IMMUNOLOGY PROGRAM
STUDENT RETREAT

Sugar Land Town Square Marriott
Sugar Land, TX

May 15th-16th 2015

THE GRADUATE SCHOOL OF
BIOMEDICAL SCIENCES
GSBS
SYNERGY IN SCIENCE

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
**ITINERARY**

**Friday, May 15th**

10:00 am – 10:30 am  
Arrive at Sugar Land Marriott

11:00 am – 1:00 pm  
Poster Session  
- Mini-Posters  
- Full Posters  

12:00 pm – 1:00 pm  
Lunch (served during poster session)  

1:00 pm – 1:45 pm  
Student Talks  
- Krit Rithipichai  
- Ashvin Jaiswal  
- Alexandria Plumer

1:45 pm – 2:15 pm  
Coffee Break/Group Picture

2:15 pm – 3:00 pm  
Student Talks  
- Stephanie Dorta-Estremera  
- Todd Bartkowiak  
- Felix Nwajei

3:00 pm – 4:30 pm  
Games

4:30 pm – 5:30 pm  
Keynote Speaker  
Dr. Todd A. Fehniger, M.D./Ph.D  
Washington University School of Medicine

6:00 pm – 8:00 pm  
Dinner at *Escalante’s*

8:00 pm –  
Free for the night

**Saturday, May 16th**

8:00 am – 10:00 am  
Breakfast  

10:00 am – 10:30 am  
Awards
Our laboratory focuses on basic and translational NK cell biology, as well as lymphoma genomics. Basic NK Cell Biology: Natural killer (NK) cells are innate immune lymphocytes that are important for defense against infection, which also mediate anti-tumor responses. Projects will advance our fundamental knowledge of how NK cells develop, mature, and function. These studies will provide insight into how NK cells may play role in thwarting both infectious disease and malignant transformation. Translational NK Cell Research: Translational research integrates the basic and clinical science arenas. Here, we take new basic findings in NK cell biology and apply them in pre-clinical or clinical studies. Alternatively, we investigate NK cell numbers, phenotype, and function in correlative studies from patients being treated on clinical trials. Lymphoma Genetics: Lymphoma is a cancer of immune lymphocytes, most commonly B cells. We collaborate with the Washington University lymphoma clinical team and The Genome Institute at Washington University to use next-generation sequencing to identify novel mutations in lymphoma patients, and correlate these mutations with clinical outcomes.
Redefining BTLA’s function to redesign the TIL manufacturing strategy

Krit Ritthipichai¹,², Cara Haymaker¹, Minying Zhang¹, Lidiya Obertas³, Roza Nurieva³, Patrick Hwu¹, and Chantale Bernatchez¹

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(No abstract body disclosure due to pending IP)

Identifying Mechanisms of Acquired Resistance to Immunotherapy

Ashvin Jaiswal¹,², Midan Ai¹, Casey Ager¹,², Todd Bartkowiak¹,², Dhwani Haria¹, Pratha Budhani¹, Michael Davies³, David Hong⁴, James P Allison¹,², and Michael A. Curran¹,²

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Immunotherapies are shown to be very efficient in generating antitumor immune responses, but in some animals tumors still relapse. The molecular mechanisms which drive these tumor relapses are unclear. We are investigating the molecular mechanisms of tumor immune evasion in response to various immunotherapies in mouse models of melanoma. We hypothesize that tumors evolve resistance under immunotherapeutic pressure mediated by changes in gene expression which can be selected and propagated through serial passage and then identified and targeted. We are evolving immunotherapy resistant strains of B16 melanoma by using in vivo serial passage under immunotherapy induced selective pressure. Using gene expression profiling, we are studying the genetic mechanisms employed by melanoma tumor cells to evade the range of immunotherapies. Through multiple in vivo passages, we have already selected a B16 melanoma tumor line that has evolved almost 100% resistance to combination co-inhibitory blockade. After each passage under selective pressure of combination blockade, we have observed visible signs of increased angiogenesis correlating with enhanced therapy resistance. We are investigating the underlying mechanisms of resistance and neo-angiogenesis. By determining the pathways engaged by these tumors to develop resistance to tumor-specific immunity, we hope to hope to identify key new targets for immunotherapeutics interventions in melanoma patients.
Epithelial inducible resistance to viral and bacterial pneumonia

