Live Like Bella Pediatric Cancer Biomedical Research Proposal Review

Grant# 22L01

30014

PI: Hall, Matthew

Institution: Baptist Health South Florida

Personalizing radiotherapy dose using genomic markers of radiosensitivity to predict tumor response and normal tissue toxicity in pediatric malignancies

Targeted therapies are increasingly used to personalize cancer care based on an individual patient's tumor genome. While radiotherapy (RT) technologies have advanced over time, patients largely receive the same empirical dose based on their diagnosis and the risk of normal tissue complications. To date, RT has not been adjusted based on inherent biological differences of patients or their tumors. In adults, we developed a radiosensitivity index (RSI) to stratify radiosensitive and radioresistant tumors and the genomic-adjusted radiation dose (GARD) to quantify the optimal RT dose for individual patients to achieve local disease control. RSI and GARD were studied in >20 adult tumor types including glioma, sarcoma, breast and lung cancers and were both prognostic and predictive of local tumor control and survival across multiple tumor types. These measures are derived from a multigene expression panel to predict tumor radiosensitivity and understand normal tissue complication probability (NTCP) by accounting for biological heterogeneity within patients and tumors. RSI and GARD were validated in adults but have never been studied in pediatric cancers. We aim to perform the first evaluation of RSI and GARD in childhood cancers. We will collect pathology specimens of 200 pediatric and young adult patients treated with curative intent RT between 2014-2020, including medulloblastoma, ependymoma, germinoma, rhabdomyosarcoma, and Ewing sarcoma cohorts. After informed consent is obtained, tumor specimens will be assayed using the Affymetrix Hu-RSTA-2a520709 microarray, which provides data on 25,000 genes. RSI and GARD will be calculated for each patient using the RSI formula validated in adults. Using robust long-term follow-up data collected in our survivorship and late effects clinics on up to 90% of our treated patients, we will examine whether RSI and GARD are correlated with local control and survival outcomes. As a critical exploratory objective, we also have prospective data for 50 brain tumor patients who received RT and had regular volumetric MRIs to measure brain substructure volumes and late effects assessments. We seek to correlate anatomic changes in the brain and neurocognitive/neuroendocrine outcomes with results of the gene microarray. If validated, RSI and GARD may enable the personalization of RT dose, where the optimal prescription will reliably achieve cure and minimize risk of normal tissue injury. Childhood cancer survivors experience significant risk of morbidity and death after treatment and reducing these effects is imperative. In selected tumors that are radiosensitive, de-escalation may be feasible where patients can receive lower RT doses and maintain high cure rates while reducing the risk of late toxicities. In radioresistant tumors, clinicians may be able to dose escalate in selected patients to improve local control and survival.

