Poster #1

Structure Guided Approach Leads to the Development of 51-106, a Small Molecule Inhibitor Targeting MAP3K1 in Pancreatic Cancer

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In response to diverse cellular cues, MAP3K1, a mitogen-activated protein kinase, participates in various cancer signaling networks including the NFκB, JNK, ERK, and p38 pathways. Functioning as a signaling kinase in these oncogenic pathways, MAP3K1 contributes to tumor growth and metastasis. Additionally, Higher MAP3K1 transcript level in pancreatic cancer patients is associated with poorer (50% vs. 15%) 5-year survival, suggesting MAP3K1 is an attractive therapeutic target for cancer. We recently reported the discovery of a quinoxaline analog as a selective MAP3K1 inhibitor (2022, PNAS). Structure-guided design using MAP3K1 AlphaFold and Schrödinger GLIDE led to 51-106 that was predicted to have improved affinity for MAP3K1 through the formation of an orthogonal multi-polar interaction. Profiling 51-106 using the KiNativ™ platform in a cellular matrix revealed that 51-106 was indeed a selective ATP-competitive MAP3K1 inhibitor with improved potency. Follow up studies showed that 51-106 blocked TNFα-induced MAP3K1-IKKβ-mediated NFκB activity. Phosphoproteomics analysis following MAP3K1 inhibition by 51-106 showed a dose dependent decrease in NPM1 T199 phosphorylation indicating NPM1 as a novel substrate of MAP3K1. NPM1 plays a critical role in DNA damage repair; consistently we observed a dose dependent S-phase arrest upon MAP3K1 inhibition by 51-106, suggesting a dysfunctional DNA damage response. Treatment of pancreatic cancer cell lines with the MAP3K1 inhibitor 51-106 inhibited cell growth and migration. In combination studies, 51-106 synergistically inhibited growth with gemcitabine in LSL-KrasG12D/+, LSL-Trp53R172H/+, Pdx1-Cre (KPC) cell lines *in vitro* and in KPC syngeneic orthotopic implantation mouse model of pancreatic cancer *in vivo*. In summary, we used structure-guided design to develop an improved MAP3K1 inhibitors. Our study is the first to identify NPM1 as a member of MAP3K1 signaling, and these results warrant the investigation of MAP3K1 inhibition as a therapeutic option in cancer.

Poster #2

DESIGN, BIOLOGICAL EVALUATION, AND COMPUTER-AIDED ANALYSIS OF DIHYDROTHIAZEPINES AS SELECTIVE ANTICHLAMYDIAL AGENTS

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Chlamydia trachomatis (CT) causes the most prevalent, reportable sexually transmitted bacterial disease in the United States. Treatments for chlamydia are based on broad spectrum antibiotics that, although effective, can disrupt the microbiome and increase the risk for the development of antibiotic resistance. Consequently, the lack of drug selectivity is one of the main challenges of current chlamydia pharmacotherapies. CT transitions between two developmental forms: an infectious elementary body and a replicative reticulate body. The metabolic needs of CT are controlled, among others, by cylindrical proteases and their chaperones (e.g. ClpPX). It has been shown that dihydrothiazepines can disrupt CT-ClpPX function in *S. aureus*. Based on this precedent, we synthesized a dihydrothiazepine library and characterized its antichlamydial activity using a modified semi-high throughput screening assay. Then, we selected a set of the active compounds and demonstrated their ability to inhibit ClpX ATPase activity *in vitro*, supporting the ClpX as a target. Further, our lead compound (**5a**) did not present activity against other bacteria, including those from the human microbiota. Besides the promising selectivity profile, the molecule showed acceptable cytotoxicity, and no mutagenic potential. Also, **5a** was stable in human serum and in simulated gastric and intestinal fluids. Additionally, using machine learning methods, we generated a 2D-QSAR model to better elucidate the SAR and used this model to guide the design of compounds with superior potency, indicating that our model can be used as a support tool in the identification of potent antichlamydial molecules. In conclusion, this study suggests the dihydrothiazepines represent a starting point for the development of new and selective antichlamydial drugs.

