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Expedited evaluation of hereditary hematopoietic malignancies in the setting of stem cell transplantation

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Data Sharing Statement: Genomic data are available on request

Running head: Pre-transplant evaluation for hereditary blood cancers

Author Contributions:
MWD conceived the study; MWD, LEC, and GWR collected and analyzed the data; MWD, LEC, GWR, SK, ASD, MTN, AAP, EN, LAG, RAL, OO, WS, MRB cared for the patients; DG, SD performed molecular pathology testing and reporting; GWR and MWD drafted the manuscript; all authors edited the manuscript.

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Increasingly, it is recognized that many patients with blood cancers harbor germline variants that increase cancer risk.[1] For example, 14% of acute myeloid leukemia patients (AML) in the BEAT AML study had germline variants associated with hereditary hematopoietic malignancies (HHMs) despite an older age at diagnosis (median: 72 years).[2] Moreover, 7% of myelodysplastic syndrome (MDS) patients shared deleterious germline variants with their matched-related stem cell donors (MRDs).[3] Clinical complications, particularly graft failure and donor-derived malignancies, can occur when an MRD with an HHM-related germline variant is unknowingly used.[4] Accurately and promptly diagnosing an HHM reduces the risk of these complications.[5-8]

There are several obstacles to efficiently diagnosing HHMs in the transplant setting.[9] First, physicians must recognize patients at risk for HHMs. Clinical suspicion may be obscured by the adult age of onset of some HHMs, which mimics many sporadic malignancies. Contemporary family structures are also smaller, which may reduce the family history "signal" of an HHM.[10] HHM diagnosis also typically necessitates the sequencing of germline DNA free of hematopoietic tissue. One common approach is to sequence DNA from cultured skin fibroblasts. This approach, however, may take 2-3 months.[11] Particularly for patients evaluated late in the transplant planning course, this timeline presents challenges that may delay transplant, putting patients at risk of relapse. For patients with bone marrow failure, we have historically been hesitant to delay transplant for HHM evaluation given risks of clinical deterioration from infectious or hemorrhagic complications.

Finally, we have cared for patients who received care in the community before completing pre-transplant evaluations at our center. For these patients, initial suspicion for HHMs occurred in the weeks before transplant, raising concerns that delaying transplant for HHM evaluation could worsen outcomes and cause geographic disparities in transplant availability.[12]

These tensions led us to develop novel techniques for HHM evaluation in the transplant setting. Our approaches facilitated timely transplantation with ideal outcomes, as no patients have experienced graft failure, HHM-related transplant complications, or donor-derived malignancies after

more than a year of follow-up. To inform the development of similar programs at other centers, we provide examples in which HHM risk was promptly recognized and mitigated.

We reviewed all patients undergoing stem cell transplant at the University of Chicago since we implemented clinical HHM testing in 2014. We extracted data from transplant patients who underwent expedited HHM evaluations. We grouped these approaches into four categories (**Supplementary Figure S1**).

Transplant recipients in Group 1 had potentially incidental germline variants detected via tumor-only genomic profiling. These patients (n=3) did not have personal or family histories concerning for an HHM, so we quickly determined if potentially incidental variants were of germline origin.[13] We performed tumor-only sequencing in these patients during a morphologic remission and did not perform dedicated germline testing. This diagnostic maneuver differed from our standard procedure, as we typically do not perform tumor-only sequencing in remission. These patients are in the blue “variant-informed” box in **Supplementary Figure S1.**

Group 2 had striking personal and family histories but negative HHM testing. Given our concern for an HHM not detected by contemporary techniques, we prioritized MUDs to avoid using cells from MRDs with undiagnosed HHMs. The yellow “high-risk” box represents these patients (n=5) in **Supplementary Figure S1**.

Group 3 had HHMs diagnosed early in their clinical course and have not yet proceeded to transplant, but are undergoing HHM-focused donor evaluation. These patients (n=2) are in the gray “personalized” box in **Supplementary Figure S1**.

Group 4 had personal or family histories concerning for HHMs, but their anticipated transplant dates would not allow for skin fibroblast testing. We instead performed "donor-focused” HHM evaluations by sequencing DNA from each donor’s saliva, peripheral blood, or DNA previously provided for human leukocyte antigen (HLA) testing. This approach, particularly using DNA collected for HLA testing, enabled rapid turnaround times by avoiding additional visits for donor DNA collection. For this group, HHM evaluation on the index patient (transplant recipient) was not completed before

transplant. This group also included patients who had matched unrelated donor (MUD) transplants because a MRD without the variant in question was not available. These patients (n=12) are in the green “donor-focused” box in **Supplementary Figure S1**.

