haematologica
Journal of the Ferrata Storti Foundation

Universal germline genetic testing in patients with hematologic malignancies using DNA isolated from nail clippings

by Ozge Ceyhan-Birsoy, Elise Fiala, Satshil Rana, Margaret Sheehan, Jennifer Kennedy, Zarina Yelskaya, Vikas Rai, Yirong Li, Ciyu Yang, Donna Wong, Ivelise Rijo, Jacklyn Casanova-Murphy, Joshua Somar, Nikita Mehta, Hyeonjin Park, Silvana Ostafi, Kanika Arora, Angelika Padunan, Mark D. Ewalt, Umut Aypar, Panieh Terraf, Maksym Misyura, Sofia Haque, Gerald G. Behr, Tamanna Haque, Maria Sulis, Mark B. Geyer, Christopher Forlenza, Meghan C. Thompson, Maria Carlo, Alicia Latham, Ying Liu, Ahmet Zehir, Rose Brannon, Michael Berger, Luis A. Diaz, Ahmet Dogan, Marc Ladanyi, Kseniya Petrova-Drus, Khedoudja Nafa, Kenneth Offit, Maria Arcila, Zsofia K. Stadler, Michael F. Walsh, and Diana Mandelker

Received: February 2, 2024.
Accepted: March 26, 2024.

Citation: Ozge Ceyhan-Birsoy, Elise Fiala, Satshil Rana, Margaret Sheehan, Jennifer Kennedy, Zarina Yelskaya, Vikas Rai, Yirong Li, Ciyu Yang, Donna Wong, Ivelise Rijo, Jacklyn Casanova-Murphy, Joshua Somar, Nikita Mehta, Hyeonjin Park, Silvana Ostafi, Kanika Arora, Angelika Padunan, Mark D. Ewalt, Umut Aypar, Panieh Terraf, Maksym Misyura, Sofia Haque, Gerald G. Behr, Tamanna Haque, Maria Sulis, Mark B. Geyer, Christopher Forlenza, Meghan C. Thompson, Maria Carlo, Alicia Latham, Ying Liu, Ahmet Zehir, Rose Brannon, Michael Berger, Luis A. Diaz, Ahmet Dogan, Marc Ladanyi, Kseniya Petrova-Drus, Khedoudja Nafa, Kenneth Offit, Maria Arcila, Zsofia K. Stadler, Michael F. Walsh, and Diana Mandelker. Universal germline genetic testing in patients with hematologic malignancies using DNA isolated from nail clippings.
Haematologica. 2024 Apr 4. doi: 10.3324/haematol.2024.285055 [Epub ahead of print]

Universal germline genetic testing in patients with hematologic malignancies using DNA isolated from nail clippings

*Ozge Ceyhan-Birsoy[1], *Elise Fiala[2,3], Satshil Rana[1], Margaret Sheehan[2], Jennifer Kennedy[2], Zarina Yelskaya[1], Vikas Rai[1], Yirong Li[1], Ciyu Yang[1], Donna Wong[1], Ivelise Rijo[1], Jacklyn Casanova[1], Joshua Somar[1], Nikita Mehta[1], Hyeonjin Park[1], Silvana Ostafi[1], Kanika Arora[1], Angelika Padunan[2], Mark D. Ewalt[1], Umut Aypar[1], Panieh Terraf[1], Maksym Misyura[1], Sofia Haque[4], Gerald G. Behr[4], Tamanna Haque[2], Maria Sulis[2,3], Mark B. Geyer[2], Christopher Forlenza[2,3], Meghan C. Thompson[2], Maria Carlo[2], Alicia Latham[2], Ying Liu[2], Ahmet Zehir[1], Rose Brannon[1], Michael Berger[1], Luis A Diaz Jr[2], Ahmet Dogan[1], Marc Ladanyi[1], Kseniya Petrova-Drus[1], Khedoudja Nafa[1], Kenneth Offit[2], Maria Arcila[1], Zsofia K. Stadler[2], Michael F. Walsh[2,3], Diana Mandelker[1]

[1] Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2] Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[3] Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4] Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

*O C-B and EF contributed equally as co-first authors.

#ZKS, MFW, and DM contributed equally as co-last authors.

Running head: Genetic testing in hematologic malignancies

 # Corresponding authors:

Diana Mandelker, MD, PhD

Email: mandelkd@mskcc.org

Michael F. Walsh, MD, FAAP, FACMG

Email: walshm2@mskcc.org

Zsofia K. Stadler, MD

Email: stadlerz@mskcc.org

Data sharing statement: All de-identified genomic results for the patients in this study are available in the cBioPortal for Cancer Genomics at http://cbioportal.org/msk-impact.

Manuscript word count: 2050

Acknowledgement

Not applicable

Funding

This work was partly supported by the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, the Precision, Interception and Prevention Program; the Robert and Kate Niehaus Center for Inherited Cancer Genomics; V Foundation; and the National Institutes of Health (NIH)/National Cancer Institute (NCI) Cancer Center Support Grant (P30 CA008748). Figure 1D was created partly with BioRender.com.

Authors' contributions

Study conception and design: OC-B, EF, ZKS, MW, DM; Acquisition of data: All authors; Data analysis and interpretation: OC-B, EF, SR, KP-D, MA, ZS, MW, DM; Writing of manuscript: OC-B, EF, ZS, MW, DM; Review and final approval of manuscript: All authors.

DECLARATIONS:

Ethics approval and consent to participate

All patients in the study provided written informed consent for genetic testing under an institutional review board-approved protocol (12-245) as part of the MSK Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT; ClinicalTrials.gov identifier, NCT01775072) paired tumor-blood DNA sequencing test.

