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Evaluation of patients and families with concern for predispositions to hematologic malignancies within the Hereditary Hematologic Malignancy Clinic (HHMC)

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Abstract

Introduction—Although multiple predispositions to hematologic malignancies exist, evaluations for hereditary cancer syndromes (HCS) are underperformed by most hematologist/

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oncologists. Criteria for initiating HCS evaluation are poorly defined, and results of genetic testing for hereditary hematologic malignancies have not been systematically reported.

Patients/Methods—From April 2014 to August 2015, 67 patients were referred to the Hereditary Hematologic Malignancy Clinic (HHMC). Referral reasons included (1) bone marrow failure or myelodysplastic syndrome in patients > 50 years, (2) evaluation for germline inheritance of identified *RUNX1*, *GATA2*, or *CEBPA* mutations on targeted next-generation sequencing panels, (3) strong personal and/or family history of malignancy. Cultured skin fibroblasts were utilized for germline DNA in all patients with hematologic malignancy.

Results—Eight (12%) patients were clinically diagnosed with a HCS; 4 patients with *RUNX1*-related familial platelet disorder (FPD)/AML, and one patient each with dyskeratosis congenita, Fanconi anemia, germline *DDX41*, and Li-Fraumeni Syndrome (LFS). Two patients with concern for FPD/AML and LFS, respectively, had *RUNX1* and *TP53* variants of unknown significance. Additionally, four patients with prior HCS diagnosis (1 LFS, 3 FPD/AML) were referred for further evaluation and surveillance.

Conclusion—In this HHMC-referred hematologic malignancy cohort, HCS was confirmed in twelve patients (18%). HCS identification provides insight for improved and individualized treatment, and screening/surveillance opportunities for family members. The HHMC has facilitated HCS diagnosis, and advocates that with increased clinical awareness of hematologic malignancy predisposition syndromes, more patients who may benefit from evaluation can be identified. Mutation panels intended for prognostication may provide increased clinical suspicion for germline testing.

Keywords

predisposition; germline; MDS; AML; inherited; HCS

Introduction

Hereditary cancer syndromes (HCS) such as Li-Fraumeni Syndrome (LFS), hereditary non-polyposis colorectal cancer (HNPCC), and hereditary breast and ovarian cancer syndrome (HBOC) are well-established familial syndromes within solid tumor oncology. The identification of heterozygous germline mutations in the well-annotated cancer susceptibility genes *TP53*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *BRCA1* and *BRCA2* impact treatment decisions of an affected patient directly, and identify individuals who may benefit from potentially life-saving screening and prevention approaches among affected family members. There are currently over 200 hereditary cancer susceptibility syndromes defined, estimated to account for 5–10% of all cancer diagnoses.(1, 2)

Within hematologic malignancies, there is increasing appreciation for the genetic basis of certain inherited bone marrow failure (BMF) syndromes such as Fanconi anemia (FA) and telomere syndromes including dyskeratosis congenita (DC).(3) BMF syndromes have been characteristically diagnosed in children, although approximately 10% of patients with FA and a majority of short telomere syndrome patients are diagnosed as adults.(4–8) Despite critical therapy implications such as avoidance of DNA crosslinking agents, high-intensity regimens, and informing appropriate sibling selection for stem cell transplantation (SCT),

HCS evaluation among patients with hematologic malignancies is thought to be routinely underperformed, particularly among adults. Within the past decade, nearly a dozen adult-onset inherited leukemia and myelodysplastic syndrome (MDS) predisposition syndromes have been defined (Table 1). Although considered quite rare, the low level of clinician awareness of these syndromes, coupled with the frequently insufficient family history obtained during the routine care of patients with hematologic malignancies, suggests these syndromes are considerably under-diagnosed. Furthermore, among patients with strong family histories of hematologic malignancies who *are* tested for currently known hereditary predisposition syndromes, many lack an identifiable genetic cause, suggesting that additional pathogenic loci remain to be discovered.(9)

Moreover, with the growing importance of next-generation sequencing (NGS) technologies to evaluate for prognostically-significant or targetable genomic alterations in patients with hematologic malignancies, detecting mutations that could be of either somatic or germline origin (e.g. *RUNX1*, *CEBPA*, *GATA2*) has become increasingly available and commonplace. Given the difficulty of obtaining a germline “control” in the hematologic malignancy population, determination of whether the NGS-identified mutation is somatically-acquired or inherited is not straightforward; yet accurate classification is critical for optimal patient management.

The Hereditary Hematologic Malignancy Clinic (HHMC) was established for the dedicated evaluation and monitoring of patients with hematologic malignancies and suspected germline predisposition to malignancy. We herein report our experience and initial results from the first 18 months of genetic counseling and evaluation.

Patients and Methods

Patient Selection

From April 2014 to August 2015, 67 patients between the ages of 13 to 80, from 65 unique families, were referred to the HHMC at The University of Texas M.D. Anderson Cancer Center (UTMDACC). Approximately 500 patients with AML and 350 with MDS were referred to the Department of Leukemia at UTMDACC during this same time period. For manuscript clarity given the varied reasons for HHMC referral, the primary reason for HHMC referral has been apportioned to one of the following groups/cohorts, which are detailed separately within the manuscript:

- A. Diagnosis of BMF, aplastic anemia (AA) or MDS in patients 50 years of age, or any age with concerning clinicopathologic features of a bone marrow failure syndrome.(7, 10)
- B. Evaluation for germline inheritance in patients with hematologic malignancies and 1 known mutation(s) of *RUNX1*, *GATA2*, or *CEBPA* on a targeted NGS MDS/AML panel
- C. A hematologic malignancy patient with multiple primary malignancies and/or a strong family history of cancer(s) at early age

- D.** A hematologic malignancy patient with one or more first-degree relatives, or 2 second-degree relatives, with hematologic malignancies.
- E.** Referral for evaluation, counseling and continued follow-up for patients with a previously identified HCS with associated risk for hematologic malignancy.

Sequencing of UTMDACC Patient Samples

NGS-based CLIA-approved targeted mutation analysis of 28 genes implicated in hematologic malignancies is routinely performed on DNA from bone marrow aspirates in all MDS and AML patients referred to UTMDACC as previously described.(11, 12) The coding sequence of *ABL1*, *ASXL1*, *BRAF*, *DNMT3A*, *EGFR*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *KIT*, *KRAS*, *MDM2*, *IKZF2*, *JAK2*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PTPN11*, *RUNX1*, *TET2*, *TP53* and *WT1* are sequenced. *CEBPA* analysis is performed separately as previously described via Sanger sequencing.(13) Identified sequence variants are classified based on analytic findings and curated reference databases (e.g. COSMIC, dbSNP). Known germline polymorphisms, defined by membership in dbSNP build 138/1000 genomes dataset and/or presence in >20% of our patient population were excluded.

