The National Institutes of Health’s Medical Research Scholars Program

Hematology & Oncology Scholars Abstracts 2015-2017
Yajie "Julie" An, Class of 2017

School: Northeast Ohio Medical University
Mentor: Bradford J. Wood, M.D., Director, Center for Interventional Oncology; Chief, Interventional Radiology, NIH Clinical Center
Institute: Clinical Center (CC)
Research: Does a Negative Prostate mpMRI Rule-Out Clinically Significant Cancer?

Prostate multiparametric MRI (mpMRI) has significantly changed the paradigm of prostate cancer evaluation. However, the utility of systematic prostate biopsy in the presence of a negative mpMRI is still uncertain. In a retrospective database analysis, we evaluated the histopathologic results from systematic 12-core biopsies performed in patients with a negative prostate mpMRI.

Under an IRB-approved protocol, we queried our prostate mpMRI database for men who underwent systematic 12-core biopsy within a year of a negative prostate mpMRI. Clinicopathologic features were analyzed and stratified by biopsy history. Negative predictive value (NPV) was calculated for detection of any cancer (Gleason Score ≥ 6) and clinically significant cancer (Gleason Score ≥7). Regression analysis was performed to identify outcome predictors.

Overall, 114 men met the inclusion criteria. Median age and PSA in this cohort were 61 (IQR 57-67) years and 5.5 (IQR 3.6-8.7) ng/ml, respectively. The NPV of mpMRI for clinically significant cancer was 96.5% (95%CI 93.1 to 99.9%) overall. NPV for significant cancer in biopsy naïve (n=20), prior negative biopsy (n=53), and prior positive biopsy (n=41) cohorts was 100%, 100%, and 90%, respectively. No significant predictors were identified for detection of prostate cancer in this cohort.

Negative prostate mpMRI has an excellent NPV for clinically significant cancer and serves as a useful modality for stratifying risk in patients with suspicion of prostate cancer. Patients with negative mpMRI can avoid unnecessary prostate biopsy, thereby decreasing the detection of clinically insignificant cancer and avoiding morbidity due to overtreatment.

Full Length Publications:
- An JY, Sidana A, Choyke PL, Wood BJ, Pinto PA, Turkbey B. Multiparametric magnetic resonance imaging for active surveillance of prostate cancer. Balkan Medical Journal. [Under review]
- An JY, Sidana A, Holzman SA, Biaccio JA, Choyke PL, Wood BJ, Turkbey B Pinto PA. Does a negative prostate mpMRI rule-out clinically significant cancer? [Under review]

Abstract Publications:

Travel to Professional Meetings:
Angela Wei Chen, Class of 2017

School: University of Pittsburgh School of Medicine
Mentor: Bradford Wood, M.D., Director, Center for Interventional Oncology, Chief of Interventional Radiology Section
Institute: Clinical Center (CC)
Research: Tremelimumab combined with subtotal locoregional therapy induces systemic tumor response in patients with unresectable multifocal hepatocellular carcinoma

Tremelimumab, a monoclonal antibody against T lymphocyte surface receptor CTLA-4, inhibits B7-CTLA-4-mediated downregulation of T-cell activation. Ablation has been shown to induce a peripheral immune response to enhance the effect of anti-CTLA4 treatment in patients with advanced HCC. The purpose of this study was to describe changes in the radiographic appearance of hepatocellular carcinoma treated with tremelimumab in combination with limited ablation.

A retrospective review of imaging was conducted for seven consecutive patients (age 70.8 ± 4.2, all male) with multifocal discrete HCC (Childs Pugh A/B7; BCLC B/C; ECOG 0/1; post-sorafenib) treated on clinical trial with tremelimumab (10 mg/kg/IV q4 weekly x6 doses) followed by cryoablation (n=4), microwave (MWA) (n=1) or radiofrequency ablation (RFA) (n=2) of a single lesion on day 36. Changes in size, density, and enhancement pattern of lesions were summarized.

Mean cross-sectional imaging follow-up was 160 days. Of the seven ablated tumors, four increased in size, two decreased, and one remained unchanged. Four showed increased enhancement, one showed decreased enhancement, and two remained unchanged. 18/42 (43%) of the ablation-naive remote tumors demonstrated growth at the first post-ablation imaging interval. At subsequent imaging of 15/18 lesions, 7 regressed in size while 6 progressed and 2 remained stable. The majority of remote tumors (67%) showed increased enhancement, compared to 15% with decreased enhancement, and 18% with unchanged enhancement.

In a subset of patients receiving immune checkpoint inhibition therapy with supplemental limited ablation, a majority of ablation-naive tumors showed initial growth, followed by subsequent shrinkage. This imaging response may initially be misclassified according to RECIST criteria. Although speculative, initial increased enhancement could be a consequence of immunocyte infiltration which has been previously documented in a subset of responding patients who underwent repeat biopsy.

