Year 2017-2018 Live Like Bella Pediatric Cancer Research Grants

Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Jonathan Licht, MD	University of Florida	Identification Of Therapeutic Targets And Pathways In Relapsed Childhood Acute Lymphocytic Leukemia Associated With NSD2 Mutation	Background: Our laboratory has studied NSD2, a histone lysine methyltransferase initially identified by its rearrangement and aberrant overexpression in t(4;14)- associated multiple myeloma (MM). Overexpression of NSD2 leads to shifts in chromatin modification, gene expression and cell growth. We described a point mutation (E1099K) in the enzymatic domain of NSD2, present in 10-20% of cases of relapsed pediatric acute lymphoblastic leukemia (ALL). The mutation was found in 1% of the leukemia cells of a child at diagnosis and in 100% at relapse, suggesting that the mutation allows cells to persist in the face of therapy. NSD2 or the genes it affects must be targeted to prevent early ALL relapse. Preliminary data: The NSD2 E1099K mutation enhanced the rate of H3K36 dimethylation in vitro. Using gene editing we removed the NSD2 mutation from three ALL cell lines. Cell lines harboring E1099K exhibit increased H3K36 dimethylation and reduced H3K27 trimethylation. This led to up-regulation of a set of genes (~400) associated with neural and stromal lineages, not normally expressed in blood cells. This abnormal gene expression program correlates with aggressive biology. Mutant NSD2 cells exhibit reduced apoptosis and enhanced proliferation, clonogenicity, adhesion, and migration. In mouse xenografts, mutant NSD2 cells are more lethal and brain invasive. NSD2 mutant cells were resistant of glucocorticoids commonly used to treat childhood ALL as well as chemotherapy agents. Hypothesis: These data lead to our hypothesis that NSD2 drives expression of genes that stimulate cell growth and therapy resistance in childhood ALL. We will study the molecular basis of therapy resistance and identify critical NSD2 target genes. Our specific aims will be: 1-Determine how NSD2 mutation causes résistance to therapy. We will determine, how NSD2 mutation affects response to drugs used to treat ALL including: DNA damage inducers (doxorubicin), microtubule disrupters (vincristine); depletion of asparagine (L-asparaginase), proteasome inhibitors (bortezomib) and

			glucocorticoids. We will determine how NSD2 mutation affects DNA damage sensing and repair processes and the function of the glucocorticoid receptor to induce cell death. 2-Determine which NSD2 targets drive leukemia relapse- We will create a library of guide RNAs designed to inactivate genes regulated by mutant NSD2 (~400 genes; 6 guides/gene). NSD2 mutant cells will be transduced with CAS9 and a lentiviral guide RNA library. The initial composition of the library is determined by next generation sequencing shortly after transduction. The cells are allowed to growth for two weeks and the cells are again harvested, and guide library sequences and sequenced. Genes whose disruption blocks cell growth will have their guides be present in lower numbers. The screen will also be performed after injection of ALL cells into mice. We will collect leukemia cells from the marrow, spleen liver and brain to see if specific genes are required for brain invasion. We will further determine if disruption of NSD2 target genes re-sensitizes cells to therapy. 3- Determine epigenomic susceptibilities driven by NSD2 mutation that may represent new therapeutic targets. Cells in which the NSD2 mutation has been removed and parental controls will be screened for susceptibility to inactivation of epigenetic regulators using a focused guide RNA library and a panel of epigenetic inhibitors.

Year 2017-2018 Live Like Bella Pediatric Cancer Research Grants

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Cristina Fernandez-Valle	University of Central Florida	Synergistic FAK and PI3K combinatorial targeting for NF2 Schwannomas	Neurofibromatosis causes tumors to grow on nerves throughout the body (schwannomas) and in the brain (meningiomas and ependymomas). Neurofibromatosis type 2 (NF2) affects around 1 in 25,000 individuals worldwide. NF2 is caused by mutations in the Neurofibromatosis Type 2 gene (NF2) that encodes a tumor suppressor called merlin. A diagnostic criteria for this disorder is the development of schwannomas on both hearing and balance nerves; these tumors are called vestibular schwannomas or acoustic neuromas. NF2 patients develop multiple schwannomas on other nerves, and multiple brain and spinal tumors such as meningiomas and ependymomas. Most patients develop symptoms in their teenage years or during early childhood. Tumor control early in life is critical for maintaining a quality of life and preventing malignancies. Currently, potential treatments available for NF2-associated tumors are surgery, chemotherapy, and radiation therapy. However, due to location some tumors are inoperable and there are no Food and Drug Administration (FDA) approved drug therapies that shrink or stop the growth of schwannoma tumors. Undoubtedly, there is a great need for pharmaceuticals to prevent tumors from growing and shrinking or slowing the growth of existent tumors. The development of NF2 drug therapies has been challenged by lack of relevant merlin-deficient Schwann cell lines, animal models and clear druggable target because merlin lacks enzymatic activity. To address these deficits, our lab has created a panel of human and mouse cell models of the disease, developed high-throughput viability and high-content multi-parametric assays, conducted multiple screening campaigns of compound libraries using our Schwann cell lines, and optimized a sciatic nerve allograft mouse model in immune-deficient mice. We performed an unbiased chemical genomics approach and identified several PI3K and PI3K/mTOR inhibitors that selectively reduce viability NF2 model cells compared to control cells. However, kinome analysis of NF2 model cells chronically exposed to PI3K

