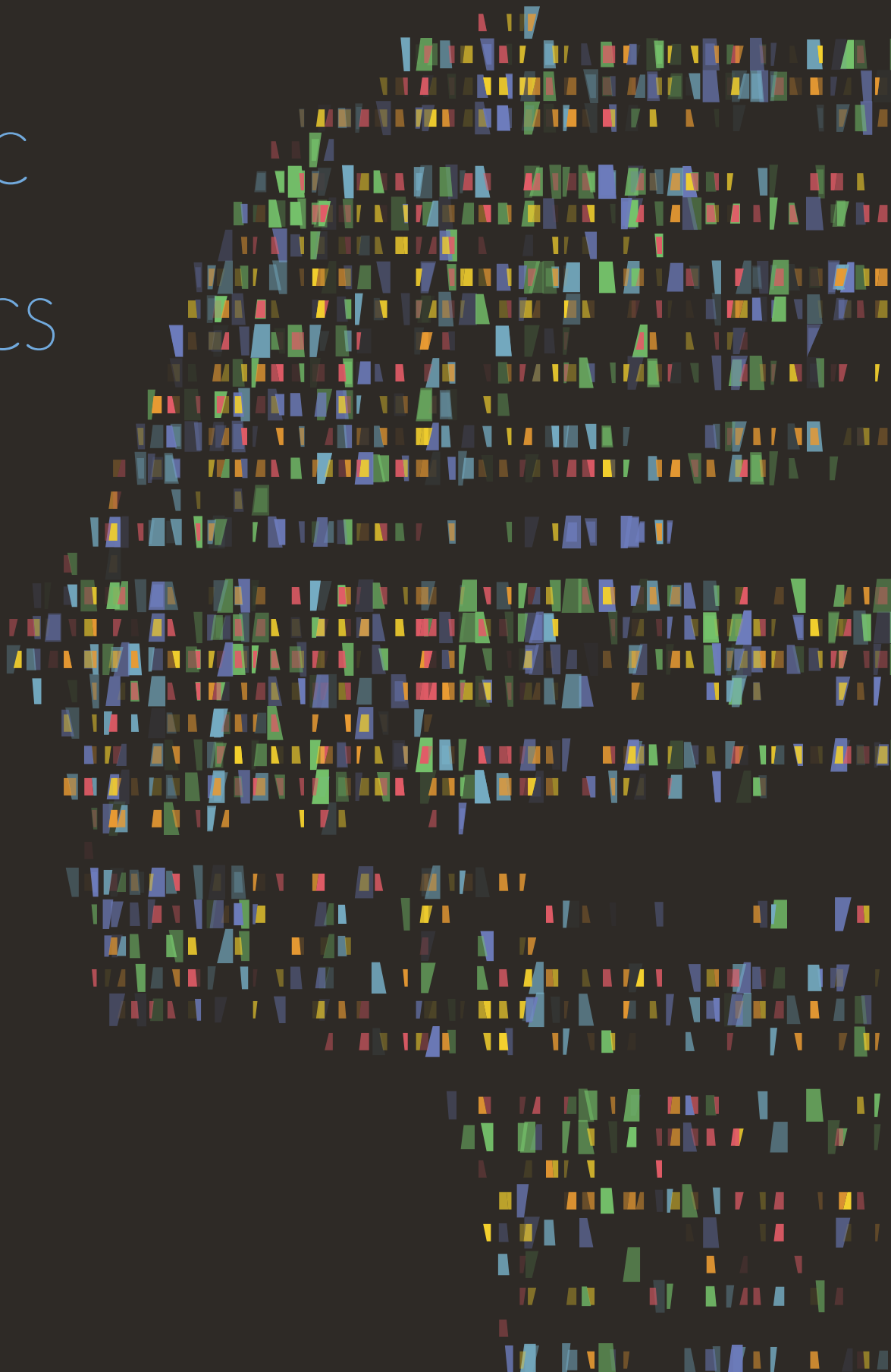
PEDIATRIC CANCER GENOMICS REPORT



Children's Mercy
KANSAS CITY

LOVE WILL.

WELCOME

This year's Children's Mercy Pediatric Cancer Genomics Report focuses on what, until now, has been a far-off distant future dream – Cancer Genomics. **IT IS HERE!** And now cancer genomics is rapidly redefining our understanding of cancer, and in turn our treatment of cancer.

