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1. Tumor mutational burden and coexisting actionable mutations in Biliary Tract Cancers (BTC).

Reham Ali

Mutations in DNA repair pathway were identified in 13% of Biliary Tract Cancers (BTC). High Tumor mutational burden (TMB) tumors including melanoma, lung cancer and those with microsatellite instability (MSI-H) are associated with susceptibility to immune blockade using checkpoint inhibitors. TMB data in BTC is limited and its association with actionable somatic mutation (mut) profiles in BTC is unknown.

Comprehensive genomic profiling (CGP) of 309 formalin fixed paraffin embedded (FFPE) tissue blocks of BTC patients with a hybrid capture of all coding exons of 236 cancer related genes and 47 introns of 19 genes frequently rearranged in cancer was done using FoundationOne. Base substitutions, indels, gene fusion/rearrangements, TMB, and MSI status were assessed. TMB was calculated by counting mutations across a 1.25Mb region and classified into high (TMBH; ≥ 20 mut/Mb), intermediate (TMBI; 6 - 19mut/Mb) and low (TMBL; < 6mut/Mb). MSI (MSI-H) and Stable (MSS) status was assigned by a computational algorithm examining 114 intronic homopolymer loci. Patients with TMB > = 6 mut/Mb (N = 60) were included in the clinical correlative portion of this study.

Sixty patients with TMB ≥ 6 were identified out of 309 patients of which 9 (15%) were TMBH and 51 (85%) were TMBI. These included 3 (5%) MSI-H and 18 (30 %) MSS. The median age was 59 years (range: 29-86), 35 (58%) were females, majority of cases were intrahepatic cholangiocarcinoma (n = 31; 52%) and 28 (47%) presented with advanced disease at diagnosis. Twenty three (38%) patients had received radiation therapy, 28 (47%) surgery and 3 (5%) received immunotherapy. Most frequent coexisting mut seen was T53 (N = 35; 58%). APC mut was seen in 7 (12%) patients. DNA repair pathway muts (MSH6, BRCA1, BRCA2, ATM, MLH1, or MSH2 genes) were identified in 78% of TMBH versus 16% in TMBI cases (p < 0.0001). Frequency of PIK3CA mut differed significantly between TMBH and TMBI (44% vs 10%, p < 0.0001).Patients with TMBI had a significantly better median OS (110 weeks) as compared to TMBH (43 weeks) (p = 0.003).

A better understanding of TMB and associated actionable mutations in BTC may be of value for the management of BTC patients with targeted agents and immunotherapy.
2. Towards personalized structural analysis of peptide-HLA complexes for T-cell-based immunotherapy

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Immunotherapy represents a promising new avenue for cancer treatment, making use of the patient's own immune system to identify and eliminate cancer cells. The key molecule in this context is the class I human leukocyte antigen (HLA) receptor, which is capable of binding peptides derived from intracellular proteins and displaying them at the cell surface. The recognition of these peptide-HLA (pHLA) complexes by cytotoxic T-cells is the cornerstone of cellular immunity, enabling the elimination of infected or tumoral cells. T-cell-based immunotherapies against cancer, which leverage this mechanism, can greatly benefit from experimental and computational structural analyses of pHLA complexes. In fact, several attempts have been made to use molecular docking for such computational structural analyses. However, these attempts have been hindered by the fact that resolving pHLA complex structure remains too challenging for even state-of-the-art docking tools. To overcome these limitations, we developed an incremental meta-docking approach for structural prediction of pHLA complexes. Previous docking-based methods applied in this context used specific constraints or assumptions to reduce the complexity of this prediction problem, at the expense of generality. Our strategy, by contrast, makes no initial assumptions and can potentially be used to predict binding modes for any pHLA complex. Our method has been tested in a re-docking experiment, to reproduce the binding modes of 25 pHLA complexes whose crystal structures are available (i.e., whose binding modes are known). Our dataset includes some of the most prevalent human HLA receptors, bound to peptides with significantly different conformations. The average error for our predictions was only 1.92 angstroms, which is considered a valid reproduction. This study is a proof of concept that incremental docking strategies can lead to general geometry prediction of pHLA complexes, which in turn can be used to improve existing efforts to provide personalized T-cell-based immunotherapy against cancer.
3. Adenosine Receptor Gene and Protein Expression in the Non-Human Primate Eye

Krista Beach, Baskar Arumugam, Li-Fang Hung, Earl Smith, Lisa Ostrin

Adenosine receptor (ADOR) antagonists have been shown to slow progression of myopia (nearsightedness) by increasing scleral collagen fibril diameter and stiffening the sclera. However, the mechanism of action and the distribution of the four ADORs in the primate eye are unknown. This study examined the distribution of the ADORs in the normal monkey eye.

Eyes were enucleated from two male Rhesus monkeys (age 150 days). Eyecups were dissected into five petals. Two petals were separated into layers and flash-frozen for RNA analysis. RNA was analyzed using SYBR™ Green chemistry and custom-designed primers. Gene expression in ocular tissues was compared to gene expression in brain tissue, where ADORs are known to be abundant. The remaining petals were fixed, cryoprotected, flash-frozen, and cryosectioned. Sections were post-fixed, and immunostained (primary antibodies: ADORA1, ADORA2a, ADORA2b, and ADORA3 all at 1:100; secondary antibodies: AlexaFluor488, 1:200).

ADORAb gene expression was upregulated in the retina and choroid compared to brain. ADORA3 expression was upregulated in the choroid but not retina. ADORA1 and ADORA2a were equivalent in retina and brain and were downregulated in the choroid compared to the brain. All four ADORs showed high immunoreactivity in the ganglion cell layer and moderate immunoreactivity in photoreceptors. ADORA1 staining was highest in the retinal nerve fiber layer (RNFL), but absent in the choroid and sclera. ADORA2a staining was high in the RNFL and outer choroid, and moderate in scleral fibroblasts and retina. ADORA2b staining was high in isolated scleral fibroblasts, moderate in the synaptic layers of the retina, and absent in the choroid. ADORA3 staining was conspicuous in photoreceptor processes, and was moderate in the RNFL, the choroid, and in scleral fibroblasts.

Immunohistochemistry indicated differential patterns of expression of the four adenosine receptors in the retina, choroid, and sclera of the normal non-human primate eye, and RNA analysis confirmed these patterns in retina and choroid. The presence of ADORs in scleral fibroblasts may suggest a direct mechanism by which ADOR antagonists may prevent myopia.
4. Doppel expression in vascular endothelial cells promotes angiogenesis

Naze Gul Avci

In the central nervous system (CNS), the interaction between endothelial cell and extracellular environment is essential for the development of the new blood vessels and the brain and generates critical responses for CNS functions including pathologies such as cancer. However, little is known about how endothelial cells interacts with neurovascular cells and regulates the formation of a functional blood brain barrier and promotes angiogenesis. The GPI-linked cell surface protein Doppel, encoded by prion protein doublet (Prnd), is expressed in the vascular endothelium of the developing brain but is subsequently turned off in adult cerebral blood vessels. Recent studies have shown that Prnd expression is reactivated during GBM-induced angiogenesis, but its role is not fully understood. In this study, our hypothesis is that Doppel in vascular endothelial cells regulates angiogenesis and tumor development. Using pLOC lentivirus, Doppel was expressed in primary brain microvascular cells (HBMVECs). Inducing Doppel overexpression enhanced endothelial cell proliferation and migration in vitro. RPPA and western blot analysis of HBMVECs overexpressing Doppel regulate phosphorylation of angiogenic markers including AKT, ERK1/2, Src. Prmd knockout mouse and N-tva/RCAS tumor models were used for Doppel-dependent angiogenesis and microvasculature development in vivo. Laminin and CD31 stainings showed intense angiogenic vascular growth in wild type neo-natal and adult mice. Together, our preliminary data may suggest an important endothelial cell-specific role for Doppel in regulating signaling pathways during vascularization and angiogenesis.
5. Galectin-8 recognizes Mycobacterium tuberculosis-containing phagosomes to control infection

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Studies of the important human pathogen *Mycobacterium tuberculosis* (Mtb) have shown that during macrophage infection, permeabilization of the phagosome allows for cytosolic sensing of bacterial DNA. Recognition of this danger signal by the host leads to targeting of a population of Mtb to the selective autophagy pathway for killing. However, even without cytosolic DNA sensing, a sizeable portion of intracellular Mtb is targeted for killing by selective autophagy. This observation led us to investigate whether additional cytosolic danger signals are detected during Mtb infection. Our experiments reveal that the carbohydrate-binding protein galectin-8 is recruited to the Mtb-containing phagosome in an ESX-1 secretion system-dependent manner. This provides some of the best evidence to date that the Mtb-containing phagosome is extensively permeabilized, and its contents are readily accessible to cytosolic host proteins. These same galectin-8-positive Mtb phagosomes are decorated with selective autophagy markers (ubiquitin, LC3, etc.), suggesting a role for galectin-8 in marking or targeting Mtb for destruction in autolysosomes. Importantly, overexpression of galectin-8 leads to increased Mtb killing, and we are currently investigating the mechanisms by which galectin-8 may enhance selective autophagy and/or destruction of Mtb in autolysosomes. Together these data suggest that galectin-8 is a key sensor that detects damage to the Mtb-containing phagosome and promotes killing through selective autophagy.
6. Combinatorial Targeting of Leukemia and Stromal Cells Overcomes Fms-like Tyrosine Kinase 3 (FLT3) Inhibitor Resistance in Acute Myeloid Leukemia (AML)

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One-third of AML patients harbor FLT3 mutations (internal tandem duplications-ITDs or point mutations). FLT3-ITD patients have shorter remissions, early relapses and are refractory to salvage chemotherapy. Responses with single agent FLT3-ITD inhibitors have been short, due to acquired resistance: point mutation acquisition, MAPK activation by FLT3-ligand secreted by bone marrow (BM) stromal cells (MSCs), etc. Our previous data with E6201 (dual MEK1-FLT3 inhibitor) confirmed that E6201 could overcome stroma-mediated resistance, improved survival in vivo, and was active against secondary acquired FLT3-point mutations. The receptor for stromal SDF1α (CXCR4) is highly expressed in FLT3-ITD AML blasts, and is essential for leukemia-stroma interactions. Abolition of its surface expression in FLT3-ITD AML improves survival in mouse model, corroborating the critical role of CXCR4 in survival of FLT3-ITD AML. Thus, we hypothesized that combinatorial targeting of FLT3, MAPK and CXCR4 by E6201 and BL-8040 (peptidic CXCR4-antagonist) would synergistically improve therapeutic outcomes in FLT3-mutant AML.

In a leukemia/MSC co-culture system, the combination of E6201 (100nM) and BL-8040 (20uM) showed synergistic cytostatic (CI=0.04) and cytotoxic (CI=0.6) effects. In a disseminated, FLT3-ITD AML xenograft model, the combination of E6201 and BL-8040 markedly reduced tumor burden (by bioluminescence imaging) compared to single agents. While BL-8040 had single agent anti-leukemia activity, the combination with E6201 was statistically significantly (P-value<0.0001) more effective in reducing tumor burden, evidenced by reduced human CD45+ and GFP+ cells in BM and spleen (by flow cytometry and immunohistochemistry), compared to single agents. To verify BL-8040-mediated receptor occupancy, CXCR4 surface expression was quantified in double positive cells, showing a significant reduction in CXCR4 expression in both BL-8040 single agent and combination groups. The BL-8040 and E6201 combinatorial treatment also significantly improved survival in the mouse model (P-value<0.01).