Competing interests

MB declares consulting fees (AstraZeneca, Eli Lilly, Paige.AI) unrelated to this work and intellectual property rights (SOPHiA GENETICS) related to MSK-IMPACT. ZKS declares that immediate family member serves as a consultant in Ophthalmology for Adverum, Genentech, Neurogene, Novartis, Optos Plc, Outlook Therapeutics, and Regeneron outside the submitted work. ZKS serves as an Associate Editor for JCO Precision Oncology and as a Section Editor for UpToDate. MCT declares research funding (to the institution) (AstraZeneca, Beigene, Nurix Therapeutics, Genentech, Abbvie, Genmab), consulting/advisory boards (AstraZeneca, Beigene, Janssen, Pharmacyclics, LOXO Oncology at Lilly), honoraria (Philips Group Oncology Communications, VJHemOnc, Curio Science LLC, Dava Oncology (travel support and honoraria), Brazilian Association of Hemotherapy and Cellular Therapy (ABHH), MJH Life Sciences, Massachusetts Medical Society, Peerview, and other (Nurix therapeutics (travel support)). AZ is currently employed at AstraZeneca. DM declares consulting fees (AstraZeneca) unrelated to this work. All other authors declare no conflict of interest.

While identifying hereditary cancer predisposition in individuals with hematologic malignancies (HM) is critical for optimal clinical management [1-5], genetic testing for these individuals in limited, in part, by the difficulty in obtaining DNA suitable for germline analysis. Peripheral blood, saliva and buccal swabs are often contaminated with malignant cells in patients with HM [6]. Cultured fibroblasts obtained through skin biopsies are used for germline testing of patients with HM, although the invasive nature of the procedure and the multiple weeks-long culturing time before testing can be performed limits the utility of this specimen.

Nail clippings represent a non-invasive source of DNA that can be used for germline testing [7,8]. Here, we describe a novel, clinically validated, and New York State Department of Health approved next generation sequencing assay for germline testing of patients with HM using DNA isolated from nail clippings. We describe the institution-wide implementation of this assay, the initial clinical findings in this cohort, and the clinical benefit experienced by these patients due to expanded genetic testing.

Genomic DNA was isolated from peripheral blood, saliva, and FFPE tissues as described [9,10]. For DNA isolation from nail clippings, 10 fingernails or 10 toenails from the patient were collected submitted to the laboratory as long thin strands in a dry sterile container. 20mg of fingernail or toenail clippings (~3 thin slivers) were pulverized using BeadBlaster 24 instrument (Benchmark Scientific, NJ). Samples were lysed by heating at 56°C and collected using QIAamp DNA investigator kit (Qiagen, Valencia, CA). DNA samples were normalized to yield up to 250ng input and maximized to 55ul in TE Buffer. The median genomic DNA yield in 505 samples that were extracted was 569ng. Extracted genomic DNA was sheared using Covaris E200 instrument (Covaris, Woburn, MA) and libraries were prepared as described [9,10]. Briefly, sequence libraries were prepared using KAPA HTP protocol (Kapa Biosystems, Wilmington, MA) and Biomek FX system (Beckman Coulter, Brea, CA) through a series of enzymatic steps including shearing of double-stranded DNA, end repair, A-base addition, ligation of dual-barcoded sequence adaptors, and low-cycle PCR amplification. Multiple barcoded sequence

libraries were pooled and captured using our custom-designed biotinylated probes (Integrated DNA Technologies, Coralville, IA). Analysis of 505 nails samples showed that 1% (5/505) of samples had coverage (read depth) <150X, the minimum threshold for this assay, and were considered insufficient for germline analysis. Variant calling, annotation, and interpretation were performed as described [9-11]. Briefly, variants called using MuTect and Genome Analysis Toolkit (GATK) Haplotypecaller were filtered based on 25% variant allele fraction for single nucleotide variants (SNVs) and 15% for insertions/deletions (indels) and 20X coverage thresholds. Copy number variants (CNVs; deletions and duplications of single or multiple exons) in the target genes were captured and assessed using a validated in-house developed pipeline.

To first evaluate whether nails could provide a source of DNA suitable for germline testing, the variant allele fraction (VAF) of 2398 somatic variants in 82 cancer susceptibility genes (Germline MSK-IMPACT-Heme, Supplementary Table 1) were interrogated in the nails from 2611 patients who received MSK-IMPACT-Heme (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets for hematologic malignancies) [11]. Seven (0.29%) of 2398 somatic variants were found to have a VAF >20% in the nails (n=1 for *CALR, CHEK2, MPL, PMS2,* and *RUNX1* and n=2 for *ETV6*), six of which were from one patient with a myeloproliferative neoplasm (MPN) and fibrotic bone marrow, while the remaining variant was in a patient with myelodysplastic syndrome (MDS), suggesting that caution may be warranted for performing germline testing using nail DNA in patients with a fibrotic bone marrow in the absence of a matched tumor . Therefore, only 2/2611 (0.08%) patients tested had somatic contamination in the nails that could potentially confound germline analysis in the absence of a matched tumor, and in 99.92% of cases, nail DNA was deemed suitable for germline testing.

For validation of Germline MSK-IMPACT-Heme as a genetic testing assay, its performance was compared to that of the clinically validated panel MSK-IMPACT [9] (used for germline testing in patients with solid tumors) for 68 genes shared by the two assays. Coverage (read depth at each target position) and variant calling were analyzed in blood specimens from

21 individuals run on MSK-IMPACT to specimens from the same individuals (nail clippings (n=14), saliva (n=11), and/or blood (n=19)) run on Germline MSK-IMPACT-Heme. Each target exon and adjacent splice sites (+/-20bp) had equivalent coverage. There was 100% concordance in variant calling using Germline MSK-IMPACT-Heme and MSK-IMPACT for 1482 variants (1355 single nucleotide variants (SNVs), 109 insertion/deletions (indels), and 18 large deletions and insertions (copy number variants; CNVs)), including variants with a range of clinical significance classifications (Figure 1A-C).

