Presenter	Info	Lab PI	Category	Title	Funding Source and Year	Abstract
Iden, Marissa, PhD	Research Scientist II	Rader, Janet, MD	Translational	Defining HPV integration sites of unknown significance in invasive cervical cancer	NIH/NCI R01CA095713 (JSR) and NIH/NCI R01CA193343 (MJF)	Introduction: Integration of viral DNA into the host genome is thought to drive invasive and metastatic cervical cancer, yet little is known about how specific HPV integration events may alter the surrounding human DNA and activate and/or contribute to cervical carcinogenesis. Objective: The objective of this study was to begin to identify and fully characterize the genomic landscape of HPV integration events that drive cervical carcinogenesis in a large cohort of tumors from invasive cervical cancer (ICC) patients. Methods: We combined TCGA-'omics' data with short- and long-read sequencing of HPV-enriched DNA from matched samples to better understand the impact of HPV integration sites on disease pathogenesis. Results: Short-read sequencing of HPV-enriched tumor DNA provided highly accurate data, confirming all TCGA chimeric sites and identifying additional intergenic integrations. Our novel long-read sequencing approach provided high spatial resolution of HPV integration events and demonstrated the highly complex nature of these events. Conclusions: Our data demonstrate the success of our novel workflow that combines innovative sequencing technologies with TCGA data to define HPV integration sites, their underlying mechanisms, and associated clinical outcomes. Significance: Women with advanced or recurrent invasive cervical cancer (ICC) soon develop resistance to platinum-based chemotherapy and >90% die within 2 years. Moreover, few ICC biomarkers exist, as classic histologic type, tumor grade, and even HPV status are not useful in disease subtyping. Thus, it is critical to identify novel targets for new drugs to treat cervical cancer. Funding source: NCI R01CA095713 (JSR) and NCI R01CA193343 (MJF)
Izaguirre-Carbonell, Jesus, PhD	Postdoctoral Fellow	Zhu, Nan, PhD	Translational	Critical Role of Jumonji Domain of JMJD1C in MLL-rearranged leukemia	NIH/NCI R00CA168996	Introduction: JMJD1C, a member of the lysine demethylase 3 (KDM3) family, is aberrantly expressed in mouse mixed lineage leukemia (MLL) -AF9 leukemia stem cells (LSC) and in human MLL-rearranged leukemias (MLLr). We have shown previously that JMJD1C is required for self-renewal of AML LSCs but not normal hematopoietic stem cells (HSCs). Objective: The domains within JMJD1C that promote LSC self-renewal are unknown. We intended to uncover which JMJD1C domains are required for leukemia cell survival. Methods: We used clustered regularly-interspaced short palindromic repeats (CRISPR)/ CRISPR-associated protein-9 nuclease (Cas9) negative selection screening to elucidate the relevance of each domain. We subsequently performed in vivo studies to validate our findings. 10XGenomics single cell sequencing was used to study molecular mechanisms in leukemia. Finally, we performed genome-wide profiling by ChIP-seq to study JMJD1C enzymatic activity. Results: CRISPR negative selection screening identified the catalytic Jumonji domain (JmjC) and Zinc finger domain (ZFD) of JMJD1C as required for leukemia cell survival. Mutating JmjC and ZFD impaired colonegenic activity and proliferation, increased apoptosis and induced differentiation of MLL-AF9 leukemia cells. Furthermore, mutating these domains significantly prolonged survival of recipient mice in a mouse model of MLL-AF9. Single cell seq revealed increased activation of RAS/ mitogen-activated protein kinase (MAPK), Janus kinase (JAK)- signal transducer and activator of transcription (STAT) pathway in cells harboring the JmjC sgRNA.. We discovered that up-regulation of interleukin 3 (IL-3) receptor genes mediates increased activation of IL-3 signaling upon JMJD1C loss or mutation. Along the line, we observed resistance to JMJD1C loss in MLLr AML bearing activating RAS mutations suggesting that RAS pathway activation confers resistance to JMJD1C loss. Finally, we found that histone lysine 36 methylation (H3K36me) is a marker for JMJD1C activity at gene loci Conclusions: We discovered the functional importance of the JMJD1C JmjC domain in AML leukemogenesis and a novel interplay between JMJD1C and the IL-3 signaling pathway. Significance: Our results provide rational for the development of small molecule inhibitors against JmjC catalytic domain. Moreover, it underscores the importance of individualized therapy taking into consideration the mutation spectrum of the patients.