We are analyzing signaling pathways in stroma-induced resistance using mass-spectrometry-based flow cytometry and western blotting of MSCs and AML cells grown alone and/or together. Since, both E6201 and BL-8040 have been studied in clinical trials individually with no hint of overlapping toxicity, this combination potentially presents a unique therapeutic strategy to simultaneously target FLT3 and stroma to overcome the resistance developed through MAPK and CXCR4 signaling in FLT3-mutant AML.
7. Nanofluidic drug-eluting seed for sustained intratumoral immunotherapy in triple negative breast cancer

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Conventional systemic immunotherapy administration often results in insufficient anti-tumor immune response and adverse side effects. Delivering immunotherapeutics intratumorally could maximize tumor exposure, elicit efficient anti-tumor immune response, and minimize toxicity. To fulfill the unmet clinical need for sustained local drug delivery to avoid repeated intratumoral injections, we developed a nanofluidic-based device for intratumoral drug delivery called the nanochannel drug eluting seed (nDES). Similar to brachytherapy seed insertion, the nDES is intratumorally implanted using a trocar method, and offers a clinical advantage of eluting immunotherapeutic drugs. The nDES utilizes nanochannels to passively control the diffusion of molecules, thereby resulting in constant and sustained drug delivery, without the need for pumps or further intervention by clinicians. In this study, we utilize the nDES to deliver immunotherapeutics intratumorally in the 4T1 orthotopic murine mammary carcinoma model, which recapitulates triple negative breast cancer. We demonstrate that nDES-mediated intratumoral release of agonist monoclonal antibodies, a-OX40 and a-CD40, results in potentiation of local and systemic anti-tumor immune response, as indicated by increased tumor infiltrating lymphocytes and macrophages in the tumor and spleen. Further, nDES-immunotherapy treated mice showed significant inhibition of tumor growth compared to control mice. We also demonstrate reduced toxicity via liver pathology analysis in nDES-immunotherapy treated mice compared to systemically-treated mice. Collectively, our study validates the nDES as an intratumoral drug delivery platform for sustained immunotherapeutic release to activate local and systemic anti-tumor immune response. This approach could be applied to a broad spectrum of therapeutic agents and extended for treatment of other cancers.
8. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer.

Julienne Carstens

The exact nature and dynamics of pancreatic ductal adenocarcinoma (PDAC) immune composition remains largely unknown. Desmoplasia is suggested to polarize PDAC immunity. Therefore, a comprehensive evaluation of the composition and distribution of desmoplastic elements and T cell infiltration is necessary to delineate their roles. Here we develop a novel computational imaging technology for the simultaneous evaluation of eight distinct markers, allowing for spatial analysis of distinct populations within the same section. We report a heterogeneous population of infiltrating T lymphocytes. Spatial distribution of cytotoxic T cells in proximity to cancer cells correlates with increased overall patient survival. Collagen-I and αSMA+ fibroblasts do not correlate with paucity in T cell accumulation, suggesting that PDAC desmoplasia may not be a simple physical barrier. Further exploration of this technology may improve our understanding of how specific stromal composition could impact T cell activity, with potential impact on the optimization of immune modulatory therapies.
9. Studying Protein Structure through Hydrogen Exchange and Conformational Sampling

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A protein's function is known to be modulated by changes in its three-dimensional structure. Studying this structure-function relationship requires gathering information about the protein's conformational space, i.e., the space of all possible states of the protein. Some information can be obtained experimentally, using techniques such as X-ray crystallography. Various computational methods, such as molecular dynamics, are also used to obtain structural information. However, experimentally observing and computationally modeling large proteins remain critical challenges for structural biology.

Our work aims to address these challenges by combining experimental and computational techniques to overcome their respective shortcomings. At one end of the experimental spectrum, X-ray crystallography yields atomic-resolution structural models, but presents strong limitations in terms of cost and applicability. At the other end of the spectrum, hydrogen-exchange monitoring is cheap and easier to implement, but cannot produce structural models because of its low resolution. To mitigate this issue, one side of our coupled approach consists of developing computational methods to complement such low-resolution experimental techniques. As these computational methods suffer from the curse of dimensionality when applied to large proteins, the other side of our coupled approach consists of guiding them with experimental data.

Our group has historically leveraged robotics-inspired techniques to model and analyze protein structure, by implementing coarse-grained conformational sampling methods to explore a protein's conformational space. We have developed a computational framework, named Structured Intuitive Move Selector (SIMS), which integrates sampling-based path-planning algorithms with the well-established Rosetta library for protein modeling.

Here, we present three outcomes of our coupled approach combining SIMS on the computational side and hydrogen exchange on the experimental side. First, we argue that using coarse-grained conformational sampling of protein structure improves the fit between computationally-generated conformations and experimental hydrogen-exchange data. Second, we show that our approach allows analyzing the variability of a protein's native state described by crystallographic and hydrogen-exchange data. Finally, we explain how to obtain an atomic-resolution structural model of a protein state for which only hydrogen-exchange data is available.
10. Does Intention Predict Well Physical Activity among Cancer Survivors?: The Intention-Behavior Gap

Dalnim Cho

**Background:** The Theory of Planned Behavior, which is one of the most cited theories in the domain of health, contends that behavioral intention is a central predictor of action. However, meta-analyses have demonstrated discrepancies between intention and health behaviors in many populations, which have not yet been systematically reviewed among cancer survivors. The present study conducted a meta-analysis to examine the intention-behavior association among cancer survivors.

**Methods:** Systematic search located 21 articles (25 tests, \( n = 4,153 \)), which met inclusion criteria. Correlations that correct only sampling error and those that correct measurement error (reliability of intention measures) in addition to sampling error were calculated using the random-effects method. Effect size was also separately calculated in four subgroups: 1) retrospective/cross-sectional studies; 2) prospective studies; 3) studies with shorter time since diagnosis; and 4) those with longer time since diagnosis.

**Results:** All studies that investigated relationships between intention and health-related behaviors were with respect to physical activity. Sample-weighted correlation \( (r_s) \) between intention and physical activity was .41 (medium effect size). After correcting measurement and sampling error, effect size \( (\rho) \) remained medium-to-large (.44). Intention-physical activity relations differed across subgroups of the sample, although confidence intervals (CIs) somewhat overlapped: the \( r_s \) was .50 (9 tests; 95% CI=.42 to .59) in retrospective/cross-sectional studies, whereas the \( r_s \) was .27 (15 tests; 95% CI=.10 to .45) in prospective studies. Also, the \( r_s \) was .48 in survivors with longer time since diagnosis (9 tests; 95% CI=.40 to .57), whereas the \( r_s \) was .29 in those with shorter time since diagnosis (8 tests; 95% CI=.09 to .50).

**Conclusion:** Results demonstrate that there is an intention-physical activity gap among cancer survivors. Increasing intention to potentiate an exercise intervention in this population might not be enough, and therefore, additional techniques will be required. The effect size variations across subgroups might be taken into account to develop interventions (i.e., increasing intention might be more effective in those with longer time since diagnosis), although cautious interpretation is required because of overlapping CIs and small number of studies in the analysis.
11. The role of miR17-92 in regulating thymus fate during development

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The thymus develops bilaterally from third pharyngeal pouch (3\textsuperscript{rd} pp) endoderm, which is initially patterned into separate thymus and parathyroid specific domains. The transcription factors FOXN1 and GCM2, expressed in 3\textsuperscript{rd} pp thymus-fated or parathyroid-fated domains respectively, are required for organ development, but do not specify organ fate. We are investigating the molecular pathways required to generate a functional thymus from 3\textsuperscript{rd} pp endodermal progenitors. We recently reported that \textit{Tbx1}, which is required for parathyroid development, negatively regulates thymus development by suppressing \textit{Foxn1} expression. We are currently using gain- and loss-of-function genetic models to test the hypothesis that \textit{Tbx1} expression in 3\textsuperscript{rd} pp endoderm is regulated by \textit{microRNA (miR)} 17-92. We find that TBX1 expression is expanded and FOXN1 expression is reduced in the 3\textsuperscript{rd} pp endoderm of \textit{miR17-92}\textsuperscript{null} embryos and that thymus organogenesis is severely impaired. We are currently targeting deletion of floxed \textit{miR17-92} to the endoderm using \textit{Sox17}\textsuperscript{Cre} to determine if the null phenotype is endoderm intrinsic. Similarly, \textit{Sox17}\textsuperscript{Cre} is being used to activate an \textit{R26miR17-92}\textsuperscript{OE} allele to determine the consequences of overexpressing \textit{miR17-92} in the 3\textsuperscript{rd} pp endoderm. These studies will clarify the role of \textit{miR17-92} in regulating \textit{Tbx1} expression and provide insight into molecular mechanisms that regulate 3\textsuperscript{rd} pp patterning and thymus organogenesis.

Qiao Chu and Qian Lu

Previous research indicates that expressive writing focused on emotional disclosure improves psychological well-being among Non-Hispanic White healthy and clinical populations. However, few studies have been conducted among Asian American breast cancer survivors. Because Asian culture tends to discourage emotional expression in comparison to American culture, it is likely that emotional disclosure would be less beneficial for the Asian American population. Moreover, Asian immigrants’ acculturation to American culture may also influence the efficacy of expressive writing. Ninety-six female Chinese American breast cancer survivors (37-77 years, $M_{age} = 54.5, SD_{age} = 7.9$) participated in a three-week expressive writing intervention program and were randomly assigned to three groups. Participants in the control or cancer-fact group wrote about their cancer diagnosis and treatment experience for three weeks. Participants in the self-regulation group wrote about their deepest thoughts and feelings on Week 1, their most stressful experience and coping strategies on Week 2, and the positive thoughts and feelings they experienced on Week 3. Participants in the emotional disclosure group wrote about their deepest thoughts and feelings for three weeks. Post-traumatic stress symptoms (PTSS) were measured at baseline, 1 month, 3 months, and 6 months following the intervention. We found that PTSS tended to decrease from baseline to post-intervention for the cancer-fact group but not for the self-regulation or emotional disclosure groups, although the changes of PTSS did not reach statistical significance. Interestingly, participants’ level of acculturation to American culture moderated the effect of expressive writing on avoidance symptoms ($p = .023$) and arousal symptoms ($p = .032$). At 3 months and 6 months following the intervention, highly acculturated participants benefited more (i.e., lower PTSS) from emotional disclosure than cancer-fact writing whereas less acculturated participants benefited more from cancer-fact writing than emotional disclosure. These findings have important implications for future research and intervention programs targeting the minority population in American society in that the design of an intervention should consider not only participants’ original cultural background but also participants’ level of acculturation to the mainstream culture.
13. Diversity and composition of the gut microbiome are associated with differential responses to anti-PD1-therapy in melanoma patients.

Vancheswaran Gopalakrishnan,

**Background:** Melanoma therapy has benefitted greatly from immune checkpoint blockade, although responses are variable and not always durable. Recent evidence in murine models suggests that modulation of the gut microbiome may enhance responses to immune checkpoint blockade. However, this has not been investigated in patients.

**Methods:** We collected buccal (n=105) and stool (n=53) samples from a cohort of anti-PD-1 treated metastatic melanoma patients (n=112). Patients were classified as either Responders (R) or Non-responders (NR) based on RECIST criteria. 16S rRNA, and whole-genome shotgun sequencing was performed to characterize the diversity, composition and functional capabilities of the microbiomes. Immune profiling via a 7-marker IHC panel of CD3, CD8, PD-1, PD-L1, Granzyme B, RORγT and FoxP3, flow cytometry and cytokine analysis were also performed in available tumors and serum samples at baseline.

**Results:** We observed significant differences in the diversity and composition of the gut microbiome in R versus NR to PD-1 blockade at baseline, but no clear differences were seen in buccal microbiomes. Specifically, R had a significantly higher alpha diversity compared to NR (p=0.017), and the Ruminococcaceae family of the Clostridiales order was enriched in R whereas the Bacteroidales order was enriched in NR. Immune profiling demonstrated significantly increased immune infiltrates in baseline tumor samples of R, with a positive correlation between CD8 T-cell density and abundance of specific bacteria enriched in R. Low diversity was also associated with elevated levels of chronic inflammation markers in the serum at baseline. Lastly, we saw a distinct metabolic signature in the gut microbiome with synthetic processes predominating in R and degradative processes predominating in NR.

**Conclusion:** Differences exist in the diversity and composition of the gut microbiome in R vs NR to anti-PD-1 therapy and these microbiota could bridge the gap between host metabolism and anti-tumor immunity. These results have far-reaching implications and suggest that modifications to the gut microbiome could potentially enhance therapeutic responses to immune checkpoint blockade.
14. Caspase-3 substrate for non-invasive imaging of apoptotic cell death by PET/CT.

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Regulation of cell death through apoptosis is critical for proper development and homeostasis of multicellular organisms. Apoptosis is generally triggered by either DNA damage or signaling through death receptors. Cell death signals result in the activation of executioner caspases which cleave hundreds of intracellular substrates and commit the cell to apoptotic death. Caspase-3 is a critical executioner caspase activated by both intrinsic and extrinsic signals. The preferred substrate sequence of caspase-3 is Asp-Glu-Val-Asp (DEVD) with cleavage occurring after the C-terminal aspartic acid. Currently, there is a lack of diagnostic options for observing acute cellular apoptosis from injury or cancer therapies. The ability to rapidly and non-invasively measure apoptotic cell death can inform future medical intervention.