The clinical testing cohort consisted of 240 consecutive patients, unselected based on current genetic testing criteria, prospectively consented to germline analysis by their treating physicians as part of MSK-IMPACT-Heme between 6/2022-7/2023 (Supplementary Table 2), 210 of whom had a diagnosis of an HM (or aplastic anemia) and 30 individuals who received testing as part of an evaluation for possible HM. Individuals with a prior allogeneic stem cell transplant were excluded. Nail samples were used for individuals with myeloid and T-cell malignancies (212/240 (88%)), and for individuals with B-cell lymphomas, saliva samples were also acceptable for germline testing. The most common diagnoses of patients with HM included Mature B-Cell Neoplasms (MBN; n=48), MPN (n=42), Acute Myeloid Leukemia (AML; n=35), MDS (n=23), B-Lymphoblastic Leukemia/Lymphoma (BLL; n=15) (Supplementary Figure 1). Nail specimens were used in 212 individuals and saliva specimens were used in 28 individuals. Of the 240 individuals, 38 (15.8%) had pediatric-onset (<18 years old) and 202 (84.2%) had adult-onset disease (Supplementary Table 2). The median turnaround time from sample accessioning to report sign-out was 10 workdays.

Germline pathogenic and likely pathogenic variants (gPVs) in hereditary cancer predisposition genes were identified in 17.1% (41/240) of individuals and in 16.7% (35/210) of the patients with a diagnosis of an HM (Table 1, Supplementary Figure 1). Six of 41 (14.6%) patients with gPVs had exon-level deletions. The positivity rate was 17.3% (35/202) in adult patients and 15.8% (6/38) in pediatric patients. In patients with a clinical diagnosis of HM, the

highest rate of gPVs was in patients with MPN (26.2% (11/42)), followed by BLL (20% (3/15)) (Supplementary Figure 1).

Eight of 240 (3.3%) patients had gPVs in high penetrance genes known to be associated with predisposition to HMs. Three individuals in our cohort (3/240, 1.3%) had biallelic variants in genes associated with rare autosomal recessive (AR) syndromes (Figure 2) that all confirmed to be in *trans* by parental testing. The first patient (Figure 2A-C) had a history of cytopenias and was diagnosed at age 25 with MDS that progressed to AML-MR (myelodysplasia related) (Figure 2B). Germline MSK-IMPACT-Heme revealed two *FANCA* gPVs, leading to a diagnosis of Fanconi anemia. A chromosomal breakage study with DEB on cultured fibroblasts was positive, consistent with Fanconi anemia, and the patient underwent reduced intensity conditioning for treatment of his AML. The second patient (Figure 2D-E) was diagnosed with ataxia telangiectasia at the age of four years after presenting with neurological symptoms. At age 13 years, the patient developed diffuse large B-cell lymphoma. Previous genetic testing had identified a single *ATM* pathogenic variant (c.8103_8104delAA (p.Ile2702Argfs*15)). Germline MSK-IMPACT-Heme identified this variant and a second pathogenic deletion of *ATM* exons 62-63. The third patient (Figure 2F-H) developed a large mediastinal mass at age 12 years, which was diagnosed as T-cell lymphoblastic leukemia/lymphoma. Germline MSK-IMPACT-Heme identified two *MSH6* gPVs, and the patient was diagnosed with constitutional mismatch repair deficiency (CMMRD). Subsequent colonoscopy revealed multiple polyps. Both parents were found to have Lynch syndrome.

Five patients had gPVs in autosomal dominant (AD) high penetrance genes associated with predisposition to HM (two with *DDX41*, one each with *ETV6, RUNX1*, and *POT1*). The patient with an *ETV6* gPV (Figure 2I) had thrombocytopenia with onset in her 30s and was diagnosed with chronic myelomonocytic leukemia at age 59 years. The variant segregated in the patient's daughter, who had thrombocytopenia. A pathogenic *RUNX1* multi-exon deletion was identified in a patient with a previous diagnosis of idiopathic thrombocytopenia purpura,

who developed mixed phenotype acute leukemia and simultaneously uterine leiomyosarcoma at age 47 years (Figure 2J). The patient with a *DDX41* p.Gln306* gPV was diagnosed with AML at age 56 years and had a second somatic *DDX41* oncogenic variant (p.Arg525His) identified in the matched bone marrow specimen (Figure 2K-L). The patient with *POT1* p.Ile78Thr variant had a history of papillary thyroid carcinoma at age 25 years, *JAK2* p.Val617Phe-positive polycythemia vera diagnosed at 32, and multiple small papillary renal cell carcinomas diagnosed at 44, consistent with descriptions of the *POT1* phenotype [12]. Additionally, 20% (6/30) of individuals tested for suspicion of a HM were also found to carry gPVs (Table 1). Further studies on the benefit of genetic testing in individuals with hematologic abnormalities but no cancer diagnosis may be warranted.

The majority of individuals with gPVs (56.1% (23/41)) had variants in homologous recombination pathway genes not currently known to predispose to HM (*CHEK2* (n=7), *BRCA1* (n=5), *ATM* (n=5), *BRCA2* (n=2), *PALB2* (n=2), *FANCA* (n=1), and *FANCC* (n=1)). While variants in these genes have been reported in patients with HM [13,14], larger cohorts of patients with different tumor types are required to determine if they confer an increased risk for these cancer types. Additionally, a 28-year-old patient with CLL had an *MSH6* variant leading to a diagnosis of Lynch syndrome, a 56-year-old patient with CLL/SLL had an *SDHB* variant and was recently diagnosed with renal cell carcinoma and paraganglioma, and two patients without known personal or family history of schwannoma had *LZTR1* variants associated with schwannomatosis. These results suggest that expanded genetic testing approaches in individuals with hematologic malignancies are likely to identify hereditary predisposition to a broad spectrum of cancer susceptibility syndromes.

