differences to non pPCL MM. 2) Determine whether genetic changes pertinent to pPCL could be explored as therapeutic targets to improve the dismal prognosis of this patient population. METHODS/STUDY POPULATION: Samples from overall 19 pPCL patients that presented to the Myeloma Center, UAMS between 2000-2018 were used for this study. We performed gene expression profiling (GEP; Affymetrix U133 Plus 2.0) of matched circulating peripheral PCs and bone marrow (BM) PCs from 13 patients. Whole exome sequencing (WES) was performed on purified CD138+ PCs from BM aspirates from 19 pPCL patients with a median depth of 61x. CD34+ sorted cells, taken at the time of stem cell harvest from the same 19 patients, were used as controls. Translocations and mutations were called using Manta and Strelka and annotated as previously reported. Copy number was determined by Sequenza. RESULTS/ANTICIPATED RESULTS: 1) GEP from the BM and circulating peripheral PCs showed that the expression patterns of the two samples from each individual clustered together, indicating that circulating PCs and BM PCs in pPCL result from the same clone and are biologically clearly related. 2) The clinical characteristics from the patient cohort used for WES analysis were as follows: median age was 58 years (range 36–77), females accounted for 74% (14/19), an elevated creatinine level was found in 78% (14/18) and an elevated LDH level in 71% (10/14). All patients presented with an ISS stage of III. Median OS of the whole dataset was poor at 22 months, which is consistent with OS from previously reported pPCL cohorts. 3) Primary Immunoglobulin translocations were common and identified in 63% (12/19) of patients, including MAF translocations, which are known to carry high risk in 42% (8/19) of patients [t(14;16), 32% and t(14;20), 10%] followed by t(11;14) (16%) and t(4;14) (10%). Furthermore, 32% (6/19) of patients had at least one MYC translocation, which are known to play a crucial role in disease progression. 4) The mutational burden of pPCL consisted of a median of 98 non-silent mutations per sample, suggesting that the mutational landscape of pPCL is highly complex and harbors more coding mutations than non-pPCL MM. 5) Driver mutations, that previously have been described in non-pPCL MM showed a different prevalence and distribution in pPCL, including KRAS and TP53 with 47% (9/19) and 37% (7/19) affected patients respectively compared to 21% and 5% in non-PCL MM. PIK3CA (5%), PRDM1 (10%), EP300 (10%) and NF1 (10%) were also enriched in the pPCL group compared to previously reported cases in non-pPCL MM. 6) Biallelic inactivation of TP53 – a feature of Double Hit myeloma - was found in 6/19 (32%) samples, indicating a predominance of high risk genomic features compared to non-pPCL MM. Furthermore, analysis of mutational signatures in pPCL showed that aberrant APOBEC activity was highly prevalent only in patients with a MAF translocation, but not in other translocation groups. DISCUSSION/SIGNIFICANCE OF IMPACT: In conclusion we present one of the first WES datasets on pPCL with the largest patient cohort reported to date and show that pPCL is a highly complex disease. The aggressive disease behavior can, at least in part, be explained by a high prevalence of MAF and MYC translocations, TP53 and KRAS mutations as well as bi-allelic inactivation of TP53. It is of interest that only KRAS but not NRAS mutations are highly enriched in pPCL. From all highly prevalent genomic alterations in pPCL, only KRAS mutations offer a potential for already available therapeutically targeting with MEK inhibitors, which should be further explored.

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Hemoglobin selectively inhibits renal proximal tubular uptake of proteins: Implications for vitamin D deficiency and kidney disease in sickle cell disease

