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

It is being increasingly recognized that many patients with blood cancers harbor germline variants that increase cancer risk.[1] For example, 14% of patients with acute myeloid leukemia in the BEAT AML study had germline variants associated with a hereditary hematopoietic malignancy (HHM) despite an older age at diagnosis (median, 72 years).[2] Moreover, 7% of patients with a myelodysplastic syndrome shared deleterious germline variants with their matched-related stem cell donors (MRD).[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 HHM in the transplant setting.[9] First, physicians must recognize patients at risk of an HHM. Clinical suspicion may be obscured by the adult age of onset of some HHM, which mimics many sporadic malignancies. Contemporary family structures are also smaller, which may reduce the family history "signal" of an HHM.[10] The diagnosis of an HHM 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 transplantation, putting patients at risk of relapse. For patients with bone marrow failure, we have historically been hesitant to delay transplants for an HHM evaluationgiven the 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, the initial suspicion of an HHM occurred in the weeks before transplantation, raising concerns that delaying the transplant in order to carry out an evaluation for an HHM 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 transplantation 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 (*Online 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 that raised concerns of an HHM, so we quickly determined whether potentially incidental variants were of germline origin.[13] We performed tumor-only sequencing in these patients during a morphological 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 shown in the blue "variant-informed" box in *Online Supplementary Figure S1*.

Group 2 had striking personal and family histories but were negative in HHM testing. Given our concern about an HHM not detected by contemporary techniques, for these patients we prioritized matched unrelated donors (MUD) to avoid using cells from MRD with undiagnosed HHM. These patients (N=5) are shown within the yellow "high-risk" box in *Online Supplementary Figure S1*.

Group 3 had been diagnosed as having an HHM early in their clinical course and had not yet proceeded to transplant, but were undergoing HHM-focused donor evaluation. These patients (N=2) are in the gray "personalized" box in *Online Supplementary Figure S1*.

Group 4 had personal or family histories that raised concerns about an HHM, 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 the transplantation. This group also included patients who received 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 *Online Supplementary Figure S1*.

All patients underwent genetic counseling before germline testing and provided informed consent to Institutional Review Board-approved research protocols at the University of Chicago. All research was conducted according to the Declaration of Helsinki. R Studio version 2023.09.0 and GraphPad Prism version 8.0 were used for data analysis and visualization. All variants of interest are listed in *Online Supplementary Table S2*.

We classified the patients into four groups (Tables 1 and 2, *Online 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 fre-

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

Pt #, disease (age in years)	MRD candidate (age in years)	HHM gene	HHM testing method	HHM result	Final donor	HHM evaluation
GROUP 1						
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 on cultured SF from 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
GROUP 2						
Pt 4, JMML (7)	NA	*PALB2* (somatic panel)	HHM/ immunodeficiency NGS panel on cultured SF from index patient	*PALB2* p.I156fs*11	MMUD	121 days
Pt 2, AML (24)	Sibling with history of AA	Unknown (general HHM phenotype)	HHM panel on cultured SF	Negative	MUD	41 days
Pt 6, t-MN (69)	NA	*TP53* (somatic panel)	HHM panel on cultured SF	Negative	MUD	68 days
Pt 7, ALL (22)	NA	Unidentified	HHM panel on cultured SF	Negative	MUD	71 days
Pt 8, t-MN (68)	Sibling with history of DLBCL, t-MN	Unidentified	HHM panel on cultured SF	Negative	SCT pending	65 days
GROUP 3						
Pt 9, AML (73)	NA	*DDX41* (somatic panel)	HHM panel on cultured SF	*DDX41* p.Ala191Thr	SCT pending	72 days
Pt 10, t-MN (65)	NA	*BRCA1*	Prior commercial testing for HBOC syndrome	*BRCA1* p.Q1777P*fs	SCT pending	NA

Suspected hereditary hematopoietic malignancy (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. Group 1 comprises 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. Group 2 contains patients and MRD who are suspected of having an HHM based on a strong personal and/or family history, but with negative germline testing. These patients received stem cells from matched unrelated donors. Group 3 consists of patients in whom an HHM was identified identified early in the clinical course but have not yet proceeded to transplantation; they represent “ideal” timelines for HHM evaluation and are used as examples of “control” timelines. Pt #: patient number; MRD: matched related donor; AML: acute myeloid leukemia; VAF: variant allele frequency; NGS: next-generation sequencing; SF: skin fibroblasts; SAA: severe aplastic anemia; JMML: juvenile myelomonocytic leukemia; NA: not applicable; MMUD: mismatched unrelated donor; AA: aplastic anemia; MUD: matched unrelated donor; t-MN: therapy-related myeloid neoplasm; ALL: acute lymphoblastic leukemia; DLBCL: diffuse large B-cell lymphoma; SCT: stem cell transplantation; HBOC: hereditary breast and ovarian cancer.

quency changes during induction therapy.[13] In each patient, the potential germline variants disappeared at remission, confirming their somatic origins (Table 1, *Online Supplementary Figure S1*).