			inhibitors revealed that cells compensated by consistently increasing activity of focal adhesion kinase (FAK) and SRC kinase family members. Rewiring of kinase networks occurs in cancer cells developing drug resistance to monotherapies. Moreover, an exploratory combination drug screen identified the PI3K inhibitor GSK-2126458 and the FAK inhibitor, TAE226, as highly synergistic and selective for merlin deficient Schwann cells. These results suggest that targeting the FAK/SRC pathway in combination with PI3K inhibitors should provide sustained inhibition of merlin-deficient Schwann cell proliferation and/or survival. To advance these findings, we propose to screen the effectiveness of PI3K pathway and FAK/SRC inhibition (alone and in combination) in multiple mouse and human merlin-deficient Schwann cell lines, and to study in vivo the best synergic combination (individual and combined drugs), using our orthotopic allograft model of NF2 schwannomas. We expect to obtain the necessary preclinical data to support their potential use in NF2.To optimize translation into clinical trials, the in vivo preclinical study will be designed and conducted in consultation with our collaborators, Drs. Smith and Aguilar-Bonilla, the pediatric neuro-oncologists at the Arnold Palmer Hospital for Children in Orlando.

Year 2017-2018 Live Like Bella Pediatric Cancer Research Grants

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David Robbins, PhD	University of Miami	Novel Regulators of SHH-driven Medulloblastoma	Medulloblastoma is the most common malignant pediatric brain cancer, 30% of which is driven by mutations in the Sonic Hedgehog signaling pathway. Improved therapies for medulloblastoma patients have significantly increased five-year survival. Still, current treatments often require radiation and chemotherapy, which can have devastating and long-term effects, including physical and cognitive defects, and secondary malignancies in the later part of their lives. Thus, there remains a pressing public health need to develop innovative, targeted therapies against such pediatric malignancies. To date, the majority of inhibitors that block Sonic Hedgehog signaling target the pathway activator Smoothened, which regulates the levels and activity of the Gli family of transcription factors. One such compound, vismodegib, is FDAapproved for a type of skin cancer, basal cell carcinoma, and is now undergoing clinical evaluation in medulloblastoma patients. However, rapid tumor recurrence, due to acquired mutations or inherent resistance, has already been frequently observed in medulloblastoma patients treated with vismodegib. Such observations underscore the critical need to identify inhibitors that act downstream of Smoothened, ideally on Gli family members themselves. However, as there are few examples in the clinic of drugs that act directly on transcription factors, we have instead focused on the identification of druggable proteins that in turn regulate Gli activity. Consistent with this strategy, we provide preliminary data showing that the methylation of Gli proteins can modulate their activity or stability, providing a novel way to regulate Gli activity by targeting these Gli methylases. We hypothesize that these regulators of Gli activity will provide novel ways to modulate its activity, and to attenuate the growth of Sonic Hedgehog driven medulloblastoma. The goal of this proposal is to elucidate the mechanism of action of this set of novel Gli regulators, determine their role in regulating medulloblastoma growth ex vivo, and demonstrate their functional roles in vivo using mouse and patient derived orthotopic mouse models of medulloblastoma.

