Pediatric Insights

BEST CHILDREN'S HOSPITALS U.S.News & WORLD REPORT CANCER 2019–20

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An Update From the **Division of Pediatric Hematology/Oncology** and the **Division of Blood and Marrow Transplantation and Cellular Therapies**

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UPMC CHILDREN'S HOSPITAL OF PITTSBURGH

New Findings In Ewing Sarcoma Cell Biology

Kelly M. Bailey, MD, PhD, is a sarcoma specialist in the Division of Pediatric Hematology/Oncology at UPMC Children's Hospital of Pittsburgh who is interested in sarcoma biology and the impact of the EWS-FLI1 fusion oncoprotein on the Ewing sarcoma tumor microenvironment. Dr. Bailey's research is focused on Ewing sarcoma and its metastatic pathways, and how microenvironmental stresses can affect both primary and metastatic disease states and progression. Determining why certain individuals' cancers behave more aggressively is of primary importance for Dr. Bailey, given the fact that relapsed and refractory cases are challenging to treat successfully.

Dr. Bailey's early research efforts were recognized by the Alex's Lemonade Stand Foundation, when they awarded her one of their 2016 Young Investigator Grants. The three-year, $150,000 award is funding Dr. Bailey's research project titled "Micro-environmental Regulators of Ewing's Sarcoma Metastasis." In March 2018, Dr. Bailey was awarded an Emerging Scientist Grant from the Children's Cancer Research Fund® (CCRF) to help further her research into the signaling pathways that cause some Ewing sarcoma cells to become more aggressive and less sensitive to chemotherapy. The $100,000 award from the CCRF is designed to support young investigators to develop independent research. In October of this year, Dr. Bailey was awarded a Hyundai Hope on Wheels® grant that will help further her sarcoma research (see Page 11 for additional details).

Dr. Bailey also is a part of the recently formed New Agents for Ewing Sarcoma Task Force of the Children's Oncology Group (COG). Dr. Bailey is one of 10 investigators comprising the task force.

Ewing Sarcoma and the EWS-FLI1 Fusion Oncoprotein: New Findings

The vast majority (75 to 80 percent) of Ewing sarcoma tumor cells express the EWS-FLI1 fusion oncoprotein (transcription factor). EWS-FLI1 is an abnormal protein not found in normal cells. It is created through a chromosomal abnormality and linkage between chromosomes 11 and 22, the fusion of which created the abnormal fusion oncoprotein.

Dr. Bailey's new paper[1] in the journal *Oncotarget* details her team's work and findings into how the EWS-FLI1 fusion oncoprotein is formed and how it can influence or mediate the way T cells can or cannot recognize the Ewing sarcoma tumor cells and target them for immune response and ultimate destruction. Dr. Bailey's research specifically looked at the effect of EWS-FLI1 on CD8-positive T cells and the cytokine interferon-gamma.

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Fertility Preservation for Pediatric Oncology and Hematology Patients:

Looking Beyond the Disease to the Future

Erika Friehling, MD, Assistant Professor and Fellowship Director in the Division of Pediatric Hematology/Oncology at UPMC Children's Hospital of Pittsburgh, is passionate about her work and that of the Hospital in helping young cancer patients preserve their potential for future fertility after being cured of their disease.

Pediatric patients survive cancer more frequently now than in decades past, a testament to the steady advance in research and treatments that continue to provide cures and allow these young individuals to live full, normal lives. Unfortunately, the efforts to cure an individual's cancer are not without side-effects, some long term. The impact on fertility is one such side-effect for many individuals; various forms of chemotherapy and radiation, and some surgeries are detrimental to the reproductive organs, impairing fertility.

"There is data in the literature indicating that the vast majority of patients and families want fertility-related education ahead of time before they start treatment for cancer. The data also suggest that as oncologists, we need to do a better job of dealing with this aspect of patient care at the time of the initial diagnosis," says Dr. Friehling.

It is a very emotional time for patients and families. Many serious conversations take place rapidly upon diagnosis. Patients, families, and caregivers alike must deal with many competing priorities. Discussion of future fertility often takes a back seat to other pressing concerns.

"Our goal with the Oncofertility Preservation Program at UPMC Children's is to improve this dynamic. Two years ago, we instituted a fertility consultation service at UPMC Children's. Our group of experts in the field of oncofertility can bring a clear understanding of the options available to patients, and at the same time, be a resource for our oncology colleagues," says Dr. Friehling.

A treating physician, caring for a newly diagnosed patient with Hodgkin's lymphoma, does not need to focus on discussing fertility preservation options. Dr. Friehling's team takes on this responsibility to meet and educate the patient and family about infertility risks of the treatment and to explore fertility preservation options.

"The oncofertility group also keeps up to date on the current literature surrounding fertility preservation and how to best counsel and educate both patients and families, guiding them through the process so that the primary oncologist can focus on the cancer care at that moment. Our consultation service removes the barriers to getting this information and increases access for patients and families.," says Dr. Friehling.

Continued on Page 3

About the UPMC Children's Division of Pediatric Hematology/Oncology

Under the leadership of **Linda M. McAllister-Lucas, MD, PhD**, division chief and professor of pediatrics at the University of Pittsburgh School of Medicine, UPMC Children's Division of Pediatric Hematology/Oncology boasts the largest and most comprehensive care center in western Pennsylvania, eastern Ohio, and northern West Virginia for pediatric and young adult patients with all forms of cancer and disorders of the blood. The Division is part of UPMC Hillman Cancer Center.

Research and Clinical Trials

The Division supports an extensive research program of basic science, translational investigations, and clinical trials. This work is collectively dedicated to uncovering new insights and knowledge with respect to how and why cancers develop and spread, and to developing the next generation of therapies.