It was not too long ago that many thought cancer might be caused by viruses. We now know that cancer is, in fact, the result of one of our own cells gone awry—like a copy machine stuck on copy. The resulting mistakes in our instructions, better known as our genes, are mutations that drive these cancer cells to divide uncontrollably.

The result is these cancer cells take over the space our normal cells and organs need to do their jobs, spreading and wreaking damage throughout the body. Much of our new understanding of this process is being generated by our own researchers here at Children's Mercy. Their work clearly shows that what may appear to be similar between adults and children is not. Cancer has vastly different origins, different mutations, and different paths in children as compared to adults.

In adults, it generally takes decades of cumulative mutations to finally result in enough change in those genes to turn the cell into a cancer cell. But in children, the mutations are few. Sometimes just a single mutation is powerful enough to turn a cell cancerous, and can do so in a child in a much shorter time span, even as young as a newborn.

In my 30-plus years in the field of childhood cancer, I have seen many changes resulting in many improvements for our young patients. Yet, in just the past five to 10 years, with the advent of genomics, there has been an explosion of new discoveries resulting from the critical research labs in cancer centers and universities, like those in the Children's Mercy Research Institute, and from the bedsides of children fighting the battle for their lives.

At times it seems like we are stepping out of a thick, dark forest into a bright, vast clearing that illuminates the causes of, and the therapeutic approaches for, cancer. It is such a bright light that we are still adjusting to it. But we are now seeing our future in the therapy for childhood cancer more clearly. We are so fortunate to have these capabilities here at Children's Mercy. Both the research focus and clinical application of this new medical approach in our Cancer Genomics program benefits children in our region, and as you'll read, nationally and internationally.

In this journey of better understanding cancer, and particularly childhood cancer, we have entered a new era of discovery that is creating a new era of understanding. With the research and clinical application that is ongoing in the Children's Mercy Cancer Genomics Program and worldwide, we will be, in the coming few years, learning how to better apply this understanding with more precise and rational therapy.

We are already seeing in select cancers that this is proving to be a safer way to achieve higher cure rates for our children. Join us in the following pages as we describe these research discoveries and how we are starting to apply them for our children who have cancer. But stay tuned, this is only the beginning.

ALAN S. GAMIS, MD, MPH

Associate Division Director, Section of Oncology; Professor of Pediatrics, University of Missouri-Kansas City School of Medicine; Clinical Professor of Pediatrics, University of Kansas School of Medicine

TO LEARN MORE ABOUT CANCER GENOMICS AND OUR COMPREHENSIVE CANCER CENTER SEE OUR CANCER CARE REPORT AT

childrensmercy.org/cancercarereportFY19

CANCER REGISTRY 2018

The Cancer Registry at Children's Mercy Kansas City plays a vital part in the surveillance of cancer in our pediatric population. The Cancer Registry is a HIPAA-compliant confidential database comprised of malignant cancers, benign brain tumors and other specified benign tumors. The database is operated under the guidance of the Cancer Care Committee. Data collected, which includes diagnosis, treatment, recurrence and survival, is standardized for state and national comparisons.

Following each patient's cancer status is a very important part of Cancer Registry data collection. Knowing outcomes of each cancer patient can assist care providers with determining best treatment methods and long-term effects of cancer treatment. Therefore, follow-up letters inquiring about a patient's cancer status are sent out yearly. Parents and older patients are encouraged to contact the registry by secure email at cancerregistry@cmh.edu to discuss follow-up.

During 2018, the Cancer Registry added 209 patients to the database. Of these patients, there were 188 patients who were diagnosed with malignancies and benign central nervous system tumors. There were 21 patients added to the registry as having benign reportable conditions. These conditions are collected at the request of the Cancer Care Committee for surveillance purposes and are not required to be reported outside our facility. Please see the frequency by diagnosis chart for a breakdown of cancers.

209

Patients added to the Cancer Registry database during 2018.