Johnson, Alexander	Medical Student	Knight, Jennifer, MD	Translational	Beta-adrenergic signaling and Rap1 expression and prenylation during hematopoietic cell transplantation: a randomized controlled trial of propranolol	National Cancer Institute (NCI) Contract Nos. HHSN261200800001E and R01 CA188871 and the NCI Network on Biobehavioral Pathways in Cancer; the National Center for Advancing Translational Sciences, National Institutes of Health (NIH), through Grant Numbers UL1TR001436 and KL2TR001438; and the Laura Gralton Philanthropic Fund.	Introduction: Preclinical research suggests that the sympathetic nervous system (SNS) innervates the bone marrow microenvironment, promoting the dissemination of hematopoietic malignancies via ß-adrenoreceptor–mediated pathways. Hematopoietic cell transplantation (HCT) recipients represent a population exposed to conditions of chronic ß-adrenergic signaling given the adverse immunological and social health risk factors they experience as part of their cancer treatment. ß-adrenergic signaling regulates multiple cellular processes that contribute to the initiation and progression of cancer. Specifically, past studies have demonstrated the role of ß-adrenergic signaling in upregulating genes involved in inflammation and downregulating genes involved in the antiviral response, resulting in increased relapse and decreased disease-free survival in HCT recipients. Furthermore, in vitro studies have shown that ß-adrenergic receptors on tumor cells increase the risk of metastatic spread by inhibiting the function of a small GTPase called Rap1, which normally functions to anchor tumor cells together through the process of prenylation. Rap1 regulates many cellular responses, and numerous studies have implicated Rap1 activation, dysregulation and cellular levels in a variety of cancers. Because of the diverse yet intersecting roles that Rap1 and ß-adrenergic signaling have in neoplastic processes, further investigation into how ß-adrenergic signaling modulates Rap1 levels and function is required. Objective: The objective of this study was to assess whether ß-blocker administration to individuals undergoing HCT increased Rap1 prenylation or decreased total Rap1 levels. Methods: We conducted a randomized controlled trial evaluating whether the nonselective ß-antagonist propranolol was effective in increasing Rap1 prenylation or decreasing total Rap1 levels in 25 individuals receiving an autologous HCT for multiple myeloma. Propranolol was administered for 1 week prior to and 4 weeks following transplant. Blood was collected at baseline, Day -2, and Day +28. Western Blot analysis of recipient's blood samples was conducted in order to determine levels of prenylated and total Rap1 in circulating polymorphonuclear cells. Twelve participants were randomized to the intervention and 13 to the control. Results: Relative to the control group, propranolol-treated patients did not show significantly increased levels of prenylated Rap1 or decreased levels of total Rap1. In both the control and propranolol-treated groups, Rap1 was almost entirely prenylated. There was a strong positive correlation between the two isoforms of Rap1 (Rap1A and Rap1B) in the control and propranolol-treated groups at baseline ($r=0.716$, $p=<0.0001$) and at different time points throughout the length of the study ($r=0.735$ to 0.856, $p=<0.0001$-0.0155). There was a significant association between platelet engraftment days and baseline Rap1B expression in both control and propranolol-treated groups ($p=0.0188$). There were no significant associations between neutrophil engraftment days and total Rap1 levels in both subject groups. Transplant did not significantly alter total Rap1 levels in either treatment group or overall. Conclusions: Peri-HCT propranolol administration does not detectably increase Rap1 prenylation or decrease total Rap1 levels in HCT recipients. This may suggest that ß-adrenergic signaling does not significantly affect the cell-cell anchoring function of Rap1 (via altering prenylation) or its total level. However, as there was minimal unprenylated Rap1 detected, the current analyses may not have captured the time period during which unprenylated Rap1 exists. Although this study suggests that ß-adrenergic signaling does not have a significant effect on Rap1 protein levels or function, future work investigating alternate timing and potential upstream ß-adrenergic-mediated changes in Rap1 gene expression is warranted.