Clinical Programs and Services

- Adolescent and Young Adult Oncology
- Pediatric Solid Tumors
- Hemophilia
- Hemostasis and Thrombosis
- Leukemia
- Sickle Cell Disease
- Neuro-Oncology
- Pediatric Cancer Survivorship Clinic
- Mario Lemieux Lymphoma Center for Children and Young Adults
- Cancer Predisposition Program
- Bone Marrow Failure
- Immunocytopenias
- Melanoma

Fertility Preservation *Continued from Page 2*

Multidisciplinary By Design

The oncofertility team at UPMC Children's consists of a multidisciplinary team of pediatric oncologists, neuro-oncologists, bone marrow transplant physicians, endocrinologists, adolescent gynecologists, the experts in fertility preservation at UPMC Magee-Women's Hospital, and the Fertility Preservation Program in Pittsburgh run by UPMC Magee researcher and fertility expert Kyle Orwig, PhD.

"From a technology standpoint, we have implemented a consultation process in the electronic medical record such that for any newly diagnosed cancer patient, the treating clinicians can order the consult, and a request then goes directly to our team for follow-up. Again, this takes the burden off of the primary oncologist and improves patient access to this information," says Dr. Friehling.

The Consult Service: How it Works

Oncofertility specialists will first review the patient's treatment plan. Is the patient going to receive chemotherapy? If so, which medications and how much? The same questions are asked for radiation therapy and surgery. The team also assesses how ill the patient is currently and how much time can be spent on efforts to preserve fertility before anti-cancer treatment begins.

"Our goal is to provide every family with fertility education and to discuss the risks of infertility based on the specific treatment plan," says Dr. Friehling.

Dr. Friehling's team will discuss the applicable fertility preservation options available to both boys and girls. Sperm banking is one option for post-pubescent males, and the oncofertility program will help coordinate the logistics for this process. There also exist potential options for egg freezing for post-pubescent females. There are also two experimental protocols available to patients at UPMC Children's for pre-pubertal and even post-pubertal children. One option is ovarian tissue cryopreservation where either a piece of an ovary or a full ovary will be removed and frozen for potential use in the future. The other research protocol involves testicular cryopreservation, utilizing either tissue from a biopsy or an entire testicle. While these are experimental protocols, work is progressing to bring the technologies to the clinic that will make these frozen tissues viable in the future for reimplantation, hopefully restoring fertility potential to the child after they have been cured of their cancer.

"We have patients come to Pittsburgh from around the country and the world to take part in these experimental protocols being run by Dr. Orwig, and that so far are showing great promise for the future. It is inspiring and rewarding to be able to offer the potential to preserve these young patients' fertility for the future when they are ready to start a family. Time will tell if we are successful, but again the work done so far is incredibly promising," says Dr. Friehling.

More Than Just Cancer

While pediatric cancers account for the majority of consults that the oncofertility program performs, fertility preservation is also of concern for patients diagnosed with other conditions. Hematologic diseases, such as sickle cell disease, can, in some cases, be treated with bone marrow transplantation, a therapy that can render patients infertile. Several metabolic disorders and immune deficiencies also can require treatment with bone marrow transplantation, which can lead to loss of fertility. Dr. Friehling's group can consult on these cases, as well.

Program Growth and Success

UPMC Children's sees approximately 180 new cases of pediatric cancer per year. Not all of these patients will receive treatments that put into jeopardy a patient's future fertility. However, Dr. Friehling's group can consult on all oncology cases in order to educate the patient and family regardless of the type of disease, prognosis, or potential risk to fertility that treatments pose.

"Our program is unique in that we have the capabilities for multiple fertility preservation options for patients, as well as the dedicated consultation process that is key to providing access to all the options available. We are a national leader on this front and routinely consult with other institutions about our experiences with the programs that we have implemented," says Dr. Friehling.

We have seen the growth of not only the number of consultations but also the number of fertility preservation procedures performed for patients at UPMC Children's. In 2016 there were 11 sperm banking/freezing procedures performed. In 2017, 22 individuals pursued one of the options available (sperm cryopreservation, testicular cryopreservation, oocyte freezing, ovarian cryopreservation). So far, in 2019, there have been 26 cases of fertility preservation services, with projections to reach 30 or more cases before the end of the year.

It is not yet known if the experimental preservation options will one day prove successful in restoring fertility. We value the opportunity to preserve reproductive tissue for a future time when the technologies prove themselves successful. We are hopeful that the potential for fertility can be preserved for survivors of childhood cancer.

New Melanoma Trials Open

Two new clinical trial opportunities are open at UPMC Children's Hospital of Pittsburgh in the Division of Pediatric Hematology/Oncology for young patients with melanoma.

The first trial, "A Multicenter, Open-label Phase 1b Study of the Combination of Binimetinib and Encorafenib in Adolescent Patients With Unresectable or Metastatic BRAF V600-mutant Melanoma," will use a combined targeted therapy for advanced melanoma. This same regimen has been shown to be well-tolerated and efficacious in adults with melanoma in previous studies.

This international, multicenter study is now open and recruiting patients at UPMC Children's. UPMC Children's is the first site in the United States to open for this trial, and only two other U.S. sites are currently planning participation (Houston and Baltimore).

Brittani Seynnaeve, MD, **MS**, assistant professor of pediatrics, director of the pediatric melanoma program, and associate fellowship program director in the Division of Pediatric Hematology/Oncology at UPMC Children's is the site principal investigator for this study.

Full details and trial information can be found at ClinicalTrials.gov (NCT03878719).

KEYNOTE-716 Study

The second new study for pediatric melanoma patients is the "Adjuvant Therapy With Pembrolizumab Versus Placebo in Resected High-risk Stage II Melanoma: A Randomized, Double-blind Phase 3 Study (KEYNOTE-716)."