We have developed novel caspase-3 substrates for non-invasive imaging of cellular death by fluorescence and PET-CT. Fluorescent substrates were cleaved by caspase-3 in vitro and accumulated in cisplatin treated cells, but not in caspase inhibitor-treated or untreated cells. Cold radiotracer derivatives were synthesized with peptide coupling followed by click chemistry and were screened for in vitro caspase hydrolysis by HPLC. The substrate 2MP-TbD-MeTE[¹⁸F], with both the highest in vitro caspase-3 cleavage rates and best caspase specificity, was then further developed as a PET/CT tracer. 2MP-TbD-MeTE[¹⁸F] was obtained at with high radiochemical yield and specific activity. This radiotracer accumulated in cisplatin-treated ovarian cancer cells in a caspase- and cisplatin concentration-dependent fashion. In addition, this probe accumulated in the liver of a Jo2-induced hepatic cytotoxicity model as compared to untreated controls. Our data demonstrates that 2MP-TbD-MeTE[¹⁸F] can provide immediate pharmacodynamic readout of acute apoptosis. This tracer could be used to model and treat apoptosis-related diseases or as a diagnostic for monitoring and directing treatment of acute injury.
15. Exercise ameliorates effects of pediatric cranial radiation therapy

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Cranial radiation therapy (CRT) is one of the most effective treatments available for pediatric brain cancer. However, use of CRT in a pediatric population results in neuronal stem cell destruction and damage to surrounding healthy tissue and vasculature required for normal functioning. As a result, survivors of pediatric brain cancer suffer from lifelong behavioral and social deficits. Given evidence of beneficial exercise effects in several neurodegenerative disorders, we used exercise as a radiomitigator along with our model of cranial CRT and assessed the behavior of rats using 5-choice serial reaction-time task (5-CSRTT) to test executive function and impulsivity and MRI to assess imaging signatures of the brain.

The study consisted of 4 groups, i.e. sedentary-SHAM (SS), S-CRT, exercise-S (E-S), and E-CRT. Fischer 344 rats were cranially irradiated at 31d with 5x4Gy x-ray. Ten days post-CRT, they were run freely in a wheel for 2hrs a day for 7w. Training for 5CSRTT begins at 3m post-CRT and rats are tested at 6m, 9m and 12m post-CRT. At the end of 5CSRTT, brains were collected for MRI (9.4T Bruker Avance BioSpec Spectrometer) and histology to look at myelin, gliosis and microglia.

CRT rats have lower body weight and size compared to SHAM rats. In 5CSRTT, S-CRT rats took a higher number of trials to meet stage 5 criteria of the task compared to SS rats. Most interestingly, E-CRT rats took a significantly lower number of trials to meet stage 5 criteria compared to S-CRT rats. MRI showed brain volumes of RT rats were significantly lower than SHAM, however exercise mitigated some deficits. ES rats had significantly higher brain volume compared to SS. There was no observed change in fractional anisotropy; however, there was an increase in fiber number with exercise. Myelin staining showed CRT affected the corpus callosum fiber tracts. The widths of these tracks were significantly thinner in RT rats compared to SHAM. Exercise abrogated these differences and E-CRT rats had significantly higher fiber thickness compared to S-CRT. Our results indicate effectiveness of exercise as a potential radiomitigator.
16. Ethanol upregulates Munc13-1: A possible implication in presynaptic physiology and alcoholism

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Munc13-1, a pre-synaptic protein plays an essential role in glutamatergic synapic vesicle priming in hippocampal neurons. Munc13-1 binds to alcohol and modulates alcohol self-administration in Drosophila. However, it is not known whether alcohol has any direct effect on the Munc13-1 expression and glutamate release. Here, we examined the effects of ethanol in different model systems. First, primary hippocampal neurons were treated with ethanol and we found that ethanol upregulated the expression of Munc13-1. We detected Munc13-1 expression together with unaltered vesicular glutamate transporter1 (VGLUT1), a marker of glutamatergic neurons, suggesting a possible role of Munc13-1 in maintaining glutamatergic synapses. To further extend our findings in vivo, we treated both wild type (Wt) C57BL/6 and heterozygous Munc13-1 knockout (Munc13-1+/-) mice with alcohol for 6 weeks (drinking in the dark paradigm, DID) and found that ethanol induced upregulation of Munc13-1 expression in VGLUT1 i.r. neurons in hippocampus and cerebellum of Wt mice, whereas, expression of VGLUT1 remain unaltered. Furthermore, ethanol also compensated for the loss of Munc13-1 expression in Munc13-1+/- mice. Interestingly, down regulated Munc13-1 expression was not rescued by ethanol in the striatum. Moreover, ethanol consumption interfered with the expression of glutamatergic NMDA and metabotropic receptors both at postsynaptic and presynaptic neurons. Taken together, we demonstrated ethanol-induced, brain region specific alterations of Munc13-1 and glutamate receptors, possibly interfering the glutamatergic neurotransmission. We conclude that Munc13-1 could be an essential target in alcoholism.
17. Functional Characterization of a Neomorphic Platelet-Derived Growth Factor Receptor Alpha Driver Mutation

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Genomic aberrations are the key drivers of tumorigenesis. Large scale genome sequencing projects reveal massive genomic mutations which consist of “drivers” that have functional consequences in promoting cancer progression and pathologically neutral “passenger”. Importantly, specific mutations do not only lead to gain (hypermorphic) or loss (hypomorphic) of protein function, but also gain-of-novel function (neomorphic) that could alter therapeutic liabilities. Thus, identification and functional characterization of neomorphic driver mutations are critically important in devising effective therapeutic strategies. By using our functional genomic assessment pipeline, we characterized 18 platelet-derived growth factor receptor alpha (PDGFRA) mutations identified from MD Anderson Cancer Center patients of different cancer types. We identified a strong activating extracellular domain mutation, Y288C, from glioblastoma that promoted cell survival and proliferation of Ba/F3 and MCF10A independent of IL-3 and EGF/insulin, respectively. Further, Y288C stimulated cell proliferation and conferred resistance to apoptosis in the absence of ligand and serum stimulation. In addition to proliferation, Y288C enhanced cell motility when compared with wild-type (WT). Although the majority of the Y288C protein was incomplete glycosylated, the mutation did not abolish cell surface expression and immunofluorescence staining and membrane fractionation showed a gain of additional localization of Y288C protein at the endoplasmic reticulum (ER) as well as Golgi apparatus which enhanced the stability of the receptor by preventing ligand-mediated degradation. Cell surface Y288C protein induced Akt and ERK activation under PDGF stimulation. Strikingly, Y288C was covalently dimerized at the ER and Golgi apparatus and constitutively activated STATs in addition to Akt and ERK signaling. Imatinib and other PDGFR inhibitors had limited activity against Y288C. In contrast, inhibition of Akt, ERK, and STAT3 signaling by specific siRNAs significantly impeded cell proliferation, suggesting that targeting Akt, ERK, or STAT3 signaling may be a better therapeutic strategy for patients with this mutation. Our data supports the importance of understanding the functional activities and neomorphic effects of mutations for precision cancer therapy.
18. Overcoming the immunosuppressive effects induced by chemother-apy and radiation by targeting the immune microenvironment

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Besides directly promoting tumor cell death, chemoradiotherapy (CRT) has been shown to induce anti-tumor immunity; however, increasing evidence suggests that CRT also negatively impacts immune response by promoting immunosuppressive mechanisms such as accumulation of Treg and MDSC. Thus, there is an urgent need for therapeutic strategies capable of reversing the immunosuppressive effects of solid tumors thereby rendering them more susceptible to chemoradiotherapy.

Toward this goal, we have developed a therapeutic regimen using two existing drugs, a selective iNOS inhibitor, L-NIL, and cyclophosphamide (CTX). These drugs inhibit the tumoral infiltration of MDSCs and Tregs, respectively. Furthermore, while CTX/L-NIL and CRT singlet treatments induce only modest tumor regressions, the combination of CRT and CTX/L-NIL display synergistic inhibition of tumor growth and significant survival improvements.

Thus, we hypothesized that CTX/L-NIL could reverse CRT-induced immunosuppressive effects by coordinate immune gene expression, leading to enhanced infiltration of a diverse pool of antigen-specific CD8 cytotoxic T cells.

To test this hypothesis, we optimized the MTEC tumor model, a murine oropharynx epithelial cell line transformed with HPV16 oncogenes (E6 and E7) and H-ras. This model was selected as a model of HPV-related head and neck cancer and its response to CRT has previously been shown to depend on an intact immune response.

Using this model, we will analyze immune-related gene expression changes induced after CTX/L-NIL treatment by NanoString Technologies’ immune profiling panel and observed an increase in CD8 T cells score and genes related to T cell function. These quantitative changes in CD8 T cell activity and niche were further confirmed by flow cytometry. Therefore, we anticipate that CTX/L-NIL therapy will induce a change of CD8 T cell niche, in favor of tumor antigen-specific effector CD8 T cells.

The increased in tumor-infiltrating activated CD8 T cells associated with beneficial responses suggests that CTX/L-NIL can enhance susceptibility of immune-refractory tumors to CRT. Our overall goal is to develop a simple therapeutic approach which can render solid tumors receptive to the anti-tumor immune response induced by chemoradiotherapy.
19. Targeting Nucleolin for better survival in diffuse large B-cell lymphoma

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Introduction: Diffuse large B cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL) and approximately 30% of the patients develop relapsed/refractory disease. ABC-DLBCL is characterized by chronically active B-cell receptor (BCR) signaling that can be modulated by Bruton’s tyrosine kinase (BTK) activity. Thus, BTK serves as an attractive therapeutic target in this type of B-cell malignancy. In phase I/II clinical trials, of single agent ibrutinib elicited an overall response rate of 68% in patients with relapsed/refractory MCL and 37% in ABC-DLBCL. However, in spite of these encouraging results, responses are generally incomplete due to drug-resistant mutants, acquired resistance is common. In this study, we have identified a compensatory pathway responsible resistance to ibrutinib and leads to survival benefits in ABC-DLBCL’s

Methods: Ibrutinib resistance (IR) in DLBCLs, syngeneic resistant cell lines were generated based on their sensitivity to ibrutinib by invitro culture of parental cell lines (PT) for prolonged period of time. Analysis of differentially expressed genes/key pathways were carried out and efficacy of targeted therapy for IR cells were determined.

Results: In comparison to PT versions of these cell lines, IR cells did not form clumps in suspension cultures, displayed irregular cell morphology, elevated colony formation. Western blots and gene expression profile data showed increased expression by IR cell lines of IAP family members survivin, cIAP2, and oncogenic BCL2. Reduced BTK expression, and enhanced PI3K-Akt activity was identified in IR cell lines. Analysis of PI3K isoforms revealed up-regulation of PI3Kβ with decreased expression of PTEN. Given the enhanced PI3K isoform expression with IR, we treated IR cell lines with PI3K inhibitor, and observed reduced survival and tumor growth in xenografts. Moreover PI3K inhibitor it showed a strong synergy with CHOP components.

In summary, our results showed a novel switch from chronic to tonic BCR dependency of ABC-DLBCL for their survival after gaining of ibrutinib resistance. The compensatory PI3K/Akt/mTOR axis was identified in IR-DLBCL, which provided BCR signaling independent survival benefits. By targeting the PI3K/Akt/mTOR axis with PI3K inhibitor in combination with CHOP might provide alternative approach for a better outcomes in ibrutinib-resistance cases.
20. 3D Local White Matter Integrity Analysis in Psychosis Spectrum Youths

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**Introduction**: Psychosis is a spectrum of disorders with many different etiologies or origins with most familiar terms such as schizophrenia. The psychosis prodrome is a prolonged phase with subthreshold symptoms preceding clinical diagnosis. It usually happens in youths and 20%-30% of the cases can be converted to psychosis spectrum (PS) [1-2]. Therefore, early identification of disorder is critical for optimizing therapeutic interventions. Although cognitive impairment is one of the core features in PS, whether such deficits are present in youths with less severe symptoms is unknown. Neuroimaging can be utilized to help establish the connections between phenotypic imaging biomarkers with the symptom-based classifications. Previous studies showed both structural and functional brain abnormalities in PS youths with traditional structural and functional MRI [3-4]. However, it is rarely known whether the integrity of white matter (WM) is affected by the disease. Here, we apply our newly developed diffusion-weighted framework, autoMATE (automated Multi-Atlas Tract Extraction), and novel statistical analysis algorithm, FADTTS (Functional Analysis of Diffusion Tensor Tract Statistics), to demonstrate the alterations of 3D profiles of diffusion derived parameters such as fractional anisotropy (FA) between PS children and typically developing (TD) comparators in a large-scale study.

