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<th>Presenter</th>
<th>Info</th>
<th>Lab PI</th>
<th>Category</th>
<th>Title</th>
<th>Funding Source and Year</th>
<th>Abstract</th>
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<td>Iden, Marissa, PhD</td>
<td>Research Scientist II</td>
<td>Rader, Janet, MD</td>
<td>Translational</td>
<td>Defining HPV integration sites of unknown significance in invasive cervical cancer</td>
<td>NIH/NCI R01CA095713 (JSR) and NIH/NCI R01CA193343 (MJF)</td>
<td>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 &gt;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)</td>
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<td>Izaguirre-Carbonell, Jesus, PhD</td>
<td>Postdoctoral Fellow</td>
<td>Zhu, Nan, PhD</td>
<td>Translational</td>
<td>Critical Role of Jumonji Domain of JMJ1C in MLL-rearranged leukemia</td>
<td>NIH/NCI R00CA168996</td>
<td>Introduction: JMJ1C, 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 JMJ1C is required for self-renewal of AML LSCs but not normal hematopoietic stem cells (HSCs). Objective: The domains within JMJ1C that promote LSC self-renewal are unknown. We intended to uncover which JMJ1C 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 JMJ1C enzymatic activity. Results: CRISPR negative selection screening identified the catalytic Jumonji domain (JmJC) and Zinc finger domain (ZFD) of JMJ1C as required for leukemia cell survival. Mutating JmJC and ZFD impaired clonogenic 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 JMJ1C loss or mutation. Along the line, we observed resistance to JMJ1C loss in MLLr AML bearing activating RAS mutations suggesting that RAS pathway activation confers resistance to JMJ1C loss. Finally, we found that histone lysine 36 methylation (H3K36me) is a marker for JMJ1C activity at gene loci Conclusions: We discovered the functional importance of the JMJ1C JmJC domain in AML leukemogenesis and a novel interplay between JMJ1C 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.</td>
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Introduction: Preclinical research suggests that the sympathetic nervous system (SNS) innervates the bone marrow microenvironment, promoting the dissemination of hematopoietic malignancies via β-adrenoreceptor–mediated pathways. Hematopoietic cell transplantation (HCT) recipients represent a population exposed to conditions of chronic β-adrenergic signaling given the adverse immunological and social health risk factors they experience as part of their cancer treatment. β-adrenergic signaling regulates multiple cellular processes that contribute to the initiation and progression of cancer. Specifically, past studies have demonstrated the role of β-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 β-adrenergic receptors on tumor cells increase the risk of tumor migration and survival. β-adrenergic signaling upregulates 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 β-adrenergic signaling have in neoplastic processes, further investigation into how β-adrenergic signaling modulates Rap1 levels and function is required.

Objective: The objective of this study was to assess whether β-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 β-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 β-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 β-adrenergic signaling does not have a significant effect on Rap1 protein levels or function, future work investigating alternate timing and potential upstream β-adrenergic-mediated changes in Rap1 gene expression is warranted.

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 Girollon Philanthropic Fund.

Introduction: Preclinical research suggests that the sympathetic nervous system (SNS) innervates the bone marrow microenvironment, promoting the dissemination of hematopoietic malignancies via β-adrenoreceptor–mediated pathways. Hematopoietic cell transplantation (HCT) recipients represent a population exposed to conditions of chronic β-adrenergic signaling given the adverse immunological and social health risk factors they experience as part of their cancer treatment. β-adrenergic signaling regulates multiple cellular processes that contribute to the initiation and progression of cancer. Specifically, past studies have demonstrated the role of β-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 β-adrenergic receptors on tumor cells increase the risk of tumor migration and survival. β-adrenergic signaling upregulates 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 β-adrenergic signaling have in neoplastic processes, further investigation into how β-adrenergic signaling modulates Rap1 levels and function is required.

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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 β-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 β-adrenergic signaling does not have a significant effect on Rap1 protein levels or function, future work investigating alternate timing and potential upstream β-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: 2Z104976

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: We performed a time course ex vivo model of KRAS activation in a rat pancreatic ductal cell line. 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 immuno precipitation 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: Our results indicate that 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: 2Z10496 NIH R01 Urrutia
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<tr>
<th>Name</th>
<th>Designation/Institution</th>
<th>Study Type</th>
<th>Description</th>
<th>Methods</th>
<th>Results</th>
<th>Conclusions</th>
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<tbody>
<tr>
<td>Lu, Tongtong</td>
<td>Graduate Student</td>
<td>Translational</td>
<td>Intraoperative imaging of breast tumor margins using a UV fluorescence scanning microscope. 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 reduces patient anxiety, optimize cosmesis, avoid delay for adjuvant therapy, and decrease costs associated with additional surgeries. FDA approved MarginProbe, a fluorescence imaging device, has shown promise in improving surgical margins. 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, were 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.</td>
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<td>Singavi, Arun, MD</td>
<td>Instructor, MD</td>
<td>Translational</td>
<td>Secondary Myeloid Malignancies after Autologous Stem Cell Transplantation for Multiple Myeloma are associated with a Distinct Mutational Profile. 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, MYH9, 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.</td>
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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 kappa‐Β 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: The IL6Myc model 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]
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<th>Research Scientist I</th>
<th>Translational</th>
<th>A Novel Immunotherapy Overcomes Antigen Escape and Prevents Relapse</th>
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| Xin, Gang, PhD        |               | 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, |

| Director, Bioinformatics/Assistant Professor | Translational | Process for molecular modeling and through next-generation sequencing of clinical cases |  |
| Zimmermann, Michael, PhD |               | 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 |