This study will investigate the efficacy of the immunotherapy agent pembrolizumab in fully resected, localized high-risk stage II melanoma. This investigation is designed to determine if an increase in recurrence-free survival can be obtained by using pembrolizumab in patients aged 12 to 17 years. Dr. Seynnaeve will lead this study when it opens at UPMC Children's.

This multicenter, international trial is a two-part study to evaluate the safety and efficacy of pembrolizumab (MK-3475) compared to placebo in participants with surgically resected high-risk stage II melanoma. Participants in Part 1 will receive either pembrolizumab or placebo in a double-blind design for up to 17 cycles. Participants who complete 17 cycles of pembrolizumab and experience disease recurrence may be eligible to receive additional cycles of pembrolizumab in Part 2 in an open-label design.

Complete details of the trial are available at ClinicalTrials.gov (NCT03553836).

A corollary study is currently open at UPMC Hillman Cancer Center for patients 18 years and older. **John Kirkwood, MD**, Usher Professor of Medicine, Dermatology, and Translational Science, principal investigator of the melanoma and skin cancer SPORE, and co-leader of the Melanoma and Skin Cancer Program at UPMC Hillman Cancer Center is the site principal investigator of the trial for adult patients under the same NCT trial number.

"That's Pediatrics" Research Podcast Series

UPMC Children's Hospital of Pittsburgh medical podcast series for physicians, scientists, and other health care professionals features compelling interviews with the hospital's leading researchers and clinicians discussing innovative basic, translational, and clinical research. New episodes are released every two weeks.

Current episodes of "That's Pediatrics" from **hematology/oncology** and **BMT-CT faculty** and related topics include:

- *A New Look at Bone Marrow Transplantation and Cellular Therapies* with **Paul Szabolcs, MD**

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That's PEDIATRICS

UPMC | CHILDREN'S HOSPITAL OF PITTSBURGH

MIBG Treatment for Neuroblastoma Arrives at UPMC Children's Hospital of Pittsburgh

UPMC Children's Hospital and the Division of Pediatric Hematology/Oncology have recently finished the building and deployment of a dedicated metaiodobenzylguanidine (MIBG) treatment room for use with patients who have neuroblastomas.

Neuroblastoma (the second most common extracranial solid tumor) typically develops in the nerve tissue of the adrenal glands but also may occur in the pelvis, neck, or chest. Neuroblastomas often migrate to the chest, bones, and bone marrow, and most diagnoses of the disease occur at an advanced stage with distant metastases. Most cases of neuroblastoma are diagnosed in very young children — neonates up to the age of 5. These tumors comprise approximately 7.5 percent of all childhood cancers.

MIBG is a unique radio-labeled iodine product used in the treatment of neuroblastoma that is specifically taken up by these kinds of tumor cells, leading to highly focused and targeted radiotherapy to combat the disease.

"There are very few places in the United States that have dedicated MIBG rooms. They are very costly to build and require significant capital expenditures. Also, construction times and building plans are very detailed and specific because of the radioactive nature of the treatment," says **Jean M. Tersak, MD**, professor of medicine, principal investigator at UPMC Children's for Children's Oncology Group (COG) trials conducted on-site, and founder of the Survivorship Clinic in the Division.

Patients treated for neuroblastoma with MIBG become and remain radioactive for three to four days after the treatment. The MIBG room must be completely lead-lined to prevent the escape of ionizing radiation, and the shielding must extend into the floor and piping that leads out of the room down to the lower floors of the hospital.

"At present, MIBG is a therapy used for children with relapsed or refractory disease, but it is now being evaluated to become a first-line therapy for neuroblastoma. Additionally, providing this new treatment modality in our oncology armamentarium to our patients and having the MIBG room on-site will allow us to conduct additional investigator-initiated research projects, and to take part in future multicenter clinical trials," says Dr. Tersak.

Neuroblastoma Research and Clinical Trials

Dr. Tersak and UPMC Children's have a long history of research and clinical trials investigating various aspects of neuroblastoma. UPMC Children's, as part of the COG, continues to take part in multicenter clinical trials investigating various therapeutic agents at differing disease stages to improve global outcomes and survival rates for neuroblastoma.

"We will take part in a pilot study using dinutuximab, which has previously been used as consolidation after bone marrrow transplant, and give this agent during the original induction of therapy to determine its effects on tumor burden and survival rates," says Dr. Tersak.

Dinutuximab, a monoclonal antibody, was previously studied in the ANBL0032 study, which led to its FDA approval as a first-line therapy for neuroblastoma in 2015. UPMC Children's participated in this original multi-center study through COG, with Dr. Tersak serving as site principal investigator.

Dr. Tersak and UPMC Children's also are part of the Late Effects After High-Risk Neuroblastoma (LEAHRN) study (ALTE15N2) through COG that is investigating late effects of high-risk neuroblastoma therapies that include multi-agent chemotherapy, surgery, bone marrow transplant, radiation therapy, and antibody therapy. However, with the increasing number of survivors, there is a lack of understanding of how these new treatment modalities may affect future health and quality-of-life.

"To be sure, there are organ toxicities related to the heart and hearing, but we do not have a firm or clear understanding at present of possible pulmonary toxicities or the effects on the endocrine system and consequent growth and development," says Dr. Tersak.

The LEAHRN trial opened in June 2017 and continues to enroll patients who were previously treated for neuroblastoma.

In March, The Children's Hospital of Pittsburgh Foundation received a $1 million gift from Stephanie McMahon, WWE chief brand officer, and Paul "Triple H" Levesque, WWE executive vice president, Talent, Live Events & Creative, to establish the family centered MIBG Therapy Suite at UPMC Children's.

UPMC Children's has the only pediatric MIBG therapy suite in western Pennsylvania.

