Cancer Predisposition Program Genetic Testing: Analysis of Genetic Testing Outcomes and Procedures

by

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The multi-gene panel is the most utilized genetic test for germline cancer predisposition and has been found to lead to greater changes in clinical management than single-gene genetic testing. These panels are dynamic, varying in size, testing capabilities, and consistency. A retrospective chart review was conducted from the UPMC Children's Hospital of Pittsburgh, Cancer Predisposition Program to explore how these multi-gene panels are being utilized in a pediatric and young adult clinical setting to determine if changes in the size and scope of these panels have impacted clinical care. A review of the corresponding results of multiple rounds of genetic tests was also obtained. Our findings indicate a trend of multi-gene panels growing larger over time with the addition of more genes and found that the yield of actionable test results improved over multiple rounds of testing. This shapes a discussion about the potential to retest individuals who have had previously negative or uninformative testing for panels that have changed in size over time. A lack of homogeneity between genetic testing labs and panels over time was appreciated by this study which signifies a need to discuss the equity of genetic testing panels in the clinical setting within the scope of public health.

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Preface

I would like to thank my committee chair, Dr. Julia Meade and my committee members, Dr. Andrea Durst and Elena Kessler, MS, CGC for their continued mentorship and guidance not only through this project but also in my genetic counseling training.

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1.0 Introduction

There are gaps in knowledge and clinical guidance for pediatric and young adult patients in the cancer predisposition field. In the adult oncology patient population, there is uncertainty about who to offer genetic testing to and how to manage patients with non-conclusive genetic test results (Culver et al., 2013), (Chern et al., 2019). As genetic testing capabilities expand, genetic testing labs are offering a variety of multi-gene panels for common genetic indications, however these panels often bear few similarities (Roloff et al., 2021). More information about genetic testing guidelines is particularly important as part of the management of treating children and young adults who have had a personal history of cancer.

The main goal of this project is to analyze the genetic testing information from the UPMC Children's Hospital of Pittsburgh Cancer Predisposition Program to help inform this body of knowledge about testing and guidance for pediatric and young adult populations. Through a retrospective chart review of genetic tests and test outcomes for patients in the Cancer Predisposition Program, we will be able to compare the test outcomes of panels over time as well as the clinical utility of multiple stages of testing.

This information can be helpful in a clinic utilization management setting in order to identify the testing that yields the most changes to patient management and thus patient health outcomes. This can also help inform medical providers or genetic counselors who make decisions about what type of testing may be most useful for their patients. Genetic counselors may use this information to provide better anticipatory guidance about testing strategies. In addition, identification of variants on newer panels that may have been missed on outdated testing can

provide patients with a diagnosis and variant to identify in other family members who may be at an increased risk for cancer.

Our study aims to identify patterns in genetic testing outcomes:

1. Analyze how the cancer genetics panels and tests have changed over time.
    a. How has the size of the panels and tests changed over time?
    b. What type of results did these tests yield (positive, negative, or variant of uncertain significance)?
    c. How have the results from these tests changed in conjunction with the size of the panels?
2. Analyze test results of patients who have had multiple rounds of genetic tests completed in a stepwise manner.
    a. How do the end results of multiple rounds and steps of genetic testing compare to the results from primary genetic testing efforts?

2.0 Manuscript

2.1 Background

Only within the past few decades have we begun to understand the genetic basis predisposing some people to certain types of cancers. The Human Genome Project accelerated the research capabilities in the cancer genetics field, and more cancer susceptibility genes have been identified and continue to be discovered every year (Wheeler & Wang, 2013). There are specific cancer predisposition syndromes that disproportionately affect children and young adults (i.e., Rhabdoid Tumor Predisposition Syndrome), (Biswas et al., 2016), (Nesvick et al., 2018), (Eaton et al., 2010), (Kratz et al., 2021). Studies have shown that approximately 8.5 - 10% of childhood cancers are due to an underlying genetic predisposition (Mody et al., 2015), (Zhang et al., 2015). For tumors of the central nervous system, this number is closer to 15% but can be as high as 50% for specific tumor types, such as choroid plexus carcinoma, which is a brain cancer that is associated with Li Fraumeni Syndrome (Patil et al., 2022). Testing children with cancer for genetic predisposition is generally well-accepted and of great interest to families. However, there are many children who could benefit from genetic testing but are not referred to the proper care team to discuss or facilitate genetic testing (Brozou et al., 2018), (D'Aquila et al., 2023). While we understand that children with cancer are at risk of having an underlying cancer predisposition syndrome, researchers have reported a lack of literature about clinical guidelines and surveillance strategies for cancer prevention in the pediatric population (Kratz et al., 2021). Screening recommendations for common pediatric cancer predisposition syndromes, such as leukemia predisposition syndromes, Li Fraumeni, and neural tumor syndromes, were reviewed by the

Pediatric Cancer Working Group of the American Association for Cancer Research (AACR) in 2017 (Brodeur et al., 2017). This group created recommendations for cancer surveillance when a patient's risk of developing cancer by the age of 20 years is 5% or greater as well as provided generalizable recommendations.

There are several options for germline genetic testing in the pediatric oncology clinical setting. Initially, genetic testing for germline mutations was limited to single gene panels. Early research of cancer predisposition genes identified associations between retinoblastoma and structural variants of the RB genes (Dulbecco, 1986). These discoveries along with the Human Genome Project helped advance cancer susceptibility research (Wheeler & Wang, 2013). In 1994, BRCA1 was discovered and linked to Hereditary Breast and Ovarian Cancer susceptibility, followed by BRCA2 in 1995 (Miki et al., 1994), (Wooster et al., 1995). As the knowledge base of cancer predisposition genes and genetic testing methods has evolved, the current most utilized genetic testing method for germline cancer predisposition syndromes is now the multi-gene panel (Crawford et al., 2017). Testing multiple genes helps to prevent pathogenic variants from being missed by single-gene testing and has been found to lead to greater changes in clinical management (Casasanta et al., 2018), (Desmond et al., 2015). These panels are dynamic and can vary in size and testing capabilities. One study found pathogenic variants associated with cancer predisposition syndromes in adult women that had a prior history of negative BRCA1/ BRCA2 sequencing (Crawford et al., 2017). These variants were identified by subsequent multi-gene panel test, and now it is recommended that people with a history of prior negative testing with only BRCA1/2 with HBOC indications be evaluated for a multi-gene panel (Crawford et al., 2017). Roloff et al. (2021) examined the differences and similarities between eight commercially available panel tests that were designed for hereditary hematopoietic malignancies (HHMs). Of the 82 genes associated

with HHMs, only 4 genes were consistently covered by the panels across all 8 of the labs they examined. Inconsistencies between genetic testing, labs, and lack of guidelines for providers who order genetic testing panels for pediatric and AYA populations confusing and difficult (Roloff et al., 2021).

The Cancer Predisposition Program at the UPMC Children's Hospital of Pittsburgh provides clinical care for patients with suspected or known cancer predisposition conditions and their families. This study took a phenotype- first view of the genetic test results as they were from a subset of patients from this program that had a clinical suspicion for a cancer predisposition syndrome. This study explores the results from a variety of multi-gene panels and other genetic tests that were ordered in this clinical setting and how these individual panels have changed over time. This study also explores the yield of variant results (positive, negative, variants of uncertain significance) for patients that underwent multiple rounds of genetic testing.

2.2 Methods

The study participants are patients of the UPMC Children's Hospital of Pittsburgh, Cancer Predisposition Program and were seen between November 1st, 2018 and November 30th, 2022. This study was approved by the University of Pittsburgh Institutional Review Board (Appendix A). Patients were included in this study if they were within a pediatric and young adult population age range of 39 years of age and below and evaluated in the clinic for a personal history of cancer, tumors, or clinical features suspicious for a cancer predisposition syndrome. Patients were not included in the study if they had Down Syndrome, a molecularly confirmed cancer predisposition

syndrome, or if targeted variant testing was being completed to confirm a cancer predisposition syndrome that had been previously identified in other family members.

A retrospective chart review was conducted for a total of 162 patients and all data collected was recorded in a Microsoft Excel spreadsheet and Excel was also used for descriptive statistical analysis. Demographic information about the sex and age of these patients at the time of genetic testing and at the time of their diagnosis was extracted. Diagnostic information such as clinical indication and tumor pathology was reviewed to categorize the patients' clinical features into four groups: CNS tumor, liquid tumor, solid tumor, or other. Some patients had tumors that fit into more than one category, so they were categorized by the characteristics of their primary tumor diagnosis.

Scanned records were reviewed to collect information about the genetic tests that were ordered. Names of the genetic test completed with the corresponding testing lab, dates of collection, dates of reporting, age at testing, panel size, and genetic testing results were obtained. Some genetic testing labs allowed clinicians to add additional genes for sequencing or deletion and duplication studies to their panels. For patients with orders for add-on genes, these genes were classified as an additional, or a second round of genetic testing separate from the primary panel. The names of the labs were removed and designated as Labs A- H for the purposes of this study, and the number of times genetic testing was ordered for a single patient was noted. The number of genes covered by panels that were ordered in the clinic between 2018 and 2022 was compared with the current number of genes covered by the labs in the current year, 2023, which were posted on the lab's website.

Clinic notes and family pedigrees were also examined to collect information on family history of cancer. Patients were noted to have a family history of cancer if they reported at least

one family member within three degrees of relation to the proband that had any diagnosis of cancer. Patients who did not have a family history of cancer documented were noted as not having a family history of cancer.

All genetic testing results were reviewed to determine whether the findings were on- target with the diagnosis and if they were clinically actionable. Variants were on-target if they were found within a gene that is known to be associated with the patient's clinical presentation regardless of whether that variant was classified as pathogenic, likely pathogenic, or a variant of uncertain significance. Variants were classified as off-target if they were found in genes that were unrelated to the patient's clinical presentation. Whether a variant found was relevant to the clinical presentation of a patient was determined either through the genetic test report or web based clinical tools such as Online Mendelian Inheritance in Man (OMIM®) or ClinVar. Clinic notes were also reviewed to determine if the results from the testing were clinically actionable. Results were classified as actionable if they led to changes in the patient's clinical management, such as increased surveillance, referral to another provider, or led to recommendations for familial testing. The results were classified as not- actionable if they did not change or inform the patient's clinical care.