All patients underwent genetic counseling before germline testing and provided informed consent to IRB-approved research protocols at the University of Chicago. All research was conducted per the Declaration of Helsinki. R Studio Version 2023.09.0 and GraphPad Prism v.8.0 were used for data analysis and visualization. All variants of interest are listed in **Supplemental Table S2**.

We classified the patients into four groups (**Table 1, Table 2, Supplementary Figure S1**). In the first group of patients (n=3) without family histories of cancer or blood disorders, potentially incidental HHM-related germline variants in CEBPA, RECQL4, and TERT were identified on tumor-only sequencing. We analyzed variant allele frequency changes during induction therapy.[13] In each patient, the potential germline variants disappeared at remission, confirming their somatic origins (**Table 1, Supplemental Figure S1**).

The second group of five patients had a negative HHM evaluation, but we used MUDs based on a high suspicion of an undiagnosed HHM. For these patients, the median time from skin biopsy to HHM result was 68 days (range: 41-121 days, **Figure 1A**). One patient (patient 4) carried a germline PALB2 pathogenic variant. This variant was discordant with their phenotype, and we continued to have a high suspicion for an HHM with an undetectable germline driver. This patient received a MUD transplant and continues to do well 97 days after transplant.

The fourth group (n=12) received expedited transplant clearance via sequencing of DNA from donor HLA samples (n=5), saliva (n=1), or blood (n=4). Two patients did not have MRDs without the HHM-related variant in question. For these patients, we used MUDs. Patient 9 carried a germline *PALB2* variant associated with hereditary solid tumors, but without association to HHMs. This variant was identified in a potential MRD. There are theoretical risks of stem cell mobilization in donors with germline variants in genes related to DNA repair, but these remain unproven.[14] Since no unrelated or

alternative donor was available for the patient, this MRD was used. The patient engrafted as expected and remains free of donor-derived complications 4.2 years after transplant.

For MRDs in whom we sequenced a known variant identified in the index patient, the median time from sample collection to test result was 12 days (range: 3-64, **Figure 1A**). For index patients with a concerning personal and/or family history, but for whom sequencing of cultured skin fibroblast DNA was not feasible due to time constraints, we performed next-generation sequencing on donor DNA with a median turnaround time of 26 days (range: 12-39 days, Figure 1A). Details of this sequencing panel are in **Supplementary Table S1**.

Our donor-focused HHM screening approach enabled us to significantly reduce turnaround time for HHM evaluations. While the median turnaround of an HHM evaluation with cultured skin fibroblasts was 64 days, the median turnaround with donor-focused sequencing was 14 days ($p < 0.05$). Expedited HHM evaluation enabled us to sequence donors before results from recipients' cultured skin fibroblasts returned (**Figure 1A**). This approach was particularly helpful for patients with bone marrow failure who were at high risk for clinical deterioration from infectious or hemorrhagic complications (patients 13 and 17). For these patients, our HHM evaluations took 12 and 24 days, respectively (**Table 2**).

Importantly, we observed highly variable timelines to transplant after HHM results returned. This post-HHM evaluation/pre-transplant period was often longer than the turnaround time of our HHM evaluations. This delay reflects the many variables that stall stem cell transplant (Figure 1B), but our expedited approaches removed HHM evaluations as a source of delays.

At a median follow-up of 451 days post-transplant, none of our patients experienced graft failure, transplant-related morbidity, or donor-derived malignancies. Patient 14 died 197 days after transplant from non-relapse-related respiratory failure secondary to pneumonia.

Here, we describe methods for expediting HHM evaluations in urgent transplant situations that prohibited sequencing DNA from cultured skin fibroblasts. Using a combination of tumor mutation dynamics, donor-focused HHM screening, rigorous donor selection, and clinical inference, we

screened each patient for an HHM and cleared them for transplant. While ongoing research seeks to further characterize a growing spectrum of HHM phenotypes, we caution against the reflexive exclusion of donors harboring pathogenic variants that have not been clearly implicated in HHMs, which in our series included *BRCA1/2, PALB2,* and a *FANCE* heterozygous carrier. The field currently lacks clear consensus surrounding the use of known carriers of pathogenic or likely pathogenic mutations as stem cell donors, especially for BRCA1/2,[15] and decision-making surrounding these donor candidates varied amongst physicians at our center. Nevertheless, in our study, after a median follow-up of more than one year, all patients in this study have been free of graft failure, HHM-related transplant morbidity, and donor-derived malignancies. Our approaches to performing expedited HHM evaluations may benefit other physicians involved in caring for patients at risk for HHMs who are being considered for stem cell transplant.