"MIBG is now at a point where it can offer true benefits to our patients with relapsed or unresponsive neuroblastoma," says **Linda McAllister-Lucas, MD, PhD**, chief, Division of Pediatric Hematology/Oncology at UPMC Children's. "With this gift from Stephanie McMahon and Paul "Triple H" Levesque, we are now able to translate this therapy into life-saving clinical care."

Stephanie and Paul have been supporters of UPMC Children's for many years. They established Connor's Cure, a fund in honor of 8-year-old WWE fan, Connor Michalek, who battled medulloblastoma—a rare tumor that affects the brain and spinal cord.

To date, Connor's Cure has raised nearly $3 million and expanded its partnership with Children's Foundation to gain an important ally — The V Foundation for Cancer Research. Today, through The V Foundation's grant-making process, funds raised by WWE in support of Connor's Cure help to fuel promising cancer research studies at leading medical facilities throughout the country.

Division News and Recent Publications

Division Fellows to Present at ASH

Two fellows from UPMC Children's Hospital of Pittsburgh Division of Pediatric Hematology/Oncology will present abstracts at the 2019 American Society of Hematology annual conference that will be held December 7-10, 2019 in Orlando Florida.

Division fellow **Meghan McCormick, MD**, (left, top) will present on the topic of: Levofloxacin Prophylaxis Is Effective and Cost-Effective in Pediatric Patients with Acute Myeloid Leukemia.

Fellow **Archana Ramgopal, DO**, (left, bottom) will also present at the conference. Her abstract will discuss: Morbidity, Mortality, and Healthcare Utilization in a National Cohort of Pediatric Patients with Hemophagocytic Lymphohistiocytosis Who Received Hematopoietic Stem Cell Transplant.

New Paper Examines Solid Organ Transplant After Childhood Cancer Treatment

A new study[1] published in October in *The Lancet Oncology* from the Childhood Cancer Survivor Study sheds light on the incidence and risk factors surrounding the need for solid organ transplantation after receipt of treatment for a childhood cancer. UPMC Children's Hospital of Pittsburgh Division of Pediatric Hematology/Oncology was one of the participating sites in the multicenter study.

Main findings of the study showed that while the overall incidence of solid organ transplant is low in pediatric cancer survivors, insults to various solid organs through various treatment modalities does increase the risk of long-term damage that could lead to end-organ failure and the need for transplant. Survival rates of pediatric cancer survivors who underwent solid organ transplant led the study group to conclude that transplantation should be considered for those individuals who are five years or more post-treatment and are suffering from end-organ failure.

Jean M. Tersak, MD, professor of medicine and principal investigator of the Children's Oncology Group (COG) trials conducted at UPMC Children's served as the site investigator for the study and is also a co-author on the paper.

[1] *Dietz AC, Seidel K, Leisenring WM, Mulrooney DA, **Tersak JM**, Glick RD, Burnweit CA, Green DM, Diller LR, Smith SA, Howell RM, Stovall M, Armstrong GT, Oeffinger KC, Robison LL, Termuhlen AM. Solid Organ Transplantation After Treatment for Childhood Cancer: A Retrospective Cohort Analysis From the Childhood Cancer Survivor Study. Lancet Oncol. 2019 Oct; 20(10): 1420-1431. Epub 2019 Aug 27.*

Study Examines Behavioral Late Effects in Pediatric Cancer Survivors

Late effects of cancer treatment continue to be of significant concern to all cancer survivors and their caregivers, particularly young adolescent survivors. Late effects can arise in a host of organs and systems and can impact quality-of-life, morbidity, and mortality years after the successful treatment of a cancer.

New research co-authored by **Robert Noll, PhD**, a pediatric psychologist at the University of Pittsburgh School of Medicine who works extensively with pediatric cancer patients and their families, investigated the prevalence of symptoms of anxiety and depression many years after cancer treatment in adolescents (18 years of age).

The study, published in the journal *Psycho-Oncology,* followed children who received care at Cincinnati Children's Hospital Medical Center to determine if survivors exhibit more symptoms of anxiety and depression, and more evidence of psychiatric disease, comparing pediatric cancer survivors to peers from their schools when they were first diagnosed and had no severe chronic illness.

The main findings of the paper suggest that these young adult cancer survivors do not experience more symptoms of depression or anxiety based on reports from the survivors and their caregivers. Learn more about the study through the following reference: D'Souza AM, Devine KA, Reiter-Purtil J, Gerhardt CA, Vannatta K, Noll RB. Internalizing Symptoms in AYA Survivors of Childhood Cancer and Matched Comparisons. *Psycho-Oncology*. 2019 Jul 20. E pub ahead of print.

More About Dr. Noll

Dr. Noll is a pediatric psychologist in the Department of Pediatrics at the University of Pittsburgh School of Medicine. He has a rich background in research, clinical care, and teaching. After graduating from Ohio University with a BBA, Dr. Noll spent five years as a fighter pilot in the U.S. Navy where he flew more than 170 combat missions in Vietnam and received two Distinguished Flying Crosses, four Individual Air Medals, and four Navy Commendation Medals. After discharge from the U.S. Navy, Dr. Noll spent a year at the University of California at Berkeley where he received a psychology major. He then completed his graduate studies at Michigan State University, where he earned a doctorate in clinical child psychology with a developmental psychology minor.

Dr. Noll's research has focused on gaining a better understanding of the impact of medical challenges for children, their parents, and siblings. While Dr. Noll's work has included studies examining the functioning of children with sickle cell, arthritis, hemophilia, migraines, fibromyalgia, and neurofibromatosis, children with cancer and their families have been the center point of his research and teaching career. Dr. Noll has published 150+ peer-reviewed papers in scientific journals, including more than 50 that focus on pediatric cancers. Along with this academic focus, Dr. Noll has been extremely involved in the mentoring of junior faculty, fellows, and graduate students. Dr. Noll has been the primary or co-primary mentor for six career development awards, and four of his previous fellows currently have National Institutes of Health (NIH) funding.

