UNIVERSITY OF CALIFORNIA, IRVINE

# Evaluation of Somatic Genetic Testing as a Screening Tool to Detect Hereditary Cancer Predisposition in Patients Diagnosed with Myeloid Malignancies

THESIS

submitted in partial satisfaction for the requirements
for the degree of

MASTER OF SCIENCE

in Genetic Counseling

by

Rachel Collier

Thesis Committee:
Professor Fabiola Quintero-Rivera, Chair
Health Sciences Associate Clinical Professor Kathryn Singh
Adjunct Professor Pamela Flodman

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**DEDICATION**

To

my future patients

and to all the advocates who make this work meaningful

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**ABSTRACT OF THE THESIS**

Evaluation of Somatic Genetic Testing as a Screening Tool to Detect Hereditary Cancer Predisposition in Patients Diagnosed with Myeloid Malignancies

By

Rachel Collier

Master of Science in Genetic Counseling

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Professor Fabiola Quintero-Rivera, Chair

A referral to genetic counseling is commonly made for patients with many types of cancer, given that cancers are hereditary in 5-10% of cases (National Institutes of Health, 2017). However, patients with myeloid malignancies are not often provided with genetic services despite emerging evidence that these cancers are hereditary about 13% of the time (Yang et al., 2022). This study explored the use of somatic genetic testing as a screening tool for hereditary cancer predisposition in patients diagnosed with myeloid malignancies, in the hopes of providing more evidence that this is an appropriate way to identify patients in need of genetics services. The study involved 110 participants whose medical records and somatic genetic test results were analyzed for criteria that indicate a need for genetic counseling and germline genetic testing. Furthermore, this study investigated the current landscape of genetics professionals' involvement in the care of patients with myeloid malignancies at a single institution. Results from this study showed that 62 out of 110 patients (56%) met criteria for germline genetic testing to assess hereditary cancer risk based on the National Comprehensive Cancer Network 2023 guidelines for AML/MDS and the 2022 European LeukemiaNet guidelines (Döhner et al.,

2022; National Comprehensive Cancer Network, 2023). However, none were referred for genetics services on the basis of their diagnosis of myeloid malignancy. Additionally, of those 62 patients, 9 (15%) would not have met those criteria without considering the results of their somatic genetic testing, all of which included genetic variants that could be germline in origin, and as such, would be clinically relevant. This indicates a need for greater awareness among providers (hematologic oncologists, genetic counselors, molecular pathologists, and molecular geneticists) concerning the current recommendations for germline genetic testing for patients with myeloid malignancies and somatic genetic testing. The findings of this study show that utilization of somatic genetic test results is merited as a resource and screening tool for determining whether a patient should receive genetic counseling and consideration of genetic testing.

# I. INTRODUCTION

## 1.1 Overview of Genetic Counseling

Genetic counselors are specialized medical professionals who are trained in genetics and counseling (National Society of Genetic Counselors, n.d.). They provide services including risk assessment, genetics education, coordination of genetic testing, and results disclosure. Genetic counselors instruct patients about the benefits and limitations of genetic testing, facilitate decision-making, prepare patients to receive results, coordinate genetic testing, and assist in the interpretation and communication of test results. They also assist patients by identifying resources, such as support groups or specialist referrals, based on the results of genetic testing as well as personal and family history. The main goal of a genetic counselor is to empower patients to make independent decisions about their healthcare by providing them with the necessary education and psychosocial support (Patch et al. 2018).

Genetic counselors can work in a variety of subspecialties including prenatal, pediatric, oncology, and internal medicine (adult) genetics, among others. In an oncology setting, genetic counselors may see patients who have been diagnosed with cancer or patients who have a family history of cancer and thus have a higher chance of carrying a DNA variant that increases their cancer risk. Genetic counselors provide important information that other providers may not have time to share, including background education about the genetic basis and cause of cancer, what a cancer diagnosis means for future cancer risk on a personal or familial level, or psychosocial counseling and emotional support.

## 1.2 Cancer and Carcinogenesis

Cancer is a disease wherein cells of the body begin to divide rapidly and no longer adhere to normal cell regulators of growth and maturity. There are many types of cancer, many of which can be categorized into groups such as sarcoma, carcinoma, leukemia, and lymphoma. Sarcoma affects soft tissue and connective tissue. Carcinomas can form in many parts of the body, but are most commonly seen in the breast, lung, prostate, and colon. Leukemia affects the bone marrow while lymphoma affects the lymphatic system. Each cancer is distinct and may have vastly different causes, prognoses, and treatment requirements depending on location and level of progression.

Regardless of the site of origin, cancerous cells can spread, or metastasize, to other parts of the body through blood vessels or the lymphatic system. Locations to which the cancer spreads are known as metastasis sites, and common metastasis sites are the brain, blood, lung, and liver. Generally, cancers (metastatic or localized) are treated using chemotherapy, radiation therapy, surgery, hormone therapy, antibody therapies, stem cell transplantation, or any combination of the above. Approximately 40% of all people will be diagnosed with cancer at some point in their lifetime, and cancer is the second leading cause of death in the United States (National Institutes of Health 2017). While most cancers are caused by some combination of environment, random chance, and other factors—these types of cancers are referred to as sporadic cancers—only a small subset of cancers is hereditary.

Hereditary cancers are caused by one or more inherited genetic changes that are passed through a family. It is currently believed that 10 to 15% of all cancer diagnoses are hereditary, which in many cases can be confirmed through genetic testing. The results of genetic testing can help a patient clarify their risk of developing cancer based on whether

they carry any harmful variants in cancer-related genes (National Institutes of Health, 2017).

With the exception of sex-linked genes for chromosomal males (46,XY), and mitochondrial DNA, our genes are in pairs, with one copy inherited from the mother and the other from the father. The body employs various mechanisms for copying and repairing DNA that can become damaged over the course of a person's lifespan, as well as introducing normal variation into the population—but when these processes go wrong, damaging variants (also known as mutations or pathogenic variants) may occur and can disrupt gene function and proper protein production. These variants can occur spontaneously due to DNA damage from carcinogens or environmental exposures, or they can be inherited. Cell regulators are under genetic control, and it is the disruption of these genetic regulators that causes cancer. Thus, cancer is considered a genetic disease, although cancer predisposition is not always inherited.

Although many genetic factors can play into both cell growth and tumorigenesis, there are two main types of genes that regulate cell growth and development—tumor suppressor genes and oncogenes. Tumor suppressor genes, as the name suggests, function to limit cell growth. Examples of cancer predisposition syndromes that are driven by variants in tumor suppressor genes include Lynch Syndrome and Hereditary Breast and Ovarian Cancer Syndrome. Pathogenic variants in tumor suppressor genes are generally loss of function and are inherited in an autosomal dominant fashion. One working copy of the gene is sufficient to regulate cell growth, but if that copy is rendered nonfunctional by a pathogenic variant, epigenetic silencing, or other loss of function event caused by environmental or other factors, there would be no functional copies of the gene and cancer

may develop. Tumorigenesis occurs due to the loss of functional alleles at a combination of tumor suppressor gene sites (the specific genes vary between cancer types and individual tumors) and the activation of other genes that drive cell growth. Individuals who are born with a pathogenic variant in a tumor suppressor gene begin with a "head start" towards tumorigenesis, as one allele is already nonfunctional and the person is more likely to end up with two silenced, mutated, or otherwise nonfunctional copies of that gene. This simplified description of tumorigenesis, called the two-hit hypothesis, was first proposed by Alfred Knudson in his research on retinoblastoma and has since been accepted as a mechanism of carcinogenesis for many cancers caused by variants in tumor suppressor genes (Knudson, 1971). In contrast to this autosomal dominant model of hereditary cancer predisposition, people with autosomal recessive hereditary cancer predisposition syndromes would be born with a pathogenic variant in each copy of the relevant gene. This is the case in several disorders including *MUTYH*-polyposis and Constitutional Mismatch Repair Deficiency Syndrome (CMMRD).

The other class of cell regulators are called proto-oncogenes, which promote cell growth. When mutated, these are called oncogenes and are drivers of tumorigenesis, as they permanently activate cell growth pathways. Syndromes caused by pathogenic variants in proto-oncogenes are less common but include Multiple Endocrine Neoplasia type 2 (*RET* proto-oncogene) and Hereditary Papillary Renal Cell Carcinoma (*MET* proto-oncogene), among others.

### 1.3 Types of Genetic Testing

There are many types of genetic testing that can have important implications for medical management. Germline DNA testing—performed on the DNA that the person

inherited upon their conception, which is found in every cell of their body including reproductive cells—can provide information on a person's hereditary predisposition to cancer and/or indicate a need for familial testing. There are several forms of germline genetic testing that could be ordered based on a patient's specific personal or family history; testing methodologies include both gene panel testing (which analyzes many genes simultaneously) and single gene analysis, among other types of genetic testing. Once the testing is complete, a patient may receive a negative result, meaning that no pathogenic changes were identified in the analyzed genes. A positive result may occur, meaning that a pathogenic or likely pathogenic variant was detected. Additionally, variant(s) of uncertain significance (VUS) may be identified, wherein the effect of the identified genetic change is unknown. VUSs are generally treated as a negative result until enough scientific evidence is gathered to determine their significance; it is estimated that approximately 90% of oncology-related VUSs are later reclassified as benign (Mersch et al. 2021). Additionally, germline testing can provide information on a person's future cancer risk by showing if a person is at increased risk for other hereditary cancers. Lastly, germline testing can inform risks for family members. If a person has a germline variant it is likely that one of their parents, and potentially other family members as well, carry the variant and so genetic testing is recommended for relatives. However, in cases where the patient's biological parents are *not* found to carry the same variant in non-germ cell tissues (i.e. blood, saliva), it is possible that 1) the variant was a new, or *de novo,* change in that patient, or 2) the variant is inherited, but is only found in *some* of the cells in the parent's gonads (gonadal mosaicism).

In contrast to germline genetic testing, some providers may order *somatic* genetic testing. Somatic cells are all cells in the body except reproductive cells, and any pathogenic variants acquired in somatic tissue during a person's life will not be passed down to their offspring. Somatic variants are generally limited to the isolated population of cells in which they occur and their progenitor cells. Somatic genetic testing analyzes the DNA of cells in a patient's tumor, which would include both germline and acquired DNA variants that occurred as part of tumorigenesis. Somatic testing (via Next Generation Sequencing, or NGS) is commonly ordered by oncologists and is most often used to help providers make decisions about the prognosis, treatment and management of an individual's cancer, such as determining which medications, therapies, or clinical trials a person is eligible for. In contrast, as described above, germline genetic testing can provide information on that person's or their family members' future cancer risks. Both kinds of genetic testing are used in precision medicine in oncology, allowing patients and providers to discuss and select care that is specific and relevant to them based on available therapies and their inherited genetic cancer risks (Spector-Bagdady et al., 2022).

The results of somatic genetic testing should include, in theory, any germline genetic alterations the person carries. It is known that the results of somatic testing can occasionally identify variants that are present in germline DNA and as such would be relevant for assessing personal and familial cancer risk. In some cases, somatic testing on tumor tissue can be paired with a sample representative of germline tissue (this will vary between some cancer types, testing labs, and ordering provider preference) to clarify which variants are somatic and which are germline. It should also be noted that some laboratories intentionally filter out germline variants identified on somatic testing, which is

important to be aware of when assessing somatic testing (Li et al., 2020). Concordance between somatic test results and the composition of actual germline DNA occurs most often in two situations: when the phenotype of the variant fits the personal or family history of the patient, or when the variant allele frequency or variant allele fraction (VAF) is in the 40% to 60% range (Baliakas et al., 2019). According to Strom, 2016, the VAF is "the percentage of sequence reads observed matching a specific DNA variant divided by the overall coverage at that locus". In a germline sample, this ratio should be 50% or 100%, as a person with a heterozygous germline variant should have one wild type allele in addition to their allele with the variant, or they may have no wild type allele due to mutations or deletions acquired during tumorigenesis (He et al., 2019). Thus, the VAF should remain above 30-40% for germline variants, although it may change throughout tumor formation. The closer to 50% and the more consistent in its percentage a VAF is in different samples for the same individual (i.e. samples taken at different dates), the more likely it is that the results of somatic testing are reflective of germline origin. However, there are many other reasons why a VAF may be elevated. For example, loss of heterozygosity (LOH) can impact VAF. LOH is the "loss of one parent's contribution to the cell. It is a common form of allelic imbalance by which heterozygous somatic cells become homozygous because one of the two alleles gets lost" (Dutra). LOH and other somatic variants such as deletions/duplications may be causing the VAF to be higher than anticipated as no wild type alleles are present. A new variant may occur early in tumor formation and so may be found throughout the tumor, also resulting in a high VAF on testing. The VAF of a clinically actionable variant may also be much lower than 50% due to low tumor purity and presence of treatment-induced variants, as well as the method of tissue preparation and the

sequencing platform used (Shin, Hyun-Tae, et al., 2017). Additionally, certain germline variants, such as large insertions or deletions (INDELs), may cause preferential amplification or capture of normal homologue, resulting in < 50% VAF for germline variants (He et al., 2019). It follows that germline variants may have low VAFs on somatic testing for the same reasons; thus, although a variant is more likely to be germline if the VAF is closer to 50%, many variants with low VAFs may still warrant further investigation.

From a broader clinical standpoint, somatic testing may incidentally identify variants that could indicate the need for a referral to genetics and/or confirmation by germline genetic testing on a sample that is representative of germline DNA. The only way to differentiate between a somatic and a potentially germline variant seen on somatic testing is to pursue germline confirmatory testing (Li et al., 2020). If a positive result is identified on somatic testing and certain other criteria are met (as detailed later in this discussion), germline testing may be indicated. Although most variants identified on somatic testing are acquired during tumorigenesis, referral for germline testing based on a somatic test result is recommended by current guidelines for patients with many cancer types, including breast cancer, colon cancer, myeloid malignancies, and others (Döhner et al., 2022; National Comprehensive Cancer Network, 2023). However, it has not been universally adopted as part of standard practice in many clinics.

### 1.4 Myeloid Malignancies and Hereditary Cancer

Hematologic malignancies comprise a group of cancers that affect the blood and bone marrow, and include lymphoma, plasma cell myeloma, and leukemia. In a healthy state, stem cells in the bone marrow should begin the process of hematopoiesis by becoming myeloid and lymphoid progenitor cells, which in turn differentiate into many

types of immune cells. Myeloid malignancies (cancers that affect the progenitors of myeloid cells) include "clonal proliferative diseases with shared but diverse phenotype characteristics; this classification includes (1) the myeloproliferative neoplasms (MPNs), polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF); (2) chronic myeloid leukemia (CML); (3) myelodysplastic syndrome (MDS); and (4) acute myeloid leukemia (AML)" (Sud et al., 2020). In 2016 the World Health Organization recognized the hereditary nature of some hematologic malignancies (Arber et al., 2016). In 2017, a recommendation for germline genetic testing was added to the National Comprehensive Cancer network's guidelines on managing MDS, and a recommendation for germline testing for those with AML was added in 2021 (National Comprehensive Cancer Network, 2017, 2021; Obrochta & Godley, 2018). Though there was no mention of a recommendation for germline genetic testing or hereditary predispositions in the 2010 edition, ELN recommendations in 2017 cited the updated WHO guidelines on germline genetic testing and hereditary hematologic malignancies and were updated with more comprehensive information on germline genetic testing and genes relevant to hereditary syndromes in 2022 (Döhner et al., 2010, 2017, 2022).