**Methods**: Study participants were selected from the Philadelphia Neurodevelopmental Cohort [5], including 183 PS children (Male: 85, Female: 138; age: 185.7+/−31.9 months) and 259 TD children (Male: 121, Female: 138; age: 191.9+/−31.6 months). Diffusion-weight MRI was acquired with 64 diffusion-weighted volumes with b = 1000 s/mm² and 7 volumes with no diffusion. autoMATE [6] can automatically label whole-brain tractography into anatomically well-defined 18 major WM tracts, including anterior thalamic radiations, cingulums, corticospinal tracts, inferior fronto-occipital fasciculi, inferior longitudinal fasciculi, fornix, left arcuate fasciculus, and six segments of the corpus callosum projecting to the frontal lobes, precentral gyri, postcentral gyri, superior parietal lobes, temporal lobes, and occipital lobes. Here, we extracted these 18 WM tracts from each of the diffusion scans with autoMATE. autoMATE also can establish point-to-point correspondence for each tract over the entire cohort. The FA values at those corresponding points were then interpolated to build the 3D profiles, respectively. Then we performed a novel statistical analysis algorithm, FADTTS, a functional analysis method that associated diffusion parameters with a set of covariates of interests, such as age, sex, and diagnostic status [7]. It models the diffusion parameters as a mathematical function of the fiber point locations, instead of isolated values in the general linear model. Therefore, it generates more statistical power to interpret the results.

**Results**: Figure 1 shows the 3D FA profiles of the top three tracts that has the largest alterations in terms of the percentage of the fiber points whose p-value < 0.05 (false discovery rate [FDR] corrected) between PS and TD youths. The –log10 p-values correspond to the color bar. Redder colors indicate greater differences. The tracts were the corpus callosum section that connects postcentral gyri, right inferior fronto-occipital fasciculus, and right inferior longitudinal fasciculus, and the significant percentages were 27%, 24%, and 18%, respectively. The abnormalities of these tracts have been reported in patients with schizophrenia in previous studies [8]. However, our analysis was able to identify the specific 3D locations where the alterations occurred.

**Conclusion**: Here, we presented a large-scale study to examine the integrity of the major WM tracts in PS youths. The 3D locations of the FA alterations between the groups were identified. The proposed pipeline can also be extended to examine WM integrity in other neurological and neuropsychiatric diseases.
21. Harnessing additional binding sites to improve the binding specificity and activity of protein targeted ligands

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Multisite binding ligands consist of multiple copies of monomers that are connected through a linker. These ligands exhibit improved affinity to their target through avidity effect. However, these linker regions have not been used to find additional binding sites on the protein surface. These additional binding sites can potentially improve the ligand’s i) specificity towards protein by identifying target specific allosteric sites ii) affinity by forming multiple contacts with protein. Therefore, we plan to employ this strategy in two different applications. In first application, we will enhance specificity of heterobivalent ligand towards cancer specific EphrinA3 (EphA3) kinase by identifying EphA3 kinase specific allosteric sites using the linker. Our approach involves i) optimizing the length of linker connecting kinase inhibitor and allosteric peptide in heterobivalent ligand ii) then using linker to find additional binding hot spots by employing a combination of molecular modelling and combinatorial chemistry methods. So far, we identified an optimized the linker length by screening eleven heterobivalent ligands with different linker lengths using ELISA-like assay. In second application, we will improve the cell killing activity of peptide-peptoid hybrid targeting overexpressed plectin protein on cancer stem cells (CSC) by employing linker to i) simultaneously target multiple homologous subdomains ii) identify additional binding pockets in between subdomains using combinatorial chemistry. So far, we have identified the range of effective linker length needed to kill CSCs by screening five homodimers with different linker lengths using MTS assay.
22. Loss of phospholipase PLA2G6/PARK14 disrupts ceramide metabolism, retromer function, and causes a progressive loss of neuronal function

Guang Lin

Mutations in PLA2G6 cause neurodegenerative disorders in human, including autosomal recessive Infantile Neuroaxonal Dystrophy (INAD), Atypical NAD and early onset Parkinsonism (PARK14). Here we show that loss of iPLA2-VIA, the fly homologue of PLA2G6, reduces lifespan, increases bang sensitivity and affects synaptic transmission. Phospholipases typically hydrolyze glycerol phospholipids. Surprisingly, loss of iPLA2-VIA does not affect the phospholipid composition of brain tissue but rather disturbs sphingolipid metabolism. The resulting increase in ceramides and sphingolipids impairs the function of the retromer and lysosomes. Reducing the levels of ceramides with myriocin alleviates lysosome stress and suppresses neurodegenerative phenotypes. Similarly, enhancing retromer function with a drug (R55) or overexpressing key retromer components (Vps26 or Vps35), suppresses the loss of iPLA2-VIA-induced neurodegeneration. Our data indicate that loss of iPLA2-VIA disrupts sphingolipid metabolism and impairs retromer function via a negative feedback loop leading to the demise of neurons.
23. Dynamic Regulation of Nuclear Proteolysis Controls Cell Cycle Gene Expression in Multiple Myeloma

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The dynamic interaction of transcription factors (TFs) and co-regulators with promoters and enhancers allows cells to continuously adjust gene expression. Whereas the composition and binding of TFs at genomic sites is the focus of a widespread research effort, relatively little is known about how these complexes are being removed by the ubiquitin-proteasome system (UPS).

Multiple myeloma (MM), the second most common hematopoietic malignancy, has become a model disease for drugs that interfere with the UPS through either blocking or facilitating protein elimination. The proteasome inhibitor Velcade, for instance, has become first-line treatment in myeloma. Yet, our knowledge of how myeloma cells are killed by this drug is incomplete. Our research is focused on defining how proteolysis regulates transcriptional dynamics in this disease and how this impacts Velcade sensitivity.

The analysis of a panel of 264 MM patient dataset revealed that patients with high expression levels of genes necessary for centromere formation present a poorer survival when they were treated with Velcade. Following proteasome inhibition in the MM.1S cell line, we used next generation sequencing to identify unique gene clusters and evaluate epigenetic changes. Our findings reveal that a particular subset of genes involved in centromere formation and sister chromatid segregation are associated with nuclear protein turnover and transcriptionally repressed by proteasome inhibition. We thus screened six major repressor complexes in the patient dataset mentioned above and found only one corepressor and its corresponding deacetylase to be associated with significantly better patient survival. Proteasome inhibition also increased the recruitment of this particular deacetylase at the promoters of the centromere-forming genes. We are currently validating the mechanism of its stabilization by proteasome inhibition, with a particular emphasis on cell cycle gene regulation, to better understand how precise spatial proteosomal degradation at specific genomic locations impacts myeloma proliferation.

This research project will contribute to our understanding of epigenetic and transcriptional dynamics in MM. With our focus on the continuously changing abundance of TF and corepressors at cell cycle promoters, we seek to unlock new attractive pathways for molecular therapy, as well as identify more specific targets for treatment compared to blunt proteasome inhibition.
24. Genetic deletion of Snail or Twist inhibits endothelial–to–mesenchymal transition and attenuates vascular damage leading to amelioration of kidney fibrosis

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Kidney fibrosis consists of the progressive scarring of the kidney caused by a maladaptive tissue repair response to persistent damaging insults which initiate a cascade of events resulting in inflammatory pathway activation, tubular cell death, impaired microcirculation, myofibroblasts accumulation and extensive extracellular matrix deposition, eventually leading to the loss of renal functionality. One of the hallmarks of kidney fibrosis is vascular damage. It is characterized by defective angiogenesis, microvasculature rarefaction and vascular leakage, which lead to hypoxia and induction of profibrotic responses. Renal endothelial cells acquire mesenchymal features through a process known as endothelial–to–mesenchymal transition (EndMT). EndMT has been shown to contribute to a small fraction of interstitial myofibroblasts, however the functional consequences of EndMT in kidney fibrosis have not been explored. We generated novel mouse models that enable genetic ablation of EndMT through deleting two mesenchymal-driving transcription factors specifically in endothelial cells. Mice with Snail and Twist conditional knock–out alleles (Snail\textsuperscript{L/L} and Twist\textsuperscript{L/L}, respectively) were bred with mice expressing Cre recombinase under a tamoxifen–inducible, endothelial–specific, promoter (Cdh5-Cre\textsuperscript{ERT2}) to obtain wild-type and Snail (Snail\textsuperscript{End–cKO}) or Twist (Twist\textsuperscript{End–cKO}) conditional knock-out mice (cKO). When kidney fibrosis was induced by unilateral ureteral obstruction, fibrotic kidneys from Snail\textsuperscript{End–cKO} and Twist\textsuperscript{End–cKO} mice displayed an improved fibrotic response, characterized by increased number of healthy tubules, reduced collagen deposition and reduced myofibroblasts accumulation compared to wild-type fibrotic kidneys. We found a similar improvement of the fibrotic outcome in a folic acid-induced nephropathy model. Importantly, we observed that inhibition of EndMT decreased vascular leakage in the peritubular vessels and reduced hypoxia in the tubular epithelial cells. Collectively our results demonstrate that EndMT functionally contributes to the development of kidney fibrosis by affecting endothelial cell barrier properties. Targeting EndMT may represent a new effective therapeutic strategy for kidney fibrosis.
25. A Cell-Based Screen for Poor Prognosis Ovarian Cancer Chemotherapy

**Background and Significance:** LIN28, an RNA binding protein, promotes tumorigenesis by suppressing the biogenesis of the tumor suppressor let-7 microRNA family. LIN28A expression correlates with poor prognosis and promotes drug resistance in ovarian cancer patients, making the LIN28-let-7 pathway an attractive target for chemotherapy. Also, LIN28A inhibition caused regression of established xenograft tumors. The overall goal of this study was to identify compounds that restore let-7 levels in LIN28A positive ovarian cancer cells that hold promise for improving prognosis for patients bearing LIN28A positive tumors.

**Methods:** To identify pharmacologically active small molecules, we developed a cell-based dual luciferase let-7 reporter in the LIN28A-positive ovarian cancer cell line, OVK-18. Elevated let-7 decreases nanoluciferase whose expression is regulated by the incorporation of the 3' UTR of the *HMGA2* gene that has multiple let-7 binding sites. We performed a primary screen and counterscreen (to rule out false positive hits) of approximately 3000 small molecules comprising the Prestwick and LOPAC\textsuperscript{1280} Libraries. The top hits for each library were validated for restoration of let-7 levels by RT-qPCR.

**Results:** We obtained a Z score of 0.7, indicating that the assay was robust enough for screening. The screens identified Temozolomide, Nifedipene and Ketoprofen as lead molecules for LIN28A chemotherapy. By RT-qPCR, we determined that exposure to these compounds at a dose of 10 \( \mu \text{M} \) for 72 hours resulted in a near two-fold increase in let-7 levels. Furthermore, by western blot, we found a dose-dependent decrease in the downstream targets of let-7 such as HMGA2, c-MYC, Cyclin D1 as well as LIN28A.

**Conclusion and Discussion:** This research is the first study to utilize a cell-based screen to target LIN28A-positive cancer cells with small molecules that may hold promise as a new therapy. This system represents a valuable tool for cancer drug discovery because it overcomes constraints of biochemical screens.