2.3 Results

2.3.1 Patient Demographics and Disease Categorization

The patients in this study were all diagnosed with cancer and have all had genetic testing through the Cancer Predisposition Program at the UPMC Children's Hospital of Pittsburgh.

Demographic and genetic testing information was collected for the 162 total patients that comprised this study (Table 1). Of the total 162 patients, 75 were female (46.3%) and 87 were male (53.7%). Of the disease classification, 70 patients were categorized as having a CNS Tumor (43.2%) of which some prevalent indications were glioma and medulloblastoma. There were 45 patients categorized as having a “Solid Tumor” (27.8%) with sarcoma, carcinoma, and kidney tumors (I.e. Wilms tumors) among the most prevalent indications. Forty-four patients were categorized as having a “Liquid Tumor” (27.2 %), with acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) as two of the most prevalent indications. The three patients classified as “Other Tumor” (1.9%) were diagnosed with Langerhans cell histiocytosis (LCH), odontogenic keratocyst, and an adrenocortical adenoma.

Table 1: Patient Demographics and Disease Categorization

	Total (N= 162)
Age at Diagnosis (years)	
Mean	8.66
Median [min, max]	8 [0, 24]
Age at Testing (years)	
Mean	11.21
Median [min, max]	12 [0, 38]
Sex	
Female	75 (46.3%)
Male	87 (53.7%)
Family History of Cancer	
Positive History	135 (83.3%)
Negative History	27 (16.7%)
Disease Categorization	
CNS Tumor	70 (43.2%)
Solid Tumor	45 (27.8%)
Liquid Tumor	44 (27.2 %)
Other Tumor	3 (1.9%)

The average age of the patient's cancer diagnosis was 8.66 years with the youngest age of diagnosis recorded as 0 years, in an infant with a prenatal suspicion for a tumor, and the oldest age of diagnosis was 24 years with a median age of 8 years (Table 1). The average age at the time of testing was 11.21 years with the youngest age at testing as 0 years and the oldest age at testing of 38 years with a median age of 12 years. Of the 162 patients, 135 patients reported having a family history of cancer (83.33%) and 27 reported that they had no family history of cancer (16.67%).

2.3.2 Genetic Testing Ordered

All 162 patients underwent an initial round of genetic testing, a secondary round of testing was ordered on 39 occasions, and a third round of testing was ordered on 7 occasions **(Table 2).** This translates to 123 patients who had only one genetic test performed (75.9%), 32 patients who had two genetic tests performed (19.8%), and 7 patients who had three genetic tests performed (4.3%).

Table 2: Results from Multiple Rounds of Genetic Testing

Round of Testing	Pathogenic/ Likely Pathogenic Variants	Variants of Uncertain Significance
1st Round	38 (47.5%)	42 (52.5%)
2nd Round	6 (60.0%)	4 (40.0%)
3rd Round	2 (50.0%)	2 (50.0%)
Total Variants Identified	46	48

A total of 210 genetic tests were ordered during these rounds of testing. There were 6 SNP microarray tests ordered (2.9%), 6 whole exome sequencing tests ordered (2.9%), and 29 single gene tests ordered (13.8%). Multi-gene panels were the most frequently ordered test; there were 34 unique panel tests that were ordered a total of 146 times (69.5%) (Table 2). This excludes the number of custom panel tests which were ordered a total of 23 times (10.9%). Of the multi-gene panels, there were eight labs from which the tests were ordered designated as Labs A-H.

Table 3: Multi-Gene Panels (Excluding Custom Panels) and Frequency Ordered

	Total Panel Orders: 146
Lab A: Comprehensive Hereditary Cancer Panel Plus	26
Lab B: Multi-Cancer Panel	19
Lab A: Hereditary Leukemia Panel Plus	19
Lab B: Nervous System/ Brain Cancer Panel	12
Lab B: Pediatric Nervous System/ Brain Tumors Panel	9
Lab B: Rhabdoid Tumor Predisposition Syndrome Panel	7
Lab B: Pediatric Solid Tumors Panel	6
Lab B: Sarcoma Panel	3
Lab B: RASopathies and Noonan Spectrum Disorders Panel	3
Lab A: Bone Marrow Failure Syndrome Panel (Hematology) Plus	3
Lab B: RASopathies Comprehensive Panel	3
Lab B: Common Hereditary Cancers Panel	3
Lab B: Melanoma Panel	3
Lab A: Primary Immunodeficiency Panel Plus	3
Lab B: Hereditary Paraganglioma-Pheochromocytoma Panel	2
Lab G: Neuroblastoma panel	2
Lab C: Methylation and High-Resolution Copy Number Analysis for Chromosome 11p15.5; reflex to CDKN1C seq	2
Lab B: Basal Cell Nevus Syndrome Panel	2
Lab A: Hereditary Endocrine Cancer Panel Plus	2
Lab B: Renal/ Urinary Tract Cancers Panel	2
Lab A: Comprehensive Hematology and Hereditary Cancer Panel Plus	2
Lab B: Familial Neuroblastoma Panel	1
Lab D: Meningiomatosis Panel by NGS	1
Lab B: Fanconi Anemia Panel	1
Lab A: Macrocephaly/ Overgrowth Syndrome Panel (Malformations) Plus	1
Lab B: Constitutional Mismatch Repair- Deficiency Panel	1
Lab E: Pediatric Tumor Panel	1
Lab A: Noonan Syndrome Panel Plus	1
Lab F: Comprehensive Lymphoma and Immunodeficiency Panel	1
Lab B: Schwannomatosis Panel	1
Lab E: Comprehensive Neurofibromatosis Panel	1
Lab B: Primary Immunodeficiency Panel	1
Lab G: Wilms Tumor Panel	1
Lab H: Wilms Tumor Panel	1

Total Unique Panels: 34

2.3.3 Most Frequently Ordered Panels

The four most frequently ordered panels were the Lab A: Comprehensive Hereditary Cancer Panel Plus (26 orders), the Lab B: Multi-Cancer Panel (19 orders), the Lab A: Hereditary Leukemia Panel Plus (19 orders), and the Lab B: Nervous System/ Brain Cancer Panel (12 orders). These top four most frequently ordered panels in combination make up 76 orders and account for about half of all panel orders (52.0%) (Table 3).

For Lab A: Comprehensive Hereditary Cancer Panel Plus, the average number of genes covered by the panel over the 26 times it was ordered was 156 genes. The most common indication for using this panel was for a liquid tumor (11 patients), followed by 10 patients with a CNS tumor, and five patients with a solid tumor (Figure 1). It is important to note that some test reports identified and reported more than one variant. This panel identified seven pathogenic/ likely pathogenic variants (Figure 2) in the following genes: PTEN, PALB2, RECQL4, ATM, ERCC3, and CHEK2. There were 13 negative reports and 12 variants of uncertain significance identified (Figure 2).

Figure 1: Testing Indications for Frequently Ordered Panels

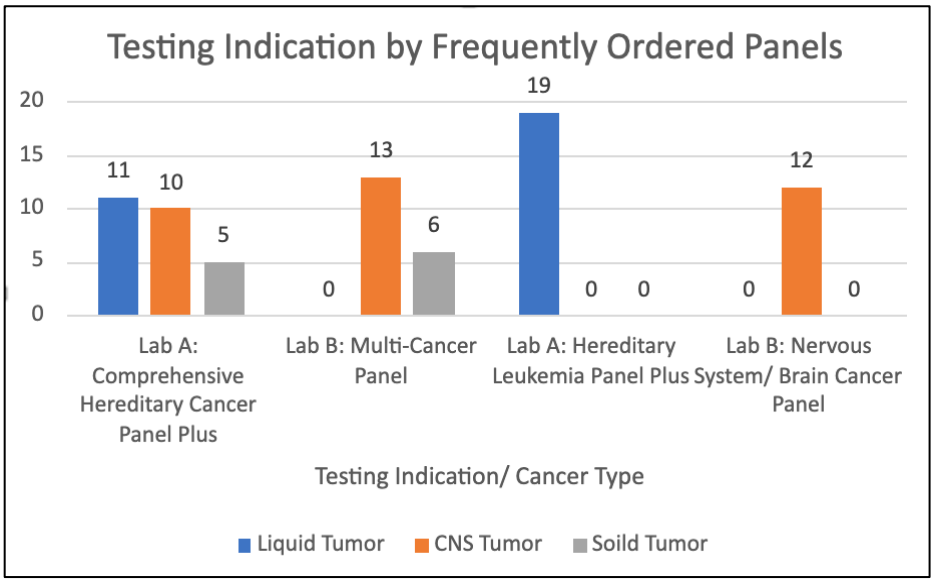
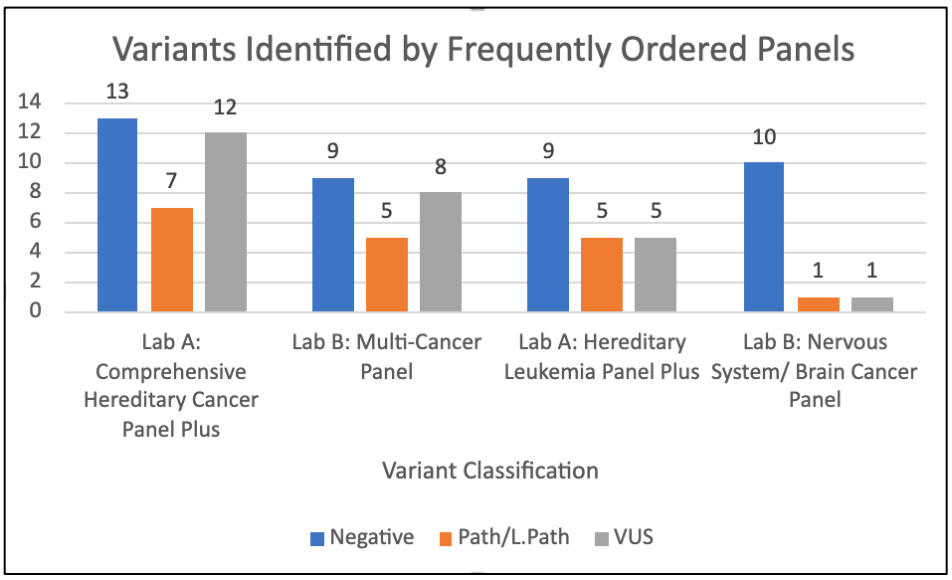


Figure 2: Frequently Ordered Panels and the Variants they Identified



The size of Lab Bs: Multi-Cancer Panel remained unchanged during the 19 times it was ordered and covered 84 genes. The most common indication for using this panel was for a CNS tumor (13 patients) followed by 6 patients with a solid tumor (Figure 1). This panel identified five

pathogenic/ likely pathogenic variants (Figure 2) in the following genes: ATM, MUTYH, PTEN, SMARCA4, and SDHA. There were nine negative reports and eight variants of uncertain significance identified (Figure 2).