Grant# 22L02

30005

PI: Westmoreland, Tamarah

Institution: Nemours Childrens Hospital

Treatment of Diffuse Intrinsic Pontine Glioma with the Oncolytic Zika Virus

Diffuse intrinsic pontine glioma (DIPG) is a devastating brainstem tumor affecting 150-300 children in the US per year. Despite advances, treatment for this aggressive tumor is not effective. Consequently, the median survival for children with DIPG is less than a year. Insights into novel DIPG therapies are found in related tumor research.Our research team published neuroblastoma cells are permissive to Zika virus. One neuroblastoma cell line was not susceptible because it lacked the cell surface protein CD24. With CD24 expression, these cells became sensitive to virus-induced killing. We validated a survival advantage in mice baring human neuroblastoma treated with Zika virus. Our findings in neuroblastoma may apply as a therapy for DIPG. While Zika viruses are potentially teratogenic to a fetus, 80% of infections in children and adults are asymptomatic. If symptomatic, symptoms are mild and self-limiting without long-term sequelae. Thus, wild-type Zika viruses may be tolerated as oncolytic therapies.Because of the common lineage between neuroblasts and glial cells, we found that a single Zika virus injection killed >90% of multiple DIPG cell lines. In a pilot mouse study, we successfully treated DIPG tumors with Zika virus with >90% cell death. As a result, we are pursuing the Live Like Bella grant to characterize the oncolytic killing of DIPG cells by Zika virus and expand our in vivo model by achieving the following aims:Aim 1: Assess the in vivo murine xenograft modeling of the antitumor effects of Zika virus on diffuse intrinsic pontine glioma (DIPG). We will assess Zika virus as a treatment for DIPG in vivo in tumors both subcutaneously and orthotopically. We will use cultured DIPG cells and patient-derived cells to create tumors in immunodeficient nude mice. The tumor-bearing mice will be treated to determine if Zika viruses kill the tumor in vivo, exploring a multiple injection protocol to further optimize the process. DIPG tumor volume will be monitored with calipers and measured histologically at experiment end. We predict DIPG xenograft tumors will respond to Zika virus treatment with tumor burden reduction or elimination with a multiple injection protocol. Aim 2: Determine if cultured DIPG cells express the cell surface glycoprotein, CD24, and determine if CD24 protein expression is required for Zika virus-induced DIPG cell lysis. Cell surface CD24 expression will be measured as described in our published paper (Mazar et al., 2018). Given that CD24 is expressed in gliomas and that DIPG cells are susceptible to Zika virus-induced lysis, we anticipate that CD24 is expressed in DIPG.To determine if CD24 protein expression is required for Zika virus-induced DIPG cell lysis, CD24 expression will be reduced using a proven anti-CD24 small interfering RNA (siRNA) targeted against CD24. Once reduced, we will treat the DIPG cells with Zika virus and measure cell viability using direct visualization via bright field examination, cytotoxicity assays, and cell proliferation assays. Based on our publications, we expect that CD24 will be expressed in DIPG cells and be required for Zika virus-induced DIPG cell lysis.Due to our neuroblastoma experience and our data with DIPG cells and mice, we are confident that Zika viruses will have significant oncolytic activity in DIPG. Completing these animal studies will open the door to phase 1 trials for the novel therapeutic administration of Zika virus as a treatment for children with DIPG.

Grant# 22L03

30012

PI: Licht, Jonathan

Institution: University of Florida

Elucidation and targeting of epigenetic changes resulting in glucocorticoid resistance in pediatric acute lymphoblastic leukemia

Background: Glucocorticoids (GC) are a major component of therapy of pediatric acute lymphoblastic leukemia (ALL). Early relapse of ALL is associated with mutations/deletion of NR3C1 (glucocorticoid receptor, GR), NR3C2 (mineralocorticoid receptor-MR) and NSD2 (histone methyltransferase). We hypothesize that these mutations affect therapeutic response to GC. GR and MR both bind GC and enter the nucleus to alter gene expression. Both proteins may mediate the therapeutic effect of GC in ALL. With Live Like Bella support, in a new Cancer Discovery paper we characterized the NSD2 p.E1099K mutation of ALL using CRISPR-edited cell lines and patient-derived-xenografts (PDX). The mutation drove invasive oncogenic behavior and GC resistance of ALL by disrupting the balance of histone H3 lysine 36 and lysine 27 methylation required for normal gene regulation. NSD2 p.E1099K led to aberrant, repressive histone H3 lysine 27 methylation at the NR3C1 promoter, blocking GR expression in response to GC, resulting in loss of genome-wide GR binding and a failure to activate pro-death genes. Treatment of NSD2 mutant cell lines with EZH2 inhibitors decreased histone methylation at NR3C1, allowing its induction by GC to reverse GC resistance in cell lines and patient specimens. EZH2 inhibition also increased the efficacy of GC therapy in NSD2 wild-type cells. Based on these findings we proposed a trial of vincristine, EZH2 inhibitor and GC for relapsed ALL to the Children's Oncology Group. The role of other NSD2 mutations found in ALL is uncertain. Patient derived cells with NSD2 p.G1246S mutation were resistant to GC while a sample with NSD2 p.T1150A was sensitive. NSD2 p.E1099K upregulated NR3C2 while repressing NR3C1, suggesting an interplay between GR and MR signaling in ALL. Furthermore, NR3C1 and NR3C2 deletions are mutually exclusive in ALL and examination of the Depmap database indicates that lower expression or loss of the genes encoding GR or MR is associated with decreased sensitivity of ALL cell lines to GCs. Based upon these findings we will: Aim 1: Determine how NSD2 mutations affect the phenotype and therapeutic response of ALL. We will contrast the GC sensitivity, gene expression (RNA-Seq) and epigenetic changes (ChIP-Seq for NSD2/H3K36me2, EZH2/H3K27me3, EP300/H3K27ac; ATAC-Seq for chromatin accessibility) driven by the recurrent NSD2 mutations of ALL (E1099K, G1246S and T1150A). This will be performed using isogenic gene edited cell lines and validated in specimens from collaborator Richard Lock who leads an NCI funded bank of ALL specimens maintained as mouse xenografts. We will determine how each of these mutations affect genes related to invasive behavior as well as expression of the GR. Aim 2: Determine the role of MR in the response of ALL to glucocorticoids. While repression or deletion of GR has been studied in ALL, the role of MR has not been explored. We will determine how GC response of ALL cells is affected by knockout of MR expression and determine genome wide binding patterns of the MR in ALL cells. Aim 3 Develop methods to augment GC response of ALL. EZH2 inhibitors augment the activity of GC in NSD2 mutant and wild type ALL cell lines. We will further determine other ways to modulate gene expression and augment GC response in the face of NSD2 mutations or loss of GR or MR expression, including the use of histone deacetylase inhibitors, activators of histone acetyl transferase and DNA methylation inhibitors.