Kerketta, Romica, PhD	Postdoctoral Fellow	Urrutia, Raul, MD	Translational	The Early Epigenomic Landscape of Oncogenic Kras Signaling in Pancreatic Cancer	NIH: 22104976	Introduction: Pancreatic ductal adenocarcinoma (PDAC) develops through accumulation of genetic alterations, with the KRAS oncogene being the earliest genetic mutation found, which drives the progression of preneoplastic pancreatic intraepithelial neoplasia (PanIN) lesions into carcinoma. Direct targeting of the KRAS gene has been clinically unsuccessful and the downstream impact that the constitutive activation of KRAS has on chromatin remains unknown. Objective: Our objective was to identify chromatin events downstream of oncogenic KRAS which can be clinically targeted. For this purpose, we investigated the earliest changes at the transcriptomic and epigenomic levels that occur following activation of this oncogene. Methods: Our in vitro pancreatic cell model was derived from a genetically engineered mouse, carrying a doxycycline-inducible KRASG12D transgene. At different time points following doxycycline treatment, western blot was used to evaluate levels of oncogenic KRAS. Subsequently, RNA, cross-linked chromatin and DNA were isolated for next generation sequencing (NGS). These NGS technologies included RNA sequencing (RNA-seq) for gene expression, chromatin immunoprecipitation sequencing (ChIP-seq) of a series of histone marks to assess active and silenced chromatin, and reduced representation bisulfite sequencing (RRBS) for DNA methylation. After sequencing, advanced bioinformatics tools were used to process, integrate and analyze changes in the gene pathways and epigenetic landscape. Results: Induction of oncogenic KRAS was confirmed by western blot using a G12D specific antibody. RNA-seq data indicated that following KRAS induction, genes involved in the regulation of epithelial to mesenchymal transition (EMT) and metabolic pathways were downregulated, while genes involved in KRAS signaling and cellular proliferation were upregulated. ChIP-seq revealed an increase in the deposition of histone marks associated with enhancers/super-enhancers (H3K27ac and H3K4me1), activated promoters (H3K4me3), and regions silenced by polycomb (H3K27me3). Integration of RNA-seq and ChIP-seq data demonstrated that up- or down-regulated genes also had corresponding alterations of the H3K27ac and H3K4me3 activating histone marks near their promoters. DNA methylation levels of several CpG islands were also altered following KRAS induction. Conclusions: Based on our results, exposure to oncogenic KRAS induced pancreatic cells to acquire a more epithelial-like phenotype with increased proliferation, which coincides with changes in the transcriptome and epigenome. RRBS indicated that KRAS induction resulted in differentially methylated regions across the genome. Through the analysis of histone marks, we observed a marked increase in active enhancers and super-enhancers, as measured by H3K27ac and H3K4me1 peaks, implicating the role of histone acetyltransferases as downstream epigenetic modulators of oncogenic KRAS signaling. Significance: Oncogenic KRAS modulates specific gene expression patterns and biological pathways. These changes are achieved by different epigenomic regulators, many for which drugs are being tested in clinical trials. Thus, these epigenomic modifications serve as potential therapeutic targets for mitigating the progression of pancreatic cancer. Funding source: 2210496 NIH R01 Urrutia

Lu, Tongtong	Graduate Student	Yu, Bing, PhD	Translational	Intraoperative imaging of breast tumor margins using a UV fluorescence scanning microscope	GHR funding; We Care Fund, MCW, Department of Surgery	Introduction: In 2018, it is estimated that there were over 265,000 women with newly diagnosed breast cancer in the United States, of whom about 50-75% underwent breast conserving surgery (BCS or lumpectomy). The goal of BCS is to completely remove the tumor with a rim/margin of normal breast tissue while preserving as much of the normal tissue as possible. Positive margin status (cancer cells found on the surface of the specimen) is a predictor of higher rates of local recurrence. Intraoperative margin detection helps to complete tumor excision at the first operation, which not only reduces the risk of recurrence but also reduce patient anxiety, optimize cosmesis, avoid delay for adjuvant therapy, and decrease costs associated with additional