Plumer AK1,2, Leiva MM2, Kirkpatrick CT2, Pantaleon J2, Kulkarni V2, Evans SE1,2
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Pneumonia is the leading cause of premature death and disability in both healthy and immunocompromised patients. Our laboratory has previously demonstrated that we can broadly protect against pneumonias caused by a wide range of pathogens by pretreating mice with a novel combination of inhaled Toll-like receptor agonists (Pam2-ODN). This protection is characterized by a heretofore undescribed synergistic interaction of two ligands that would be anticipated to signal in an additive manner, resulting in both enhanced host survival and reduced pathogen burden. Moreover, previous in vivo and in vitro work revealed that lung epithelial function is necessary and sufficient for this inducible resistance. For example, when we disrupt Toll-like receptor signaling in the lung epithelium, Pam2-ODN-induced protection against lethal viral pneumonias is completely abrogated. Depletion of macrophages and neutrophils does not similarly impair inducible resistance. Subsequent studies revealed that immortalized mouse and human lung epithelial cell lines could be protected against viral infections in vitro by Pam2-ODN. Investigating whether similar protection could be induced in primary mouse and human epithelial cells, we observed a dose-dependent reduction of viral NP gene with Pam2-ODN treatment. SAA3, an acute-phase apolipoprotein is the most synergistically upregulated gene in epithelial cells following Pam2-ODN treatment. SAA3 is a purported antimicrobial peptide, and we have shown that it induces direct bacterial killing at physiological conditions in vitro. Preliminary in vivo studies show that SAA3−/− mice show a mild impairment of Pam2-ODN-induced protection. To determine whether the synergistic induction of SAA3 is essential to Pam2-ODN-induced protection, a larger study is necessary to confirm the phenotype. This information can help us both understand the mechanisms underlying inducible resistance and may help its clinical translation through biomarker identification.

Interferon γ-Producing Natural Killer Cells Stimulate Humoral Autoimmunity

Stephanie M. Dorta-Estremera1,2, Xinfang Huang3, Jingjing Li2, Wei Cao1,2
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In systemic autoimmunity, dysregulation of diverse immune cell types is thought to contribute to the production of autoantibodies. However, whether and how natural killer (NK) cells participate in the development of humoral autoimmunity is uncertain. In an inducible autoimmune model, we have observed heightened autoantibody development in mice containing dysregulated NK cells that hyper-produce interferon γ (IFNγ). Intriguingly, pathogenic IgG isotypes, e.g. IgG2a and IgG2b, constitute the self-reactive immunoglobulin repertoire, indicating a likely TH1 polarization. Given that type I IFN, i.e. IFNα/β, operates upstream of NK-IFNγ axis, we determined the role played by type I and type II IFN during autoimmune B cell development in vitro and in vivo. Interestingly, the addition of both IFNs enhanced autoantibody production by cultured autoimmune B cells. Moreover, co-culture of NK cells from autoimmune-prone mice enhanced the production of autoantibodies. Mice lacking neutrophils or reactive oxygen species display exaggerated IFNγ production by NK cells, enhanced T and B cell activation, and consequently autoantibody production. Conversely, IFNγ-deficient mice had defective germinal centers and failed to license autoimmunity. Hence, our findings suggest that IFNγ-producing NK cells may play an important role in promoting the development of humoral autoimmunity.