Grant# 22L04

30019

PI: Copik, Alicja

Institution: University of Central Florida

Edited Natural Killer cells as an immunotherapeutic approach for the treatment of pediatric cancers

The survival rates of pediatric cancer patients have greatly increased over the last decades with advances in technology and improvements in multimodal therapy and combination chemotherapies. According to the American Cancer Society from 2010-2016, the 5-year relative survival for childhood and adolescent cancer was 84%. This improvement, however, can largely be accounted for with increased cure rates of pediatric patients with more benign and localized disease. Children with high-risk tumors, metastatic disease at diagnosis, or those with progressive, refractory, or relapsed disease still have only modest improvement and many a dismal prognosis. For example, the prognosis of children with high-risk neuroblastoma at diagnosis is less than 50%. There is a critical need for new treatment modalities that are effective in these high-risk patients. Immune checkpoint blockade has been a highly successful tool for controlling malignancy in many cancer types and has revolutionized treatment of advanced cancers in adults. This drug class blocks receptors that signal to suppress the immune system from acting against cancers cells. Blocking these inhibiting proteins allows the body to better respond and kill cancer cells. Recently, there has been interest in the use of checkpoint inhibitors in pediatric oncology. While testing has been limited, there have been a dozen Phase I/II clinical trials testing checkpoint inhibitors in the treatment of resistant, refractory, or relapsed pediatric cancer. There are, however, several challenges to overcome before checkpoint inhibitors are standard of care for pediatric cancers. Unlike many adult cancers, most pediatric cancers are considered immunologically “cold.” This means there are not many immune cells present in and around the tumor, so it is difficult for the body to respond. This, combined with the fact that many of the current targets for blockade are not abundant in pediatric solid tumors makes it difficult for current immune checkpoint inhibition strategies to be effective. Currently available immune checkpoint inhibitors target the adaptive immune system to generate an antitumor response. However, this response depends on an intricate interplay between both the adaptive and the innate immune cells. Natural Killer (NK) cells are an important part of the innate immune response. They have an inherent ability to recognize and kill cancer cells and recruit other components of the immune system to further direct complete elimination of cancer. We hypothesize that this has the potential to turn "cold tumors", common in pediatric cancers, "hot" to greatly improve treatment outcomes. Recently, combination therapies using NK cells have shown antitumor responses against pediatric cancer cell types and have been used in a phase I clinical trial for treating younger patients with brain tumors. NK cells orchestrate the innate and adaptive immunity and play a pivotal role priming the immune system and setting the stage for successful response to cancer immunotherapy. NK cell-based therapeutics can provide a viable solution to increase the success of immunotherapeutic strategies in pediatric cancers. This project aims to develop clinically translatable NK cell-based immunotherapeutic strategies that harness both the innate and adaptive immune response to increase the response rate and lower the relapse rate in pediatric cancer patients.