surgeries. FDA approved MarginProbe, fluorescence imaging, light-sheet microscopy, nonlinear microscopy, optical coherence tomography, optical spectroscopy, and photoacoustic microscopy have all been studied for intraoperative assessment of breast tumor margins. However, a margin tool that is capable of imaging all six margins of large lumpectomy specimens with both high resolution and fast speed (within 20 minutes) is yet to be developed. Objective: A margin tool that is capable of imaging all six margins of large lumpectomy specimens with both high resolution and fast speed (within 20 minutes). Methods: Deep ultraviolet (UV) light allows simultaneous excitation of multiple fluorophores at a penetration depth of only a few micrometers, and thus generating surface fluorescence images. We have developed an UV fluorescence scanning microscope (UV-FSM) for slide-free, high-resolution and rapid examination of freshly excised tumor specimens during BCS. The UV-FSM uses a deep UV LED for oblique back illumination of freshly excised breast tissues stained with propidium iodide and Eosin Y and motorized XYZ stages for mosaic scanning. Results: Fluorescence images of pure, grade 2 invasive ductal carcinoma (IDC) tissue, a mixed sample of grade 1, invasive lobular carcinoma (ILC) and normal tissue, are captured by a color CCD camera and then stitched together using Fiji (ImageJ). Conclusions: Both ILC and IDC images showed excellent contrasts from that of the normal cells in color, tissue texture, and cell density and shapes. Significance: These contrasts have been consistently observed in all samples (n=9) we have imaged so far, and thus may be utilized either qualitatively by a trained surgeon or quantitatively by a computer algorithm to detect positive margins of lumpectomy specimens during BCS. Funding source: GHR funding; We Care Fund, MCW, Department of Surgery
Singavi, Arun, MD	Instructor	Hari, Parameswaran, MD	Translational	Secondary Myeloid Malignancies after Autologous Stem Cell Transplantation for Multiple Myeloma are associated with a Distinct Mutational Profile	Sequencing of tissue samples was supported and completed by the Genomic Sciences and Precision Medicine Center at the Medical College of Wisconsin	Introduction: Patients (pts) diagnosed with multiple myeloma (MM) are at risk for developing secondary myeloid malignancies (SMM). The etiology of SMM-associated genetic alterations (GAs) is unclear. We hypothesized that the GAs present in MM-associated SMMs would have a distinct profile compared to de novo or other therapy related myeloid malignancies Objective: For MM pts who develop SMM, compare GAs at Autologous Stem Cell Transplant (ASCT) and at diagnosis of SMM; assess for presence of previously reported deleterious myeloid GAs. Methods: We retrospectively identified 9 MM pts with SMM post-ASCT. Autograft cells and SMM Fresh Frozen Plasma Extraction (FFPE) samples underwent whole exome sequencing. GAs with known clinical significance and high or moderate impact on the gene-encoded protein were included for analysis. Data was analyzed using SAS software. From literature review, we identified 89 reported known GAs (kmGAs) in myeloid malignancies. Results: 9 pts with MDS/AML were included (age 56-71) - 8 Auto samples and 9 SMM samples available. All pts received MM-directed induction therapy prior to ASCT (44% and 55% with lenalidomide/thalidomide or bortezomib containing regimens, respectively). Lenalidomide maintenance was utilized in 60% of pts. Of 118,614 GAs in all samples, 2074 GAs were included. Average mutational burden was similar between Auto and SMM samples. kmGAs in the ATM, FLT3, GATA2, GNAS, IDH1, JAK3, MRE11A, MYH9, NF1, NOTCH1, and SETBP1 genes were detected in this cohort. For paired samples (matched Auto and SMM samples for each pt), 1173 GAs with kmGAs of GATA2, SETBP1, and ATM were present. GATA2 and SETBP1 were present in 3 and 5 Auto samples, and 4 and 6 SMM samples, respectively. SETBP1 and GATA2 were present in paired samples for 3 and 1 pt, respectively. By contrast, 1667 GAs in the ATM, FLT3, GATA2, GNAS, IDH1, JAK3, MRE11A, NF1, NOTCH1, SETBP1 genes were present in SMM samples but not in their paired Auto sample. Of note, GATA2 and SETBP1 were present in 3 and 2 SMM pts, respectively, but not in their Auto sample. Variant allele frequency (VAF) for GATA2 was 0.33 in Auto and 0.30 in SMM. VAF for SETBP1 was 0.45 in Auto and 0.44 in SMM. Though TP53 GAs were