The second group of five patients had a negative HHM evaluation, but we used MUD based on a strong 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 the patient's phenotype, and we continued to have a strong suspicion of an HHM with an undetectable germline driver. This patient received a MUD transplant and continues to do well 97 days after transplantation.

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 a MRD without the HHM-related variant in question. For these patients, we used MUD. Patient #9 carried a germline *PALB2* variant associated with hereditary solid tumors, but without association with an HHM. 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 transplantation.

For MRD 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

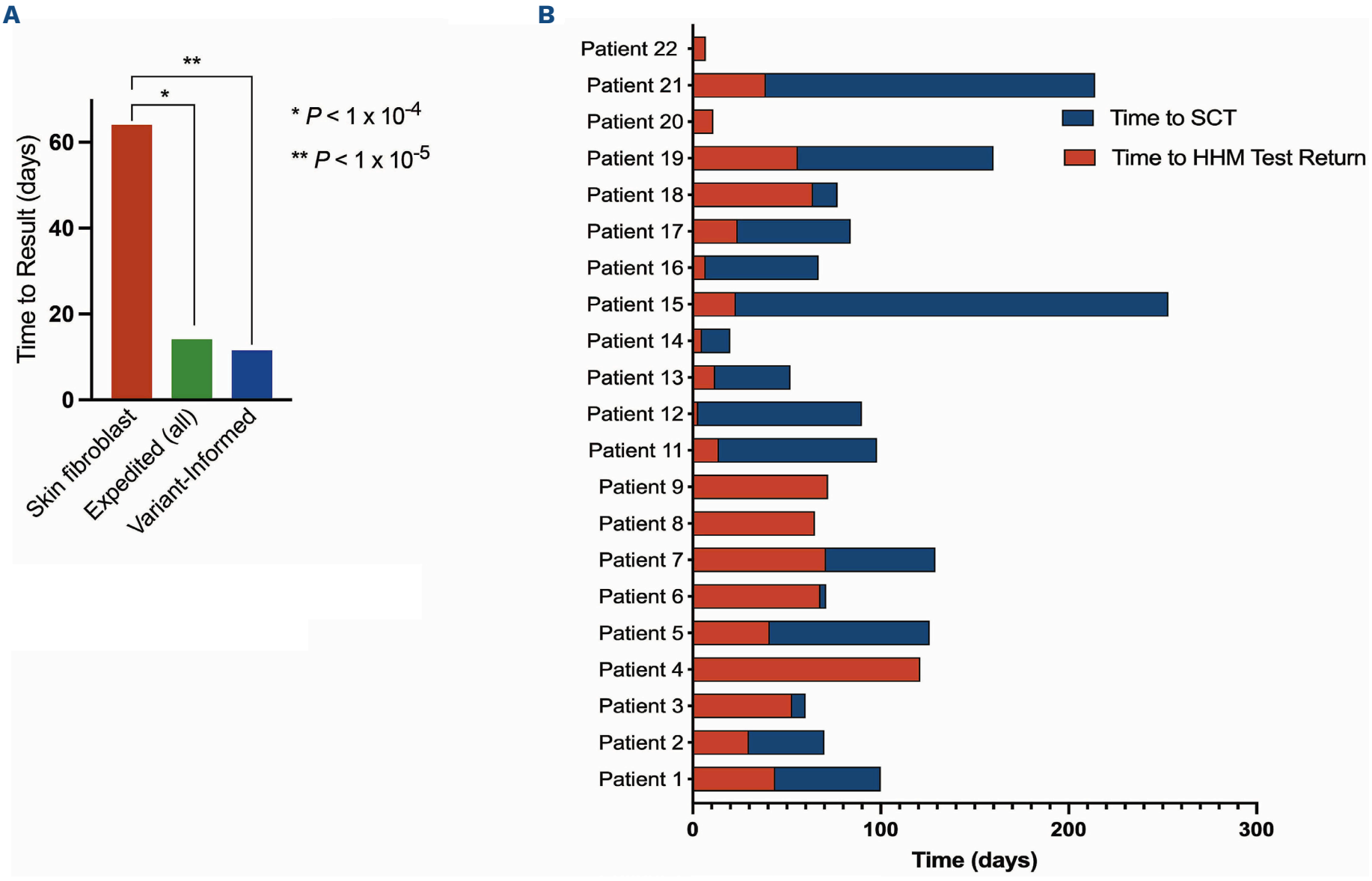

Figure 1. Turnaround time for hereditary hematopoietic malignancy (HHM) test results using cultured skin fibroblasts, an expedited HHM evaluation approach, or a variant-informed HHM evaluation approach. (A) Turnaround times for HHM testing are shown for patients evaluated using the "classic" approach 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 the fraction of a potentially incidental germline variant allele 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 HHM evaluation and time to transplantation for patients in the cohort. Of note, patients #8 and #9 had not undergone stem cell transplantation prior to publication. In one patient (patient #4), HHM results returned after a matched unrelated donor stem cell transplant was pursued. SCT: stem cell transplantation.

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

Pt #, disease, (age in years)	MRD candidate (age in years)	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 MRD	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 on cultured SF from patient; single blood PB testing from MRD	*FANCA* p.His913Pro	Haploidentical	24 days
Pt 18, AML (70)	NA	*MLH1* (known) *TP53* (somatic panel)	Pt with known Lynch syndrome, HHM panel on SF revealed Li Fraumeni syndrome	*MLH1* p.Val612del; *TP53* exon 1 deletion	MUD	64 days
Pt 19, AML (46)	NA	*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 on PB	*FANCE* heterozygous carrier	Haploidentical	39 days
Pt 22, SPTCL (52)	Child 1 Child 2	*HAVCR2* (homozygous)	Prior commercial testing: HLH panel on PB	*HAVCR2* p.Tyr82Cys homozygous (patient); Child 1 confirmed heterozygote on PB single gene testing	SCT canceled	7 days