Despite KLRG1 expression, highly cytotoxic ThEO/TcEO polarized T cells efficiently establish immunologic memory
Targeting immune inhibitory (CTLA-4, PD-1) or stimulatory (OX-40, 4-1BB) co-receptors has proven a potent anti-cancer therapeutic strategy, eliciting robust anti-tumor T cell responses and compelling pre-clinical and clinical data in a variety of cancers. We have previously shown in pre-clinical models that 4-1BB agonist antibodies engender strong anti-tumor T cell responses, imparting enhanced survival benefit in addition to evoking durable tumor regression. This impressive anti-tumor effect is due, in part, to the unique ability of 4-1BB co-stimulation to differentiate a novel subset of T cells that acquires a highly cytotoxic effector profile typically only seen in NK cells. This subset—dubbed ThEO (CD4+) or TcEO (CD8+)—is driven by the T-box transcription factor Eomesodermin, and is further characterized by expression of the inhibitory receptor KLRG1. We show here that, despite their strong effector function and expression of several co-inhibitory receptors (PD-1, TIM-3, KLRG1), the ThEO/TcEO phenotype establishes immunological memory and recalls with high frequency upon secondary tumor challenge with potent anti-tumor effector function. Further, these cells manifest characteristics of central memory (Tcm), effector memory (Tem), as well as stem cell memory (Tscm), and reside in multiple memory niches. These findings provide mechanistic insight into the process of memory formation and give evidence for the capacity of ThEO/TcEO cells to prevent tumor recurrence.

Immune response toward brain metastasis depends on the neuronal chemokine fractalkine—CX3CR1 receptor axis

Felix Nwajei1,3, Figen Beceren-Braun1, Konrad Gabrusiewicz2, Meenakshi Shanmugasundaram1, Anna Zal1, Wuenye Wu4, Amy Heimberger2, and Tomasz Zal1
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4. Carleton College, Northfield, Minnesota, USA

The migratory dynamics of immune cells is critical for anti-tumor immune surveillance. However, how this process is regulated in brain tumors is poorly understood. We found that MCA-fibrosarcoma cancer cells formed lethal tumors in the brain of immune deficient Rag-KO mice but not in immune competent mice, suggesting adaptive immune surveillance. In immune competent multi-color fluorescent reporter mice, longitudinal intravital imaging via cranial windows revealed initial engraftment and growth of MCA cancer cells that was followed by tumor regression in concordance with T cell infiltration. Intratumoral T cell numbers and spatial localization correlated with that of CD11c+ dendritic cells. Depletion of CD11c+ DCs led to a dramatic decrease in T cell numbers and in T cells paralysis in tumor foci. Interestingly, MCA tumors progressed in the brains of mice deficient in CX3CR1—the unique receptor for fractalkine, the neuronal chemokine—coinciding with impeded recruitment of T cells to the micrometastases and altered intratumoral T cell motility patterns. Furthermore, MCA brain metastases also progressed in mice with a partial CX3CR1 deficiency further indicating that optimal signaling from fractalkine was critical for the immune surveillance of MCA metastases in the brain. Our results reveal a role for the adaptive immunity in brain metastasis surveillance and implicate tumor-associating CD11c+ DCs and fractalkine in regulating this process.

Students Presenting Full Posters

Soluble IL-15 complexes are generated after CD40 stimulation and VSV infection independent of Type I IFN signaling

Scott M. Anthony1,2 and Kimberly S. Schluns1,2
Interleukin (IL)-15 is a cytokine that promotes the development and homeostasis of a group of lymphocytes but is also significantly up-regulated in response to immune stimulation and during inflammation. Previous studies show that TLR stimulation and total-body irradiation (TBI) induce soluble IL-15Rα/IL-15 complexes (sIL-15), which are capable of mediating agonistic IL-15 effects in a paracrine manner. The effects of elevated IL-15 are well characterized, yet little is known about the signals inducing these complexes. We report that treatment with αCD40 Ab but not α41BB Ab led to systemic increases in sIL-15 complexes in vivo. BATF3 KO mice, which are deficient in CD8+ DCs, were impaired in the generation of sIL-15 complexes in response to αCD40 treatment, indicating an important role for CD8+ DCs in the generation of sIL-15 complexes in vivo. Interestingly, although CD40 stimulation or VSV infection enhanced systemic IFN-α protein expression, Type I IFN signaling was not required for the induction of sIL-15 complexes during either event. This is in contrast to the requirement for IFNR signaling in generating sIL-15 complexes after TBI or poly I:C. CD40 signaling during VSV infection was also not required for sIL-15 complex generation, as blockade with αCD40L exhibited no effect. Overall, this study indicates that sIL-15 complexes are induced in vivo by both type I IFN dependent and independent pathways.