188

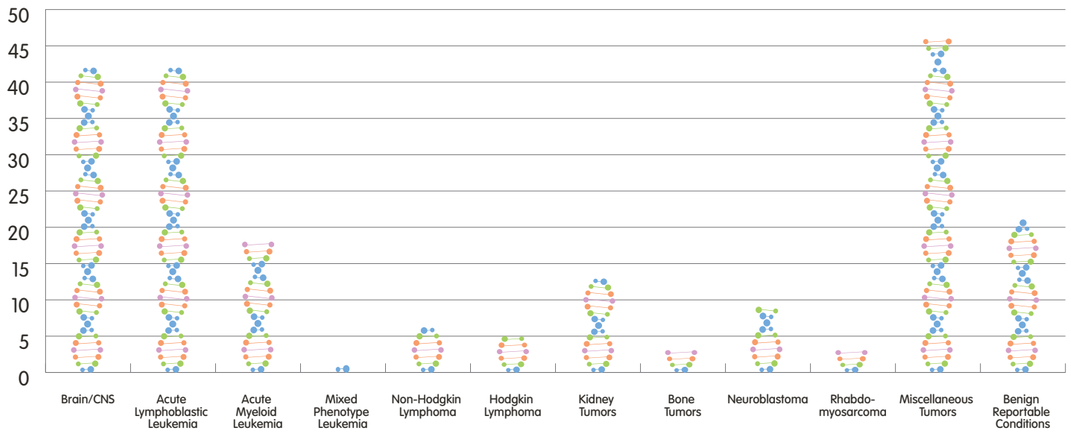
Patients diagnosed with malignancies and benign central nervous system tumors during 2018.

21

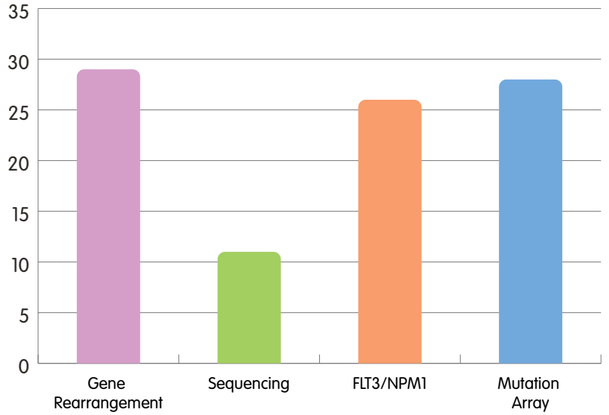
Patients added to the Cancer Registry as having benign reportable conditions during 2018.