Poster #3

Investigation of Claudin-1 Inhibitors for Treatment of Drug-Resistant Metastatic Colorectal Cancer

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Colorectal cancer (CRC) is the second most lethal cancer and the third most prevalent malignant tumor worldwide. An estimated 1.8 million new cases and 860,000 deaths from CRC are reported annually. It is the third most common cancer and the third cause of cancer-related deaths in the United States, with 151,030 new cases and 52,580 deaths estimated to occur in 2022. Tumor metastasis and resistance to chemotherapy are the key factors responsible for the high fatality rate of CRC. Therapeutic methods for treating metastatic CRC are limited or insufficient and are further complicated by drug resistance. Claudin-1 protein is a component of tight junctions in epithelial cells, including those found in the lining of the colon. It plays a critical role in the formation and maintenance of tight junctions, which are essential for regulating the passage of molecules between cells. Overexpression of claudin-1 in CRC leads to an increase in cell adhesion, which can contribute to the development and progression of the disease. Studies show that high levels of claudin-1 are associated with poor prognosis in CRC patients and targeting claudin-1 may have therapeutic potential for the treatment of CRC. We report the discovery and optimization of small molecules that inhibit claudin-1 dependent CRC progression.

Poster #4

The effects of novel GGDPS inhibitor RAM2061 on pre-osteoclasts and mature osteoclasts in vitro

Molly E Muehlebach, Sarah A Holstein MD/Ph.D – University of Nebraska Medical Center

Multiple myeloma (MM) accounts for 10% of all hematological malignancies and leads to over 13,000 deaths per year in the United States. This disease results from proliferation of malignant plasma cells which overcrowd the bone marrow causing bone destruction and displacement of healthy hematopoietic cells. It is commonly characterized by overproduction and secretion of monoclonal protein, renal disfunction, immunosuppression, and lytic bone disease. Our lab has developed a novel geranylgeranyl diphosphate synthase (GGDPS) inhibitor (GGSI) as a means for disrupting Rab GTPases involved in intracellular trafficking processes. GGDPS catalyzes the synthesis of isoprenoid donor geranylgeranyl diphosphate (GGPP) which is required for proper localization and function of Rab proteins. Our lab has established our lead GGSI, RAM2061, an α-methyl homoneryl triazole bisphosphonate, to inhibit Rab geranylgeranylation in MM cells, causing disruption of intracellular trafficking leading to accumulation of monoclonal protein in the ER. This leads to the activation of ER stress responses and subsequent apoptosis of the cells. As mentioned, osteolytic lesions are a common side effect of MM due to overactivation of osteoclasts induced by the malignant plasma cells. Whether GGSI treatment alters osteoclast activity directly is not yet known. Therefore, current efforts are now focused on characterizing the effects of RAM2061 in RAW264.7 cells, an osteoclast precursor cell line. We found that in RAW264.7 undifferentiated cells, RAM2061 cytotoxicity increased over time in a concentration-dependent manner. Also in the undifferentiated cells, we have determined RAM2061 to disrupt geranylgeranylation at concentrations as low as 33 nM. At higher concentrations (200 and 400 nM), we observed robust activation of the unfolded protein response pathway as well as caspase cleavage. These effects were attenuated with the addition of exogenous GGPP, consistent with the observed effects of RAM2061 being the result of cellular depletion of GGPP levels. We have also begun experiments looking at the effects of RAM2061 in fully differentiated, mature osteoclasts. Preliminary results have shown that RAM2061 decreases osteoclast activity and may also inhibit osteoclast differentiation. Next steps include further characterization of RAM2061 on mature osteoclast differentiation and inhibition and elucidating the mechanisms behind these results.