**Manipulating Natural Killer Cell KIR Populations and NK Licensing ex vivo During Stimulation and Propagation**

**Jolie Schafer**1,2, Vladimir V. Senyukov2, Dean A. Lee1,2

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The Natural Killer (NK) cell receptor repertoire is molded through interactions with its environment. It is currently unclear whether licensing and molding of the KIR repertoire are dependent on each other. Furthermore it is unknown if human NK cells can undergo a re-licensing process when their environment is altered, as has been described following murine adoptive transfer. We investigated whether NK cells forced to interact with HLA ligands different from the original environment will alter both licensing and the KIR repertoire. NK cells were isolated from peripheral blood of donors with known HLA and KIR genotypes and expanded in the presence of the EBV-transformed B cell line 721.221 expressing no HLA, or expressing HLA B*5801, C*0702, or C*0401 to provide inhibitory signaling through KIR3DL1, KIR2DL2/3 and KIR2DL1, respectively. After expansion in vitro for 2 weeks in the presence of IL-2, and IL-15, NK cells were phenotyped by flow cytometry for expression of KIR3DL1, KIR2DL2/3 and KIR2DL1. Additionally the effector functions of NK cells expanded in different HLA environments were determined, wherein degranulation of KIR+ subsets were compared to determine if licensing was altered post expansion. Expansion of NK cells in an environment in which the inhibitory ligand (HLA) for a specific KIR was present decreased the percentage of NK cells expressing the inhibitory KIR, thus increasing the percentage of NK cells responsive to targets missing their inhibitory ligand compared to NK cells grown in a HLA negative environment. The effect of the HLA environment on the re-licensing of NK cells during ex vivo expansion is still being investigated.

**Regulation of Autoimmune Germinal Center Reactions in Lupus-Prone BXD2 Mice by Follicular Helper T Cells**

**Young Uk Kim**1,2, Hoyong Lim2, Ha Eun Jung2, Rick A. Wetsel1,2, Yeonseok Chung1,2

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BXD2 mice spontaneously develop autoantibodies and subsequent glomerulonephritis, offering a useful animal model to study autoimmune lupus. Although initial studies showed a critical contribution of IL-17 and Th17 cells in mediating autoimmune B cell responses in BXD2 mice, the role of follicular helper
T (Tfh) cells remains incompletely understood. We found that both the frequency of Th17 cells and the levels of IL-17 in circulation in BXD2 mice were comparable to those of wild-type. By contrast, the frequency of PD-1+CXCR5+ Tfh cells was significantly increased in BXD2 mice compared with wild-type mice, while the frequency of PD1+CXCR5+Foxp3+ follicular regulatory T (Tfr) cells was reduced in the former group. The frequency of Tfh cells rather than that of Th17 cells was positively correlated with the frequency of germinal center B cells as well as the levels of autoantibodies to dsDNA. More importantly, CXCR5+ CD4+ T cells isolated from BXD2 mice induced the production of IgG from naïve B cells in an IL-21-dependent manner, while CCR6+ CD4+ T cells failed to do so. These results together demonstrate that Tfh cells rather than Th17 cells contribute to the autoimmune germinal center reactions in BXD2 mice.

NKp46 as a Marker for Enabling Outbred Spontaneous Large Animal Models for NK Cell Therapy