Emerging research indicates that the percentage of myeloid malignancies caused by hereditary germline variants is higher than previously known, and a hereditary predisposition to myeloid malignancy is increasingly recognized in the literature (Sud et al., 2020). Furthermore, this percentage is comparable to or greater than that of other cancers commonly associated with hereditary cancer syndromes (National Institutes of Health, 2017). According to Yang et al. (2022), as many as 13.6% of myeloid malignancies occur due to a hereditary predisposition. Stubbins et al. (2022) note that germline variants may

be identifiable in 5-10% of patients with this type of malignancy. In a population of families selected based on a family history of two or more family members with MDS/AML, the percentage of families who carry a relevant genetic variant may be as high as 57% (Rio-Machin et al., 2020).

The importance of performing germline genetic testing on individuals who are diagnosed with myeloid malignancies is underscored by the changes made to the recently released 5th Edition of the WHO classification of hematologic malignancies. The 5th edition classifies myeloid malignancies that arise from germline genetic predispositions as "Secondary Myeloid Neoplasms" and recognizes that variants in several genes confer an increased risk of myeloid malignancies (Khoury et al., 2022). These genes include those associated with syndromes that carry other non-hematological risks and complications such as Fanconi Anemia, telomere biology disorder, and Schwachman-Diamond syndrome, among others. Other genes, such as *RUNX1*, *ANKRD26*, *CEBPA*, and *DDX41* cause non-syndromic myeloid malignancies (WHO 5th Edition). *RUNX1* is notable for its prevalence among those who undergo germline testing for myeloid-malignancy-associated genes and has been known to be associated with AML for the last 20 years, although many other genes are more recently discovered (Rio-Machin et al., 2020). Table 1 details some of the genes considered relevant to a hereditary predisposition to myeloid malignancies (see a full list of the relevant genes considered in this study in Appendix A). Although many of these genes are commonly somatically mutated in AML, they would be relevant to a patient's clinical picture if a variant is found to be germline (Desai et al., 2018).

**Table 1. Excerpt of Relevant Gene List (adapted from the National Comprehensive Cancer Network)**

| Gene | Direct Relevance - Malignancy | Additional Relevance- Malignancy | Additional Relevance - Hematologic | Additional Relevance - Other | Syndrome |
|---|---|---|---|---|---|
| *ANKRD26* | AML | MDS, AML | Thrombocytopenia, platelet dysfunction | | |
| *BLM* | MDS | AML, MDS | | Pre- and post- natal growth restriction, photosensitive skin changes, immunodeficiency, insulin resistance, microcephaly, hypogonadism, high-pitched voice, early onset multiple cancers | Bloom Syndrome |
| *BRCA1* | MDS | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *BRCA2* | MDS | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *CEBPA* | AML | AML | | | |
| *CHEK2* | Myeloid Predispositions | Myeloid neoplasms, CHIP predisposit | Breast Cancer, Colon cancer | | |
| *DDX41* | AML | MDS, AML, CMML | Monocytosis | Solid tumor predisposition | |
| *DNAJC21* | MDS | AML, MDS | Bone marrow failure | Pancreatic insufficiency, skeletal abnormalities | Shwachman-Diamond |
| *EFL1* | MDS | AML, MDS | Bone marrow failure | Pancreatic insufficiency, skeletal abnormalities | Shwachman-Diamond |
| *ELANE* | MDS | AML, MDS | Neutropenia | | Congenital Neutropenia |
| *ERCC6L2* | MDS | MDS, AML | Marrow failure | Skeletal/cardiac abnormalities, neurological defects, erythroleukemia | |
| *ETV6* | AML | MDS, AML, CMML, B-All, Myeloma | Thrombocytopenia, platelet dysfunction | | |
| *FANCA* | MDS | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *KRAS* | MDS | AML, MDS | | Syndromic findings and other cancer | RASopathies |
| *MBD4* | AML | AML | | Colon polyps | |
| *MECOM/E* | AML | MDS, AML | Bone marrow failure, B-cell deficiency | Radioulnar synostosis, clinodactyly, cardiac malformations, renal malformations, hearing loss | |
| *PTPN11* | MDS | AML, MDS | | Syndromic findings and other cancer | RASopathies |
| *RUNX1* | AML | MDS, AML, T-cell ALL | Thrombocytopenia, platelet dysfunction | | |
| *SAMD9* | AML | MDS, AML | Pancytopenia | Normophosphatemic familial tumor calcinosis, MIRAGE syndrome, ataxia | |
| *SAMD9L* | AML | MDS, AML | Pancytopenia | Normophosphatemic familial tumor calcinosis, MIRAGE syndrome, ataxia | |
| *TP53* | MDS | AML, MDS | ALL | Adrenocortical carcinoma, brain and breast cancer, chorid plexus carcinoma, other cancers | Li-Fraumeni Syndrome |

## 1.5 A New Indication for Cancer Genetic Counseling

This new data highlights that patients with myeloid malignancies may benefit from genetic counseling services that would provide an opportunity to learn about risks for future cancers and/or risks to family members. “Genetic counselors are ideally placed to educate and support other healthcare professionals delivering genomic medicine” and are the appropriate healthcare professionals to consult when germline genetic testing is needed (Kohut et al., 2019). In the 2022 NCCN Guidelines for MDS, there is a section outlining the need for and structure of genetic counseling for those at risk of heritable hematologic malignancy predisposition syndromes, underscoring the importance of providing genetics services to this class of patients (National Comprehensive Cancer Network, 2022). Currently, cancer-focused (specialized) genetic counselors see patients for a wide variety of indications, including personal and family history of breast, ovarian, endometrial, pancreatic, and prostate cancers, as well as many others. However, due to the

novelty of gene classifications for myeloid malignancies, complicated logistics of germline testing for blood cancers, and lack of awareness of why a patient may need these services, very few patients with myeloid malignancies receive accurate and comprehensive genetic testing after receiving their diagnosis (Döhner et al., 2022).

One of these barriers is the difficulty of performing germline testing on a patient with an active hematologic malignancy. Germline genetic testing is traditionally performed by collecting a saliva, buccal swab, or blood sample; however, these sample types are not appropriate for patients with myeloid malignancies, as the samples will likely be contaminated with tumor cells/DNA and may lead to a higher rate of false positive results. Instead, "germline variant testing should only be performed on non-neoplastic tissues that do not carry a risk of blood contamination, such as cultured skin fibroblasts from a skin biopsy" (National Comprehensive Cancer Network, 2022). Additionally, for patients who had an allogenic stem cell transplant as part of their treatment, blood, saliva, or buccal samples cannot be used because they are contaminated by donor DNA. This requirement for sample type is a barrier to testing for a variety of reasons such as difficulty in obtaining the sample, as skin punch biopsies are an additional invasive procedure to coordinate (Furutani & Shimamura, 2017), as well as the cost of additional steps needed for this type of testing. Maintenance of the sample after collection is time and resource-intensive, as the skin fibroblast cells need to be cultured. This additional time taken for cell growth, potentially causes false positives due to the acquisition of somatic variants during culturing, in addition to the time already required for performing genetic testing, can delay the diagnosis (Desai et al., 2017; Furutani & Shimamura, 2017). Some genetics laboratories do not have the ability to culture skin fibroblast samples so appropriate laboratories would

need to be chosen prior to ordering testing (Yang et al., 2022). Skin punch biopsies are usually performed by a dermatologist, surgeon, medical geneticist, or other specialist trained to perform the procedure. Despite these challenges, a study of 23 patients with leukemia reported that 100% of participants expressed willingness to undergo a skin punch biopsy if needed and noted that the sample type would not be a deterrent (Beecroft, 2018).

Consistent and practical methodologies need to be developed for providers to identify patients who should consider genetic testing. The 2022 ELN guidelines state that "because the treatment plan for many patients with AML includes allogeneic stem cell transplant and relatives are the preferred donors, testing for germline risk alleles should be performed as early as possible during clinical management", further emphasizing the importance of this assessment (Döhner et al., 2022). In the 2017 ELN guidelines, genetic counseling is specifically recommended for cancer patients found to carry germline mutations, and other genetics organizations broadly recommend counseling prior to testing (Döhner et al., 2017; American College of Obstetricians and Gynecologists' Committee on Genetics, 2017; Li et al., 2020). This recommendation should increase access to genetic counseling services for those who need it while eliminating unnecessary expenditure of resources associated with providing genetic counseling for individuals who may not benefit from it.

### 1.6 Supporting Data for the use of Somatic Genetic Testing as a Screening Tool

Currently, referrals to cancer genetics clinics are often made based on a person's family history. Obrochta and Godley (2018) suggest that one of the following criteria should be met before considering genetic testing for a hematologic malignancy: "(i) the

patient has had multiple primary cancers; (ii) if there is at least one other case of hematopoietic malignancy in the family within two generations; or (iii) the pedigree warrants clinical genetic testing due to clustering of solid tumors". However, according to the 2022 ELN guidelines authored by Döhner et al., genetic testing should be considered if any of the following clinical criteria are met, regardless of age (Table 2).

**Table 2. Germline Genetic Testing Criteria for Hereditary Myeloid Malignancies (Adapted from Döhner et al., 2022)**

| **Clinical features** |
|---|
| Personal history of ≥2 cancers, 1 of which is a hematopoietic malignancy (order does not matter) |
| Personal history of a hematopoietic malignancy plus:<br>• Another relative within two generations with another hematopoietic malignancy, or<br>• Another relative within two generations with a solid tumor diagnosed at age 50 or younger, or<br>• Another relative within two generations with other hematopoietic abnormalities |
| Presence of a deleterious gene variant in tumor profiling that could be a germline allele, especially if that variant is present during remission* |
| Age of diagnosis of hematopoietic malignancy at an earlier age than average (eg, MDS diagnosed ≤ 40 y) |
| Germline status of a variant is confirmed by: |
| Its presence in DNA derived from a tissue source not likely to undergo somatic variant frequently (eg, cultured skin fibroblasts or hair follicles) AND at a variant allele frequency consistent with the germline (generally considered between 30-60%), or |
| Its presence in at least two relatives at a variant allele frequency consistent with the germline |
| *Certain gene alleles (eg, *CHEK2* I200T and truncating *DDX41* variants) are overwhelmingly likely to be germline and should prompt consideration of germline testing when identified even once |

Other somatic variants may prompt germline testing—for example, some Ashkenazi Jewish founder mutations in *BRCA1*/*BRCA2* are likely to be germline, and "7-11% of AML with biallelic *CEBPA* mutations have a germline mutation" (Taskesen et al., 2011). The latest guidelines from NCCN also note that patients found to have a "high variant allele frequency (>30%) mutation associated with AML predisposition" should pursue germline genetic assessment (National Comprehensive Cancer Network, 2022). Given this set of indications and the specific recommendation to pursue germline testing when certain variants are seen on tumor profiling, somatic genetic testing may be an appropriate tool to determine which patients with hematologic malignancies should be referred for germline testing.

The practice of using somatic test results as a screening tool for germline testing is already utilized for other cancer types, such as pancreatic and breast cancer, and has been proven to be useful, with as high as a 30% overall germline-positive confirmatory rate after a positive somatic test result across diverse cancer types (Lincoln et al., 2020). In fact, though the use may vary by cancer type and diagnostic laboratory workflow, some clinical genetic testing labs offer the option to test both tumor tissue and germline DNA concurrently (Tempus, 2023).

The National Comprehensive Cancer Network (NCCN) guidelines recommend that somatic testing be ordered for all patients with myeloid malignancies (AML Guidelines, National Comprehensive Cancer Network, 2021). Somatic testing on a patient suspected to have myeloid malignancy is comprised of a bone marrow biopsy or blood draw, and the test results are currently used to accurately diagnose disease and inform treatment decisions. The use of somatic genetic test results as a screening tool for germline variants in patients with myeloid malignancies has already been suggested in the literature and has

been shown to be useful in identifying some patients with germline variants (Baliakas et al., 2019; Drazer et al.., 2018). One source supports adapting already-performed somatic genetic testing as a screening tool for germline testing because "prognostication on DNA derived from malignant cells is able to identify patients with germline syndromes" (Obrochta & Godley, 2018). Another study showed that about 21% (n=74) of somatic tests done on 360 patients with hematologic malignancies had results with a high likelihood of germline concordance, and 24% of those with this high likelihood carried a germline variant relevant to their cancer risk (Drazer et al., 2018).

The objective of this study was to investigate the feasibility an" utility of using somatic genetic Next Generation Sequencing (NGS) results as a screening tool for patients with myeloid malignancy who may need germline genetic testing by analyzing the characteristics of the individuals who are positive for pathogenic and likely pathogenic variants. Although we postulate that some of the individuals identified will also be found to have a personal or family history of cancer, it is hypothesized that some patients *without* personal or family history may carry germline-suspicious variants, which is clinically useful in identifying individuals at risk for hereditary hematologic malignancy predisposition. This project will seek to corroborate existing data and spread awareness of the possible hereditary nature of some hematologic malignancies.

# II. METHODS

## 2.1 IRB approval

This research study was reviewed by the University of California, Irvine, Institutional Review Board. The research protocol was reviewed under the "expedited" category due to entailing "no more than minimal risk" to participant subjects. The application was submitted on June 23, 2022. After undergoing revisions, UCI IRB #1452 was initially approved on August 31, 2022, and an amendment was approved on November 16, 2022.

## 2.2 Data Collection

A retrospective chart review was conducted to collect the results of somatic NGS testing that was performed at UCI (somatic test names: PANHEME and MYEL75). The Experimental Tissue Shared Resource Facility (ETR) HS# 2012-8716, a shared resource of the Chao Family Comprehensive Cancer Center and the Department of Pathology and Laboratory Medicine, searched the Pathology LIS database CoPath and provided the list of medical record numbers (MRNs) associated with the PANHEME and MYEL75 test panels with a report date between June 9, 2020 and August 31, 2022. These dates were chosen because a comprehensive somatic genetic testing panel for hematological malignancies was made available at UCI beginning on June 9, 2020 and IRB approval for study number 1452 was granted on August 31, 2022.

The CoPath data query was conducted through several searches (by diagnosis as listed in the "Final Diagnosis" section of the patient's linked pathology report) as follows: search AML, exclude CML; search AMML, MDS AML CMML exclude ALL; search MDS, exclude CML; search MPN exclude ALL; search MPN exclude CML. CML was intentionally

excluded as current guidelines do not include germline testing for CML and known genes associated with hereditary myeloid malignancies are not thought to increase the risk of CML (Baliakas et al., 2019; AML Guidelines, National Comprehensive Cancer Network, 2021). Lymphoid malignancies were also excluded, given that a hereditary component is not well understood for that cancer type (Cerhan & Slager, 2015).

**2.3 Protection of Patient Privacy and Risk/Benefit Analysis**

During the study, data was viewed only by the research team and all data was coded and de-identified prior to statistical analysis. The privacy risks of the research were determined to be reasonable relative to the anticipated benefits of the research because no personal health information was accessed outside of the secured UCI Health HIPAA-compliant platform. Additionally, a code was used to link subject identifiers with the information needed for analysis. The code was destroyed at the earliest possible opportunity upon completion of this project.

Every precaution was taken to avoid the small risk that the information could be handled incorrectly (e.g. shared with a person not on the research team) by keeping information on secure servers and ensuring that only members of our team had access to the information. The privacy of all patients whose charts were analyzed was preserved. Furthermore, some individuals may benefit from this study by having the option to consider germline genetic testing, as their ordering providers were made aware that they should consider receiving genetic counseling services through the provider letter, as detailed below.