Currently, Dr. Noll is the past-chair of the Behavioral Science Committee within the Children's Oncology Group and is responsible for the mentoring and development of a number of scholars in the United States. He also is involved with four ongoing NIH-funded studies related to children with cancer.

Ewing Sarcoma Cell Biology *Continued from Page 1*

Ewing sarcoma cells can dynamically express high or low levels of the EWS-FLI1 protein, and there can exist much intratumor cell heterogeneity with respect to EWS-FLI1 protein levels. Recent research in the field has that varying levels of EWS-FLI1 can significantly influence cellular behavior, with those in low states highly prone to metastasize.

Previous work by Dr. Bailey also has shown that Ewing sarcoma cells with low levels of EWS-FLI1 tend to overexpress ICAM-1, a protein that aids in T cell activation and interaction between tumor cells and T cells.

"Our original hypothesis was that since low EWS-FLI1 cells overexpress ICAM-1, T cells would be better able to recognize these cells and target them for destruction. However, our research actually uncovered the opposite effect from what we expected to see. Although these tumor cells do express more ICAM-1, they appeared to be more resistant to the T cell response," says Dr. Bailey.

The reason for this contradictory response appears to be because the low-state EWS-FLI1 Ewing sarcoma cells upregulate both PD-L1 and PD-L2. With more PD-L1 and 2 available on a tumor cell, it can bind to the PD-1 and 2 receptors on the T cell and reduce or turn off the immune response, thereby evading detection.

Dr. Bailey's paper, however, goes on to show that when they blocked PD-L1 expression in the tumor cells with low levels of EWS-FLI1, they were able to increase the efficacy of the T cell response compared against tumor cells with higher levels of EWS-FLI1.

Tumor Heterogeneity and the Tumor Microenvironment Are Key to Better Understanding Ewing Sarcoma

Two things about Ewing sarcoma are firmly understood: There exist great levels of heterogeneity within the tumor and within the individual cells of the tumor, and that very little is currently understood about the micro-environment of these tumors and how these factors influence growth, mechanisms of metastasis, and suppression or evasion of the immune response.

"The overarching goal of our research is to better understand these aspects of Ewing sarcoma, how and why there is so much cellular variability in these tumors, and the methods by which we can work on improving therapies that target all of these important pathways that make some cells more responsive to therapy and others less so," says Dr. Bailey.

The work is challenging for these reasons, and also compounded by the fact that there are no good animal models currently available for Ewing sarcoma, partly because of the heterogeneity that exists, and partly because the field has yet to understand fully the exact cell of origin in these tumors and what other factors or influences on cells at a specific point in development lead to tumorigenesis.

An Initial Step

For Dr. Bailey, this paper and its findings are an initial step to understanding how EWS-FLI1 might be regulating tumor cell immunogenicity and the cell's response to interferon-gamma. While it may be the first step, new research is already underway that will continue to advance Ewing sarcoma cell biology. Dr. Bailey indicates that future work will look more specifically at the role of interferon-gamma and other aspects of the immune response to these tumors.

References

[1] Bailey KM, Julian CM, Klinghoffer AN, Bernard H, Lucas PC, McAllister-Lucas LM. EWS-FLI1 Low Ewing Sarcoma Cells Demonstrate Decreased Susceptibility to T-Cell-Mediated Tumor Cell Apoptosis. *Oncotarget*. 2019; 10(36): 3385-3399.

Further Reading

Hawkins AG, Julian CM, Konzen S, Treichel S, Lawlor ER, Bailey KM. Microenvironmental Factors Drive Tenascin C and Src Cooperation to Promote Invadopodia Formation in Ewing Sarcoma. *Neoplasia*. 2019 Sep 12; 21(10): 1063-1072. Epub ahead of print.

Venier RE, Maurer LM, Kessler EM, Ranganathan S, McGough RL, Weiss KR, Malek MM, Meade J, Tersak JM, Bailey KM. A Germline BARD1 Mutation in a Patient With Ewing Sarcoma: Implications for Familial Testing and Counseling. *Pediatr Blood Cancer.* 2019 Sep; 66(9): e27824. Epub ahead of print.

Bailey K, Cost C, Davis I, Glade-Bender J, Grohar P, Houghton P, Isakoff M, Stewart E, Laack N, Yustein J, Reed D, Janeway K, Gorlick R, Lessnick S, DuBois S, Hingorani P. Emerging Novel Agents for Patients With Advanced Ewing Sarcoma: A Report From the Children's Oncology Group (COG) New Agents for Ewing Sarcoma Task Force. *F1000Res.* 2019 Apr 15; 8. pii: F1000 Faculty Rev-493. doi: 10.12688/f1000research.18139.1. eCollection 2019. Review.

Bailey KM. Prospective Investigation of Drug Resistance: An Approach to Understanding and Optimizing the Clinical Benefit of Targeted Agents in Ewing Sarcoma. *Oncotarget.* 2018 Dec 18; 9(99): 37270-37271.

UPMC Physician Resources

For the latest news, events, videos, and free CME courses presented by UPMC clinicians and researchers, visit **UPMCPhysicianResources.com/Pediatrics**.

Bone Marrow Failure Syndromes — *Presented by Steven Allen, MD*

Hot Topics in Pediatric Hematology and Oncology — *Presented by A. Kim Ritchey, MD*

Adolescent and Young Adult Cancer Treatment — *Presented by Louis Rapkin, MD*

Video Rounds

Video Rounds is a series of short, informative, and educational videos created for physicians and covering a variety of medical and surgical disciplines. Current topics in ***oncology*** *include:*

Inherited Cancer Predisposition Syndromes *with Julia Meade, MD*

Blood Disorders in the Pediatric Population *with Jake Cooper, MD*

Trends and Advances in Pediatric Leukemia *with A. Kim Ritchey, MD*

About The Division of Blood and Marrow Transplantation and Cellular Therapies

The Division of Blood and Marrow Transplantation and Cellular Therapies (BMT-CT), led by Paul Szabolcs, MD, Division chief and professor of Pediatrics and Immunology at the University of Pittsburgh School of Medicine, places special emphasis on the development and use of reduced-intensity/toxicity transplant regimens for a range of non-malignant conditions that has attracted pediatric and adult patients from more than 20 states in the U.S.