Abstract Publications:

Travel to Professional Meetings:

Awards:
Medical School: University of Utah School of Medicine
Mentor: Brigitte Widemann, M.D.; Jack Shern, M.D.; John Glod, M.D./Ph.D., Center for Cancer Research, Pediatric Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Outcomes and Mechanisms of Disease Progression in MEN2B Patients with Advanced Metastatic Medullary Thyroid Carcinoma Treated with Vandetanib

Childhood medullary thyroid carcinoma (MTC) generally arises from a germline mutation in the RET proto-oncogene. Children with advanced MTC are treated with RET-targeting inhibitors such as vandetanib. We sought to characterize outcomes, vandetanib tolerability, patterns of disease progression, and clonal evolution of childhood MTC. We monitored toxicities, disease burden, and natural history of patients taking vandetanib for advanced MTC (NCT00514046, NCT01660984). Where feasible, germline/tumor samples were analyzed by genome and transcriptome sequencing.

Seventeen patients [8 male; 13 (9-17)* years] enrolled; 16 had a RET p.M918T germline mutation. Patients received vandetanib for 5.5 (0.1-9.2+)* years. Treatment is ongoing in 8 patients. Best response was partial response in 10, stable disease in 6, and progressive disease in 1 patient. Response duration was 5.1 (0.6-8.6+)* years. Six patients died from disease 2.1 (0.4-4.3)* years after stopping vandetanib. Progression free survival was 6.2 years (95% CI 3.0-na) and overall survival was 7.9 years (95% CI 5.9-na). No patients discontinued vandetanib for toxicity.

Panel DNA sequencing (12 samples, 7 patients) identified a change in DNA copy number (CN) from 10.1±1.9% in primary tumors to 29.7±9.4% in metastatic lesions (p = 0.002) and a somatic RET p.L790F mutation as potential mechanisms of disease progression. Whole exome and transcriptome sequencing of sequential tumors from one patient (4 samples) confirmed accumulation of CN aberrations over time and showed genome-wide changes in RNA expression. Whole exome and genome sequencing was also used to examine clonality of MTC tumors (7 samples, 2 patients). Evolutionary models, k-means, and heirarchial clustering predicted relationships between lesions within individual patients, and suggest that some lesions may be new primary tumors rather than metastatic MTC.

We found that vandetanib is safe and results in sustained responses in children with advanced MTC. We propose that genomic instability may have a role in MTC progression and suggest that some lesions may be new primary tumors rather than MTC metastases.

*median (range)

Abstract Publications:
*Equal contribution

Travel to Professional Meetings:
- Rare Tumors Initiative Symposium, National Institutes of Health, Bethesda, MD; Oct. 6-7, 2016 *Participated in the Clinical Trials Study Section.
- American Society for Pediatric Hematology and Oncology Annual Meeting, Montreal, Canada; Apr. 26-29, 2017.
- American Society for Clinical Oncology, Chicago, IL; June 1-6, 2017.

Awards:
- Best Student/Post-Bac Poster: Pediatric Oncology Branch Annual Research Round-Up.
Jeffrey Lin, Class of 2017

School: David Geffen School of Medicine at UCLA
Mentor: Andrea Apolo, M.D., Chief, Bladder Cancer Section; NIH Lasker Clinical Research Scholar, Genitourinary Malignancies Branch
Institute: National Cancer Institute (NCI)
Research: Combined Fluodeoxyglucose (FDG) and Sodium Fluoride (NaF) Positron Emission Tomography/Computed Tomography (PET/CT) Study in Patients with Metastatic Genitourinary Tumors (mGU) Treated with Cabozantinib + Nivolumab +/- Ipilimumab

18F-NaF PET/CT has reemerged as a valuable imaging method for detecting osseous metastasis, and 18F-FDG PET/CT is well-established to detect metastatic disease, particularly soft tissue disease. These scans are typically done on separate days to clear the radiotracer; however, there are data to support the clinical utility of obtaining both scans sequentially on the same day.

We conducted a single-arm, multicenter, phase I trial of cabozantinib, ipilimumab, and/or nivolumab in patients with mGU. Patients had a FDG PET/CT followed within 1-hour by a NaF PET/CT at baseline and at 8 weeks. We captured the number and location of metastatic lesions on FDG and on combined FDG/NaF. The concordance of bone disease was compared between the FDG and combined FDG/NaF scans.

49 patients with mGU had combined FDG/NaF scans. Median age of subjects was 58 years and 85% were male. 784 lesions were detected on the FDG scans with the majority found in bone (29.5%), lymph node (29.2%) and lung (27.7%). A patient-based analysis revealed that of the 49 patients, the majority of patients had lymph node (77.8%), lung (46.7%), and bone (35.6%) disease. For the combined FDG/NaF scans, 396 lesions were detected, with the majority of lesions found in the spine (27%) and rib (26%). A concordance analysis analyzing how well FDG or NaF could detect metastatic bone disease found that of 405 lesions analyzed, FDG detected 62% of the lesions, and NaF detected 94% of the lesions.

Combination FDG/NaF scans adequately detected metastatic disease in mGU patients treated with targeted therapy and immunotherapy. Most lesions were found in bone, lymph node, and lung, and most bone lesions were found in the spine and rib. NaF detected more bony lesions than FDG alone. There were no instances of where the uptake from the prior FDG scan affected the determination of metastatic disease in the sequential NaF scan.