**2.4 Chart Review and Data Entry**

The list of MRNs was pulled from CoPath LIS by a member of the ETR team on November 21, 2022 and data collection began at that time. The list originally contained 381 patients, but upon further review, only 146 of them had a PANHEME or MYEL75 NGS test report in their chart. The remaining 235 charts were excluded. The data points from EPIC were collected for each patient using the following methodology. The chart was opened, and it was confirmed to be the correct patient. The somatic NGS test (PANHEME or MYEL75) was found in the media tab of their chart by filtering to items marked as "Pathology Report". The test information was coded, and each test was saved on the secure server, pending review by other members of the research team. Information on the personal and family history, as well as the diagnosis of the patient's current hematologic malignancy, were found by searching in the Problem List and various clinic notes from the Hematologic Oncologist or Emergency Department provider. Each data point was validated for accuracy through an additional chart review. Patient-specific variables that were collected included type of hematologic malignancy, any personal or family history of cancer (including cancer type, age of diagnosis, and relationship to patient if available in the chart), bone marrow transplant type and date, whether the patient was referred to genetic counseling or underwent germline testing, whether the patient has clinical features consistent with having a germline pathogenic variant in a relevant gene, insurance, and zip code. However, the data on zip code and insurance type were not included as part of the final data analysis given the challenges associated with interpreting the data and extracting accurate clinical information. Test-specific variables that were collected include test number, test date, sample type, ordering provider, number of variants reported on test, and number of qualifying test reports. Variant-specific variables that were collected include

gene, transcript, cDNA [c.] and protein amino acid [p.] nomenclature, variant allele frequency/fraction (VAF) and Tier. Tier is a classification of a variant's clinical significance that is determined by the genetics laboratory based on published guidelines from the American College of Medical Genetics and Genomics, American Society of Clinical Oncology, and College of American Pathologists (Li et al., 2017). Tier IA is defined as a variant of "Strong Clinical Significance, Level A Evidence (FDA approved therapy or practice guideline in patient's tumor type)", Tier IB is "Strong Clinical Significance, Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)", Tier IIC is "Potential Clinical Significance, Level C Evidence (FDA approved therapy or practice guideline in other tumor type, evidence from multiple small published studies, or based on availability of investigational therapies)", and Tier IID is Strong Clinical Significance, Level D Evidence (case reports or other preclinical studies).

Through the chart review, some patients were found to have a diagnosis of ALL or another cancer type not included in this study. These charts were excluded, resulting in a total of 110 patients who met criteria to be included in the study. Using both 2023 NCCN guidelines for AML, MDS, Breast/Pancreas, and Colon, as well as the 2022 ELN guidelines, an assessment was made for each patient to determine whether they meet criteria for germline genetic testing based on personal and/or family history even *before* considering the results of their somatic testing. For the specific ELN criterion stating that a patient should have testing if the age of diagnosis was earlier than average, an age of 50 was used as the cutoff given a median age of diagnosis of 68 years for patients with AML (National Cancer Institute).

## 2.5 Collation of Relevant Gene List

Variant data was extracted from somatic test reports. Up to ten reports, and up to ten variants on each report, were coded for each patient. Each patient had either the MYEL75 (75 genes) or the PANHEME (225 genes) panel performed. A full list of genes included on each panel can be found in Appendix B. Analysis of individual genetic variants was conducted using the full "Relevant Gene List", which was curated from NCCN guidelines (see Appendix A). Further literature review was conducted on the specific variants in genes flagged for review to identify if any of these variants warranted germline testing. First, variants were sorted based on the clinical relevance of the associated gene. Only those variants in genes that would be clinically actionable or relevant in a germline state were considered further. Second, the study team considered multiple databases, including GnomAD, ClinVar, and Cosmic, to gather additional information on these variants and make an assessment to the relevance of the variant. The variants' classification in ClinVar had the most impact on variant classification, namely, if a variant was absent in ClinVar, or present in a germline or unspecified state as pathogenic or likely pathogenic, then that provided strong evidence that the variant was worth pursuing on germline testing. Additionally, each variant was searched for in GnomAD (population database) and if it was found at a high frequency (greater than 1%) then that provided evidence against pursuing the variant on germline testing. Lastly, the variants were searched for in the Cosmic database. If variant was confirmed as germline in Cosmic, then that provided additional evidence towards pursing germline follow up, but if there were only entries on somatic status (especially if there were 50 or more somatic entries) then that would provide some evidence against pursuing germline follow up. For all variants that were

determined to be potentially of germline origin based on ClinVar and Cosmic data, the corresponding hematopathology report of the same sample was reviewed to determine the patient's disease status at the time of NGS testing, specifically whether they were in remission or had persistent disease based on blast percentage determined by flow cytometry results. For individuals with more than one somatic test report (i.e., testing performed on more than one date), the reports were compared to determine whether the potential germline variant was identified in each test. Only variants that were always seen at a VAF over 30% were considered for final review. If a patient had NGS testing done in both bone marrow and blood collected within 24 hours, only the bone marrow results were included. All of this data was integrated and assessed to determine whether a variant merited further investigation, with the level of evidence being the strongest for ClinVar data, and weakest for Cosmic. All assessments were reviewed and agreed upon by three or more members of the study team.

**2.6 Statistical Analysis**

The IBM Statistical Package for the Social Sciences (SPSS) Statistics version 29 (IBM SPSS Statistics for Windows) and Microsoft Excel were used for data analysis. Descriptive statistics included a frequency assessment for relevant variables.

**2.7 Provider Contact**

After data analysis, patients whose variants were flagged as possibly germline were noted. The provider who ordered the genetic test that contained the variant of interest was contacted by a licensed genetic counselor, using a standardized letter to recommend consideration of germline genetic testing for their patient. The providers of deceased individuals (including those participants for whom there was evidence in the medical

record that they passed away in between this study's data collection and the date that the letters were sent) were not contacted given the limitations of contacting the patient's family members and the anticipated low yield of testing surviving relatives. The letter can be found in Appendix D.

## II. RESULTS

### 3.1 Demographics

There were 110 patients whose charts were eligible for study inclusion. Of those, 54 (49%) were female and 56 (51%) were males; 50 (45%) of patients were deceased as of the data collection start date. The average age was 62 years with a range of 18 to 96 and a median age of 67 (Figure 1).

**Figure 1**

*Age Range of 110 Participants*

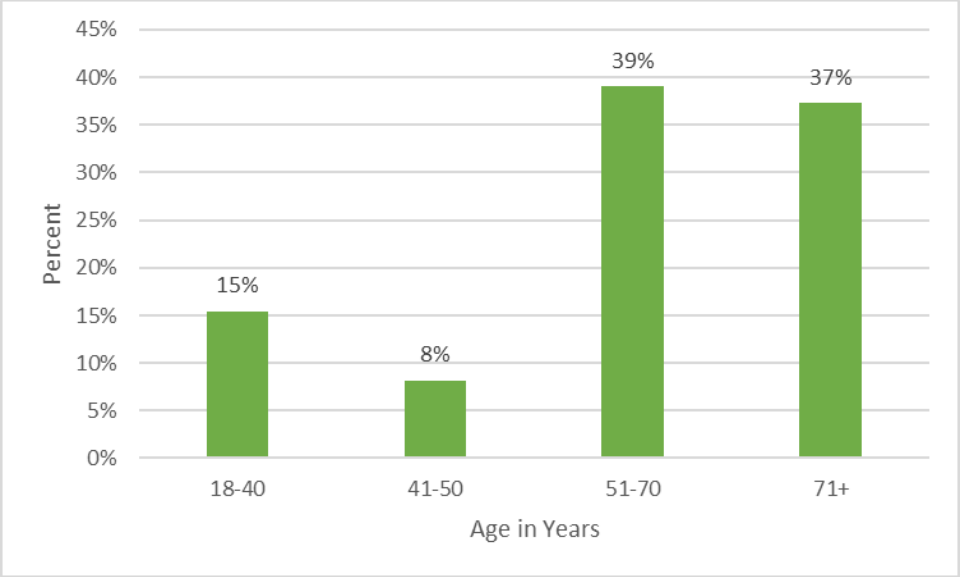


**Table 3**

*Diagnoses of Participants*

| Hematologic Malignancy Type | Count of Patients | % | Age Range (years) | Median Age (years) | Mean Age (years) |
|---|---|---|---|---|---|
| Acute Myeloid Leukemia (AML) | 93 | 84% | 19-96 | 66 | 62 |
| Myelodysplastic Syndrome (MDS) | 11 | 10% | 18-81 | 66 | 61 |
| Myeloproliferative Neoplasms (MPN) | 3 | 3% | 31-75 | 32 | 46 |
| Myelofibrosis | 2 | 2% | 57-63 | 60 | 60 |
| Polycythemia Vera (PV) | 1 | 1% | 25 | - | - |

Ninety-three individuals (84%) were diagnosed with Acute Myeloid Leukemia and the other 15% were diagnosed with other disorders (Table 3). It should be noted that two of the 11 patients with MDS listed in Table 3 may have had therapy-related MDS, and as such, it is unlikely that their cancer had a hereditary component. However, they were still included in the study given that they had NGS performed during the specified time frame and were diagnosed with a myeloid malignancy.

Seventeen out of 110 participants (15%) underwent allogenic bone marrow transplant (BMT). Researchers correlated date of sample collection with the date of BMT to determine if any patients had a relevant variant that disappeared after BMT, as that would affect our interpretation of that variant's likelihood of being of germline origin, but this was not observed. Three patients underwent allogenic BMT from related donors as treatment for a hematologic malignancy prior to our study period. We retained these individuals in the study because their donors were related and thus any potentially germline findings would be relevant for their family members, but none of these findings were identified.

Fourteen individuals underwent allogenic BMT during the study period; however, none of these individuals had any potentially germline variants detected prior to, or after, BMT.

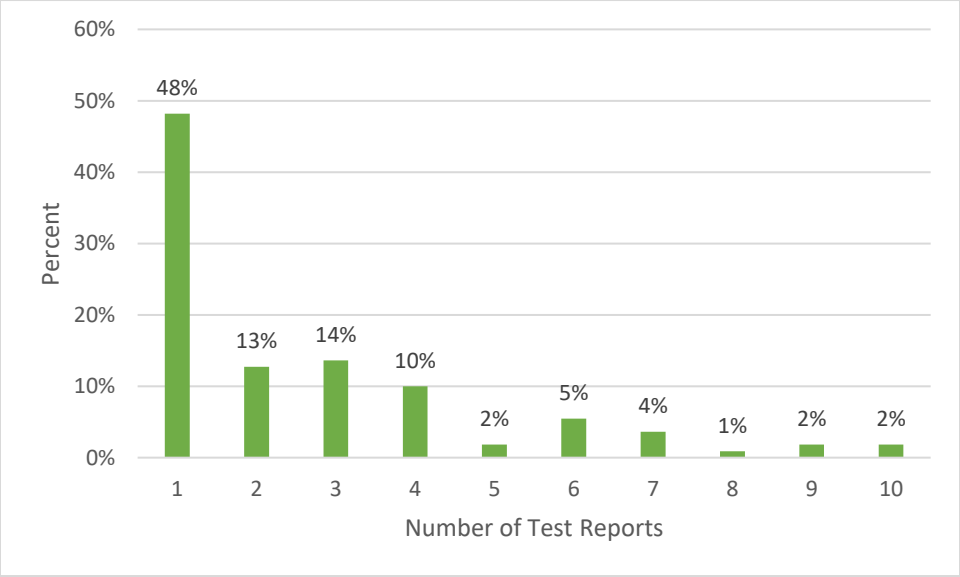
Two patients out of the original 110 patients in the cohort had undergone previous germline genetic testing but were not referred to genetic counseling based on their diagnosis of a myeloid malignancy. One of those two patients was previously referred to medical genetics for evaluation and testing based on personal history of muscle weakness and another received cancer genetic testing based on their family history of cancer or other personal history of non-hematological cancer from another provider before they were diagnosed with a myeloid malignancy. A referral to genetic counseling and/or for additional genetic testing would have been appropriate for these patients, as records indicate that the testing performed did not cover genes associated with hereditary predisposition to myeloid malignancies.

### 3.2 Somatic NGS Test Reports

From the 110 patients in the cohort, there were 290 somatic NGS test reports; the average number of reports per patient was 2.6 (Figure 2). About half of the patients (n=53; 48%) had only one test report available for review, and 26 of those individuals were deceased at the start of data collection. Of the remaining 57 patients, 40 had 2-4 test reports (18 of whom were deceased) and 17 patients (6 of whom were deceased) had 5 or more test reports.

**Figure 2**

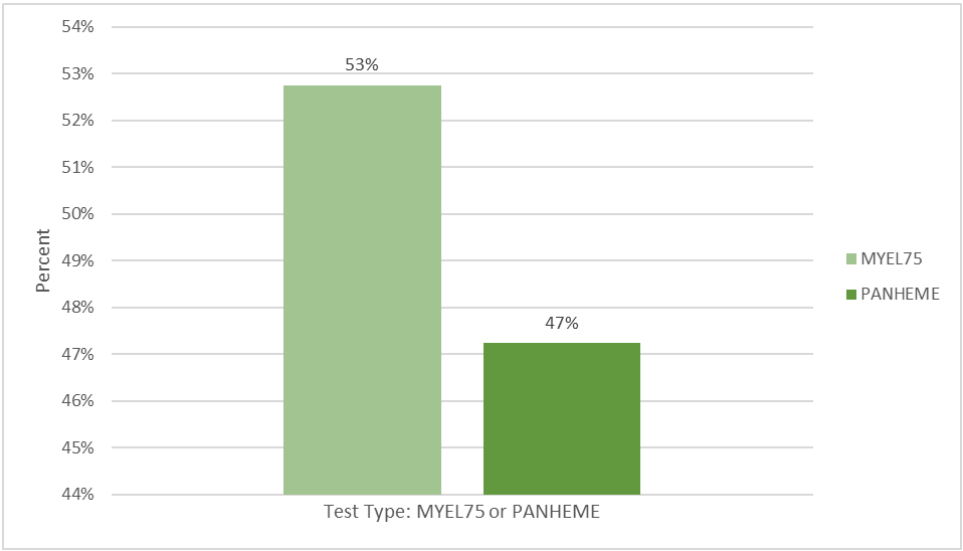
*Number of NGS Reports per Patient*




Of the 290 reports, 233 (80%) were performed in 2021 or 2022, with the other 57 (20%) performed in 2020. Regarding the testing methodology, 153 tests (53%) were run on the MYEL75 panel, and 137 tests (47%) were run on the PANHEME panel. As noted in the methods section, the MYEL75 panel included only 75 genes, while the PANHEME panel was expanded to include an additional 150 genes; thus, if a patient had both panels performed, it is possible that a variant identified on PANHEME may have not been detectable on a prior MYEL75 test for this patient, if the variant was in a gene not included on MYEL75 panel. This could affect the study team's interpretation of the significance of that patient's genetic variants so a comparison was made and it was determined that none of the additional 150 genes included on PANHEME were identified on any of the test reports in this cohort, and as such, the study team's interpretation of the significance of genetic variants was unaffected by the type of test performed.

**Figure 3**

*NGS Test Type*




The sample type varied, with 245 (84%) of the tests performed using a bone marrow sample, and 45 (16%) utilizing a blood sample (Figure 3). Two patients did not have NGS run on bone marrow at any point during the study period, three patients had NGS run on bone marrow prior to (but not after) they had a blood sample, and nineteen had NGS run on bone marrow at a later time. Of those who utilized a blood sample at any point during their treatment, 39 were diagnosed with AML, 1 with Myeloproliferative Neoplasm, 4 with Myelodysplastic Syndrome, and 1 with myelofibrosis. There were 54 distinct providers who ordered the NGS panels.