Year 2017-2018 Live Like Bella Pediatric Cancer Research Grants

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Matthew Hall, MD	Miami Cancer Institute, Baptist Health South Florida	The Impact of radiation Dose on Brain Morphology, Volumetric Changes, Endocrine Function, and Neurocognitive Function Following Cranial Radiation Therapy in Children with Brain and Skull Base Tumors	Radiotherapy (RT) is a critical component of treatment for many children with central nervous system (CNS) tumors. Although >75% of children with brain tumors are cured, childhood cancer survivors are at substantial risk of treatment-related toxicities and their associated morbidities. Dosimetric studies have correlated the risk of neurologic, cognitive, and endocrine deficits with irradiation of specific brain regions in children. The relationship between morphometric changes in the brain after cranial RT and the development of neurocognitive, endocrine, and behavioral toxicities has not been systematically analyzed. To date, the effects of brain radiation have been studied in relatively few substructures and mostly in regions receiving higher doses. Low dose radiation exposure also has profound implications on brain development and function, and its effects are poorly understood. The brain is highly interconnected and is endowed with exquisitely radiosensitive stem cells responsible for neuro-regeneration. Radiation damage to one brain region can potentially affect brain development more broadly. This has important implications for RT planning and the potential for understanding its long-term effects on brain development. In this project, we propose a novel Phase 2 multi-institutional clinical trial to measure changes in brain morphology and substructure volumes in children following cranial RT and to correlate these effects with delivered dose. We also seek to quantify substructure dose-volume relationships with the incidence of RT toxicities. All children who receive conventional or proton RT to the brain are eligible. Morpho-volumetric changes within irradiated and unirradiated brain regions will be quantified on magnetic resonance imaging (MRI) using a novel software package. This sophisticated and validated tool can automatically delineate and accurately measure >50 separate brain regions and compare them to a normal age-adjusted population. Neurocognitive testing, endocrine status, and health-related quality of life (HRQOL) will

			be recorded at baseline and at pre-specified time points after RT. The primary objective is to measure the magnitude of morphometric changes in the brain after RT over time and correlate these changes with radiation dose. We hypothesize that measurable volumetric changes will occur in the brain following exposure to high, low, and even no RT. We further expect these changes to be different in different structures. For example, the dose response for morphometric changes in white and grey matter will be different in the developing brain. Secondary objectives include: • To correlate morphometric changes with the development of late treatment-related effects, including hormonal and neurocognitive deficiencies and HRQOL. • To measure cytokine levels and biomarkers in the blood before and after RT and analyze potential associations with radiation-induced toxicities and volumetric changes in the brain. This collaboration joins three high volume pediatric centers treating a significant proportion of children with brain tumors in South Florida. Our combined clinical and research infrastructure provides the patients and institutional resources needed to pursue this important inquiry. With a reliable model incorporating significant contributory parameters, clinicians can develop RT planning strategies to minimize risks after cranial RT and significantly reduce complications.

Year 2017-2018 Live Like Bella Pediatric Cancer Research Grants

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Diana Azzam, PhD	Florida International University	Personalized Ex Vivo Drug Screening and Genomics Profiling to Guide Individualized Treatments for Children with Relapsed or Refractory Solid Tumors and Leukemias	The major challenge in the clinical management of pediatric cancers is the development of effective and targeted therapies for the 30-40% of children with certain types of leukemias and solid tumors, in whom no remission occurs or who suffer relapse. Children with recurrent and refractory cancers often have very limited standard therapy options and are suffering from various treatment-related complications. Despite increased efforts in whole genome screening of pediatric cancers, much work remains to be done in characterizing germline and somatic mutations with matching drugs and appropriate targeted treatment. We have previously implemented and validated a combined functional drug screening and genomics platform to tailor treatments to individual patients with adult refractory cancers. This personalized approach enabled clinical application of individualized treatment for refractory patients with no alternative options. Here, we intend to adopt this approach in refractory pediatric cancers to guide clinical decision making and provide novel therapeutic options for children, selecting drugs from a library of FDA-approved oncology drugs to offer patients the safest possible chance of achieving remission and ultimately cure of their disease. In this pilot feasibility study, we intend to enroll chemorefractory or relapsed patients with all types of cancers where tumor tissue would be available for drug screening and genomic profiling. Using our robust high throughput ex vivo drug sensitivity assay and combining it with mutation analysis, we will be able to create a compendium of drug responses in individual patients, match actionable mutations with selective targeted therapies and clinically apply individual treatment for refractory patients with no alternative options.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Q.X. Amy Sang, PhD	Florida State University	Engineering Human Childhood Brain Malignant Rhabdoid Tumor Organoids	Atypical teratoid rhabdoid tumor (ATRT) is the deadliest type of human pediatric cancer of the central nervous system. It is responsible for half of all pediatric brain cancer deaths. Despite of research being done using animal models, there is no efficacious therapy specific for treating ATRT patients, partially due to lack of compelling human ATRT models to test potential therapeutics. To address this unmet scientific and medical need, our collaborative team of cancer cell biologists and biomedical engineers will build a human organoid model to better recapitulate human ATRT development. Over 90% of patients have biallelic inactivation of tumor suppressor gene SMARCB1 (*hSNF5/INI1*). Since SMARCB1 is a component of the SWI/SNF chromatin remodeling complex, the cause of ATRT could be related to defective chromatin configuration and uncontrolled cancer cell proliferation. The central hypothesis to be tested is that human pediatric brain rhabdoid tumor is originated from early neural progenitor cells after the inactivation of the SMARCB1 tumor suppressor; thus, deleting the SMARCB1 gene in early neural progenitor cells could generate a rhabdoid tumor model for therapeutic evaluation. Our goal is to develop a novel 3dimensional organoid model that mimics human pediatric brain rhabdoid tumor formation. The state of art CRISPRCas9 gene editing and stem cell technologies will be utilized to generate this novel human pediatric brain cancer model for drug screening and evaluation. Furthermore, we will identify the affected genes, proteins, and signaling pathways upon the loss of SMARCB1 gene in this cancer model to identify biomarkers and drug targets for future drug development. This is a high-risk, high-reward, and highly innovative pilot project that may lead to major breakthrough in human pediatric brain cancer research.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

