Molecular Genetic Analysis of Pediatric Oncology patients who have a family history and/or medical history that suggest a germline mutation using Comprehensive Genetic Sequencing

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BACKGROUND

Although cancer in children is rare, it is the leading cause of death by disease past infancy among children in the United States [Ward et al, 2014]. Historically, the proportion of pediatric cancers associated with an inheritable genetic mutation was generally unknown. With recent advancements in genomic sequencing and analysis, improved understanding of this critical risk factor has been achieved. Not only has the repertoire of known mutations increased, but also the estimate of pediatric cancer incidence related to an inheritable genetic syndrome [Kuhlen et al, 2015].

A recent report published by the St. Jude-Washington University Pediatric Cancer Genome Project showed that 4-30% of all pediatric oncology cases are associated with germline mutations. They further reported that 'pathologic' or 'likely pathologic' germline variants across 31 known cancer predisposition genes were detected in 8% of these patients; 16% of patients with solid tumors, 8.6% with brain tumors, and 3.9% with leukemia [Walsh et al, 2014]. Likewise, a recent study evaluating pediatric cancer survivors at a follow-up clinic determined that 29% of patients were considered appropriate for genetic follow-up/referral, with 'family history of cancer' being the most commonly cited reason at 61% of this group [Knapke et al, 2012].

Evidence abounds for the role of germline mutations in development of some pediatric cancers. While significant realization has been brought about as of late, the field still calls for continued research and understanding. Inheritable syndromes that lend susceptibility to cancer development may in fact be far more prevalent than we currently recognize.

The goal of this study is to use comprehensive genetic sequencing to identify previously unrecognized germline mutations that convey susceptibility to cancer. We will sequence a trio of patient, parent and relative (those who also developed early-onset cancer), then perform a linkage analysis between these individuals. This will allow us to isolate germline mutations that segregate with the cancer. The realization of such genes, and examination of their function and activity, will

enable deeper insight into the pathogenesis of hereditary cancer. This understanding will ultimately allow for improved screening, counseling, treatment, and clinical decision making.

STUDY OBJECTIVE

To identify new candidate genes in the setting of germline mutations that could account for increased cancer predisposition within a family. Such genes and mutations would be outside of those already known, such as *TP53*. Our aim is to study a small, well-defined group of Pediatric Oncology patients and their families, whose family history is strongly suggestive of a germline mutation.

STUDY METHODS

General design

Through comprehensive genetic sequencing, we will look for novel mutations and pathogenic variants in cancer-associated genes in the germline of patients and family members. We will then perform linkage analysis to assess if these mutations segregate with the cancers within each family.

Study subjects and recruitment

The Pediatric Oncology group at CUMC will be queried to identify patients and families who meet the inclusion criteria listed below. The primary oncologist will be consulted prior to contacting families to verify that they may be approached. Either the primary oncologist or the study team will reach out initially to the family, a decision at the discretion of the primary oncologist.

After initial outreach, participants will be recruited and counseled by Pediatrics Hematology-Oncology staff, Medical Genetics and Genetic Counselors during a face to face visit or by phone. The families will be invited to undergo sequencing involving at least 3 key members: patient, parents and relatives (if said relative is not one of the parents).

The target accrual for this study is 20 subjects.

Inclusion criteria:

- Pediatric patient (age 0-30 years) diagnosed with a malignant tumor who also has one of the following:
  - A first-degree relative with cancer diagnosed before age 40.
  - More than one second-degree relative with cancer diagnosed before age 40.
  - Any positive family history of pediatric cancer outside of patient.

-or-

- Any clinical presentation that raises concern for genetic predisposition to develop cancer such as atypical age of diagnosis, tumor type, tumor behavior, response to treatment or a second tumor that is not fully explained by previous therapies.

Consent and enrollment

Patients who meet the inclusion criteria for the study will be enrolled after consent is obtained. All study subjects will be assigned a unique identification number, which will allow linkage of clinically relevant information with the sequencing data generated from the study.

Patients who have had genetic testing performed through the PIPseq program will also be targeted for enrollment. Only patients and family members who have undergone whole exome sequencing, and who have consented to leftover samples and sequencing data being used for research purposes will be included in this study.

Sample and data collection

In order to perform genetic testing, a sample will be obtained from the patient (either blood or tissue for the purposes of this protocol). A serum sample will also be obtained from the parent and relative, if appropriate. For serum samples, we will collect 4 cc of blood for any participant >5 years of age and 2 cc of blood for children <5 years of age.

Although samples from parents and relatives will be sequenced, they are not considered research subjects because their samples will serve as references during the sequencing process. This comparison is essential in order to differentiate between heritable and de novo genetic mutations.

Clinical data (age, diagnosis, prior therapy with response and pertinent family history) will be collected from institutional medical records.