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OBJECTIVES/SPECIFIC AIMS: While Hb-induced toxicity has been assumed to cause PT dysfunction, the development of tubular proteinuria from this dysfunction in SCD patients is not well understood. We previously found that free Hb, at concentrations predicted to be present chronically and during hemolytic crisis in the tubular filtrate of SCD patients, impairs uptake of albumin by PT cells via direct competition for binding to megalin and cubilin receptors. The purpose of this study is to further evaluate the consequences of increased filtered Hb concentrations on vitamin D reabsorption and activation. METHODS/STUDY POPULATION: We have developed a PT cell culture model that closely mimics in vivo PT cell structure, morphology, and endocytic capacity. Using this model, we treated cells with physiologic levels of cell-free Hb estimated in SCD and measured protein endocytosis and toxicity/oxidative stress. Endocytosis of fluorescently-tagged DBP and RBP were evaluated and quantified by confocal imaging and spectrofluorometry. Cellular toxicity and oxidative stress were assessed by measuring aconitase activity and accumulation of mitochondrial reactive oxygen species. RESULTS/ANTICIPATED RESULTS: PT cell uptake of DBP was significantly inhibited by both concentrations of Hb estimated to be filtered into the tubule lumen under chronic conditions (0.6μM Hb; 39% inhibition) and hemolytic crisis (≤20μM Hb; up to 92% inhibition) in SCD patients. ****$p<0.0001$ by one-way ANOVA, Dunnett's multiple comparisons test. PT cell uptake of RBP was minimally affected by the same concentrations of Hb that profoundly inhibited internalization of DBP. RBP uptake was not significantly inhibited by all concentrations of Hb tested except the estimated hemolytic crisis maximum concentration (20μM; 27% inhibition). RBP uptake inhibition at 20μM Hb treatment was dramatically less than DBP uptake inhibition under the same treatment condition (27% RBP inhibition vs 92% DBP inhibition). *$p<0.05$ by one-way ANOVA, Dunnett's multiple comparisons test. Mitochondrial oxidative stress, measured as a decrease in aconitase activity, was significantly increased in cells exposed to Hb (~43% aconitase activity reduction after 72h 20μM Hb treatment, and ~11% aconitase activity reduction after 72h 1μM Hb treatment). *$p<0.05$, **$p<0.01$ by one-sample t-test of the differences between treatment and untreated control conditions. We are currently assessing changes in PT cell vitamin D hydroxylase expression levels and vitamin D metabolism after exposure to chronic and hemolytic concentrations of Hb. Because oxidative stress has been previously reported to affect hydroxylase expression and activity, we expect to find a decrease in vitamin D hydroxylase expression and/or activity, resulting in decreased vitamin D activation. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that competition for megalin/cubilin binding between Hb and normally-filtered proteins, including DBP, may be the primary cause of tubular proteinuria in SCD patients. This inhibition appears to be selective for proteins that are largely α-helical in structure, such as albumin and DBP. Understanding the structural basis for Hb competition with filtered proteins for PT uptake could identify biomarkers to detect tubular proteinuria in SCD patients prior to the onset of kidney disease. This may also help develop therapeutic compounds that would selectively inhibit

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Hb binding to megalin/cubilin receptors. We have developed a highly sensitive fluorescence-based assay to test for such compounds. Inhibition of DBP uptake and vitamin D metabolism in the PT could lead or contribute to vitamin D deficiency. To our knowledge, our study is the first to suggest a mechanism for vitamin D deficiency commonly observed in SCD patients. Ongoing studies focus on measuring vitamin D metabolism in both cell and mouse models of SCD.

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Identification of exhaustive markers in cytotoxic T-cells to guide immune modulation in hepatocellular carcinoma ex vivo