**Global Impact of Research:** Overall, this study is the requisite first step to a new drug class with effectiveness not only for poor prognosis ovarian cancers but also \(~15\%\) of other cancers characterized by LIN28 expression.
Chemotherapy-induced peripheral neurotoxicity is among the most common dose-limiting side-effects of cancer treatment, which not only hampers effective cancer therapy, but also greatly reduces the quality of life for cancer survivors. Currently, there is no FDA-approved treatment available. Histone deacetylase 6 (HDAC6) is a microtubule-associated deacetylase whose function includes regulation of α-tubulin-dependent intracellular mitochondrial transport. Inhibition of HDAC6 has been shown protective in several neurological disorders. Here we examined the effect of HDAC6 inhibition on established cisplatin-induced peripheral neuropathy and cisplatin-induced cognitive impairments in mice. We used a novel HDAC6 inhibitor ACY-1083, which readily crosses the blood-brain barrier and shows 260-fold selectivity towards HDAC6 versus other HDACs. The HDAC6 inhibitor was injected intraperitoneally for 2 weeks after termination of cisplatin treatment. Our results demonstrate that HDAC6 inhibition completely reversed already existing cisplatin-induced mechanical allodynia, spontaneous pain, numbness, and cognitive deficits. It also restored the loss of intra-epidermal nerve fiber density in cisplatin-treated mice. Mechanistically, treatment with the HDAC6 inhibitor increased α-tubulin acetylation. In addition, HDAC6 inhibition restored the cisplatin-induced reduction in mitochondrial bioenergetics and mitochondrial content in the tibial nerve, indicating increased mitochondrial transport. At a later time point, dorsal root ganglion mitochondrial bioenergetics also improved. Cisplatin-induced changes in mitochondrial morphology and bioenergetics in the synaptosomes were also restored by the HDAC6 inhibitor. Our results demonstrate that pharmacological inhibition of HDAC6 completely reverses established cisplatin-induced peripheral neuropathy and cognitive impairments, and the protective effects were associated with normalization of mitochondrial function. These results are especially promising because HDAC6 inhibitors are currently used in clinical trials as an add-on cancer therapy, highlighting the potential for clinical translation of our findings.
The FDA has begun to expand the approved uses of immune checkpoint blockade antibodies targeting CTLA-4 and PD-1. Blocking either checkpoint has shown significant responses in patients with cancer. While αPD-1 therapy has a greater response rate according to RECIST criteria, recent reports have suggested responses to αPD-1 may not be as durable as αCTLA-4. 25% of patients who responded to αPD-1 have tumor relapse within 24 months. In contrast, the 3-year survival rate of patients treated with αCTLA-4 is at least ~25% suggesting a durable response. The mechanism which leads to relapse following αPD-1 treatment in tumor models is not clear. The goal of this project is to understand how immunotherapies shape memory T-cell formation and how that relates to the mechanism of tumor relapse.

To test whether αCTLA-4 or αPD-1 can induce a better memory T-cell response, mice were vaccinated with irradiated B16F10 murine melanoma cells and treated αCTLA-4 or αPD-1. Mice were re-challenged with B16F10 after 80 days. Although both αCTLA-4 and αPD-1 improved tumor rejection compared with controls, αCTLA-4 treated mice exhibited superior tumor control compared to αPD-1 suggesting the memory T-cell response mediated by αCTLA-4 is more durable. To test if this memory response is antigen-specific, mice were re-challenged with unrelated MC38 or 3LL cancer cells. The antibody treated groups did not show improved antitumor effect compared with vaccine control. To test whether the frequency of memory T-cells recruited to the re-challenged tumor could affect memory T-cell response, antigen-specific pmel-1 T-cells were infused to mice following vaccination with αCTLA-4 or αPD-1. Our result suggested that there were more tumor-infiltrating pmel-1 T-cells in the αCTLA-4 treated group compared to the αPD-1 treated group. In order to augment the durability of αPD-1 treatment, αPD-1 was combined with αCTLA-4 following vaccination. The combined treatment group has superior antitumor response compared to that with αPD-1 and overlapped with the αCTLA-4 treated group during re-challenge, indicating that the effect of the combined treatment is dominated by αCTLA-4.
28. Inhibiting Sphingosine Kinase 2 Mitigates Mutant Huntingtin-Induced Neurodegeneration

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Huntington disease (HD) is the most common inherited neurodegenerative disorder. It has no cure. The protein huntingtin causes HD, and mutations to it confer toxic functions to the protein that lead to neurodegeneration. Thus, identifying modifiers of mutant huntingtin-mediated neurotoxicity might be a therapeutic strategy for HD. Sphingosine kinases 1 (SK1) and 2 (SK2) synthesize sphingosine-1-phosphate (S1P), a bioactive lipid messenger critically involved in cell survival. Here, we found that SK2 is nuclear in primary neurons and, unexpectedly, when overexpressed, SK2 is neurotoxic in a dose-dependent manner. SK2 also promotes DNA double-strand breaks in cultured primary neurons. We found that SK2 is hyperphosphorylated in the brain samples from a model of HD, the BACHD mice. These data suggest that the SK2 pathway may be a part of a pathogenic pathway in HD. ABC294640, an inhibitor of SK2, reduces DNA damage in neurons and increases survival in two neuron models of HD. Our results identify a novel regulator of mutant huntingtin-mediated neurotoxicity and provide a new target for developing therapies for HD.
Co-existence and functional interdependence of angiotensin II type 2 receptor and receptor Mas in obese Zucker rat kidney.

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The actions of angiotensin II type 2 receptor (AT₂R) and the receptor Mas (MasR) are complex but show similar pro-natriuretic function; particularly AT₂R expression and natriuretic function are enhanced in obese/diabetic rat kidney. In light of previous reports, we tested hypothesis that AT₂R and MasR are interdependent to produce natriuresis in obese rats due to potential physical interaction. Infusion of AT₂R agonist C21 (5 µg/kg/min) in obese Zucker rats (OZR) increased urine flow (UF) and urinary Na-excretion (UNaV) which were attenuated by simultaneous infusion of the AT₂R antagonist PD123319 (50 µg/kg/min) or the MasR antagonist A-779 (50 µg/kg/min). Similarly, infusion of MasR agonist Ang-(1-7) (110 fmol/kg/min) in OZR increased UF and UNaV, which were attenuated by simultaneous infusion of A-779 or PD123319. Dual labeling of AT₂R and MasR in OZR kidney slices revealed four-fold colocalization of AT₂R and MasR (9.83 ± 2.29 vs. 2.50 ± 1.00 dual labeled cells per 1600 µm²) compared with lean rats in which AT₂R is not natriuretic. Moreover, the AT₂R also was found to be co-immunoprecipitated with MasR in cortical homogenate of OZR. Immunoblotting of AT₂R and MasR after disulfide cross-linking with zero length oxidative cross-linker cupric-phenanthroline in cortical homogenate and glucose (25 mM)-treated HK-2 cells shifted the bands upward with overlapping migration for their complexes which were sensitive to β-mercaptoethanol. Collectively, results implicate –SH group and hyperglycemic oxidative stress in physical interaction and functional interdependency between AT₂R and MasR, which will be critical for the low expressing receptors to exert natriuretic response in obesity.
30. Prematurity Blunts Feeding-Induced Stimulation of Translation Initiation Signaling and Protein Synthesis in Muscle of a Neonatal Piglet Model

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Background: Preterm infants commonly experience extrauterine growth faltering. Growth restriction during the neonatal period may contribute to short- and long-term morbidities. Objective: To determine how preterm birth alters the protein anabolic response of muscle to feeding in a neonatal piglet model.

Methods: Piglets delivered by Cesarean section at term (112 d) or preterm (103 d) gestation were immediately fitted with a jugular catheter for delivery of total parenteral nutrition (240 mL/kg/d). Following a 4 hr fast at 3 d of age, pigs were fasted one additional hour or fed an enteral elemental meal (4 groups: PF, preterm fasted; PP, preterm postprandial; TF, term fasted; TP, term postprandial). Flooding dose of L[4-³H]phenylalanine was injected at 30 min relative to the meal for measurement of protein synthesis, and tissues collected at 60 min.

Results: Birth weight was lower (P<0.01) in preterm (n=48, 4 litters) vs. term pigs (n=42, 4 litters). Body weight gain was also lower (g/kg/d; P=0.01) in preterm vs. term pigs despite equivalent nutrient delivery. Gestational age at birth (GAB) did not affect fasting plasma glucose or insulin, but plasma glucose and insulin rose more slowly and reached a lower peak after a meal in preterm vs. term pigs. Feeding increased longissimus dorsi (LD) muscle fractional protein synthesis rates in both GAB groups, but the response was lower in preterm vs. term pigs (PF=TF<PP<TP; P<0.01). Muscle PKB phosphorylation followed a similar pattern (PF=TF<PP<TP; P<0.01), indicating blunted insulin signaling pathway activation in preterm pigs. Formation of active eIF4E·eIF4G complex, and phosphorylation of S6K1 and 4EBP1 in LD also reflected this pattern (PF=TF<PP<TP; P<0.01), demonstrating less robust translation initiation signaling in preterm vs. term fed pigs. LC3II to total LC3 ratio in LD was decreased (P<0.01) by feeding regardless of GAB, indicating reduced autophagy-lysosome system activation. There were no differences between groups in LD MuRF1 or atrogin-1 abundance, indices of ubiquitin-proteasome system activity.

Conclusions: Prematurity decreases weight gain and feeding-induced stimulation of muscle protein synthesis due to a reduction in translation initiation signaling in a piglet model. These mechanistic data will be used to design future translational studies aimed at improving lean growth in premature infants.
31. RSV Gene Copy Number Patterns are Virus-Specific and Do Not Follow a 3’ to 5’ Gradient

Felipe-Andres Piedra

Respiratory syncytial virus (RSV) is the leading microbial cause of severe lower respiratory tract illness in young children and the elderly worldwide. RSV is a non-segmented negative-sense RNA (NNS) virus. Its genome has one promoter and encodes ten genes each with their own transcriptional start and end sites. Early experiments showed that transcription in RSV is obligatorily sequential (the gene closest to the promoter is transcribed first, followed by its downstream neighbor and so on) and it has been widely assumed that transcript abundances follow a 3’ to 5’ gradient, decreasing in proportion to distance from the 3’ promoter.

We infected a variety of cell lines in vitro with viruses belonging to four different RSV genotypes and measured copy numbers (CNs) by quantitative real-time polymerase chain reaction (qPCR) of 5 RSV genes: NS1, NS2, N, G, and F. Patterns of RSV gene CNs were virus-specific and conserved in nasal wash samples collected from infected cotton rats. We found no evidence of a 3’ to 5’ gradient in gene expression; in fact, the G gene, located roughly halfway between the 3’ and 5’ ends of the RSV genome, was most abundant for three of the four viruses tested. Measurements of transcript stabilities from experiments in which a viral polymerase inhibitor was used to halt RSV transcription showed that the transcripts measured have roughly equal half-lives, indicating that the relative abundance of G and, more generally, the RSV gene CN patterns observed are not related to differences in mRNA stabilities. We propose a model incorporating known bidirectional scanning by viral polymerases for a new gene start site after terminating transcription plus variable gene start site ‘stickiness’ to account for the observed patterns of RSV gene CNs and the absence of a 3’ to 5’ gradient in gene expression.
Improving gemcitabine/cisplatin-mediated immune responses by suppressing COX-2 activity

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Chemotherapeutic agents are standard therapy in the neoadjuvant setting for treating muscle invasive bladder cancer (MIBC). Our previous studies utilizing PDX models from MIBC patients revealed that chemotherapy-treated tumors become progressively unresponsive after multiple treatment cycles and indicated tumor-derived prostaglandin E2 (PGE2) as a key mediator of tumor repopulation. PGE2 is a well-known immunosuppressive factor that negatively affects cytotoxic T lymphocyte (CTLs) functions, an important mediator of chemotherapy anti-tumor effects. Therefore, we sought to investigate how chemotherapy-induced PGE2 alters tumor immune cell infiltration and whether PGE2 depletion improves chemotherapeutic response in an immunocompetent MIBC mouse model. Here we show that treatment of murine MIBC tumors with Gemcitabine/Cisplatin (GC) for 48 hours followed by flow cytometry resulted in an increase of polymorphonuclear myeloid derived suppressor cells (PMN-MDSCs). However, co-administration of Celecoxib, a Cox-2 inhibitor, reversed PMN-MDSC expansion in both peripheral blood and tumor. Additionally, Celecoxib-mediated suppression of PGE2 increased the expression of inflammatory cytokines (i.e. IFNg, IL6) and improved CTL infiltration in the tumor. Moreover, Celecoxib administration impaired PMN-MDSC’s ability to suppress CD8+ T cell proliferation in vitro by reducing the expression of immunosuppressive genes (i.e. Arg2, Nos3). Taken together, our results suggest an important role for PGE2 in modulating anti-tumor immunity in MIBC that can be readily exploited therapeutically with FDA approved drugs.
33. Target identification and activity validation of a cancer stem cell (CSC)-specific peptidomimetic antagonist

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A small subset of highly proliferative cancer cells, termed cancer stem cells (CSC) resist traditional treatments, and drive both relapse and metastasis. Cancer treatments that target CSCs directly will be essential to fully eliminating cancer. The conventional drug development approaches rely on prior knowledge of disease specific biomarkers to develop drugs to target them. Applying such strategies for targeting CSCs, however, face many challenges due to the paucity of true known CSC biomarkers, incomplete understanding of CSC biology and heterogeneous expression of known biomarkers/ potential drug targets. Therefore, alternative drug discovery tools are needed to identify drug-leads that can effectively target CSCs.