Lab As: Hereditary Leukemia Panel Plus, was ordered a total of 19 times (Table 3). The panel covered 41 genes all except one time it was ordered when it covered 42 genes. All 19 orders were for patients with a liquid tumors (Figure 1). This panel identified five pathogenic/ likely pathogenic variants in the following genes: BRCA2, ETV6, GATA2, and PMS2. There were nine negative reports and five variants of uncertain significance identified (Figure 2).

Lab Bs: Nervous System/ Brain Cancer Panel was ordered a total of 12 times (Table 3). The average number of genes covered by the panel over the times it was ordered was 29 genes. All 12 orders were for patients with a CNS (Figure 1). This panel identified pathogenic/ likely pathogenic variants in one gene (TP53). There were ten negative reports and one variant of uncertain significance identified (Figure 2).

2.3.4 Multi-Gene Panel Size Change Over Time

Of the 34 unique multi-gene panels that were ordered, twelve of these panels were found to have changed in size over the six-year study time period (Table 4). Two of the panels, the Lab B: RASopathies Comprehensive Panel and the Lab H: Wilms Tumor Panel, had been discontinued by the labs and appear to be replaced by larger gene panels that addressed broader patient indications. Also, Lab Hs: Wilms Tumor panel had the same name as a panel offered by another lab; the Lab G: Wilms Tumor Panel. The Lab G: Wilms Tumor panel was ordered once in 2018 and the Lab H: Wilms tumor panel which was ordered once in 2022 (Table 3). The Lab H panel

was noted to cover 28 genes in 2022 and the Lab G panel covered 5 genes in 2018 before being discontinued by the lab.

Table 4: Net Change in Multi-Gene Panel Sizes

Multi-Gene Panel Name/ Lab:	2018	2019	2020	2021	2022	2023	Net change
Lab A: Primary Immunodeficiency Panel Plus	-	274 genes	298 genes	-	336 genes	336 genes	**+ 62 genes**
*Lab A: Comprehensive Hereditary Cancer Panel Plus	-	146 genes	154 genes	154 genes	160 genes	160 genes	**+ 14 genes**
Lab B: Pediatric Solid Tumors Panel	48 genes	48 genes	-	53 genes	54 genes	54 genes	**+ 6 genes**
Lab B: Common Hereditary Cancers Panel	43 genes, 47 genes	-	47 genes	-	-	47 genes	**+ 4 genes**
*Lab B: Nervous System/ Brain Cancer Panel	-	27 genes	27 genes	27 genes	29 genes	29 genes	**+ 2 genes**
*Lab A: Hereditary Leukemia Panel Plus	-	41 genes	41 genes	41 genes	42 genes	42 genes	**+ 1 gene**
Lab B: Renal/ Urinary Tract Cancers Panel	-	24 genes	25 genes	-	-	25 genes	**+ 1 gene**
Lab B: Sarcoma Panel	-	-	-	28 genes	29 genes	29 genes	**+1 gene**
Lab B: Pediatric Nervous System/ Brain Tumors Panel	-	26 genes	26 genes	26 genes	-	29 genes	**+ 3 genes**
Lab A: Comprehensive Hematology and Hereditary Cancer Panel Plus	-	-	348 genes	348 genes	-	369 genes	**+ 21 genes**
Lab B: Fanconi Anemia Panel	-	-	-	17 genes	-	15 genes	**-2 genes**
Lab B: Noonan Syndrome Panel Plus	-	-	-	35 genes	-	36 genes	**+ 1 gene**

* These panels were in the top four most frequently ordered panels for this patient group.
- These panels were not ordered during the year listed.

The number of genes that these panels grew by was variable; however, a pattern was discerned with the larger panels showing the greatest change over time (Table 4). The three largest panels had the greatest number of new genes added, with the Lab A: Primary Immunodeficiency Panel Plus showing the greatest number of new genes added over time (62 genes) (Table 4). Four panels showed a net increase of only 1 gene (Lab A: Hereditary Leukemia Panel Plus, Lab B: Renal/ Urinary Tract Cancers Panel, Lab B: Sarcoma Panel, Lab B: Noonan Syndrome Panel Plus) (Table 4).

As primary immunodeficiency diseases (PIDs) can predispose people to cancer, primary immunodeficiency multi-gene panels are used in the cancer predisposition program setting (Kebudi et al., 2019). The Lab A: Primary Immunodeficiency Panel Plus grew by the largest number of genes and was ordered in 2019, 2020, and 2022. In 2019, this panel covered 274 genes, and by 2020, the panel covered 24 additional genes to cover 298 genes (ALPI, ARHGEF, ARPC1B, ATP6AP1, C17ORF62, DBR1, DNASE2, ELF1, FCH01, IL23R, IL2RB, IL6ST, LIG1, POLD1, POMP, PRG4, RELA, RIPK1, SLC39A7, SPPL2A, SRP54, TGFB1, TNFRSF9, TTC37). By 2022, the panel had grown by an additional 38 genes and the panel covered 336 genes in total and remained unchanged in 2023 (AP3D1, B2M, C5, C6, C7, C8A, C8B, C9, CCBE1, CD4, CDC42, COG6, CYP27A1, DGAT1, DIAPH1, DNASE1L3, DSG1, FANCA, FAT4, FCGR3A, G6PC, GUCY2C, HAVCR2, HMOX1, IL6R, IL7, IRF4, IRF7, NFE2L2, OBFC1, POLA1, PSMB4, RANBP2, RELB, SEC61A1, TAZ, TLR3, UBA1).

The Lab A: Comprehensive Hereditary Cancer Panel Plus grew from 146 genes in 2019 to 154 genes in 2020 with the addition of eight genes (EFL1, EFL1, FAM111B, GALNT12, MSH3, RB1, RECQL, RPS20, SAMD9). Six more genes were added to the panel by 2022 (CTNNA1, GPR101, HAVCR2, KIF1B, SMARCE1, TRIP13) and the panel was unchanged in 2023.

The Lab B: Pediatric Solid Tumors Panel was ordered in 2018, 2019, 2021, and 2022. In 2018 and 2019, the panel covered 48 genes. By 2021, five genes had been added (AIP, EXT1, EXT2, LZTR1, REST) and by 2022 one additional gene (POT1) was added to the panel. The Lab B: Common Hereditary Cancers Panel was ordered in 2018 and 2020. During 2018, the panel size grew from covering 43 genes to 47 genes and four genes were added (CDK4, CTNNA1, MSH3, NTHL1). When this test was ordered again in 2020, it remained stable at 47 genes.

The Lab B: Nervous System/ Brain Cancer Panel was ordered in 2019, 2020, 2021, and 2022. In 2019, 2020, and 2021, the panel covered 27 genes. By 2022, two more genes were added to the panel (NBN, POT1) and it remains unchanged in 2023.

The Lab B: Pediatric Nervous System/ Brain Tumors Panel was ordered in 2019, 2020, and 2021 and covered 26 genes. When compared with the panel that is offered in 2023, the panel had added three genes (NBN, POT1, SMARCA4). The Lab A: Comprehensive Hematology and Hereditary Cancer Panel Plus was ordered in 2020 and 2021. For both of those years, the panel covered 348 genes. By 2023, the panel has grown by 21 genes and now covers 369 genes (C6ORF25, CD59, CECR1, CTLA4, CTNNA1, GLRX5, GPR101, HAVCR2, HK1, HMOX1, KCNN4, KIF1B, NAF1, OBFC1, PGK1, RPL26, SMARCE1, STAT3, TBXAS1, TRIP13, ZCCHC8).

There were four panels that exhibited a single gene addition. The Lab A: Hereditary Leukemia Panel Plus grew from sequencing 41 genes in 2019, 2020, and 2021, to sequencing 42 genes in 2022 with the addition of one gene (HAVCR2). The Lab B: Renal/ Urinary Tract Cancers Panel was ordered in 2019 and 2020. In 2019, the panel covered 24 genes and by 2020, one gene (REST) had been added to the panel. The Lab B: Sarcoma Panel was ordered in 2021 and 2022. In 2021, this panel covered 28 genes and by 2022 one additional gene (POT1) was added to the

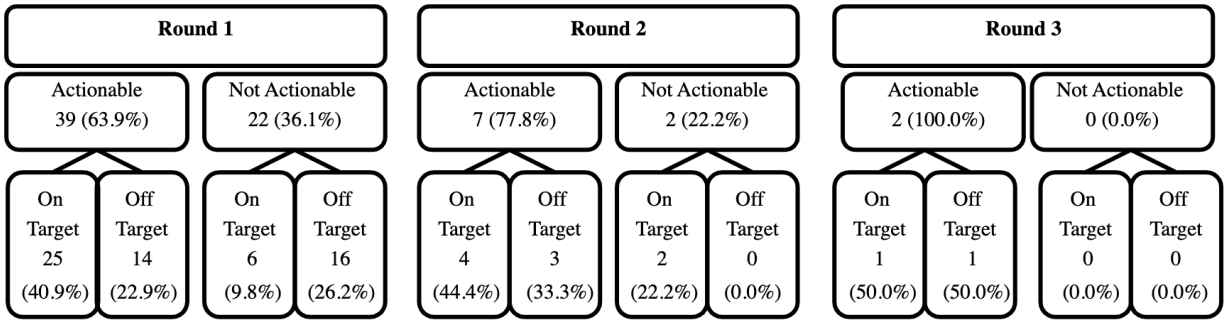
panel. The Lab B: Noonan Syndrome Panel Plus was ordered once in 2021 (Table 3) and covered 35 genes. In 2023, the same panel covered an additional gene (BMP2).