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Table 1. Overview of patients and matched related donor candidates undergoing testing for hereditary hematopoietic malignancy (HHM) syndromes in the setting of stem cell transplant.

Pt #, disease, age	MRD Candidate	HHM Gene	HHM Testing Method	HHM Result	Final Donor	HHM evaluation
Pt 1, AML, 18	Sibling (14)	*TERT*	VAF dynamics tumor NGS	Negative	MRD	64 days
Pt 2, AML, 24	Sibling (21)	*CEBPA*	HHM Panel NGS from cultured skin fibroblasts on index patient	Negative	MRD	40 days
Pt 3, SAA, 22	Sibling (24)	*RECQL4* (uncultured SF)	HHM/Immunodeficiency panel on cultured SF from index patient	*RECQL4* variant (heterozygous)	MRD	53 days
Pt 4, JMML, 7	n/a	*PALB2* (somatic panel)	HHM/Immunodeficiency NGS panel on cultured SF from index patient	*PALB2* p.I156fs*11	MMUD	121 days
Pt 5, SAA, 37	Sibling with history of AA	Unknown (general HHM phenotype)	HHM panel from cultured SF	Negative	MUD	41 days
Pt 6, t-MN, 69	n/a	*TP53* (somatic panel)	HHM panel from cultured SF	Negative	MUD	68 days
Pt 7, ALL, 22	n/a	Unidentified	HHM panel from cultured SF	Negative	MUD	71 days
Pt 8, t-MN, 68	Sibling with history of DLBCL, t-MN	Unidentified	HHM Panel from cultured SF	Negative	SCT pending	65 days
Pt 9, AML, 73	n/a	*DDX41* (somatic panel)	HHM panel from cultured SF	*DDX41* p.Ala191Thr	SCT pending	72 days
Pt 10, t-MN, 65	n/a	*BRCA1*	Prior commercial testing for HBOC syndrome	*BRCA1* p.Q1777P*fs	SCT pending	n/a

Suspected HHM-related genes are frequently first identified via standard-of-care somatic tumor sequencing. HHM workup is further clarified by personal and/or family history. Results of germline testing and the methodology used to identify HHM-associated genes are shown. Patients for whom HHM evaluation was triggered by the potentially incidental identification of a pathogenic/likely pathogenic germline variant on tumor genomic profiling, but an expedited HHM evaluation was pursued without culturing skin fibroblasts due to transplant time constraints are shown in **blue (Group 1)**. **Group 2** (**yellow**) contains patients and MRDs with a strong suspicion for an HHM based on strong personal and/or family history, but with negative germline testing. These patients received

stem cells from matched unrelated donors (MUDs). Patients for whom HHMs were identified early in the clinical course are highlighted in **grey (Group 3)** but have not yet proceeded to transplant. Patients in grey represent “ideal” timelines for HHM evaluation and are used as examples of “control” timelines. Abbreviations: AML, acute myeloid leukemia; HHM, hereditary hematopoietic malignancy; MRD, matched related donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; CR, complete response; NGS, next generation sequencing; PGV, pathogenic germline variant; SCT, stem cell transplantation; SAA, severe aplastic anemia; BMF, bone marrow failure; PB, peripheral blood; SF skin fibroblasts; MDS, myelodysplastic syndrome.