Grant# 22L05

30018

PI: Parks, Griffith

Institution: University of Central Florida College of Medicine

Oncolytic virus in combination with NK cells for treatment of pediatric cancers

Pediatric cancers are a leading cause of death in children past infancy in the US, and it is estimated that world-wide a child is diagnosed with cancer every 2 minutes. There are major challenges in treatment of many pediatric cancers, since the cell biology and immunology of childhood cancers can differ substantially from that of adult cancers. This raises the important question of how to modify therapies such as those based on oncolytic (cancer lysing) viruses and immune-based therapies for more effective treatment of pediatric cancers. Natural killer (NK) cells are an integral part of the innate immune system that play pivotal roles in clearance of tumor cells as well as viral infections. We have developed a method for specific expansion of human NK cells that yields vastly superior PM-21 NK cells, with ~10-fold to 100-fold higher cytotoxicity than NK cells generated with previous methods. Our published work has also shown that cancer cells infected with an oncolytic virus produce signals which enhance the ability of PM-21 NK cells to kill virus infected targets, but importantly these signals stimulate NK cells to also kill neighboring non-infected cancer cells. Our oncolytic virus treatment can also modulate the surface of infected cancer cells to reduce expression of immune-suppressive molecules which can inhibit immune cell killing of cancer cells. Taken together, our long history of defining unique properties of our oncolytic virus and PM21 NK cells lead us to testing the hypothesis that treatment of pediatric cancers would be greatly improved by combining these therapies. This project, we will utilize our established approaches and reagents to test important pediatric tumor cell types for their ability to respond to single treatment or combined treatment with oncolytic virus and/or PM-21 NK cells. In the long term, our results will provide a foundation of new information on novel treatments that can impact the burden of childhood cancers.

Grant# 22L06

30013

PI: Deleyrolle, Loic

Institution: University of Florida

Co*-opting *TME lactate signal to benefit T cell therapy

Complex alterations of energy pathways have been described in cancers and originate from the Warburg hypothesis, which postulates that the majority of cancer cells derive their energy from aerobic glycolysis. This specific metabolic reprogramming of strong engagement in the glycolytic pathway is a hallmark of high-grade glioma (HGG). As part of their high glycolytic rate, HGG secrete metabolic byproducts such as lactate, which is thought to act as an important oncometabolite and immunosuppressor. It is now well recognized that HGG cell energetics strongly dictate the metabolic landscape of the tumor microenvironment (TME) supporting tumor development and growth. The TME is a complex network of diverse cellular compartments where tumor cells interact with a variety of non-neoplastic cells including immune cells, which represent key components of the tumor milieu. The metabolic specificities of HGG can determine fates and functions of neoplastic cells but also of immune cells creating specific niches, which play critical roles in restricting anti-tumor responses. Notwithstanding the presence of immune cells in HGG, the TME is globally immunosuppressive. Immune

evasion and metabolic reprogramming are now well-recognized hallmarks of cancer and are considered to be functionally linked. Immune cells also possess defined metabolic characteristics and requirements and the tumor milieu metabolic status tightly controls the function of immune cells. Understanding and exploiting the mechanisms of these immunosuppressive metabolic conditions has promise for improving anti-tumor immunity and may help in developing novel immunotherapies. Specifically, lactate produced in the TME, as a result of cancer cell metabolic rewiring, participates in immune escape via restricting T lymphocyte activity through the inhibition of their proliferation and cytokine production. Capitalizing on our current knowledge of tumor metabolism and how metabolic pathways affect immune response, this project proposes to test an innovative therapeutic modality based on reprograming the metabolic qualities of anti-tumor immune cells to enhance immunotherapy for the treatment of HGG. We hypothesize that co-opting lactate signal may be a useful approach to overcome metabolically driven tumor-imposed immunosuppression and for developing efficient immunotherapies. This project will test the efficacy of lactate receptor genetic engineering in T cells in the context of adoptive cell therapy to treat HGG. The main innovation of this project is the integration of fundamental concepts of tumor and immune metabolism in the design of T cell therapy. The major impact of our study is that successfully completed it will demonstrate that immunometabolism represents a viable and critical target for the development of new cancer therapies to treat brain tumors, especially HGG and will validate a clinically applicable method to overcome treatment resistance to adoptive cellular therapy in brain tumors.