Historically, these have been applied to inborn errors of immunity and inherited metabolic disorders such as mucopolysaccharidosis syndromes (MPS) and leukodystrophies.

Clinical research within the Division has led to new trial designs that recently have been approved by the U.S. Food and Drug Administration (FDA) and Institutional Review Board (IRB) of the University of Pittsburgh to bring a cure or alleviate advanced cases of certain autoimmune disorders such as Crohn's disease (CD) and systemic sclerosis (SSc). Another exciting new protocol also received FDA and IRB approval, whereby all sickle cell disease (SCD) patients who may benefit from allogeneic transplants would be able to find partially matched healthy unrelated stem cell donors.

Toward these objectives, the BMT-CT Division collaborates with pediatric and adult gastroenterology, rheumatology, cardiology, and pulmonary specialists from UPMC and the University of Pittsburgh, to form CD, SSc, and SCD disease-specific task forces led by Dr. Szabolcs. Pediatric and adult candidates who have traveled from distant locations in the United States are being screened and treated.

UPMC Children's is the only entity in the world currently performing tandem cadaveric lung and bone marrow transplantation for both pediatric and adult patients diagnosed with a primary immunodeficiency with progression to end-stage lung disease. Early favorable experience has now led to new indications that are focusing first on combined bone marrow and lung failure related to a diagnosis of idiopathic pulmonary fibrosis.

Division Faculty and Staff

Paul Szabolcs, MD — Division Chief

Randy Windreich, MD — Clinical Director and Fellowship Director

Jessie Barnum, MD

Craig Byersdorfer, MD, PhD

Beth Carella, DO

Elizabeth O'Brien Stenger, MD, MSc

Meghan Frost, NP

Amy Hatley, MSN, CRNP

Nicole Hogue, PA-C

Emily Mansmann, MPAS, PA-C

Patricia McLendon, DNP, CRNP

Tammy Spight, PA-C

CAR T Cell Therapy Comes to UPMC Children's

Chimeric Antigen Receptor T cell (CAR T cell) therapy has proven to be, for a subset of patients and diseases, a revolutionary, curative, and durable therapy.

UPMC Children's Hospital of Pittsburgh began preparations for performing CAR T cell infusions for select pediatric patients in late 2017, with live administrations of the therapy beginning in February of this year. UPMC Children's is currently treating pediatric and young adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia (B-cell ALL) up to the age of 25, with Kymriah® from Novartis.

Randy Windreich, MD, clinical director and fellowship director in the Division of Blood and Marrow Transplantation and Cellular Therapies, oversees the CAR T cell program at UPMC Children's.

"Significant investment and infrastructure were needed to bring CAR T cell therapy to UPMC Children's. Our Division, with the assistance and support of hospital administration, opted for the ability to do all the cell processing and counts on site, which dramatically speeds up the process for our patients," says Dr. Windreich.

Dr. Windreich also notes that with the infrastructure now in place, UPMC Children's will be in position to offer institutional clinical trials in CAR T cell, as well as participating with Children's Oncology Group (COG) trials in the future and expanding patient access to potentially novel therapies.

As of the time of this writing, UPMC Children's has treated three patients with CAR T cell infusions. The first two patients were children and the third a young adult, all of whom had relapsed and refractory B-cell ALL. All three patients went into the CAR T cell therapy with varying amounts of leukemia still present, and at one month all were minimal residual disease (MRD) negative or in complete remission.

"Our first two patients have finally been able to achieve remission and sustain it for the last several months. Our third patient, who had perhaps the more difficult course, was also thrilled to hear that the CAR T cell worked and that he was in remission. So far, we are pleased with our initial results," says Dr. Windreich.

Managing the Complications of CAR T Cell: Cytokine Release Syndrome

As with any therapy, CAR T cell brings with it the potential for complications or patient reactions. One potentially severe and life-threatening complication of CAR T cell therapy is cytokine release syndrome (CRS). Essentially a massive, systemic inflammatory response, CRS poses a significant risk of morbidity and mortality.

Prior to opening CAR T cell therapy at UPMC Children's, Dr. Windreich worked with staff from the pediatric intensive care unit (PICU) to develop a management algorithm for CAR T cell patients, essentially a set of instructions to help all staff manage these patients if or when CRS arises and at differing levels of severity. Hematology/oncology physicians, BMT physicians, advanced practice providers, PICU staff, emergency department staff, pharmacy, and nursing were trained in how to manage CRS in CAR T cell patients. Neurologists have also been incorporated into the training sessions because of the potential neurotoxicity with CAR T cell therapy.

"We invested significant time in the upfront training, but we also continue to do refresher training as well for new staff members in an ongoing manner. We also have made the information readily available on our internal website for the staff. Because months can go by without a CAR T cell case, we have to keep team members at the ready," says Dr. Windreich.

Predicting who may end up with CRS or severe cases of it is ongoing. Right now, as Dr. Windreich explains, the best indicator is leukemia burden. The higher the initial burden, the higher the risk or severity of CRS. Other factors may be ongoing inflammation or infection, or other inflammatory states that could predispose patients to the complication.

Forward-Looking Perspectives on CAR T Cell Therapy

It ought to be obvious, but Dr. Windreich is hopeful that in the future CAR T cell therapies can be developed to target other types of leukemias and lymphomas, and even solid tumors for which it has so far shown little efficacy in other clinical trials and research around the country, although that work is progressing.