Continued on following page.

Patients for whom hereditary hematologic malignancy (HHM) testing was performed on samples from potential matched related donors (MRD) without the HHM evaluation having been completed 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 to induction therapy, and the risks/benefits were felt to favor deferring the transplant. Pt #: patient number; AML: acute myeloid leukemia; PB: peripheral blood; MUD: matched unrelated donor; SAA: severe aplastic anemia; BMF: bone marrow failure; HLA: human leukocyte antigen; MDS: myelodysplastic syndrome; PGV: pathogenic germline variant; NGS: next-generation sequencing; SF: skin fibroblasts; NA: not applicable; MMUD: mismatched unrelated donor; ALL: acute lymphoblastic leukemia; SPTCL: subcutaneous panniculitis-like T-cell lymphoma; HLH: hemophagocytic lymphohistiocytosis; SCT: stem cell transplantation.

a median turnaround time of 26 days (range, 12-39 days) (Figure 1A). Details of this sequencing panel are shown in *Online 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 of 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 transplantation 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 transplantation (Figure 1B), but our expedited approaches removed HHM evaluations as a source of delays.

At a median follow-up of 451 days after transplantation, none of our patients experienced graft failure, transplant-related morbidity, or donor-derived malignancies. Patient #14 died 197 days after transplantation 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 transplantation. 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 HHM, 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 among physicians at our center. Nevertheless, in our study, after a median follow-up of more than 1 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 of an HHM who are being considered for stem cell transplantation.

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https://doi.org/10.3324/haematol.2023.284584

Received: October 30, 2023.
Accepted: April 5, 2024.
Early view: April 11, 2024.

Disclosures

GWR, SK, MTN, EN, FH, DG, SD, and LEC have no conflicts of interest to disclose. AAP has received honoraria from AbbVie and BMS and research funding from Celgene/BMS, Pfizer, and Kronos Bio. ASD has participated in a speakers' bureau for CEConcepts. LAG