We performed an “unbiased” On-bead Two-color (OBTC) combinatorial cell screen using a library of peptoids, along with ALDEfluor-sorted H358 lung cancer cells to identify peptoid compounds PCS1 and PCS2 that bind specifically to the ALDH+ (aldehyde dehydrogenase) CSC subpopulation of the cell line. We validated these compounds using CSCs pulled down in multiple lung cancer lines and identified a dimeric version of PCS2, PCS2D1 that has an antagonist effect on CSC growth, colony formation, and wound healing, but no effect on remaining cancer cells from same cell line and normal bronchial epithelial cells. We then used PCS2 to pull down and identify Plectin as the cell-surface protein that PCS2 targets. Plectin is a cytosolic protein found in normal cells as a scaffolding unit to build cytoskeletal structure, but has been reported to be localized onto the cell surface in metastatic cancer cells. We found both genotypic and phenotypic correlations of cell surface plectin with CSCs. We are in the process of further validating plectin as a biomarker for CSCs via a genome-wide approaches and regulator of CSC-related phenotypes, further validating PCS2D1 as a therapeutic compound targeting CSCs, identifying the direct molecular mechanisms of the PCS2/plectin interaction, and improving the structures of PCS2/PCS2D1 to maximize this interaction. In particular, targeting cell surface Plectin is a potential new method to identify, separate, and target CSCs.
34. Identification of miRNA-mRNA regulatory modules underlying colorectal cancer molecular subtypes

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Introduction: Colorectal cancer (CRC) is a heterogeneous disease posing a challenge for accurate classification and treatment of this malignancy. Recently, an international multicenter consortium (CRC Subtyping Consortium) was established aiming at the classification of CRC patients in biologically homogeneous CRC subtypes. Four consensus molecular subtypes were identified. Our aim is to develop an analytical pipeline to search for microRNAs potentially driving CRC subtypes.

Motivation: MicroRNAs are small single-stranded non-coding RNA that function in RNA silencing and post-transcriptional regulation of gene expression. They play an important role in tumorigenesis, and understanding the regulatory mechanism of microRNAs in gene regulatory network will help elucidate the complex biological processes at play during malignancy.

Data: For this study, we obtained CRC microRNA expression data from The Cancer Genome Atlas (TCGA), and generated a matched mRNA-microRNA expression data set by integrating mRNA expression data that assembled from TCGA for the same samples. The total sample size is 222, in particular, the number of patients for different subtypes (CMS1-4) are 32, 92, 32 and 66, respectively. To validate our findings, we also used another data set: MD Anderson CRC data. The total sample size is 152, and the number of patients for different subtypes (CMS1-4) are 37, 70, 13 and 32, respectively.

Method: First find list of miRNA differentially expressed (UP or DOWN) across CMS1-4. Then find list of predicted mRNA targets for miRNA, and assess differential expression for them, or find characteristic UP and DOWN genes (mRNA) for each CMS and perform some type of GSEA to see whether the miRNA target genes are overrepresented in the UP/DOWN signature genes. Perform network analysis to find list of mRNA targeted by each miRNA. In particular, we used a novel statistical method (community detection) to perform network analysis.

Results: Using the network analysis method, we find 4 communities (groups) for the differentially expressed microRNAs across different subtypes. The functional enrichment analysis of selected mRNAs revealed that our method selected mRNAs that are significantly associated with colorectal cancer.
35. NKTR-214, an engineered cytokine, synergizes and improves efficacy of anti-cancer vaccination in the treatment of established murine melanoma tumors

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Background: IL-2 has been used as effective immunotherapy in metastatic renal cell carcinoma and melanoma, and may synergize with other cancer immunotherapies. However, toxicities associated with high dose IL-2 treatment limited its further use in anti-cancer therapies. NKTR-214 which is an engineered IL-2 cytokine, was designed to provide a non-toxic, stable and more efficient alternative to IL-2. NKTR-214 provides sustained activation of the IL-2 pathway through controlled release of active CD122-biased (IL-2Rβγ) cytokines. Prior preclinical studies demonstrated that NKTR-214 can expand tumor-infiltrating lymphocyte populations resulting in marked tumor growth suppression as single-agent and in combination with checkpoint inhibitors. In this pre-clinical study, we investigated whether NKTR-214 can promote expansion and function of vaccination-induced, tumor specific effector CD8+ T cells using the murine B16 melanoma model. We also studied how NKTR-214 impacts the localization of effector CD8+ T cells and Tregs to tumor and spleen.

Material and methods: To understand the effect of NKTR-214 on antigen-specific CD8+ T cells, we adoptively transferred naive gp100-specific TCR transgenic pmel-1 CD8+ T cells into mice bearing established subcutaneous B16 tumors, followed by vaccination (gp100 peptide + anti-CD40 mAb + TLR-7 agonist) alone or in combination with NKTR-214 or IL-2. Mice then received NKTR-214 or IL-2 every 8 days. Tumor growth, survival and T cell response in blood was monitored, and localization of effector pmel-1 CD8+ T cells and CD4+ Foxp3+ Tregs in tumor and spleen were analyzed.

Results: NKTR-214 efficiently synergized with vaccination, potently suppressing tumor growth and improving survival of mice compared to vaccination with IL-2. NKTR-214 enhanced pmel-1 CD8+ T cell numbers and decreased numbers of immune-suppressive Tregs in tumor. NKTR-214 was able to stably maintain a high ratio of pmel-1 CD8+ T cells over Tregs in tumor for >30 days. Despite the induction of very strong CD8+ T cell responses and anti-tumor activity, no gross toxicity was observed.

Conclusions: NKTR-214 synergizes with vaccination by supporting the survival, maintenance and tumor infiltration of effector CD8+ T cells without promoting the intratumoral accumulation of immune-suppressive Tregs. These preclinical results establish that NKTR-214 is highly effective in increasing CD8+ effector T cell responses with potent anti-tumor activity.
Whole exome sequencing reveals heterozygous variants in CHD4 gene in Moyamoya disease patients

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Moyamoya disease (MMD) is a progressive vasculopathy characterized by the stenosis and occlusion of the terminal portion of the internal carotid arteries and its branches, and the formation of compensatory moyamoya network of abnormal collateral vessels. Moyamoya disease can be observed with an early onset in children (brain ischemic events) and later in adults (hemorrhagic events) with a prevalence between 3.16-10.5/100,000 depending on the ethnicity. To determine new genes involved in MMD, we use whole exome sequencing in a patient cohort. We identified heterozygous new rare variants of the CHD4 gene in 8 MMD probands which are predicted to be pathogenic. CHD4 protein is a helicase and the main component of the nucleosome remodeling and histones deacetylase (NuRD) repressor complex involved in the epigenetic regulation of gene transcription, DNA repair and cell cycle progression. CHD4 participates in the remodeling of chromatin by deacetylating histones. We found that the splicing variant c.1686+1G>T leads to the skipping of 41 bp of exon 11 and a frameshift that should lead to degradation of CHD4 mRNA by NMD resulting in haploinsufficiency in the patient. The others variants are missense alterations located in key domains of the CHD4 protein that could act as dominant negative. CHD4 interacts also with pericentrin, encoded by PCNT, a gene known to be responsible of Microcephalic Osteodysplastic Primordial Dwarfism type II, which in around 20% of the cases are associated with cerebral aneurysm or MMD. We conclude that CHD4 is responsible for a Moyamoya syndrome associated with various clinical presentations and variable penetrance (among them MMD, high blood pressure, developmental delay and cardiac manifestations). These results suggest that exome sequencing can successfully identify genes and pathways involved in Moyamoya syndromes, and that our results may give insights into pathophysiological models and potential treatment strategies.
Derivatization of lipid-phosphatidylserine (PS) targeted peptide-peptoid hybrid altering non-important residues

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We recently reported a PS binding peptide-peptoid hybrid (PPS1) that has distinct positively charged and hydrophobic residue-containing regions, which displayed high specificity of binding towards a panel of lung cancer cells that express PS, but not to normal cells. Interestingly, a simple C-terminal dimeric version of PPS1, PPS1D1 displayed potent in-vitro cancer cell killing activity as well as in-vivo tumor burden effects, while the monomeric PPS1 remained inactive. Subsequently, minimum pharmacophore studies of PPS1D1 (by replacing each monomer with alanine/sarcosine, one at a time) using ELISA binding MTS cell viability assays identified that the first and fourth residues were not necessary for the binding and activity of PPS1D1. We then replaced non important fourth position (N-lys) with the substituents having varied physiochemical properties such as hydrophobic, aliphatic-alicyclic, heterocyclic, and negatively charged residues and developed a mini-library of 38 derivatives. MTS and Calcein AM cell viability assays on HCC4017 lung cancer cells indicated that fourth position of PPS1D1 is insensitive to most of the changes. In addition, shortening each monomeric sequence by dropping the methionine at first position did not affect the activity. After confirming this fourth position is absolutely not important, a unique series of PPS1 multimeric derivatives were synthesized by switching the linker from the C-terminus to this internal fourth position (N-lys). The synthesis strategy was developed employing variations of (I) the linker size, (II) the number of positively charged residues, and (III) the number of hydrophobic regions. Cytotoxicity to HCC4017 lung cancer cells showed that a minimum of two hydrophobic regions was important to retain the activity and that the shortest linker length was optimal for activity.
38. Evaluation of plectin as a cancer stem cell biomarker

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Tumors are heterogeneous and contain a small sub-population of therapy resistant cancer cells called cancer stem cells (CSC). CSCs are resistant to conventional therapies and thought to be the driving force of cancer metastasis and recurrence. Thus, eliminating CSCs is important to have a complete outcome in cancer treatments. Targeting CSCs is extremely challenging due to the paucity of CSC biomarkers. We recently applied an unbiased On-Bead Two-Color (OBTC) combinatorial assay to screen a peptoid compound library, and identified a lead compound PCS2, targeting lung CSCs. Later, the structural protein plectin was identified as the target of PCS2. Plectin is normally found in the cytosol, but in invasive metastatic cells it can be expressed on the cell surface. The dimeric version of PCS2, PCS2D1 displayed anti-CSC effects against lung cancer cell lines. Our main aim of this project is to validate plectin as a CSC biomarker and PCS2D1 as a possible antagonist of CSC activity in various types of cancer. The initial investigation includes the knockdown of plectin to confirm its importance in CSCs, western blotting to quantify plectin expression in different cancer cell types and also the validation of CSC binding to PCS2 using magnetic bead binding techniques. The biological effect of PSC2D1 was also tested using standard MTS cell viability, colony formation and wound healing assays. The initial data indicates that plectin is important for CSC functions and PCS2D1 can decrease CSC activities.
**39. p300 is a critical catabolic switch of cancer-induced cachexia**

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**Introduction:** With no cures at present, cachexia accounts for ~30% of cancer-related deaths involving profound loss of the skeletal muscle. We have shown previously that activation of C/EBPβ by p38β MAPK mediates the degradation of muscle proteins under tumour burden. Acetylation of C/EBPβ is also known to increase its transcription factor activity. However, the underlying mechanism contributing to C/EBPβ acetylation and transactivation of catabolic genes in the cachectic muscle remains unknown.

**Methods:** C2C12 myotubes transfected with p300 or PCAF siRNA or plasmids encoding C/EBPβ mutated at Lys39 were incubated with conditioned medium harvested from Lewis Lung Cancer (LLC). Inhibitors of p300 (C646) and PCAF/GCN5 (CPTH6) were added in separate experiments. LLC-bearing mice were subject to subcutaneous administration with C646 or forced expression of Lys39-mutated C/EBPβ in the muscle.