Genetic Evaluation

All patients referred to the HHMC underwent a standard assessment including evaluation, family history, and pedigree analysis by a certified genetic counselor (S.A.B., M.M.) and hematologist/oncologist with expertise in HCS (C.D.D.). Patients with a clinical phenotype suggesting a specific HCS underwent syndrome-specific genetic testing, such as chromosome breakage analysis for FA evaluation or lymphocyte telomere length (TL) by flow-FISH for DC. Patients referred for evaluation of a variant identified within our UTMDACC NGS cancer panel underwent gene or site-specific CLIA-approved germline testing performed on cultured skin fibroblasts. Clinical genetic testing performed was patient-specific, based on personal and family history, current medical knowledge, and available clinical testing. For patients from “Group D” with a family history of hematologic malignancies, clinical testing was provided using an Agilent Clinical Research Exome kit which has been iteratively revised to include the following genes (with the percentage of the coding region covered at >10X) by exome sequencing: ANKRD26 (100%), CEBPA (61%), DDX41 (98%), ETV6 (100%), GATA2 (100%), RUNX1 (92%), SRP72 (100%), and TP53 (100%).

In all patients with active hematologic malignancy or post-allogeneic transplantation, a 4mm skin punch biopsy was performed, and skin fibroblasts were cultured to obtain germline DNA. Blood or saliva specimens for germline DNA analysis were obtained for the evaluation of consented family members. Written informed consent was obtained following institutional guidelines in accordance with the Declaration of Helsinki.

Statistical Analysis

Personal and family histories, demographics, and clinicopathologic characteristics were obtained from the electronic health record and genetic counseling session results. Patient

characteristics are summarized using median (range) for continuous variables and frequency (percentage) for categorical variables.

Results

Sixty-seven patients from 65 unique families were referred to the HHMC. Evaluated patients who declined germline genetic testing (n=2) are included. Results are detailed below by reason for patient referral.

BMF/AA/MDS at young age [Group A]

Eight patients (Table 2) were referred for a history of BMF/AA/MDS at a young age, defined here as ≤ 50 years of age at diagnosis, or any age with clinical symptoms suggestive of a BMF syndrome (i.e. short stature, skeletal abnormalities, pulmonary fibrosis, abnormal skin, dentition or hair).^(7, 10) The median age was 39 (range 14–55 years), and five were female. Testing for FA was performed by diepoxybutane (DEB) induced chromosomal breakage assay, and DC by TL flow-FISH analysis and/or gene panel testing. A TL below the age-adjusted first percentile, in the setting of confirmatory clinical history, is consistent with a telomere syndrome.⁽¹⁴⁾ Existing gene panel testing identifies a causative mutation in approximately 50–70% of patients with DC (e.g. *TERT*, *TERC*), while the remaining DC patients have uninformative genetic panel testing resulting from unknown genetic cause. In this patient cohort, germline *RUNX1* testing was also performed in one patient with a lifelong history of thrombocytopenia and multiple additional family members with thrombocytopenia, MDS and AML suggestive of FPD/AML.

Three (38%) of eight Group A patients were diagnosed with a HCS. The pedigrees of these three individuals are depicted in Figure 2(a–c). One 53-year-old male with a personal history of MDS, pulmonary fibrosis, short stature, premature grey hair, whose father and brother also have idiopathic pulmonary fibrosis, had an age-adjusted TL $<1\%$ in lymphocytes and granulocytes consistent with a telomeropathy, and a pathogenic heterozygous *TERT* R865H variant on gene panel testing.⁽¹⁵⁾ A 14-year-old female with BMF diagnosed at age 11, also with congenital skeletal abnormalities including radial arm defects, was diagnosed with FA by DEB assay, with homozygous pathogenic *BRIP1* p.R798* variants on FA gene sequencing.⁽¹⁶⁾ A 54-year-old female with lifelong thrombocytopenia and easy bruising/bleeding, MDS diagnosed at age 48, and a strong family history of MDS/AML was identified to have a germline *RUNX1* p.P240Hfs deletion leading to a diagnosis of FPD/AML. In these three patients, screening for family members and appropriate selection of siblings for SCT donors has been possible.

Testing for germline status of *CEBPA*, *GATA2* and *RUNX1* mutations identified from NGS-based prognostication panel [Group B]

Germline inheritance of *CEBPA*, *GATA2* or *RUNX1* mutations lead to well-described, autosomal dominant, hematologic malignancy syndromes (Table 1) with variable penetrance.^(17–21) Seventeen patients were referred for germline investigation of *CEBPA*, *GATA2* and/or *RUNX1* mutations, after one or more of these mutations was identified from an NGS-based hematologic malignancy panel performed routinely for disease

prognostication at our institution. As germline samples in patients with hematologic malignancies are not typically obtained, standard sequencing cannot distinguish between germline or somatic nature, and some mutations identified on leukemia sequencing panels may be inherited. An emphasis for referral was placed on patients in whom mutations were present near-heterozygous (40–60%) or near-homozygous (90%) allelic frequency, and/or with the presence of multiple mutations within one of these genes. During this time period at our institution, 771 patients with MDS or AML had NGS panel testing and 88 *CEBPA* (11%), 15 *GATA2* (2%) and 105 *RUNX1* (14%) pathogenic variants were reported.

Two *CEBPA*-mutated patients (one single-mutant and one double-mutant *CEBPA* also with *GATA2* mutation), 6 *GATA2*-mutated patients, and 10 *RUNX1*-mutated patients were referred (Table 3) for germline evaluation. Upon genetic counseling, one 36-year-old male with an NGS-identified *RUNX1* p.P200fs was ascertained to have a significant family history of thrombocytopenia and leukemia. He declined germline evaluation, for fear of placing the blame of his and his sister's leukemia onto his mother, who notably shares a history of lifetime mild thrombocytopenia. Results of germline analysis by site-specific mutation testing of cultured fibroblasts in the remaining 16 patients revealed that 0/2 *CEBPA*, 0/6 *GATA2*, and 2/9 *RUNX1*-mutated patients with NGS-identified mutations were of germline origin.

Germline *GATA2* mutations lead to *GATA2* (MonoMAC) syndrome, characterized by profound B-lymphocyte, monocyte, NK, and dendritic cell deficiencies, frequent atypical infections and a propensity to develop MDS/AML.(22–25) Three patients referred for germline *GATA2* testing had experienced recurrent and disseminated viral and fungal infections and culture-negative pneumonia. Notably, flow cytometry was consistent with the anticipated phenotype of germline *GATA2* mutations, i.e. the lack of a defined monocyte population, poorly-defined lymphocyte population, and decreased frequency of NK cells with specific loss of the CD56^{bright} subset (26) (Supplemental Figure 1); yet skin fibroblast analysis for constitutional *GATA2* mutations, including evaluation for intronic variants, was wild-type in all patients. This suggests the presence of an additional unidentified germline *GATA2* mutation in these three patients, or alternatively that acquired *GATA2* mutations may impart a MonoMAC-like phenotype at the time of somatic *GATA2* acquisition.