Only one panel exhibited a decrease in genes on a panel over time; the Lab B: Fanconi Anemia Panel. This panel was ordered once in 2021, and at the time, it covered 17 genes. When compared to the 2023 version of this panel, there are three genes that were no longer offered by this panel (FANCM, RAD51C, XRCC2) and there was one gene added (UBE2T). The 2023 version of this panel offered the option of expanding the panel by seven genes that had preliminary evidence suggesting an association with Fanconi anemia. All three of the genes that were removed from the main panel offered in 2021 were listed among the preliminary evidence genes from the expanded panel in 2023.

2.3.5 Number of Pathogenic and Likely Variants Identified and Actionability

Of the 202 genetic tests that were ordered and run over the multiple rounds of genetic testing performed there were 72 reports that identified at least one variant (either pathogenic/ likely pathogenic or variant of uncertain significance) in the testing or a 35.6% yield of results for tests ordered (Figure 3). It is important to note that some tests identified and reported more than one variant. These reports were categorized as either actionable/ non-actionable and either on/ off target (Figure 3). A report was categorized as actionable/ non-actionable based on whether there were clinical decisions that were made due to the results of the report. The same reports were classified as on/ off target whether the results contained a variant in a gene that is associated with the patient's cancer phenotype.

Figure 3: Actionability of Reports per Multiple Rounds of Genetic Testing



Key: Count is by report. Categorized as actionable and on/ off target.
* Some reports identified multiple variants

In the first round of testing, there were 101 negative tests and 61 tests that identified a variant (either a pathogenic/ likely pathogenic variant or a variant of uncertain significance) (60.4%). Of the 80 total variants identified, there were 38 pathogenic or likely pathogenic variants (47.5%), and 42 variants of uncertain significance (52.5%). Of the 61 test reports that had identified at least one variant, 39 of the reports (63.9%) were classified as actionable and 25 (40.9%) of those reports were also classified as on target (Figure 3). There were 22 reports (36.0%) that were classified as not actionable.

In the second round of testing, there were 32 negative tests and nine tests that identified a variant (Figure 3). Over those nine tests, there were six pathogenic or likely pathogenic variants identified, and 4 variants of uncertain significance identified. Of the nine test reports that had identified at least one variant, seven of the reports (77.8%) were classified as actionable and four (44.4%) of those reports were also classified as on target. There were two reports (22.2%) that were classified as not actionable.

In the third round of testing, there were five negative tests and two tests that identified a variant. Over those two tests, there were two pathogenic or likely pathogenic variants identified,

and two variants of uncertain significance identified. Of the two reports that had identified at least one variant, both reports (100.0%) were classified as actionable and one of those reports (50.0%) was also classified as on target. There were no reports that were classified as not actionable.

2.3.6 Genes With Pathogenic and/ or Likely Pathogenic Variants

There were 46 pathogenic and likely pathogenic variants identified across 32 different genes (Table 5). The genes that had more than one pathogenic/ likely pathogenic variant identified were: NF1, ATM, TP53, PTEN, SDHA, ETV6, FANCA. The three genes in which the most variants were identified were NF1, ATM, and TP53 (Table 5).

Table 5: Genes with a Pathogenic or Likely Pathogenic Variant Identified

Genes with Pathogenic/ Likely Pathogenic Variants:	Number of Variants (%)
NF1	5 (10.86)
ATM	5 (10.86)
TP53	3 (6.52)
PTEN	2 (4.34)
SDHA	2 (4.34)
ETV6	2 (4.34)
FANCA	2 (4.34)
RB1	1 (2.17)
REQL4	1 (2.17)
NFKB1	1 (2.17)
BARD1	1 (2.17)
DICER1	1 (2.17)
PMS2	1 (2.17)
ERCC3	1 (2.17)
CHEK2	1 (2.17)
ARID1B	1 (2.17)
NF2	1 (2.17)
SDHB	1 (2.17)
PALB2	1 (2.17)
SMARCA4	1 (2.17)
BRCA2	1 (2.17)
STXBP2	1 (2.17)
BRCA1	1 (2.17)
APC	1 (2.17)
ALS2	1 (2.17)
SPINK1	1 (2.17)
GATA2	1 (2.17)
SUFU	1 (2.17)
IC2	1 (2.17)
VHL	1 (2.17)
KMTB2B	1 (2.17)
MUTYH	1 (2.17)
Total Variants	**46**

Not all the pathogenic/ likely pathogenic variants identified fell within a cancer predisposition gene. One patient received the Lab A: Comprehensive Hereditary Cancer Panel Plus due to a diagnosis of acute myeloid leukemia (AML). This panel identified low levels of mosaic monosomy X as well as carrier status for a variant in the ERCC3 gene. These findings did not explain the patient's diagnosis of AML.

Another pathogenic variant was identified via whole exome sequencing of a patient with a brain tumor. This patient had completed two rounds of testing; the initial test was the Lab B: Nervous System/ Brain Cancer Panel. This panel identified a variant of uncertain significance in TSC1. Whole exome sequencing was ordered for the second round of genetic testing which identified a pathogenic variant in the KMT2BB gene and provided a molecular diagnosis of KMT2B-related dystonia, which is a childhood-onset movement disorder (Abela & Kurian, 1993). The identification of this variant led to a neurology referral and the diagnosis of cerebral palsy was removed from their problem list.

Another patient received the Lab B: Common Hereditary Cancers Panel due to a congenital mesoblastic nephroma and was found to have a pathogenic variant in BRCA1 and a variant of uncertain significance in CDH1. These results were also considered to be off target and actionable as the variants identified in these genes were likely unrelated to the patient's diagnosis of a congenital mesoblastic nephroma. However, the identification of the pathogenic variant in BRCA1 prompted a discussion of familial variant testing and will change the patient’s clinical management as an adult.

2.4 Discussion

This study aimed to identify the frequency of changes in multi-gene panel genetic testing size and to examine the clinical utility of genetic testing results for patients who have undergone multiple rounds of genetic testing. A trend of panels growing over time was identified.

2.4.1 Evaluation of Multi-Gene Panels Over Time

Of the 34 unique multi-gene panels that were ordered by this clinic, 12 of these panels, or 35.3% of the total panels, had changed over time. Custom panel tests were excluded from the multi-gene panel data as they are not standardized and are subject to change depending on the ordering provider preference. Eleven of these panels had grown; three of which were in the top four most frequently ordered multi-gene panels for this patient group **(Table 3).** The Lab B: Fanconi Anemia Panel was the only panel that showed a decrease in the number of genes offered by their baseline panel test over time, however, the 2023 version of this panel did have the option of expanding to a larger panel with preliminary evidence genes that included all the genes removed. Even though the baseline version of this panel had shrunk over time, the expanded panel still has broader testing options that include more genes than the previous baseline panel offered. This means that labs may remove a gene from their baseline panel if new information indicates that it is less correlated to a clinical indication than previously thought. The option to do an expanded panel also demonstrates a trend toward larger multi-gene panels, possibly as we learn more about cancer predisposition genes are being discovered. As these multigene panels grow larger, this may lead to more identification of variants in general, particularly, variants of uncertain significance (Chang et al., 2019). It is important to consider the implications of genetic testing labs that are

shifting toward larger panels as clinics may need to be equipped to counsel patients for more variants of uncertain significance (Chang et al., 2019).

It is not common practice for labs to report changes in their panels and there was no clear indication of these changes aside from their gene list changing. It would be important for clinics to revisit the genes offered by commonly ordered panels as these panels could have grown and genetic testing offerings may become quickly outdated. During the chart review and report collecting, it was noted that there was no mention of testing reanalysis offered by the labs to test patients that had prior negative testing for panels that eventually grew over time. However, if a patient were to undergo whole exome sequencing rather than a multi-gene panel, most labs would offer the option to run a re-analysis of the results after a set period of time. This would potentially allow for the incorporation of updated testing that could cover genes that were recently found to be associated with the patient's phenotype. Whole exome sequencing is broader than a multi-gene panel and can also identify incidental findings or secondary findings unrelated to the patient's clinical presentation. It is unclear if labs that currently offer multi-gene testing will ever consider offering updated testing for patients that have had uninformative genetic testing on outdated versions of their panels.

While collecting information about the differences in the multi-gene panels, this study incidentally highlighted the lack of homogeneity between the type of tests that genetic testing labs offer described by Roloff et al. (2021). There were 143 orders for 34 unique multi-gene tests for patients whose tumor profiles fit into one of three tumor categories (solid, liquid, CNS). Two of the panels that were ordered had the same name and were offered by two different labs; Lab G and Lab H: Wilms Tumor Panels. The Lab H panel was ordered once in 2018 and covered five genes. It has since been discontinued by the lab and replaced by a larger, more comprehensive renal and

urinary cancer panel. In contrast, the Lab G panel was ordered once in 2022 and covered 28 genes. These panels with the same name offered by two different labs a few years apart had a 23 gene difference. The large variety of testing options shows how varied the genetic tests offered by different labs can be, even for similar clinical phenotypes.