Abstract Publications:
- Lin J, et.al. Combined FDG and NaF PET/CT study in patients with metastatic genitourinary tumors treated with cabozantinib and nivolumab +/- ipilimumab (CaboNivo+/-Ipi). J Clin Oncol; 2017 June 2-6; Chicago, IL; American society for Clinical Oncology (ASCO); Abstract 16017.

Travel to Professional Meetings:
- Genitourinary Cancers Symposium; Orlando, Fl; Feb. 16-18, 2017.
- American Association of Neurological Surgeons Annual Scientific Meeting, Los Angeles, CA; Apr. 22-26 2017.
- American Society of Clinical Oncology Annual Meeting, Chicago, IL; June 2-6, 2017.
Human papilloma virus (HPV) causes cervical, anal and oropharyngeal cancers. Only diseased cells express HPV viral proteins, allowing for selective immune targeting. Engineered T cells can directly recognize and kill viral antigen-expressing tumor cells. However, some tumors inactivate immune cells and evade killing. Upregulated expression of immune checkpoints in tumors can contribute to tumor immune resistance. When engaged by its ligands, PDL1 and PDL2, the checkpoint inhibitor receptor, PD1, limits the activity of T cells in peripheral tissues. The blockade of immune checkpoints can unleash the potential of the antitumor immune response.

Our lab uses engineered T cells targeting HPV proteins to treat metastatic cancers. However, these engineered T cells are not always active after infusion into patients. We developed a mouse model to understand the importance of the PD1 immune checkpoint inhibitor on T cell immunotherapy. The C57Bl/6 mouse model is syngeneic and immunocompetent. B16F0 mouse melanoma cells were retrovirally transduced to express ovalbumin, a non-self antigen, and PDL1. Tumors were implanted subcutaneously in irradiated mice. Seven days later, mice were treated with OT1 T cells that specifically target ovalbumin. Some mice also received PD1 or PDL1 blocking antibodies.

The addition of PDL1 did not change the untreated tumor growth curve, and showed a trend to decreased antitumor effects of OT1 T cells. Adding either PDL1 or PD1 blocking antibodies to the system removed the T-cell inhibitory effects of the tumors. The combination of OT1 T cells and blocking antibody caused tumor regression. These results confirm the role of the PD1 axis in tumor immune evasion. This mouse model will probe the effects of checkpoint inhibitors on T cell activation and serve as a model for testing new disruptors of the PD1 checkpoint inhibitor pathway. These insights may result in better clinical outcomes in autologous T cell therapy.

**Travel to Professional Meetings:**
The presence of the blood-brain barrier (BBB) poses a significant hindrance in delivering effective chemotherapy to malignant brain tumors. Previous studies utilizing vascular endothelial growth factor (VEGF) to induce transient permeability of the BBB have failed to investigate a role for its use in brain tumor treatment. Our study goals were to look at the effects of VEGF on brain endothelial cells and on drug delivery in animal models. In-vitro studies of brain endothelial cell junctional/cytoskeletal proteins were performed via Western blotting, immunofluorescence, and electrical cell-cell impedance. In-vivo studies involved use of rodents receiving VEGF and chemotherapy (temozolomide) to investigate the effect of VEGF on drug delivery.

Both recombinant mouse (rm) and recombinant human (rh) VEGF demonstrated effects on cell-cell adhesion, resulting in approximately a 30% decrease in electrical impedance. These effects were controlled and reversed with Bevacizumab, a human VEGF monoclonal antibody. Western blotting and immunofluorescence indicated that rmVEGF decreased the expression of junctional proteins, including claudin-5 and VE-cadherin; while altering cytoskeletal arrangement of actin filaments. Systemic administration of rmVEGF to rodents affected the integrity of CNS endothelial tight junctions, demonstrated by transmission electron microscopy. However, pre-treatment with a single dose of intravenous rmVEGF failed to demonstrate increased CNS delivery of temozolomide in healthy mice.

Our in-vitro results are consistent with the known properties of VEGF and its effects on vasculature, particularly its role in vasogenic edema. Our animal studies demonstrate that systemically administered VEGF does affect the BBB on the cellular level, but fails to improve chemotherapy penetration. This is possibly due to the renal vascular changes of systemic VEGF, which cause increased excretion of temozolomide. In summary, in-vitro VEGF studies demonstrated an impact on cellular integrity, but further animal studies are needed to evaluate the effect of direct VEGF administration on CNS vasculature, in an effort to improve chemotherapy delivery.