Grant# 22L07

30011

PI: Castillo, Paul

Institution: University of Florida

Unlocking CAR T cell efficacy against osteosarcoma using adjuvant RNA vaccine

Refractory osteosarcoma (OSA) has a dismal prognosis of < 20% long-term survival rates despite aggressive chemotherapeutic and surgical treatments. Chimeric antigen receptor T (CART) cells have shown great promise in treating B cell hematological malignancies but have not proven as effective in refractory solid tumors, such as OSA. Reasons for therapeutic failure include poor CART cell proliferation, persistence, and inefficient CART cell trafficking into the regulatory OSA tumor microenvironment (TME). Unleashing CART cell therapy against poorly immunogenic cancers requires new technologies that activate the TME, while concomitantly inducing CART cell proliferation and persistence in vivo to generate sustained cellular immunity. We have developed a novel systemic vaccination approach to rapidly mobilize CART cells and unlock their activity in preclinical solid tumor models. This approach leverages surface antigen specific mRNA (i.e., CD70, a ubiquitously expressed OSA surface antigen) loaded into lipid-nanoparticle (NP) delivery systems to simultaneously reprogram the solid TME and promote CART cell activity. When delivering CD70 as an mRNA-NP vaccine, there is regression of CD70 expressing tumors resistant to CART monotherapy. We have shown that systemic NP vaccines induce type I interferon-mediated systemic activation that upregulates CCR2 expression on T cells leading to T lymphocyte migration from circulating blood into reticuloendothelial tissues for synergistic activity with adoptive cell therapy. We propose elucidation of mechanistic underpinnings responsible for CART cell activity in murine and canine OSA disease models. The canine model has nearly 100% homology to human OSA, and will allow identification of biologic response correlates. Since these translatable NP vaccines can be expeditiously manufactured, they can be harnessed as an “off the shelf” platform to unlock CART cell activity in refractory solid tumors. This work promises to advance our

understanding of innate and adaptive pathways needed for overcoming OSA immune resistance to CART cells while broadening its application to other poorly immunogenic malignancies. The scientific premise for this work is that CART cell activity is stymied by inadequate in vivo priming, tumor trafficking and persistence within the OSA TME. We hypothesize that RNA-NPs will mediate in vivo priming, tumor trafficking, and persistence of CART cells in the periphery and OSA TME unlocking their therapeutic activity. Towards that end, our SPECIFIC AIMS will be to: Aim 1: Establish the impact of systemic vaccination on safety, immunity, and efficacy of CART cells. We will deliver a ubiquitously OSA surface antigen (CD70) as RNA-NPs in conjunction with corresponding CART cells to establish safety and to dissect the mechanisms of how targeted RNA vaccination may co-opt innate and/or adaptive (CD70 specific) pathways for enhanced CART cell trafficking and fitness into the OSA TME. Aim 2: Determine safety and immunologic activity of CART cells with and without systemic vaccination in a large animal OSA model. CART + NP combination approach will be assessed in a pet dog trial in comparison with CART monotherapy. In preparation for first-in-human trials, we will treat 14 client-owned canines (7 per arm) with OSA and investigate safety, immunologic response, and efficacy. Immune correlates for response, guided from our data in aim 1, will be assessed from serum, blood, and end-organs of animals.