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Mihaela Druta, MD	H. Lee Moffit Cancer Center and Research Institute	A Phase Ib/II Study to Evaluate the Safety, Feasibility and Efficacy of Nivolumab or Nivolumab in Combination with Azacitidine in Patients with Recurrent, Resectable Osteosarcoma	Osteosarcoma is the most common bone cancer in pediatric and young adult patients. Unfortunately, cure rates have not improved over the past 3 decades despite many efforts. Osteosarcoma is particularly difficult to cure if it recurs. In osteosarcoma genetic material, the DNA has become scrambled with tens of thousands of breaks. This is thousands of times more frequent than in most other childhood cancers and has made figuring out which of these changes matter elusive. To date, there are not clear genes that can be targeted with chemotherapy in a smarter way for osteosarcoma. There is a potential opportunity that all of these complex genetic changes may offer immunotherapy. There has been decades of observations that some osteosarcomas can stimulate the patient's immune system and the immune system can shrink or control osteosarcoma. Several trials had sought to prove that agents that stimulate the immune system could be efficacious in osteosarcoma but unfortunately have not thus far demonstrated success. The planned study builds on rapidly developing discoveries in the field of immuno-oncology, training the immune system to fight cancer. This field works to understand tumor cells, immune cells, and the environment in which these cells interact – many complicated interaction. Researchers typically focus on one aspect of this interaction. There is evidence that combining 2 agents increased the chances that the immune system would recognize and destroy osteosarcoma cells. The trial builds from this evidence and from observations in other genetically complex cancers to combine Azacytidine and Nivolumab in osteosarcoma patients that have had their disease recur. While it is often difficult to obtain access to exciting agents in pediatric cancers, we have been able to obtain access to this agent through ongoing work with Bristol-Myers Squibb and they are committed to supplying Nivolumab for this trial. We will give these compounds to patients who have had osteosarcoma come back and then proceed to surgery to remove all of

			the tumors. Both agents will be given after surgery as well to determine if they can keep osteosarcoma from coming back again. The nearly 40 patients that will be treated on this trial would be expected to have another recurrence 80% of the time without this therapy and we are hypothesizing that this treatment can more than double chances of osteosarcoma not coming back within a year from surgery. The trial will be conducted through an established framework: the National Pediatric Cancer Foundation's Sunshine Project. This network was born in Florida and is coordinated through Moffitt Cancer Center which is also the location of the principal investigator. The budget covers the Florida costs of this trial including the coordinating canter's major part in conducting this trial safely. There are currently six active Florida sites that conduct trials through this mechanism and an additional dozen sites around the nation that would participate in this trial. These trials are available to all Florida patients and to patients across the country. The Sunshine Project has a successful track record of completed trials in pediatric cancers in Florida and beyond.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Keiran Smalley, PhD	H. Lee Moffitt Cancer Center and Research Institute	Defining and Modeling Pediatric Melanoma Development	Melanoma is the deadliest form of skin cancer, accounting for ~70% of all skin cancer fatalities. Although more common in later life, melanoma is also a pediatric disease and there is evidence that rates of melanoma are increasing in children and adolescents. Potential risk factors for pediatric melanoma include xeroderma pigmentosum and the presence of large congenital nevi, as well as a family history of melanoma. Despite this, the majority of childhood melanomas are sporadic, with similar risk factors to that of adult melanoma, e.g. fair skin, blue eyes and an impaired tanning response. As a group, pediatric melanomas are highly heterogeneous and include those that are similar to adult melanomas (mostly arising post-puberty), melanomas that are associated with congenital nevi (typically prepubertal) and a subset of melanomas that exhibit Spitzoid histology (seen in both pre and post-pubertal cases). Recent years have seen major breakthroughs in our understanding of the genetic events that underlie the development of adult melanoma. In contrast, relatively little is known about molecular basis of sporadic pediatric melanoma. There is evidence that neonates and young children may be uniquely susceptible to the mutagenic effects of ultraviolet radiation from sun exposure. Studies in mice have demonstrated that a single intense sunburn at an early age, rather than in adulthood, leads to melanoma development. Although the reasons behind this are not understood, it is known that neonates have less developed immune systems, potentially allowing mutated melanocytes to evade immune detection. We hypothesize that sunburn in children, coupled with reduced immune editing, leads to the emergence of a heterogeneous mix of transformed melanocytes can rapidly develop into melanoma. At this time, there are major gaps in our understanding of these mechanisms. We do not know the age at which intense sunburn is the most harmful for melanoma development, we also know little about how immune competence shapes the mutational profile of the resulting melanoma.

