasma C3, suggesting sickle erythrocytes are prone to complement-mediated hemolysis.

We then developed a humanized SS x C3 knock-out mice, and preliminary data suggests that these mice are protected from CVF mediated ALI and death. Our data thus far suggest that complement activation in SS mice results in hemolysis, release of free heme and production of C5a, which is a potent pro-inflammatory mediator, all of which possibly play a role in ALI. These results demonstrate that inhibition of C3a or C5a production may represent pharmacological targets to treat ACS in patients with SCD.
B8. Bortezomib Significantly Enhances Gamma Delta T Cell-Mediated Lysis of Acute Myeloid Leukemia and T-cell Acute Lymphoblastic Leukemia

Authors: Story, Jamie; Zoine, Jaquelyn; Burnham, Rebecca; Hamilton, Jamie; Spencer, H. Trent; Doering, Christopher; and Raikar, Sunil

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Previous studies have shown bortezomib, a proteasome inhibitor, increases surface expression of NKG2D ligands on cancer cells and enhances their sensitivity to innate immune cell-mediated cytotoxicity. Here, we investigate the combination of bortezomib and γδ T cells as a novel therapy for acute myeloid leukemia (AML), a hematologic malignancy in which CAR-based cellular immunotherapy has been challenging. Our results showed 24 hour treatment with bortezomib significantly increased ULBP2/5/6 expression in Kasumi-1 cells and Nomo-1 cells (n = 3, p <0.05). Day 12 ex vivo expanded γδ T cells were incubated with target cells at the following effector to target (E:T) cell ratios: 0:1, 1:4, 1:2, 1:1, and 2.5:1. Total target cell death of Kasumi-1 cells was significantly increased with bortezomib treatment compared to vehicle control from 16.8% to 52.1% at a 1:4 E:T ratio, 30.0 % to 64.0% at 1:2, and 48.0% to 77.8% at the 1:1 (n = 3, p <0.05). Nomo-1 cell death was significantly increased with bortezomib and γδ T cell combination treatment, compared to vehicle control, from 8.8% to 33.7%, 13.9% to 43.6%, 23.1% to 56.5%, and 45.8% to 71.3% at the 1:4, 1:2, 1:1, and 2.5:1 E:T ratios, respectively (n = 3, p <0.05). We further validated this combination approach in two T-ALL cell lines, Jurkat and MOLT4, which also had significantly increased surface expression of ULBP2/5/6 with 5 nM bortezomib treatment (n = 3, p <0.05). The total cytotoxicity against bortezomib treated Jurkat cells significantly increased from 39.9% to 64.0% at the 1:4 E:T ratio and from to 58.1% to 76.4% 1:2 ratio compared to vehicle control treated cells (n = 3, p <0.05). The total cytotoxicity of MOLT-4 cells was also significantly increased with bortezomib treatment compared to vehicle treated cells from 19.6% to 35.5% at the 1:4 E:T ratio, 36.7% to 48.4% at the 1:2 E:T ratio, and 57.4% to 68.3% at the 1:1 E:T ratio (n = 3, p <0.05). These results provides proof-of-concept for developing a platform for effective combination therapy of γδ T cells with stress ligand inducing drugs for high-risk leukemias.
B9. Postexposure Prophylaxis to Mitigate the Neurodevelopmental Consequences of Postnatal Zika Virus Infection in Infant Rhesus Macaques

Authors: Raper, Jessica; Schoof, Nils; Richardson, Rebecca; Medina, Alejandra; Rusnak, Rebecca; Kovacs-Balint, Zsofia; Sanchez, Mar; and Chahroudi, Ann