Abstract Publications:

Travel to Professional Meetings:

Awards:
- ACEA™ Cancer and Immunotherapy Symposium, Award for best late-breaking abstract.
Ionizing radiotherapy (IR) has many applications in contemporary cancer management. Single or hypofractionated high-dose IR enhances responses to experimental immunotherapies. However, standard-of-care clinical management of many cancers involves low-dose, fractionated (daily) IR. The effect of low-dose fractionated IR on anti-tumor immunity remains unclear. This year, we compared the impact of 2 fractions of 8 Gy (hypofractionated high-dose, 48 hours apart) versus 10 fractions of 2 Gy (fractionated low-dose, daily) on tumor infiltrating lymphocyte (TIL) accumulation and activation, peripheral immune cell densities and activation, and tumor-draining lymph node (TDLN) T-lymphocyte antigen-specific IFNγ responses in T-cell inflamed syngeneic murine models of oral cavity (MOC1) and colon (MC38-CEA) carcinoma. Tumors, TDLNs and spleens were collected at 5, 10 and 20 days after the start of either IR regimen. Both IR schemas delayed primary tumor growth to a similar degree and neither regimen enhanced CD8 TIL accumulation over time. However, TIL CD107a positivity and tumor-draining lymph node T-lymphocyte antigen-specific responses were significantly increased in the high-dose IR cohort. While both IR regimens limited the accumulation of tumor-infiltrating myeloid-derived suppressor cells with tumor progression, both also resulted in increased peripheral and tumor-infiltrating T regulatory cells. Tumors that received high-dose IR also demonstrated significantly increased TIL PD-1 and tumor cell PD-L1 expression, suggesting that adaptive immune resistance may be limiting responses. Experiments combining high-dose hypofractionated or low-dose fractionated IR with systemic PD-1 mAb are underway. In addition to providing biologic insight into the effects of low-dose and high-dose IR on anti-tumor immunity, the results of these experiments will critically inform the design of clinical trials utilizing concurrent IR and PD-based checkpoint inhibition.

Full Length Publications:

Abstract Publications:

Travel to Professional Meetings:
Although treatment of chronic lymphocytic leukemia (CLL) has been advanced by introduction of targeted therapies, there remains a need for adjunct treatments capable of inducing deeper initial response, response in the setting of resistance to first-line agents, and/or CLL cure. Bispecific antibodies (bsAbs) can be used to target endogenous T cells against tumor cells via the formation of cytolytic synapses. The anti-CD19/CD3 bsAb blinatumomab is the most clinically advanced bsAb to date, and is approved for treatment of Philadelphia chromosome-negative relapsed/refractory B-cell acute lymphoblastic leukemia. However, due to its half-life of 2.1 hours, blinatumomab requires continuous intravenous dosing for efficacy. We have developed a novel anti-CD19/CD3 bsAb in the single chain-Fv Fc format (CD19/CD3-scFv-Fc). With a half-life approximately 100-fold longer than blinatumomab, CD19/CD3-scFv-Fc may be suitable for weekly dosing, which would provide a significant logistical advantage in the clinic.

We first sought to evaluate the activity of anti-CD19/CD3 bsAbs against CLL by culture of CLL peripheral blood mononuclear cells (PBMCs) with either blinatumomab, CD19/CD3-scFv-Fc, negative control HER2/CD3-scFv-Fc, or medium alone. Compared to negative control, treatment with both CD19/CD3-scFv-Fc and blinatumomab induced potent killing of CLL cells, as measured by flow cytometry (p = 0.0031 and 0.0004, respectively, n=10). This response was associated with expansion of autologous CD4+ and CD8+ T cells, as well as increase in T cell activation and granzyme B expression. We next investigated response to CD19/CD3 bsAbs in a NOD/scid/γc(null) (NSG) xenograft mouse model. Here, mice were treated weekly with either blinatumomab, CD19/CD3-scFv-Fc, or HER2/CD3-scFv-Fc after established engraftment with human CLL PBMCs. Treatment with CD19/CD3-scFv-Fc resulted in a 99.5% reduction in circulating leukemia burden compared to treatment with control (p<0.0001). However, blinatumomab failed to induce a response (p=0.82). These data support promise of CD19/CD3-scFv-Fc as a novel immunotherapy for use in CLL.

**Abstract Publications:**

**Travel to Professional Meetings:**

**Awards:**
- Best Predoctoral Fellow Pitch Award, National Heart, Lung, and Blood Institute Research Festival; June 9, 2017.
Acute myeloid leukemia (AML) is a malignancy of blood-forming stem cells characterized by a high rate of relapse despite initial therapeutic response. Persistent leukemic burden giving rise to relapse is referred to as measurable residual disease (MRD). Due to disease heterogeneity, established MRD PCR assays are only applicable to small patient subsets with recurrent cytogenetic abnormalities.

We have developed a targeted RNA-sequencing assay capable of detecting and tracking MRD in approximately 70% of cases. Our panel detects important AML wild-type gene expression signatures (WT1, PRAME, ABL), inversions (CBFB-MYH11), insertions (NPM1), and translocations (BCR-ABLp190, BCR-ABLp210, RUNX1-RUNX1T1, PML-RARA). 400 ng of RNA is extracted from peripheral blood and is reverse transcribed in the presence of primers containing 12-bp random barcode sequences to enable individual molecular labeling. Sequencing adapters are attached to targeted amplicons with 26 cycles of PCR and library quality is assessed by a Qubit DNA Fluorometer and 2100 Bioanalyzer. Libraries are sequenced on an Illumina MiSeq using V3 Reagent chemistry with 150bp single-end reads and analyzed using a custom bioinformatics pipeline.