**Results:** Conditioned medium from LLC (LCM) activated the catabolic machinery measured as MAFbx, UBR2 and LC3-II and provoked subsequent degradation of structural muscle protein MHC. These aberrant changes were blocked by p300 siRNA and the specific p300 inhibitor C646. In contrast, neither did PCAF knock down nor dual inhibition of PCAF/GCN5 with CPTH6 prevent myotube catabolism. More importantly, we identified that LCM induced a specific increase in acetylation of C/EBPβ at residue Lys39. Substitution with alanine or arginine, but not with glutamine, in an attempt to confer resistance to Lys39 acetylation recapitulated the protective effects of p300 suppression. LLC-bearing mice administered with C646 were resistant to the elevation of catabolic markers and resultant loss of skeletal muscle. Likewise, muscles over-expressing C/EBPβ with defective Lys39 acetylation did not succumb to cachectic challenge.

**Conclusion:** Our work is the first to reveal that specific Lys39 acetylation of C/EBPβ by p300, but not the GCN5/PCAF acetyltransferase family (GNAT), is critical to the development of cancer-induced muscle wasting. Thus, p300-dependent activation of C/EBPβ appears to be a distinct molecular switch of cachexia and may represent a novel intervention strategy for cachectic patients.
40. Identifying immunotherapy resistance in melanoma in vitro and in vivo by Magnetic Resonance

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Background: Imaging cancer immunotherapy is an emerging area in diagnostic imaging. Cancer immunotherapy is employed by blocking the two negative regulatory proteins of T-cell activation known as cytotoxic T-lymphocyte associated protein-4 (CTLA4) and programmed death-1 (PD1). The immunotherapy is successful in treating cancer patients of melanoma however not all patients responds. The overarching purpose of our study is to identify immunotherapy resistant patients by non-invasive real time metabolomic imaging/spectroscopy.

Methods: We have employed a two prong strategy to assess immunotherapy response; a) \textit{in vitro} high resolution Nuclear Magnetic Resonance (NMR) spectroscopy and b) \textit{in vivo} hyperpolarized \textsuperscript{13}C pyruvate magnetic resonance spectroscopy (MRS) in live mice model\textsuperscript{1}. The standard one dimensional (1D) \textsuperscript{1}H NMR with water suppression sequence was used to acquire the data on a melanoma cell line responsive to immunotherapy (B16/BL6 TMT) and a corresponding immunotherapy resistant cell line (B16/BL6 3I F4). The data was processed in Topspin 3.1 and resonances are identified using Chenomx, human metabolic database (HMDB), 2D \textsuperscript{1}H-\textsuperscript{13}C TOCSY and 2D \textsuperscript{1}H-\textsuperscript{13}C HSQC\textsuperscript{2-3}. All the data were acquired on Bruker spectrometer operating at 600 MHz proton resonance frequency equipped with triple resonance TXI (\textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N) cryogenically cooled probe. The dissolution DNP (HyperSense, Oxford Instruments) operating at 3T is employed to hyperpolarize \textsuperscript{13}C pyruvate. The \textsuperscript{13}C MR spectrum of hyperpolarized \textsuperscript{13}C pyruvate was acquired on live immunocompetent mice (BL6) models bearing melanoma tumor in flank at 7T Bruker MRI scanner.

Results: NMR metabolomics study reveals that immunotherapy responding cell line and tumors can be distinguished from resistant one. The variation in the concentration of AMP, glycine, phosphocholine, acetate, lactate and alanine molecules will serve as biomarkers to distinguish immunotherapy resistant cell lines. \textit{In vivo} magnetic resonance spectroscopy with hyperpolarized \textsuperscript{13}C pyruvate in mice bearing immunotherapy resistant and responding melanoma tumor showed significant difference in pyruvate to lactate conversion.

Discussion: Metabolism seems to play an important role in resistivity of immunotherapy in melanoma. The gene analysis showed correlation of upregulation of phosphoglycerate mutase-2 (PGAM2) to immunotherapy resistance gene involved in glycolysis. The metabolomic analysis by NMR spectroscopy and \textit{in vivo} \textsuperscript{13}C hyperpolarized MR spectroscopy also correlates with gene analysis. The key question now is whether it is possible to translate this difference into an imaging biomarker for immunotherapy resistance in the clinic.

Conclusions: NMR metabolomics and hyperpolarized MR are promising imaging tools to differentiate immunotherapy resistant and responding melanoma cell lines \textit{in vitro} and \textit{in vivo} which can potentially be extended to distinguish patients in near future.
Deciphering the cryptic communication between mantle cell lymphoma-initiating cells and bone marrow niche

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**Introduction:** Mantle cell lymphoma (MCL) represents an aggressive, incurable form of non-Hodgkin's lymphoma (NHL). The health complications associated with advanced age of MCL patients restrict treatment with intense chemotherapy. Pathogenesis of MCL has always been elusive until the recent developments made in the tissue culture system, using human mesenchymal stromal cells (hMSC), suitable for propagating patient derived MCL cells. Following the current notion that tumor-initiating cells are responsible tumor relapse and chemoresistance, we quest to identify different communications and related transporters responsible for survival and maintenance of MCL-initiating cells (MCL-ICs).

**Methods:** Patient derived MCL cells (n = 35) were co-cultured with hMSCs for long term culturing and the enriched content of MCL-ICs was analyzed by surface marker expression profile; CD34^+^CD3^-^CD45^-^CD19^-^ along with other functional assays. Exosomes and the cytokines were analyzed from the co-culture soup to identify the soluble factors that promotes survival and propagation of MCL ICs. Expression of these factors were validated by RT-PCR analysis. Hyperactive signaling pathways addicted to identify soluble factors in such co-culture system were blocked in 3 MCL cell lines to confirm their indispensable role in survival of MCL cells and respective MCL-ICs.

**Results:** Co-cultures of MCL cells with hMSCs supported the growth for over 4 weeks with constant presence of MCL-ICs. We found that exosomes and interleukins triggered an FGF/FGFR autocrine loop along with activated Notch signaling. However the extent of FGFR expression highly correlated tightly with expression of SOX11 (negative prognostic marker). Notch ligands and related key players were also observed to be dynamically regulated in the bone marrow microenvironment. Blocking of this signaling pathway with FGFR1/Notch inhibitors consistently induced early reduction in IAP family member and subsequently MCL cell death and decrease in the tumorigenesis.

**Conclusion:** We have established propagation of primary MCL in co-cultures with hMSCs for long term survival by enriched self-renewal MCL ICs. We also identified essential factors for survival of MCL-ICs that present new targets for improved MCL treatment strategies.
42. Translatome and transcriptome of the rat hippocampus differently affected after pilocarpine-induced status epilepticus (SE)

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The molecular and cellular changes underlying epileptogenesis, development of chronic epilepsy after an initial insult such as SE, are still not completely understood. Transcriptome studies explored the SE-triggered gene expression but not the genes being synthesized into proteins. Here we used the polysome profiling to identify which gene will be translated 24h after SE. SE was induced in male SD rats (32-45d old) followed by pilocarpine (320 mg/kg; i.p.) or saline 30mins later. SE was terminated by pentobarbital (30mg/Kg) after 1h. To obtain polysomes 90% of hippocampal cytosolic extracts were layered on top of 15-45% sucrose gradients, centrifuged at 35,000 rpm for 2h at +4°C and fractionated by gradient elution with real time optical detection (254nm). The translatome (mRNAs extracted from polysomal fractions) and the transcriptome (obtained from 10% extracts remainder) were then profiled with Next Generation Sequencing. Enriched pathway and gene ontology were investigated [broadinstitute.org/gsea/msigdb/annotate.jsp]. Differential expression analysis revealed 1838 differentially expressed genes (DEGs, 0.5 < fold change < 1.5 and false discovery rate < 1%) from the translatome and 1210 from the transcriptome between SE and Sham (n=3/group). Out of these DEGs 42% were specific for translatome and 12% were specific for transcriptome. The increased translation is prevalent in enzyme binding, signal transduction, response to stress, and cell motility. Immune system, extracellular matrix, transmembrane transport, axon guidance, and actin cytoskeleton were among over-represented pathways in translatome of SE, while genes related to RNA-DNA metabolism and protein synthesis machinery were seen in the transcriptome of SE. Our results suggest an increased expression of already transcribed genes 24h after SE. These translational programs require further investigation to understand acute and long-term changes related to epileptogenesis.
Cancer immunotherapy is treatment that induces the immune system to attack tumor cells and is a promising therapy for wide variety of malignancies. An exciting approach of cancer immunotherapy is adoptive T-cell therapy, wherein the natural ability of T-lymphocytes to recognize tumor antigens and kill target cells is augmented. In normal human T-lymphocytes, extensive proliferation leads to replicative senescence; a constraint in the number of times that cells can divide. Replicative senescence is characterized by a reduction in telomerase (hTERT) activity and shortening of telomeres. This is an obstacle for T-cells engineered to express chimeric antigen receptors (CAR-T) therapies because the ex-vivo clonal expansion required to achieve therapeutic doses of the CAR-T cells involves a stage of high proliferation. In this regard, an adoptive immunotherapy trial observed that telomere length of transferred lymphocytes correlated with in-vivo T-cell persistence following treatment, suggesting that telomere length and the proliferative potential of the transferred T-cells may play a significant role in avoiding replicative senescence and thereby mediating a successful clinical response. We have generated the first pre-clinical evidence that transfection of hTERT mRNA increases T-cell replicative capacity in vitro, and improves efficacy of CAR-T approach against a murine model of human B-cell malignancy. Our studies demonstrate that hTERT mRNA has great potential to improve the therapeutic benefits of CAR-T therapy. We further aim to develop a novel codon-optimized RNA-based reagent and a biomimetic nanoparticle-based delivery of hTERT mRNA that will improve cancer immunotherapies by enhancing the proliferation capacity of tumor-targeting T-cells, which can be applied to other cell therapies.
44. On-Bead Combinatorial Synthesis of Side Arms Modified Europium(III)-DOTA Complexes

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Magnetic resonance imaging (MRI) & its improved mechanistic approach Chemical Exchange Saturation Transfer (CEST) mechanism have emerged as the versatile imaging techniques in clinical medicine inducing difference in contrast due to inherent differences in water proton densities and relaxation rates between various tissue components. Effectiveness and sensitivity of a contrast in a CEST agent is highly governed by electronic effects and stereochemistry of neighboring substituents. Previously, our research group reported an on-bead combinatorial study of different di-peptoid-Europium(III)-DOTA complexes giving preliminary insight to the combined effects of physiochemical properties of substituents on CEST signal intensity. This led us to develop more focused library to identify the factors that could produce better CEST signals. Therefore, we developed a combinatorial library of 77 new on-bead Europium(III)-DOTA complexes, mainly consisting of negatively charged groups conflated with diverse stereo-electronic properties along the three DOTA ligating arms. Four different reaction types (amino acid, anhydrides, carboxylic acids and peptoid couplings) were employed to modify three side arms of DOTA via parallel synthesis on solid phase. The single CEST image of the library displayed that 21 of the 76 compounds showed stronger CEST signal than the parent compound. Out of 21 compounds, 52% carried a negative charge yielded CEST intensity in 40-80% range. Negatively charged aliphatic functionalities, preferably with less sterically hindered groups, contributed to high CEST contrast over negatively charged aromatics. Only 19% carried a positive charge including primary and secondary amines derivatives depicted not more than 40% CEST signal. We are currently screening this library targeting lung cancer cells to directly identify ‘theranostic’ agents with improved cell permeability than DOTA itself.
45. Cadherin-11 contributes to pulmonary fibrosis by regulating macrophage function

Sarah To and Sandeep Agarwal

Pulmonary fibrosis is a chronic lung disease characterized by excessive deposition of extracellular matrix by myofibroblasts leading to remodelling of the lung architecture. Cadherin-11 (Cdh11) is a cell adhesion molecule expressed on alveolar macrophages of patients with pulmonary fibrosis and mice given bleomycin. Cdh11 has been shown to be implicated in the development of pulmonary fibrosis however the mechanism by which Cdh11 regulates macrophage function to contribute to pulmonary fibrosis remains unclear. In this study, we aim to understand the role of Cdh11 in macrophage biology using macrophages from Cdh11-deficient mice. We show that Cdh11-deficient macrophages have decreased expression of the alternative activation markers Arg1, Ym1, and CD206, and reduced secretion of TGF-β1. Furthermore, we show decreased phagocytosis, proliferation, and migration by Cdh11-deficient macrophages. This is the first demonstration of a role for Cdh11 in regulation of macrophage function and suggests a possible mechanism by which Cdh11 alters fibrotic responses in the lung.
46. Effects of biologically determined high androgen and steroid levels in castrate-resistant prostate cancer (CRPC).

