Targeting isomor-specific PI3 kinase signaling for treatment of cutaneous T cell lymphoma
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OBJECTIVES/SPECIFIC AIMS: (1) Determine the anti-proliferative effect of Copanlisib (α/β PI3 kinase inhibitor) in CTCL cell lines and synergy with other anti-tumor drugs such as HDAC inhibitors. (2) Determine the effect of Copanlisib treatment on downstream targets of the PI3K/AKT/mTOR pathway.

METHODS/STUDY POPULATION: We will test the anti-proliferative effect of Copanlisib on the cell lines H9 and HH, which are well characterized for evaluating new therapies for CTCL (Netchiporouk et al., 2017). We will also test the anti-proliferative effect of Copanlisib in combination with HDAC inhibitors on CTCL cell lines. Cell Titer Glo (Promega) will be used for the proliferation assay. Briefly, Cell-Titer Glo will be added after 24, 48, and 72 hours and luminescence will be measured as a % of maximal growth. Inhibitory effect will be determined by comparison to % growth of the control cultures without Copanlisib treatment. Our next objective is to determine the effect of Copanlisib treatment on downstream targets of the PI3K/AKT/mTOR pathway using Western blot analysis. In brief, 30 μg of each lysate will be subjected to 4%–12% gradient SDS-PAGE gel electrophoresis. All primary antibodies were purchased from Cell Signaling Technology. Membranes will be washed with TBST and incubated with 1:10,000 dilution of IRDye-conjugated secondary antibody (Licor) for 1 hour. Results will be expressed as relative intensity: the intensity of each band adjusted to that of GAPDH. Experiments will be done in triplicate and t-test analysis of variance followed by multiple comparison test will be applied to compare the cell proliferation between different treatment groups.

RESULTS/ANTICIPATED RESULTS: Previous results from the Ai lab have demonstrated the importance of the PI3 kinase signaling cascade using high-throughput proliferation assays and siRNA knockdown of individual and double PI3 kinase isoforms (unpublished data). So far, I have successfully established culture of HH and H9 cells in 96 well plates (100,000 cells/200 μL). We are titrating Copanlisib at doses from 20 nm to 20 μM. Initial results suggest a promising anti-proliferative effect and currently we are optimizing the most effective pharmacologic dose. Thus, we anticipate that Copanlisib will exhibit a potent anti-proliferation activity in CTCL cell lines. H9 and HH cell lysates have been collected and preserved for Western blot analysis. We anticipate that Copanlisib treatment will significantly decrease the phosphorylation of AKT and 4EBP1, both downstream targets of PI3 kinase signaling. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings will elucidate the importance of the PI3 kinase/AKT/mTOR pathway in tumor proliferation in CTCL. Identifying the importance of specific isoforms of PI3 kinase in CTCL will allow for more targeted selection of treatment. Copanlisib targets α and δ isoforms of PI3K and is newly approved by the FDA for low-grade B cell lymphoma. Our results seek to quantify the anti-proliferative effect of Copanlisib and determine an on-target mechanism of action by investigating the drug’s effects on downstream signaling molecules of the PI3 kinase pathway. This project will elucidate the disease process of CTCL and provide important insights for its management.

The cell-cell adhesion component PLEKHA regulates the pro-tumorigenic MIR17HG long non-coding RNA in colon epithelial cells
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OBJECTIVES/SPECIFIC AIMS: The goal of this study is to test the hypothesis that the adherens junctions of colon epithelial cells regulate IncRNAs levels and function via the microprocessor and RISC complexes to suppress expression of pro-tumorigenic markers and aberrant cell behavior.