2.4.2 Discussion of Actionability of Variant Findings

As several rounds of testing were performed, the ratio of results that were on-target and actionable increased (Figure 3). This suggests that testing results were able to yield more variants that explained the patient's phenotype after multiple rounds of testing. These results may have interesting implications when considering retesting or additional testing after prior inconclusive or negative results from multi-gene panels that have changed in panel size over time. The increased ratio of on-target and actionable results may be due to our increased understanding of cancer predisposition genes or due to other factors surrounding the selection of the specific patient as a candidate for multiple tests. Only seven of the 162 patients had three rounds of genetics testing, so a larger sample size would be needed to make a more accurate assessment. The reasons for why similar tests were chosen over others or why some patients received multiple rounds of genetic testing were not explicitly stated and fell beyond the scope of this study. It was thought that some patients who received three rounds of testing may have had previously uninformative results. This may be true for some patients, but in fact, three of the seven patients had a pathogenic or likely pathogenic variant identified prior to the third round of testing. This demonstrates a lack of equitable decision making regarding the use of genetic testing in the clinical setting.

While the clinical goal is to aim for actionable, on-target genetic test results, there were cases of actionable, off-target results that were discussed in section 2.3.6 (Genes with Pathogenic

and /or Likely Pathogenic Variants). These results included pathogenic variants for genes that were unrelated to the patient's clinical presentation or pathogenic-leaning variants of uncertain significance. We had a patient who was diagnosed with KMT2B-related dystonia via whole exome sequencing, which did not explain their brain tumor, but did lead to a neurology referral and the removal of cerebral palsy from their problems list. We also had a patient with a pathogenic BRCA1 mutation that did not explain their diagnosis of congenital mesoblastic nephroma, but did result in a discussion of familial testing and will change their health management as an adult. If a result was off-target but actionable, that means that there were clinical actions made about family testing or cancer surveillance. Although these results were not “on-target”, they do provide information that can help inform the health of the patient and their family.

2.4.3 Genes with a Pathogenic/ Likely Pathogenic Variants

While all patients had a diagnosis of cancer, not all pathogenic variants found explained their clinical phenotype. The three genes in which the most pathogenic/ likely pathogenic variants were identified were NF1, ATM, and TP53. Pathogenic variants in these genes lead to Neurofibromatosis Type 1, Ataxia Telangiectasia (an autosomal recessive condition), and Li-Fraumeni Syndrome, which are all conditions that are childhood onset. Some patients, however, had incidental findings where a pathogenic variant was identified and there were clinical steps that could be taken for surveillance or familial testing, but ultimately those variants did not explain the patient’s diagnosis. One example of a case that was actionable but not on- target was one patient who was diagnosed with Acute Myeloid Leukemia. The multi-gene Lab A: Hereditary Leukemia Panel (Hematology) Plus was performed, and the patient was found to have a pathogenic variant in the BRCA2 gene. This gene is not associated with leukemia and was not thought to be the

explanation for why they developed leukemia. As there are guidelines established by the National Comprehensive Cancer Network that gives surveillance recommendations for adults with BRCA2 pathogenic variants (*NCCN - Evidence-Based Cancer Guidelines, Oncology Drug Compendium, Oncology Continuing Medical Education*, 2019), in addition to recommendations for familial variant testing, this was clinically actionable.

Overall, any pathogenic variants identified in affected children in genes that were linked to adult-onset cancers were considered to be incidental findings. These findings are linked to adult-onset cancers and thus have no recommendations for childhood screenings, however, these findings can lead to cascade targeted familial testing of the patient's adult relatives. Overall, results do not always have an established phenotype, particularly in the pediatric population. As these panels grow in size, there will be more opportunities to identify incidental findings such as these, which demonstrate a shift of genetic testing from a phenotype-first to a genotype-first approach. In a cardiovascular genetics setting, it was found that a genotype first approach identified patients with pathogenic and likely pathogenic variants that would have otherwise not met clinical diagnostic criteria (Wenger et al., 2021). The cardiovascular genetics setting is reflective of the oncology genetics setting as both can aim to identify pathogenic/ likely pathogenic variants to increase screening and prevent future detrimental health consequences. The Children's Oncology Group has practiced the genotype- first approach in the pediatric oncology setting (Li et al., 2021). They provided all patients with a newly diagnosed rhabdomyosarcoma (RMS) with whole exome sequencing and compared the results to common cancer predisposition genes. They found that some patients were affected by cancer susceptibility syndromes that were not previously associated with rhabdomyosarcoma and demonstrated the utility of broad testing.

2.4.4 Limitations

The nature of a descriptive chart reviews has its limitations and challenges. As many people contribute information to a patient's chart, there is the potential to have incomplete or incorrect information. Also, this provides us data of what this specific cancer predisposition program is offering during a limited point in time. It is however possible that the practices, data, and outcomes of this program are not generalizable to other similar programs.

A limitation of the study may be discrepancies on the categorization of cancer types. One example is that for the purposes of this project, cases of lymphoma were classified as a liquid tumor. Lymphoma arises from lymph fluid, however, it is often referred to as a solid tumor as the disease has typically progressed to form a solid tumor by the time of initial diagnosis (NCI Dictionary of Cancer Terms, 2011). In addition to possible discrepancies in classification, there were also limitations in the categorization of the classification types. Some patients had been diagnosed with multiple tumor types over their life, but their diagnostic indication was classified based on their primary tumor diagnosis.

While reviewing the multi-gene panels that were found to have changed over time, there were some years during the cancer predisposition program when a specific panel had not been ordered. This meant that during those years data may be incomplete, and it is possible that they may have changed or stayed the same. In addition, it is possible that some of the other multi-gene panels that were ordered less frequently have experienced change but were just not ordered enough for that change in the panel size to be appreciated from this data set.

In addition, while reviewing the clinical utility of ordering multiple rounds of genetic tests, only seven out of the 162 patients had completed a total of three rounds of genetic testing. A larger cohort of patients that have received more rounds of genetic testing would be needed to complete

a more accurate assessment on the precision of genetic test variants in relation to their clinical actionability and could be revisited in the future.

2.4.5 Future Studies

This study looked at a cancer predisposition clinic from the scope of practice of ordering providers; however, a version of this study could be adapted for labs to conduct internally. An introspective look within the operations of a lab could be completed to see how their own multi-gene panel tests are changing over time, what criteria is met for certain genes to be added to a panel and see how this change in panels might compare to other labs. In addition, it could be helpful to take a closer look at the newly added genes and see if the clinical guidelines for these genes are established and extend to pediatric and adult populations. This could give more insight into the lack of homogeneity between the labs and their genetic tests offered for similar clinical phenotypes and provide more insight into why certain genes are added to panels.

Future directions for this study would be to examine the genetic test type and genetic test results between patients that have a family history of cancer and those who did not. Tests ordered by tumor type category could also be used to look for patterns between results obtained from testing specific multi-gene panels. This study reviewed results of patients that had a variety of diagnoses to see how these tests are being utilized in a cancer predisposition program clinic setting. The scope of this study could be narrowed to inform the best testing options for a select group of patients and how these tests change over time. For example, a review of results could be completed for patients diagnosed with a specific tumor type who had multi-gene panel testing through the Lab A: Comprehensive Hereditary Cancer Panel Plus, the testing of the Lab B: Common Hereditary Cancers Panel, or a combination of both.

2.5 Conclusions

The goal of this study was to determine if multi-gene panels that are being utilized in a cancer predisposition program are changing over time and to look for patterns in the yield of pathogenic variants for patients who had multiple genetic tests. This study found that as children with cancer had more genetic tests, there was a higher yield of clinically actionable results. As only seven patients underwent three rounds of genetic testing, a more accurate assessment should be made using a larger patient population.

A review of the literature regarding genetic testing multi-gene panels suggests there is a need for further research on how genetic testing panels are evolving over time. This study has shown that multi-gene panels are trending toward growing larger over time with the addition of more genes. Further studies should explore why these genes are added, if clinicians and genetic counselors have tools necessary to adapt to the growth of these panels, and how added genes may affect people who had prior negative testing with smaller versions of the same test. Seeing the pattern of a higher yield of actionable results with multiple rounds of testing may have implications on decisions that healthcare providers and genetic testing laboratories may make regarding offering expanded testing for people who have had previously negative or uninformative testing for panels that have changed in panel size over time.

3.0 Research Significance to Genetic Counseling and Public Health

This study aimed to explore genetic testing outcomes in a pediatric cancer predisposition clinical setting. It highlighted one of the Ten Essential Public Health Services outlined by the CDC for Public Health Systems and Best Practices; the service to provide assurance through "equitable access (CDC, 2021). By researching how genetic tests are changing and who is being offered testing for cancer predisposition conditions, barriers to equitable access can be identified and we can further our understanding of how we should apply these tests to the public.

Another Essential Public Health Service is to "build a diverse and skilled workforce", and this study and review highlighted ways in which the workforce is struggling (CDC, 2021). There are limited guidelines for when genetic testing is appropriate, particularly for pediatric and young adult populations. In addition to limited guidelines, there is also a lack of homogeneity of the genetic testing panels offered by different genetic testing labs with regards to the size of the panels, the consistency of the genes that are offered, and the depth of coverage of the genes (Roloff et al., 2021). These inconsistencies between labs, speed at which multi-gene panels can change, and lack of guidance on genetic testing make it more challenging for health care providers and genetic counselors to parse through the genetic testing options and make decisions regarding their patients' care. With a lack of uniform guidelines and testing options, it may be difficult for providers to stay skilled at knowing which testing option is best for their patients while also providing equitable genetic testing. This research and review highlight the need for clearer guidelines and policies in place for equitable care.

Genetic counselors help comprise the "diverse and skilled workforce" that is part of the Essential Public Health Services. The results of this study suggest a trend of multi-gene panels

growing to accommodate more genes. This is important for genetic counselors to be aware of so that they may revisit the details of commonly ordered panels over time. This would also be important for genetic counselors to be aware of as they may have to accommodate for an increase in reporting of variants of uncertain significance. Increasing the size of these multi-gene panels can indicate that we will also see an increase in the number of variants of uncertain significance being reported (Lucci-Cordisco et al., 2022). It is important to consider the return of results when ordering testing; the trend of growing panels could mean that more patients will need counseling on VUS, and there may be a greater need for genetic counselors to help patients interpret and understand these variants in the context of their care.