Grant# 22L08

30010

PI: Horn, Biljana

Institution: University of Florida

Engineered Donor Graft for Pediatric Hematopoietic Cell Transplant (HCT) Recipients with Hematologic Malignancies (HM)- Florida Pediatric Bone Marrow Transplant and Cell Therapy Consortium (FPBCC) First Prospective Multicenter Trial

In 2018, Florida pediatric BMT physicians from 5 institutions founded a consortium (FPBCC) with the goal of improving pediatric hematopoietic cell transplant (HCT) outcomes in Florida. FPBCC activities over the last 3 years, described in 2 published papers and 12 meeting abstracts, have included sharing best practices during monthly consortium meetings, a consortium-wide quality improvement project, and extensive retrospective data analyses to identify priorities for prospective clinical trials. Improving survival rates of children undergoing HCT for hematologic malignancies (HM) was identified as the number one FPBCC priority. Kaplan-Meier estimates of 1-year and 2-year overall survival (OS) of 150 children who received HCT for ALL and AML in FPBCC centers during the 2015-2019 period are 68% [95% CI 60-76%] and 58% [95% CI 49-68%], respectively. This low survival is due to a large proportion (65%) of high-risk patients, defined as those receiving HLA-mismatched transplants and/or those with high-risk disease. One-year overall survival of high-risk patients was 58% [95% CI 48-68%], and 2-year survival was 50.5% [95% CI 39-62%]. High-risk patients had high non-relapse (26%) and relapse (18%) mortality, at a median follow-up of 12 months (range 0.4-52 months).Improving survival, free of relapse and chronic graft-versus-host disease (cGVHD) in children receiving HCT for HM is the objective of the proposed multi-center trial. Graft engineering consists of precisely separating hematopoietic progenitor cells obtained by apheresis into components, e.g., stem cells, T-regulatory cells (Treg), and conventional T-cells (Tcon), and then designing a graft with the optimal ratio of cell subsets. Pre-clinical and clinical research indicates that infusion of high-purity Treg preceding Tcon infusion prevents graft-versus-host disease (GVHD) and maintains anti-cancer immunity. A precision-engineered graft made in a central GMP laboratory (Orca Biosystems, Inc.) was studied in high-risk adult HM recipients. One-year GVHD-free relapse-free survival was 69% in Orca engineered graft recipients, compared with 33% in recipients

of unmodified grafts. A similar approach using Treg/Tcon and haploidentical stem cells in high-risk pediatric ALL recipients in Italy resulted in 75% 3-year leukemia-free survival. We are proposing the first US pediatric prospective multicenter clinical trial using an engineered Orca graft. The trial will include children with ALL and AML undergoing HCT and will be conducted through the consortium (FPBCC) with a track record of collaboration and effectiveness. The product will be manufactured in a central GMP laboratory and provided for the trial by Orca Biosystems Inc. Study participants will undergo a myeloablative conditioning regimen and receive the engineered graft from related or unrelated donors. Study participants will be carefully followed for adverse events, relapse, GVHD, infections, and immune reconstitution. Transplant donors will undergo a peripheral blood stem cell collection. The product will be carried to the central GMP laboratory, processed, and returned to centers for infusion within 72 hours of collection. If this trial shows similar survival the adult Orca trial and Italian pediatric study, the survival of study participants will be significantly better than the FPBCC baseline data, and this approach may become a new standard of care.

Grant# 22L09

30007

PI: Stover, Brian

Institution: University of Florida

Ultrasound elastogram assessment of liver fibrosis in children and adolescents/young adults (AYA) receiving chemotherapy or allogeneic bone marrow transplantation, and identification of risk factors for liver injury