Two patients referred for NGS-identified *RUNX1* mutations were identified to have a germline *RUNX1* mutation leading to the diagnosis of FPD/AML (Figure 2d and 1e). One patient was a 13-year-old female with lifetime mild thrombocytopenia and a concern for MDS at an outside institution. Her bone marrow biopsy at referral demonstrated abnormal megakaryocytes without morphologic criteria for MDS and a *RUNX1* duplication (c. 1098_1103dupCGGCAT). She was subsequently referred to the HHMC where her *RUNX1* duplication was recognized to be of germline origin; she continues to be monitored with clinical exam and surveillance blood work every six months. The second patient was a 46-year-old man with a presumptive historical diagnosis of ITP status-post splenectomy, whose father was deceased from CMML transformed to AML at age 77, and whose daughter was referred to our institution at age 5 for deletion 5q- MDS. Both the daughter and father had a *RUNX1* p.K194N variant detected on institutional NGS sequencing; confirmed in the father to be germline through clinical *RUNX1* sequencing. The p.K194N variant is predicted to be

deleterious by *in-silico* analysis programs (e.g. Polyphen-2 and SIFT). Functional testing of the RUNX1 p.K194N variant by luciferase reporter assay demonstrated lack of transactivation compared to wild-type *RUNX1*, confirming likely pathogenicity (Supplemental Figure 2).

Of additional interest, a 55-year-old male with myeloid neoplasm with eosinophilia and *FGFR1* gene rearrangement had two RUNX1 mutations identified on sequencing panel, a p.R201* truncating mutation and p.G387A missense variant of uncertain significance (VUS). The p.R201* was confirmed somatic, and the p.G387A was present in his germline, and clinically classified as a VUS by the testing laboratory. His brother was his SCT donor, and he was notably characterized as a poor mobilizer (<4 million/kg CD34+ cells obtained) and shared the germline p.G387A VUS.

Multiple primary malignancies or strong family history of cancers at early ages (Group C)

Thirteen patients with hematologic malignancies were referred for evaluation due to a history of multiple primary malignancies and/or a strong family history of relatives with cancer(s) (Table 4). The median age was 52 years (range 25–73). Seven of 13 patients met Revised Chompret criteria for *TP53* testing.(27) In addition, one 40-year-old female with a historical diagnosis of LFS and personal history of breast cancer and sarcoma was referred at the time of a new diagnosis of therapy-related AML (Table 5). Her germline *TP53* abnormality consisted of a deletion spanning exons 10–11, which incidentally was not identified on her leukemia NGS panel-based testing, likely due to the difficulty of identifying large deletions/duplications on NGS-based sequencing platforms not specifically designed for this purpose.

One 24-year-old male with a synchronous diagnosis of hypodiploid T-cell ALL and low-grade astrocytoma had a confirmed germline deleterious *TP53* p.R175H mutation identified. His NGS panel notably identified the p.R175H mutation with an allelic frequency reported as >90%, presumably due to somatic loss of heterozygosity (LOH). (Figure 2f)

A 44-year-old man with rhabdomyosarcoma diagnosed at age 42 treated with surgery, chemotherapy and radiation was referred to the HHMC at the time of diagnosis of therapy-related AML with complex cytogenetic abnormalities. His sister died at age 3 of an unknown malignancy, and his father died at age 43 of cancer of unknown primary. His leukemia NGS panel identified two *TP53* variants, p.R267Q and p.R156H. *TP53* testing of cultured fibroblasts demonstrated both variants present in his germline. *In-silico* analysis of p.R267Q lists this change as damaging (SIFT) and probably damaging (Polyphen), and recent analysis has demonstrated <10% *TP53* activity with this mutant.(28) Likewise, *in-silico* analysis lists p.R156H as damaging (SIFT) and probably damaging (Polyphen), with <20% *TP53* activity. Thus, a diagnosis of LFS is considered very likely and possibly related to two pathogenic germline *TP53* variants; however, this is currently unable to be clinically confirmed. One additional patient declined germline testing, and the remainder of evaluated patients tested negative for germline *TP53* abnormalities.

Patients with >1 first-degree relative or >2 2nd degree relatives with hematologic malignancies (Group D)

This diverse cohort includes patients from 11 families with MDS/AML, 2 families with *JAK2* p.V617F-mutated MPNs, and 8 families with multiple affected members having B-cell malignancies including CLL and NHLs. One of the 11 families with MDS/AML was identified to have a *RUNX1* heterozygous 5'-end deletion of at least exons 1–6 (Figure 2g). While this specific deletion has not been previously reported, other exon-level deletions spanning the RUNT homology domain of *RUNX1* are confirmed pathogenic variants leading to FPD/AML. Once again, the NGS-based AML prognostication panel performed on the index patient in this family notably failed to identify this large *RUNX1* deletion.

In the index patient from another of the 11 MDS/AML families, which includes two brothers diagnosed with diploid AML at age 64 and 67 (Figure 2h) and mother with non-Hodgkin lymphoma at age 84, a previously described pathogenic start-loss substitution in *DDX41* (c. 3G>A, p.M1I) was detected.(29)

Research-based and iterative clinically-actionable sequencing is ongoing for the remaining families. Notably *ETV6* and *DDX41* was not part of the original clinical panel evaluated in the majority of the MDS/AML families. As it is now possible to sequence these genes in a CLIA-approved manner on cultured skin fibroblasts, this is being performed in all MDS/AML families above as clinically possible.

Referral for evaluation and monitoring in patients with known/previously identified HCS Group E)

In addition to the female patient with LFS described above who was referred for additional counseling and evaluation, three adult individuals were referred for follow-up and care related to germline *RUNX1* mutations leading to FPD/AML. In all three instances, either their children or a sibling's child was first diagnosed with FPD/AML after evaluation for childhood thrombocytopenia and bleeding complications, and appropriate clinical screening of family members led to their FPD/AML diagnosis. All three have a longstanding history of mild to moderate thrombocytopenia with variable bleeding/bruising propensity. With current ages of 27, 42 and 57, they continue to be followed with laboratory work and clinical evaluation biannually.

Discussion

Over 18 months, 67 patients from 65 families with hematologic malignancies were evaluated for known or suspected hereditary cancer predisposition syndromes in a dedicated clinic, providing several important and valuable insights. First, while overall rare, eight adolescents and adults (12%) were diagnosed with a HCS by the HHMC within this time interval (Figure 2), offering evidence that with improved awareness of hereditary hematologic malignancies, more patients who may benefit from genetic counseling and testing can be referred, and careful attention to clinical and pathologic information can identify high-risk patients and families.

