Pediatric Cancer Research Group Supported by the Nebraska Legislature (LB 417) in collaboration with The Fred and Pamela Buffet Cancer Center & Children's Hospital and Medical Center

This year, the Pediatric Cancer Research Group hosted the third annual Pediatric Cancer Research Group Symposium on August 26, 2020. The Symposium, co-sponsored by the Child Health Research Institute and the Nebraska Coalition to End Childhood Cancer, was attended in virtual fashion due to the pandemic by over 170 participants. This year the agenda included focused presentations on water quality research and a review of return to learn protocols for Nebraska's children with chronic medical conditions. A panel discussion among Nebraska State Senators allowed for a review of next steps and action plans for both of these specific projects.

Pilot funding provided by the Legislature through the Pediatric Cancer Research Group again led to external NIH funding in 2020, with the success of Dr. Sidharth Mahapatra's K12 award and Drs. Coulter and Mahato's R01 award. Furthermore, the Pediatric Cancer Research Group was able to retain developed talent, by adding Dr. Matt Kling, PhD to the core lab. Dr. Kling successfully completed his PhD project on Ewing's Sarcoma exosomes in 2020, and will augment the skill set available in the core lab.

Finally, the Pediatric Cancer Research Group broadened the scope of clinical research by expanding projects in a number of areas. These include evaluating virtual reality technology to decrease the need for sedation in radiation oncology treatments, utilizing a robotic seal to complete physical therapy evaluations in children who are immunocompromised, and evaluating the use of a dedicated social worker to improve the coping strategies of patients and families undergoing stem cell transplantation.

A report of the current composition of the Pediatric Cancer Research Group and an accounting of the utilization of the 1.8 million dollars transferred from the General Fund for pediatric cancer research infrastructure at the University of Nebraska follows:

Current Program Personnel:

Program Director
Don Coulter, M.D., University Tower 5117, Lab: Wittson Hall 4027

Research Team Members
Janina Baranowska-Kortylewicz, Ph.D., Radiation Oncology, Lab: Epp. Science 10025
(Radiation Biology)
Shantaram Joshi, Ph.D., Genetics, Cell Biology and Anatomy, Lab: Wittson Hall 2010A
(Tumor biology/Immunology)
Christopher Shaffer, D.Pharm, Pharmacy Practice, Lab: COP 4050 (Pharmacology)
J. Graham Sharp, Ph.D., Genetics, Cell Biology and Anatomy, Lab: Wittson Hall 4027
(Tumor Biology)
Joyce Solheim, Ph.D., Eppley Institute, Lab: Eppley Science Hall 8006 (Immunology)
Jon Vennerstrom, Ph.D., Pharmaceutical Sciences, COP 3042
(Drug synthesis/Discovery)

Research Team Members (continued):

Kishore Challagundla, Ph.D., Biochemistry and Molecular Biology, DRC 1 7032 (Tumor biology/Experimental Therapeutics)
Rhongshi Li, Ph.D., Pharmaceutical Sciences, COP , ESH 8009
(Drug synthesis/discovery)
Joseph Vetro, Ph.D., Pharmaceutical Sciences, COP 3033 (Drug delivery)
Guangshun (Gus) Wang, Ph.D., Pathology and Microbiology, Wittson 4005
(Drug Development)
Erin McIntyre, M.S., Lab Manager, PCRP Lab: Wittson 4057, 4059
Gracey Alexander, M.S., Research Technologist, PCRP Lab: Wittson 4057, 4059
Nagendra Chaturvedi, Ph.D., Senior Postdoctoral Research Associate, Lab: Wittson 2010A
Sutapa Ray, Ph.D. Assistant Professor, Lab: Wittson Hall 4027
Daryl Murry, Pharm D, College of Pharmacy (Drug Development/Analysis)
Angie Rizzino, Ph.D. Professor, Eppley Cancer Institute (Tumor Biology)
Sidharth Mahapatra, MD, Ph.D. Assistant Professor, Pediatrics (Brain tumors)
Shinobu Watanabe-Galloway PhD., Associate Professor, College of Public Health
(Epidemiology)
Hamid Band Ph.D., Professor, Eppley Cancer Institute (Tumor Biology)
Gargi Ghosal Ph.D., Assistant Professor, Genetics, Cell Biology and Anatomy
(Tumor Biology)
Ram Mahato Ph.D., Professor and Chair, Department of Pharmaceutical Sciences
(Drug Development)
So-Youn Kim, Ph.D., Assistant Professor, Department of OB/GYN (Fertility Preservation)
Alan Kolok Ph.D., Director Center for Environmental Health and Toxicology (University of Idaho)
Kate Hyde Ph.D., Assistant Professor, Eppley Cancer Institute, BCC 6.12321 (Leukemia)
Shannon Buckley, Ph.D., Assistant Professor, GCBA, BCC 10.12397 (Leukemia)
David Oupicky, Ph.D., Pharmaceutical Sciences, DRC1 (1009) (Drug Delivery)
Corey Hopkins, Ph.D., Pharmaceutical Sciences, COP3015 (Drug synthesis/Discovery)
Kishor Bhakat, Ph.D., GCBA, BCC.1012392 (DNA Repair)