To determine assay sensitivity and limits of detection, we spiked in RNA extracted from leukemia cell lines K-562 and Kas-1 into RNA from a healthy individual. Quantification of AML-specific transcripts (K562: PRAME, WT1, BCR-ABLp210 and Kas-1: RUNX1-RUNX1T1) was detectable in a linear relationship at all points in a dilution series spanning 1pg-800ng of leukemia input. 10,000 copies of ABL1 (control gene) were used as a baseline for healthy-donor expression. We then localized and quantified a pathognomonic 4bp insertion in exon 12 of NPM1 transcripts at all inputs in a dilution series of 20-400 ng RNA from blood extracted from patient samples. Ongoing work involves tracking MRD in a cohort of 50 clinically-annotated AML patients with blood samples available at longitudinal time points.

**Full Length Publications:**

**Abstract Publications:**

**Travel to Professional Meetings:**
- American Society of Hematology (ASH) Annual Meeting, Atlanta, GA; Dec. 9-12, 2017.

**Awards:**
- Best Predoctoral Fellow Pitch Award, National Heart, Lung, and Blood Institute Research Festival; June 9, 2017.
Acute myeloid leukemia (AML) is an aggressive malignancy with a poor prognosis and limited treatment options. Chimeric antigen receptor expressing T (CART) cell therapy has been highly successful against refractory B cell acute lymphoblastic leukemia. Effective use of CART cells in AML has not yet been established. Here, we evaluated the pre-clinical potency and efficacy of anti-CD123 and anti-CD33 CART cells against AML.

Anti-CD33 and anti-CD123 CART cells were developed using CD33 and CD123 single chain fragment variable sequences and cloning into a third-generation lentiviral plasmid containing a CD8 transmembrane domain, a co-stimulatory signaling domain (either CD28 or 41BB) and a CD3-zeta domain. CART surface expression on T cells was measured by flow cytometry, staining with allophycocyanin (APC)-conjugated anti-FLAG for anti-CD123 CART cells or APC-Protein L/streptavidin for anti-CD33 CART cells. Enzyme-linked immunosorbent assay kits for interleukin-2 (IL2) and interferon-gamma (IFN-gamma) were used to assess in vitro CART functionality; 10^5 tumor cells were co-incubated with 10^5 transduced CART cells or mock T cells for 16 hours in 96-well plates. Killing assays using IncuCyte live cell analysis were completed to assess killing potency of CART; 10^5 tumor cells were co-incubated with 10^5 transduced CART cells or mock T cells for 36 hours in 96-well plates.

Anti-CD33 and anti-CD123 CART cells show in vitro functionality through robust production of IL2 and IFN-gamma against various AML cell lines. Anti-CD33 CART cells produced 2000 pg/mL of IL2 when co-incubated with THP1 cell line (derived from a one-year-old male patient with relapsed AML). Anti-CD33 CART cells also produced about 2000 pg/mL of IFN-gamma against THP1 and also MOLM14 (derived from a 20-year-old male with M5 AML). Anti-CD123 CART produced about 2500 pg/mL of IFN-gamma against both MOLM14 and THP1, and 1000 pg/mL of IL2 against MOLM14. CART cells targeting both antigens showed in vitro killing activity against MOLM14 and THP1 AML cell lines.

CART cell therapy targeting surface antigens CD33 and CD123 in AML is promising. Pre-clinical AML models indicate in vitro activity of anti-CD33 and anti-CD123 CART cells against AML, showing lysis of leukemic cells and continued presence of CART cells. Further experiments are needed to characterize any associated toxicity of anti-CD33 and anti-CD123 CART cells.

Abstract Publications:

Travel to Professional Meetings:
• American Society of Clinical Oncology, Chicago, IL; Jun. 2-6, 2017.
Giacomo C. Waller, Class of 2017

School: Emory University School of Medicine  
Mentor: Richard Childs, M.D., R.A.D.M., U.S.P.H.S., Senior Investigator, Laboratory of Transplantation Immunology, Hematology Branch; Clinical Director, Division of Intramural Research, NHLBI  
Institute: National Heart, Lung, and Blood Institute (NHLBI)  
Research: Development of an Optimized Toolkit for High-Efficiency Lentiviral Genetic Modification of Human Natural Killer Cells

Natural Killer (NK) cells are a subset of immune cells with antiviral and antitumor activity that have the innate ability to lyse tumor cells without the need to recognize specific MHC-bound peptide sequences. Infusion of large numbers of NK cells, expanded in vitro, represents a promising immunotherapeutic intervention in cancer. To augment the function and homing of NK cells with expression of particular genes, we developed and optimized a protocol for lentiviral transduction of primary human NK cells. Stimulation of NK cells with IL-2 alone for two to four days was found to be necessary and sufficient to achieve high transduction efficiency, while the addition of other cytokines had negligible or transient effects. Identical off-the-shelf lentiviral constructs with eight different promoter sequences driving expression of EGFP were examined, and three of the promoters were found to consistently facilitate transduction efficiencies in the range of 25-60% with minimal loss of expression over two weeks of culture in our clinical-grade feeder-based in vitro expansion protocol. Transduced and expanded primary NK cells did not show functional deficiencies in degranulation, IFNγ, or TNFα production. To permit identification, tracking, and isolation suitable for scalable use under GMP, constructs expressing both the gene of interest and a truncated CD34 marker are being examined, both as 2A-fusions and expressed from independent promoters. The goal of this research is to enable the production of large numbers (>10^10) of uniformly modified NK cells suitable for infusion in our clinical NK cell treatment protocol.