Second, many of the referred patients have a striking family history of hematologic malignancies, yet no known HCS was identified, supporting the notion that additional germline genetic aberrations exist which remain to be identified. Much insight can be gained regarding function and importance of involved genes and pathways through these discoveries. The 700-kb duplication leading to overexpression of *ATG2B* and *GSKIP*, increasing progenitor sensitivity to thrombopoietin (TPO) and leading to increased risk of MPN exemplifies this opportunity.(30) Available clinical-based screening must be iterative and continually updated based on available evidence to incorporate validated discoveries. Panel-based genetic testing (e.g. <http://dnatesting.uchicago.edu/>) is clinically available and provides a cost-effective approach incorporating known relevant genes. In patients and families for whom a predisposition syndrome is suspected but available clinical testing is negative, consideration of research-based testing and ongoing periodic surveillance is advisable.

Third, HCS identification is not only beneficial for the patient with regards to informing appropriate treatment strategies and selection of sibling transplant donors, but may also benefit family members. Screening potentially-affected individuals within confirmed HCS families can provide clarity and surveillance opportunities for affected yet asymptomatic family members. Current expert opinion guidelines for carriers recommend a bone marrow biopsy at diagnosis, biannual physical exam and CBC, and repeat bone marrow evaluation at the time of any changes from baseline.(31) Evaluation for clonal hematopoiesis in patients with predispositions to hematologic malignancies, which can for example be detected in >80% of asymptomatic germline *RUNX1* carriers by age 50, may also provide utility as a mechanism of disease surveillance.(32)

Fourth, patients with presumed somatic mutations identified on NGS-based cancer panels may instead have a germline event, and evaluation of the patient must consider this possibility. Germline inheritance may be more likely in situations of biallelic mutations or near-heterozygous or homozygous allelic frequency, the latter due to somatic LOH. This is an area of ongoing research; notably the “threshold” allelic frequency to warrant germline evaluation is not standardized and further research is needed. It is essential to remember that NGS-panel testing often fails to identify large deletions/duplications, unless specific assay designs and bioinformatics pipelines are utilized. Mutation panels intended for somatic testing for prognostication purposes are *not* definitive for the germline analysis of patients, and at best can only be used to supplement high clinical suspicion to initiate germline analysis.

Fifth, several patients with ostensibly somatic acquisition of *GATA2* mutations experienced frequent atypical infections including disseminated viral and fungal infections and flow cytometry results consistent with monoMAC, suggesting a *GATA2*-specific clinical phenotype, regardless of constitutional or acquired nature. To the best of our knowledge, this is the first report describing a cellular deficiency phenotype in patients without identified constitutive *GATA2* mutations.

Finally, the method of obtaining germline DNA is of paramount importance for patients with hematologic malignancies. Blood is not acceptable due to the inability to distinguish

germline from somatic mutations, and saliva is not recommended due to frequent tumor contamination.(33) Skin fibroblasts are the most appropriate germline controls. In our clinic to date, only two patients declined germline evaluation, neither due to the procedural requirement, suggesting this is not a significant burden for most patients. We did not experience any 4mm punch biopsies leading to insufficient fibroblast culture growth or DNA procurement, suggesting even smaller biopsies in the future may be sufficient. The time required to attain results from a skin biopsy must be considered, as it typically requires 6–8 weeks from the time of procedure to obtaining results.

In conclusion, four new patients/families were diagnosed with FPD/AML, and one patient each with DC, FA, LFS and germline DDX41-related MDS/AML. Additionally, we identified two patients with germline VUS results, one each in *RUNX1* and *TP53*, in patients/families with strong clinical concern for these syndromes. Improved understanding of the genetic basis of hereditary cancer predisposition syndromes provides additional diagnostic information, insights into treatment strategies, and more accurate evaluation and risk assessments to family members. Surveillance of affected yet asymptomatic family members will lead to improved understanding of the mechanisms of cancer progression and should be the focus of improved surveillance and prevention techniques.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Garber JE, Offit K. Hereditary cancer predisposition syndromes. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2005 Jan 10; 23(2):276–92. [PubMed: 15637391]
2. Ngeow J, Liu C, Zhou K, Frick KD, Matchar DB, Eng C. Detecting Germline PTEN Mutations Among At-Risk Patients With Cancer: An Age- and Sex-Specific Cost-Effectiveness Analysis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015 Aug 10; 33(23):2537–44. [PubMed: 26169622]
3. Collopy LC, Walne AJ, Cardoso S, de la Fuente J, Mohamed M, Toriello H, et al. Triallelic and epigenetic-like inheritance in human disorders of telomerase. *Blood*. 2015 Jul 9; 126(2):176–84. [PubMed: 26024875]
4. Alter BP. Cancer in Fanconi anemia, 1927–2001. *Cancer*. 2003 Jan 15; 97(2):425–40. [PubMed: 12518367]
5. Savage SA, Alter BP. Dyskeratosis congenita. *Hematology/oncology clinics of North America*. 2009 Apr; 23(2):215–31. [PubMed: 19327580]
6. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. *Blood*. 2009 Jun 25; 113(26):6549–57. [PubMed: 19282459]