Pt #, disease, age	MRD Candidate	HHM Gene	HHM Testing Method	HHM Result	Final Donor	HHM evaluation
Pt 11, AML, 48	Sibling (37)	*BRCA2*	*BRCA2* single gene testing of potential MRD saliva sample	*BRCA2* p.Arg2520X	Haplo-cord	14 days
Pt 12, AML, 46	Sibling (49)	*BRCA1*	*BRCA1* single gene testing of potential MRD PB	*BRCA1* p.C61G	MUD	3 days
Pt 13, SAA, 50	Sibling 1 (53) Sibling 2 (55)	Unknown (general HHM phenotype)	Comprehensive BMF panel on HLA samples from potential MRDs	Negative	MRD	12 days
Pt 14, AML, 50	Sibling (49)	*CEBPA* (somatic panel)	*CEBPA* single gene testing of potential MRD PB sample	Negative	MRD	5 days
Pt 15, MDS, 71	Child 1 (43) Child 2 (46)	*DDX41* (somatic panel)	*DDX41* single gene testing of MRD buccal swab	*DDX41* 2.4 kb deletion in patient and Child 2	MRD	23 days
Pt 16, AML, 34	Sibling (38)	*PALB2*	MRD known *PALB2* PGV carrier, confirmed on PB single gene NGS	*PALB2* p.Ser254Ilefs*3	MRD	7 days
Pt 17, SAA, 8	Parent	*FANCA* (somatic panel)	BMF panel from cultured SF from patient; single blood PB testing from MRD	*FANCA* p.His913Pro	Haploidentical	24 days
Pt 18, AML, 70	n/a	*MLH1* (known) *TP53* (somatic panel)	Pt with known Lynch Syndrome, HHM panel on SF revealing Li Fraumeni Syndrome	*MLH1* p.Val612del; *TP53* exon 1 deletion	MUD	64 days
Pt 19, AML, 46	n/a	*CHEK2* (somatic panel)	Confirmation of PGV via SF HHM testing after incidental finding on somatic NGS	*CHEK2* p.I200T	MMUD	56 days
Pt 20, ALL, 57	Sibling (56)	*IKZF1* (somatic panel)	Comprehensive BMF panel on HLA sample from potential MRD	Negative	MRD	11 days
Pt 21, AML, 50	Child (25)	*FANCE*	Hereditary Myeloid Malignancy Panel from PB	*FANCE* heterozygous carrier	Haploidentical	39 days
Pt 22, SPTCL, 52	Child 1 Child 2	*HAVCR2* (homozygous)	Prior commercial testing: HLH panel from PB	*HAVCR2* p.Tyr82Cys homozygous (patient); Child 1 confirmed heterozygote on PB single gene testing	SCT canceled	7 days

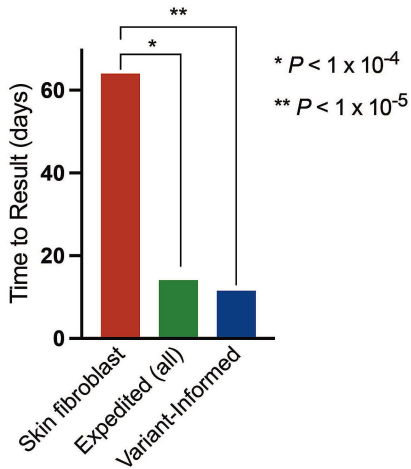
Table 2. Donor-focused expedited evaluation for hereditary hematopoietic malignancy.

Patients for whom HHM testing was performed on samples from potential matched related donors (MRDs) without completing HHM evaluation on the index patient (stem cell recipient) before