Abstract Publications:

Travel to Professional Meetings:
- American Society of Hematology 59th Annual Meeting. Atlanta, GA; Dec. 9-12, 2017. [Planned]
Inherited bone marrow failure syndromes (IBMFS) are a diverse set of genetic disorders characterized by the bone marrow’s inability to generate hematopoietic stem cells (HSCs). Allogeneic HSC transplantation and gene therapy offer potential cures but reduced numbers of matched donors and lack of gene-corrected autologous HSCs limit these treatment options. With the development of induced pluripotent stem cell (iPSC) technologies emerges the concept of generating iPSCs from an individual patient, correcting the defect using gene-specific targeting for safe integration of the therapeutic transgenes (e.g. CRISPR/Cas9), and differentiating the disease-free iPSCs into transplantable HSCs. However, unlike their in vivo counterparts, human HSCs derived from iPSCs are incapable of efficiently reconstituting long-term hematopoiesis after transplantation in xenograft animal models, hampering full exploitation of the therapeutic potential of iPSC-derived cell products.

Here we successfully used an in vitro monolayer differentiation scheme to generate CD34+CD45+ Hematopoietic Stem/Progenitor Cells (HSPCs) from healthy donor-derived iPSCs for mouse engraftment studies. We further validated that these monolayer derived cells failed to engraft following NSG mouse transplantation. High throughput single-cell RNA sequencing (Drop-Seq) analysis was then used to compare single-cell transcriptomes of phenotypically-defined ex vivo-generated HSPCs to their matched primary donor-derived HSPCs. These investigations provided preliminary insight into the genetic programs that uniquely define both candidate populations. Through utilization of these techniques, we are better able to generate transplantable human HSCs with multi-lineage, long-term reconstitution potential for gene and cell therapies of inherited HSC disorders.
DNA methyltransferase 3A (DNMT3A) is a member of the DNA methyltransferase family primarily involved in de novo gene methylation. Mutations in DNMT3A have been associated with a wide range of hematological malignancies, most frequently acute myeloid leukemia (AML). Research suggests DNMT3A mutations produce a pre-leukemic state, rendering cells vulnerable to secondary oncogenic mutations and malignant transformation. This concept is supported by genome-sequencing data from over 10,000 healthy individuals in which the presence of clonal hematopoiesis driven by somatic mutations, most commonly DNMT3A, was associated with an increased risk of developing leukemia and all-cause mortality.

The mechanisms by which DNMT3A mutations contribute to malignant transformation have not been delineated, although a propensity of mutated cells to self-renewal has been postulated. The goals of this study were thus to determine the transcriptional and biological effects of DNMT3A knockout (KO) which contribute to leukemogenesis. To do this, we generated DNMT3A KO human cell lines using the novel gene-editing technology CRISPR/Cas9. We successfully created four DNMT3A KO cell lines using K562 cells. In a growth curve analysis, we found that the DNMT3A KO cell lines exhibited significantly impaired growth compared to DNMT3A WT cells. Furthermore, we found that DNMT3A KO cells were significantly more susceptible to apoptosis and DNA damage after treatment with 5-FU. Finally, RNA-sequencing expression analysis revealed numerous differentially expressed genes and many dysregulated signaling pathways, including those of cell adhesion, apoptosis, and immune function.

We have shown that CRISPR-mediated DNMT3A KO in K562 cells can be used as a model to study the effects of DNMT3A mutation. Our data provide evidence that DNMT3A mutation alters K562 function in a global manner, consistent with its role as a DNA methyltransferase. Additional functional studies are required to elucidate the specific mechanisms by which DNMT3A mutation predisposes to leukemia.
Elizabeth J. Carstens, Class of 2016

School: University of Texas Southwestern Medical School
Mentor: Adrian Wiestner, M.D., Ph.D., Senior Investigator, Laboratory of Lymphoid Malignancies
Institute: National Heart, Lung, and Blood Institute (NHLBI)
Research: Potentiating immunotherapy by targeting complement deposited on cancer cell surfaces

Treatment of lymphoid malignancies with anti-CD20 monoclonal antibodies (mAbs) can be frustrated by the loss of cell surface CD20 through trogocytosis, creating “escape variants” that are no longer sensitive to the anti-CD20 mAb. In a clinical trial of treatment using the anti-CD20 mAb ofatumumab in chronic lymphocytic leukemia (CLL), we observed that patients with residual disease at the end of treatment frequently had these escape variants, but the variants carried the covalently bound complement activation fragments, C3d.