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Introduction: The testing of adoptive NK cell therapies in animal models has been hindered by an inability to expand clinically sufficient numbers of NK cells from species other than humans, particularly in outbred animals used as spontaneous tumor models. The comparative canine model has several advantages over murine models including spontaneous development of many human-type cancers, a complete tumor microenvironment, and similar oncogenes and gene expression signatures. However, the expansion and identification of canine NK cells has been hindered by the lack of antibodies specific to canine NK cells. We set out to fill this gap in knowledge by establishing an expansion platform for canine NK cells and by investigating the utility of NKp46 as a marker for canine NK cells. Hypothesis: We hypothesize that canine NK cells will be defined by their expression of NKp46. Methods: Canine PBMC were expanded for 21 days with K562 Clone9.mbIL21 feeder cells. Expanded canine cells were sorted and selected for NK-like phenotype based on non-expression of T-cell, B-cell, and macrophage lineage markers. NK cell receptor expression was confirmed by RT-PCR. Cytotoxicity assays were conducted using a 4-hour calcein-release assay against canine cancer cell lines. A mouse monoclonal antibody specific to canine NKp46 was generated using L-cells expressing a canine NKp46:murine CD8α fusion protein as the immunogen. Results: Our expansion platform yields a mean fold expansion of ~ 2,6000 fold NK-like cells in 21 days. Purified canine NK-like cells demonstrated elevated expression of NKG2D, NKp30, NKp44, NKp80, and DNAM-1 compared to CD3+ canine T-cells, and supported the use of NKp46 as a surface protein for positive selection. Cytotoxicity assays demonstrated that canine NK-like cells possessed the ability to kill without prior sensitization. Flow cytometry screening of the canine NKp46 antibody clones is currently underway. Discussion: This is the first report of expanding clinically-significant numbers of non-human CD3-negative NK-like cells. This expansion platform may enable the testing of NK cells in a syngeneic tumor and host environment with spontaneous tumor development and supports the use of the comparative canine model for testing NK cell therapy in a wide-variety of cancers including but not limited to osteosarcoma, melanoma, glioblastoma, and non-Hodgkin’s lymphoma. Finally, this reports the first production of a canine specific monoclonal antibody to NKp46.
Students Presenting Mini-Posters

Characterizing combination checkpoint blockade therapy with intratumoral STING activation for potent immune rejection of solid tumors with minimal toxicity

Casey Ager\textsuperscript{1,2}, Midan Ai\textsuperscript{1}, Todd Bartkowiak\textsuperscript{1,2}, Ashvin R. Jaiswal\textsuperscript{1,2}, Dhwani Haria\textsuperscript{1}, Pratha Budhani\textsuperscript{1}, and Michael A. Curran\textsuperscript{1,2}

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With the advent of checkpoint blockade as a paradigm-shifting treatment modality for patients late-stage, chemorefractory disease, there is an urgent need to optimize the clinical utility of these drugs by rationally combining them in ways that enhance antitumor immunity without exacerbating side effects. We have proposed a novel combination of therapeutic antibodies - αCTLA-4, αPD-1, and α4-1BB – which we believe will synergize therapeutically while simultaneously ameliorating the side effects associated with each therapy in isolation. Additionally, we believe that augmenting this cocktail with intratumoral activation of the innate cytosolic nucleic acid detector STING will further bolster tumor rejection by repolarizing suppressive tumor myeloid populations, thus stimulating productive antigen-presenting activity. To address this hypothesis, I have investigated the efficacy of these therapies in an established bilateral ectopic model of prostate cancer (TRAMP-C2), and have shown that intratumoral STING activation displays some capacity to cooperate with local checkpoint blockade to cure both injected and distal, uninjected lesions. Mechanistically, I have studied the effect of STING agonist c-di-GMP in phenotypically repolarizing tumor-derived macrophages (TAMs) and MDSCs in vitro through upregulation of costimulatory B7-1 and B7-2 molecules. Analysis of the side effect profiles of these therapies in combination suggests an unexpected capacity for c-di-GMP to reduce hepatotoxicities associated with checkpoint blockade. Thus, combining αCTLA-4, αPD-1, and α4-1BB with STING agonist c-di-GMP may constitute a potent therapeutic cocktail capable of safely linking innate and adaptive immune activation within tumors for robust antitumor immunity.

Oncogenic signalling in cancer inhibits cytotoxic T-cells through modulation of both antigen presentation and co-inhibitory molecule expression

Sherille Bradley\textsuperscript{1,2}, Zeming Chen\textsuperscript{3}, Jahan S. Khalili\textsuperscript{2}, Shujuan Liu\textsuperscript{3}, Tania Rodriguez-Cruz\textsuperscript{2}, Brenda Melendez\textsuperscript{1,2}, Michael A. Davies\textsuperscript{2}, Jennifer A. Wargo\textsuperscript{2}, Patrick Hwu\textsuperscript{2}, Gregory Lizee\textsuperscript{1,2}