In this report, we describe the development of a novel high-throughput germline testing assay for individuals with HMs using nail DNA. Currently, DNA from cultured fibroblasts obtained through skin biopsies is the most commonly used specimen type for germline testing in patients with active HM, although the invasive nature of the procedure and the multiple weeks-

long culturing time make it challenging to utilize on a large scale and deliver results in a timely fashion. In a recent study, the median turnaround time for fibroblast culture alone was 28 days and 5% of skin biopsies failed to culture, resulting in the need for an additional biopsy [15]. The median turnaround time of 10 workdays for our test was substantially lower than the amount of time needed for a fibroblast culture and testing time in a typical skin biopsy test. Our rapid and noninvasive germline testing approach allows for an institution-wide initiative towards universal genetic testing for patients with HMs. Remarkably, in the first 240 consecutive patients with HM who consented to genetic testing, a similar proportion tested positive for hereditary cancer susceptibility (17.1%) as in our patients with solid tumors (16.7%) tested under the same IRB protocol [16]. Even in this initial cohort, germline findings resulted in therapeutic considerations, initiation of surveillance for additional malignancies, and identification of at-risk relatives.

One limitation of this study is that the panel used does not contain all genes associated with HM predisposition, for example bone marrow failure and immunodeficiency syndromes. Inclusion in this study required referral by the treating physicians and patient consent; therefore, under or over-representation of some predisposed patients cannot be ruled out. Additionally, prior allogeneic stem cell transplant was part of the exclusion criteria, as a recent study showed that 22% of nail specimens in these individuals had some level of chimerism, with graft vs. host disease being a risk factor for this chimerism [17]. However, if nail DNA can be compared to a pre-transplant host specimen by chimerism analysis using short tandem repeats or similar methodology, and the nail DNA is found to be free of donor contamination, it would be suitable for germline analysis. Despite these limitations, our study shows the feasibility of large-scale genetic testing for individuals with HMs using DNA derived from nail clippings. This advance will both allow for larger scale genetic testing studies to help determine which patients with HMs are most likely to benefit from routine genetic testing and provide an opportunity to improve the availability and efficiency of clinical germline testing for individuals with HMs in the future.

LIST OF ABBREVIATIONS: HM: hematologic malignancies; NGS: next-generation sequencing; SNV: single nucleotide variant; indel: insertion/deletion variant; CNV: copy number variant; MBN: Mature B-Cell Neoplasms; MPN: Myeloproliferative Neoplasms; AML: Acute Myeloid Leukemia; MDS: Myelodysplastic Syndrome; BLL: B-Lymphoblastic Leukemia/Lymphoma; MDS/MPN: Myelodysplastic/Myeloproliferative Neoplasms; HL: Hodgkin Lymphoma; MTNN: Mature T and NK Neoplasms; PCM: Plasma Cell Myeloma; HDCN: Histiocytic and Dendritic Cell Neoplasms; ALAL: Acute Leukemias of Ambiguous Lineage; gPVs: germline pathogenic and likely pathogenic variants.

REFERENCES

1. Porter CC, Druley TE, Erez A, et al. Recommendations for Surveillance for Children with Leukemia-Predisposing Conditions. Clin Cancer Res. 2017;23(11):e14-e22

2. Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. Blood. 2017;130(4):424-432

3. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719

4. University of Chicago Hematopoietic Malignancies Cancer Risk Team. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood 2016;128(14):1800-1813

5. Walsh MF, Chang VY, Kohlmann WK, et al. Recommendations for Childhood Cancer Screening and Surveillance in DNA Repair Disorders. Clin Cancer Res. 2017;23(11):e23-e31

6. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. N Engl J Med. 2015;373(24):2336-2346

7. Barbashina V, Salazar P, Ladanyi M, et al. Glioneuronal tumor with neuropil-like islands (GTNI): a report of 8 cases with chromosome 1p/19q deletion analysis. Am J Surg Pathol. 2007;31(8):1196-1202

8. Kakadia PM, Van de Water N, Browett PJ, et al. Efficient identification of somatic mutations in acute myeloid leukaemia using whole exome sequencing of fingernail derived DNA as germline control. Sci Rep. 2018;8(1):13751

9. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based

Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn. 2015;17(3):251-264

10. Cheng DT, Prasad M, Chekaluk Y, et al. Comprehensive detection of germline variants by MSK-IMPACT, a clinical diagnostic platform for solid tumor molecular oncology and concurrent cancer predisposition testing. BMC Med Genomics. 2017;10(1):33

11. Ptashkin RN, Ewalt MD, Jayakumaran G, et al. Enhanced clinical assessment of hematologic malignancies through routine paired tumor and normal sequencing. Nat Commun. 2023;14(1):6895

12. DeBoy EA, Tassia MG, Schratz KE, et al: Familial Clonal Hematopoiesis in a Long Telomere Syndrome. N Engl J Med. 2023;388(26):2422-2433

13. Lampson BL, Gupta A, Tyekucheva S, et al. Rare Germline ATM Variants Influence the Development of Chronic Lymphocytic Leukemia. J Clin Oncol. 2023;41(5):1116-1128

14. Stubbins RJ, Asom AS, Wang P, et al. Germline loss of function BRCA1 and BRCA2 mutations and risk of de novo hematopoietic malignancies. Haematologica. 2024;109(1):351-356

15. DeRoin L, Cavalcante de Andrade Silva M, Petras K, et al. Feasibility and limitations of cultured skin fibroblasts for germline genetic testing in hematologic disorders. Hum Mutat. 2022;43(7):950-962

16. Ceyhan-Birsoy O, Jayakumaran G, Kemel Y, et al. Diagnostic yield and clinical relevance of expanded genetic testing for cancer patients. Genome Med. 2022;14(1):92

17. Krystel-Whittemore M, Petrova-Drus K, Ptashkin RN, et al. Cell-free DNA from nail clippings as source of normal control for genomic studies in hematologic malignancies. Haematologica. 2024 March 7 [epub ahead of print]

Table 1: Summary of patients with germline pathogenic/likely pathogenic variants in hereditary cancer predisposition genes