### 3.3 Gene Variants Identified by Somatic NGS

Out of the 110 patients in the cohort, 42 (38%) had a variant in a gene that would be clinically relevant if found to be germline (i.e. “relevant genes”) and so were flagged for further review. There was a total of 51 distinct genetic variants found within the test

reports of those 42 patients. These variants occurred in the following eight relevant genes: *CEBPA, ETV6, GATA2, KRAS, NF1, PTPN11, RUNX1*, and *TP53.*

These 51 distinct variants underwent further review as described in the Methods section. There were seven distinct variants that did not warrant a recommendation for germline testing based on the review, as they were found in the GnomAD population database at a high frequency or were classified benign/likely benign germline variants by at least 2 clinical laboratories in the ClinVar database. The other 44 variants were determined to be relevant by the study team as described in the methods. Of those 44 variants, 13 were determined to warrant germline follow up based on all available data, including the VAF and clinical status and blast percentage. VAFs ranged from 34.2% to 83.8%.

**Table 4**

*Frequency of 13 distinct variants potentially of germline origin*

| Patient # | Variant Information | VAF (%) | | | ClinVar: Absent, OR present as likely pathogenic or pathogenic | GnomAD: Absent, OR present at <1% frequency | Cosmic: Absent, OR confirmed germline |
|---|---|---|---|---|---|---|---|
| | | VAF | VAF | VAF | | | |
| **2** | *TP53*, NM_001126113.2, c.700T>A (p.Y234N) | 63 | - | - | Present as P or LP | Absent | Somatic |
| **9** | *CEBPA*, NM_004364.3, c.232delC (p.L78Wfs*82) | 43 | - | - | Absent | Absent | Somatic |
| **11** | *RUNX1*, NM_001754.4, c.497G>A (p.R166Q) | 46.1 | - | - | Present as P or LP | Absent | Somatic |
| **51** | *CEBPA*, NM_004364.3, c.1009_1010dupAC (p.L338Rfs) | 79.7 | - | - | Absent | Absent | Confirmed Germline |
| **201** | *KRAS*, NM_033360.2, c.38G>A (p.G13D) | 34.2 | 50.4 | - | Present as P or LP | Absent | Somatic |
| **276** | *PTPN11*, NM_002834.3, c.794G>A (p.R265Q) | 37 | - | - | Present as P or LP | Present at a frequency of 0.00003235 | Somatic |
| **302** | *TP53*, NM_000546.5, c.747G>C (p.R249S) | 45.2 | - | - | Present as P or LP | Absent | Confirmed Germline |
| **311** | *TP53*, NM_000546.5, c.536A>G (p.H179R) | 79.1 | - | - | Present as P or LP | Present at a frequency of 0.000003979 | Confirmed Germline |
| **328** | *RUNX1*, NM_001754.4, | 39.7 | - | - | Absent | Absent | Confirmed Germline |

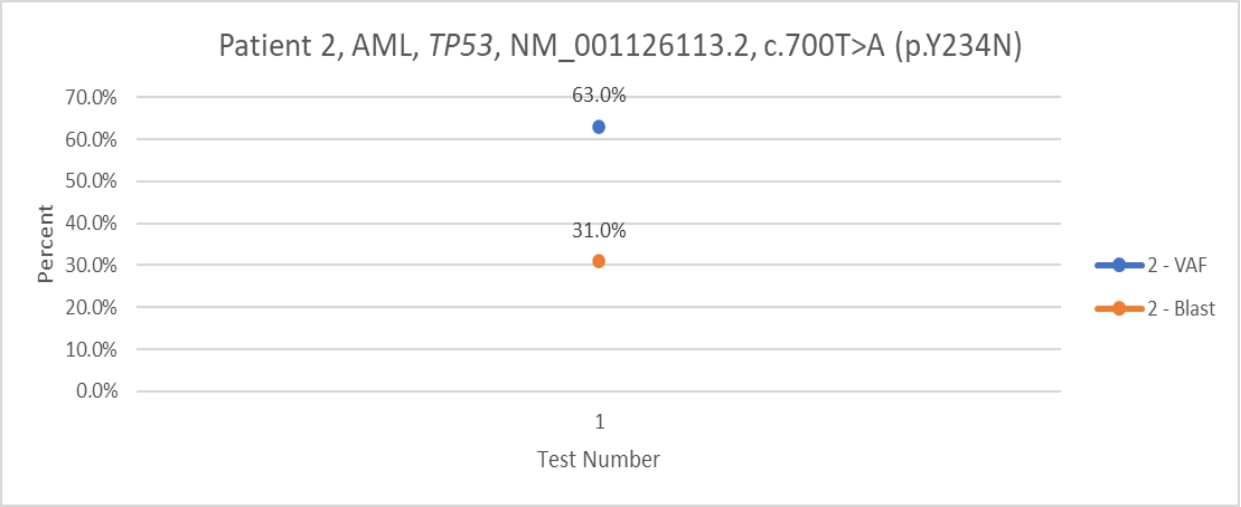
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | c.503_504ins14 (p.R169Kfs*12) | | | | | | |
| **352** | *TP53*, NM_000546.5, c.395A>G (p.K132R) | 73.2 | 83.8 | - | Present as P or LP | Absent | Somatic |
| **357** | *KRAS*, NM_004985.3, c.38G>A (p.G13D) | 34.8 | - | - | Present as P or LP | Absent | Somatic |
| **364** | *TP53*, NM_000546.5, c.537T>G (p.H179Q) | 78 | - | - | Present as P or LP | Absent | Confirmed Germline |
| **369** | *TP53*, NM_000546.5, c.919+1G>A (p.?) | 79.4 | - | - | Present as P or LP | Absent | Somatic |

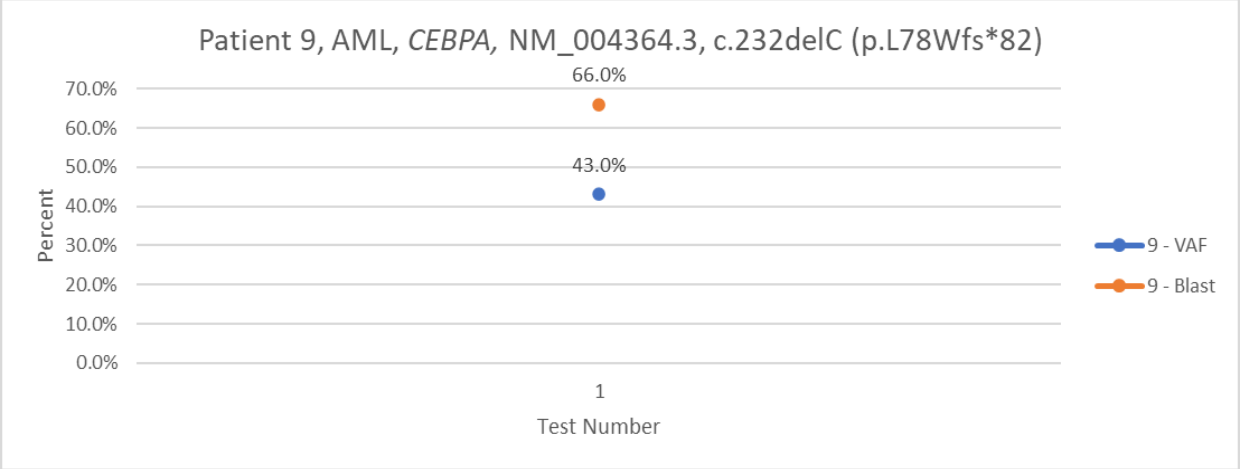
*"-" Patient may have had one or more NGS tests, but the variant was not seen an additional time, or other NGS tests were not performed within the study period. See Figure 4 for additional details on total number of tests completed and associated blast percentages.*

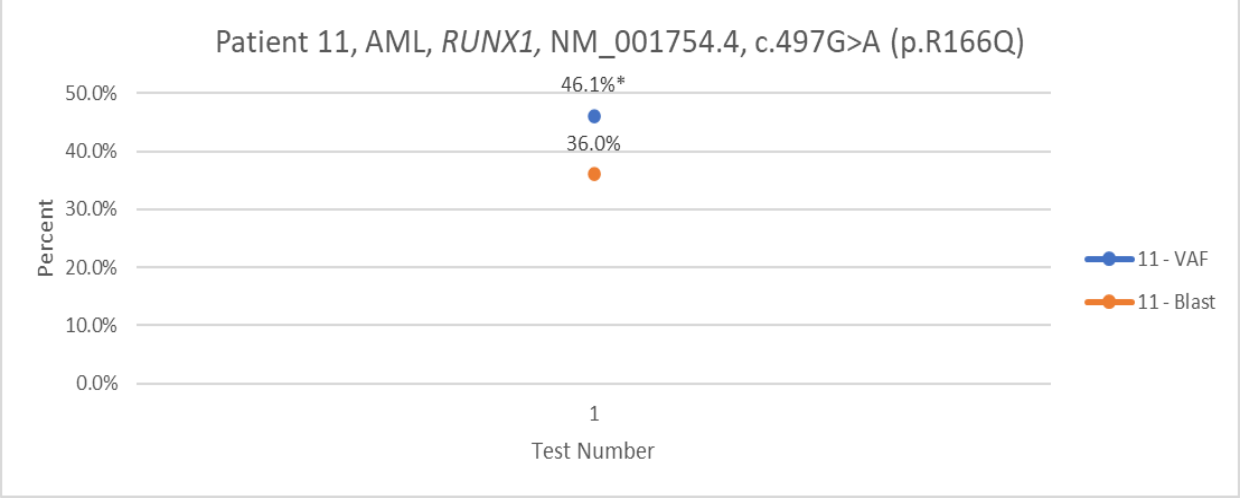
*See Appendix C for information on the Tier classification for each variant.*

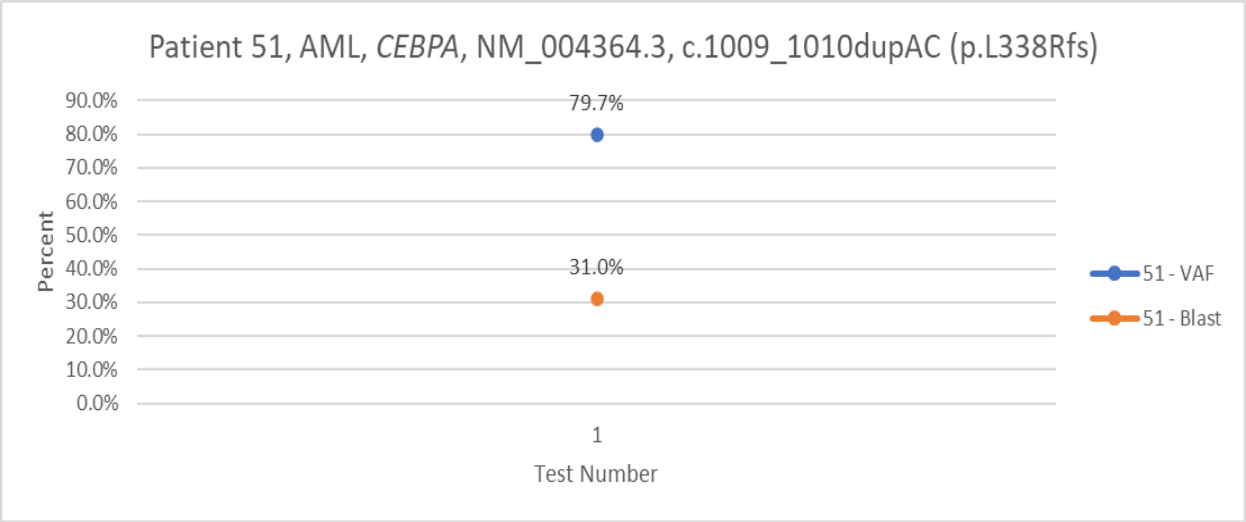
**Figure 4**

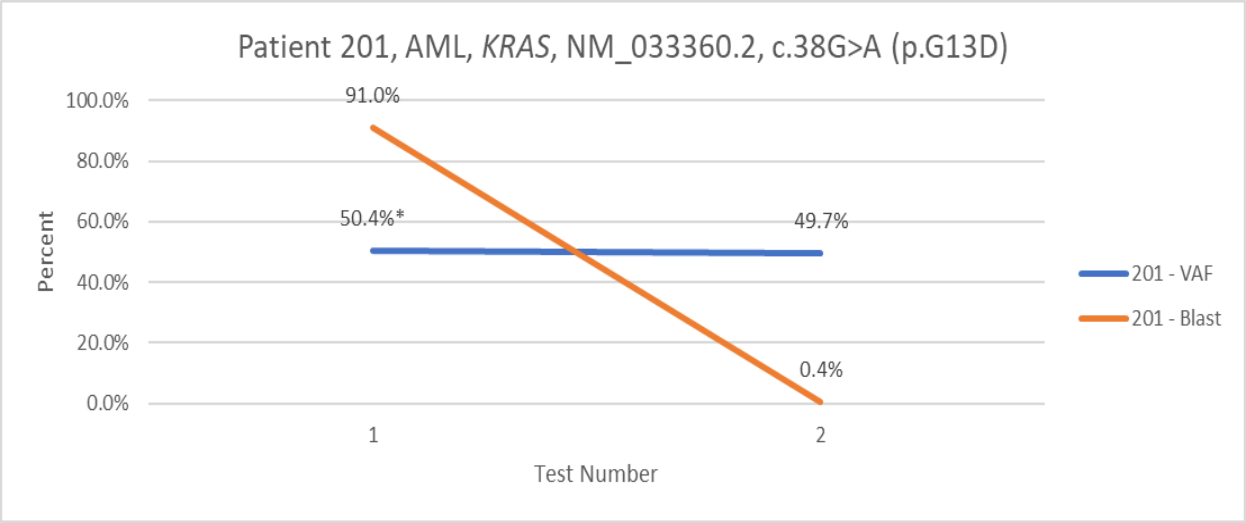
*Comparison of VAF and Blast Percentage for patients with potentially germline variants*