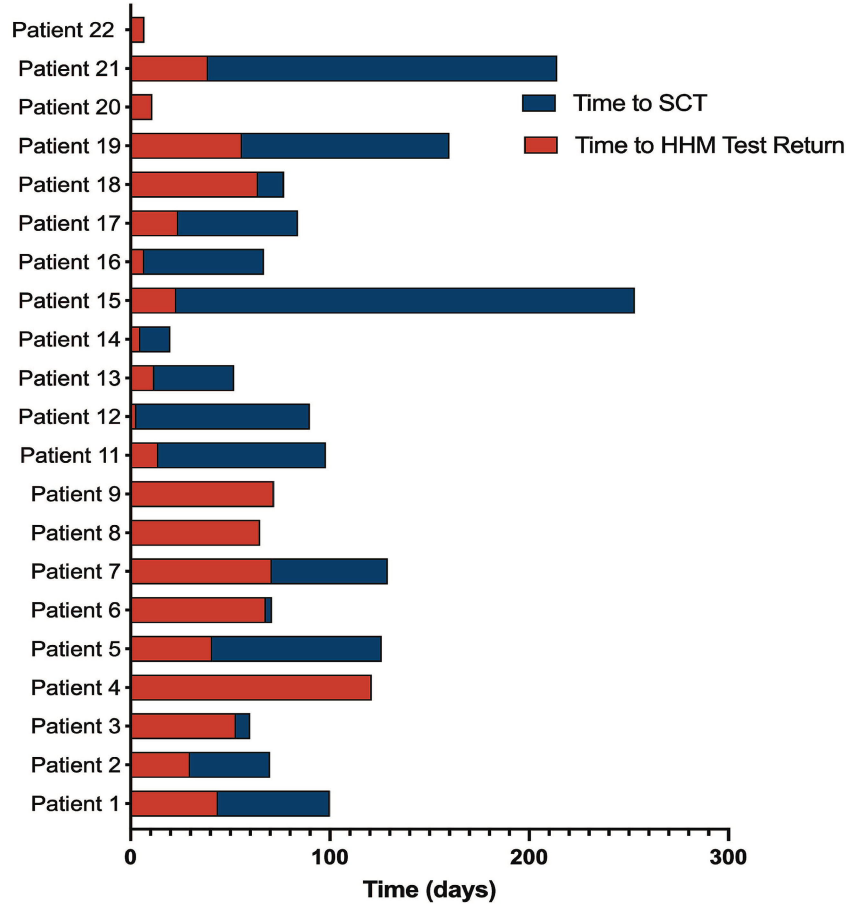
transplant are shown. The potential MRD for patient 11 carried the pathogenic familial *BRCA2* variant, and a haplo-cord cell source was chosen to reduce the theoretical risks of donor mobilization in a patient with a *BRCA2* variant. The donor candidate for patient 15 was negative for the *DDX41* variant in question and was used as an MRD. For patient 17, a haploidentical transplant from an MRD was used as no alternative sources were available. For patient 21, a haploidentical transplant from an MRD was used as the MRD was a heterozygous carrier for the *FANCE* variant in question. For patient 15, the transplant was canceled after the patient experienced an exceptional clinical response with induction therapy, and the risks/benefits were felt to favor deferring the transplant. Abbreviations: AML, acute myeloid leukemia; HHM, hereditary hematopoietic malignancy; MRD, matched related donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; CR, complete response; NGS, next generation sequencing; PGV, pathogenic germline variant; SCT, stem cell transplantation; SAA, severe aplastic anemia; BMF, bone marrow failure; PB, peripheral blood; SF skin fibroblasts; MDS, myelodysplastic syndrome; SPTCL, Subcutaneous panniculitis-like T-cell lymphoma; HLH, hemophagocytic lymphohistiocytosis; HBOC, hereditary breast and ovarian cancer.

Figure 1. Turnaround time for hereditary hematopoietic malignancy (HHM) test results using cultured skin fibroblasts, an expedited HHM evaluation approach, or a variant informed HHM evaluation approach. (A) Turnaround times for HHM testing are shown for patients evaluated using the "classic" approach of sequencing DNA from cultured skin fibroblasts, an "expedited" HHM evaluation approach, or a "variant informed" HHM evaluation approach. Expedited approaches included any non-cultured skin fibroblast-based testing approaches, such as using a donor-directed HHM evaluation. "Variant-informed" approaches used changes in a potentially incidental germline variant's allele frequency during induction therapy to clear the index patient of an HHM. Of note, these patients did not otherwise have concerning family histories. **(B)** Bar graph demonstrating the duration of hereditary hematopoietic malignancy evaluation and time to transplant for patients in the cohort. Of note, patients 8 and 9 did not receive stem cell transplantation prior to publication. In one patient (Patient 4), HHM results returned after a matched unrelated donor stem cell transplant was pursued.

A.


**
*
* $P < 1 \times 10^{-4}$
** $P < 1 \times 10^{-5}$
Time to Result (days)
0
20
40
60
Skin fibroblast
Expedited (all)
Variant-Informed

B.


Patient 22
Patient 21
Patient 20
Patient 19
Patient 18
Patient 17
Patient 16
Patient 15
Patient 14
Patient 13
Patient 12
Patient 11
Patient 9
Patient 8
Patient 7
Patient 6
Patient 5
Patient 4
Patient 3
Patient 2
Patient 1
Time to SCT
Time to HHM Test Return
0
100
200
300
Time (days)

SUPPLEMENTARY MATERIALS FOR:

Expedited evaluation for hereditary hematopoietic malignancies in the setting of stem cell transplantation

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CONTENTS:

Figure S1.