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Background: The differential dose dependent effects of steroids and androgens have gained interest with the relevance of bipolar therapy (BPT) in CRPC. Steroid concentrations at various tumor microenvironments can be high. This impact has not been fully elucidated. Methods: We investigated the effect of high dose (HD) androgen and other steroids on prostate cancer (PCa) and prostate stromal (WPMY-1) cell lines. Cell viability assays (MTT and flow cytometry) were performed in the presence and absence of biologically relevant increased steroid levels. Immunoblots were used to determine androgen receptor (AR) expression. Results: The addition of HD epitestosterone (E)(0 vs 3ug/dL, p<0.001), progesterone (P)(0 vs 5nM, p<0.001), testosterone (T)(0 vs 3ug/dL, p=0), cortisol (C)(0 vs 30ng/gL, p<0.001) and pregnenolone (Preg)(0 vs 8nM, p<0.001) decreased viability (MTT assay) in LNCaP and WPMY-1 cells in charcoal stripped serum (CSS) media after 72 hours, but not in PC3 or 144-13 cell lines. There were no apoptosis rate differences in flow cytometry. Growth curves over time suggested that viability differences were already present during the first 6 hours or earlier, which might suggest the implication of nongenomic effects as well. AR expression in these cell lines increased after the addition of HD steroids. On addition of fresh CSS media, LNCaP and WPMY-1 cells previously exposed to HD steroids for 24 hours, displayed less viability compared to the non-exposed cells, mimicking BPT. Steroid metabolome in cell lines using liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) after the addition of C13-progesterone suggested that there was no meaningful metabolism of C13-progesterone after 24 hours. Conclusions: Biologically determined HD steroids were antiproliferative on some PCa and stromal cells. Bipolar steroid administration decreased PCa and WPMY-1 cell viability over placement to CSS alone, suggesting it might potentiate the effect of androgen deprivation therapy in some patients. Further understanding of the steroid receptor and ligand profile in PCa will lead to new treatment of PCa.
47. Targeting cyclin-dependent kinase 9 enhances radio-sensitization and identifies AXL as a novel downstream target in esophageal adenocarcinoma

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Background: Despite aggressive standard of care, the 5-year survival rate of esophageal adenocarcinoma (EAC) patients is 20-30%. Inherent chemoradioresistance renders poor prognosis. Improvements in patient outcome are limited by lack of an efficient targeted-therapy. There is an urgent need for novel radiosensitizing therapeutic strategies. Cyclin dependent kinase 9 (CDK9) is over expressed in EAC. CDK9 transcriptionally regulates proteins and processes common to radiation-induced injury. Synergy and shared biomarker(s) between CDK9 inhibition and radiotherapy are unknown.

Methods: In vitro response to CDK9 inhibition was assessed by clonogenic assay, 53BP1 foci formation, cell-cycle and apoptosis. Synergistic effects between CDK9-inhibitors and ionizing-radiation in heteroxenografts and PDX models were performed to investigate CDK9 mediated sensitization to radiotherapy. CDK9 dependent signaling was determined by RPPA, qPCR, WB and IHC.

Results: A highly specific CDK9-inhibitor, BAY1143572, radiosensitized 3 radiation-naive and 1 radiation-resistant EAC cells and significantly prolonged DNA damage in 3 EAC cells. CDK9-inhibitors enhanced radiation-induced G2/M by 35% and apoptosis by 12% compared with either treatment in EAC cells. BAY1143572 and radiation reduced median tumor volume in FLO1 (p= 0.002) and SKGT4 (p=0.02) murine xenografts by 81% at D21 post treatment compared to 42% with BAY1143572 or 63% with radiation alone. BAY1143572 delayed tumor growth by 48% in patient derived xenograft (PDX) from a highly chemoresistant EAC and, with concomitant radiotherapy, significantly inhibited PDX by 78% at D21 after treatment (p<0.003). RPPA identified Axl, as possible candidate biomarker of CDK9 inhibition. In vitro, BAY1143572 and radiation each reduce AXL mRNA and protein levels, but together the reduction was significantly enhanced (52%, p=0.006). AXL expression is greatly reduced in xenograft tumors treated with BAY1143572 and radiation compared with radiation alone (p=0.03).

Conclusion: Targeting CDK9 significantly enhances radiation effects in EAC, and AXL is a potential biomarker to assess effects of CDK9-inhibitors with and without radiotherapy. Adjuvant administration of BAY1143572 could be a potent new therapeutic approach for improving the efficacy of radiotherapy against EAC, thus promoting repurposing of BAY1143572.
48. The role of cadherin-11 in a fungal proteinase model of induced airway hyperreactivity.

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Asthma is a chronic lung disease that inflames and narrows the airways. It affects more than 300 million people globally. Cadherin-11(cad11) is a cellular adhesion molecule that has been shown to play a role in lung fibrosis. During the development of lung fibrosis, cad11 is expressed in injured epithelial cells, alveolar macrophages, and fibroblasts. Expression of cad11 has also been described on smooth muscle cells where it regulates tissue contractility. Given that all of these cell types are involved in the development of asthma, we hypothesize that cad11 may also play a role in asthma. To address this question, cad11 wild type (WT) and deficient (KO) mice were challenged in a fungal proteinase model of asthma. Purified proteinase from *Aspergillus mellitus* (PAM) was administered intranasally to WT and KO mice and then airway hyperreactivity (AHR) was measured using whole body plethesmography. Indeed, AHR was significantly decreased in KO mice related to WT mice. Total cell counts in the lungs and expression of inflammatory cytokines tended to be decreased in the KO; however changes were insignificant. This indicates that cad11 may be acting on another cell type. Interestingly, preliminary data measuring tracheal rings bathed in IL-13 from KO mice had less contraction than WT mice. Together these data implicate cad11 in the development of AHR and suggest that cad11 on the smooth muscle cell may be an important target for asthma therapy.
Gas exchange in the lung alveoli requires the formation of a dense vascular network via alveolar angiogenesis, which often fails in premature births. Classical morphological studies attribute alveolar angiogenesis to a unique process termed intussusceptive angiogenesis, whereby existing vessels are split by trans-endothelial pillars to form new vessels. Compared to the better-studied sprouting angiogenesis that depends on a Vascular endothelial growth factor A (Vegfa) gradient and Notch-mediated tip-stalk cell specification, intussusceptive angiogenesis is poorly understood on the cellular and molecular levels. Here we show that endothelial cells undergoing intussusceptive angiogenesis in the lung depend on the same VEGFA signal as those undergoing sprouting angiogenesis, but adopt a distinct cellular morphology. We found that AT1 cells, instead of alveolar type 2 (AT2) cells, are the unexpected source of VEGFA. Specific deletion of Vegfa in AT1 cells, but not AT2 cells, reduces vessel surface area without a large change in cell number and proliferation rate. Mosaic deletion of Vegfa in AT1 cells lead to juxtaposed normal and simplified vasculature, suggesting that VEGFA acts locally. Interestingly, RNA-seq analyses of purified endothelial cells from control and Vegfa mutant lungs identified several down-regulated genes that are expressed in the tip cells during sprouting angiogenesis. Immunostaining confirmed these results and suggested the existence of distinct subsets of endothelial cells in the lung. Genetic cell labeling revealed that instead of forming filopodia as tip cells do in sprouting angiogenesis, lung endothelial cells acquire a web-like structure with attenuated center, consistent with the formation of transluminal pillars that are characteristic of intussusceptive angiogenesis. Unraveling the distinct cellular and molecular responses to VEGFA during intussusceptive versus sprouting angiogenesis will add a new dimension to vascular biology, and will offer new insights to pulmonary diseases with underdeveloped vasculature such as bronchopulmonary dysplasia.
50. Sustained PGC1β activation induces skeletal muscle loss via apoptosis and autophagy

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Muscle wasting is prevalent in many diseases, necessitating a full understanding of this phenomenon for therapeutics. We report that skeletal muscle-specific overexpression of nuclear receptor co-activator PGC1β in transgenic (PGC1β-TG) mice decreases whole body weight, lean mass and muscle size, resulting from repression of myofiber size and number. Myofiber loss is linked with a wide-scale induction of classical apoptosis (e.g., Casp3, Casp6, Casp9 and Apaf1) and autophagy (e.g., Becn1, Map1lc3b, Atg5, Ctss and Atp6v1g2) related genes by PGC1β in the muscle. Consequently, PGC1β induces a profound histologically measureable increase in apoptosis and autophagy in the skeletal muscles. The induction of muscle wasting by PGC1β is associated with stimulation of transcriptional factors (e.g., E2f1, Atf3, Stat1, and Stat3) known to cause apoptosis and autophagy. Therefore, PGC1β is a negative gene regulator of muscle mass, and could be a potential target for deterring muscle loss.
51. B Cell Repertoire in Response to Gammaherpesvirus Infection

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Gammaherpesviruses (γHV) establish lifelong latency in B lymphocytes. Chronic infection with γHV such as Kaposi's sarcoma-associated herpesvirus (KSHV), Epstein-Barr virus (EBV), and murine gammaherpesvirus 68 (MHV68) is associated with the development of lymphoproliferative diseases, lymphomas and other types of cancer. Because of narrow host tropism of both EBV and KSHV, and limitations in the study of human γHV in vivo, mouse model has been developed. MHV68 infects laboratory mice and provides ideal model for analysis of the acute and chronic pathogenesis. Additionally, infection may be tracked by usage of recombinant MHV68-YFP virus.

After primary infection in mucosal tissue γHV evades effectively immune response and persists in host's cells indefinitely. Early studies identified that viral particles are found nearly exclusively in B lymphocytes that have undergone a germinal center (GC) reaction, including memory B cells. Processes that take place in GC, such as somatic hypermutation (SHM) and class switch recombination (CSR), shape the generation of high-affinity antibody-secreting plasma cells and memory B cells. It is still unclear what impact γHV infection has on B cell differentiation, affinity maturation and clonal proliferation.

Our study encompasses analysis of B cell repertoire of MHV68 infected (YFP+) and uninfected (YFP-) GC cells. We used next-generation sequencing strategy to study variable regions of antibodies expressed by different B cell populations. This method enables us to determine the usage of variable (V), diversity (D) and joining (J) genes, mutation rate and isotype usage. Additionally, we have deeper insight into VDJ junction sequences (complementarity-determining region 3 - CDR3) of each repertoire and its possible overlap. Our preliminary data indicate that population of MHV68 infected B cells is characterized by distinct usage of V genes and high selection of unique clones. Moreover, both populations of infected and uninfected cells share CDR3 variants only on minimum level. In summary, despite the fact that both analyzed antibody repertoires originate from one organism, they represent distinct pools of GC cells.
52. Mechanical properties of COF-1

Martha Suarez-Villagran, Tiago Botari, John Miller, Leonardo Dantas

Research on two-dimensional (2D) crystals has revealed materials with exceptional electronic, thermal, optical, and mechanical properties. A variety of single layered structures have been synthesized since graphene’s isolation in 2004; examples include, phosphorene, and the transition metal dichalcogenides. Among the atomically thin crystals, we also find some members of the covalent organic framework (COF) family. Covalent organic frameworks are porous materials ingeniously constructed from organic building units, thus creating promising structures for gas storage applications. In addition to studies determining the available surface area of COFs, studies have also targeted the electronic properties of the 2D family members. On the other hand, the mechanical properties of COFs beyond the elastic regime are still unknown. In this work, we combined first principles and reactive molecular dynamics (MD) simulations to characterize the mechanical properties of COF-1. We found that this material presents an anisotropic response to strain. In addition, for both pulling directions, we found two regimes of response to external strain. By analyzing the MD trajectories, we found that softer and harder responses were related to reversible changes in the microstructure (pore size) of COF-1, as the strain increased. We have also found that this process leads to a considerable increase in the surface energy density of the material. Finally, we determined, for both armchair and zigzag orientations, the toughness, ultimate tensile strain, and ultimate tensile stress of the 2D COF-1 membranes.