Age at HM Dx (yrs)	Sex	Clinical Dx	Disease group	Pathogenic variants	Other malignancy
62	M	MZL	MBN	APC c.3920T>A (p.Ile1307Lys), P	No
27	F	ET	MPN	APC c.3920T>A (p.Ile1307Lys), P	No
27	M	MPN	MPN	APC c.3920T>A (p.Ile1307Lys), P	No
28	M	CLL/SLL	MBN	APC c.3920T>A (p.Ile1307Lys), P MSH6 c.3226C>T (p.Arg1076Cys), LP	No
65	F	MCL	MBN	ATM c.2192dupA (p.Tyr731*), P BLM exons 6-12 deletion, LP	BC (41); RCC (46)
70	F	CML	MPN	ATM c.7271T>G (p.Val2424Gly), P	CEAD(37); ANSC (50); TC (52)
60	M	CML	MPN	ATM c.8103_8104delAA (p.Ile2702Argfs*15), P	MEL (56)
40	F	MPN	MPN	ATM c.901+1G>A, P	No
13	M	DLBCL	MBN	ATM exons 62-63 deletion, P ATM c.8103_8104delAA (p.Ile2702Argfs*15), P	No
60	M	Myelofibrosis	MPN	BLM exons 13-14 deletion, P	No
23	F	B-Cell Lymphoma	MBN	BRCA1 c.2635G>T (p.Glu879*), P	No
50	F	Cytopenia	MDS Workup	BRCA1 c.2800C>T (p.Gln934*), P	HGSOC (47)
64	F	AML	AML	BRCA1 c.4327C>T (p.Arg1443*), P	BC (41)
40	M	MPN	MPN	BRCA1 c.68_69delAG (p.Glu23Valfs*17), P	No
49	F	B-Cell Leukemia	BLL	BRCA1 exons 7-8 deletion, P	No
54	F	CML	MPN	BRCA2 c.4276dupA (p.Thr1426Asnfs*12), P	BC, TC
77	M	Cytopenia	MDS Workup	BRCA2 c.5073dupA (p.Trp1692Metfs*3), P	PC (68)
1	M	Mixed Phenotype AL	ALAL	CHEK2 c.1100delC (p.Thr367Metfs*15), P	No
45	M	AML	AML	CHEK2 c.1100delC (p.Thr367Metfs*15), P	(Male) BC (62)
46	F	MDS/MPN	MDS	CHEK2 c.1283C>T (p.Ser428Phe), P	No
16	F	HL	HL	CHEK2 c.1421G>A (p.Arg474His), LP	No
62	M	AML	AML	CHEK2 c.444+1G>A,, P	No
23	F	ET	MPN	CHEK2 c.470T>C (p.Ile157Thr), P	No
51	M	MDS/MPN	MDS/MPN	MUTYH c.1187G>A (p.Gly396Asp), P	No
33	M	Stem cell donor	MDS Workup	DDX41 c.415_418dupGATG (p.Asp140Glyfs*2), P	No
56	F	AML	AML	DDX41 c.916C>T (p.Gln306*), P	No
59	F	CMML	MDS/MPN	ETV6 c.1196G>A (p.Arg399His), LP	No
25	M	MDS	MDS	FANCA exons 18-22 deletion, P FANCA c.367C>T (p.Gln123*), P	No
62	F	Lymphadenopathy	MPN Workup	FANCC c.456+4A>T, P	No
37	F	DLBCL	MBN	LZTR1 c.2062C>T (p.Arg688Cys), LP	No
31	F	MPN	MPN	LZTR1 c.2407delG (p.Val803Serfs*10), LP	No
58	F	Cytopenia	MDS Workup	MPL c.1653+1delG, P	PNET (54)
20	M	AML	AML	MSH3 c.1625dupT (p.Leu542Phefs*12), LP	No

12	M	TCL	MTNN	MSH6 c.3261dupC (p.Phe1088Leufs*5), P MSH6 c.3724_3726delCGT (p.Arg1242del), LP	No
66	F	CH	MDS Workup	MUTYH c.1187G>A (p.Gly396Asp), P	BC (62)
0	F	BLL	BLL	PALB2 c.3323delA (p.Tyr1108Serfs*16), P	No
53	M	CLL/SLL	MBN	PALB2 c.509_510delGA (p.Arg170Ilefs*14), P	No
32	M	PV	MPN	POT1 c.233T>C (p.Ile78Thr), LP	TC (34); RCC (44)
47	F	Mixed Phenotype AL	ALAL	RUNX1 exons 3-9 deletion, P	ULMS (47)
56	M	CLL/SLL	MBN	SDHB c.642+2T>G, LP	PGNG (56); RCC (56)
5	M	BLL	BLL	TYK2 c.691C>T (p.Arg231Trp), LP	No

Dx, diagnosis; yrs, years; M, male; F, female; Het, heterozygous; P, pathogenic; LP, likely pathogenic

MZL, Marginal Zone Lymphoma; ET, Essential Thrombocythemia; MPN, Myeloproliferative Neoplasm, MCL, Mantle Cell Lymphoma; CML, Chronic Myeloid Leukemia; DLBCL, Diffuse Large B-Cell Lymphoma; AML, Acute Myeloid Leukemia; CMML, Chronic Myelomonocytic Leukemia; TCL, T-Cell Leukemia; PV, Polycythemia Vera; AL, Acute Leukemia; BC, Breast Cancer; RCC, Renal Cell Carcinoma; CEAD, Cervical Cancer; ANSC, Anal Squamous Cell Carcinoma; TC, Thyroid Cancer; MEL, Melanoma; OC, Ovarian Cancer; PC, Prostate Cancer; PNET, Pancreatic neuroendocrine tumor; ULMS, Uterine Leiomyosarcoma; PGNG, Paraganglioma

FIGURE LEGENDS:

Figure 1: Validation of Germline MSK-IMPACT-Heme panel and overview of MSK-IMPACT-Heme workflow.