Although Zika virus (ZIKV) typically causes mild or no symptoms in adults, infection during pregnancy can result in a spectrum of disease in infants, including birth defects and neurodevelopmental disorders identified in childhood. While intense research has focused on prenatal ZIKV infection, the consequences of postnatal infection in early life are understudied. Using a highly clinically relevant rhesus macaque (RM) model, we have shown that ZIKV infection during infancy negatively impacted brain development resulting in long-term behavioral, cognitive and motor impairments. Considering that ZIKV has infected individuals in 92 countries and is endemic in many areas, it is important to investigate whether a postexposure prophylaxis could mitigate the negative neurodevelopmental consequences of postnatal ZIKV exposure. Using our established postnatal ZIKV RM model, we investigated whether an antiviral treatment could limit viral dissemination into the CNS and alleviate the impact of ZIKV on the developing brain. Three infant RMs received 14-day Sofosbuvir (SOF, 15mg/kg p.o.) treatment starting at 3 days post-infection (dpi). ZIKV+SOF infant RMs were monitored longitudinally for their immune response to ZIKV and SOF treatment, as well as assessing their behavioral, cognitive, motor, and brain development. ZIKV+SOF RMs cleared the virus at a similar rate to ZIKV-infected infant RMs, such that ZIKV was below detection by 7 dpi. Despite similar viral clearance, ZIKV+SOF RM exhibited social behavior more similar to age- and rearing-matched uninfected controls. Similar to controls, ZIKV+SOF infant RMs exhibited caregiver attachment and prosocial behaviors. However, emotional assessments and neuroimaging suggest an intermittent phenotype. For example, ZIKV+SOF infant RMs exhibited the species-typical response of freezing during the profile condition of the human intruder task, but their level was lower than controls. At 3 months of age, ZIKV+SOF RMs had normal lateral ventricle volumes, but exhibited smaller amygdalae, hippocampi, and total white matter volume compared to controls. The current data suggests that antiviral treatment may help ameliorate some, but not all, of the neurodevelopmental consequences associated with early postnatal ZIKV infection. Further assessments are needed to determine degree that postexposure treatment can alleviate the cognitive and motor impacts of postnatal ZIKV infection.
Factor VIII (FVIII) replacement in hemophilia A can be complicated by neutralizing anti-FVIII IgG alloantibodies that can actively block FVIII activity and prevent optimal replacement efficacy. Currently, no prophylactic therapy prevents inhibitor development, likely due to poor understanding of key immune regulators governing inhibitor formation.

In contrast to other model antigens, inhibitor formation occurs only following multiple FVIII exposures both in humans and mouse models. This suggests that early exposure events may prime subsequent development of long-lasting antibodies. Despite previous studies suggesting that CD4 T cells play an important role in inhibitor development, their timing and overall role in this key immune response remains incompletely understood. Thus, defining the role of CD4 T cells in inhibitor development is important if novel therapeutics for inhibitor prevention are to ever be realized.

As no tools exist to study FVIII specific CD4 T cells, we engineered the model antigen, OVA, into the B domain site of B-domain-deleted FVIII (HOVA) for tracking antigen specific CD4 T cells. HOVA had equivalent FVIII activity and immunogenicity to recombinant FVIII. Additionally, the OVA CD4 T cell epitope in HOVA was functional, as proliferation of CD4 T cells from OTII mice was observed following culture with HOVA in vitro. Surprisingly, no proliferation or activation of OTII CD4 T cells was detected in hemophilia A mice immunized with 1-2 HOVA injections. However, in previously highly immunized mice, 1 HOVA injection induced significant proliferation and activation of OTII CD4 T cells.

Understanding initiating immune events in the CD4 T cell-dependent process of FVIII inhibitor development is paramount to the development of novel therapies to prevent inhibitor formation in hemophilia A. HOVA is a unique immunologic tool for examining the FVIII specific CD4 T cell response following FVIII exposure. Using this tool, we found that FVIII specific CD4 T cell proliferation requires more than 2 prior exposures to HOVA, consistent with the observation that multiple FVIII exposures are required prior to inhibitor development. These findings provide an important clue to early steps in the development of FVIII inhibitors, with further studies needed to elucidate the mechanisms underlying this phenomenon.
All basic science posters provided by abstract authors are available in the following pages. Available posters are labeled P10 - P11.
Bortezomib Significantly Enhances γδ T Cell-Mediated Lysis of Acute Myeloid Leukemia and T-Cell Acute Lymphoblastic Leukemia

Jamie Y. Story, B.A.1,3, Jaquelyn T. Zoino, B.S.2,3, Rebecca E. Ryan, B.S.1,3, Jamie A. Hamilton2,3, H. Trent Spencer, Ph.D.1,3, Christopher B. Doering, Ph.D.1,3 and Sunil S. Raikar, M.D.3