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Two major hallmarks of cancer are (1) the frequent presence of MAPK pathway-activating oncogenic mutations and (2) immune suppression within the tumor microenvironment. We show here that oncogenic BRAF kinase, mutated to a constitutively active form (V600E) in nearly half of cutaneous human melanomas, can drive the suppression of melanoma-specific cytotoxic T lymphocyte (CTL) antitumor activity through multiple mechanisms. BRAF(V600E) signaling in melanoma cells leads to a rapid re-distribution of MHC class I (MHC-I) molecules from the tumor cell surface into endocytic compartments, a process that we show is dependent upon a highly conserved serine phosphorylation site within the MHC-I cytoplasmic tail. In addition, oncogenic BRAF also induces melanoma cell IL-1 production that can upregulate the expression of programmed death (PD)-1 ligands PD-L1 and PD-L2 on tumor-associated fibroblasts, which in turn mediate suppression of CTL function. Thus, oncogene activation and immune suppression can be considered intimately related hallmarks of cancer.

Genetic and non-genetic approaches to modifying receptor acquisition and formation during Natural Killer cell differentiation from induced pluripotent stem cells
Selena Hernandez1,2, and Dean A Lee1,2

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Natural Killer (NK) cells have potent cytotoxic responses to tumors, but the tumor microenvironment may provide mechanisms to evade the immune system such as expression of TGF-beta and ADAM17, which suppress NK cell function directly and through the down regulation of activating, lead to reduced cytotoxicity thereby allowing tumor escape. ADAM17 is a transmembrane and secreted matrix metalloproteinase that regulates the density of cell surface molecules on lymphocytes by cleaving receptors from the membrane. Expression of ADAM17 can induce tumor formation, and can directly cleave pro-immune receptors such as CD16 and IL15Ra on NK cells. CD16 is FcRIII-gamma receptor that binds to the Fc portion of IgG antibodies mediating target cell killing through the induction of antibody-dependent cell mediated cytotoxicity (ADCC). Blocking ADAM17 has been shown to upregulate CD16 and restores cytotoxicity in a suppressive microenvironment. TGF-beta suppresses NK cell cytotoxicity directly and through downregulation of activating receptors such as NKG2D. TGF-beta is a secreted protein present in most cells but overproduced by many cancer cells resulting in immune evasion. We previously demonstrated that NK cells could be derived in large numbers from induced pluripotent stem cells (iPSC). Here, we propose that genetic modification of induced pluripotent stem cells (iPSC) will allow generation of NK cells resistant to the fore mentioned inhibiting effects by the tumor cell. iPSCs will be differentiated first into CD45+CD34+ hematopoietic progenitor cells and then cultured on a feeder system of OP9 mouse stromal cells expressing DLL1 that will induce NK cell differentiation. Knockout of TGF-beta receptor and ADAM17 will occur at the pluripotent stem cell phase prior to differentiation into multipotent progenitor cells. I therefore hypothesize that knocking out TGF-beta receptor and ADAM17 will maintain the cytotoxicity of NK cells and prevent tumor evasion.
Improving Identification and Prediction of Peptide Targets for Cancer Immunotherapy
Kyle Jackson$^{1,2}$, Jason Rosvik$^2$, David Hawke$^3$, Amjad Talukder$^2$, Brenda Melendez$^{1,2}$, Sherille Bradley$^{1,2}$, Greg Lizee$^{1,2}$

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Cytotoxic lymphocytes target infected or transformed cells via recognition of peptide-HLA complexes presented on the surface of all nucleated cells. Therefore predicting and discovering peptides upregulated on tumor cells relative to normal cells can lead to identification of novel targets for cancer immunotherapy. Mass spectrometry coupled with high pressure liquid chromatography is a powerful high-throughput method for identifying peptides eluted from tumor cells. This method, coupled with MHC prediction algorithms, can be used to predict and identify possible peptides for use in therapy. However there are several limitations with this approach including confidently predicting real peptides from large elutions, poor HLA prediction algorithms and identifying appropriate tumor specific targets. Therefore we are interested in improving the prediction and identification of peptide targets and in better understanding properties associated with immunogenic epitopes. In pursuit of this I have constructed lentiviral vectors expressing HLA-A03, HLA-A11, and HLA-A24, highly expressed HLA alleles in the global population which are not well studied. I transduced and overexpressed these alleles into a melanoma cell line, purified them by flow cytometry, purified HLA molecules from the cell surface and sent peptides for mass spectrometry analysis. We are still in the process of analyzing these data but we have found peptides from multiple HLA alleles for SLC45A2, a previously identified melanoma antigen that can now be targeted on multiple HLA alleles for different patients. We are also in the process of looking at other eluted peptides as potential tumor associated targets for use in immunotherapy. Lastly we are interested in using the high-confidence HLA specific peptide lists generated from this elution to create positive and negative selection methods to predict and identify peptides from patient peptide elution datasets that otherwise would not be pursued as peptide targets.
Cross-presentation is a source of neo-antigens for multiple myeloma immunotherapy