7. Alter BP. Diagnosis, genetics, and management of inherited bone marrow failure syndromes. Hematology/the Education Program of the American Society of Hematology American Society of Hematology Education Program. 2007;29–39. Epub 2007/11/21. eng. [PubMed: 18024606]
8. Stanley SE, Armanios M. The short and long telomere syndromes: paired paradigms for molecular medicine. Current opinion in genetics & development. 2015 Jul 28;33:1–9. [PubMed: 26232116]
9. Churpek JE, Lorenz R, Nedumgottil S, Onel K, Olopade OI, Sorrell A, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. Leukemia & lymphoma. 2012 Jul 9. Epub 2012/06/14. Eng.
10. Babushok DV, Bessler M. Genetic predisposition syndromes: when should they be considered in the work-up of MDS? Best practice & research Clinical haematology. 2015 Mar; 28(1):55–68. [PubMed: 25659730]
11. Luthra R, Patel KP, Reddy NG, Haghshenas V, Routbort MJ, Harmon MA, et al. Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring. Haematologica. 2014 Mar; 99(3):465–73. [PubMed: 24142997]
12. Ok CY, Patel KP, Garcia-Manero G, Routbort MJ, Fu B, Tang G, et al. Mutational profiling of therapy-related myelodysplastic syndromes and acute myeloid leukemia by next generation sequencing, a comparison with de novo diseases. Leukemia research. 2015 Mar; 39(3):348–54. [PubMed: 25573287]
13. Patel KP, Ravandi F, Ma D, Paladugu A, Barkoh BA, Medeiros LJ, et al. Acute myeloid leukemia with IDH1 or IDH2 mutation: frequency and clinicopathologic features. American journal of clinical pathology. 2011 Jan; 135(1):35–45. [PubMed: 21173122]
14. Alter BP, Rosenberg PS, Giri N, Baerlocher GM, Lansdorp PM, Savage SA. Telomere length is associated with disease severity and declines with age in dyskeratosis congenita. Haematologica. 2012 Mar; 97(3):353–9. [PubMed: 22058220]
15. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proceedings of the National Academy of Sciences of the United States of America. 2007 May 1; 104(18):7552–7. [PubMed: 17460043]
16. Levitus M, Waisfisz Q, Godthelp BC, de Vries Y, Hussain S, Wiegant WW, et al. The DNA helicase BRIP1 is defective in Fanconi anemia complementation group. J Nature genetics. 2005 Sep; 37(9):934–5. [PubMed: 16116423]
17. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. The New England journal of medicine. 2004 Dec 2; 351(23):2403–7. [PubMed: 15575056]
18. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nature genetics. 1999 Oct; 23(2):166–75. Epub 1999/10/03. eng. [PubMed: 10508512]
19. Owen CJ, Toze CL, Koochin A, Forrest DL, Smith CA, Stevens JM, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood. 2008 Dec 1; 112(12):4639–45. [PubMed: 18723428]
20. Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nature genetics. 2011 Oct; 43(10):929–31. [PubMed: 21892158]
21. Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nature genetics. 2011 Oct; 43(10):1012–7. [PubMed: 21892162]
22. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014 Feb 6; 123(6):809–21. [PubMed: 24227816]
23. Mir MA, Kochuparambil ST, Abraham RS, Rodriguez V, Howard M, Hsu AP, et al. Spectrum of myeloid neoplasms and immune deficiency associated with germline GATA2 mutations. Cancer medicine. 2015 Apr; 4(4):490–9. [PubMed: 25619630]

24. Ganapathi KA, Townsley DM, Hsu AP, Arthur DC, Zerbe CS, Cuellar-Rodriguez J, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. *Blood*. 2015 Jan 1; 125(1):56–70. [PubMed: 25359990]
25. Wlodarski MW, Hirabayashi S, Pastor V, Stary J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics and prognosis of GATA2-related myelodysplastic syndromes (MDS) in children and adolescents. *Blood*. 2015 Dec 23.
26. Mace EM, Hsu AP, Monaco-Shawver L, Makedonas G, Rosen JB, Dropulic L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. *Blood*. 2013 Apr 4; 121(14):2669–77. [PubMed: 23365458]
27. Tinat J, Bougeard G, Baert-Desurmont S, Vasseur S, Martin C, Bouvignies E, et al. 2009 version of the Chompret criteria for Li Fraumeni syndrome. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009 Sep 10; 27(26):e108–9. author reply e10. [PubMed: 19652052]
28. Soussi T, Leroy B, Taschner PE. Recommendations for analyzing and reporting TP53 gene variants in the high-throughput sequencing era. *Human mutation*. 2014 Jun; 35(6):766–78. [PubMed: 24729566]
29. Lewinsohn M, Brown AL, Weinel LM, Phung C, Rafidi G, Lee MK, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. *Blood*. 2016 Feb 25; 127(8):1017–23. [PubMed: 26712909]
30. Saliba J, Saint-Martin C, Di Stefano A, Lenglet G, Marty C, Keren B, et al. Germline duplication of ATG2B and GSKIP predisposes to familial myeloid malignancies. *Nature genetics*. 2015 Aug 17.
31. Churpek JE, Lorenz R, Nedumgottil S, Onel K, Olopade OI, Sorrell A, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. *Leukemia & lymphoma*. 2013 Jan; 54(1):28–35. [PubMed: 22691122]
32. Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germline and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood*. 2015 Oct 22.
33. Heinrichs S, Li C, Look AT. SNP array analysis in hematologic malignancies: avoiding false discoveries. *Blood*. 2010 May 27; 115(21):4157–61. [PubMed: 20304806]

Clinical Practice Points

- Multiple predispositions to hematologic malignancies exist, yet evaluation for hereditary cancer syndromes (HCS) in patients with hematologic malignancies are underperformed by most hematologist/oncologists.
- In patients with hematologic malignancy, reasons for referral to genetic counseling should include: bone marrow failure or myelodysplastic syndrome in patients < 50 years, strong personal and/or family history of malignancy, and testing for germline status of mutations (i.e. *CEBPA*, *GATA2*, and *RUNX1*) identified on next-generation sequencing (NGS) panels intended for prognostication.
- Patients with presumed somatic mutations identified on NGS-based cancer panels may instead have a germline event.
- Cultured skin fibroblasts are the most appropriate germline DNA source for patients with hematologic malignancies.
- HCS Identification can inform appropriate treatment strategies and selection of sibling donors, and may also provide screening and surveillance opportunities for affected family members.
- Clinically available HCS testing is limited to the current state of knowledge, thus evaluation and genetic testing of patients with concern for a HCS must include periodic surveillance and iterative testing.
- With improved awareness of hematologic malignancy predisposition syndromes, more patients who may benefit from genetic counseling and testing can be identified; careful attention to personal and family history and pathologic data can identify high-risk patients and families.

Figure 1.

Flow diagram of patients referred to the Hereditary Hematologic Malignancy Clinic (HHMC) including primary reason for referral and brief summary of findings

BMF indicated bone marrow failure; AA: aplastic anemia; MDS: myelodysplastic syndrome; HCS: hereditary cancer syndrome; MPN: myeloproliferative neoplasm; CLL: chronic lymphocytic leukemia; NHL: non-Hodgkin lymphoma

Figure 2.

Pedigrees of patients/families with a hereditary cancer syndrome identified. a) Dyskeratosis Congenita (germline TERT p.R865H), b) Fanconi anemia (homozygous BRIP1 p.R798*), c) FPD/AML (germline RUNX1 p.P240Hfs deletion), d) FPD/AML (germline RUNX1 c.1098_1103dupCGGCAT duplication), e) FPD/AML (germline RUNX1 p.K194N); f) Li-Fraumeni Syndrome (germline TP53 p.R175H), g) FPD/AML (germline RUNX1 gene deletion spanning exons 1–6), h) germline DDX41 (p.M1I)
Arrowhead indicates the index patient referred to the HHMC. NOS (green): hematologic malignancy, not otherwise specified; AML (red): acute myeloid leukemia; EOS:

eosinophilia; thrombocytopenia (light blue): long-standing history of thrombocytopenia; MPN (yellow): myeloproliferative neoplasm; MDS (red): myelodysplastic syndrome; AA (red): aplastic anemia.