Poster #5

Practical and efficient gram-scale synthesis of BTE-EN1: A heterobifunctional anticancer agent

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3(4-fluorophenyl)chroman-4-one (KBU2046) binds to the interface of HSP90b/CDC37 interface and inhibits osteoclast (OC) mediated bone destruction. Bisphosphonates are the most widely prescribed and used medications for the treatment of osteoporosis. Three oral bisphosphonates (alendronate, risedronate, and ibandronate) are approved by the U.S. Food and Drug Administration (FDA). A heterobifunctional compound generated by conjugating alendronate (bis) and KBU2046 through a linker yields, Dual-Acting Bone-Defender (DABD) which was more effective than KBU2046 + bis or the individual components. The reported synthesis of a DABD BTE-EN1 required 11 steps and had an overall yield of 0.7%, which is not suitable for scale-up. To enable the large-scale synthesis of BTE-EN1, here we optimized the route and the process. The optimized route / process resulted in reducing the number of synthetic steps from 11 to 8 and increased the overall yield from 0.7% to 11% (an ~16-fold increase in efficiency).

Poster #6

Combination Therapy of Geranylgeranyl Diphosphate Synthase Inhibitor and Fatty Acid Synthase Inhibitor in Multiple Myeloma

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Multiple myeloma (MM) is characterized by accumulation and proliferation of plasma cells in the bone marrow (BM). It is the second most common hematological malignancy and accounts for approximately 13% of all blood cancers. Geranylgeranyl diphosphate synthase (GGDPS) is a key enzyme in isoprenoid pathway that leads to Rab-mediated protein trafficking which is crucial for the proliferation of MM cells. Inhibiting the GGDPS by geranylgeranyl diphosphate synthase inhibitor (GGSI) causes the disruption of protein geranylgeranylation which hinders intracellular protein trafficking resulting in apoptosis. Similarly, fatty acid synthase (FASN) is a pivotal enzyme involved in neoplastic lipogenesis and has been regarded as an important factor for tumor growth and survival. Many FASN inhibitors have been studied in several types of cancers for their anticancer effects. Multiple myeloma remains an incurable malignancy despite significant therapeutic advances. Most new cancer therapies comprise of a combination of two or more anti-cancer agents. Moreover, both FASN and GGDPS are overexpressed in MM cells making them excellent targets for combination studies for MM treatment. In the past, it has been demonstrated that a GGSI (RAM2061) successfully disrupted Rab proteins leading to apoptosis of MM cells. Here, we hypothesize that combining FASN inhibitors with RAM 2061 will allow synergistic or additive effect between the two promising pathways and increase anti-MM efficacy. Fatty acid synthase inhibitors, orlistat, TVB 3166, TVB 2640 and cerulenin showed cellular toxicity for myeloma cell lines (RPMI 8226 and MM.1S) at 24, 48 and 72 hours in micromolar range. Further, combination studies revealed that treatment of RAM 2061 and some FASN inhibitors have either synergistic or additive effects on MM cells at various concentrations. Combination of fatty acid synthase inhibitor and RAM2061 show potential to become a standard therapeutic approach for the treatment of MM due to its synergistic or additive effects. Further studies are needed to fully define the underlying mechanism of FASN inhibitors on multiple myeloma cells.

Poster #7

Intramuscular administration of the host-derived immunostimulant CPDI-02 increases protection of outbred mice against dermal MRSA infection in a curative setting.

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Staphylococcus aureus is the most common cause of bacterial skin and soft tissue infections (SSTIs) and the leading cause of hospital-associated infections in the United States. The abundance of *S. aureus* infections is partly due to the use of virulence to avoid the host immune system and the development of bacterial resistance to antibiotics. These behaviors are most noticeable with methicillin-resistant strains of *S. aureus* (MRSA). An alternative to antibiotics, Host-Directed Therapeutics (HDTs), avoid the pitfalls of antibiotics by targeting the host for immune system stimulation rather than targeting the pathogen. This mechanism of action avoids the selective evolutionary pressure, which leads to antibiotic resistance and helps the host immune system overcome the bacterial virulence factor-based immunosuppression. CPDI-02, a second-generation analog of the C-terminal of Human complement C5a, can selectively stimulate CD88 on mononuclear phagocytes over polynuclear phagocytes and other inflammation-promoting cells. This activity allows CPDI-02 to serve as an HDT, stimulating the host's innate immune response to fight infection without neutrophil and inflammation-mediated toxicity. CPDI-02 reduces the bacterial burden within bacterial-induced abscess wounds when intramuscularly administered six hours post-challenge. It also reduces the time to resolve the infection-induced abscess in a murine dermal MRSA infection model. CPDI-02 induces this therapeutic effect by interacting with host circulating mononuclear phagocytes and promoting infiltration of these cells into the abscessed tissue. The in vivo activity suggests that CPDI-02 can move the timeline of mononuclear phagocyte involvement in response to infection earlier than in untreated infections. The earlier involvement of mononuclear phagocytes in infection, in turn, reduces damage to host tissue which comes from the invading bacteria and the host's inflammatory response.