Alex Perakis\textsuperscript{1,2}, Haley Peters\textsuperscript{1}, Pariya Sukhumalchandra\textsuperscript{1}, Mao Zhang\textsuperscript{1}, Lisa St. John\textsuperscript{1}, David Hawke\textsuperscript{1}, Jeffrey Molldrem\textsuperscript{1,2}, Gheath Al-Atrash\textsuperscript{1,2}

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Despite advances in its management, multiple myeloma (MM) remains incurable and accounts for 20% of deaths from hematologic malignancies. Immune modulating drugs and immunotherapy have been shown to be beneficial in MM patients, highlighting the importance of the immune system in MM. One example of this involves the use of cytotoxic T-lymphocytes (CTL) that eliminate cancer cells by recognizing tumor-specific antigens (TSA) that are presented on tumor cell surface human leukocyte antigen (HLA) class I molecules. \textit{Endogenous} TSA are classically processed by tumor cells via the canonical antigen processing pathway that leads to their presentation on HLA class I, which causes them to become targets for killing by CTL. However through a mechanism known as \textit{cross-presentation}, \textit{exogenous} antigens also can be taken up from the tumor microenvironment and presented on HLA class I molecules. We have identified cross-presentation as a novel mechanism by which MM presents exogenous antigens, leading to an expanded repertoire of tumor antigens that can be targeted. Here we show that cross-presented antigens may be a novel immunotherapeutic strategy in MM. Using U266 multiple myeloma cells, we tested whether we could elute novel cross-presented peptides off the cell surface. Using an immunoprecipitation and mass spectrometry approach and have identified a handful of novel cross-presented peptides that appear to be promising targets.

Preclinical development of tumor infiltrating lymphocyte therapy for ovarian cancer

Donastas Sakellariou-Thompson\textsuperscript{1,2}, Cara Haymaker\textsuperscript{2}, Patrick Hwu\textsuperscript{2}, Chantale Bernatchez\textsuperscript{2}

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Immunotherapy has become an effective cancer therapy, particularly in the case of adoptive T-cell therapy. This treatment exploits the presence of tumor infiltrating lymphocytes (TIL) by exponentially expanding their numbers \textit{ex vivo} and re-infusing them into the patient. With the effectiveness of TIL therapy already well established in multiple phase II studies in melanoma, there is a push to translate it to other malignancies such as ovarian cancer (OvCa). The presence of TIL is correlated with greatly increased survival in OvCa suggesting that TIL effectively control the disease and providing a rationale to test TIL therapy in this disease setting. To test the feasibility, we characterized the immune component of OvCa, explored the ability to achieve TIL outgrowth & expansion, and tested their functionality. We found there is a robust, activated T-cell infiltrate that we can grow from OvCa tumor samples obtained pre- and post- chemotherapy. The addition of an agonistic anti-41BB antibody to the TIL culture augmented all TIL outgrowth as well as skewed the population towards mainly CD8+ T cells. Importantly, it rescued growth in cultures that did not grow with the standard culture method of IL-2 alone. Next, we found that we could rapidly expand the TIL to clinically relevant numbers with measurable anti-tumor capabilities of TIL post-expansion in the context of re-directed killing assays. In conclusion, while further \textit{in vivo} studies are needed, the initial \textit{in vitro} data suggest that TIL therapy for OvCa is feasible and could be a successful therapeutic option in the future.
We would like to thank Allison Heller, Janice Whiting, Misty Hajek and Todd Bartkowiak for their assistance in planning this year’s retreat, in addition to the Sugar Land Town Center Marriott for being kind hosts. Thank you also to our Program Director Ben Zhu for your support of this retreat.