Another Essential Public Health Service is to investigate, diagnose, and address health hazards and root causes. The CDC reports that malignant cancers are the second leading cause of death of people aged 1- 44 years old in the U.S (CDC, 2020). Of these people that are diagnosed with cancer, up to 10% of these cases are due to a genetic cancer predisposition (Van Cott, 2020). Identifying cancer predisposition syndromes can address these root causes and improve the life expectancy of affected and at-risk individuals, however, there can be barriers to who receives testing. It was unclear why some patients received multiple rounds of genetic testing and others did not; this is an example of inequitable access to genetic testing in the clinic space. These findings support the need to have clinical guidelines standards that encourage the practice of equitable genetic testing.

4.0 Public Health Essay: Analysis of Genetic Testing Guidelines

4.1 Background

Cancer is caused by genetic mutations that lead to uncontrolled growth of cells. For most people, these genetic mutations are somatic and are confined to the cancer cells themselves, however, others have a genetic predisposition to develop cancer due to genetic mutations in their germline cells. It is thought that up to 10% of cancer cases are caused by germline pathogenic variants that can be passed down through families (Mody et al., 2015), (Zhang et al., 2015). Other factors, such as exposures over our lifetime and our lifestyles, can also influence those risks.

There are two different categories of genetic tests that can be offered to with cancer patients: somatic or germline genetic testing. Somatic testing tests the genetic information of the tumor tissues to identify genetic variants that have been acquired. Germline genetic testing looks for variants in the unaffected tissues, such as a blood or saliva sample, that can cause someone to have a genetic predisposition. We would expect these genetic variants to be found in all tissues and cells of the body and for there to be a risk that these variants would be inherited from the patients' parents or be able to be passed to the patients' children. This information can provide more information for the patient and their family members to better understand risks for cancer. Some germline genetic cancer predisposition syndromes have guidelines for screening, medication, and care. Currently, not everyone who has a cancer diagnosis is offered these genetic tests.

As our knowledge of genetics grows, the genetic testing panels that are offered to patients seeking information about their cancer predisposition status have changed as well. Currently, we

do not have standardized genetic testing guidelines that apply to all adolescent and young adult (AYA) and pediatric patients diagnosed with cancer.

4.2 Genetic Testing for Pediatric and Young Adult Populations

Historically, cancer has been thought of as a "disease of aging", however, cancer in young people defies this narrative and raises suspicion for a genetic etiology (Aunan et al., 2017). When it comes to who should have genetic testing, some may rely on the presence of a family history of cancer to factor into that decision. Increasingly, it is found that family history is a poor predictor of a germline cancer predisposition, and that genetic testing should be broad and unbiased (Zhang et al., 2015). A study in 2015 ran whole genome sequencing and whole exome sequencing tests on 1120 patients aged 20 and below who were diagnosed with cancer. Of the 1120 patients, 8.5% or 95 people were found to have either a pathogenic or likely pathogenic variant in a cancer predisposition gene (Zhang et al., 2015).

This research highlights that children with cancer are at risk of having a genetic predisposition to cancer and raises questions as to what criteria are important for patients to meet to be recommended the option to do genetic testing. One study out of a pediatric oncology group provided whole exome sequencing to all children with diagnosed rhabdomyosarcoma (Li et al., 2021). This study found that some children were testing positive for pathogenic and likely pathogenic variants in cancer predisposition genes that were not associated with rhabdomyosarcoma. Of the 95 patients identified to have a pathogenic variant in the Zhang study, only 40% had a family history of cancer (2015). As more than half of these patients do not have a

family history of cancer, genetic analysis could be considered an important discussion for all children and young adults with cancer regardless of family history.

Once a cancer predisposition variant is identified, genetic testing of family members for the same variant is important, particularly in the pediatric and adolescent to young adult population. Most cancer predisposition variants are inherited in an autosomal dominant manner, meaning that all first-degree relatives would be at up to a 50% risk of inheriting the same variant. If a proband is identified as having a cancer predisposition variant, there is a risk to other family members of having the same cancer predisposition variant as well. Familial variant testing is important for others to understand their own risks for cancer and can be particularly important in the cases of some hematological malignancies such as leukemia or lymphoma. For example, if a child with a germline mutation is to receive a bone marrow transplant from a sibling, it can be important to know the genetic status of the siblings to see if it is an option to avoid transplantation of bone marrow that has the same germline mutation (Furutani & Shimamura, 2017).

Another factor that can make choosing a test more difficult for providers is the multitude of different genetic testing panels and labs. One study examined the differences between genetic testing panels and laboratories between eight commercially available panel tests that covered genes associated with hereditary hematopoietic malignancies (HHMs). The study concluded that of the 82 genes associated with HHMs, only four genes were consistently covered by the panels across all eight of the labs (Roloff et al., 2021). In addition to inconsistencies with genes covered, there are also inconsistencies with the types of samples that labs accept for testing. The same study found that 40% of the testing labs accepted peripheral blood which is an inappropriate sample type for evaluation of a patient with a hematological malignancy (Roloff et al., 2021).

With many different testing options and labs, providers may look towards guidelines for guidance in navigating germline testing options. However, few guidelines for cancer genetic testing in the pediatric and AYA populations have been developed. Providers must decide between many commercially available genetic tests to determine what type of genetic test is appropriate for their patient with little standardized guidance. Inconsistencies between genetic testing, labs, and the lack of genetic testing guidelines for providers make decisions surrounding genetic testing for pediatric and AYA populations confusing and difficult.

With guidelines to initiate genetic testing being limited, it is even more difficult to determine if it is advisable for patients to be re-evaluated through additional genetic testing over time as our knowledge of genetics changes. Because our understanding of genetics is rapidly changing, and it is possible to gain new information by revisiting an old test or by doing updated testing. One genetic test where it is common practice for labs to offer re-evaluation over time to account for changes in our understanding of genetic variants is while exome sequencing. One study looking at the diagnostic yield of whole exome sequencing reanalysis found that of the 174 whole exome sequencing tests performed, a molecular diagnosis was able to be provided for 14 of those tests upon reanalysis one to two years later (Liu et al., 2021). Genetic testing is dynamic, and further genetic examination can improve diagnostic yield.

While it is common for labs to offer reanalysis for whole exome sequencing, it is uncommon for labs to offer retesting of genetic testing panels, which as shown in Chapter 2 were the most commonly ordered genetic tests for pediatric cancer patients at UPMC Children's Hospital of Pittsburgh. While that analysis showed that additional testing can be helpful in identifying actionable and on-target genetic variants in that clinic's population, this has also been shown in the adult population. Two genes that are widely known to be linked to hereditary breast

and ovarian cancer are BRCA1 and BRCA2. One study offered repeat testing via a multigene panel to people who had a negative BRCA1 and BRCA2 testing and had initially qualified for testing due to a family history or a personal history of breast cancer. Of the 122 people retested, 13 were identified to have pathogenic variant (11%) and 11 of those people had variants that were deemed clinically actionable (Yadav et al., 2017). When genetic testing is negative, it is worth re-examining other options for testing to see if new knowledge about gene and disease associations or broader panels are available to shed light on a potential molecular diagnosis. As larger and more comprehensive multigene panels are developed and replacing outdated panels, and as whole exome sequencing is becoming more widely available, it is unclear when it is advisable for patients to be re-evaluated through genetic testing, if at all. Having resources such as guidelines for scenarios where it is unclear whether it is advisable for a pediatric patient to receive genetic testing or when a negative test result should be accepted and when more genetic testing is warranted may help providers facing these tough clinical decisions. The Pediatric Cancer Working Group of the American Association for Cancer Research (AACR) in 2017 collaborated on uniform recommendations for cancer surveillance of cancer predisposition syndromes (Brodeur et al., 2017). These guidelines are helpful to guide care in the context of a known cancer predisposition syndrome but they do not provide information on which patients should receive genetic testing to confirm a diagnosis of a cancer predisposition syndrome based on a phenotype- first approach to a molecular diagnosis. These guidelines resemble those established by the National Comprehensive Cancer Network (NCCN) which are commonly referenced to provide guidance on genetic testing. We wanted to interrogate what genetic testing guidance they offered for the pediatric and AYA population.

4.3 The National Childhood Cancer Registry, SEER Program, and National Comprehensive Cancer Network

Data registries, while difficult to create and maintain, serve as important tools to give researchers, particularly those interested in public health, access to reliable data (Pop et al., 2019). One commonly referenced data registry is the Surveillance, Epidemiology, and End Results (SEER) program. This program was established by the National Cancer Institute (NCI) after the National Cancer Act was passed in Congress in 1971 (*Surveillance, Epidemiology, and End Results (SEER) Program Contributors | U.S. Cancer Statistics Data Visualizations Tool Technical Notes | CDC*, 2022). The SEER program has since expanded and now collects data from the 22 SEER registries which are located across the United States. SEER collects data related to patient demographics, such as age, sex, and ethnic background. They also collect information to profile the cancer such as the stage of the cancer at diagnosis, morphology, and location of the tumor, and they collect statistics on mortality from the National Center for Health Statistics that can be used to understand survival rates and ages at diagnosis (*Surveillance, Epidemiology, and End Results (SEER) Program Contributors | U.S. Cancer Statistics Data Visualizations Tool Technical Notes | CDC*, 2022). The SEER registry is accessible to researchers through web-based program access. The SEER*Explorer application allows researchers to have access to the data and to apply different statistical analysis to the data. The SEER explorer tool allows for statistical analysis of data for the cancer site, cancer type, incidence rates, race and ethnic background, sex, age, and stage at diagnosis. These data points can be presented in graphical form or as a data table for download and use.