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Background
- Relapse still remains a clinical challenge in the field of childhood leukemia
- Allogeneic hematopoietic stem cell transplantation (HSCT) is only realistic chance at a cure for relapsed AML and T-ALL, but requires patients to be in remission
- CAR T cell therapy has been difficult to adopt in both leukemias due to on-target, off-effect
- Other immunotherapies need to be explored for AML and T-ALL
- γδ T cells are an attractive candidate for cancer immunotherapy
- Can induce cell lysis through engagement with stress antigens on tumor cells, such as natural-killer group 2, member D (NKGD2) ligands
- Our lab previously developed a serum free GMP method for ex vivo expansions of γδ T cells (γδ T02)
- Certain drugs can increase stress antigens on cancer cells
- Bortezomib previously shown to increase NKGD2 ligands on AML cells
- Limited benefit from addition of bortezomib to standard chemotherapy in clinical setting for AML

Hypothesis
We hypothesize a more effective approach is a combination of bortezomib with ex vivo expanded γδ T cells, potentially as a bridge to transplant by inducing remission

Research Design
- Incubated Kasumi-1, Nomo-1, Jurkat, and MOLT-4 cells with 2.5, 5, and 10 nM of bortezomib over 48 hours to determine optimal drug treatment for increase in NKGD2 ligands’ surface expression
- Treated Kasumi-1 and Jurkat cells with 5 nM bortezomib and Nomo-1 cells with 10 nM bortezomib or vehicle control for 24 hours prior to a 4 hour in vitro cytotoxicity assay with ex vivo expanded γδ T cells at various effector to target (E:T) ratios
- Targeted measured cell death by flow cytometry via 7-AAD and Annexin V staining
- Assess whether bortezomib treatment had negative effects on γδ T cells
- Treated γδ T cells with 1-5 nM bortezomib for 24 hours, which is range of expected plasma concentration for 24 hours after bortezomib injection in adults
- Incubated GFP+ target cells with γδ T cells at lower E:T ratios over 48 hours to assess if bortezomib treatment accelerated target cell death over a longer time period

Results
- Figure 1: Expansion of γδ T Cells from healthy donor PBMCs. (A) Healthy donor PBMCs were cultured in serum-free medium over a 10-day period. Cultures were supplemented with 500 U/ml of IL-2 and 10 nM of β2-microglobulin and either 0, 5, 10, or 20 nM of bortezomib. Cells were depleted of γδ T cells on day 5 and 10 nM of bortezomib was used for the 3 remaining days. γδ T cells over the course of expansion for one donor. (B) Graph depicting cultured γδ T cell number at each time point. (C) Flow cytometry was performed every 3 days to monitor the percentage of γδ T cells over the expansion. Live cells were gated and an E:T ratio of 5:1. (D) γδ T cell percentage was determined by CTC FACS. (E) Flow cytometry was performed every 3 days to monitor the percentage of γδ T cells over the expansion. Live cells were gated and an E:T ratio of 5:1. (F) γδ T cell percentage was determined by CTC FACS.
- Figure 2: Expression of NKGD2 ligands (ULBP2/5/6) in AML cell lines and primary AML patient samples. (A) Baseline protein expression of NKGD2 ligands on AML cell lines was assessed by gating on Annexin V–/7-AAD– cells and analyzing ULBP1, ULBP2, ULBP5, and ULBP6 protein expression on a 4 hour cytotoxicity assay with γδ T cells. (B) Bar graph showing expression of ULBP2/5/6 ligands (ULBP2, ULBP5, and ULBP6) in AML cell lines and primary AML patient samples. (C) Surface expression of ULBP2/5/6 in MOLT-4 cells peaked between 12 and 24 hours at all doses. (D) There was a significant increase in ULBP2/5/6 expression at 24 hours of treatment with bortezomib or vehicle. AML cells were incubated with day 12 ex vivo expanded γδ T cells at the 1:4, 1:2, and 1:1 E:T ratios (n = 3, p = 0.001, 0.014, and 0.0002, respectively). Significance was determined using two-way ANOVA with Dunnett’s multiple comparisons test.
- Figure 3: Expansion of γδ T Cells from healthy donor PBMCs. (A) Healthy donor PBMCs were cultured in serum-free medium over a 10-day period. Cultures were supplemented with 500 U/ml of IL-2 and 10 nM of β2-microglobulin and either 0, 5, 10, or 20 nM of bortezomib. Cells were depleted of γδ T cells on day 5 and 10 nM of bortezomib was used for the 3 remaining days. γδ T cells over the course of expansion for one donor. (B) Graph depicting cultured γδ T cell number at each time point. (C) Flow cytometry was performed every 3 days to monitor the percentage of γδ T cells over the expansion. Live cells were gated and an E:T ratio of 5:1. (D) γδ T cell percentage was determined by CTC FACS. (E) Flow cytometry was performed every 3 days to monitor the percentage of γδ T cells over the expansion. Live cells were gated and an E:T ratio of 5:1. (F) γδ T cell percentage was determined by CTC FACS.
- Figure 4: Expression of NKGD2 ligands (ULBP2/5/6) in AML cell lines and primary AML patient samples. (A) Baseline protein expression of NKGD2 ligands on AML cell lines was assessed by gating on Annexin V–/7-AAD– cells and analyzing ULBP1, ULBP2, ULBP5, and ULBP6 protein expression on a 4 hour cytotoxicity assay with γδ T cells. (B) Bar graph showing expression of ULBP2/5/6 ligands (ULBP2, ULBP5, and ULBP6) in AML cell lines and primary AML patient samples. (C) Surface expression of ULBP2/5/6 in MOLT-4 cells peaked between 12 and 24 hours at all doses. (D) There was a significant increase in ULBP2/5/6 expression at 24 hours of treatment with bortezomib or vehicle. AML cells were incubated with day 12 ex vivo expanded γδ T cells at the 1:4, 1:2, and 1:1 E:T ratios (n = 3, p = 0.001, 0.014, and 0.0002, respectively). Significance was determined using two-way ANOVA with Dunnett’s multiple comparisons test.

