



John T. Butler, MLS (ASCP)

Proposal: Imaging how nanovesicles contribute to drug resistance in AML

Award: \$34,500

Childhood leukemia is the most common form of pediatric cancer, and although the survival rates for many types of leukemia are favorable, Acute Myelogenous Leukemia (AML) remains one of the most aggressive and lethal cancers in children. While most children achieve remission after induction of chemotherapy, the cancer eventually returns in over 40% of children, and with increased resistance to cancer drugs. AML is a cancer originating from abnormal white blood cells in the bone marrow, the principal site of blood-cell production. When AML cells grow in an uncontrolled manner, they successively displace and impair the growth of normal blood cells within the bone marrow. In doing so AML modifies the bone marrow in ways that protect the leukemia from chemotherapy, foster drug resistance and prompt relapse. AML cells accomplish this in part by releasing small nano-sized packages of cargo, called extracellular vesicles (EVs), which act as a transport device to shuttle cancer-specific regulatory factors to bystander cells. EVs target specific cell types using an address-like code on their surface. The AML-EVs find their recipient cells, bind to their surface and enter the cells, delivering cargo from the cancer cell directly into healthy cells. This delivery of regulatory cargo causes healthy cells to stop their normal activity to instead support cancer growth. We previously showed that the release of EVs alone has the potential to decrease the production of healthy blood cells. Here, we focus on the role of EVs in reprogramming the bone marrow to protect leukemia cells from cancer drugs, and heighten the risk of relapse. Specifically, we will determine how the EVs change the function of healthy bone marrow to enable leukemia growth. By better understanding how AML-EVs dock on the cell surface, where inside a recipient cell they deliver their cancer supporting cargo, and what the molecular targets of the cargo are, the process can be therapeutically disrupted. Our long-term goal is to reverse drug resistance and improve the survival of children suffering from AML.

Monika A. Davare, PhD

Proposal: New drug discovery for effectively targeting Ewing's sarcoma

Award: \$28,750

Ewing's Sarcoma (EwS) is the second most common type of primary bone cancer in children and young adults between ages 10 and 20. When diagnosed before this cancer has spread to other organs (i.e., metastasized), approximately 70% of these children go into remission, but only after aggressive, high-dose toxic chemotherapy, and frequently, limb amputations. In short, survival comes with the heavy toll of limb loss and long-term health consequences. Notably, if at diagnosis, Ewing's sarcoma is metastasized, or the patient is an older adolescent (15 – 19 yrs), a durable cure is very difficult to achieve

using our current treatment modalities, and only 30-50% of children survive despite treatment. Given the toxicity and continuing unfavorable prognoses for many EwS patients, it is imperative to develop novel, therapeutic strategies. Our overall goal is to develop new, effective agents to target and treat Ewing's sarcoma for improving survival rates as well as long-term quality of life for surviving children.

To accomplish this goal, we recently performed a large scale, unbiased screening experiment to identify previously unknown compounds that have potent activity against EwS cells. Using this method, we identified a promising new drug lead for EwS, and synthesized a number of novel structural analogs based on this drug lead. The new structural analogs exhibit about 1000-fold greater potency to rapidly induce EwS cell death. We hypothesize that this new lead compound called MN141 is potent agent to target EwS that will exhibit a high degree of efficacy and selectivity compared to current chemotherapy strategies. The primary aims of this proposal are to further develop MN141 as a therapeutic agent for treatment of Ewing's sarcoma by (a) discovering its cellular target using biochemical experiments, and (b) validating the anti-tumor efficacy using two independent patient-derived EwS tumor cell lines. This pre-clinical drug development work will position us to move efficiently into clinical studies in the near future.

Christina Lancioni, MD

Proposal: Boosting Babies' Immunity through Toll-like Receptor-2

Award: \$71,161

Newborn infants and neonates are uniquely vulnerable to severe infection and respond poorly to vaccines. Infection is a leading cause of death during the neonatal period (defined as the first 28 days of life), and causes 36% of all neonatal deaths worldwide¹. This vulnerability to infection results from evolutionary conserved mechanisms designed to dampen the immune system of the developing baby in utero, in order to protect against excessive inflammation that could trigger miscarriage or premature birth. However, once a baby is born, several key components of the immune system remain restricted and cannot produce the inflammation necessary to protect against invading pathogens. During the neonatal period, a critical component of the immune system, CD4+ "helper" T cells, are considered incapable of becoming activated to produce sufficient quantities of the cytokine interferon-gamma, to fight infection. Interferon-gamma is a key pro-inflammatory molecule of the immune system. However, the Principle Investigator for this proposal, Dr. Lancioni, recently published the exciting finding that the bias against activation and production of interferon-gamma by neonatal CD4+ T cells can be overcome by stimulating these cells through a cell surface receptor called Toll-like Receptor-2 (TLR-2)². These findings demonstrate that neonatal CD4+ T cells are not incapable of interferon-gamma production as once believed, but require unique stimulation to reach the pro-inflammatory potential of adult CD4+ T cells. We hypothesize that TLR-2 stimulation induces a cascade of regulatory events that results in de-methylation of the interferon-gamma gene and other genes related to CD4+ T cell activation. These genes can subsequently be read by the cellular machinery, allowing TLR-2 stimulation to act as a unique "key" that unlocks the pro-inflammatory potential of neonatal CD4+ T cells. The goals of this proposal will determine how TLR-2 stimulation

of neonatal CD4+ T cells “unlocks” cellular activation and interferon-gamma production by these cells, and will lead to new immune-based targets to fight bacterial infections, design more efficacious vaccines, and save the lives of neonates.

Daniel Marks, MD, PhD & Oleh Taratula, PhD

Proposal: Novel Nanomedicine-Based Therapeutic Approach For Treatment of Muscular Dystrophy

Award: \$51,750

Muscular dystrophies (MD) are a collection of muscle disorders that are characterized by a loss of muscle strength and size, with the relentless decline in motor function severely diminishing a child’s quality of life and ultimately leading to death. We have developed a safe and nontoxic therapy that combats muscle wasting by increasing muscle size and strength. We have combined the use of nanoparticles (tiny particles capable of delivering many therapeutic cargos) and messenger RNA (the genetic blueprint that cells use to manufacture numerous proteins). The combination of these two technologies gives us the ability to increase a protein inside the body that is responsible for muscular growth and strength. To date, we have developed such a drug delivery platform and have tested it in both cell culture and healthy wild-type mice with extremely promising results. Our goal is to evaluate toxicity and efficacy of the proposed therapeutic approach in muscular dystrophy mouse models. This project results from an unusual but productive collaboration between a pediatric physician scientist (Marks) and an expert in nanoparticle chemistry (Taratula). We sincerely believe that by bringing together these two very different scientific disciplines, we will come up with creative solutions to complex