FREQUENCY OF DIAGNOSIS BY DISEASE TYPE

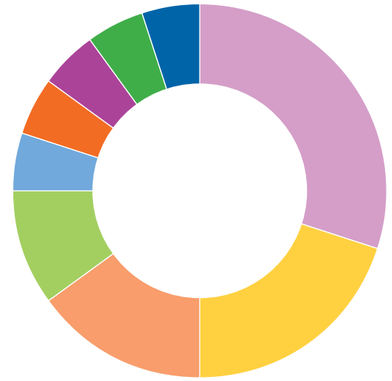
2018 PATIENTS



CASES WITH MOLECULAR TESTING



GENES WITH MUTATIONS FOUND

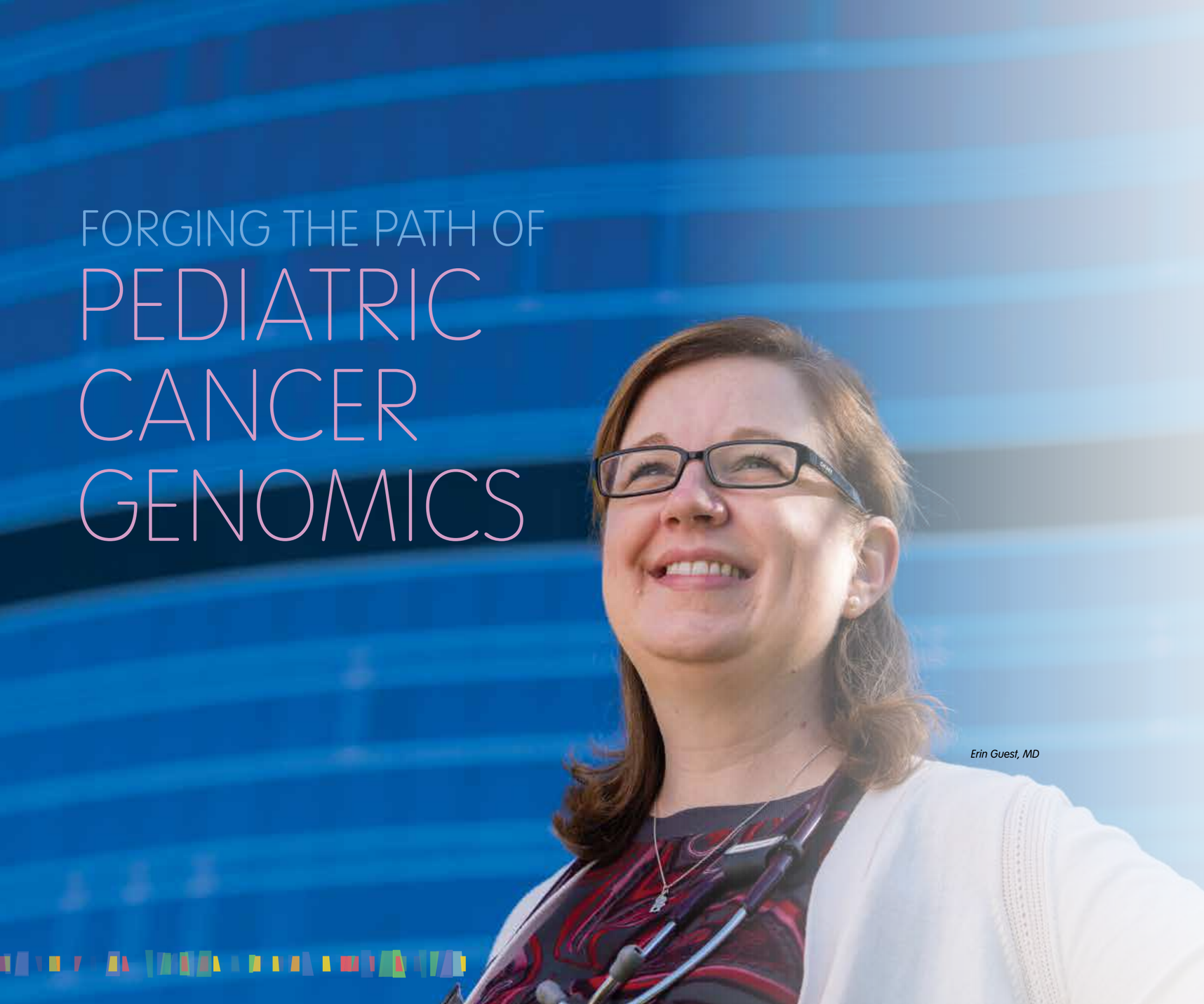


IGH/TRG FLT3 NPM1 BRAF BCOR

NRAS PIK3CA PTEN SMO

Nearly 100 molecular tests were performed on cancer samples in 2018. Testing revealed important molecular changes in many genes including *FLT3, NPM1, BRAF, BCOR, NRAS* and *SMO*. This information helped guide patient diagnoses, prognoses and therapies.

FORGING THE PATH OF PEDIATRIC CANCER GENOMICS



Erin Guest, MD

When it comes to cancer, as with many pediatric diseases, children are not simply little adults. Unlike adult cancer, childhood cancers are not typically associated with numerous genetic alterations. As a result, the molecularly targeted therapies designed to treat adults with cancer are not often beneficial to children. Further, childhood cancer is rare and many of the mutations discovered in the cancer cells are actually in the germline, within all cells in the body.

In addition, genomic research has taught us that pediatric cancers are diverse and that each patient's case is unique. This presents a major challenge in breaking down the data in a meaningful way to guide and individualize treatments. The Children's Mercy Cancer Center Genomics Program, established in 2014, consists of a team of clinicians and researchers with the common goals of incorporating genomics into our everyday clinical treatments and research programs to improve the outcomes for our patients.

The Cancer Genomics Program serves as a bridge between the Genomic Medicine Center and the Division of Hematology/Oncology/Blood and Marrow Transplant at Children's Mercy. Program members include pediatric oncologists, molecular and anatomic pathologists, cytogeneticists, clinical geneticists, genetics counselors, bioinformaticians, and other sub-specialists with particular interests in cancer diagnostics and research.