Poster #8

Overcoming resistance mechanisms to CDK4/6 inhibitor treatment using CDK6-selective PROTAC

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Hormone receptor-positive (HR+) breast cancer constitutes approximately 75% of all breast cancer patients. Endocrine therapies are often the first-line treatment. However, with the prolonged utilization of endocrine therapies in either case of nonmetastatic or metastatic breast tumors, there are signs of resistance. Cyclin-dependent kinases (CDKs), a family of kinases that require cyclin binding to be activated, are involved in the regulation of the cell cycle and transcription. CDKs, such as CDK4 and CDK6, play an essential role in cell cycle regulation. CDK4/6 is activated upon binding to D-type cyclin and triggers a phosphorylation cascade. In HR+ breast cancer, this pathway can be stimulated by estrogen binding to estrogen receptors, which in turn enhances cyclin D expression. This results in the persistent activation of the CDK4/6-cyclin D complex, which then leads to the phosphorylation of Rb and the subsequent release of E2F, a transcription factor. Once activated, E2F allows the cell to proceed from the G1 to the Sphase of the cell cycle. Inhibition of CDK4/6 has been shown to trigger cell cycle arrest,inhibit tumor growth, and induce apoptosis. Currently, there are three FDA-approved CDK4/6 inhibitors (CDK4/6i), Palbociclib, Ribociclib, and Abemaciclib. Similar to endocrine therapies, prolonged treatment with CDK4/6i results in resistance. One mechanism for an acquired resistance toward CDK4/6i treatment is the overexpression CDK6 proteins. A novel therapeutic approach known as proteolysis-targeting chimeras (PROTACs) can be employed to address this biological problem, with the goal of selectively degrading CDK6. PROTACs are heterobifunctional molecules that use the ubiquitin-proteasome system to selectively degrade targeted proteins. The Natarajan lab and others have previously reported the development of CDK6-selective PROTACs.The aim of this project is to utilize a CDK6-selective PROTAC to reduce the levels of CDK4/6i-induced overexpressed CDK6 proteins in CDK4/6i-resistant breast cancer cell lines. Here we will present our preliminary studies that led to the establishment of HR+ CDK4/6i-resistant breast cancer cell lines and show that the combined treatment of CDK4/6i with the CDK6 selective PROTAC synergistically inhibits the growth of breast cancer cells.

Poster #9

Structure-guided design of E3 ligase FBXO21 binders for the treatment of Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML) is a hematological malignancy caused by aberrant hematopoietic progenitor cell differentiation and proliferation. AML accounts for ~35% of adult and ~20% of pediatric leukemias, with adults having a 5-year survival rate of ~28%. The ubiquitin E3 ligase FBXO21, a member of the F-box protein family, was recently identified as an important regulator of AML pathogenesis but is not required for steady-state hematopoiesis. FBXO21 overexpression in patients is associated with poor prognosis, this coupled with its limited role in normal hematopoiesis makes FBXO21 a viable target for AML treatment. Moreover, knockdown of FBXO21 leads to apoptosis and differentiation of AML cells. In this study, we hypothesize that targeting the substrate binding domain of FBXO21 will lead to AML cell death without affecting normal cells. We used Schrödinger GLIDE and the available AlphaFold FBXO21 structure to dock the FBXO21 substrate (EID1)-derived peptide (Phe-Ile-Glu-Glu-Leu-Phe). Analyses of the docking results show that the glutamic acid side chains of the EID1 peptide make hydrophilic and hydrophobic contacts with the side chain of FBXO21 residues lysine (K167, K168), serine (S210), and leucine (L209). Through iterative design and docking into the EID1 binding pocket, we identified a bi- or terphenyl backbone with two carboxylic acids that will have the optimal blend of hydrophobic and hydrophilic modules to mimic EID1 binding to FBXO21. Here we will present our preliminary studies which include optimization of Suzuki coupling conditions to generate the first set of biphenyl and terphenyl analogs to be evaluated as FBXO21 binders.