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Table 1

Inherited Syndromes Predisposing to Adult-Onset Hematologic Malignancy

Syndrome	Gene	Inheritance	Hematologic Malignancy Risk	Other hematologic abnormalities	Non-hematologic complications
Familial platelet disorder with propensity to myeloid malignancies	<i>RUNX1</i>	AD	MDS/AML/T-cell ALL	Thrombocytopenia, bleeding propensity, aspirin-like platelet dysfunction	Eczema
Thrombocytopenia 2	<i>ANKRD26</i>	AD	MDS/AML	Thrombocytopenia, bleeding propensity	None described
Familial AML with mutated <i>CEBPA</i>	<i>CEBPA</i>	AD	AML	None	None described
Familial AML with mutated <i>DDX41</i>	<i>DDX41</i>	AD	MDS/AML, CMML	None	None described
Thrombocytopenia 5	<i>ETV6</i>	AD	MDS/AML, CMML, B-cell ALL, multiple myeloma	Thrombocytopenia	Possible risk for solid tumors
Familial MDS/AML with mutated <i>GATA2</i>	<i>GATA2</i>	AD	MDS/AML/CMML	None or Neutropenia, monocytopenia (MonoMAC syndrome)	Lymphedema, sensorineural hearing loss, extra-genital warts, other
Telomere syndromes (dyskeratosis congenita)	<i>TERC, TERT, CTC1, DKC1, NHP2, NOP10, RTEL1, TINF2, WRAP53, ACD, PARN</i>	AD, AR	MDS/AML	Macrocytosis, aplastic anemia	Pulmonary, hepatic fibrosis; head, neck, ano-genital SCC
Familial aplastic anemia with <i>SRP72</i> mutation	<i>SRP72</i>	AD	MDS	Aplastic anemia	Hearing loss
Familial B- cell ALL with <i>PAX5</i> mutation	<i>PAX5</i>	AD	ALL	None	None described
Germline <i>SH2B3</i>	<i>SH2B3</i>	AR	ALL	None	None described
Li-Fraumeni syndrome	<i>TP53</i>	AD	ALL (esp hypodiploid cytogenetics), therapy-related myeloid disorders	None	Multiple solid tumors (breast, sarcoma, brain)
Fanconi anemia ²	<i>FANCA, FANCB, FANCC, BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, BRIPI, FANCL, FANCM, PALB2, RAD51C, SLX4</i>	AR	MDS/AML	Aplastic anemia	Head, neck, anogenital SCC, developmental delay, skeletal and renal anomalies

AD=autosomal dominant, AR=autosomal recessive, MDS=myelodysplastic syndrome, AML=acute myeloid leukemia, ALL=acute lymphoblastic leukemia, CMML=chronic myelomonocytic leukemia, SCC=squamous cell carcinoma

¹These DC genes are typically diagnosed in infants/children with DC

The majority of individuals with Fanconi anemia are diagnosed in childhood; however, 10% of patients lack classic birth defects and present in adulthood²

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Table 2

Patients referred to the HHMC for BMF/MDS/AA at young age

Age/Sex	Dx and Age at Dx	PMHx	Family History	Cytogenetics	Testing Performed
44/M	Bone marrow failure/hypocellular MDS	Gilbert's Syndrome	Not significant	46,XY[20]	DEB assay negative 10% age-adjusted telomere length
27/F	Anemia age 12 PNH diagnosis age 22	History of sessile serrated adenoma	Father with CRC age 34 with history of multiple polyps Both paternal grandparents with colon cancer	46,XX[20]	DEB assay negative DC panel negative MLH1, MSH2, MSH6, PMS2, EPCAM negative
35/F	Hypocellular MDS age 32	Abnormal teeth, premature grey	Not significant	45,XX,-5,der(7);17(p10;q10),+mar[3] 46,XX[26]	DEB assay negative DC panel negative
53/M	MDS age 50	Ankylosing spondylitis Pulmonary Fibrosis Premature gray hair	Brother with pulmonary fibrosis age 47, father age 50	46,XY,+1,der(1;21)(q10;q10)[1]/46,XY[19]	<1% telomere length Heterozygous TERF1 R865H mutation
14/F	Anemia since birth Bone marrow failure age 10	Congenital hip dysplasia, absent thumbs bilaterally and absent right radius	Brother with aortic stenosis at birth Consanguinity present	46,XX[20]	DEB assay positive Homozygous BRIP1 p.R798*
58/M	MDS age 55 AML age 58	Premature gray, abnormal nails and dentition, short stature	Not significant	46,XY,del(11)(q13q23)[10]/46,idel,del(20)(q11.2q13.3)[2]/46,XY[8]	DEB assay negative Telomere length normal
53/F	MDS age 48 AML age 51	Longstanding easy bruising	1 sister AML age 6, 1 sister MDS age 48, 1 brother with NHL and 1 brother AML age 31. 3 of 4 first maternal cousins with AML	46,XX,t(2;22)(p23;q13.1), del(7)(q22q32)[20]	DEB assay negative Germline RU/NXI c. 719delC p.P240Hfs*14
19/F	MDS age 18 s/p SCT Relapsed MDS age 19	Developmental delay, distal symphalangism, short stature, congenital hip malformations	Not significant	40-45,XX,-3,add(5)(q13),-6,-7,-14,-15,-18,-21,+0-4mar[cp20]	DEB assay (of skin fibroblasts) negative

BMF = bone marrow failure, AA = aplastic anemia, MDS = myelodysplastic syndrome, SCT = stem cell consultation, PNH = paroxysmal nocturnal hemoglobinuria, DEB = diepoxybutane, NHL = non-hodgkin lymphoma, N/A = not applicable