Genomic medicine traverses many different disciplines and the Cancer Genomics Program is designed to intertwine with and support the genomics needs of all of the other cancer center programs, including the Leukemia and Lymphoma Program, the Brain Tumor Program, the Adolescent and Young Adult Program, Experimental Therapeutics, Stem Cell Transplant, and many more.

The Cancer Genomics Program supports clinical genomics testing for direct patient care, a variety of research efforts in genomics, and translational projects that tie patient outcomes to genomic testing. In its research functions, the program relies upon the Children's Mercy Research Institute (CMRI) for support. In addition, the Children's Mercy Cancer Center, the CMRI and the Cancer Genomics Program are all fortunate to have incredibly generous philanthropic donors who have made this work possible.

We have grown our clinical cancer genomics capabilities extensively, and we now perform most sequencing testing in our on-site Genomic Medicine Center lab. It's important to note that the interpretation of cancer genomics test results is challenging and requires extensive knowledge and skill.

Genetic mutations, also known as variants, that are specific to cancer cells are often not present uniformly within the cancer. Sometimes variants are at such a low level that it can be difficult to distinguish a real mutation from the background error rate inherent to the sequencing process.

Our specialized team of molecular oncologists interprets each child's cancer sequencing result in the context of his/her cancer diagnosis and germline sequencing data. Next, we review the results in our multidisciplinary molecular tumor board. The child's oncologist discusses the clinical diagnosis and the team of experts reviews the sequencing findings to determine if other therapies might be beneficial. In doing so, we take an individualized approach to every patient and we incorporate all available data into the treatment recommendation.

As mentioned above, germline mutations play an important role in the development of childhood cancer. Germline mutations may be inherited or may occur *de novo*, during the child's early development. For this reason, genetic counseling and testing are especially important to determine if a germline mutation contributed to the child's risk of developing cancer, and to discuss the risk to other family members, including siblings.

Certain germline mutations are associated with known risks for specific, often very rare types of cancer. For example, germline mutation of the TP53 gene results in Li Fraumeni syndrome, with increased risks for solid tumors, brain tumors, and leukemias throughout life, from early childhood to adulthood. Li Fraumeni and many other childhood cancer predisposition syndromes have established guidelines for cancer screening and early detection, which have been shown to result in better chances of survival.

Erin McBride

We work closely with our clinical genetics providers to accomplish testing, counseling, and screening when appropriate. Our Surveillance for Predisposition to Tumors (SPoT) clinic is dedicated to providing a home for patients to coordinate the recommended screening tests. We see patients annually for visits and work closely with their primary care doctors and other subspecialists to ensure that each child receives the recommended care based on his/her diagnosis.

OUR VISION FOR THE CANCER GENOMICS PROGRAM IS THAT EVERY CHILD WITH CANCER IS GIVEN THE OPPORTUNITY TO PARTICIPATE IN CLINICAL TESTING AND TO CONTRIBUTE TO RESEARCH.

Research is critical to gain understanding of the significance of sequencing results. To build a robust foundation of new knowledge, the Cancer Genomics Program has partnered with the Children's Mercy Oncology Biorepository, also known as the Tumor Bank, to sequence every single cancer case in the bank.

Patients and their parents are given the opportunity to donate blood, bone marrow and tumor tissue remaining after a biopsy to the Tumor Bank. The specimens are taken to the CMRI for isolation of DNA, RNA, live cells and other components, and then portions are transferred to the Genomic Medicine Center for sequencing. The patient's personal identifiers are removed. The result is a broad data set that includes most of the cancers diagnosed at Children's Mercy, giving us the ability to compare changes in the cancer cells from diagnosis to relapse, if relapse occurs.

Investigators can utilize the data to design and test new treatment approaches on the banked cells. The de-identified sequencing data will soon be shared in an online public database, empowering collaborative world-wide sharing of cancer genomics data toward a common goal of improving molecularly targeted treatments. In addition to our sequencing of Tumor Bank clinical samples, our Cancer Genomics Program supports a variety of other cancer-specific research projects. Our largest project so far has involved orthogonal sequencing of leukemia samples from infants with acute lymphoblastic leukemia (ALL).