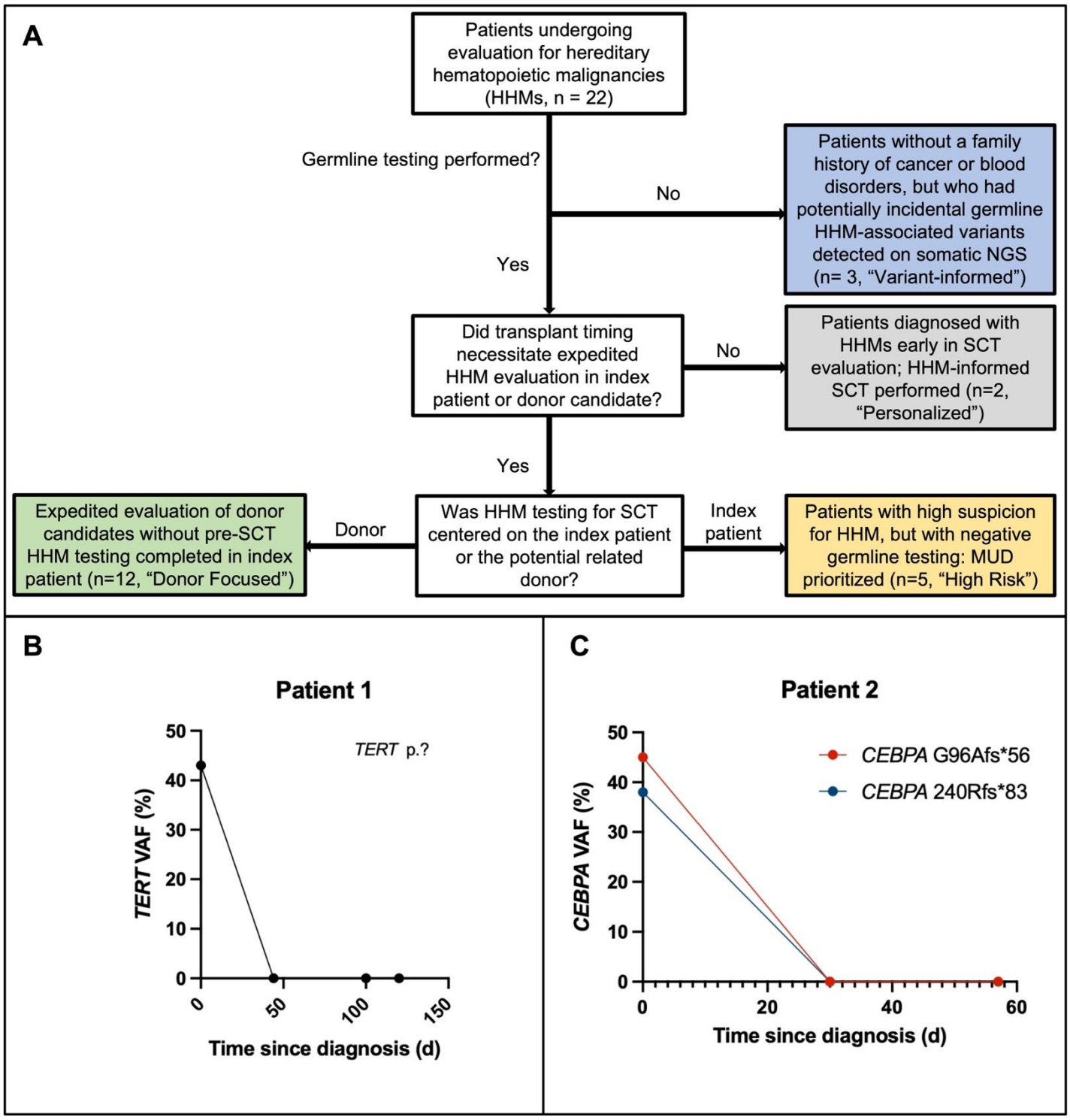

Figure S1. (A): Flow diagram for classification of patients and donor candidates undergoing expedited evaluation for hereditary hematopoietic malignancies in the setting of stem cell transplant. (B & C): longitudinal variant allele frequency (VAF) measurements for genes that raised suspicion for an HHM on diagnostic somatic tumor sequencing in patients 1 (B)

and 2 (B). These patients did not have high-risk family histories that were concerning for HHMs. The disappearance of detectable mutations with induction therapy strongly suggested these potentially incidental germline variants were of somatic origin. Therefore, transplantation was not delayed while formal HHM testing was performed. HHM: hereditary hematopoietic malignancy.