Conclusions
- ULBP2/5/6 surface expression significantly increases with 24 hour bortezomib treatment at 5 or 10 nM in AML and T-ALL cells
- Administering γδ T cells 24 hours after bortezomib treatment in humans should not negatively affect their in vivo cytotoxicity against cancer cells
- Bortezomib treatment significantly increased cytotoxicity of ex vivo expanded γδ T cells at low E:T ratios
- Target cell death >30% was seen at 1:4 E:T ratio in all cell lines
- Bortezomib treated cells were killed faster than vehicle control treated cells in a 24-48 hour cytotoxicity assay
- Demonstrates feasibility of γδ T immunotherapy with stress antigen inducing drugs, such as bortezomib, in the AML and T-ALL setting without having to develop antigen specific immunotherapies

Future Directions
- Investigate whether bortezomib treatment enhances NKGD2 ligand surface expression on primary cancer cells from AML and T-ALL patients
- Determine if bortezomib treatment enhances susceptibility of primary AML and T-ALL cells to γδ T-cell mediated cytotoxicity in an in vitro based cytotoxicity assay
- Perform in vivo experiments to investigate whether bortezomib and γδ T cells combination decreases tumor burden and/or increases survival in mice
- Inject luciferase-expression Nomo-1 and Jurkat cells into NSG mice and treat with vehicle control, bortezomib alone, γδ T cells alone, or bortezomib + γδ T cells
- Measure tumor burden via bioluminescence imaging and overall survival

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P10
**INTRODUCTION**

FVIII replacement in hemophilia A can be complicated by the development of neutralizing anti-FVIII IgG antibodies (inhibitors)1-3. These inhibitors can actively block FVIII activity and prevent optimal replacement efficacy4. Despite their significant clinical implications, there are currently no prophylactic therapies to prevent inhibitor development. This is likely in part due to a poor understanding of the key immune regulators governing inhibitor formation.

In contrast to other model antigens, inhibitor formation occurs only following multiple FVIII exposures both in humans and mouse models. This suggests that early exposure events may prime subsequent development of long-lasting antibodies. In particular, as CD4 T cells facilitate IgG formation, it is possible that each FVIII exposure event may propagate a CD4 T cell response to a threshold necessary to generate an optimal IgG response. Despite previous studies suggesting that CD4 T cells play an important role in inhibitor development5-7, their timing and overall role in this key immune response remains incompletely understood. Thus, defining the role of CD4 T cells in inhibitor development is important if novel therapeutics for inhibitor prevention are to ever be realized.

**MATERIALS AND METHODS**

**FVIII**

FVIII-A FVIII-B FVIII-D

**FVIII-BDD**

FVIII-A FVIII-B FVIII-D

**HOVA**

FVIII-A FVIII-B FVIII-D

**HOVA-BDD**

FVIII-A FVIII-B FVIII-D

**MATERIALS AND METHODS**

**MATERIALS AND METHODS**

**HOVA**

FVIII-A FVIII-B FVIII-D

**RESULTS**

**RESULTS**

**CONCLUSIONS**

**REFERENCES**

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**REFERENCES**