This sequencing work has identified mutations gained at relapse in infants with ALL who are enrolled in a Children's Oncology Group clinical trial. Ongoing work with additional infant ALL samples is looking at epigenetic changes within the patients' cells before and after treatment with azacitidine. To date, we have collaborated with researchers at Johns Hopkins University, Children's Hospital of Philadelphia, The University of Kansas, Children's National Medical Center, among other institutions, and we welcome new research partnerships.

Our vision for the Cancer Genomics Program is that every child with cancer is given the opportunity to participate in clinical testing and to contribute to research. Although the field of cancer genomics is still relatively new, this information-gathering phase is a critical step and we consider it important to broadly study and compare sequencing results across all childhood cancer types. Our program is poised to lead the way in generating and sharing data, improving outcomes for our patients now and in the future.

ADVANTAGES OF LOCAL SEQUENCING

Midhat Farooqi, MD, PhD

The Human Genome Project—an international scientific research collaboration that undertook sequencing of the entire human genome—took 13 years, 20 laboratories across the globe, and roughly 3 billion dollars to complete. In this context, it is astounding to consider that "local" genome sequencing performed by a hospital laboratory could even be a possibility, but, amazingly, less than a decade after completion of the Human Genome Project, it became very much a reality!

Today, multiple human genomes can be sequenced by a single laboratory in just a few days at a technical cost of about $1,000 per genome. Of course, this cannot be done in just any laboratory. It requires investment in a state-of-the-art facility, the presence of special sequencing equipment, availability of significant computational resources, and hiring of specialized personnel to manage the entire process.

We are fortunate that Children's Mercy Kansas City was at the forefront of this movement, investing in the Genomic Medicine Center a decade ago. We are even more fortunate that the hospital has supported it strongly ever since. The Genomic Medicine Center is a world-wide leader in genomic medicine and has received international recognition for providing whole genome sequencing and clinically relevant results for critically ill infants, all within a few days. The Genomic Medicine Center is now bringing genomic medicine to every cancer patient at Children's Mercy, both clinically and through its research initiatives via the Tumor Bank and the Children's Mercy Research Institute Biorepository.

By utilizing the Genomic Medicine Center's existing infrastructure, we are easily able to perform whole genome sequencing for pediatric cancer patients in-house. For such patients, this includes sequencing the genome of both their cancer cells (to look for changes that drive tumors), as well as their healthy cells (to look for inherited changes that increase the risk of developing cancer).

Testing patients in-house allows us to not only offer this dual sequencing but also to do it via the most comprehensive method of sequencing available, whole genome. Many other laboratories offer sequencing of a few cancer-related genes and sequence only the tumor sample by itself. Such testing makes it difficult to identify which genetic changes are truly specific to a tumor versus those that may be inherited and increase risk of cancer for a patient and their family.

Making this distinction is critically important since: 1) tumor-specific genetic changes can be used to match tumors to precision therapies that attack only cancer cells and spare healthy cells (unlike traditional chemotherapy which affects both cell types) and 2) inherited changes that confer predisposition to cancer are necessary to examine in the pediatric setting because these mutations are found in 5-15% of children with disease, much higher than what was previously believed.

Finally, in-house sequencing allows us to have much more control over the quality of sequence data that is generated. This is essential for clinical sequencing where strict quality control and validity of results is the highest priority. In-house sequencing also supports local researchers because they can drop by, ask questions, and see the process first hand. In turn, this fosters additional stronger research collaborations. Overall, in the end, doing local sequencing brings us closer to the goal of precision medicine: "finding the right drug for the right patient at the right time," as informed by genetic results.

IDENTIFYING THE DIFFERENCES IN CANCER CELLS

Other than identical twins, all individuals have a unique genetic makeup. This is due to certain sequence differences in their DNA, specific to them, that are carried by all of their body's cells. Cancer cells, in addition to carrying all of the genetic variants seen in normal cells, have additional genetic changes that differentiate them from normal cells and which help define such cells as cancerous.

In clinical cancer sequencing, it is critical to distinguish genetic variants carried only by cancer cells ("somatic" changes) versus those carried by all cells ("germline" changes) because somatic changes can help guide precision therapy. In other words, this information can help find a drug or agent that specifically targets the genetic differences found in cancer cells, and ultimately does not affect normal cells.