has received royalties from UpToDate. MRB has provided consultancy and/or advisory board services for Kite/Gilead, Novartis, Bristol Myers Squibb, CRISPR Therapeutics, Autolus, In8bio, Sana Biotechnology, Chimeric Therapeutics, Arcellx, and Achieve Clinics and has received honoraria from Bristol Myers Squibb, Kite/Gilead, Novartis, Incyte, Servier, Sanofi, and ADC Therapeutics. OO has acted as a consultant for AbbVie, Impact Biomedicines, Celgene, Novartis, BMS, Taiho Pharmaceutical, CTI, Treadwell Therapeutics, and Celgene and has received research support (for her institute) from Celgene, Daichii Sankyo, Uncyte, Astex Pharmaceuticals, NS Pharma, Abbvie, Janssen Oncology, OncoTherapy Science, Agios, AstraZeneca, CTI BioPharma Corp, Kartos Therapeutics, and Aprea AB. PAR has provided consultancy and/or advisory board services for AbbVie, Genmab, ADC Therapeutics, Pharmacyclics, Novartis, Bristol Myers Squibb, Kite/Gilead, Nurix Therapeutics, Nektar Therapeutics, Takeda, Intellia Therapeutics, Calibr, Xencor, Fate Therapeutics, and Tessa Therapeutics. RAL has acted as a consultant/advisor for Ariad/Takeda, Celgene/BMS, CVS/Caremark, Epizyme, Immunogen, Novartis, Servier, Actinium Pharmaceuticals, Kling Biotherapeutics, Curis, Jazz Pharmaceuticals, AbbVie, and Rigel; has received clinical research support from Astellas, Cellectis (institutional), Daiichi Sankyo (institutional), Forty Seven/Gilead, Novartis, and Rafael Pharmaceuticals (institutional); and has received royalties from UpToDate. WS has acted as a consultant/advisor for Amgen, Astra Zeneca, Syndax, Adaptive Biotechnologies, Jazz Pharmaceuticals, Agios, Kite, Kura Oncology, GlaxoSmithKline, MorphoSys, Pfizer, and Servier; has received honoraria from Jazz, Pfizer, AbbVie, and UpToDate; has received travel support from Pfizer; and has sat on a data safety monitoring board or advisory board for Newave. MWD is a scientific consultant for Argenx.

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, and MRB cared for the patients. DG and SD performed molecular pathology testing and reporting. GWR and MWD drafted the manuscript. All authors edited the manuscript.

Acknowledgments

We thank our patients and their family members who participated in research on hereditary hematopoietic malignancies.

Funding

This work was supported by the Edward P. Evans Foundation (MWD), the National Institutes of Health K12 Paul Calabresi award (MWD, LEC), the National Institutes of Health Loan Repayment Program (GWR), and the National Institutes of Health T32 Training Grant (GWR).

Data-sharing statement

Genomic data are available on request.

References

1. Roloff GW, Drazer MW, Godley LA. Inherited susceptibility to hematopoietic malignancies in the era of precision oncology. JCO Precis Oncol. 2021;5:107-122.
2. Yang F, Long N, Anekpuritanang T, et al. Identification and prioritization of myeloid malignancy germline variants in a large cohort of adult patients with AML. Blood. 2022;139(8):1208-1221.
3. Feurstein S, Trottier AM, Estrada-Merly N, et al. Germ line predisposition variants occur in myelodysplastic syndrome patients of all ages. Blood. 2022;140(24):2533-2548.
4. Rolles B, Meyer R, Begemann M, et al. DDX41 germline variants causing donor cell leukemia indicate a need for further genetic workup in the context of hematopoietic stem cell transplantation. Blood Cancer J. 2023;3(1):73.
5. Alter BP. Inherited bone marrow failure syndromes: considerations pre- and posttransplant. Blood. 2017;130(21):2257-2264.
6. Lahtinen AK, Koski J, Ritari J, et al. Clinically relevant germline variants in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2023;58(1):39-45.
7. Díaz de Heredia C, Bierings M, Dalle J-H, Fioredda F, Strahm B. Fanconi's anemia and other hereditary bone marrow failure syndromes. In: The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies [Internet]. 7th ed. Cham (CH): Springer; 2019. Chapter 78.
8. Gadalla SM, Sales-Bonfim C, Carreras J, et al. Outcomes of allogeneic hematopoietic cell transplantation in patients with dyskeratosis congenita. Biol Blood Marrow Transplant. 2013;19(8):1238-1243.
9. O'Connor TE, Shaw R, Madero-Marroquin R, Roloff GW. Clinical considerations at the intersection of hematopoietic cell transplantation and hereditary hematopoietic malignancy. Front Oncol. 2023;13:1180439.
10. Tawana K, Drazer MW, Churpek JE. Universal genetic testing for inherited susceptibility in children and adults with myelodysplastic syndrome and acute myeloid leukemia: are we there yet? Leukemia. 2018;32(7):1482-1492.
11. 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):950962.
12. Delamater PL, Uberti JP. Geographic access to hematopoietic cell transplantation services in the United States. Bone Marrow Transplant. 2016;51(2):241-248.
13. Kraft IL, Godley LA. Identifying potential germline variants from sequencing hematopoietic malignancies. Blood. 2020;136(22):2498-2506.
14. Fresa A, Sica S. Should the BRCA1/2-mutations healthy carriers be valid candidates for hematopoietic stem cell donation? Hered Cancer Clin Pract. 2021;19(1):22.
15. Shah PD, Nathanson KL. BRCA1/2 mutations and de novo hematologic malignancies: true, true and not clearly related. Haematologica. 2024;109(1):21-22.