<u>New Projects:</u>

Population Research:

1. *Continued evaluation of the epidemiology of pediatric cancer in Nebraska*
   The University of Nebraska College of Public Health has previously evaluated the incidence of pediatric cancer within the State, and identified a number of counties where the incidence of pediatric cancer exceeds our state-wide average. Dr. Watanabe – Galloway and her team will now use that data set to investigate the electronic health record at both Children's Hospital Medical Center and Nebraska Medicine to evaluate demographic data about pediatric cancer patients from specific areas. It is hoped that this date will assist in understanding the type of tumors originating in specific parts of the state. This information will be key in

the development of future care models for children with cancer and other chronic diseases in rural Nebraska

Translational Science Research:

1. *Pharmacokinetic evaluation of MP1 alone and combined with Temsirolimus in balb-c mice*

   MP1 is a derivative of Marino pyrroles, a group of natural products modified in the laboratory of Dr. Rongshi Li. Preliminary work has shown that MP1 has anti-tumor activity against Neuroblastoma cells in culture. This study, combining the expertise of Dr. Li with Dr. DJ Murry, will continue the evaluation of this exciting compound developed at UNMC by investigating its pharmacokinetic properties in mice when compared to Temsirolimus. The data will provide further information which could lead to an early phase trial in patients.

2. *Primary Human Cell lines in AML*

   Dr. Buckley and her research team have investigated a number of proteins important in the development of acute myelogenous leukemia, the second most common leukemia in children. This project will allow Dr. Buckley to use AML cells obtained from pediatric patients to evaluate for a select number of target proteins, and improve the understanding of how these proteins can be targeted by new drugs in development.

3. *Regulation of B7H3 by MYCN in Neuroblastoma*

   Neuroblastoma is the most common extra-cranial solid tumor in children, and has a varied clinical presentation. Immunotherapy has become a novel treatment for patients with high risk neuroblastoma, however it is ineffective in a number of patient due in part to B7H3 protein, an immune checkpoint molecule that inhibits the function of natural killer cells. This project led by Dr. Challagundla seeks to understand the function and regulation of B7H3 in neuroblastoma and further classify how it inhibits the ability of immunotherapy to be effective for patients.

Clinical Research Focus:

1. *Metabolic bone health in childhood cancer survivors*

   While 80 % of children will survive their pediatric cancer, 75 % of survivors go on to have chronic medical conditions. Among the most common of these are impacts on the health of bones. Dr. Acquazzino, the Director of the Survivorship clinic at Children's Hospital and Medical Center, has assembled a research team to investigate the impact of exercise on the overall bone health of survivors of pediatric tumors.

2. *Psychological needs of children undergoing stem cell transplant*

   Stem cell transplantation is a highly specialized procedure that can be curative for childhood cancer patients with resistant diseases. However, the toxicity of stem

cell transplant can require long hospitalizations which can have a negative impact on the coping abilities of the child and the family. This project led by Dr. Rebecca Swanson will identify the benefit of dedicated social work support to improve the overall care of the patient and family.