Most clinical cancer sequencing assays looking for these changes in a cancer's genetic makeup test only the tumor sample, making it difficult to differentiate "somatic" changes from "germline" ones. We recently questioned how many genetic variants found by clinical cancer sequencing tests done at outside laboratories for pediatric cancer patients that had been seen at Children's Mercy actually found changes that were truly tumor-specific (somatic) versus in all cells (germline).

We did this by resequencing both tissue from the patient's tumor, along with normal cells found in the same patient's peripheral blood. We found that only 11 of the 41 variants (27%) found by outside laboratories were actually somatic, which would imply that only certain therapies would truly be precise and specifically target tumor cells. This study's importance was recognized when it was one of a few selected to be presented as a poster and as a talk at a large international scientific meeting held in Washington, D.C. by the Association for Molecular Pathology.

Erin Guest, MD
Midhat Farooqi, MD, PhD

CANCER GENOMICS RESEARCH

LEUKEMIA/LYMPHOMA

Acute lymphoblastic leukemia in infants

Sadly, only about one in three infants less than 1 year of age with acute lymphoblastic leukemia (ALL) survives despite intensive therapy. Erin Guest, MD, is leading an international clinical trial with the Children's Oncology Group (COG) to test the safety of the addition of azacitidine, an epigenetic demethylating agent, to chemotherapy for infants with ALL.

Blood and bone marrow samples from infants enrolled in this trial and in a prior COG trial for infants with ALL are being studied in the Genomic Medicine Center at Children's Mercy. In collaboration with researchers at Johns Hopkins University, we are performing in-depth sequencing analyses to discover biomarkers that better predict response to treatment and to determine the biological basis of resistance to chemotherapy after relapse. We presented our early findings in abstract form at the annual meeting of the American Society of Hematology.

Measuring response to epigenetic treatment

Epigenetic medicines are designed to alter the expression of genes by turning them off or on, thereby leading to cancer cell death and/or vulnerability to chemotherapy. The Children's Mercy Cancer Center partnered with the Therapeutic Advances in Childhood Leukemia & Lymphoma consortium to study samples from patients enrolled in a trial for treatment of relapsed leukemia. Samples were sequenced to determine the pharmacodynamic changes in DNA methylation and histone acetylation. The results show evidence of potent anti-leukemia effects by the epigenetic drugs. This work has been submitted for publication in conjunction with the overall trial results.

Single-cell sequencing in infantile and T-cell acute lymphoblastic leukemia/lymphoma

Not all cancer cells are alike. One patient's cancer can contain multiple distinct sub-clones. This makes cancer difficult to target with a single approach because some sub-clones may be resistant to certain therapies. At Children's Mercy, we are utilizing powerful

sequencing strategies that analyze individual cancer cells for changes in gene expression. We are taking this innovative approach with all of our patients with newly diagnosed or relapsed leukemia, and we are particularly focused on determining the sub-clones that explain resistance to treatment in infant ALL and childhood T-cell ALL, because both of these cancers are extremely difficult to cure after relapse.

CENTRAL NERVOUS SYSTEM TUMORS

Genomics of high-grade gliomas of the brain

Brain tumors are the most common solid tumor in children, as well as the most common cause of solid tumor-related deaths in childhood. Why they arise and what predisposes them to be "high-grade" and aggressive versus "low-grade" and more benign in nature is not completely understood. This study is comprehensively analyzing genomic changes found in high-grade glial brain tumors, such as copy number changes, chromosomal abnormalities, expression differences and genetic sequence alterations, and correlating such findings to specific patient characteristics, therapy responses and outcomes. This project aims to reveal novel genomic changes in these tumors, as well as novel associations between genetic alterations and specific clinical findings.

SOLID TUMORS

Genetic and epigenetic targets for treatment in solid tumors

Children with solid tumors that do not respond to standard therapy or relapse despite standard therapy, have a very poor prognosis. This is largely due to the limited number of treatment options available. This project supports a national clinical trial where children with relapsed/refractory solid tumors, including neuroblastoma, osteosarcoma and Wilms tumor, among others, are treated with a novel immune therapy that works to help control disease. Unfortunately, not all patients respond to this treatment. This study aims to comprehensively profile the genomes of tumors being studied by the clinical trial, both before and after immune therapy, to look for specific genetic markers that correlate with therapeutic response. The project has a special emphasis on detecting certain chemical changes to DNA (epigenetic modifications) that are known to play a key role in tumor response to immune-based cellular therapies. Overall, the study's goal is to use genetic information to guide and support use of a novel targeted therapy for pediatric patients with relapsed/refractory solid tumors.