(a) Each target exon and adjacent splice sites (+/-20bp) of 68 genes that were common between MSK-IMPACT-Heme and MSK-IMPACT had sufficient (>20X) and equivalent coverage in tested blood, saliva, and nail specimens. **(b)** Representative copy number plots demonstrating the detection of a single exon (*MSH2* exon 1) deletion and an intragenic multi-exon (*MSH3* exon 3-23) deletion in validation of CNV calling using DNA isolated from nail specimens by Germline MSK-IMPACT-Heme. **(c)** Representative detection of a small deletion (*CHEK2* c.1100delC) variant by Germline MSK-IMPACT-Heme NGS panel and Sanger sequencing using DNA isolated from nail specimens. **(d)** Overview of MSK-IMPACT-Heme workflow. Patients referred by their clinical providers are consented for paired tumor-normal sequencing and germline analysis. A sample for a source of normal DNA (nail or saliva) and a sample for a source of tumor DNA (blood, bone marrow, lymph node, or other tissue) are collected. DNA is extracted from the samples, sequence libraries are prepared and captured using MSK-IMPACT-Heme probes. Sequencing is performed and data is analyzed through a custom bioinformatics pipeline to detect and annotate variants. Results are interpreted, classified, and reported by clinical molecular geneticists and molecular pathologists. Reports are transferred to patient electronic medical records. Figure 1D was created partly with BioRender.com.

Figure 2: Patients with germline pathogenic/likely pathogenic variants in genes associated with predisposition to HM.

(a) Patient with biallelic germline pathogenic/likely pathogenic variants (gPVs) in *FANCA* diagnosed with Fanconi anemia. Pedigree of the patient with biallelic *FANCA* gPVs (left panel)**,** a representative core biopsy involved by Acute Myeloid Leukemia-Myelodysplasia Related is shown (H&E, 10X original magnification) with hypercellularity, decreased maturing trilineage hematopoiesis, and increased numbers of blasts in clusters (H&E, 40X original magnification) accounting for 50% of cellularity (highlighted by CD34 immunohistology, 10X original magnification) (middle panel), and right-sided aortic arch identified in the patient (right panel). **(b)** Patient with biallelic gPVs in *ATM* diagnosed with ataxia telangiectasia. Pedigree of the patient with biallelic *ATM* gPVs (left panel). A bone marrow biopsy revealed hypercellular marrow (H&E, 4X original magnification) extensively involved by an atypical lymphoid infiltrate (H&E, 4X original magnification) with large-size neoplastic mature B-cells (H&E, 40X original magnification and CD20 immunohistology 20X original magnification) and a background of numerous reactive T-cells (CD3 immunohistology 20X original magnification), overall consistent with diffuse large B-cell lymphoma (right panel). **(c)** Patient with biallelic gPVs in *MSH6* diagnosed with Congenital Mismatch Repair Deficiency. Pedigree of the patient with biallelic *MSH6* gPVs (left panel). Colonoscopy four months after the initial presentation revealed several colonic polyps, including hyperplastic, tubular adenomas, and a tubulovillous adenoma (H&E, 2X original magnification) showing villous architecture and columnar cells showing focal high-grade dysplasia (H&E, 20X original magnification) (middle panel), and brain MRI of the patient showing nonspecific hyperintensities (right panel). **(d)** Pedigree of the patient with *ETV6* gPV. The *ETV6* gPV was identified in the patient with a history of CMML and thrombocytopenia and segregated in her daughter with a history of thrombocytopenia. **(e)** Pedigree of the patient with

RUNX1 gPV. **(f)** Pedigree (left panel) and NGS data of the patient with *DDX41* c.916C>T (p.Gln306*) gPV. The germline *DDX41* c.916C>T (p.Gln306*) variant was detected in the nail and bone marrow DNA of the patient and the somatic *DDX41* c.1574G>A (p.Arg574His) variant was detected in the bone marrow DNA sample of the patient at 13% variant allele fraction (VAF) (right panel).