"It will be interesting to see if we can harness CAR T cell therapy or some variant of it in the future to treat the myeloid leukemias and T cell leukemias. It has so far proven very difficult to get a normal myeloid or T cell to recognize abnormal ones and mount a response, but I think we will eventually crack that code. Immunotherapies are the future right now. We need to work on expanding their abilities and efficacies for more diseases and more patients."

BMT-CT Division Researcher Craig Byersdorfer Awarded New NIH R01 Grant

Craig A. Byersdorfer, MD, PhD, assistant professor of medicine in the Division of Blood and Marrow Transplantation and Cellular Therapies at UPMC Children's was awarded a National Institutes of Health (NIH) R01 grant to continue his studies on T cell-mediated graft-versus-host disease in allogeneic hematopoietic stem cell transplantation (alloHSCT). Dr. Byersdorfer's new grant (1R01HL14556-01) will investigate the role of the AMP-activated protein kinase in graft-versus-host disease (GVHD) causing cells.

alloHSCT is a curative therapy for many life-threatening conditions, but its benefits are often overshadowed by the risks of acute GVHD where donor T cells attack and destroy tissues in the host, notably the skin, liver, and gastrointestinal tract. Targeting the specialized metabolism of alloreactive T cells represents one way to overcome the risks of GVHD, but still retain the benefits of immune reconstitution and graft-versus-tumor effects.

Dr. Byersdorfer's new investigation will probe the mechanisms of why alloreactive T cells lacking the AMP-activated protein kinase cause less GVHD. The end goal will be to translate these findings into human T cells in an effort to design better therapies for GVHD and comparable T cell-mediated immune disorders.

Technical Abstract

New therapies to distinguish pathogenic T cells from T cells mediating beneficial immune responses are necessary to improve the safety and applicability of alloHSCT. Previous work by Dr. Byersdorfer's team, along with current preliminary data suggest that targeting alloreactive T cell metabolism may allow for this selective intervention. Specifically, the data demonstrate that the deletion of AMPK in donor cells mitigates GVHD but still preserves lymphopenia-driven immune reconstitution and T cell-driven graft-versus-tumor (GVT) effects.

Mechanistically, Dr. Byersdorfer's data further suggest that lower rates of GVHD result from a decreased sensitivity of AMPK knock-out (KO) cells to the effects of pro-inflammatory cytokines. From this, the research team has formed the central hypothesis that AMPK is activated early posttransplant in a tissue-specific fashion, increasing local T cell sensitivity to pro-inflammatory cytokines. In the absence of AMPK, inflammatory signals are blunted, stabilizing regulatory T cell (Treg) development and decreasing effector responses. These changes mitigate GVHD, while GVT responses are unaffected because increased cytokine sensitivity is unnecessary for inducing leukemia-directed cytotoxicity and because leukemia clearance occurs at sites where AMPK activation is less pronounced.

Dr. Byersdorfer's team will test this hypothesis in the new study with three specific aims. The first aim will determine the location and temporal necessity of AMPK by eliminating AMPK in T cells at defined times posttransplant and quantitating AMPK activation in cells recovered from multiple tissues simultaneously. The relationship between AMPK activation and cytokine sensitivity also will be defined by measuring cytokine responses following stimulation with an array of AMPK agonists.

The second aim of the study will elucidate mechanisms linking AMPK deficiency to improved GVHD and decreased cytokine sensitivity by comparing the GVHD potential of single KO cells to cells lacking both AMPK and the interleukin-6 (IL-6) receptor. In addition, mass spectrometry will be used to measure phosphorylation of novel AMPK target proteins in cytokine-stimulated and alloreactive T cells. The final aim of the study will determine the GVHD and GVT potential of AMPK-deficient human T cells after decreasing AMPK levels using CRISPR/Cas9 gene editing and short hairpin RNA transduction, followed by transplantation of modified cells into xenogeneic models of GVHD and immunodeficient models of GVT.

These studies will deepen the understanding of AMPK activation, how this activation impacts cytokine sensitivity, and whether these findings can be translated into human cells. If successful, Dr. Byersdorfer's studies will define a novel mechanism linking energy sensing to T cell effector function that will likely extend beyond GVHD to include the robust and sustained activation of any T cell, including during autoimmunity and following solid organ transplantation.

BMT-CT Division News

The Blood and Marrow Transplantation and Cellular Therapies Division at UPMC Children's Hospital of Pittsburgh is one of the leading centers in the United States and globally advancing new and safer treatment approaches for numerous medical conditions. Below are recent updates from the Division: speaking engagements, new grants, awards, and recent publications.

Speaking Engagements

Division Chief **Paul Szabolcs, MD**, was invited to give numerous lectures and presentations in 2019. Among his invited speaking engagements were the following:

"A Longitudinal Analysis of TCRß Repertoire Diversity in Immune Tolerance of Pediatric Patients Post-UCBT: T Cell Clonal Deletion Over Clonal Anergy." Presented at the 45th European Society for Blood and Marrow Transplantation annual meeting in Frankfurt, Germany, on March 25, 2019.

"Induction of Immune Tolerance by Allogeneic Transplantation." Delivered at the Children's University Hospital Tubingen in Tubingen, Germany, on March 28, 2019.

"New Challenge of Immunotherapy: Tolerance and Immunity, Mechanisms and Proposals." Presented at the Universidad Autonoma de Madrid in Madrid, Spain, on June 17-19, 2019.

"Combined Lung + Bone Marrow Transplant: Progress and Challenges." Lecture delivered at the Major Complications of Dyskeratosis Congenita/Telomere Biology Disorders meeting in Boston, Massachusetts, on September 12-13, 2019.