Another data exploration tool like SEER*Explorer is the National Childhood Cancer Registry Explorer, or NCCR*Explorer. The National Cancer Institute established a program

similar to SEER, the Childhood Cancer Data Initiative (CCDI), to focus on data and research surrounding childhood cancers (*About the NCCR*Explorer*, n.d.). The NCCR*Explorer functions as a part of the Childhood Cancer Data Initiative and provides data collected through the Virtual Pooled Registry Cancer Linkage System (*About the NCCR*Explorer*, n.d.). The NCCR registry has demographic, incidence, and survival rate statistics for people diagnosed with cancer within the adolescent and young adult (AYA) age groups 0 -39 years old. The data is standardized based on International Classification of Childhood Cancers (ICCC) and the NCCR*Explorer tool allows for statistical analysis of incidence and survival data based on sex, race or ethnicity, age, and cancer site or type. The data used is also secondary data in the National Childhood Cancer Registry which includes data from 23 NCCR registries, including SEER data, and is reported to be representative of 66% of all U.S. children, adolescents, and young adults ages 0-39 (*About the NCCR*Explorer*, n.d.).

The National Comprehensive Cancer Network (NCCN) is a non-profit organization that consists of 32 cancer centers. This organization is recognized for creating clinical standard practice guidelines for oncology, or The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). The NCCN Guidelines are used to help healthcare providers make decisions regarding the treatment and management of people with cancer. These guidelines are created by 1,700 clinicians and researchers and are reviewed annually by faculty at the 32 NCCN Member Institutions (*About Clinical Practice Guidelines*, n.d.). These guidelines are stated to apply to 97 percent of cancers that are affecting patients in the United States (*About Clinical Practice Guidelines*, n.d.). When it comes to genetic testing in cancer, whether it be for direct somatic tumor testing or germline testing for cancer susceptibility variants, the NCCN guidelines are a resource that providers have regularly utilized.

4.4 Research Questions

In order to inform the body of knowledge about genetic testing recommendations for pediatrics, adolescents, and young adults, more information was needed to understand what information is currently available. There is currently limited research that assesses the availability of genetic testing guidelines for the pediatric and adolescent and young adult (AYA) population compared to general adult population of cancer patients and their family members.

Research Questions:

1. What are the most commonly found cancer sites that are found in young people?
2. Do the NCCN guidelines provide genetic testing guidance for these?
3. How does the availability of NCCN guidelines of genetic testing for adults compare to genetic testing guidance that is specific for pediatric patients?

4.5 Methods

The NCCR*Explorer application was used to identify the three most commonly diagnosed categories of cancer that affect young people. To generate these results, the NCCR*Explorer application variables selected were for “Female and Males”, “All Races, and ages <40”. These variables were analyzed by “incidence” as the statistic to explore by “compare cancer sites”. This output data is presented in the form of graphs and tables were analyzed further by age group.

As of December 2022, the NCCN site contains a page with a list of guidelines that are specific to 62 different types of cancers. Data was collected by reviewing each of the 62 guidelines to see if they met the following criteria for analysis of germline genetic testing.

- Did the guidelines mention germline genetic testing?
- If yes, did they provide specific guidance for genes and methods for analysis?
- If yes, did they provide guidance for testing pediatric or adolescent and young adult (AYA) populations?

For each of the guidelines, the text was reviewed. If the guidelines met the criteria for a category, it was documented with the value of "1" and if they did not meet the criteria for a category, it was documented with a "2". This created a binary system to be able to quantify and compare the groups. This data was then organized by categories of common cancer sites identified from the NCCR*Explorer data. The initial data collection was the only time a binary system was used. Groups of guidelines that related to these common cancer sites were reviewed. Then, a count was taken of guidelines that met each of the aforementioned germline genetic testing criteria was organized within these common cancer site groups. The following tables and figures outlining NCCN guidelines represent a count of the guidelines which did or did not meet their corresponding criteria.

4.6 Results

4.6.1 Cancer Site Information

Through the NCCR*Explorer site data, a histogram was produced that compared the incidence of cancer by cancer sites in both females and males of all races under 40 years of age. "Epithelial Neoplasms and Melanomas" had the highest incidence at a rate of 289.1 per 1,000,000. Further investigation reviewing the 5-Year Age-Adjusted Incidence Rates from 2014-1018 of

"Epithelial Neoplasms and Melanomas" in the NCCR*Explorer revealed that the distribution of incidences of Epithelial Neoplasms and Melanomas was higher for the age range between "20 and 39 years" (incidence rate of 289.1 per 1,000,000) than "19 and below" (incidence 24 per 1,000,000). Likewise, "germ cell tumors" were found to have an incidence rate of 49.1 per 1,000,000 in the 20-to-39-year age range and was much lower for 19 years and below age range with an incidence of 11.7 per 1,000,000. As these categories were not highly representative of the adolescent age group, they were excluded from further analysis. The next three common cancers sites that were more representative of both adolescent and young adults were lymphomas (incidence rate of 60.9 per 1,000,000), leukemias (incidence rate of 51.6 per 1,000,000), and central nervous system neoplasms (incidence rate of 31.9 per 1,000,000) (Table 6).

Table 6: Prevalence of Cancer Sites per 1,000,000 People <40 Years Old

Cancer Sites	Rate per 1,000,000 people Ages <40 years
Lymphomas	60.9
Leukemias	51.6
Central Nervous System Tumor	31.9

4.6.2 Comparison of Genetic Testing Guidance for Adults and Pediatrics

The second portion of this study reviewed the NCCN Guidelines. Of the 62 total cancer guidelines, only four were labeled as specific to pediatric-specific cancers (Figure 4). These guidelines specific for the adolescent and young adult group were: Pediatric Acute Lymphoblastic Leukemia, Pediatric Aggressive Mature B-Cell Lymphomas, Pediatric Central Nervous System Cancers, and Pediatric Hodgkin Lymphoma.

After this information was collected, all 62 NCCN guidelines were reviewed, and the results were organized by guidelines that pertained to each of the three most prevalent cancer sites in pediatric and AYA populations identified through the NCCR*Explorer (Lymphomas, Leukemias, and Central Nervous System (CNS) neoplasms). There were six NCCN guidelines that pertained to types of Lymphomas; two of which were specified to pediatric population. None of the six guidelines mentioned germline genetic testing (Table 7).

Figure 4: Adult vs Pediatric Specific NCCN Guidelines

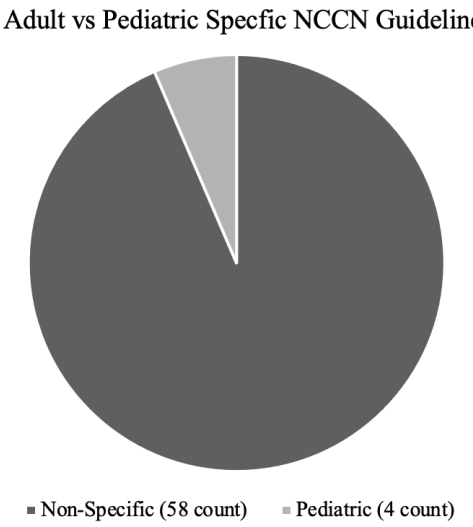


Table 7: NCCN Guidelines on Lymphomas and the Presence of a Discussion on Germline Testing

Lymphoma NCCN Guidelines	Germline Genetics Mentioned (count)	Germline Guidance (count)	AYA or Peds genetic Testing Recommendations (count)
B- cell Lymphomas	0	0	0
Hodgkin Lymphomas	0	0	0
Pediatric Aggressive mature b-cell Lymphomas	0	0	0
Pediatric Hodgkin Lymphoma	0	0	0
T-cell Lymphomas	0	0	0
Waldenstrom Macroglobulinemia/ Lymphoplasmacytic Lymphoma	0	0	0

There were six NCCN guidelines that pertained to types of Leukemias; one of which were specific to pediatric patients (Table 8). Only the guideline on Acute Lymphoblastic leukemia gave guidance about germline testing, while the pediatric specific counterpart guide for Pediatric Acute Lymphoblastic leukemia did not mention germline genetic testing.

Table 8: NCCN Guidelines on Leukemia and the Presence of a Discussion on Germline Testing

Leukemia NCCN Guidelines	Germline Genetics Mentioned (count)	Germline Guidance (count)	AYA or Peds genetic Testing Recommendations (count)
Acute Lymphoblastic Leukemia	1	1	0
Acute Myeloid Leukemia	1	0	0
Chronic Lymphocytic leukemia/ small LL	0	0	0
Chronic Myeloid Leukemia	0	0	0
Hairy Cell leukemia	0	0	0
*Pediatric Acute Lymphoblastic Leukemia	1	0	0

There were two guidelines that pertained to types of Central Nervous System Neoplasms; one of which was specific to pediatric population (Table 9). Both guidelines mentioned germline genetic testing, however, neither gave specific guidance. In the pediatric version of the guidelines, the recommendation stated that germline genetic testing should be strongly considered for pediatric populations.

Table 9: NCCN Guidelines on Central Nervous System (CNS) Neoplasms and the Presence of a Discussion on Germline Testing

Central Nervous System (CNS) Neoplasms NCCN Guidelines	Germline Genetics Mentioned (count)	Germline Guidance (count)	AYA or Peds genetic Testing Recommendations (count)
CNS Cancers	1	0	0
Pediatric CNS Cancers	1	0	1

Overall, it was noted that 33 of the 62 of the guidelines (53.22%) mentioned germline genetic testing but only 18 of the 62 of the guidelines (29.0%) provided guidance for genes of interest and genetic methods for analysis.

4.7 Discussion

The NCCN guidelines provide far less guidance for care specific to pediatric populations than those generalized to adults; only four of the 62 NCCN guidelines were labeled as for the pediatric population. These guidelines were for pediatric Acute Lymphoblastic Leukemia, pediatric Aggressive Mature B-Cell Lymphomas, pediatric Hodgkin-Lymphoma, and pediatric central nervous system cancers (Appendix B). From the NCCR*Explorer data, we found that the three most common cancers sites in people under 40 years of age were found to be lymphomas, leukemias, and central nervous system neoplasms (Table 6). While these pediatric NCCN guidelines do cover some of the types of cancers within these three main categories of cancer that a clinician may encounter in this population, only one of these specific pediatric guidelines mentioned germline genetic testing (Pediatric Acute Lymphoblastic Leukemia) (Table 8). We expected there to be more guidance on germline genetic testing as the rate of leukemia in pediatric and AYA populations is higher than other cancer sites (Table 6).