To test whether targeting C3d can eliminate escape variants after anti-CD20 therapy, we collected blood samples from CLL patients before (day 1) and 24 hours after administration of ofatumumab (day 2). A human IgG1 mouse chimeric mAb specific to C3d, developed in the lab, specifically bound and effectively killed CLL escape variants from day 2 through complement dependent cytotoxicity (CDC), NK cell mediated antibody dependent cellular cytotoxicity (ADCC), and phagocytosis. Importantly, non B lymphocytes were neither bound nor killed by the anti-C3d mAb, consistent with the highly targeted and selective deposition of C3d on CD20+ cells by ofatumumab.

Transfer of peripheral blood mononuclear cells obtained from CLL patients on day 2 of ofatumumab treatment into NSG mice and subsequent treatment with anti-C3d mAb led to significant reductions in tumor burden in both the peripheral blood and spleen compared to infusion of isotype control. In a second model, HBL2 cells, a CD20+ mantle cell lymphoma line, were xenografted into SCID mice. Mice were treated with either isotype, anti-CD20 mAb (ofatumumab or rituximab) or the combination of anti-CD20 and anti-C3d mAb. Addition of anti-C3d antibody extended time to tumor development and also prolonged survival significantly over CD20 targeting alone (median survival 34 days vs 79 days, p<0.0001). Targeting complement may represent a strategy that is universally combinable with other anti-tumor mAbs to circumvent the development of resistance through antigen escape.
Leukocyte adhesion deficiency (LAD) type I is a genetic immunodeficiency characterized by loss of expression of CD18 integrin. We hypothesized that disruption of the CXCR4 chemokine receptor in human hematopoietic (CD34+) cells could lead to an engraftment advantage for such cells, and serve as a platform for treatment of patients with LAD type I using autologous hematopoietic progenitor/stem cells (HPCs). Our goal was to disrupt the CXCR4 locus while adding the corrected CD18 gene in human CD34+ HPCs, thereby correcting the LAD lesion while also giving the gene-corrected cells an engraftment advantage. We evaluated the efficacy of several methods of introducing the Cas9 endonuclease enzyme into human CD34+ cells in order to disrupt CXCR4. We found that non-integrating lentiviral vectors were able to transduce non-hematopoietic cells but were unable to transduce CD34+ HSCs, our ultimate target. We explored a high salt method to directly deliver the Cas9 protein; however, CD34+ cells were unable to survive long-term under conditions of high osmolarity. We then investigated a Cas9-KRAB-based silencing method which recruits protein methylation to induce inhibition. This resulted in some decrease in CXCR4 expression. Finally, we found that the most successful method of knocking down CXCR4 expression was by electroporating synthetic guide RNA (sgRNA) along with Cas9 protein into CD34+ cells.

Travel to Professional Meetings:
Acute myeloid leukemia (AML) is an aggressive cancer of immature myeloid cells that has a 25% five-year survival rate. Although 75% of patients who are treated with high-intensity induction therapy achieve complete remission (CR), half will relapse within two to three years. These data highlight a significant need for better therapies.

Clinical trials recently established that adoptive natural killer (NK) cell therapy can mediate regressions of hematologic malignancies. However, NK cells expanded \textit{ex vivo} for adoptive transfusion shed CD16, attenuating their ability to mediate antibody-dependent cellular cytotoxicity (ADCC). Additionally, clinical data suggest that patients with B cell malignancies treated with rituximab respond better when they are homozygous for the high-affinity (HA) 158-V CD16 polymorphism (present in 10% of people) due to enhanced ADCC against antibody-treated targets.

The novel anti-CD123 monoclonal antibody, CSL362, targets chemoresistant leukemic stem cells that are responsible for disease relapse in patients who have achieved CR. Since AML patients could benefit from targeted antibody therapy combined with adoptive NK cell infusions, we investigated whether mRNA electroporation (EP) of expanded NK cells with HA-CD16 mRNA would enhance their capacity to mediate ADCC \textit{in vitro} against CSL362-treated AML cells.

Human NK cells isolated from healthy-donor peripheral blood were expanded \textit{ex vivo} for 12-16 days using irradiated feeder cells in media with interleukin-2 (IL-2). EP of NK cells was performed using the MaxCyte GT® Transfection System. Standard four-hour chromium-release assays were performed with a CD123-bright AML cell line, MOLM-14.

Baseline NK cytotoxicity against MOLM-14 was high (70%); adding CSL362 did not significantly increase NK cell killing of tumors. EP of NK cells reduced baseline AML killing, which increased with the addition of CSL362 but was inferior to non-electroporated NK cells. Other methods of genetic modification must be tested to explore further possibilities of augmenting NK-cell killing of CSL362-treated AML targets.
Cancer cells harboring p53 mutations have been shown to exhibit gain of function (GOF) properties including microenvironment effects that are permissive to tumor growth and spread. However, it remains uncertain how these GOF effects are elicited by such mutant p53 isoforms. Current efforts investigate whether GOF properties are elicited through exosomes, particularly in regard to macrophages.

Exosomes are 30-100 nm vesicles shed by all cells that carry bioactive cargo including protein, RNA, DNA, and lipids. Furthermore, exosome uptake has been shown to produce active effects in recipient cells. Exosomes are becoming increasingly implicated in cancer effects on the microenvironment.