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OBJECTIVES/SPECIFIC AIMS: Objective: apply checkpoint inhibitors that are specific to the exhaustive markers expressed on tumor CD8+ T-cells ex vivo in order to improve cytokine release and cytotoxic function in comparison to two control groups: (1.) T-cells that receive no antibodies; (2.) T-cells that receive standard inhibition with PD-1 and CTLA-4 antibodies only. Long-term objective: provide personalized medicine in the treatment of HCC by using checkpoint inhibitors that are specific to the receptors expressed by an individual tumor. METHODS/STUDY POPULATION: The study population includes patients undergoing liver transplantation or surgical resection for HCC. Two grams of tumor, two grams of healthy liver tissue at least one centimeter from the tumor margin, and 50 milliliters of blood will be obtained. Solid tissue will be mechanically and enzymatically disrupted and CD8+ T-cells will be isolated from all sites. Using flow cytometry, the expression of surface receptors PD-1, CTLA-4, LAG-3, TIM-3, BTLA, CD244, and CD160 will be categorized in each tissue to identify which receptors are upregulated in the tumor microenvironment. Up to three antibodies specific to the upregulated receptor(s) on the tumor T-cells will be applied per specimen. The experimental arm will receive these antibodies and co-stimulation with CD3/CD28 and will be compared to two controls. One control will receive only CD3/CD28, and the other will receive CD3/CD28 in addition to the standard combination of PD-1 and CTLA-4 inhibitors. From each condition, flow cytometry will be used to assess the mean production of interleukin-2, tumor necrosis factor-α, interferon-γ, granzyme B, and perforin expression as an assessment of T-cell function. RESULTS/ANTICIPATED RESULTS: Preliminary data from the peripheral blood of healthy controls confirms that the developed flow cytometry panels effectively identify the surface receptors and cytokine production of CD8+ T-cells. Two patients have successfully been enrolled in this study. It is predicted that T-cells extracted from the tumor will express more inhibitory receptors than normal liver or peripheral blood and will have increased function after they are targeted with checkpoint inhibitors that are specific to the inhibitory surface receptors they express. DISCUSSION/SIGNIFICANCE OF IMPACT: HCC is the second leading cause of cancer-related death worldwide and therapeutic options are limited for patients who are not surgical candidates. T-cells are a critical component of the anti-tumor response to HCC. However, T-cells can develop an exhausted phenotype characterized by up-regulated inhibitory receptors (PD-1, CTLA-4, LAG-3, TIM-3, CD-244, CD-160, BTLA) and decreased function, allowing for immune escape. Clinical trials using combined checkpoint inhibition with PD-L1 and CTLA-4 antibodies have been considered a breakthrough for patients with advanced HCC, as up to 25% show an objective tumor response. The explanation for the varied susceptibility to checkpoint inhibition remains unknown and is hypothesized to be secondary to inconsistencies in the expression of surface inhibitory receptors. Although inhibitory receptor expression has been shown to be upregulated under conditions of hepatitis and/or HCC, there has been no single study to effectively investigate the expression of all known inhibitors in order to better explore the interplay between them. It will be of great academic interest and clinical purpose to evaluate individual receptor expression and engage the correlating antibodies given the possibility of synergism between receptors and the need for a more profound anti-tumor T-cell response in HCC.

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Identification of host-microbial interaction networks that mediate intestinal epithelial barrier function in necrotizing enterocolitis

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OBJECTIVES/SPECIFIC AIMS: The central goal of this proposal is to characterize the mechanisms that mediate success or failure of immature intestinal barrier in necrotizing enterocilitis. METHODS/STUDY POPULATION: To do this, I will utilize stem cell derived human intestinal organoids (HIOs), an innovative model of the immature intestine, and a cohort of bacterial isolates collected from premature infants who developed NEC to interrogate the cause-effect relationship of these strains on maintenance of the intestinal barrier. I hypothesize that the epithelial response to bacterial colonization is strain-dependent and results in differences in inflammatory signaling that shape epithelial barrier function in the immature intestine. RESULTS/ANTICIPATED RESULTS: Preliminary data shows that colonization of HIOs with different bacteria leads to species-specific changes in barrier function, and some species selectively damage the epithelial barrier while others enhance epithelial barrier function. I have identified key inflammatory signals that serve as central drivers of intestinal barrier function. DISCUSSION/SIGNIFICANCE OF IMPACT: Characterization of this process is expected to substantially advance scientific understanding of early events in NEC pathogenesis and lead to new opportunities for targeted therapeutic intervention to accelerate barrier maturation or prevent hyperinflammatory reactivity in the neonatal intestine. The research proposed in this application represents an entirely novel approach to studying host-microbial interactions in the immature. Conceptually, this novel translational approach will help to define the pivotal role of colonizing bacteria in initiating epithelial inflammation in NEC patients.

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Identification of Immune Cell Profiles and Molecular Pathways in Inflammatory Bowel Disease Driving Non-Response to Biologic Therapy

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OBJECTIVES/SPECIFIC AIMS: Inflammatory Bowel Disease (IBD) is a chronic, life-long condition characterized by inflammation of the intestine that greatly affects an individual's quality of life. While

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