Table 3

Referral for germline evaluation of presumed somatic mutations

Age/Sex	NGS Panel	Variant %	Alteration	Cytogenetics	Heme Malignancy & Significant PMHx	Family History of Malignancy	Germline Mutation?
69/F	CEBPA	3.7%	c.146del p.Pro49fs	46,XX[20]	AML dx age 69 s/p SCT	Brother with AML age 48, unknown details	N
	NPM1	2.7%	c.860_863dupTCTG p.W288fs				
	TET2	29.4%	c.1793del p.N598fs				
	TET2	27.4%	c.4063_4087del p.A1355fs				
59/F	CEBPA	35.9%	c.946_948dupGAG p.E316dup	46,XX[20]	AML dx age 59	Father died of pancreatic cancer age 33. Paternal cousin with AML in 50s	N
	CEBPA	27.3%	c.68dupC p.H24fs				
	GATA2	36.3%	c.962T>A p.L321H				
	TET2	32.5%	c.4145A>G p.H1382R				
55/M	GATA2	52.1%	c.1169_1177del p.K390_G392del	46, XY [20]	ITP at age 21	None	N
	DNMT3A	46.6%	c.2645G>A p.R882H		Burkitt Lymphoma age 43		
	PTPN11	4.7%	c.182A>T p.D61V		MDS/MPN age 55		
					Recurrent viral infections including HSV pneumonia		
62/M	GATA2	48.1%	c.1168_1170del p.K390del	47,XY,+1,der(1;7) (q10;p10),+8[20]	MDS dx age 62	None	N
	GATA2	49.8%	c.573dupG p.S192fs*9		Hospitalized for PNA at age 57		
	IKZF2	46.5%	c.1404T>G p.L468M		Monomac Flow		
	TET2	49.4%	c.5738G>A p.G1913D				
78/M	GATA2	50.64	c.1172_1175del p.E391fs*85	46,XY[20]	Prostate cancer s/p XRT age 76.	Brother with unknown leukemia age 77, deceased	N
	ASXL1	47.8%	c.2967dupT p.E990*		MDS/MPN classified as atypical CML		
	TET2	50.2%	c.4048G>A p.E1350K				
80/F	GATA2	52.1%	c.973_974insAT p.M325fs*2	46,XX[20]	Hx of rheumatoid arthritis s/p IMiDs	Sister with post-menopausal breast cancer	N
	NRAS	45.5%	c.34G>C p.G12R		MDS/MPN-U		
57/M	GATA2	49.36%	c.1168A>G p.K390E	47,XY,+8[20]	Thrombocytopenia for 20 yrs	None	N
	KIT	49.5%	c.2089C>A p.H697N		MDS/MPN classified as atypical CML		

Age/Sex	NGS Panel	Variant %	Alteration	Cytogenetics	Heme Malignancy & Significant PMHx	Family History of Malignancy	Germline Mutation?
Multiple recurrent viral infections Culture-negative necrotic skin ulcers Monomac Flow							
36/M	RUNX1	45.5%	c.593_596dupATGG p.P200fs*	46,XY[20]	Thrombocytopenia and abnormal platelet function tests since age 10	Sister with ALL deceased age 14 Mother with lifetime thrombocytopenia	Declined germline testing
	EGFR	10.1%	c.1231C>T p.P411S		AML dx age 36		
46/M	RUNX1	50.4%	c.582A>C p.K194N	46,XX[28] 46,XY,del(11)(q1 3q23)[2]	Thrombocytopenia since age 13 Mild dysplasia but no MDS diagnosis	2 daughters with thrombocytopenia; one with MDS age 6	RUNX1 confirmed as germline
	RUNX1	39.1%	c.601C>T p.R201*	46,XX,t(8;13)(p1 1.2;q12)[19] 46,XY[1]	Myeloid neoplasm with eosinophilia and FGFR1 gene rearrangement detected by FISH	Brother poor SCT mobilizer with same G387A VUS. Another brother with sarcoma in his 30s, alive	Germline RUNX1 G387A VUS
71/F	RUNX1	26.3%	c.593A>G p.D198G	46,XX[20]	MDS No preceding cytopenias	None	N
	RUNX1	54.6%	c.796C>T p.Q266*				
54/M	ASXL1	63.0%	c.1900_1922del p.E635fs				
	RUNX1	48.0%	c.602G>A p.R201Q	46,XX,t(9;22)(q3 4;q11.2)[20]	PH+ ALL	None	N
13/F	RUNX1	46.0%	c.1098_1103dupCGGCAT	46,XX,inv(9)(p12 q13)[20]	Known thrombocytopenia since infancy	N (limited paternal information)	RUNX1 Confirmed as germline
	TET2	47.0%	p.I366_G367dup c.1598T>C p.M533T	*constitutional chromosomal polymorphism			
45/M	RUNX1	46.4%	c.497G>A p.R166Q	47,XY,+8[10] 48,idem,+9[8] 46,XY[2]	Thrombocytopenia for 3 yrs prior to MDS age 45	Mother with thrombocytosis	N
	DNMT3A	38.3%	c.2644C>T p.R882C				
	IDH1	33.6%	c.394C>T p.R132C				
	IDH2	14.1%	c.419G>A p.R140Q				
	ASXL1	48.0%	c.4493C>T p.T1498M				
58/M	RUNX1	6.0%	c.602G>A p.R201Q	46,XY,dup(11)(q 13q23)[6]/46,XY[14]	MDS	None	N
	RUNX1	40.8%	c.820del p.Q274fs				

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Age/Sex	NGS Panel	Variant %	Alteration	Cytogenetics	Heme Malignancy & Significant PMHx	Family History of Malignancy	Germline Mutation?
55/M	RUNX1	5.0%	c.595G>T p.G199W	46,XY,del(20)(q1 1.2q13.3)(6)/46,XY[14]	Therapy-related MDS age 55 History of PH+ ALL age 53	None	N
	RUNX1	6.0%	c.806-2A>T				
	RUNX1	22.2%	c.819_835del p.I273fs				
57/M	RUNX1	41.1%	c.402dupT p.G135fs	46,XY[20]	MDS dx age 56	Brother with HCV and HCC	N
	NRAS	23.7%	c.35G>A p.G12D				

Table 4

Referral for history of multiple primary malignancies and/or family history of relatives with cancer at early ages

Age/ Sex	Cancer History	Family History	Somatic Mutation Testing	Cytogenetics	Met Revised Chompret Criteria?	Germline Mutation?
58/M	Testicular seminoma age 10 months (tx surgery only)	Son with astrocytoma age 5	JAK2- V617F	46,XY[20]	No	TP53 Negative
	Papillary thyroid cancer age 44 tx RAI	Mother died age 63 of lung cancer				
46/F	Essential thrombocytosis/MPN age 44	Maternal aunt with bone cancer age 50	N/A	46,XX,t(9;22)(q34;q11.2)[19] 46,1dem,t(5;16)(q31;p13.1)[1]	Yes	TP53 Negative MMR testing also negative
	Rectal cancer age 32	Father lung cancer age 68				
48/M	Cervical carcinoma in situ age 34	Paternal grandmother with rectal cancer age 75	N/A	N/A	No	TP53 Negative
	Bronchioalveolar carcinoma age 35					
68/F	CML age 45		TP53 c.507_530del p.M169_P177delin sI 68.3% allelic frequency	43~46,XX,del(5)(q13q35),+6,+9,-10, der(11)dup(11)(q23q24) add(11)(q24)dup(11)(q23q24) add(11)(q24),der(11)t(5;11)(q13;p15)dup(11)(q23q24) add(11)(q24)dup(11)(q23q24)add(11)(q24),-13,der(13;17)(q10;q10), der(16)add(16)(p13.3)ins(?:11)(?:q23q24)x2add(11)(q24),+19,add(21)(p11.2),+1~2mar[cp18]	No	TP53 Negative
	Hodgkin Lymphoma age 15	2 paternal uncles with unknown cancers in 50–60s				
40/F	s/p XRT CML age 29 s/p SCT		ALL sample: KRAS c.38G>A p.G13D Glosarcoma: BRAF c.1799T>A p.Y600E	46,XX,add(8)(p21),-9,i(9)(q10),t(14;19)(q32;p13),+der(7)(9:?) (p13:?) [18]	Yes	MLH1, MSH2, MSH6, PMS2, EPCAM, NF1, TP53 all negative
	Meningioma age 47 s/p resection	Sister lymphoma age 32				
	Papillary and follicular thyroid cancer age 48	Sister melanoma age 35	Pre-B ALL age 37			
	Colorectal cancer age 52 s/p surgery and chemoXRT					
	Osteosarcoma age 63 s/p surgery and chemotx		Pre-B ALL age 37			
	Acute myeloid leukemia age 68					