Molecular pathology procedures

Only surplus specimens or biopsies obtained at the time of routine, clinically necessary diagnostic or therapeutic procedures will be used. Specimens will be only be utilized after all assurances are made that usage for the purposes of this study will not deplete the tissue required for diagnostic studies or clinical care.

Genome sequencing, analysis of all datasets and interpretation of results will be performed by the Columbia University Laboratory for Personalized Genomic Medicine.

No statistical measures will be used in the study.

Reporting results

Patients and their parents will be given the opportunity to opt in or out of receiving results for subject-specific data only. Results from sequencing of parents' and/or relatives' genomes will not be returned. If parents and/or relatives desire further information or testing based on the knowledge of a subject's results, they will be counseled separately to obtain genetic testing.

Through the consent process, patients and their parents will be able to opt in or out of receiving various elements of results. This includes receiving all results, only cancer-associated results (omit secondary findings) or no results. Similarly, patients and their parents will be able to opt in or out of transmitting various elements of results to the EMR. This also includes transmitting all results, only cancer-associated results (omit secondary findings) or no results.

Any positive findings will be reviewed by Medical Genetics. Return of results will be done in conjunction with Medical Genetics and Genetic Counselors.

RETENTION

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). Clinical records, including consent forms that document clinical intervention, clinical diagnostic procedures or research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy.

CONFIDENTIALITY OF STUDY DATA

Names, Medical Record Numbers, and any characteristics or codes that could identify an individual will be removed. Subject confidentiality will be ensured through de-identification procedures. This means that subjects will be assigned a unique identifier. All analyses conducted for this project will be performed on data that has no subject identifiers. Information about patients who have been sequenced will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Only the principle investigator and study team will be allowed to access identified study data. To safeguard the PHI, any PHI collected, and/or subsequent data generated from the study results, will be stored on the CUMC IT approved MC Domain network drive P (Network Storage System ID 3959). If data is stored or transported on an end-point device, the end-point device will be encrypted and protected with a strong password.

Any publication of information related to patient-related material will not include any identifying data.

PRIVACY PROTECTION

Collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is collected. Any information collected during this study that can identify a subject by name will be kept as confidential as possible. However, complete confidentiality cannot be promised. Despite all of our efforts, unanticipated problems, such as a stolen computer may occur, although it is highly unlikely. Research findings will not be part of a medical record and will not be released to anyone without

the consent of the participant. Access to names and personal identifying information will be strictly controlled by the principal investigator. Genetic results will only be released to the physician caring for the participant upon the participant's request. Participants are protected against genetic discrimination in employment and health insurance by the Genetic Information Non-Discrimination Act (GINA).

LOCATION OF STUDY

All samples will be obtained in the clinical care areas of the New York Presbyterian Hospital. Investigations using the materials will be conducted in the laboratories of the Department Pediatrics investigators or PGM.

COMPENSATION AND COSTS TO SUBJECTS

No compensation will be offered to subjects participating in this study, but they will incur no additional costs based on participation in this study.

RISKS AND BENEFITS

Loss of confidentiality is a potential risk of this study. Strict guidelines will be maintained to protect patient confidentiality. Furthermore, loss of confidentiality will be addressed by assigning each patient a unique ID code. This unique ID code and corresponding medical record numbers will be stored on a password protected server (Network Storage System ID 3959). Computers used to access the protected server are stored in locked rooms at CUMC. Any publication of information related to patient related material will not include any identifying data.

There exists the risk of genetic discrimination. Participants will be advised that results will not be reported to any medical insurance, life insurance, or other insurance forms.

There is small risk of pain during the blood draw and, rarely, a risk of bruising at the site of venipuncture.

This research is not designed to help the patient directly, although it may. In some instances, we

may be able to provide information about specific genetic alterations responsible for the participant's familial cancers, predict future risk or recurrence if a genetic etiology is identified, change management with the goal of maximizing outcomes and/or decreasing adverse effects, and eventually design a tumor screening strategy.

Benefits to society may include further understanding of the genetic underpinnings of several familial cancers and the ability of comprehensive genetic sequencing to diagnose them. This understanding can lead to significant improvements in the diagnosis, prevention, and treatment of several familial cancers suspected to be genetic.

References:

1. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics. *A Cancer Journal for Clinicians*. 2014; 64(2):83-103.

2. Kuhlen M, Borkhardt A. Cancer susceptibility syndromes in children in the area of broad clinical use of massive parallel sequencing. *European Journal of Pediatrics.* 2015; 174(8): 987-997.

3. Walsh M, Wu G, Edmonson M, Gruber TA, Easton J, Yergeau D, Vadodaria B, et al. Incidence of germline mutations in cancer-predisposition genes in children with hematologic malignancies: a report from the pediatric cancer genome project, 2014.

4. Knapke S, Nagarajan R, Correll J, Kent D, Burns K (2012) Hereditary cancer risk assessment in a pediatric oncology follow-up clinic. Pediatr Blood Cancer 58:85–89.