Table S1. Genes analyzed for donor-only sequencing.

Genes analyzed for donor-only sequencing.
AIP, ALK, ANKRD26, APC, APOA1, APOA2, ARID1A, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BTK, CARD11, CASP10, CASR, CBL, CD27, CD40LG, CD70, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CEBPA, CHEK2, CSF3R, CST3, CTLA4, CTNNA1, CTPS1, DDX41, DICER1, DIS3, DIS3L2, DOCK8, EGFR, EPCAM, ERCC6L2, ETV6, FGA, FH, FLCN, GATA2, GPC3, GREM1, GSN, HOXB13, HRAS, IKZF1, ITK, JAK2, KDM1A, KIT, LYZ, MAGT1, MAX, MBD4, MECOM, MEN1, MET, MITF, MLH1, MPL, MRTFA, MSH2, MSH3, MSH6, MUTYH, NAF1, NBN, NF1, NF2, NPAT, NPM1, NTHL1, PALB2, PAX5, PDGFRA, PGM3, PHOX2B, PIK3CD, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, PTPN11, RAD50, RAD51C, RAD51D, RASGRP1, RB1, RBBP6, RBM8A, RECQL4, RET, RTEL1, RUNX1, SAMD9, SAMD9L, SDHA, SDHAF2, SDHB, SDHC, SDHD, SH2B3, SMAD4, SMARCA4, SMARCB1, SMARCE1, SRP72, STAT3, STK11, SUFU, TERC, TERT, TET2, TMEM127, TNFRSF9, TP53, TSC1, TSC2, TTR, UNC13D, USP45, VHL, WAS, WRN, WT1

Table S2. Variants analyzed. Variants in HHM-related genes.

Patient	Variant	UChicago Interpretation	ClinVar Classification	dbSNP
Patient 1	*TERT* c.1951-1G>A, p.? NM_198253.3	P	N/A	N/A
Patient 2	*CEBPA* c.287_311del (p.G96Afs*56); c.707_713dup, (p.A240Rfs*83) NM_004364.3	P	N/A	N/A
Patient 3	*RECQL4* c.1132-1G>A, p.? NM_004260.3	LP	LP	rs751503394
Patient 4	*PALB2* c.466_467del, p.I156Ffs*11 NM_024675.4	P	P/LP	rs876659405
Patient 5	Unknown		N/A	N/A
Patient 6	*TP53* c.997dup, p.R333Pfs*4 NM_000546.6	P	N/A	N/A
Patient 7	Unknown		N/A	N/A
Patient 8	Unknown		N/A	N/A
Patient 9	*DDX41* c.571G>A, p.? NM_016222.4	P	N/A	N/A
Patient 10	*BRCA1* c.5329dup, p.Q1777Pfs*74 NM_007300.4	P	P	rs80357906
Patient 11	*BRCA2* c.7558C>T, p.Arg2520* NM_000059.3	P	P	rs80358981
Patient 12	*BRCA1* c.181T>G, p.C61G NM_007294.4	P	P	rs28897672
Patient 13	N/A	N/A	N/A	N/A
Patient 14	*CEBPA* p.Q312dup; p.V95fs*62 NM_004364.3	N/A	N/A	N/A
Patient 15	*DDX41* 2.4 kB deletion NM_016222.3	P	N/A	N/A
Patient 16	*PALB2* c.758dup, p.S254Ifs*3 NM_024675.4	P	P/LP	rs515726126
Patient 17	*FANCA* c.2738A>C , p.H913P NM_000135.4	P	P/LP	rs1302083447
Patient 18	*MLH1* c.1835_1837 (p.Val612del) NM_000249.3 *TP53 exon 1 deletion* NM_000546.5	LP / P	N/A	N/A
Patient 19	*CHEK2* c.470T>C, p.I157T NM_007194.4	LP	P/LP	rs17879961
Patient 20	*IKZF1* loss	P	N/A	N/A
Patient 21	*FANCE* c.1111C>T , p.Arg371Trp NM_021922.2	P (heterozygous)	P/LP	rs775076977
Patient 22	*HAVCR2* c.245 A>G, p.Tyr82Cys NM_032782.5	VUS	VUS	rs184868814