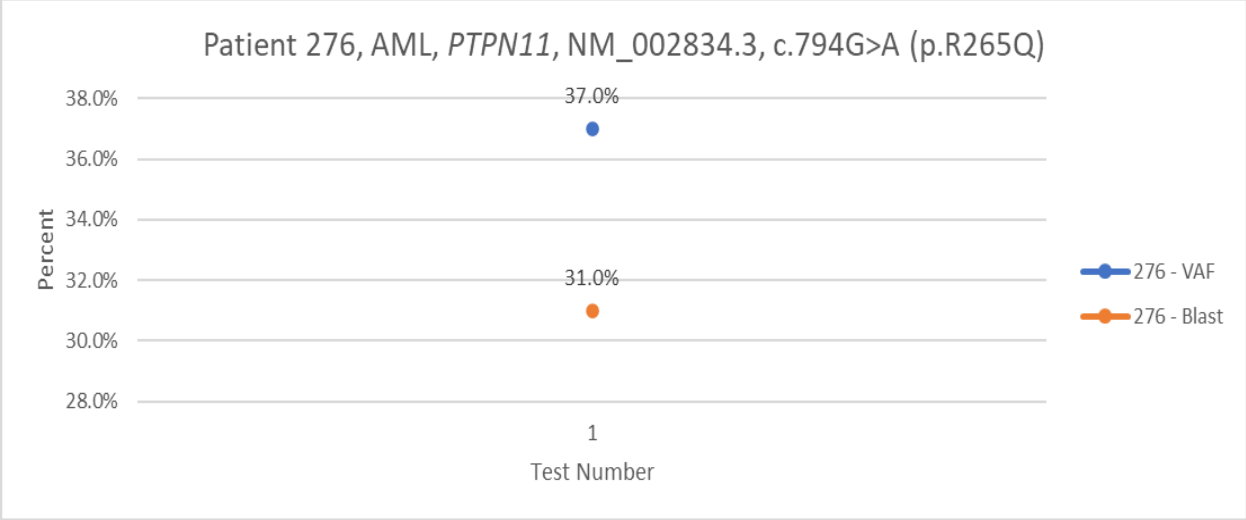


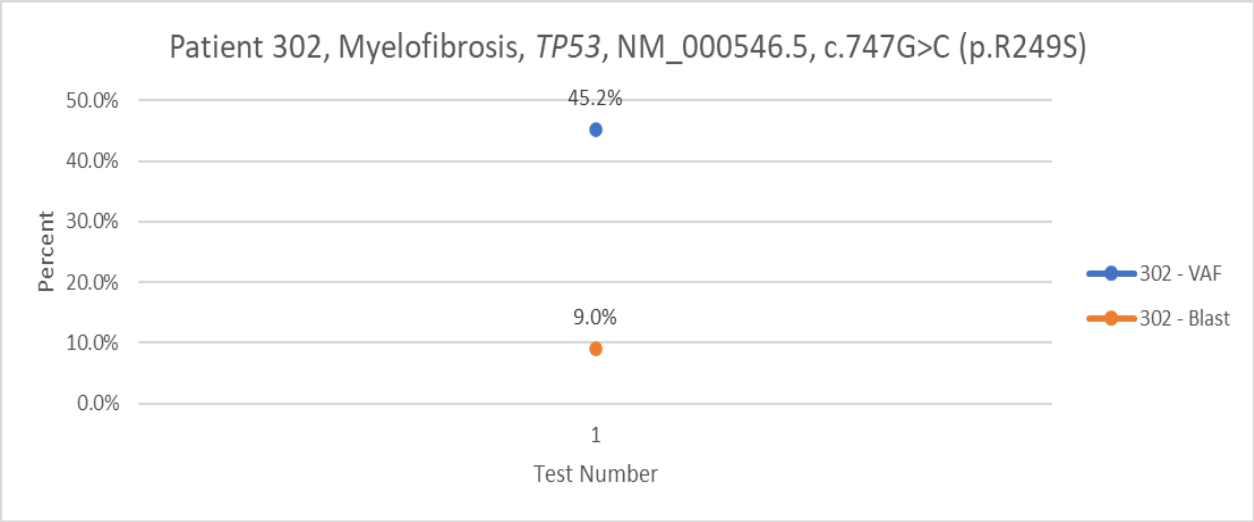



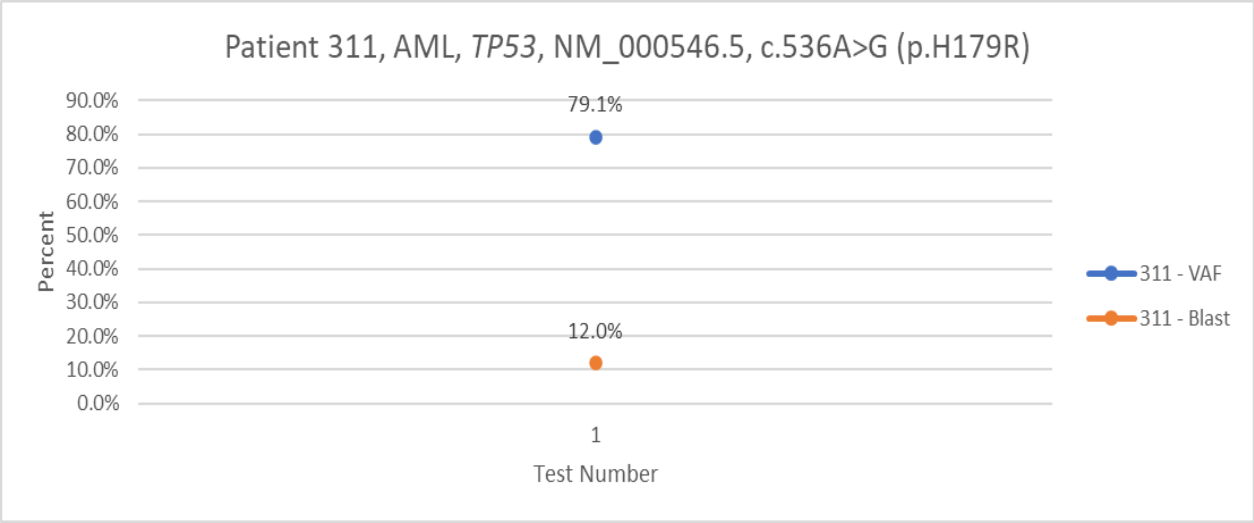



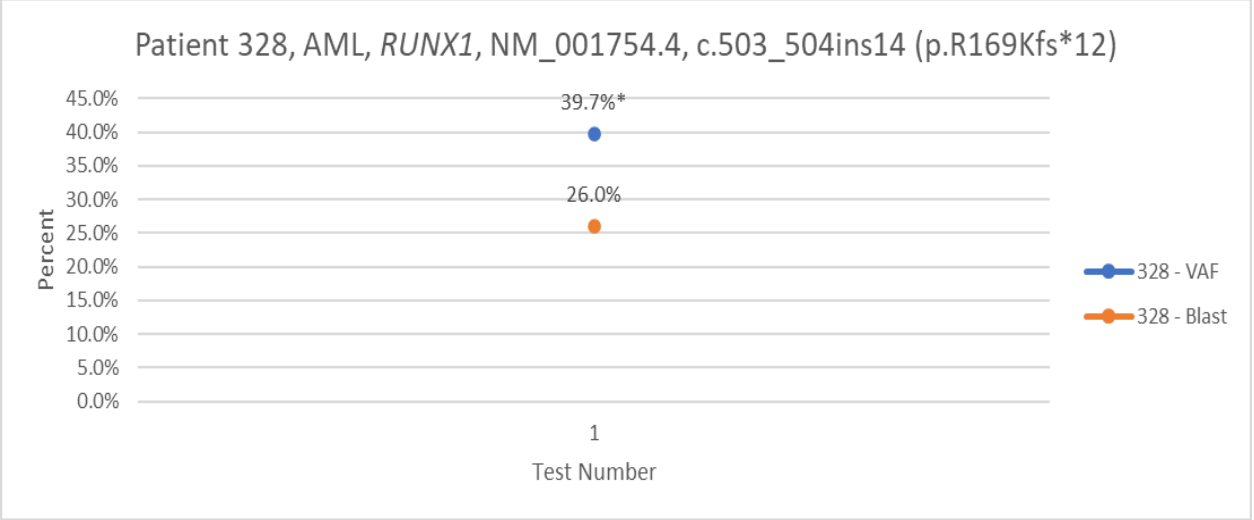

Patient 51, AML, *CEBPA*, NM_004364.3, c.1009_1010dupAC (p.L338Rfs)
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percent
79.7%
31.0%
1
Test Number
51 - VAF
51 - Blast

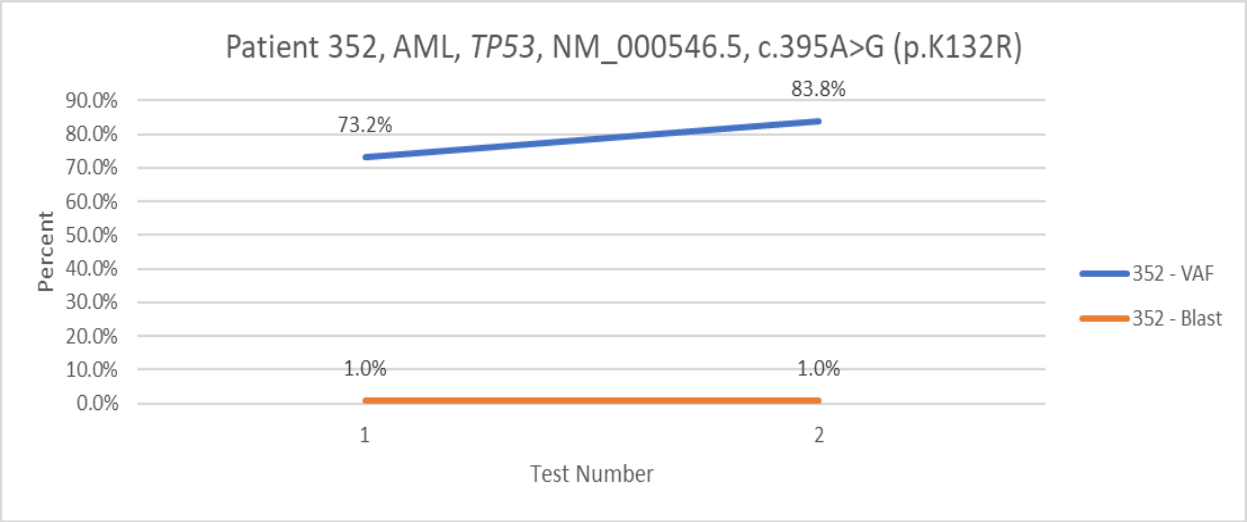

Patient 201, AML, *KRAS*, NM_033360.2, c.38G>A (p.G13D)
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Percent
91.0%
50.4%*
49.7%
0.4%
1
2
Test Number
201 - VAF
201 - Blast

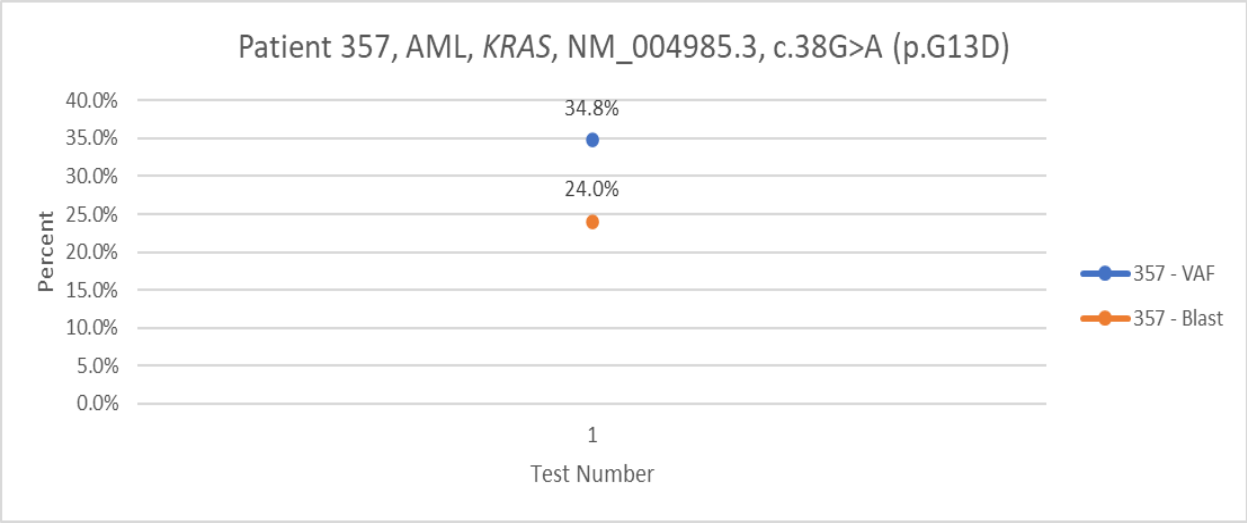

Patient 276, AML, *PTPN11*, NM_002834.3, c.794G>A (p.R265Q)
38.0%
36.0%
34.0%
32.0%
30.0%
28.0%
Percent
37.0%
31.0%
1
Test Number
276 - VAF
276 - Blast

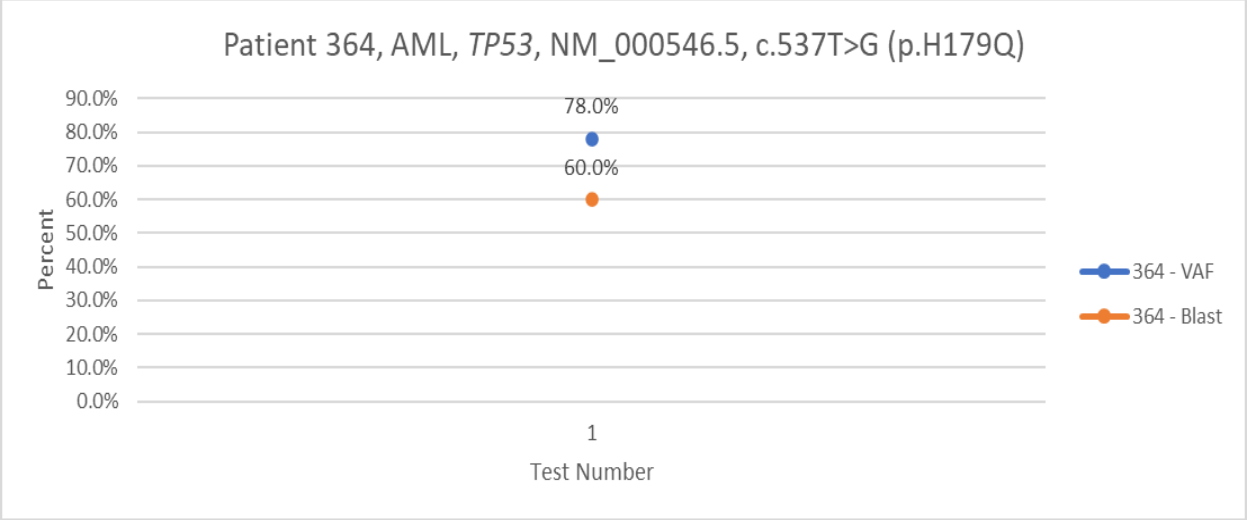

Patient 302, Myelofibrosis, *TP53*, NM_000546.5, c.747G>C (p.R249S)
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percent
45.2%
9.0%
1
Test Number
302 - VAF
302 - Blast

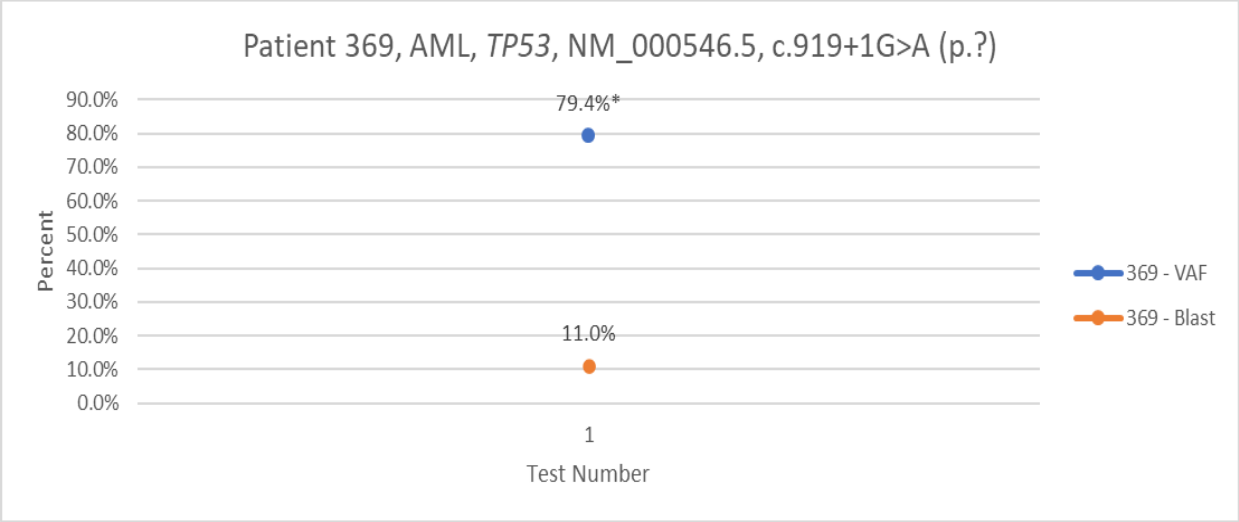

Patient 311, AML, *TP53*, NM_000546.5, c.536A>G (p.H179R)
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percent
79.1%
12.0%
1
Test Number
311 - VAF
311 - Blast