found in the SMM samples, none were considered of high clinical significance. None of the pts harbored 17p abnormalities at diagnosis of MM, however 2 developed it at SMM. Conclusions: GATA2 and SETBP1 alterations (mostly frameshift) were seen in majority of our patients - both persistent from Auto and as new mutations with SMM. SETBP1 is an oncogene and implicated in myeloid malignancies. Nonetheless, within this limited cohort, we demonstrate that the mutational profile for pts with SMM is distinct from de-novo myeloid malignancies and the average mutational burden did not change from pre-transplant to the development of SMM. Significance: Targeted sequencing for the presence of these alterations, along with kmGAs, at diagnosis of MM is planned. This limited cohort suggests a distinct mutational profile for these pts with SMM. Funding source: Sequencing of tissue samples was supported and completed by the Genomic Sciences and Precision Medicine Center at the Medical College of Wisconsin

Sun, Fumou	Project Appointment Student	Janz, Siegfried	Translational	Osteolytic disease in IL-6 and Myc dependent mouse model of human myeloma	NIH/NCI R01CA151354	Introduction: Myeloma bone disease (MBD) is an important unmet medical need characterized by focal and generalized bone loss causing severe pain, pathological fractures, instability of the vertebral column, and medullary cord / spinal nerve root compression. MBD is a disease-defining feature of multiple myeloma, the second most common blood cancer in the United States. MBD is poorly recapitulated in genetically engineered mouse models (GEMMs) of human myeloma developed in the past. Objective: To address this shortcoming, we determined onset, incidence and severity of osteolytic disease in a new GEMM of human myeloma that was recently developed in our laboratory. The model is designated IL6Myc. It relies on deregulated expression of human IL-6 and mouse c-Myc to drive myeloma-like neoplasms and MBD-like disease in transgenic mice on the genetic background of BALB/c. Our long-term goal is to validate and use this model for preclinical studies on human MBD. Methods: We used whole-body ex vivo ¬µCT imaging to analyze MBD-like changes in IL6Myc-transgenic mice. Parameters of bone loss, such as bone volume and trabecular space and thickness were determined with the assistance of the BoneJ software tool. Bone-eating osteoclasts in tissue sections were enumerated using cytochemical detection of tartrate-resistant acid phosphatase (TRAP). ELISA was employed to measure serum levels of soluble receptor activator of nuclear factor kappa-B ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG). The abundance of IL-17 producing T helper cells (Th17) in the bone marrow was determined using flow cytometry. Results: We found that IL6Myc mice that harbor primary myeloma-like plasma cell tumors (PCTs) exhibit a pattern of skeletal decay that mimics important features of human MBD. Osteolytic disease was detected in 10 of 10 PCT-bearing IL6Myc mice and was more pronounced in long bones than axial skeleton and skull (Figure 1). Mechanistically, MBD-like changes in mice were caused, at least in part, by increased osteoclast-dependent bone resorption that led to heightened serum levels of TRAP and RANKL and reduced serum levels of OPG. Just like in patients with myeloma, bone disease in mice was associated with increased numbers of Th17 cells in the bone marrow. Conclusions: The main finding of this study is the pronounced proclivity of double-transgenic IL6Myc mice to MBD-like disease. The IL6Myc model holds great promise for the elucidation of the natural history of MBD and the design and testing of new approaches to the treatment and prevention of bone loss in patients with myeloma. Significance: IL6Myc-dependent bone disease occurs in a tumor microenvironment that contains a fully intact immune system. This makes the mouse model ideal for translational myeloma studies, especially for the preclinical development of immunotherapies that play a growing role in the clinical management of myeloma including MBD. Funding source: National Cancer Center - R01CA151354 [Janz]