			Our group has unique experience in the surgical management and pathological characterization of pediatric melanocytic neoplasms as well as basic science expertise in melanoma biology and mouse modeling. We will leverage this expertise, and our bank of highly annotated clinical samples, to perform one of the most comprehensive genomic analyses to date of pediatric melanoma. This genomic information will be used to inform the development of new genetic mouse models of pediatric melanoma, which we will then use to determine the link between age of burning sun exposure and melanoma development, and to identify other potential mutagenic drivers. Innovative single cell RNASeq technology and immunophenotyping will be used to define the role of the immature immune system in permitting the outgrowth of more genetically and transcriptionally diverse melanocyte populations that lead to melanoma development. The role of sunscreen in limiting the phenotypic diversity of transformed melanocytes will also be evaluated. Together, we expect this work to provide important new insights into how interactions between agerelated UVinduced mutagenesis and immune cell function cooperate to drive pediatric melanoma development. We further expect to define the critical age at which pathogenic sun exposure occurs and whether sun screen use can protect against this.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Jatinder Lamba, PhD	University of Florida	Pharmacogenomics and Toxicities of Thiotepa, Busulfan and Fludarabine in Pediatric HSCT Recipients	Hematopoietic cell transplant (HCT) is a complex, potentially lifesaving procedure for a number of pediatric malignancies. Acute lymphoblastic leukemia (ALL), diagnosed in approximately 3500 children annually in the US is the most common indication for pediatric HSCT with 400 children receiving HSCT for ALL annually. Based on results of studies published at the beginning of this century, total body radiation (TBI)based conditioning regimens are considered the standard of care for children with ALL undergoing HCT. TBI is related to many late effects and its avoidance in young HCT recipients is critical to prevent well-defined long-term toxicities. In 2018, the Pediatric Blood and Marrow Transplant Consortium (PBMTC) initiated a multi-institutional prospective clinical trial using a non-TBI based conditioning regimen including busulfan, fludarabine and thiotepa (BuFluTT) for children with ALL undergoing HCT (PBMTC ONC1701, EndRAD Trial, NCT03509961). The treatment arm of the trial will enroll 70 subjects. While multidrug conditioning aims to avoid TBI-related late-effects, little is known about the effect of variations in genes that metabolize these drugs (pharmacogenomics/PGx) on clinical outcomes including engraftment, relapse, and acute drug-related toxicities. Interpatient variation in the pharmacokinetics (PK) of drugs can result in the unpredictable occurrence of adverse events and toxicity and affect therapeutic efficacy. Although many factors contribute to alterations in PK, inherited variability in the expression of genes involved in drug metabolism and transport play a key role. For the drugs utilized in the EndRAD trial, PK data will be available, providing a great opportunity to explore the impact of PGx on drug exposure and clinical outcomes. Thiotepa requires metabolic conversion by several hepatic enzymes to the active species, including cytochrome P450s (CYP2B6, CYP3A).Busulfan undergoes extensive metabolism by several glutathione S transferases (GSTs) in the liver. Fludarabine requires transport by numerous influx transporters and

			intracellular conversion to the triphosphate form to inhibit DNA and RNA synthesis. Selected known single nucleotide polymorphisms (SNPs) within key candidate genes involved in the metabolism and disposition of BuFluTT will be genotyped using Quant studio. Additionally, GSTM1 gene deletion will be evaluated in these patients by gene sequencing analysis. We have preselected SNPs based on their functional impact on gene expression or activity and abundance in population. Overall SNPs with evidence of potential clinical relevance, occurring with the minimum allele frequency of greater than 10% have been selected for genotyping and statistical analysis. SNPs will be evaluated for their impact on drug clearance. The relationships between PGx, PK, disease relapse and adverse drug-related events will be explored. The effect of PGx and alterations in drug exposure (area under the curve) will be performed using standard nonlinear mixed effect modeling (NONMEM) and previously established population PK models for drug clearance. The associations with clinical endpoints will be evaluated by appropriate logistical and linear regression analyses. This study will expand our knowledge about the impact of PGx on efficacy and treatment-related morbidities of the BuFluTT conditioning regimen and has the potential to improve both acute and long-term survivorship for pediatric patients undergoing HCT for ALL.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