Neuro-Oncology Focus:

1. *Sonic Hedgehog inhibitors and FACT inhibition in Medulloblastoma*
   Medulloblastoma is the most common brain tumor of childhood, and new therapeutics which can cross the blood brain barrier are needed for the treatment of this disease. This project led by Dr. Mahato seeks to expand on the work that was recently funded by the NIH to combine sonic hedgehog inhibitors with other compounds to develop a new therapeutic option for patients and their families.

Continuing Research:

Translational Science Research:

1. *NET targeted RNAi therapy in high risk Neuroblastoma*
   Neuroblastoma is the most common extra-cranial solid tumor in children and requires multimodal therapy with surgery, chemotherapy, radiation therapy and immunotherapy. Still overall survival for patients with High Risk Disease approaches 60 %, and that survival comes with a number of long-term impacts. Dr. Vetro is continuing his investigations into interfering RNA that is packaged into a nano-particle and attached to MABG, an analog of MIBG which is a protein avidly absorbed by neuroblastoma cells.

2. *Exosomes secreted under Hypoxia Enhance Aggressiveness in Ewing's Sarcoma*
   This project led by Dr. Shantaram Joshi will continue investigations into the molecular mechanisms by which low oxygen tension leads to increased resistance and metastasis in Ewing's Sarcoma cells. Under these hypoxic condition, Dr. Joshi and his team have identified that Ewing's Sarcoma cells excrete small pockets of cellular information called exosomes. These exosomes are then able to change the microenvironment surrounding the cancer cell into a more suitable environment for growth and metastasis. A better understanding of the contents and function of these exosomes may lead to further clarity regarding resistance mechanisms in Ewing's Sarcoma.

3. *Deciphering the role of mitochondrial electron transfer flavoproteins in AML*
   When compared to patients with Acute Lymphocytic Leukemia, patients with Acute Myeloid Leukemia experience relapses 50 % of the time and overall lower survival rates. A better understanding of the proteins within these cancer cells is necessary to allow for higher remission rates. Dr. Buckley and her team have identified a set of proteins differentially expressed in AML cells, specifically ETFA and ETFB. A further investigation of these proteins

and their role in tumor initiation and progression may lead to novel therapeutic targets for these patients.

4. *Evaluation of Fusion Genes in Ewing's Sarcoma*
   Ewing 's sarcoma is an aggressive tumor of bone or soft tissue, primarily affecting children and adolescents. The EWSR-FL1 fusion gene represents a key driver of oncogenesis in Ewing's sarcoma, but is not the only fusion product that is involved in the development of the tumor. This project led by Dr. Vellichirammal seeks to evaluate multiple fusion genes in Ewing's Sarcoma and determine their response to a number of different chemotherapeutic agents. This project is co-funded by a grant supplied by the Fred and Pamela Buffet Cancer Center Pilot Grant Program.

5. *Targeting USP1 in Ewing's Sarcoma*
   Using a quantitative PCR array, Dr. Ghosal and her team identified USP1, a deubiquitinase that functions in both the DNA damage and replication stress responses, as being overexpressed in EWS cell lines compared to its cell of origin, the human mesenchymal stem cell. Importantly, we found USP1 to be essential for EWS cell line proliferation, as growth of these cell lines is arrested with knockdown of USP1. This project is co-funded by a grant supplied by the Fred and Pamela Buffet Cancer Center Pilot Grant Program.

6. *Novel Therapeutics for bone tumors*
   Osteosarcoma and Ewing's Sarcoma represent the two most common bone tumors in children. Dr. Holstein and her group have developed a novel family of compounds called geranylgeranyl diphosphate synthase (GGDPS) inhibitors which they hope to develop as new treatments for these bone diseases. This project is co-funded by a grant supplied by the Fred and Pamela Buffet Cancer Center Pilot Grant Program.

Clinical Research Focus

1. *Using robotic animal assistance for physical therapy in pediatric patients*
   Physical therapy plays an important role in the health of children with chronic disease. Multiple tools are used to assist in the completion of these important functions, including therapeutic animals like dogs. Unfortunately, many pediatric patients with cancer are immunosuppressed, and interactions with live animals may pose an infectious risk. Dr. Hetland is using a robotic seal named HARPO to perform these important interactions with children, and identify if the robotic animals can provide improved physical therapy sessions over live animals.