Urrutia, Guillermo, MD	Postdoctoral Fellow	Lomberk, Gwen, PhD	Translational	Cell Cycle Dependent Inhibition of G9a Induces Cell Death via Replication Catastrophe in Pancreatic Cancer	NIH/NCI R01CA178627 and Advancing a Healthier Wisconsin Endowment (AHW)	Introduction: Pancreatic ductal adenocarcinoma (PDAC) presents a significant health burden and is the third leading cause of cancer-related deaths in the United States. Despite significant efforts to develop better therapeutics to treat PDAC, the 5-year survival rate for patients has improved only marginally. Thus, there remains an urgent need to further understand the molecular mechanisms underlying PDAC development to identify innovative therapeutic targets. Our laboratory is focused on utilizing epigenetic inhibitors for this purpose. Objective: The current study focuses on an innovative therapeutic approach for the treatment of PDAC based on leveraging the arrest of malignant cells at a time that shows increased sensitivity for the use of certain epigenomic inhibitors. Specifically, we sought to combine inhibition of Checkpoint kinase 1 (Chk1) and the G9a histone methyltransferase by using the small molecule inhibitors Prexasertib and BRD4770, respectively. In this way, we targeted simultaneously two pathways that are necessary for the stability of replication forks. Methods: The antitumor effects and molecular mechanisms of the combination were assessed on well-established L3.6 and patient derived PDAC cell lines by a variety of in vitro and in vivo models and assays, including cellular and clonogenic growth, FACS cell cycle analysis, BrdU incorporation strategies, comet assay, western blot, immunofluorescence, as well as xenografts and immunohistochemistry. Results: Using live cell imaging, we found that the growth of PDAC cells, both L3.6 and primary cell lines from PDAC patients, is reduced by the combined inhibition of Chk1 (Prexasertib) and G9a (BRD4770), achieving a synergistic effect. This result was recapitulated by clonogenic assays. Combination treatment led to a highly efficient induction of cell death that could not be reversed through addition of the pan-caspase inhibitor Z-VAD-FMK, indicating that the main mechanism involved in this process is not caspase-dependent. To determine the underlying cellular mechanism, we performed FACS analysis of cells treated with the combination which demonstrated a significant increase of cells in S-phase, as well as a substantial sub-G1 fraction, confirming cell death during this cell cycle stage. Reduced BrdU incorporation of treated cells further supported these observations. To understand the molecular mechanism, we evaluated the extent of DNA damage as measured by alkaline comet assay, confirming generation of ssDNA breaks. We found that in response to Chk1 and G9a combined inhibition PDAC cells activate the ATR-Chk1 axis, but fail at the Chk1 checkpoint leading to replication stress, as confirmed by ssDNA generation and phosphorylation of ATR, RPA32, CHK1 and H2A.X proteins. In vivo treatment of subcutaneous pancreatic cancer xenografts demonstrated that combined targeting of these pathways reduces tumor growth, which involves a reduction in proliferation observed by Ki67 staining along with an increase in the replication stress response and overall DNA damage. Conclusions: Our results demonstrate that targeting the epigenetic regulator G9a in combination with inhibition of the DNA damage response checkpoint offers a novel therapeutic approach for pancreatic cancer through triggering DNA replication catastrophe. Significance: There remains an urgent need to improve therapeutic approaches for the treatment of PDAC. Here, we demonstrate a synergistic effect in the combined targeting of Chk1, a key regulator of cell cycle transition in the DNA damage response pathway, and G9a, an epigenetic regulator of histone methylation necessary for reforming chromatin during DNA replication. The novelty of this approach relies on the fact that, in contrast to most studies which are based on the use of epigenomic drugs to modulate gene expression, we timed inhibition, through combination with checkpoint inhibitors, at a stage of the cell cycle to exert a highly

