

CR2-Crry (C3 inhibitor that blocks all activation products), anti-C5 mAb (blocks C5a and MAC), C5aRA (blocks C5a-C5a receptor interaction), or anti-C7 mAb (blocks MAC). Study endpoints were P7 or P14. **RESULTS/ANTICIPATED RESULTS:** Following GMH, CR2-Crry treatment decreased MAC deposition on RBC and additionally decreased heme oxygenase-1 expression, heme deposition, and iron-induced inflammation measured at P7. In support of a specific role for the MAC, anti-C7 mAb treatment resulted in similar outcomes and was similarly protective. Anti-C7 mAb treatment also reduced hydrocephalus development at a later time point (P14). A similar result was obtained using C7 deficient mice and with anti-C5 mAb treatment. On the other hand, no protective effect was seen with C5aR blockade, and double knock out of C3aR/C5aR also did not provide protection, indicating no role for the anaphylatoxins C3a and C5a and their receptors expressed on leukocytes and endothelial cells in exacerbating deteriorating outcomes. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our data indicate a key role for the MAC in RBC induced hemolysis after GMH which serves as a driver of inflammation and early GMH pathogenesis. We further show that we can effectively increase precision by targeting solely the MAC complex acutely. Future work will be undertaken to determine temporal roles of individual complement activation products.

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Defining the impact of short-chain fatty acids on the gut-urinary axis in a naturally occurring canine model of calcium oxalate urolithiasis

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OBJECTIVES/GOALS: Short-chain fatty acids (SCFAs) exert protective effects against calcium oxalate (CaOx) urinary stone formation in experimental rodent models, yet these effects are not understood in natural stone formers. This study will define the impact of SCFAs on stone risk factors along the gut-kidney axis in a natural canine model of CaOx stone disease. **METHODS/STUDY POPULATION:** A randomized, placebo-controlled, clinical trial will be performed using a crossover study design. Twenty dogs that are natural CaOx stone formers will be fed a standardized diet and randomized to receive either a daily prebiotic fiber (inulin) that stimulates SCFA production or a placebo. We will perform fecal shotgun metagenomics and SCFA quantification before and after each intervention (four timepoints) to identify how inulin and SCFAs enrich or deplete pathways relevant to stone formation within the gut microbiome. RT-qPCR will be performed to determine the effects of SCFAs on intestinal oxalate transporter gene expression (SLC26A3 and SLC26A6). At each timepoint, urinary shotgun metagenomics and quantification of urine biochemical profiles used to predict stone risk will also be performed. **RESULTS/ANTICIPATED RESULTS:** We anticipate that prebiotic stimulation of SCFAs with inulin will reduce stone risk factors along the gut-urinary axis in a natural canine model of urinary stone disease. Specifically, we anticipate that prebiotic stimulation of SCFAs will 1) modify gut and urinary microbial communities to promote pathways considered protective against stone formation, 2) alter the expression of oxalate transporters (SLC26A3, SLC26A6) to reduce net oxalate absorption, and 3) reduce stone-promoting metabolites (e.g., oxalate) in the urine. **DISCUSSION/SIGNIFICANCE OF IMPACT:** By defining the impact of prebiotic fibers and SCFAs on the gut-urinary axis in a natural animal model of CaOx

urolithiasis, we will lay the foundation for novel nutritional strategies to prevent CaOx stone disease in both humans and animals.

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Development of small molecules targeting an epigenetic modulator for pediatric neuroblastoma

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OBJECTIVES/GOALS: The bromodomain PHD finger transcription factor (BPTF) is an oncogenic driver of neuroblastoma. Our objective is to pioneer the discovery of the first class of chemical compounds that engage the PHD finger of BPTF and inhibit its biological function in cellulo, thereby establishing first-in-class chemical probes for this epigenetic reader. **METHODS/STUDY POPULATION:** Our previous work has identified a collection of small molecules that engage BPTF PHD in vitro. Following structure-activity relationships analysis, candidates will be used in a neuroblastoma cell model to validate BPTF PHD interaction in cellulo and predict therapeutic potential. Nanoluciferase bioluminescence resonance energy transfer (NanoBRET) will be used to confirm BPTF PHD engagement by compounds. Selective toxicity in neuroblastoma cells upon inhibitor treatment will be gauged by comparing cell growth and viability in the IMR-32 cell line against the HEK293 cell line. Treated HEK293 cells will be subjected to the assay for transposase-accessible chromatin (ATAC) and RNA sequencing methods to monitor changes in chromatin structure and transcriptional signatures against untreated cells. **RESULTS/ANTICIPATED RESULTS:** We hypothesize that compounds with low micromolar potency for BPTF PHD in vitro will engage the target in cellulo and displace NanoLuciferase tagged protein from its HaloTagged® peptide binding partner. Additionally, we anticipate that our inhibitors will show cytotoxicity for IMR-32 cells with limited effects on HEK293 cells. We envision that inhibitor treatment in HEK293 cells will correlate with reduced chromatin exposure, suggesting that blocking the BPTF-histone interaction via PHD finger inhibition hinders the remodeling of transcriptionally silent heterochromatin into a transcriptionally active state. Finally, we expect that inhibitor treatment will result in diminished gene expression of oncogenic transcription factors, including N-Myc, a biomarker of neuroblastoma. **DISCUSSION/SIGNIFICANCE OF IMPACT:** These first-in-class chemical probes for BPTF PHD will enable further investigation of BPTF and high-risk neuroblastoma progression, as well as its role in other diseases. In addition, these compounds will serve as a platform for the development of new anticancer agents that may improve outcomes for children that suffer from high-risk neuroblastoma.

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Fetal natural killer cells play an essential immunoprotective role in preventing the onset of symptomatic congenital cytomegalovirus

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OBJECTIVES/GOALS: Congenital cytomegalovirus (cCMV) continues to be the primary infectious cause of fetal anomalies. The role