2. *A pilot study of 'Immersive Education' Utilizing Virtual Reality Software for Children Undergoing Radiation Therapy and MRI*
   Children receiving radiation therapy or imaging with MRI are expected to hold still for extended periods of time. Often the age of the child inhibits their ability to be compliant and sedation is required to complete the

therapy. Pediatric sedation is a process with inherit risk, and increasing the proportion of children who can participate in procedures without sedation would decrease cost and increase quality. This project led by Rebecca Swanson DNP, utilizes virtual reality and an education session with a child life specialist in an attempt to decrease the number of children who require sedation for their treatment.

Neuro-Oncology Focus:

1. *SOX2 Regulation of Sonic Hedgehog driven Medulloblastoma*
   Dr. Rizzino and his team of investigators have previously identified SOX2 as a regulator gene responsible for quiescence in medulloblastoma cells, which is one established mechanism by which cancer cells avoid the toxic impact of chemotherapy. An existing grant is evaluating inhibitors of SOX2 as possible adjuvant therapeutic options for patients with medulloblastoma, and this project seeks to further understand the downstream impacts of SOX2, which may lead to other novel therapeutics for patients with medulloblastoma.

2. *Sonic Hedgehog Inhibitors as novel therapy in Medulloblastoma*
   Medulloblastoma is the most common brain tumor of childhood, and new therapeutics which can cross the blood brain barrier are needed for the treatment of this disease. This project led by Dr. Mahato seeks to develop a category of drugs that target Sonic Hedgehog, a molecule important in the development of medulloblastoma cancer cells.

3. *Genetic and Pharmacologic targeting of STAT3 in Medulloblastoma*
   Medulloblastoma is the most common brain tumor in childhood, and treatment currently involves surgical resection, chemotherapy and radiation therapy. These modalities can have long term impacts for the 60 – 70 % of children who will survive the disease. Dr. Sutapa Ray is investigating STAT3, and its role in maintaining medulloblastoma growth. By inhibiting STAT3 with both genetic and pharmacologic approaches, Dr. Ray hopes to develop therapies that will sensitize Medulloblastoma cells to our current treatment approaches, thereby improving outcomes and the quality of life for these patients.

4. *Identification of tumor suppressor genes on chromosome 17p13.3 in non SHH/WNT subgroup medulloblastoma*
   Dr. Sidharth Mahapatra is elucidating the impact of tumor suppressor genes on chromosome 17 on the development of medulloblastoma using a combination of *in silico* (RNA sequencing), *ex vivo* (patient samples), and *in vitro* (established cell lines) techniques. This research has the potential to identify novel therapeutic targets that could then be administered using nano technology developed by fellow Pediatric Cancer Research Group member Dr. Joe Vetro.

Successful Extramural Funding:

1. NIH R01 (R01HD096042): Mechanism of premature ovarian insufficiency in females receiving chemotherapy. Principal Investigator: So-Youn Kim, PhD

2. NIH R01 **(**R01NS116037): Overcoming Resistance Mechanisms in Hedgehog and Myc-amplified Medulloblastoma. Principal Investigators: Don Coulter, MD and Ram Mahato, PhD

3. NIH K12 (K12HD047349): Establishing miR-1253 mediated potentiation of cisplatin cytotoxicity in Group 3 Medulloblasroma. Principal Investigator: Sidharth Mahapatra, MD, PhD

Published Manuscripts:

1. Kanchan RK, Perumal N, Atri P, Venkata RC, Thapa I, Klinkebiel D, Donson AM, Perry D, Punsoni M, Talmon G, Coulter DW, Boue' DR, Snuderl M, Nasser MW, Batra SK, Vibhakar R, Mahapatra S. MiR-1253 exerts tumor suppressive effects in medulloblastoma via inhibition of CDK6 and CD276 (B7-H3). Brain Patho. 2020 Mar 7.

2. Kortylewicz ZP, Coulter DW, Han G, Baranowska-Kortylewicz J. Norepinephrine-Transporter-Targeted and DNA-Co-Targeted Theranostic Guinidines. J Mec Chem 2020 Mar 12;63(5): 2051-2073

3. Kortylewicz ZP, Coulter DW, Han G, Baranowska-Kortylewicz J. Radiolabeled (R)-(–)-5-iodo-3'-(i)O(/i)-[2-(ε-guanidinohexanoyl)-2-phenylacetyl]-2'-deoxyuridine: a new theranostic for neuroblastoma. J Labelled Comp Radiopharm. 2020 Mar 9.