METHODS/STUDY POPULATION: To test this hypothesis, we used colon epithelial cancer cell lines. We performed RNA-seq following knockdown of PLEKHA7, a key component of the adherens junctions, to identify changes in IncRNA expression and downstream mRNA levels. We confirmed junctional localization of affected IncRNAs from the RNA-seq and those that we found in our preliminary study by using in situ hybridization (ISH). RESULTS/ANTICIPATED RESULTS: RNA-seq identified junction-associated IncRNAs whose expression levels are regulated by PLEKHA7. The top upregulated IncRNA upon PLEKHA7 depletion was MIR17HG, an oncogenic host transcript of a cluster of miRNAs. These mature miRNAs also co-precipitate with PLEKHA7. PLEKHA7 knockdown results in increased levels of MIR17HG, but only a subset of its host miRNAs (miR-19a/b). Notably, miR-19a and miR-19b are highly upregulated in colon cancer. Our data suggest that 2 PLEKHA7-associated miRNAs, miR-20a and miR-372, mediate suppression MIR17HG. Re-expression of PLEKHA7 in aggressive colon cancer cells that lack PLEKHA7 suppressed expression of MIR17HG as well as anchorage independent growth of these cells. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data point towards a novel mechanism of IncRNA regulation that tethers epithelial tissue integrity with pro-tumorigenic cell transformation. Reducing elevated MIR17HG levels in cancer cells might be a therapeutic approach to suppress the tumorigenic behavior of cells that have lost their junctional integrity and homeostasis. Identify a network of miRNA-miRNA-IncRNA interactions that could be exploited for further mechanistic studies, as well as for diagnostic and therapeutic purposes in the future.

The direct effect of trimethylamine N-oxide (TMAO) on cardiac muscle contractile mechanics
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OBJECTIVES/SPECIFIC AIMS: The objective of this study was to determine if trimethylamine N-oxide (TMAO) alone could acutely alter cardiac contractile function on a beat-to-beat basis.

METHODS/STUDY POPULATION: CD1 adult mouse hearts were harvested, attached to a force transducer, oxygenated, and paced within an organ bath. Changes in contractility were measured after pipetting or reverse perfusing TMAO through the aorta via a modified Langendorff apparatus to facilitate TMAO delivery into the myocardium. To determine if our findings translated to the human heart, we performed contractility experiments using human right atrial appendage biopsy tissue retrieved during cardipulmonary bypass procedures. To investigate whether TMAO alters contractile rate, in a separate series of experiments, the atria and sinoatrial node of isolated hearts were kept intact to allow for spontaneous beating without artificial pacing and were treated with TMAO or vehicle. In addition, intracellular calcium measurements were performed on spontaneously beating embryonic rat cardiomyocytes after TMAO or vehicle treatment.

RESULTS/ANTICIPATED RESULTS: We found acute exposure to TMAO, diluted into the organ bath, increased average contraction amplitude 20% and 41% at 300 μM and 3000 μM, respectively (p < 0.05, n = 6). Langendorff reverse perfusion of mouse hearts ex vivo with 300 μM TMAO generated an even greater response than nonperfusion peripheral exposure and increased isometric force 32% (p < 0.05, n = 3). Consistent with what we observed in mouse hearts, incubation of human atrial muscle tissue with TMAO at 3000 μM increased isometric tension 31% compared with vehicle (p < 0.05, n = 4). TMAO treatment (3000 μM) also increased average beating frequency of ex vivo mouse hearts by 27% compared with vehicle (p < 0.05, n = 3) and increased the spontaneous beating frequency of primary rat cardiomyocytes by 13% compared with vehicle treatment (p < 0.05, n = 4). DISCUSSION/SIGNIFICANCE OF IMPACT: TMAO, at pathological concentrations, directly increases the force and rate of cardiac contractility. Initially, these inotropic and chronotropic effects may be adaptive, however, chronic increases in isometric tension and beating frequency can promote cardiac remodeling and heart failure. Further translational studies are needed to understand the intricate relationship between the microbiome, kidneys, and heart and to examine if TMAO represents a therapeutic target for reducing cardiovascular mortality in CKD patients.