Patient 328, AML, *RUNX1*, NM_001754.4, c.503_504ins14 (p.R169Kfs*12)
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Percent
39.7%*
26.0%
1
Test Number
328 - VAF
328 - Blast


Patient 352, AML, *TP53*, NM_000546.5, c.395A>G (p.K132R)
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percent
73.2%
83.8%
1.0%
1.0%
1
2
Test Number
352 - VAF
352 - Blast


Patient 357, AML, *KRAS*, NM_004985.3, c.38G>A (p.G13D)
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Percent
34.8%
24.0%
1
Test Number
357 - VAF
357 - Blast


Patient 364, AML, *TP53*, NM_000546.5, c.537T>G (p.H179Q)
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percent
78.0%
60.0%
1
Test Number
364 - VAF
364 - Blast




**indicates that blood was used for the NGS sample type*

*Note that patient 352 had an additional NGS report run on a blood sample, with a sample collection date that was one day prior to the NGS run on bone marrow (Test Number 1, Panel 11, Figure 4). This NGS result on blood had a low VAF (12%), thus it was excluded given the result in the bone marrow sample is the most representative.*

Of the 13 variants listed in Table 4, two were identified in patients who underwent more than one NGS test, meaning that there was additional data available for review when assessing the potential for germline origin. The NGS reports were reviewed to determine whether the laboratory recommended follow up testing based on the variant's likelihood of being germline. The research team's review of the NGS reports was done after the independently assessment of each variant using ClinVar, Cosmic, and GnomAD, as detailed in the Methods section. Five of the 13 variants (38%) listed in Table 4 were in test reports that either provided a reference to the available information in ClinVar related to that variant or included a brief discussion of what the related hereditary syndrome would be if

the variant was of germline origin, but did *not* contain any specific recommendation for confirmatory testing or genetic counseling.

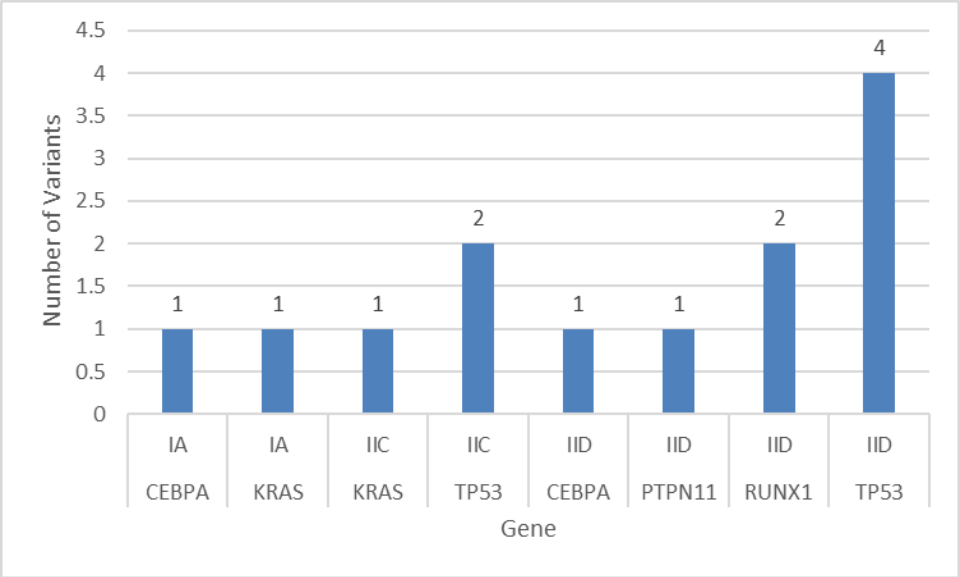
One variant, determined by the research team to be unlikely to be germline (and as such, this variant is not included in Table 4) had a specific recommendation for confirmatory germline testing (Patient # 337): "*A GATA2 missense variant is identified at an allelic fraction of 50-55% (cannot exclude germline origin). Germline testing is recommended."* However, this variant was seen at a VAF of 45.1% on this NGS report. Notably, this variant was excluded as it was not reported on two subsequent tests performed for this patient and this patient did not have BMT.

The other eight variants listed in Table 4 had no mention of germline potential origin in their associated NGS report. However, the research team did identify entries in ClinVar for four of these eight variants; those entries were submitted to ClinVar before the final NGS report was issued and so should have been included in the lab's assessment of the ClinVar data for that variant.

Of these 13 variants (Figure 5), 2 (15%) were classified by the UCI laboratory as Tier IA, none were classified as Tier IB, 3 (23%) were classified as Tier IIC, and 8 (62%) were classified as Tier IID (Li et al., 2017). There was suggestive evidence that there may be an association between variant type (potentially germline or not) and tier classification (Fisher's exact test, $p = 1.0$). However, this association did not reach statistical significance in this sample.

**Figure 5**

*Distribution of Tier Classification for variants potentially of germline origin*




*This is a representation of the number of potentially germline variants reported in each of the genes, and in which tier each variant was classified.*

### 3.4 Criteria Met

Using the 2022 ELN criteria, along with NCCN criteria for other indications for genetic testing, 52 patients (49%) from the original cohort of 110 met 2022 ELN criteria for genetic testing based on personal and/or family history of cancer (i.e. *without considering* the results of their somatic genetic testing). Twenty-four (44%) of those individuals are deceased. Four (8%) of those 52 individuals also carry a variant on somatic NGS that warrants germline follow up testing. One additional patient (#136) met criteria for germline genetic testing, but based only on 2023 NCCN criteria for Breast/Pancreas/Colon and not based on their personal or family history of myeloid malignancy. It should be noted that 50 of the 110 patients had insufficient data in their medical record to make an accurate

assessment of the family history; of those, 24 patients already met criteria for testing based on personal history factors or somatic test result but the other 26 did not meet any criteria. Missing data included age of onset of cancers in family members, as well as presence or absence of cancers other than hematologic malignancies in the family.

**Table 5**

*Criteria for Consideration of Germline Testing*

*Number of Patients who meet ANY guideline based on Personal or Family History OR somatic NGS result*

| **Meets criteria for germline genetic testing based on personal *or* family history per ELN or NCCN guidelines for Hereditary Myeloid Malignancies, HBOC, or Lynch, OR somatic NGS results** | **Count** |
|---|---|
| No | 48$ |
| Yes | 62 |
| **Total** | 110 |

*Number of Patients who meet NCCN or ELN guidelines based on Personal or Family History of Myeloid Malignancy*

| **Meets criteria for germline genetic testing based on personal *or* family history per ELN or NCCN guidelines for Hereditary Myeloid Malignancies** | **Count** |
|---|---|
| No | 58* |
| Yes | 52 |
| **Total** | **110** |

*Number of Patients who met guidelines based on Personal History, ELN guidelines*

| **Meets criteria for germline genetic testing based on *personal* history per ELN guidelines** | **Count** |
|---|---|
| No | 61 |
| Yes | 49 |
| **Total** | **110** |

*Number of Patients who meet guidelines based on Family History, ELN guidelines*

| **Meets criteria for germline genetic testing based on *family* history per ELN guidelines** | **Count** |
|---|---|
| No | 102+ |
| Yes | 8 |
| **Total** | **110** |

*Number of Patients who meet guidelines based on Personal or Family History, NCCN* guidelines *for Hereditary Breast and Ovarian Cancer Syndrome or Lynch Syndrome*

| **Meets criteria for germline genetic testing based on personal *or* family history per NCCN guidelines for HBOC or Lynch Syndrome** | **Count** |
|---|---|
| No | 101& |
| Yes | 9 |

| | |
|---|---|
| **Total** | **110** |

*Number of Patients who meet ELN guidelines based on somatic NGS results*

| **Meets criteria for germline genetic testing based on somatic NGS results** | **Count** |
|---|---|
| No | 97 |
| Yes | 13 |
| **Total** | **110** |

Note: These individuals may meet criteria for multiple reasons

$31/48 had insufficient family history information

*31/58 had insufficient family history information

+50/102 had insufficient family history information

&47/101 had insufficient family history information

Of the 57 patients who did not meet criteria for testing based on personal or family history of cancer, nine met criteria for germline NGS based on the results of their somatic NGS testing (Table 6). This underscores the utility of using somatic NGS as a screening tool, as this method identifies patients who are eligible for germline NGS that may be missed if providers only consider personal or family history.

**Table 6**

*Characteristics of variants potentially of germline origin detected in patients who do not meet 2022 ELN or 2023 NCCN criteria for germline genetic testing based on personal or family history*

| Patient # | Variant Information | VAF (%) | | | Clinvar: Absent, OR present as likely pathogenic or pathogenic | GnomAD: Absent, OR present at <1% frequency | Cosmic: Absent, OR confirmed germline |
|---|---|---|---|---|---|---|---|
| | | VAF | VAF | VAF | | | |
| **9** | *CEBPA*, NM_004364.3, c.232delC (p.L78Wfs*82) | 43 | - | - | Absent | Absent | Somatic |
| **11** | *RUNX1*, NM_001754.4, c.497G>A (p.R166Q) | 46.1 | - | - | Present as P or LP | Absent | Somatic |
| **201** | *KRAS*, NM_033360.2, c.38G>A (p.G13D) | 34.2 | 50.4 | - | Present as P or LP | Absent | Somatic |
| **276** | *PTPN11*, NM_002834.3, c.794G>A (p.R265Q) | 37 | - | - | Present as P or LP | Present at a frequency of 0.00003235 | Somatic |
| **302** | *TP53*, NM_000546.5, c.747G>C (p.R249S) | 45.2 | - | - | Present as P or LP | Absent | Confirmed Germline |
| **311** | *TP53*, NM_000546.5, c.536A>G (p.H179R) | 79.1 | - | - | Present as P or LP | Present at a frequency of 0.000003979 | Confirmed Germline |
| **352** | *TP53*, NM_000546.5, c.395A>G (p.K132R) | 73.2 | 83.8 | - | Present as P or LP | Absent | Somatic |
| **357** | *KRAS*, NM_004985.3, c.38G>A (p.G13D) | 34.8 | - | - | Present as P or LP | Absent | Somatic |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **364** | *TP53*, NM_000546.5, c.537T>G (p.H179Q) | 78 | - | - | Present as P or LP | Absent | Confirmed Germline |

*See Appendix C for information on the Tier for each variant. "-" Patient may have had one or more NGS tests, but the variant was not seen an additional time, or other NGS tests were not performed in the study period.*

In summary, when considering the 2023 NCCN guidelines for AML/MDS, Breast/Pancreas, and Colon, as well as the 2022 ELN guidelines, 62 out of 110 patients (56%) meet criteria for germline genetic testing, either based on personal or family history or NGS result, to assess hereditary cancer risk for myeloid malignancies. Thirty-five of them are still living and could benefit from a referral to genetic counseling services. Importantly, of the 13 patients who had a variant identified on somatic testing that warrants germline follow up testing, 9 (69%) of those did not meet criteria for any other reason than their NGS result. No patients were referred to genetic counseling or received germline genetic testing based on their diagnosis of a myeloid malignancy per detailed chart review.

# IV. DISCUSSION

Genetic counseling and germline genetic testing are indicated for certain individuals with cancer diagnoses or a significant family history of cancer. These services are traditionally offered to patients diagnosed with various primary cancers including breast, ovarian, pancreatic, prostate, and colon cancers. Although some cancers, such as hematologic malignancies, have not historically been considered to have a hereditary component, recent research shows that myeloid malignancies (a subset of hematologic malignancies) are hereditary about 13% of the time—a proportion that is comparable to or exceeds that of other "hereditary" cancers listed above (Yang et al., 2022). This highlights a potential gap in care for individuals diagnosed with myeloid malignancies. The specific objective of this study was to investigate the feasibility and utility of using somatic NGS test results as a screening tool for determining which patients with myeloid malignancy may need germline genetic testing by analyzing the characteristics, both of variant itself and personal/family history, of those individuals who had positive NGS results. A retrospective chart review of 110 patients was conducted to collect the results of somatic NGS testing for patients with myeloid malignancies that was performed at UCI from June 9, 2020 and August 31, 2022. Data including demographic information, somatic NGS results, pertinent medical history, and any available family history were coded for further analysis.

## 4.1 Genetic Counseling Services and Utility of Somatic Testing as a Screening Tool

As noted above, a gap in care was hypothesized to exist for patients diagnosed with myeloid malignancies given the recent advances in understanding the hereditary nature of these cancers. This study sought to define that gap by determining whether genetic counseling services were offered to a cohort of UCI Health patients with myeloid

malignancies and somatic variants that suggest possible germline predisposition. It was found that none of the patients were referred to genetics or had germline genetic testing based on either their clinical diagnosis or their somatic NGS results at UCI. No evidence was found in the medical record of any patient education on the topic or consideration of genetic counseling. There were two patients who had germline genetic test results available for review, but these patients were referred for reasons unrelated to their history of hematologic malignancy. This underscores a need for increased attention from a genetics perspective to patients with myeloid malignancies, which could include providing education for hematologic oncologists and other medical professionals as to when to refer patients, as well as education for genetic counselors to increase awareness of the need for germline testing. Reputable resources such the ELN and NCCN guidelines detail key features for identifying and managing families at increased risk for inherited myeloid malignancy syndromes from a clinical standpoint.

Additionally, this study aimed to investigate methods that could increase uptake of recommended germline genetic testing. These methods include increased awareness of and adherence to ACMG recommendations for germline genetic testing (Li et al., 2020). A greater emphasis could be placed on the utility of somatic genetic testing as a tool to screen for patients who may benefit from germline genetic testing. This study investigated its utility and found that 13/110 individuals (12%) had a variant that was potentially of germline origin and so warranted further investigation and consideration of germline testing. Four of those 13 individuals already met criteria for germline testing due to their personal or family history. This strongly suggests that somatic NGS is an appropriate screening tool, as it validates the findings of other screening methods while providing

additional clinical information to identify other individuals who may benefit from germline testing. Given that there is evidence to support the use of somatic NGS as a screening tool, laboratories could consider adding to the somatic test reports when appropriate a recommendation for consideration of further genetic counseling and/or germline testing, with language highlighting the potential for germline origin per ACMG recommendations (Drazer et al., 2018; Obrochta & Godley, 2018; Li et al., 2020). Additionally, genetic counselors, molecular geneticists, and/or pathologists could participate in hematologic tumor boards to present/discuss relevant variants of potential germline origin.