Xin, Gang, PhD	Research Scientist I	Cui, Weiguo, PhD	Translational	A Novel Immunotherapy Overcomes Antigen Escape and Prevents Relapse	R01	Introduction: One of the most common causes of resistance to adoptive cell transfer (ACT)-based immunotherapy is antigen escape, a mechanism exploited by cancer cells to allow the outgrowth of tumor variants that lose ACT-targeted antigens. According to recent estimates, antigen loss accounts for up to 40% of reported relapses in CAR T cell therapy. The ultimate strategy to overcome this challenge includes expansion of the immune response directed against new tumor antigens that were not initially targeted by ACT. Such a phenomenon, known as antigen spreading, has served as an important component of effective cancer vaccines and checkpoint blockade immunotherapies, however it is rarely achieved at a meaningful level in the setting of ACT therapy. Objective: The purpose of this study is to capitalize on this mechanism in order to combat antigen escape and improve ACT efficacy using our recently developed reenergized ACT (ReACT) therapy. ReACT combines ACT with a bacterial vaccine, which is known to effectively induce Batf3-driven dendritic cells (DCs), which specialize in cross-priming. We hypothesize that the initial ReACT therapy will mediate tumor destruction and result in the release of tumor antigens, which can in turn be taken up by infection-activated DCs to prime secondary tumor-specific T cells and expand antigen coverage. Methods: To simulate the phenomenon of antigen escape, we employed CRISPR-Cas9 technology to generate a B16-F10 tumor cell line that lacks expression of ACT-targeted antigen, gp100. One week after inoculation with this tumor, mice were treated with ReACT or ACT alone. Results: Compared to ACT alone therapy, ReACT induced significant tumor regression and even eradicated the tumor in 60% of mice (Figure 2). Using an elegant murine model to track endogenous tumor-reactive T cells, we provide solid evidence to show that this protection was mediated through a ReACT-recruited endogenous antitumor immune response against new tumor antigens. Mechanistically, our study further reveals that antigen spreading relies on ReACT-boosted Batf3-dependent DCs. More importantly, we found that ReACT therapy engages endogenous CD8 T cells to differentiate into memory cells, including tissue-resident memory cells that protect against both local and distant relapse. Conclusions: In conclusion, we have developed a promising approach to successfully overcome tumor antigen escape and provide a durable protection against recurrence. Significance: This approach highlights a previously unappreciated role of antigen spreading and holds great translational value in reducing resistance and enhancing clinical outcomes of ACT therapy. Funding source: R01,
Zimmermann, Michael, PhD	Director, Bioinformatics/Assistant Professor	Zimmermann, Michael, PhD	Translational	Process for molecular modeling and through next-generation sequencing of clinical cases	Advancing a Healthier Wisconsin	Introduction: Clinical applications of DNA-based testing using high-throughput technologies has led to the identification of a large number of novel variants, many of which lack prior clinical evidence, making their implications for the patient uncertain. For this reason, they are categorized as Variants of Uncertain Significance (VUS). We must move “beyond the base pairs” in order to gather and interpret genomics data, establish molecular mechanisms, and translate tumor genomics data into actionable knowledge. Objective: We are developing methods to better interpret genomic variants and uncover mechanisms. Methods: Computational tools for simulating the atomic-level effects of variants on protein structure and dynamics are well established, but have not achieved systematic use in clinical settings. We are applying molecular modeling and simulation to generate specific hypotheses for the molecular effects of VUS - information that is overlooked by current clinical guidelines. Because the dynamics of each protein differ from one another, we generate protein-specific metrics. We use established disease variants and polymorphisms as comparators for determining the significance and consistency of VUS-associated effects. Results: Here we present our process for molecular modeling and specific examples for VUS identified through next-generation sequencing of clinical cases presenting with undiagnosed diseases or in cancer. Functional validation using in vitro assays confirmed the effects predicted by modeling. Conclusions: We believe molecular modeling will become an increasingly important component in the process of interpreting the effects of human genetic variation. Significance: Genomic variants identified through clinical sequencing cannot be used in patient care until they can be interpreted. Thus, the tools we are developing to interpret genomic variants will be applicable to Funding source: New faculty startup and AHW