Figure 1

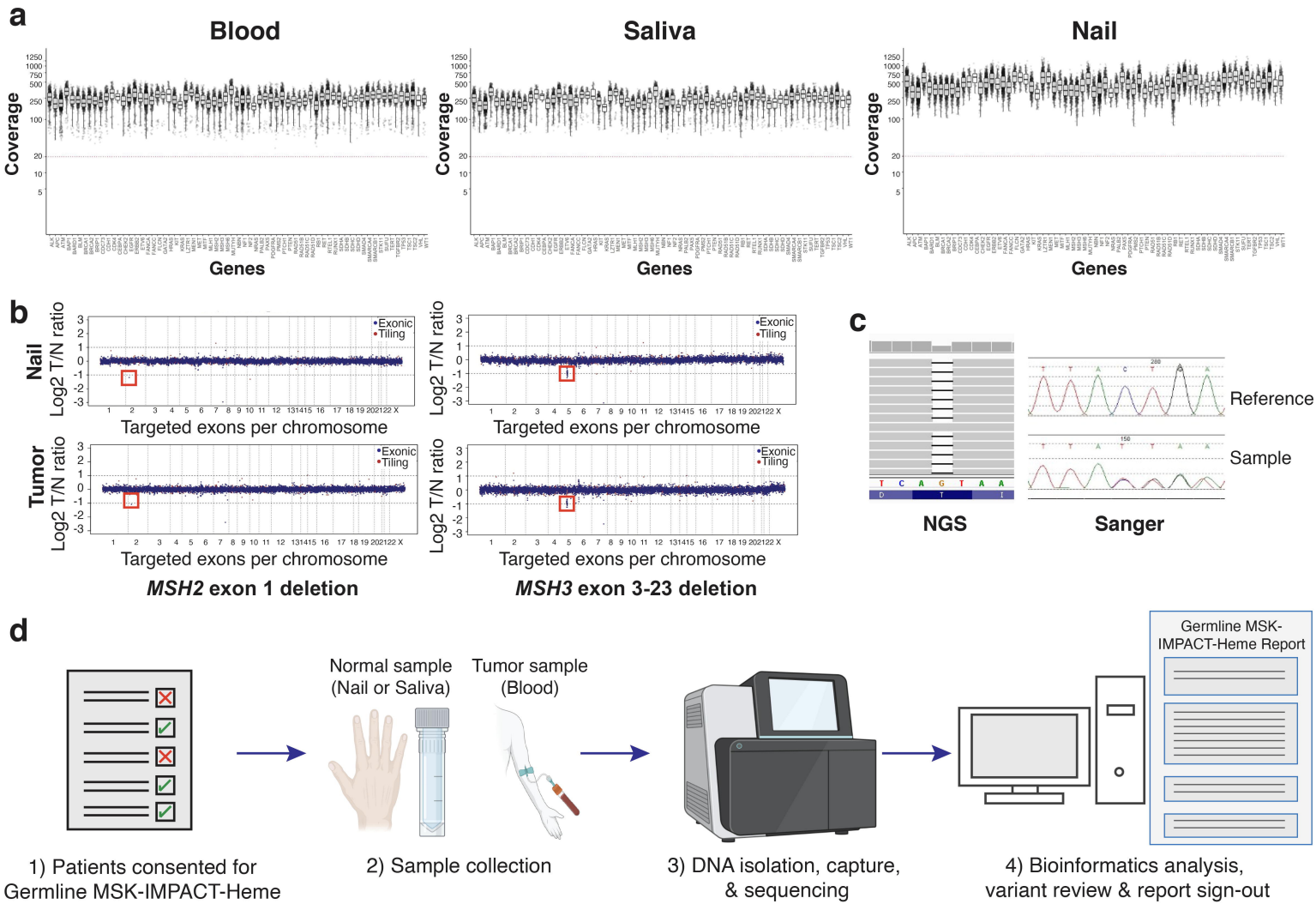
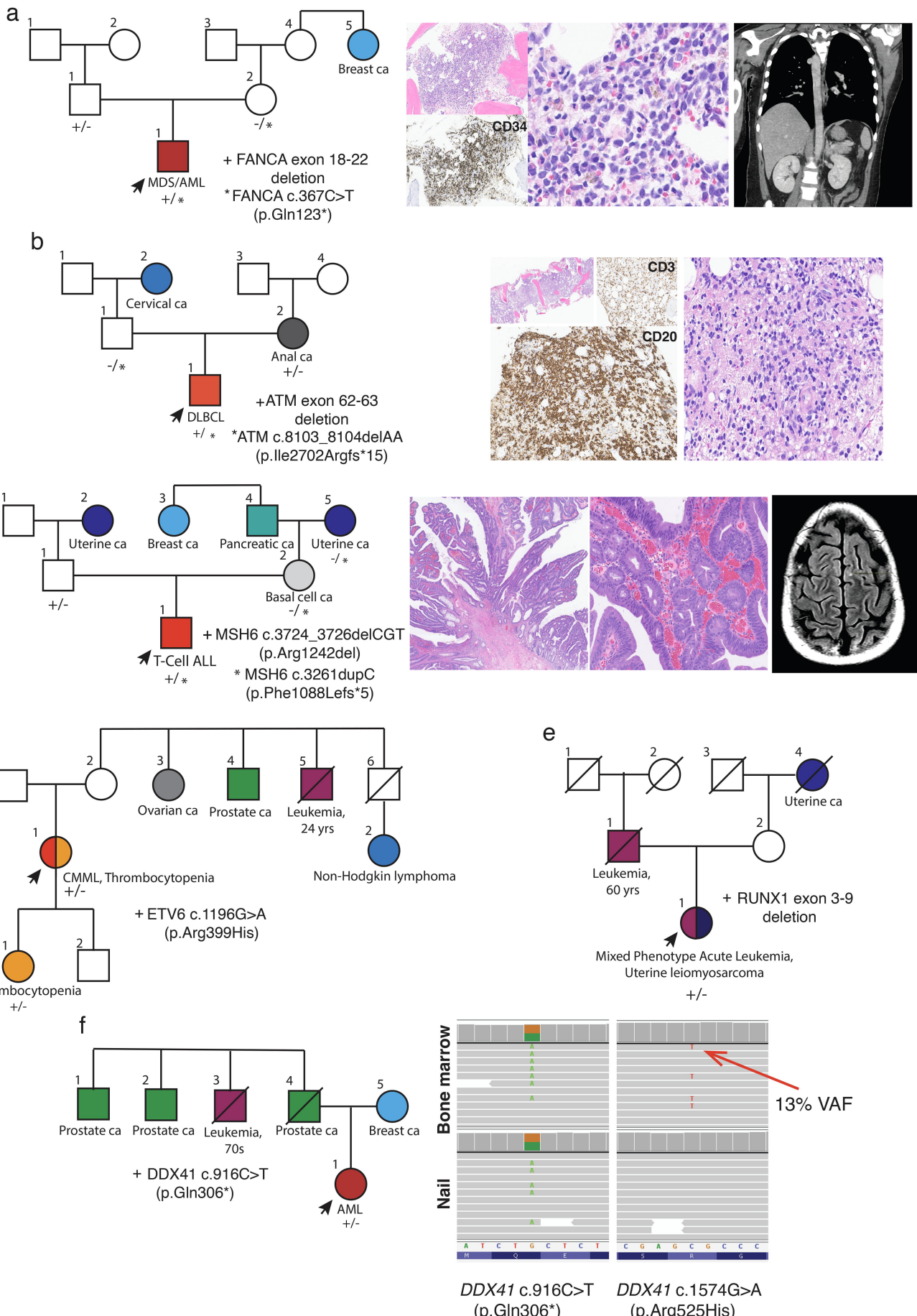

a
Blood
Saliva
Nail
Coverage
Genes
b
Nail
Tumor
Log2 T/N ratio
Exonic
Tiling
Targeted exons per chromosome
MSH2 exon 1 deletion
MSH3 exon 3-23 deletion
c
NGS
Sanger
Reference
Sample
d
Normal sample
(Nail or Saliva)
Tumor sample
(Blood)
Germline MSK-
IMPACT-Heme Report
1) Patients consented for
Germline MSK-IMPACT-Heme
2) Sample collection
3) DNA isolation, capture,
& sequencing
4) Bioinformatics analysis,
variant review & report sign-out