4. Wang Q, Kumar V, Lin F, Sethi B, Coulter DW, McGuire TG and Mahato R. ApoE Mimetic Peptide targeted nanoparticles carrying a BRD4 inhibitor for treating medulloblastoma in mice. Accepted by Journal of Controlled Release, April 30, 2020

5. Poelaert BJ, Romanova S, Knoche SM, Olson MT, Sliker BH, Smits K, Dickey BL, Moffitt – Holida AEJ, Goetz BT, Khan N, Smith L, Band H, Mohs AM, Coulter DW, Bronich TK, Solheim JC. Nanoformulation of CCL21 greatly increases its effectiveness as an immunotherapy for neuroblastoma. J Control Release 2020 Jul 22:327: 266 – 283

6. Kesherwani V, Shukla M, Coulter DW, Sharp JS, Joshi SS, Chaturvedi NK. Long non-coding RNA profiling of pediatric Medulloblastoma. BMC Med Genomics 2020 Jun 26;13(1): 87

7. Chaturvedi NK, Kling MJ, Griggs CN, Kesherwani V, Skulka M, McIntyre EM, Ray S, Liu Y, McGuire TR, Sharp JG, Band H, Joshi SS, Coulter DW. A Novel Combination Approach Targeting an Enhanced Protein Synthesis Pathway in MYC-driven (Group 3) Medulloblastoma. Mol Cancer Ther. 2020 Jun; 19 (6): 1351 – 1362.

PCRG FY2020 Expenditures

Current Research Personnel

Don Coulter, MD (PCRG Director, COG PI UNMC)	22,171
Minnie Abromowitch, MD (COG PI CHMC)	19,252
Sutapa Ray, PhD (Research Scientist)	77,259
Nagendra Chaturvedi, PhD (Research Scientist)	69,707
Zhen Ye (Graduate Research Assistant)	4,125
Erin McIntyre (Lab Manager)	57,733
Grace Alexander (Research Technologist)	46,508
Benefits	88,544
Current Personnel Subtotal	**385,299**

Core Lab Operational Costs

Operating Expenses	22,296
Supplies, Scientific Services, Publication Costs, etc.	128,543
Travel	0
Capital	34,969
Operational Costs Subtotal	**185,809**
TOTAL RESEARCH PERSONNEL AND CORE LAB FY2020	**571,107**

Awarded Projects FY2020

NET targeted RNAi therapy for Neuroblastoma	Vetro	100,000
Exosomes in Ewing's Sarcoma	Joshi/Murry/Kling	123,801
SOX2 regulation in Medulloblastoma	Rizzino	50,000
SHH inhibitor in Medulloblastoma	Mahato	50,000
Animal assisted physical therapy	Hetland	32,623
Exosomes in Ewing Sarcoma	Joshi	50,000
Targeting STAT3 in Medulloblastom	Ray	50,000
Virtual Reality use in Radiation Therapy	Swanson	9,961
Molecular Mechanisms of AML	Buckley	50,000
Targeting USP-1 in Ewing's Sarcoma	Ghosal	25,000
Novel Therapeutics in Bone Tumors	Holstein	50,000
Evaluation of Fusion Genes in Ewing's Sarcoma	Vellichirammal	75,000
TOTAL AWARDED FY2020		**615,665**

Awarded Projects FY2021

MP1 in Neuroblastoma	Li/Murry	53,668
SHH and FACT inhibition in Medulloblastoma	Mahato	50,000
Primary human cells in AML	Buckley	25,000
Metabolic Health in Cancer Survivors	Acquazzino	65,000
Epidemiology of Childhood Cancer	Watanabe-Galloway	50,000
B7H3 in Neuroblastoma	Challagundla	50,000
Psychological needs of children in SCT	Swanson	30,000
TOTAL AWARDED FY2021		**373,668**

Planned Funding FY2021

Fred and Pamela Buffet Cancer Center Pilot Project	150,000
TOTAL PLANNED FUTURE FUNDING FY2021	**150,000**
TOTAL FY2020 – FY2021	**1,710,440**