For the six NCCN guidelines developed for lymphomas, none mentioned germline genetic testing. The guidelines for pediatric Acute Lymphoblastic Leukemia, pediatric Aggressive Mature B-Cell lymphomas, and pediatric Hodgkin-Lymphoma are specific to their diagnosis; these guidelines do not encompass many other different types of liquid tumor indications. According to work by Roloff et al.(2021) we know that around 8.5% of children with cancer have a cancer predisposition syndrome. Specific types of leukemias and lymphomas are linked to cancer predisposition syndromes such as Li Fraumeni Syndrome (LFS) or primary immunodeficiency diseases (PIDs) (Schneider et al., 1993), (Kebudi et al., 2019). There is a need to expand these guidelines to provide germline genetic testing guidance on more types of leukemias and lymphomas. In some guidelines, it can be unclear if it is recommended to go forward with genetic

testing due to wording or lack of specificity. Regardless of whether there are known genes associated with a specific type of cancer, this information should be explicitly mentioned in these guidelines to guide care and address questions.

This study identified a disparity of the information about genetic testing in AYA populations compared to those offered for adult populations within the NCCN guidelines. When evaluating the NCCN guidelines for leukemias, it was noted that the pediatric specific guidelines for Acute Lymphoblastic leukemia did not include a discussion of germline genetic testing while the adult counterpart did (Table 8). However, for the guidelines for CNS neoplasms, the pediatric guidelines more explicitly gave recommendations for genetic testing recommendations than the guidelines for adult CNS neoplasms, indicating that the discussion of recommendations for germline genetic testing were not consistent throughout guidelines for the same cancer in different age groups. It is unclear if this was due to a higher prevalence of genetic predisposition to Acute Lymphoblastic leukemia in the adult patients than with pediatric patients or due to hesitancy to create genetic testing recommendations for a pediatric population. It could also be due to different panels of experts writing different sets of guidelines.

Genetic testing to screen for a cancer predisposition syndrome in affected children is generally well-accepted by families (Brozou et al., 2018). This research is flanked by the findings that although this testing would be well received, many children that could benefit from genetic testing are not referred to the proper care team to discuss or facilitate genetic testing (D'Aquila et al., 2023). This could imply a public health dilemma; cancer predisposition syndromes may be being missed in people with cancer due to a lack of germline genetic testing guidance in these national guidelines.

4.7.1 Limitations

One limitation of the data utilized in this review is the possibility that the NCCR*Explorer data over- or under- represent certain demographic groups. This data was reported to be representative of 66% of all U.S. children, adolescents, and young adults ages 0-39, but if these individuals differed significantly from the general population, there could be differences in the incidences of cancer reported and that of the larger population. The NCCN guidelines are frequently updated, and it is possible that some guidelines have more recently been updated to include more information on germline genetic testing.

While the review of the NCCN guidelines focused on discussions of germline genetic testing, the guidelines did discuss somatic testing as well. Ultimately, data regarding the discussion of somatic testing was excluded from this analysis as it fell outside the scope of this project. However, somatic genetic testing can provide indications for follow-up germline testing or may be paired with germline testing as part of the offered test. Unless this is explicitly outlined in a guideline, the decision to offer further germline genetic testing may be a provider preference and not a national recommendation, which can mean that people with cancer predisposition syndromes are being missed.

Language created another limitation in interpreting what populations were covered by some guidelines. Some language automatically included AYA groups without explicitly describing the recommendations for them. For example, a recommendation may say “all patients should…” but did not further describe if “all patients” refers to all age groups, including pediatrics. The definition of who fell under the category of “all patients” was interpreted to include the AYA in this analysis but could be open to interpretation by clinicians. We limited the scope of this

project to investigate the NCCN guidelines, however, there may be more clear guidance on germline genetic testing in other referenced guideline.

4.7.2 Future Research

The prevalence and discrepancies of genetic testing guidelines of adult and pediatric specific populations deserve further interrogation. Further studies should investigate which resources pediatric oncology providers most frequently turn to in order to learn information about recommendations for genetic testing. In addition to studying the discrepancies between these guidelines, collaborative efforts should be made to close this gap and more genetic testing guidelines that are specific to age groups should be developed.

4.8 Conclusions

A review of the NCCN guidelines for discussion of germline genetic testing found that there is a significantly larger body of information that applies to adults than guidelines that are specific toward pediatrics patients. These results highlight the complexity of the decisions that clinicians face when offering genetic testing for this young population. Without specific guidelines that offer information about whether germline genetic testing is recommended, many questions go unanswered, and this would likely result in inconsistencies in who is being offered genetic testing, when, and why.

This review implies the need for more specific guidance for germline genetic testing, particularly, more guidance specific for AYA groups. This may be informed by exploring types of

genetic tests and genetic testing outcomes for the AYA population in the cancer predisposition clinic. More research should be conducted on what types of testing yields results and how they have evolved over time, this can contribute to the body of research that can help researchers make more specific recommendations for the NCCN guidelines.

Appendix A IRB Approval University of Pittsburgh

EXEMPT DETERMINATION

Date:	January 10, 2023
IRB:	STUDY22110072
PI:	Elise Williams
Title:	Cancer Predisposition Clinic Genetic Testing: Analysis of Genetic Testing Outcomes and Procedures
Funding:	None

The Institutional Review Board reviewed and determined the above referenced study meets the regulatory requirements for exempt research under 45 CFR 46.104(d).

Determination Documentation

Determination Date:	1/10/2023
Exempt Category:	(4) Secondary research on data or specimens (no consent required)
Determinations:	• Waiver of HIPAA authorization • Children
Approved Documents:	• Data Collection Form .xlsx, Category: Data Collection;

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, Stacy Eckstein. **NOTE**: Modifications are only required if they will affect the exempt determination. It is important to **close your study when finished** by submitting a Continuing Review.

Please take a moment to complete our Satisfaction Survey as we appreciate your feedback.

Appendix B Blank Data Collection Sheet

Appendix Table 1

	A	B	C	D	E	F	G	H	I
1	Code	Sex	date of testing	age at testing	age at dx	category	indication	Family Hx	Testing Company
2									
3									
4									
5									

Appendix Table 2

	J	K	L	M	N	O
1	GT Test Name	# genes	Test Result	Variants Found	Interpretation	If VUS- recommendations?
2						
3						
4						
5						

Appendix Table 3

	P	Q	R	S	T	U
1	Age at Add. Test	Date of Add. Test	Testing Company	GT Test Name	# genes	Variants
2						
3						
4						
5						

Appendix Table 4

	V	W	X	Y	Z
1	Variants Found	Interpretation	If VUS- recommendations?	Age at Add. Test	Date of Add. Test
2					
3					
4					
5					

Appendix Table 5

	AA	AB	AC	AD	AE	AF
1	Testing Company	GT Test Name	# genes	Test results	Variants Found	Interpretation
2						
3						
4						
5						

Appendix Table 6

	AG
1	If VUS- recommendations?
2	
3	
4	
5	

Appendix C NCCN Guideline Germline Discussion Data Collection Sheet

Appendix Table 7

	A	B	C
1	NCCN Guideline Name	Germline discussion?	
2	Acute Lymphoblastic Leukemia	1	
3	Acute Myeloid Leukemia	1	
4	Ampullary Adenocarcinoma	1	
5	Anal Carcinoma	2	
6	Basal Cell Skin Cancer	1	
7	B-cell Lymphomas	2	
8	Bladder cancer	2	
9	Bone cancer	1	
10	Breast cancer	1	
11	CNS cancers	1	
12	Cervical cancers	2	
13	Chronic lymphocytic leukemia/ small LL	2	
14	Chronic Myeloid leukemia	2	
15	Colon cancer	1	
16	Dermatofibrosarcoma Protueberans	2	
17	Esophageal and Esophagogastri Junction Cancers	1	
18	Gastric Cancer	1	
19	Gastrointestinal stromal tumors (GIST)	1	
20	Gestational Trophoblastic Neoplasia	2	
21	Hairy cell leukemia	2	
22	Head and neck cancers	2	
23	Hepatobiliary cancers	1	
24	Histiocystic Neoplasms	1	
25	Hodgkin Lymphoma	2	
26	Kaposi Sarcoma	2	
27	Kidney cancer	1	
28	Malignant Peritoneal Mesothelioma	1	
29	Malignant Pleural Mesothelioma	1	
30	Melanoma: cutaneous	1	
31	Melanoma: Uvueal	1	
32	Merkel Cell Carcinoma	2	
33	Multiple Myeloma	2	
34	Myelodysplastic Syndromes	1	
35	Myeloid/ Lymphoid Neoplasms with Eos and TKF genes	2	
36	Myeloproliferative Neoplasms	2	
37	Neuroendocrine and Adrenal Tumors	1	
38	Non-Small cell lung cancer	1	
39	Occult primary	2	
40	Ovarian cancer/ fallopian tube cancer/ primary peritoneal cancer	1	
41	Pancreatic Adenocarcinoma	1	
42	Pediatric Acute Lymphoblastic Leukemia	1	
43	Pediatric Aggressive mature b-cell lymphoma	2	
44	Pediatric CNS cancers	1	

	A	B	C
45	Pediatric Hodgkin Lymphoma	2	
46	Penile cancer	2	
47	Primary cutaneous lymphomas	2	
48	Prostate cancer	1	
49	Rectal cancer	1	
50	Small Bowel Adenocarcinoma	1	
51	Small cell lung cancer	2	
52	Soft Tissue sarcoma	1	
53	Squamous Cell Skin Cancer	2	
54	Systemic Light chain Amyloidosis	2	
55	Systemic Mastocytosis	1	
56	T-cell Lymphomas	2	
57	Testicular cancer	2	
58	Thymomas and Thymic Carcinomas	2	
59	Thyroid Carcinoma	1	
60	Uterine Neoplasms	1	
61	Vulvular cancer	2	
62	Waldenstrom Macroglobulinemia/ Lymphoplasmacytic Lymphoma	2	
63	Wilms Tumor Nephroblastoma	1	

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