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Zhijian Qian, PhD	University of Florida	Molecular Basis and Treatment of Pediatric AML with Hyperexpression of EVI1	Pediatric Acute Myeloid Leukemia (AML), which is a cancer of myeloid line of blood cells, represents 1520% of all pediatric acute leukemia. Ectopic Viral Integration site 1 (EVI1) is a known oncogenic gene that cause cancer when it is aberrantly expressed in cells. EVI1 upregulation is implicated in 10 25% of primary AML in children and young adults, and has an inferior outcome with current chemotherapy regimens. However, hypomethylating agents are not currently used as standard chemotherapy for upfront treatment of AML in children and young adults. Their use is limited to the relapse setting where certain AML patients respond to them. It is not well understood what determines response to these drugs. We recently demonstrated a novel role of EVI1 as an epigenetic regulator in hematopoietic progenitor cells, and found that high EVI1 expression leads to global changes in DNA methylation in human CD34+ stem/progenitor cells. In addition, hyperexpression of EVI1 led to promoter hypermethylation and downregulation of critical genes involved in cell proliferation and differentiation in leukemia cells. More importantly, reversing DNA hypermethylation by hypomethylating agents or re-expression of the gene silenced by EVI1 inhibited growth and induced apoptosis of EVI1high human leukemia cells but not the leukemia cells with low EVI1 expression. To explore the role of EVI1 in hematopoietic stem/progenitor cells in vivo, we have established a novel mouse model in which EVI1 hyperexpression can be induced specifically in hematopoietic stem/progenitor cells. By using this newly established mouse model, human leukemia cell lines as well as primary leukemia cells from pediatric patients, we will determine the role of the critical downstream pathways that are deregulated by EVI1 as a consequence of EVI1induced DNA hypermethylation in the development of leukemia and determine whether and how hypomethylating agents work for the subgroup of EVI1high pediatric AML. Our studies advance our understanding of the biology of EVI1high pediatric AML and provide new molecular insights into therapeutic effects of hypomethylating agents in the subgroup of EVI1high pediatric AML.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

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Claudia Rodrigues, PhD	University of Miami	Novel Mechanisms of Anthracycline-Induced Cardiomyopathy	Anthracyclines are highly effective chemotherapeutic agents broadly used in the treatment of different types of pediatric and adult cancer. However, cancer survivors experience serious chronic and life-threatening effects due to its cardiotoxicity, which can lead to a decline in left ventricular function and congestive heart failure. This is especially true for pediatric patients as their survival rates have substantially improved, increasing their risk to suffer from life-threatening side effects of cancer treatment. Currently, there is no effective treatment for anthracycline-induced cardiomyopathy and heart failure. Since their discovery over 50 decades ago, the cardiotoxic effects of anthracyclines remain a significant medical problem. Cardiotoxic mechanisms are multifactorial and likely involve multiple cell types. Our long-term goal is to develop novel cardioprotective strategies to prevent or attenuate anthracycline-induced cardiotoxicity reducing the risk of heart failure. The goal of the proposed studies is to investigate a novel mechanism involved in anthracycline-induced cardiotoxicity taking in consideration remodeling changes that occur in the heart during the course of aging. We present supporting evidence of the involvement of cMyc in anthracycline cardiotoxicity and will investigate the central hypothesis that molecular switches driven by cMyc in endothelial cells trigger doxorubicin-induced cardiotoxicity and heart failure during aging. Our first aim is to investigate the role of cMyc in cells that line our blood vessels, known as endothelial cells, in anthracycline-induced cardiotoxicity. Our specific goal is to identify vascular-associated mechanisms that contribute to anthracycline-induced cardiac damage and lead to heart failure. We will test the novel hypothesis that anthracycline treatment reduces the expression of cMyc in endothelial cells triggering dysfunction and the release of soluble factors that reduce the survival of cardiac cells, which are required for proper heart contractile function. Loss of cardiac cells triggers a remodeling process

			that leads to heart dysfunction and failure. One of the main gaps in anthracycline-induced cardiomyopathy is the fact that most studies did not include aging as a risk factor. Better understanding of age-associated changes that contribute to cardiac remodeling after cancer treatment is essential, considering that pediatric patients are at risk of developing cardiomyopathy as they age. Our second aim is to identify molecular changes that contribute to anthracycline-induced cardiac remodeling during aging. We will test the novel hypothesis that distinct molecular switches are activated during early anthracycline-induced injury and throughout lifespan in the heart, triggering transition from adaptive to maladaptive cardiac remodeling, and ultimately leading to heart failure. Completion of the proposed aims will define novel mechanisms associated with anthracycline-induced cardiotoxicity, leading to the potential development of future therapeutic strategies for cardio protection in chemotherapy.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