New Grants

- In September, **Craig Byersdorfer, MD, PhD**, was awarded a Hyundai Hope on Wheels® Scholar Grant for a project on "Minimizing Cell Therapy-Associated Cytokine Release Syndrome." The $300,000 grant is one of 24 given to researchers across the country. The Scholar Senior Research Grants fund research projects designed to improve the treatment and quality of life for children with cancer. The ultimate goal of the Scholar Senior Research Grant program is to find cures for childhood cancers once and for all. Since 1998, the program has funded $115 million in research to Children's Oncology Group (COG) member institutions nationwide. UPMC Children's Division of Pediatric Hematology/Oncology researcher **Kelly Bailey, MD, PhD**, also was a recipient of one of the awards for her work with Ewing's sarcoma.

L-R – Linda McAllister-Lucas, MD, PhD; Craig Byersdorfer, MD, PhD; Kelly Bailey, MD, PhD; Mr. Tim Butler, District Director for U.S. Representative Mike Kelly.

- Dr. Byersdorfer also received a U.S. Department of Defense (DOD) grant for a new study called "Leveraging T Cell Metabolism to Improve Anticancer Immunotherapies." In this study, Dr. Byersdorfer and colleagues will be investigating metabolic pathways that may be able to augment and increase in vivo persistence of tumor-reactive lymphocytes, specifically T cells involved in antitumor responses against acute myelogenous and acute lymphoblastic leukemia. Immune cell targeting of tumor antigens offer hope for these difficult cases, but the full potential of adoptive immunotherapy remains unrealized. The innovation for this project comes from taking a recognized clinical problem (a lack of immune cell persistence) and addressing it by metabolically reprogramming an established treatment, making this innovation both conceptual and technical.

The first aim of Dr. Byersdorfer's study will elucidate metabolic changes in human T cells following constitutive activation of AMPK or expression of PPAR-δ. His laboratory has recently generated lentiviral constructs bearing either mutant AMPKγ2 sequences, which drive AMPK activation, or full-length human PPAR-δ cDNA. Lentiviral genes will be transduced into human T cells and T cell metabolism assessed following in vitro and in vivo stimulation. The second aim of the study will determine the in vivo impact of constitutive AMPK activation or PPAR-δ expression on T cell persistence and subsequent antitumor responses. CAR T cells targeting human CD19 will be transduced with a second lentivirus bearing PPAR-δ or mutant AMPKγ2. CAR T cells, with or without metabolic manipulation, will then be injected with CD19+ leukemia cells into immunodeficient mice where both T cell persistence and antitumor responses will be

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BMT-CT Division News *Continued from Page 11*

measured. In a second model, cytotoxic lymphocytes (CTLs) will be generated against the antigen PR-1. PR1-reactive CTLs will then be transduced with metabolic lentiviral constructs and injected into immuno-deficient mice, followed by assessment of CTL persistence and the ability to clear a PR1-presenting tumor cell line. In a final series of experiments, primary human T cells will be expanded in vitro against primary human AML blasts, transduced with AMPK or PPAR-δ lentiviral constructs, and then tested for the ability to eliminate primary AML cells in vivo in an immunodeficient mouse model.

These studies seek to positively impact the wider field of immunotherapy by determining whether metabolic changes, such as constitutive activation of AMPK, can improve T cell responses by prolonging their survival and thereby lessening overall disease burden. These studies also are important for cancer research in general, as the ability to modulate immune responses is likely to be a cardinal feature in multiple treatment regimens.

Other Division News

Elizabeth Stenger, MD, MSc, was elected to the Board of Directors of the International Society for Cell & Gene Therapy (ISCT).

Recent Peer-Reviewed Publications and Abstracts

Hsu AP, Donkó A, Arrington ME, Swamydas M, Fink D, Das A, Escobedo O, Bonagura V, **Szabolcs P**, Steinberg HN, Bergerson J, Skoskiewicz A, Makhija M, Davis J, Foruraghi L, Palmer C, Fuleihan RL, Church JA, Bhandoola A, Lionakis MS, Campbell S, Leto TL, Kuhns DB, Holland SM. Dominant Activating RAC2 Mutation With Lymphopenia, Immunodeficiency, and Cytoskeletal Defects. *Blood.* 2019 May 2; 133(18): 1977-1988.

Dreyzin A, Michaels MG, Vander Lugt MT, **Szabolcs P**. Oral Ribavirin for Paramyxovirus Infection After Alemtuzumab-Containing Reduced-Intensity Conditioning HCT Regimen. *Pediatr Transplant.* 2019 Mar; 23(2): e13358.

Burk CM, Coffey KE, Mace EM, Bostwick BL, Chinn IK, Coban-Akdemir ZH, Jhangiani SN, Lupski JR, Ortiz D, **Barnum JL**, Allen SW, Robertson LM, Orange JS, Chong HJ. Immunodeficiency, Centromeric Instability, and Facial Anomalies (ICF) Syndrome With NK Dysfunction and EBV-Driven Malignancy Treated With Stem Cell Transplantation. *J Allergy Clin Immunol Pract.* 2019 Sep 11. pii: S2213-2198(19)30765-2. doi: 10.1016/j.jaip.2019.08.040. Epub ahead of print.

About UPMC Children's Hospital of Pittsburgh

Regionally, nationally, and globally, UPMC Children's Hospital of Pittsburgh is a leader in the treatment of childhood conditions and diseases, a pioneer in the development of new and improved therapies, and a top educator of the next generation of pediatricians and pediatric subspecialists. With generous community support, UPMC Children's Hospital has fulfilled this mission since its founding in 1890. UPMC Children's is recognized consistently for its clinical, research, educational, and advocacy-related accomplishments, including ranking 15th among children's hospitals and schools of medicine in funding for pediatric research provided by the National Institutes of Health (FY2018) and ranking on *U.S. News & World Report's* Honor Roll of America's Best Children's Hospitals (2019–20).