The effect of antipyretics and fever on the mortality of mechanically ventilated patients
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OBJECTIVES/SPECIFIC AIMS: (1) To evaluate clinical outcomes in mechanically ventilated patients with and without fever. We hypothesize that, after adjusting for confounding factors such as age and severity of illness: (a) In septic patients, fever will be associated with improved clinical outcomes. (b) In nonseptic patients, fever
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These subsets. Cox proportional hazard models and multivariable linear regression route of measurement. Patients intubated for cardiac arrest or brain injury were temperature results are reported here. In the overall group, presence of and forward stepwise regression. Models were assessed for collinearity and residual plots were used to assure each model met assumptions. RESULTS/ANTICIPATED RESULTS: Antipyretic administration is currently undergoing analysis. Initial temperature results are reported here. In the overall group, presence of hypothermia or fever within 72 hours of intubation compared with normothermia conferred a hazard ratio (HR) of 1.95 (95% CI: 1.48–2.56) and 1.31 (95% CI: 0.97–1.78), respectively. Presence of hypothermia and fewer reduced hospital free days by 3.29 (95% CI: 2.15–4.42) and 2.34 (95% CI: 1.21–3.46), respectively. In our subgroup analysis of patients with sepsis, HR for 28-day mortality 2.57 (95% CI: 1.68–3.93) for hypothermia. Fever had no effect on mortality (HR 1.11, 95% CI: 0.694–1.76). Both hypothermia and fever reduced hospital free days by 3.39 (95% CI: 3.30–5.24) and 3.89 (95% CI: 2.46–5.32) days, respectively. DISCUSSION/SIGNIFICANCE OF IMPACT: As expected, both hypothermia and fever increased 28-day mortality and decreased hospital free days. In our sepsis subgroup, hypothermia again resulted in higher mortality and fewer hospital free days, while fever did not have a survival benefit or cost, but reduced hospital free days. Antipyretic administration complicates these findings, as medication may mask fever or exert an effect on survival. Fever may also affect mechanisms by which septic patients differently than septic patients not on mechanical ventilation. Continued analysis of this data including antipyretic administration, ventilator free days and progression to ARDS will address these questions.

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The effects of autoimmune inflammation on proliferation, differentiation, and androgen receptor signaling in adult prostate stem cells

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OBJECTIVES/SPECIFIC AIMS: The primary goal of this project is to verify findings from a murine prostatitis model in the human setting. METHODS/STUDY POPULATION: Methods include primary cell isolation and culture, FACS, adoptive transfer, 3D cell culture, histology, immunofluorescence, xenograft, and tissue recombination. The study population includes patients undergoing HoLEP or active prostate cancer due to hyperplasia or indolent bladder or prostate cancer. RESULTS/ANTICIPATED RESULTS: Having verified similar sensitivities to androgen receptor (AR) inhibitors between naive murine and human basal prostate stem cells, we anticipate that autoimmune inflammation in humans affects the response of basal prostate stem cells in a manner similar to the murine setting as well. This includes increased proliferation, increased differentiation, and decreased response to AR inhibitors. DISCUSSION/SIGNIFICANCE OF IMPACT: The identification of survival mechanisms used by basal prostate stem cells in an androgen deprived environment may give insight to the process by which prostate cancer becomes androgen independent. The effect of inflammation on proliferation, survival, and AR signaling in these cells may also provide information relevant to cancer initiation and progression.

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The microbial-derived short-chain fatty acid butyrate directly and differentially inhibits gut T helper cell subset activation and proliferation