Survival rates for cancer have improved over the last several decades, but many of these cancer survivors are left with long term side effects from treatment. Antineoplastic therapy is commonly associated with acute and often reversible hepatotoxicity, but there is little knowledge available regarding the long-term liver health of these patients. Current recommendations for follow-up of hepato-biliary late effects include annual evaluation of liver enzymes, bilirubin levels, and ferritin levels. This limited follow-up may underestimate the risk of chronic liver injury related to chemotherapy or other events during cancer treatment. This study aims to identify the incidence of liver fibrosis and/or liver cirrhosis using non-invasive ultrasound elastography in a population of children (12 years of age and older) and adolescent young adults (AYA) who received chemotherapy for treatment of cancer or during stem cell transplant. We will analyze risk factors for liver injury and explore a potential relationship between polymorphism of genes involved in chemotherapy metabolism and the risk of liver injury. This is a cross sectional study which will enroll 100 children/AYA subjects who will have a liver ultrasound elastogram and will provide blood for pharmacogenomic testing. Detailed clinical data will be collected to gather information on risk factors for liver injury/fibrosis, including a history of obesity, hepatitis, pancreatitis, endothelial liver damage, elevation in LFTs, septic episodes and blood transfusions. Data will also be collected on chemotherapy agents received to explore a relationship between polymorphism of genes involved in their metabolism and the risk of liver injury. The results of the liver elastogram will be shared with each subject and if the liver elastogram is abnormal, they will be referred to the gastroenterology service for further evaluation and treatment. Results from this study will provide data for evidence-based guidelines that can be used in long-term follow-up of the hepatobiliary system in patients who received chemotherapy. Furthermore, identifying gene variants related to increased liver toxicity of chemotherapeutic agents will contribute to knowledge required for individualized patient treatment that will decrease risks of organ injury.

Grant# 22L10

30008

PI: Salloum, Ramzi

Institution: University of Florida

Point-of-care intervention to address financial toxicity in families facing pediatric cancer

The costs of cancer treatment have increased dramatically in recent years and this trend is projected to continue over time. Patients and families are bearing more of the economic burden of cancer treatments due to inadequate health insurance coverage which has led to growing out-of-pocket expenses. Financial hardship is the economic distress that results due to cancer diagnosis or treatment. If not addressed, financial hardship may lead to financial toxicity, which is the long-term adverse impact of financial hardship on physical or mental health outcomes. The potential impact of financial hardship on a patient and their family is particularly concerning when the child is the cancer survivor. Identifying families of pediatric cancer patients who experience financial hardship and testing interventions to connect them with financial counseling is a promising strategy. Financial counselors in Florida's Cooperative Extension System are well-positioned and experienced in delivering financial counseling including health insurance navigation. Our overall objective is to test the feasibility, acceptability, efficacy, and overall implementation of a point-of-care intervention to connect families of pediatric cancer patients who experience financial hardship with targeted, family-centered financial counseling services delivered via telehealth by accredited financial counselors in the Extension System. Telehealth can facilitate the implementation of financial and health insurance counseling among families of pediatric cancer patients, allowing for a broader geographic reach. We will conduct a randomized control trial with two study arms comparing a financial counseling program delivered via a telehealth platform versus usual care patient navigation. Eligible participants will be parents of patients (ages ≤21) diagnosed with cancer who are currently being treated. This research will be conducted in pediatric oncology practices affiliated with the OneFlorida Clinical Research Consortium. OneFlorida is a collaborative, statewide clinical research network that includes 12 healthcare delivery and payer partners providing care for 64% of all Floridians, covering all 67 Florida counties. Aim 1: Adapt and refine a financial counseling program for parents of pediatric cancer patients. To inform adaptations to the existing program, we will conduct semi-structured interviews with parents of pediatric cancer patients (n=20) and pilot test the adapted program with 10 parents. Aim 2: Evaluate the feasibility, acceptability, and efficacy of the financial counseling program against usual care patient navigation in a pragmatic trial of families facing pediatric cancer (n=80). We will assess program enrollment, satisfaction, and whether the program helps families improve health insurance literacy. We will examine differences in these outcomes in specific subgroups by sex, age, race, ethnicity, rurality, and type of insurance coverage. Aim 3: Identify barriers and facilitators to implementing the financial counseling program in clinical practice using the consolidated framework for implementation research (CFIR). We will examine the health care setting context and implementation processes with families and clinical care teams throughout the trial period. Families, clinicians, health systems, and payers will be engaged across the project continuum in a stakeholder advisory committee and user groups to optimize the program's dissemination and implementation potential.