Macrophage activation exists on a spectrum, ranging from phagocytic, classically activated M1 macrophages, to M2 macrophages, which exhibit immunosuppressive effects while stimulating vasculogenesis. Our studies have shown that mutant p53 isoforms tilt macrophage phenotype toward M2, possibly enabling tumor growth and metastatic potential.

Mutant p53 was found to be more prevalent in exosomes compared to wild type p53, suggesting possible unique mutant p53 effects through exosomes. However, research continues in order to uncover p53 interactions influencing exosome cargo and release as well as influences on macrophage phenotype.
Anna Chichura, Class of 2015

School: Georgetown University School of Medicine
Mentor: Nicholas P. Restifo, M.D., Senior Investigator, Surgery Branch
Institute: National Cancer Institute (NCI)
Research: Adoptive Cell Transfer Targeting the Programmed Death-1 Receptor in Melanoma

Melanoma is currently the fifth most common cancer in the United States; the 5-year survival rate for patients with stage IV metastatic melanoma treated with dacarbazine is about 10%, but advances in immunotherapy with monoclonal antibodies have increased the 5-year survival rate to about 20%. The immunotherapy with the greatest impact on survival is adoptive cell transfer (ACT). ACT is a type of cancer immunotherapy in which antitumor lymphocytes are identified \textit{ex vivo}, expanded to large numbers, and re-infused into the same cancer patient. A major factor that limits the successful use of ACT in melanoma and other solid cancers is the identification of the diverse T cell receptor (TCR) repertoire that recognizes mutation-specific antigens expressed on tumor cells but not on essential normal tissue. It has been demonstrated that programmed death receptor-1 (PD-1) expression on CD8+ tumor infiltrating lymphocytes (TILs) accurately identifies clonally expanded tumor-reactive cells. However, it is not known if sequencing TCRs from PD-1-expressing (PD-1+) TIL will identify mutation-reactive TCRs that can mediate tumor regression \textit{in vivo}.

To test this hypothesis, we assessed the TCRs expressed by PD1+ T cells in tumor-bearing mice. We identified the sequences of paired TCR\textalpha and TCR\textbeta chains of CD3+ PD1+ TIL using a single cell sequencing based approach. TCR\textbeta deep sequencing revealed that the CD3+ tumor infiltrating T cell population was more oligoclonal than the CD3+ splenic T cell population, however there was no dominant V\textbeta clonotype that was shared among TIL from different mice. The receptors identified by TCR single cell sequencing will be retrovirally transduced into mouse T cells and adoptively transferred to tumor-bearing mice to determine if the TCRs derived from PD1+ TIL confer tumor specificity and have therapeutic potential in an ACT setting.
Amrita Karambelkar, Class of 2015

School: Icahn School of Medicine at Mount Sinai
Mentor: Richard Childs, M.D., R.A.D.M., U.S.P.H.S.; Senior Investigator, Laboratory of Transplantation Immunology, Hematology Branch; and Clinical Director, Division of Intramural Research, NHLBI
Institute: National Heart, Lung, and Blood Institute (NHLBI)
Research: Mechanistic Insights into Efficient Targeting of Ewing’s Sarcoma by Ex Vivo Expanded Natural Killer Cells: Promise for Natural Killer Cell Immunotherapy

Ewing’s sarcoma (EwS) is a cancer arising in the long bones of the extremities and axial skeleton, with 5-year relapse-free survival rates of 55% vs. 21% without and with metastasis. The dire prognosis of EwS presents an opportunity for exploring new therapeutic approaches for this disease.

Natural killer (NK) cells are highly cytotoxic immune cells involved in the innate defense against cancer. They kill tumor cells via induction of apoptosis through death receptors expressed on the target cell or via release of cytotoxic granules controlled by signals from activating and inhibitory germline-encoded cell surface receptors. Based on their ability to kill tumor cells, NK cells have been explored as therapeutic agents in settings of cell-based cancer immunotherapy. Ongoing clinical trials indicate that therapy with large numbers of ex vivo expanded NK cells is safe and can induce tumor regression in subgroups of cancer patients.

Past studies have shown that EwS cells are susceptible to NK cells. Additionally, in vitro experiments have unveiled a synergistic effect of the proteosome inhibitor bortezomib and NK cell tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) on EwS. In this study, we showed that ex vivo expanded NK cells are able to rapidly and efficiently kill EwS, primarily via release of cytotoxic granules triggered by relative lack of MHC class I on EwS, as well as signaling through the NKG2D, DNAM-1, and NKp30 NK cell receptors. Furthermore, we found that bortezomib priming of EwS resulted in increased expression of the TRAIL ligand death receptor 5 (DR5) and synergized with recombinant human TRAIL (rhTRAIL) to kill EwS. However, bortezomib pre-treatment did not result in higher NK cell lysis of EwS at four hours. This study gives us a better understanding of specific mechanisms governing NK cell targeting of EwS, which can potentially be harnessed for NK cell immunotherapy against Ewing’s sarcoma.