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David Robbins, PhD	University of Miami	Designing New Therapeutic Strategies for the Most Lethal Forms of Medulloblastoma	Brain tumors are the number one cause of cancer related deaths in children, with medulloblastoma being the most common of them. One-third of medulloblastoma patients harbor tumors associated with constitutive SONIC HEDGEHOG activity (SHH subgroup). Although the survival of most medulloblastoma patients is over 50%, a subset of SHH subgroup patients have proven resistant to standard of care therapy, resulting in rapid tumor relapse and patient death. This subset of patients is characterized by mutations, both genomic and sporadic, in the tumor suppressor gene *P53*. Thus, these pediatric medulloblastoma patients represent the highest risk group within SHH medulloblastoma, and highlight the critical clinical need for alternative treatment paradigms for these patients. We recently described a small population of WNT-dependent tumor propagating cells within such *P53* mutant medulloblastomas. Further, abrogation of WNT signaling in mice harboring *P53* mutant medulloblastomas reduced tumor growth, and increased the overall survival of these mice. Our results suggest that loss of P53 activity in SHH subgroup medulloblastoma triggers the emergence of a distinct set of targetable signaling pathways, including that driven by WNT. Thus, we hypothesize that the growth of *P53* mutant medulloblastoma will be abrogated by targeting this P53 medulloblastoma specific set of signaling pathways. The goal of this proposal is to uncover the signaling pathways that are activated by *P53* loss in medulloblastoma, determine their role in regulating medulloblastoma growth, and identify novel therapeutic strategies to target these signaling pathways.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Brian Marples, PhD	University of Miami	Maintaining Renal Function after Total Body Irradiation	Total body irradiation (TBI) is used as a preparative regimen for allogeneic hematopoietic stem cell transplantation (HSCT). However, TBI is associated with acute and chronic renal dysfunction, as well as radiation nephropathy (RN). Resident cells in the glomerulus have been linked with renal injury after TBI, but the molecular mechanisms governing RN are not understood. In cultured glomerular podocytes, we discovered that the levels of sphingomyelin-phosphodiesterase-acid-like-3b (SMPDL3b) are reduced after irradiation, and this triggers the cellular relocation of cytoskeletal proteins leading to a morphological change that alters podocyte functionality. We have associated the reduction of SMPDL3b with concurrent reduced expression of the cytoskeleton-modulator ATE1, along with increased podocyte apoptosis. C57BL/6 mice given kidney-only focal irradiation and treated with rituximab, which we demonstrated to bind SMPDL3b and protect podocyte morphology, had reduced renal dysfunction. Whereas, no radiation protection was seen when rituximab was given to our conditional podocyte-specific SMPDL3b knock-out mice. Based on these data we hypothesize that SMPDL3b and ATE1 regulate podocytopathy and renal dysfunction after kidney-only focal irradiation. The objective is to investigate the mechanistic role of SMPDL3b in renal injury after single dose TBI and fractionated TBI as this represents a standard of care for hematopoietic conditioning. Our long-term goal is to discover a molecular-based protective or mitigating strategy for RN, and potentially chemotherapy-induced nephrotoxicity.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Matteo Trucco, MD	University of Miami	Enhancing Immunotherapy through Inhibition of Carbonic Anhydrase IX to Treat Osteosarcoma	Osteosarcoma is the most common bone tumor, primarily affecting children, adolescents and young adults, with the cure rate for this cancer being stagnant for decades at about 70% for patients who have localized disease and below 30% in the fifth of patients who present with metastatic disease. There have been no significant improvements in the cure rates for metastatic or relapsed Osteosarcoma in over 40 years. Unfortunately, promising new treatments like immunotherapy, that has improved survival for several other cancers, have not worked well in sarcomas. One feature of Osteosarcoma that contributes to the poor cure rate and lack of response to therapies are pockets of low oxygen within the tumor, which program the Osteosarcoma cells to make more lactic acid, which eventually is pumped out of the tumor cells and render their surrounding acidic. One of the proteins responsible for pumping out the acid is Carbonic Anhydrase IX (CAIX). Beyond protecting the cancer cells from a buildup of acid within them, the acidic environment around the tumor prevents the immune system from attacking the cancer cells. Thus, blocking CAIX can on the one hand be detrimental to the cancer cells because acid will build-up inside them, and on the other hand allow the immune cells to attack the tumor. Since there is little to no CAIX on normal cells this strategy will be target the cancer cells and spare normal cells. Our research goal is to evaluate this strategy in Osteosarcoma in order to develop a viable strategy that can contribute to the treatment of local, and more importantly, metastatic disease. In collaboration with WeliChem BioTech, we will test the ability of their CAIX inhibitor, WBI5111, to stop the growth of Osteosarcoma in an immunocompetent mouse model of both localized and metastatic Osteosarcoma. WBI5111 will be tested in combination with immunotherapy, specifically, anti-programmed cell death protein 1 (PD1) antibody (similar to nivolumab and pembrolizumab). AntiPD1 antibodies have shown dramatic responses in melanomas and lung