Figure 2


a
Breast ca
+/-
-/*
MDS/AML
+/*
+ FANCA exon 18-22 deletion
FANCA c.367C>T (p.Gln123)
CD34
b
Cervical ca
-/*
Anal ca
+/-
DLBCL
+/*
+ATM exon 62-63 deletion
*ATM c.8103_8104delAA (p.Ile2702Argfs*15)
CD3
CD20
c
Uterine ca
Breast ca
Pancreatic ca
Uterine ca
-/*
+/-
Basal cell ca
-/*
T-Cell ALL
+/*
+ MSH6 c.3724_3726delCGT (p.Arg1242del)
* MSH6 c.3261dupC (p.Phe1088Lefs*5)
d
Ovarian ca
Prostate ca
Leukemia, 24 yrs
Non-Hodgkin lymphoma
CMML, Thrombocytopenia
+/-
+ ETV6 c.1196G>A (p.Arg399His)
Thrombocytopenia
+/-
e
Uterine ca
Leukemia, 60 yrs
+ RUNX1 exon 3-9 deletion
Mixed Phenotype Acute Leukemia, Uterine leiomyosarcoma
+/-
f
Prostate ca
Prostate ca
Leukemia, 70s
Prostate ca
Breast ca
+ DDX41 c.916C>T (p.Gln306*)
AML
+/-
Bone marrow
Nail
13% VAF
DDX41 c.916C>T (p.Gln306*)
DDX41 c.1574G>A (p.Arg525His)

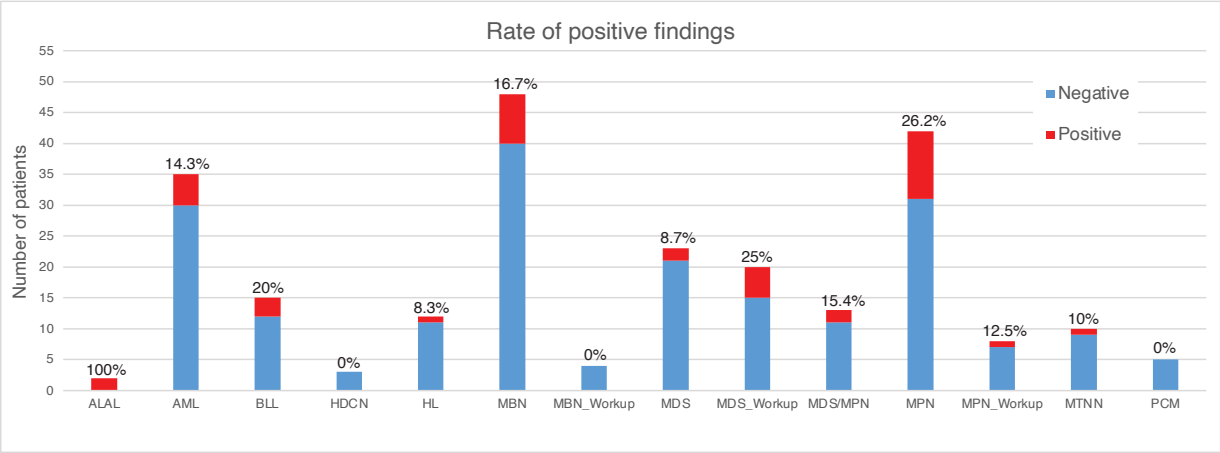
Supplementary Table 1: Genes on Germline MSK-IMPACT-Heme panel

ALK	MUTYH
ANKRD26	NBN
APC	NF1
ATM	NF2
BAP1	NRAS
BARD1	NSD1
BLM	PALB2
BRCA1	PAX5
BRCA2	PDGFRA
BRIP1	PMS2
BTK	POT1
CALR	PTCH1
CBL	PTEN
CDC73	PTPN11
CDH1	RAD51
CDK4	RAD51B
CDKN2A	RAD51C
CEBPA	RAD51D
CHEK2	RB1
DDX41	RET
EGFR	RTEL1
ERBB2	RUNX1
ETV6	SDHA
FANCA	SDHB
FANCC	SDHC
FAS	SDHD
FLCN	SH2B3
GATA2	SMAD4
HRAS	SMARCA4
IKZF1	SMARCB1
KIT	SRP72
KRAS	STK11
LZTR1	SUFU
MEN1	TERT
MET	TGFBR2
MITF	TP53
MLH1	TSC1
MPL	TSC2
MSH2	TYK2
MSH3	VHL
MSH6	WT1

Supplementary Table 2: Patient demographics

	Number of patients (n=240) (%)
Sex	
Female	103 (42.9%)
Male	137 (57.1%)
Age at HM diagnosis (years)	
<18	38 (15.8%)
18-39	44 (18.3%)
40-59	77 (32.1%)
60-79	76 (31.7%)
80-99	5 (2.1%)
Genetic ethnicity	
African/African American	15 (6.3%)
Ashkenazi Jewish	37 (15.4%)
East Asian	10 (4.2%)
European	141 (58.7%)
Native American	2 (0.8%)
South Asian	2 (0.8%)
Admixed/Other	28 (11.7%)
Unknown	5 (2.1%)

Continental-level genetic ancestries were assigned if the inferred contribution of that population to their ancestry is ≥80%. Otherwise, they were assigned as Admixed/Other. Genetic ancestry could not be inferred in five individuals.



Supplementary Figure 1. Rate of gPVs in hereditary cancer predisposition genes identified in patients with HMs. Percentage of patients with gPVs (Positive; red) are presented above each bar. Clinical diagnoses of patients with HM included Mature B-Cell Neoplasms (MBN; n=48), Myeloproliferative Neoplasms (MPN; n=42), Acute Myeloid Leukemia (AML; n=35), Myelodysplastic Syndrome (MDS; n=23), B-Lymphoblastic Leukemia/Lymphoma (BLL; n=15), Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN; n=13), Hodgkin Lymphoma (HL; n=12), Mature T and NK Neoplasms (MTNN; n=10), Plasma Cell Myeloma (PCM; n=5), Histiocytic and Dendritic Cell Neoplasms (HDCN; n=3), and Acute Leukemias of Ambiguous Lineage (ALAL; n=2).