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OBJECTIVES/SPECIFIC AIMS: A hallmark of progressive HIV-1 infection is the massive activation and depletion of the gut barrier protective CD4 T helper subsets (Th17 and Th22) in the intestinal mucosa. The loss of these cells is thought to contribute to microbial translocation and systemic immune activation that occurs during chronic infection. In addition to the loss of protective Th subsets, we previously showed that chronically HIV-1 infected individuals have an altered colonic mucosal microbiome, which is in part characterized by a lower relative abundance of bacteria that produce the short-chain fatty acid butyrate in conjunction with increased relative abundance of gram-negative pathobionts. This dysbiosis was linked to markers of mucosal and systemic immune activation in these individuals. Following up on these clinical observations, we sought to understand how a loss of butyrate might contribute to HIV-associated immune activation in these individuals. Initial studies showed that the addition of butyrate to cultured lamina propria mononuclear cells (LPMC) resulted in decreased pathobiont-driven gut T cell activation, HIV-1 infection levels and production of IL-17 and IFNy. Since the gut barrier protective Th17 and Th22 subsets are preferentially infected and depleted, which is critical to HIV-1 pathogenesis, we wanted to determine the mechanism by which butyrate modulates activation of these important Th subsets in the gut. METHODS/STUDY POPULATION: Total LPMCs or purified PD CD4 T cells were isolated from human jejunum tissue (n = 3–6), labeled with CFSE and cultured with TCR/CD28 beads to mimic APC driven T cell activation, with the addition of butyrate at physiologic doses (0–2 mM). Four days after culture, secreted cytokines (IL-17 and IFNy) levels were measured by ELISA. Cells were then short-term (4 hr) mitogenically stimulated (PMA/Ionomycin) in the presence or absence of a butyrate transport inhibitor. Total CD4 T cell activation (CD3+ /HLA-DR+ , CD25+ ), proliferation (CFSElow), and frequencies of intracellular cytokines were measured by multi-color flow cytometry. Paired t-tests were performed to determine statistical significance. RESULTS/ANTICIPATED RESULTS: Butyrate inhibited PD CD4 T cell proliferation (Day 0 vs. Day 4, p = 0.015) within total LPMCs stimulated with TCR/CD28 beads in a dose-dependent manner, with significant activity starting at 0.125 mM. Quantification of total secreted cytokines revealed that butyrate significantly decreased both IL-17 and
d-based violence and health. METHODS/STUDY POPULATION: The Empower Lab consists of 10–12 undergraduate, graduate, and medical students at a time. Students undergo a rigorous application process, and agree to volunteer 8 hours per week for at least 1 year. Students are assigned to teams, and learn research skills such as literature searches, systematic reviews, research question generation, study design, IRB procedures, database creation and management, data collection and analysis, oral and poster presentation, manuscript preparation, team collaboration and communication, advocacy, and leadership. Students start as research assistants, and can be promoted to team leader, and associate director of research. Students mentor and teach each other, and are supervised by the principal investigator (PI). A survey self-assessment is administered to lab members on entry to the lab, every 4–6 months, and upon exit. RESULTS/ANTICIPATED RESULTS: In total, 20 students have participated in the lab to date, and 12 are currently enrolled. Eighty percent of the lab members are women. The students are 45% undergraduates, 15% graduate (nursing, social work, public health), 20% medical students, and 10% not currently enrolled in school (gap year). Twenty students completed entry surveys, 11 students have completed interim surveys, and 5 students have completed exit surveys. Examination of current surveys indicates that students are gaining skills throughout the lab experience. Free-text feedback provided further insight. Currently, the lab has 5 IRB-approved studies actively recruiting participants, 4 manuscripts being written, and 3 studies in the development phase. Students have presented at three local and 2 national meetings to date. Changes have been made to the lab structure over time in order to provide clear expectations and feedback, and strengthen student performance. DISCUSSION/SIGNIFICANCE OF IMPACT: The Empower Lab is an innovative public health lab that provides opportunities for real-world research experience for students. The teamwork, collaboration, and structure of the lab permit mentoring, support, and teaching from peers, as well as from the PI. The lab increases the PI’s productivity. Students are encouraged to develop and implement their own research ideas, further encouraging independence and initiative. Although the number of surveys is limited to date, they indicate improvement in skills and confidence among lab members. The predominance of women in the lab suggests that this is a strong model for recruitment and retention of women in STEM.

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The Empower Lab: An innovative model for research experience and training for undergraduate, graduate, and medical students

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OBJECTIVES/SPECIFIC AIMS: The Empower Lab was established in 2015 with the goal of providing students with hands-on research experience in sexual and gender-