Exosomes in Ewing sarcoma

Exosomes are tiny particles that are released by tumor cells, which contain components of the original cancerous cell. Because they can be found in blood, the hope is that exosomes can be used to monitor tumor burden and serve as a valuable biomarker for disease. This study has found that exosomes produced by Ewing sarcoma cells are detectable in the blood of patients with disease. It is currently working to develop this finding into a clinically-available blood test that would be used to detect the presence of Ewing sarcoma cells earlier than an MRI or other radiology scans.

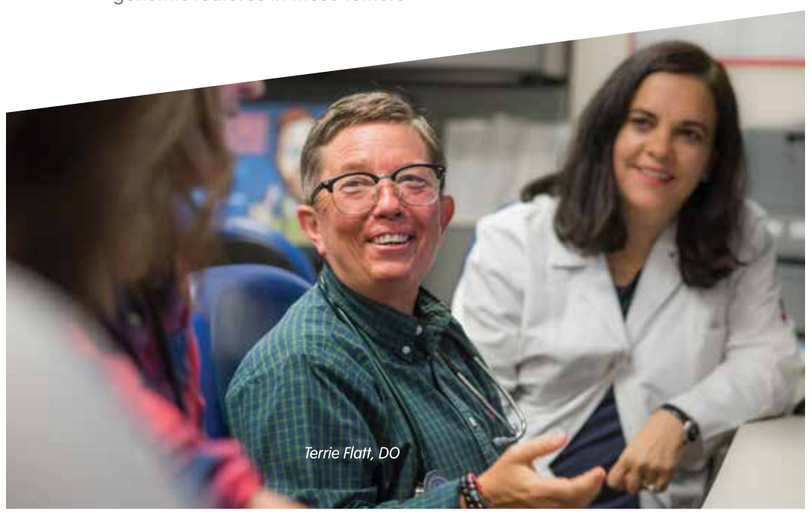
Rhabdomyosarcoma

Rhabdomyosarcoma is the most common type of soft tissue sarcoma found in children with about 400 to 500 new cases occurring in the United States each year. More than half of rhabdomyosarcomas are diagnosed in children younger than 10 years old. What causes these tumors to arise is not clearly known. This study is looking for pathological changes, chromosomal abnormalities, expression differences and genetic sequence alterations in these tumors, correlating them to specific patient characteristics, therapy responses and outcomes. The goal of this project is to reveal novel genomic features in these tumors that can help explain disease biology and also be specifically tied to relevant clinical findings.

ETHNICITY AND GENOMICS

Hispanic and Native American children diagnosed with Acute Lymphoblastic Leukemia (ALL) in the United States have the poorest outcome when compared to whites, Asians and African-Americans. Some studies have demonstrated that the higher the percentage of Native American (Pre-Columbian) ancestry among children with ALL portends worse overall and event-free survival as well as a less favorable response to end of induction therapy. The term Hispanic, as it is used in the United States, is very broad and is not specific or descriptive of actual ethnic and racial admixture. The term does not account for individual ancestry such as Mexican, Guatemalan, Peruvian, Puerto Rican ancestry or the various combinations of ancestry. As a result, knowledge regarding which Hispanic groups have the worse outcome remains unknown. The term Hispanic as used in the United States does not account for the vast diversity of European and Pre-Columbian ancestry. The vast majority of the population is an admixture of European and Native indigenous populations (Pre-Columbian ancestry) which is referred to as the Mestizo. Mestizo genetic ancestry is associated with a higher incidence and poorer outcome of ALL. Analyzing ethnic profiles of children diagnosed with ALL may be crucial to improving outcome among Hispanics in Mexico and the United States.

Terrie Flatt, DO, along with collaborating institutions in Mexico, is conducting research studies to look into genetic links between ethnicity and how children respond to treatment. By partnering with international hospitals, Dr. Flatt has established both research and clinical links that provide benefit to all.



Terrie Flatt, DO

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Children's Mercy KANSAS CITY

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