Poster #10

CHARACTERIZING 50-008, A QUINOXALINE-BASED PROTAC, AS AN ANTI-CANCER AGENT

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PROTACs (Proteolysis Targeting Chimera) are heterobifunctional molecules that form ternary complexes with an E3 ligase and a protein of interest (POI). This results in the ubiquitination of the POI and proteasomal degradation of the ubiquitinated target protein. With 18 PROTACs in clinical trials for various types of cancers, the PROTAC approach is rapidly emerging as a mainstream strategy to target various oncoproteins. **The Mitogen-Activated Protein Kinase Kinase Kinase 1 (MAP3K1)** is a serine-threonine kinase known to regulate multiple oncogenic pathways such as ERK1/2, JNK, IKKβ. In addition to its kinase activity, MAP3K1 possesses an E3 ligase domain capable of degrading downstream proteins. MAP3K1 is significantly upregulated in breast cancer tumor samples and facilitates a microenvironment that favors breast cancer metastasis. siRNA suppression of MAP3K1 in human breast cancer cell lines and MAP3K1 deficiency in mice have shown growth inhibitory and anti-metastatic effects. Various studies have reported that MAP3K1 plays a role in ERK, NF-κB_- and AP1-mediated gene transcription, thus making MAP3K1 an attractive therapeutic target. Our lab developed a quinoxaline-based PROTAC library. A phenotypic screen of this library identified 50-008 as potent inhibitor of cancer cell growth. Here **we hypothesize that 50-008 perturbs proteins downstream of MAP3K1 to induce growth inhibitory effects.** This study aims to explore the phenotypic effects and mechanism of action of a **novel MAP3K1- based PROTAC, 50-008,** as an anti-cancer agent.

Poster #11

Enhanced Tumor Retention Through Endolysosomal Trapping Evaluated in an Antagonistic NTSR1-targeted Construct for Colorectal Cancer

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Many low-molecular weight targeted radiotherapeutics (TRTs) can achieve excellent *in vivo* tumor uptake quickly after administration. However, short tumor residence times observed in many otherwise promising TRTs impedes clinical translation by limiting the deliverable therapeutic dose. We previously presented a strategy of equipping low-molecular weight TRTs with irreversible cysteine cathepsin (CC) inhibitors (e.g., E-64 analogs) to increase tumor retention. These inhibitor-incorporated constructs can form intracellular irreversible covalent bonds with endolysosomal CCs. Through endolysosomal trapping (ET), inhibitor-equipped constructs have demonstrated greater tumor retention resulting in higher therapeutic dose delivery. In this study, we incorporated an irreversible CC inhibitor into the structure of a neurotensin receptor subtype 1 (NTSR1)-targeted antagonistic TRT. NTSR1 is a G-protein coupled receptor significantly upregulated in many cancer types with paired negligible expression in surrounding normal tissues. We explore the impact of the incorporation of the ET inhibitors on the *in vitro* and *in vivo* biological performance of an NTSR1-TRT using NTSR1-positive human colon cancer (HT-29) models. **Methods:** An NTSR1-TRT containing an endolysosomal trapping inhibitor (^{177}Lu-ET1) was synthesized. ^{177}Lu-3BP-227, an NTSR1-TRT currently in clinical trials, was used as a control for comparison. *In vitro* assays using HT-29 xenograft colon cancer cells examined the NTSR1 binding, internalization and efflux, cysteine protease inhibition, and adduction formation properties of the construct. The biodistribution profile was studied in an HT-29 xenograft mouse model at 4-, 24-, 72-, and 168-hours post-injection. **Results:** NA-ET1 demonstrated 10-fold higher inhibition kinetics than the CC inhibitor alone. The inhibitor-equipped construct also demonstrated statistically similar *in vivo* NTSR1-targeting compared to ^{177}Lu-3BP-227. ^{177}Lu-NA-ET1 demonstrated high-molecular weight adduct formation in HT-29 cells at molecular weights consistent with CCs. ^{177}Lu-NA-ET1 demonstrated higher tumor retention than ^{177}Lu-3BP-227 at 24-, 72-, and 168-hours post-injection. However, the incorporation of the epoxysuccinyl peptide-based inhibitor into the structure of ^{177}Lu-NA-ET1 resulted in increased renal uptake comparatively. **Conclusion:** This research demonstrates that ET inhibitors can be successfully incorporated into an antagonistic NTSR1-targeted construct to increase tumor retention. The antagonistic construct also underwent internalization and is capable of adduct formation. Further investigation of the ET approach is warranted in NTSR1- and other receptor-targeted antagonistic TRTs.