			cancers but have been disappointing when tested in sarcomas. We hypothesize that WBI 5111 will enhance the antiPD1 antibody's ability to unmask tumors to the immune system allowing the body's defenses to destroy the cancer cells. Our research will involve focused preclinical studies in order to facilitate a quick transition from "bench-to-bedside". We plan to use state of the art animal models derived from osteosarcoma patient samples to achieve these. We will test both locally advanced as well as metastatic disease, which contribute the most to the morbidity and mortality seen in Osteosarcoma patients. Corresponding PI Matteo Truco, MD, is a pediatric oncologist specializing in pediatric sarcomas and the director of the only Pediatric Oncology Phase 1 Clinical Research program in South Florida, with extensive experience in translational research and conducting clinical trials. CoPI Sulagna Banerjee, PhD has extensive experience in basic and translational studies in cancer, including the study of hypoxia in cancers and development of new therapies. Together, this team is very well suited to execute the proposed project. Along with the enthusiastic support of WeliChem BioTech, this project will rapidly lead to a clinical trial in pediatric sarcomas and has the potential to revolutionize how we treat these challenging tumors as well as other pediatric cancers.

Year 2018-2019 Live Like Bella Pediatric Cancer Research Grants

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Mildred Acevedo-Duncan, PhD	University of South Florida	Anti-Neuroblastoma Effects of ICA-1	The challenge addressed by this proposal is the need to revolutionize therapy regimens by replacing treatments that have life-threatening toxicities with ones that are safer. Certain cancers are highly lethal tumors due to the emergence of therapy-resistant cancer cells. Protein kinase C-iota (PKCi) is an enzyme that plays a major role in resistance of cancer cells to chemotherapy. We have published that PKCi affects the cell cycle and proliferation during neuroblastoma cell growth and development. Neuroblastoma is cancer of nerves which occurs mainly in children. Overproduction of PKCs has been linked with the rapid growth rates of malignant cells. PKCi functions by attaching a phosphate group to proteins called substrates in a process called phosphorylation. Once the phosphate group is donated by PKCi, the substrate will carry out its intended function. This function may involve regulation (either increasing or decreasing the rate) of the cell cycle, cell proliferation, and cell survival. The cell cycle comprises all of the life stages of cells that are the basis for cell division, cell multiplication and cell proliferation. Therefore, large uncontrolled quantities of a PKCi that stimulates the cell cycle may phosphorylate too many substrates and cause uncontrolled cell growth and cancer formation. The goal of our research is to block PKCi with PKCi inhibitors to halt tumor progression and even destroy the tumor. We believe this to be a successful approach to battle cancer because our preliminary results demonstrate that ICA1 reduces breast cancer and glioblastoma tumor growth by 50% in mice. ICA1 decreases the proliferation of neuroblastoma in cell culture. Moreover, ICA1 should have fewer side effects than current drugs because ICA1 is highly specific in targeting just PKCi. All other current therapeutic inhibitors are non-specific and therefore highly toxic because they inhibit many nontarget enzymes. To address this health issue, the objectives are to quantify the amount of PKCi in adrenal neuroblastoma and to test different types of neuroblastoma grown in mice to determine whether ICA1 can block

			tumor growth and PKCi induced activation of cell proliferation proteins (Cdk7 and cdk2) and/or cell survival proteins (i.e., Bad). The methods that will be used are biochemical techniques and animal husbandry for the testing of ICA1. This research is important because it addresses the processes controlling cell proliferation and may reveal new approaches to help neuroblastoma patients that have PKCi dysregulation. Because the information gained from this research will provide a fundamental understanding of the mechanisms of cellular regulation it will also help us understand other malignant systems. It is conceivable that, within a decade, patients that have over production of PKCi (e.g., personalized medicine approach) may be treated with drugs (ICA1 or one of its derivatives) that block the function of PKCi for their cancers.