When reviewing the available family history data, it was found that 45% (n=50) of study participants lacked enough information in their medical record to accurately assess whether they met criteria for germline genetic testing. The family history data were gathered from the medical record from consult and progress notes written by non-genetics health care providers. This significant lack of information underscores the relevance of collecting detailed family history information, including ages of diagnosis for medical conditions. If the patient is being seen for a diagnosis of a hematologic malignancy, information on solid tumors in the personal or family history should be collected as well, given that solid tumor diagnoses are considered relevant when assessing for hereditary causes of hematologic malignancy. Without a detailed and comprehensive family history, providers will not be able to accurately assess whether a patient meets criteria for referral to genetics for cancer risk assessment or for any other indication.

### 4.2 Further Investigation of Potential Germline Variants in Relevant Genes

As mentioned, the study found that 13 individuals (12% of the total study population) had a variant that warranted germline follow up testing to assess for

hereditary hematological malignancy predisposition. The study team pursued a multistep process in order to characterize these variants as relevant, as described in the methods section of this paper.

As recommended by The University of Chicago Hematopoietic Malignancies Cancer Risk Team (2016) and The American College of Medical Genetics and Genomics (ACMG), providers could consider follow up testing for patients with any suspected germline finding, as the process of determining the significance of individual variants can be difficult (Li et al., 2020). Additionally, "studies have shown that more than 50% of germline [variants] identified with tumor testing would have been missed if germline testing had been limited to guideline-concordant care"; thus, a broader approach to germline confirmation testing may be indicated (Li et al., 2020). The NCCN criterion for AML recommends germline testing for any individuals with a variant in a clinically relevant gene that has a Variant Allele Frequency (VAF) >30%, especially if the VAF remains high after remission and without a history of transplant (National Comprehensive Cancer Network, 2023).

### 4.3 Implementation

Germline variant detection in patients with myeloid malignancy is a critical aspect of cancer care, as identifying a germline pathogenic variant can inform treatment decisions, clarify diagnoses, determine need for future screening, and ensure that appropriate therapies are utilized (The University of Chicago Hematopoietic Malignancies Cancer Risk Team, 2016; Li et al, 2020).

Identification of patients who have a germline variant is also important when considering the impact on the health of family members. If a variant is identified, family

members may undergo cascade testing to determine whether they also inherited the variant of interest. As discussed below, there are limitations to consider if the patient has passed away, but family members may still be candidates for genetic counseling and germline genetic testing (NGS) based on family history or other personal history factors. Once the patient or family members' variants have been identified, they may be able to pursue increased screening or preventive surgery as part of the management of their cancer risk. For example, if one patient was found to have a variant in *TP53* that was consistently identified on every somatic test performed (including after the patient has achieved remission), this increases the suspicion that the variant may be of germline origin. If this was confirmed to be of germline origin by follow up testing (using an appropriate sample type), significant changes would be made to the patient's medical management to mitigate the risk of cancer or identify cancers early in development. However, for variants in other genes related only to risk for hematologic malignancies "there is no consensus on optimal management of individuals diagnosed with a familial acute leukemia or MDS syndrome, so management must be individualized" (National Comprehensive Cancer Network, 2023).

Cascade testing for family members is especially relevant given those family members are often donors in the setting of a bone marrow transplant, which is a treatment often pursued for patients with hematologic malignancies. If a family member carries the same germline genetic variant and donates bone marrow to the proband, the patient would be at risk for donor-derived posttransplant malignancies, recurrent disease, or infection due to impaired immune reconstitution (Galera et al., 2018). Thus, germline genetic testing should always be pursued as early in the proband's treatment process as possible to avoid

using donor family members who may carry the same genetic variant (The University of Chicago Hematopoietic Malignancies Cancer Risk Team, 2016; Li et al., 2020).

All of this evidence serves to emphasize the fact that early identification of germline variants is clinically relevant and medically recommended for the management of patients with hematologic malignancies. Given the high percentage of this study's participants who met criteria for genetic testing based on ELN or NCCN criteria, it is imperative that clinicians begin to consider these recommendations more consistently and refer patients to genetic counseling services when appropriate.

This study found that over half of participants (62 out of 110 patients or 56%) should have been referred for genetic counseling services and consideration of germline testing. Germline genetic testing for patients with myeloid malignancies is no longer a rare event, especially considering that some professionals advocate for a "low threshold for referral to genetics" for their patients with myeloid malignancies (The University of Chicago Hematopoietic Malignancies Cancer Risk Team, 2016).

This study also found a high percentage (45%) of the cohort whose records were reviewed are now deceased, even though all participants included had somatic tests completed during the relatively recent period of June 2020-August 2022 (and many were newly diagnosed at the time of testing). According to the National Institutes of Health, the 5-year survival rate for AML is 31.7%. Our cohort ranged from less than six months since diagnosis to over two years, with a survival rate of 55%. These low survival rates emphasize the need for prompt and thorough genetic evaluation of patients diagnosed with myeloid malignancies so that informed treatment decisions can be made, and familial testing conducted while patients are still living.

### 4.4 Study Limitations and Future Research

Although this study's findings largely align with prior research in this field, there are several limitations which should be discussed. The most significant limitation is that due to time and resource constraints, as well as the fact that many participants were deceased, the research team was unable to conduct follow up testing on these individuals to obtain data on the frequency of germline mutations confirmed among those whose somatic test contained a potentially significant variant.

Another limitation exists because of the method of data extraction from the UCI CoPath Laboratory Information System (LIS); namely, that the CoPath search was based only on Final Diagnoses of AML, MPN, AMML, MDS, and CMML, and is not generalizable to all types of myeloid malignancy. Additionally, these NGS test reports are not kept in a format that is easily searchable in the electronic medical record, leading to possible errors in searching or potential issues in correctly identifying patients who belonged in this cohort.

Another important fact to consider is that less information was available for review when assessing variants for individuals who have only one somatic NGS test report (n= 53, 48%) when compared with patients who had multiple test reports. For those with a single NGS report, certain datapoints such as the consistency of a variant across multiple test reports or the trend of the VAF over time were not available. However, this situation is common in clinical practice, as "testing for germline risk alleles should be performed as early as possible during clinical management" and so additional somatic NGS reports may not always be available for review (Döhner et al., 2022). Furthermore, there is some variability in available information between patients who had tests completed on different

sample types. For example, if there are not enough blast cells circulating in the peripheral blood, a test on blood may not detect a variant present in the malignant cells in the bone marrow. Of the 45 patients who had an NGS test performed on a blood sample, 25 (56%) had multiple test reports available for review. Of those 25, one had a bone marrow result performed within a day (so the bone marrow result was used instead for interpreting the possible germline nature of variants seen on NGS) and two patients only had NGS tests run on blood samples available for review. Twenty of the 45 patients who had NGS performed on a blood sample (44%) had only that one NGS test to be analyzed, which limited the research team's ability to accurately assess variants on those tests. The research team was also limited by only viewing results collected during the study period, rather than using the time period to identify patients who were eligible and then evaluate all tests performed for that patient, regardless of the study period.

Additionally, the "Relevant Gene List" was curated from 2022 NCCN guidelines. There are additional genes listed in the 2022 ELN guidelines that were not included and so variants in these genes were not considered relevant for the purposes of this study. Those genes include *LANE, G6PC3GFI1, JAGN, TCRG1, VPS45A, SRP54, MDM4, NPM1, RPA1, Apollo, CLB, NRAS, MPL, RECQL4, NBN, WAS, CSF3R, JAK2, RBBP6*, and *TET2*. It should be noted that some of these (*CSF3R, RBBP6*, and *TET2*) are associated with emerging disorders and a clear relationship between variants in these genes and an increased risk of myeloid neoplasms is not well characterized at this time (Döhner et al., 2022).

As mentioned, about half of study participants (45%) were deceased as of the start of data collection. This complicated the process of informing their providers for several reasons. First, providers are not likely to see or act on the notification, given that the

patient is deceased. Second, the deceased patient is the most appropriate candidate for germline genetic testing and is unable to provide a sample. This reduces the effectiveness of germline genetic testing for family members for whom cascade testing would have been indicated if the proband had tested positive for a germline variant in a relevant gene.

Lastly, personal and family history information was used as one method to determine eligibility to undergo germline genetic testing. However, comprehensive family histories taken by genetics professionals were not available, and so the information coded into the dataset was comprised only of the limited family history available in the electronic medical chart (EMR) Additionally, is possible that not all personal history information was included in participant's UCI EMR, as they may have medical conditions treated elsewhere and so documentation was not available for review, although efforts were made to find all recorded and relevant information on participant's personal and family history. We cannot rule out the possibility that other participants may have met eligibility but were not identified due to the absence of information about relevant family history in their medical record; given that 45% were lacking sufficient family history it is likely that others were also eligible. As discussed previously, the involvement of a genetics professional is crucial to a thorough evaluation of a patient's personal and family history. Genetic counselors are trained to identify patterns of inheritance, consider pertinent positives and negatives, and understand the significance of a truncated family history (wherein patients have few family members)—all of which are nuances of risk assessment that other providers may miss.

This study aimed to assess the utility of somatic genetic testing as a screening tool for germline genetic testing. However, as mentioned, follow up germline testing was not pursued for patients in this cohort. An extension of this research could pursue follow up

testing for living individuals to better understand the utility of somatic testing as a screening tool. Other researchers could consider investigating the efficacy of various guidelines for screening and management of patients with confirmed hereditary myeloid malignancies. Current recommendations for screening for future cancers or for unaffected family members who test positive are limited or not available, so management must be individualized (National Comprehensive Cancer Network, 2023).

### 4.5 Conclusions

In conclusion, this study found that that 62 out of 110 patients (56%) who underwent somatic testing as part of their hematologic evaluation met criteria for germline genetic testing to assess hereditary cancer risk based on current guidelines from the National Comprehensive Cancer Network for AML, MDS, Breast/Pancreas, and Colon as well as guidelines from European Leukemia Network, but none received it at UCI Medical Center (Döhner et al., 2022; National Comprehensive Cancer Network, 2023). Of those 62 patients, 9 (15%) would not have met criteria without considering the results of their somatic genetic testing, which included genetic variants that could be germline in origin. These findings show that consideration of the results of somatic testing is a valuable screening tool to detect patients with myeloid malignancies who are eligible for referral to genetic counseling who may be missed by a traditional assessment of personal and family history. Additionally, the high percentage (45%) of participants who are now deceased (noting that the cohort was only comprised of patients who underwent somatic testing from June 2020-August 2022) emphasized the need for prompt and thorough genetic evaluation of patients diagnosed with myeloid malignancies. Further studies investigating this topic may benefit from a larger cohort and an investigation of the outcomes of

germline genetic testing. Despite study limitations, these findings underscore the importance of evaluating all patients with myeloid malignancy for features of hereditary myeloid malignancy syndromes and providing genetics counseling referrals/germline genetic testing as appropriate.

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**Appendix A. Relevant Gene List (Curated from the 2022 Version 3 National Comprehensive Cancer Network guidelines for Acute Myeloid Leukemia)**

| Gene | Additional Relevance-Malignancy | Additional Relevance - Hematologic | Additional Relevance - Other | Syndrome |
|---|---|---|---|---|
| *ACD* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *ANKRD26* | MDS, AML | Thrombocytopenia, platelet dysfunction | | |
| *ATG2B* | AML, CMML, ET | Myelofibrosis | | |
| *BACH1* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *BLM* | AML, MDS | | Pre- and post- natal growth restriction, photosensitive skin changes, immunodeficiency, insulin resistance, microcephaly, hypogonadism, high-pitched voice, early onset multiple cancers | Bloom Syndrome |
| *BRCA1* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |

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|---|---|---|---|---|
| *BRCA2* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *BRIP1* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *CBS* | AML, MDS | | Syndromic findings and other cancer | RASopathies |
| *CEBPA* | AML | | | |
| *CHEK2* | Myeloid neoplasms, CHIP predisposition | Breast Cancer, Colon cancer | | |
| *CTC1* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *DDX41* | MDS, AML, CMML | Monocytosis | Solid tumor predisposition | |
| *DKC1* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *DNAJC21* | AML, MDS | Bone marrow failure | Pancreatic insufficiency, skeletal abnormalities | Shwachman-Diamond |
| *EFL1* | AML, MDS | Bone marrow failure | Pancreatic insufficiency, skeletal abnormalities | Shwachman-Diamond |
| *ELANE* | AML, MDS | Neutropenia | | Congenital Neutropenia |

| | | | | |
|---|---|---|---|---|
| *EPCAM* | AML, MDS | ALL, lymphomas | Café-au-lait spots, CNS/GI/other tumors | CMMRD |
| *ERCC4* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *ERCC6L2* | MDS, AML | Marrow failure | Skeletal/cardiac abnormalities, neurological defects, erythroleukemia | |
| *ETV6* | MDS, AML, CMML, B-All, Myeloma | Thrombocytopenia, platelet dysfunction | | |
| *FANCA* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCB* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCC* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCD1* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCD2* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCE* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |

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|---|---|---|---|---|
| *FANCF* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCG* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCI* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCJ* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCL* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCM* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCN* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCO* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |

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|---|---|---|---|---|
| *FANCP* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCR* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCS* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCT* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCU* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANCV* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *FANQ* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *G6PC3* | AML, MDS | Neutropenia | | Congenital Neutropenia |
| *GATA1* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |

| | | | | |
|---|---|---|---|---|
| *GATA2* | MDS, AML, CMML | Monocytopenia, lymphopenia | Sensorineural deafness, immunodeficiency, cutaneous warts, pulmonary alveolar proteinosis, MonoMAC syndrome, Emberger syndrome | |
| *GFI1* | AML, MDS | Neutropenia | | Congenital Neutropenia |
| *GSKIP* | AML, CMML, ET | Myelofibrosis | | |
| *HAX1* | AML, MDS | Neutropenia | | Congenital Neutropenia |
| *KRAS* | AML, MDS | | Syndromic findings and other cancer | RASopathies |
| *LIG-4* | MDS, Lymphoid malignancy | Marrow failure | Short stature, microcephaly, combined immunodeficiency | |
| *MAD2L2* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *MBD4* | AML | | Colon polyps | |
| *MBD4* | AML, MDS | | Pre- and post- natal growth restriction, photosensitive skin changes, immunodeficiency, insulin resistance, microcephaly, hypogonadism, high-pitched voice, early onset multiple cancers | Bloom Syndrome |
| *MECOM/EVI1 Complex* | MDS, AML | Bone marrow failure, B-cell deficiency | Radioulnar synostosis, clinodactyly, cardiac malformations, renal malformations, hearing loss | |
| *MLH1* | AML, MDS | ALL, lymphomas | Café-au-lait spots, CNS/GI/other tumors | CMMRD |
| *MSH2* | AML, MDS | ALL, lymphomas | Café-au-lait spots, CNS/GI/other tumors | CMMRD |
| *MSH6* | AML, MDS | ALL, lymphomas | Café-au-lait spots, CNS/GI/other tumors | CMMRD |

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|---|---|---|---|---|
| *NAF1* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *NF1* | AML, MDS | | Syndromic findings and other cancer | RASopathies |
| *NHP2* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *NOP10* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *PALB2* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *PARN* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |

| | | | | |
|---|---|---|---|---|
| *PMS2* | AML, MDS | ALL, lymphomas | Café-au-lait spots, CNS/GI/ other tumors | CMMRD |
| *POT1* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *PTPN11* | AML, MDS | | Syndromic findings and other cancer | RASopathies |
| *RAD51* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *RAD51C* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *REV7* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *RLP15* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPL11* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |

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|---|---|---|---|---|
| *RPL23* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPL26* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPL27* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPL31* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPL35A* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPL5* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |

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|---|---|---|---|---|
| *RPS10* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS17* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS19* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS24* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS26* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS27* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |

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|---|---|---|---|---|
| *RPS28* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS29* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RPS7* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *RTEL1* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *RUNX1* | MDS, AML, T-cell ALL | Thrombocytopenia, platelet dysfunction | | |
| *SAMD9* | MDS, AML | Pancytopenia | Normophosphatemic familial tumor calcinosis, MIRAGE syndrome, ataxia | |
| *SAMD9L* | MDS, AML | Pancytopenia | Normophosphatemic familial tumor calcinosis, MIRAGE syndrome, ataxia | |
| *SBDS* | AML, MDS | Bone marrow failure | Pancreatic insufficiency, skeletal abnormalities | Shwachman-Diamond |

| | | | | |
|---|---|---|---|---|
| *SLX4* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *SRP72* | MDS | Marrow failure | Congenital sensorineuro deafness | |
| *TERC* | MDS, AML | Macrocytosis, cytopenias, aplastic anemia | Idiopathic pulmonary fibrosis, hepatic cirrhosis, nail dystrophy, oral leukoplakia, skin hypopigmentation, skin hyperpigmentation, premature gray hair, cerebellar hypoplasia, immunodeficiency, developmental delay | |
| *TERT* | MDS, AML | Macrocytosis, cytopenias, aplastic anemia | Idiopathic pulmonary fibrosis, hepatic cirrhosis, nail dystrophy, oral leukoplakia, skin hypopigmentation, skin hyperpigmentation, premature gray hair, cerebellar hypoplasia, immunodeficiency, developmental delay | |
| *TINF2* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenosis malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *TP53* | AML, MDS | ALL | Adrenocortical carcinoma, brain and breast cancer, chorid plexus carcinoma, other cancers | Li-Fraumeni Syndrome |
| *Trisomy 21* | AML, MDS | Transient abnormal myelopoesis/ ALL | | Down Syndrome |

| | | | | |
|---|---|---|---|---|
| *TSR2* | AML, MDS | Anemia and marrow erythroid hypoplasia | Cardiac abnormalities, Cathie facies, genitourinary abnormalities, cleft lip/palate, short stature; sarcomas; elevated erythrocyte adenosine deaminase | Diamond-Blackfan anemia |
| *UBE2T* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *WARP53* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenous malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |
| *XPC* | MDS, Increased myeloid malignancies and T cell lymphoblastic leukemia in people ages 7-29 | | Sensitivity to UV light, dry skin, freckling, hearing loss, poor coordination, loss of intellectual function, seizures, melanomas, squamous cell carcinomas | Bloom Syndrome |
| *XRCC2* | AML, MDS | Bone marrow failure | Short stature, skin pigmentation, skeletal abnormalities, squamous cell carcinomas, liver tumors, other solid tumors | Fanconi Anemia |
| *ZCCHC8* | AML, MDS | Bone marrow failure | Idiopathic pulmonary fibrosis, emphysema, early hair graying, osteoporosis, pulmonary arteriovenous malformation, hepatopulmonary syndrome, liver fibrosis-cirrhosis, enterocolitis, immune deficiency | Short telomere syndromes |

## Appendix B. MYEL75 and PANHEME Gene Lists

| Test | Gene |
|---|---|
| MYEL75 | ABL1 |
| MYEL75 | ANKRD26 |
| MYEL75 | ASXL1 |
| MYEL75 | ATRX |
| MYEL75 | BCOR |
| MYEL75 | BCORL1 |
| MYEL75 | BRAF |
| MYEL75 | BTK |
| MYEL75 | CALR |
| MYEL75 | CBL |
| MYEL75 | CBLB |
| MYEL75 | CBLC |
| MYEL75 | CCND2 |
| MYEL75 | CDC25C |
| MYEL75 | CDKN2A |
| MYEL75 | CEBPA |
| MYEL75 | CSF3R |
| MYEL75 | CUX1 |
| MYEL75 | CXCR4 |
| MYEL75 | DCK |
| MYEL75 | DDX41 |
| MYEL75 | DHX15 |
| MYEL75 | DNMT3A |
| MYEL75 | ETNK1 |
| MYEL75 | ETV6 |
| MYEL75 | EZH2 |
| MYEL75 | FBXW7 |
| MYEL75 | FLT3 |
| MYEL75 | GATA1 |
| MYEL75 | GATA2 |
| MYEL75 | GNAS |
| MYEL75 | HRAS |
| MYEL75 | IDH1 |
| MYEL75 | IDH2 |
| MYEL75 | IKZF1 |
| MYEL75 | JAK2 |
| MYEL75 | JAK3 |

| | |
|---|---|
| MYEL75 | KDM6A |
| MYEL75 | KIT |
| MYEL75 | KMT2A |
| MYEL75 | KRAS |
| MYEL75 | LUC7L2 |
| MYEL75 | MAP2K1 |
| MYEL75 | MPL |
| MYEL75 | MYC |
| MYEL75 | MYD88 |
| MYEL75 | NF1 |
| MYEL75 | NOTCH1 |
| MYEL75 | NPM1 |
| MYEL75 | NRAS |
| MYEL75 | PDGFRA |
| MYEL75 | PHF6 |
| MYEL75 | PPM1D |
| MYEL75 | PTEN |
| MYEL75 | PTPN11 |
| MYEL75 | RAD21 |
| MYEL75 | RBBP6 |
| MYEL75 | RPS14 |
| MYEL75 | RUNX1 |
| MYEL75 | SETBP1 |
| MYEL75 | SF3B1 |
| MYEL75 | SH2B3 |
| MYEL75 | SLC29A1 |
| MYEL75 | SMC1A |
| MYEL75 | SMC3 |
| MYEL75 | SRSF2 |
| MYEL75 | STAG2 |
| MYEL75 | STAT3 |
| MYEL75 | TET2 |
| MYEL75 | TP53 |
| MYEL75 | U2AF1 |
| MYEL75 | U2AF2 |
| MYEL75 | WT1 |
| MYEL75 | XPO1 |
| MYEL75 | ZRSR2 |
| PANHEME | BCOR |
| PANHEME | CBL |
| PANHEME | CDC25C |

| | |
|---|---|
| PANHEME | CDKN2A |
| PANHEME | CUX1 |
| PANHEME | ETV6 |
| PANHEME | EZH2 |
| PANHEME | FLT3 |
| PANHEME | IKZF1 |
| PANHEME | KDM6A |
| PANHEME | LUC7L2 |
| PANHEME | MYC |
| PANHEME | NF1 |
| PANHEME | PTEN |
| PANHEME | RAD21 |
| PANHEME | RPS14 |
| PANHEME | RUNX1 |
| PANHEME | TET2 |
| PANHEME | TP53 |
| PANHEME | U2AF1 |
| PANHEME | WT1 |
| PANHEME | ZRSR2 |
| PANHEME | ABL1 |
| PANHEME | ABL2 |
| PANHEME | AKT3 |
| PANHEME | ALK |
| PANHEME | ANKRD26 |
| PANHEME | ASXL1 |
| PANHEME | ATRX |
| PANHEME | BAX |
| PANHEME | BCL11B |
| PANHEME | BCL2 |
| PANHEME | BCL6 |
| PANHEME | BCOR |
| PANHEME | BCORL1 |
| PANHEME | BCR |
| PANHEME | BIRC3 |
| PANHEME | BRAF |
| PANHEME | BTK |
| PANHEME | CALR |
| PANHEME | CARD11 |
| PANHEME | CBFB |
| PANHEME | CBL |
| PANHEME | CBLB |

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|---|---|
| PANHEME | CBLC |
| PANHEME | CCND1 |
| PANHEME | CCND2 |
| PANHEME | CCND3 |
| PANHEME | CD79B |
| PANHEME | CDK6 |
| PANHEME | CDKN2A |
| PANHEME | CEBPA |
| PANHEME | CHD1 |
| PANHEME | CHIC2 |
| PANHEME | CIITA |
| PANHEME | CREBBP |
| PANHEME | CRLF2 |
| PANHEME | CSF1R |
| PANHEME | CSF3R |
| PANHEME | CUX1 |
| PANHEME | CXCR4 |
| PANHEME | DCK |
| PANHEME | DDX41 |
| PANHEME | DEK |
| PANHEME | DHX15 |
| PANHEME | DNM2 |
| PANHEME | DNMT3A |
| PANHEME | DUSP22 |
| PANHEME | EBF1 |
| PANHEME | EIF4A1 |
| PANHEME | EPOR |
| PANHEME | ERG |
| PANHEME | ETNK1 |
| PANHEME | ETV6 |
| PANHEME | EZH2 |
| PANHEME | FBXW7 |
| PANHEME | FGFR1 |
| PANHEME | FGFR2 |
| PANHEME | FGFR3 |
| PANHEME | FLT3 |
| PANHEME | GATA1 |
| PANHEME | GATA2 |
| PANHEME | GLIS2 |
| PANHEME | GNAS |
| PANHEME | HRAS |

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|---|---|
| PANHEME | IDH1 |
| PANHEME | IDH2 |
| PANHEME | IKZF1 |
| PANHEME | IKZF2 |
| PANHEME | IKZF3 |
| PANHEME | IL7R |
| PANHEME | JAK1 |
| PANHEME | JAK2 |
| PANHEME | JAK3 |
| PANHEME | KAT6A |
| PANHEME | KDM6A |
| PANHEME | KIT |
| PANHEME | KLF2 |
| PANHEME | KMT2A |
| PANHEME | KRAS |
| PANHEME | LUC7L2 |
| PANHEME | MALT1 |
| PANHEME | MAP2K1 |
| PANHEME | MECOM |
| PANHEME | MKL1 |
| PANHEME | MLF1 |
| PANHEME | MLLT10 |
| PANHEME | MLLT4 |
| PANHEME | MPL |
| PANHEME | MYC |
| PANHEME | MYD88 |
| PANHEME | MYH11 |
| PANHEME | NF1 |
| PANHEME | NFKB2 |
| PANHEME | NOTCH1 |
| PANHEME | NOTCH2 |
| PANHEME | NPM1 |
| PANHEME | NRAS |
| PANHEME | NT5C2 |
| PANHEME | NTRK3 |
| PANHEME | NUP214 |
| PANHEME | NUP98 |
| PANHEME | P2RY8 |
| PANHEME | PAG1 |
| PANHEME | PAX5 |
| PANHEME | PBX1 |

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|---|---|
| PANHEME | PDCD1LG2 |
| PANHEME | PDGFRA |
| PANHEME | PDGFRB |
| PANHEME | PHF6 |
| PANHEME | PICALM |
| PANHEME | PLCG1 |
| PANHEME | PLCG2 |
| PANHEME | PML |
| PANHEME | PPM1D |
| PANHEME | PRDM16 |
| PANHEME | PTEN |
| PANHEME | PTK2B |
| PANHEME | PTPN11 |
| PANHEME | RAD21 |
| PANHEME | RARA |
| PANHEME | RBBP6 |
| PANHEME | RBM15 |
| PANHEME | RHOA |
| PANHEME | ROS1 |
| PANHEME | RUNX1 |
| PANHEME | RUNX1T1 |
| PANHEME | SEMA6A |
| PANHEME | SETBP1 |
| PANHEME | SETD2 |
| PANHEME | SF3B1 |
| PANHEME | SH2B3 |
| PANHEME | SLC29A1 |
| PANHEME | SMC1A |
| PANHEME | SMC3 |
| PANHEME | SRSF2 |
| PANHEME | STAG2 |
| PANHEME | STAT3 |
| PANHEME | STAT5B |
| PANHEME | STAT6 |
| PANHEME | STIL |
| PANHEME | TAL1 |
| PANHEME | TCF3 |
| PANHEME | TET2 |
| PANHEME | TFG |
| PANHEME | TP53 |
| PANHEME | TP63 |

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|---|---|
| PANHEME | TYK2 |
| PANHEME | U2AF1 |
| PANHEME | U2AF2 |
| PANHEME | WT1 |
| PANHEME | XPO1 |
| PANHEME | ZCCHC7 |
| PANHEME | ZRSR2 |

**Appendix C. Variant Frequency and Tier, Detailed Report**

| Patient # | Gene Information | VAF (%) | | | Tier |
|---|---|---|---|---|---|
| | | VAF | VAF | VAF | |
| **2** | *TP53*, NM_001126113.2, c.700T>A (p.Y234N) | 63 | - | - | IID |
| **9** | *CEBPA*, NM_004364.3, c.232delC (p.L78Wfs*82) | 43 | - | - | IID |
| **11** | *RUNX1*, NM_001754.4, c.497G>A (p.R166Q) | 46.1 | - | - | IID |
| **51** | *CEBPA*, NM_004364.3, c.1009_1010dupAC (p.L338Rfs) | 79.7 | - | - | IA |
| **201** | *KRAS*, NM_033360.2, c.38G>A (p.G13D) | 34.2 | 50.4 | - | IA |
| **276** | *PTPN11*, NM_002834.3, c.794G>A (p.R265Q) | 37 | - | - | IID |
| **302** | *TP53*, NM_000546.5, c.747G>C (p.R249S) | 45.2 | - | - | IIC |
| **311** | *TP53*, NM_000546.5, c.536A>G (p.H179R) | 79.1 | - | - | IID |
| **328** | *RUNX1*, NM_001754.4, c.503_504ins14 (p.R169Kfs*12) | 39.7 | - | - | IID |
| **352** | *TP53*, NM_000546.5, c.395A>G (p.K132R) | 73.2 | 83.8 | - | IID |
| **357** | *KRAS*, NM_004985.3, c.38G>A (p.G13D) | 34.8 | - | - | IIC |
| **364** | *TP53*, NM_000546.5, c.537T>G (p.H179Q) | 78 | - | - | IID |
| **369** | *TP53*, NM_000546.5, c.919+1G>A (p.?) | 79.4 | - | - | IIC |

*"-" Patient may have had one or more NGS tests, but the variant was not seen an additional time. See Figure 4 for additional details on total number of tests completed and associated blast percentages.*

## Appendix D. Letter to Provider

DATE

Dear Dr. ***,

Your patient's (PATIENT NAME, DOB, MRN) medical record was reviewed as part of an IRB-approved study (STUDY NUMBER 1452) at UCI that analyzed results from somatic genetic testing in patients with myeloid malignancies. **Your patient's somatic test results identified a variant(s) that warrants a referral to genetic counseling and consideration of germline genetic testing to assess for a possible hereditary cancer predisposition.**

We strongly recommend that you refer your patient for cancer genetics services, either through the Cancer Genetics Clinic at UCI or another local provider (provider search tool available at https://findageneticcounselor.nsgc.org/).

If you choose to coordinate follow up testing yourself, please keep in mind that blood, saliva, and buccal samples SHOULD NOT be used for germline genetic testing for patients with myeloid malignancies, as tumor unique DNA will confound results. Cultured fibroblasts from a skin punch biopsy are the appropriate specimen for germline genetic testing in these individuals.

If you have questions about this research study, please contact the faculty sponsor, Kathryn Singh, at 714-456-6883 or kesingh@hs.uci.edu.

If you have questions about referring patients to Cancer Genetics Clinic at UCI please contact Lisa Marquez at 714-456-2246 or marquel3@hs.uci.edu.

Best,

Rachel Collier, BS, Genetic Counseling Graduate Student

Kathryn Singh, MPH, MS, LCGC

Faculty Sponsor

Health Sciences Associate Clinical Professor
Assistant Director, UCI Graduate Program in Genetic Counseling
Department of Pediatrics, Division of Genetic and Genomic Medicine
UC Irvine Health