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Age/ Sex	Cancer History	Family History	Somatic Mutation Testing	Cytogenetics	Met Revised Chompret Criteria?	Germline Mutation?
25/M	T-cell ALL and synchronous low- grade astrocytoma age 24	Daughter with rhabdomyosarcoma age 2	TP53 c.524G>A p.R175H NOTCH1 c.4754T>C p.L1585P NOTCH1 c.7003dupC p.L2335fs*19	1~45,XY,-1,add(1)(p13),add(1)(p36.1),add(5)(q31),der(6)add(6)(p12)dup(6)(q23q25),+7,-12,-16,del(17)(p11.2),-19,+21,+1~2mar[cp13]/46,XY[7]	Yes	Germline TP53 p.R175H mutation *93.8% allelic frequency, presumed LOH
71/F	DCIS age 57 s/p surgery and tamoxifen CLL age 66 Bilateral papillary thyroid cancer age 69 s/p RAI Pulmonary neuroendocrine tumor age 70	Brother with bronchioalveolar carcinoma age 66 Brother with testicular cancer age 64 Father with lung cancer, nonsmoker at 69	N/A	46,XX[20]	No	TP53 and PTEN negative
45/M	Rhabdomyosarcoma age 42 s/p adriamycin/ ifosfamide x 6 cycles and 50 Gy XRT and surgery Acute Myeloid Leukemia age 44	Sister died at age 3 of cancer Father died at age 43 with cancer of unknown primary	TP53 c.800G>A p.R267Q (59.2% allelic frequency) TP53 c.467G>A p.R156H (54.9% allelic frequency) TET2 c.4138C>T p.H1380Y	44,XY,del(5)(q13),add(7)(q11.2), -11,-12,-17,-17,+t,+mar[18]/44,XY,del(5)(q13q33),add(7)(q11.2),-11,-12,-17,-17,+1~2mar[cp2]/	Yes	Both TP53 mutations identified in germline and clinically classified as VUS *clinical TP53 testing in his mother initiated for additional characterization of inheritance
62/M	Liposarcoma age 24 s/p XRT and surgery Leiomyosarcoma R leg Myxoid malignant fibrous histiocytoma age 60 CLL/SLI age 60	Identical twin brother with CLL age 55 and leiomyosarcoma right leg age 58, Deceased age 61. Sister with DCIS age 49. Niece with melanoma age 12, Mother with breast cancer age 44, deceased age 46	N/A	N/A		TP53 negative
52/F	CLL age 44	Brother with sarcoma age 20 Mother with breast cancer age 36	TP53 c.743 G>A p.R248Q (17.5% allelic frequency)	46,XX[20]		TP53, BRCA1 and BRCA2 negative
28/F	Diploid AML diagnosed during pregnancy age 22	Nephew with sarcoma age 14	N/A	46,XX[20]		TP53 testing negative

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Age/ Sex	Cancer History	Family History	Somatic Mutation Testing	Cytogenetics	Met Revised Chompret Criteria?	Germline Mutation?
73/M	Gastric cancer age 45 s/p partial gastrectomy	One daughter with meningioma	TP53 c.614A>G p.Y205C (21.2% allelic frequency)	45,XX,add(2)(p12),-5,-7,t(11;17)(q13;p11.2),+mar[7]/46,XY[13]		Declined testing
	Prostate cancer age 68 s/p surgery	One brother with prostate cancer age 60	TET2 c.3765C>A p.Y1255*			
	Hodgkin lymphoma age 70 s/p chemox					
	Marginal zone lymphoma age 72					
	s/p Rituxan Myelodysplastic syndrome age 73					
57/M	CML diagnosed age 54	Sister deceased breast cancer age 37 Maternal aunt with breast cancer age 70	BRCA1/2 and TP53 tumor testing negative	46,XX,t(9;22)(q34;q11.2)[18]/46,XX[2]		TP53, BRCA1 and BRCA2 negative

Table 5

HHMC referral for patient with a previously identified hereditary cancer syndrome

Age/Sex	Significant PMHx	Family History	Previously Identified Germline Mutation
57/M	Lifetime mild thrombocytopenia ($70-90 \times 10^9/L$) Recurrent sinus infections	Daughter with lifetime severe thrombocytopenia	RUNX1 p.W279*
27/M	Lifetime mild thrombocytopenia ($80-120 \times 10^9/L$) and elective surgeries complicated by major bleeding Chronic sinusitis and recurrent ear infections Presumptive dx of ITP	Son and daughter with severe thrombocytopenia identified at age 1 and 4	RUNX1 p.T104*
40/F	Age 30: Stage II ER/PR+ invasive ductal breast cancer s/p neoadjuvant Taxotere and Xeloda followed b FEC (5-FU, epirubicin and cyclophosphamide) followed by surgery, radiation therapy and Tamoxifen. Age 35: Fibromyxoid sarcoma of R thigh s/p neoadjuvant Adriamycin/ifosfamide and XRT. Age 37: Spindle cell sarcoma R thigh s/p gemcitabine and Taxotere Age 40: Acute myeloid leukemia with complex cytogenetics	Son deceased age 6 medulloblastoma Daughter deceased age 12 metastatic adrenal gland tumor Mother deceased age 35 gastric cancer Maternal grandmother deceased age 45 gastric cancer	TP53 deletion spanning exons 10 through 11 <i>*not apparent on NGS-based AML prognostication panel</i>
42/M	Lifetime mild thrombocytopenia ($70-80 \times 10^9/L$)	Mother with AML age 60. Maternal grandmother with AML age 45. Sister and niece with lifetime thrombocytopenia	RUNX1 p.R166*