Poster #12

Evaluation of the Stability and Maximum Tolerated Dose (MTD) of an NTSR1-targeted Small Molecule Antagonist Equipped with a Cysteine Cathepsin Trapping Agent

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Introduction: Neurotensin receptor 1 (NTSR1) is a peptide-binding G protein-coupled receptor (GPCR) that is upregulated in highly malignant cancers such as pancreatic, prostate, and colorectal. Recently, an NTSR1-targeted small molecule antagonist, ^{177}Lu-3BP-227, has undergone clinical trials and demonstrated significant uptake in NTSR1 positive tumors and excellent non-target tissue clearance. However, ^{177}Lu-3BP-227 has a relatively rapid washout from the affected areas, diminishing its potential therapeutic action. Recently, we have incorporated a known irreversible inhibitor of endolysosomal proteases to extend tumor retention time and dose delivery with NTRS1-targeted constructs. In this presentation, we will discuss the radiolabeling, stability, and maximum tolerated dose (MTD) studies of our lead NTSR1-targeted candidate, ^{177}Lu-NAN-E1.

Methods: An epoxysuccinyl peptide inhibitor that selectively and irreversibly binds to the catalytic cysteine residue in cysteine proteases was incorporated into a small molecule NTSR1-targeted antagonist. Optimized radiolabeling conditions and stability, with and without a radiolytic protectant (ascorbic acid), of the ^{177}Lu-NAN-E1 were explored. For the MTD studies, ^{177}Lu-NAN-E1 was administered via tail vein injection to six CF-1 mice over a six-week period with increasing doses of radioactivity (1.47mCi, 1.67mCi, 2.08mCi, 2.62mCi, 3.43mCi, 4.50mCi). Mice were euthanized at the end of week six and a biodistribution study was performed.

Results: The optimal molar ratio for the labeling of ^{177}Lu-NAN-E1 was 50:1. At a concentration of 4.50mCi/100µL, ascorbic acid (10mg/mL) was found to substantially increase the stability of ^{177}Lu-NAN-E1 (89% intact at 24 h) relative to PBS control (7% intact at 24 h). For the MTD studies, body score and body weight showed no significant changes throughout the study. White blood cell count, lymphocytes, and neutrophils decreased in a dose-dependent manner. Significant uptake in the liver, kidney, and small intestine was observed. Based on pathology analysis, no damage was observed in the kidneys and small intestine while minor damage was observed in the liver.

Conclusions: No significant organ damage and no adverse effects due to radiotoxicity were observed. The lack of adverse effects and stability of ^{177}Lu-NAN-E1 indicates that the compound is a valid candidate for further development. Therapy studies are ongoing to investigate the therapeutic potential of ^{177}Lu-NAN-E1 in NTSR1-positive tumor models.