SUPPLEMENTARY DATA

**Supplementary Materials and Methods**

Patients and germline testing

Indications for germline testing included: a personal history of two cancers, one of which was a hematopoietic malignancy; family history of another hematopoietic malignancy within two generations of the index case; family history of a non-hematopoietic tumor diagnosed at age 50 or younger within two generation of the index case; and/or the identification of gene variants on tumor molecular profiling associated with HHMs[1]. Written informed consent was obtained according to approved institutional protocols in accordance with the Declaration of Helsinki. Single nucleotide and copy number variants were classified for pathogenicity using American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines **(Figure S1)**[2].

Next generation sequencing

Exome sequencing was performed using the Agilent SureSelect Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA, USA) that targets the exome, augmented with spike-in probes covering non-coding regions known to contain risk alleles, including the 5'UTRs of *ANKRD26*, *DKC1*, *TERC,* and *TERT*, one intronic region (c.1017+572) in *GATA2* (NM_032638.4), and two intronic regions (c.3724+78 and c.3724+139) in *RTEL1* (NM_032957.4)[1,3,4]. Sequencing was performed using Illumina NextSeq technology with 150-bp paired-end reads (Illumina, San Diego, CA, USA). The mean depth of coverage per sample was over 150X, and on average more than 96% of the targeted regions were covered at a minimum of 30×. Variants within the genes associated with hereditary hematologic malignancies (HHMs) were identified and evaluated using a validated bioinformatic pipeline. The raw variants were filtered to retain high quality variants which were annotated using the Alamut-Batch software and further filtered based on the global population frequency in 1000 Genomes project, Exome Sequencing Project, and ExAC database. Sequence data were analyzed for the presence of single-nucleotide variants (SNVs) and small insertions and deletions (indels). Variants were interpreted by a team of board-certified geneticists and genetic counselors. Single nucleotide and copy number variants were classified for pathogenicity using American College of Medical Genetics and Genomics/Association for Molecular Pathology **(Figure S1)**[2].

Figure S1. Pipeline for filtering germline variants and pathogenicity annotation
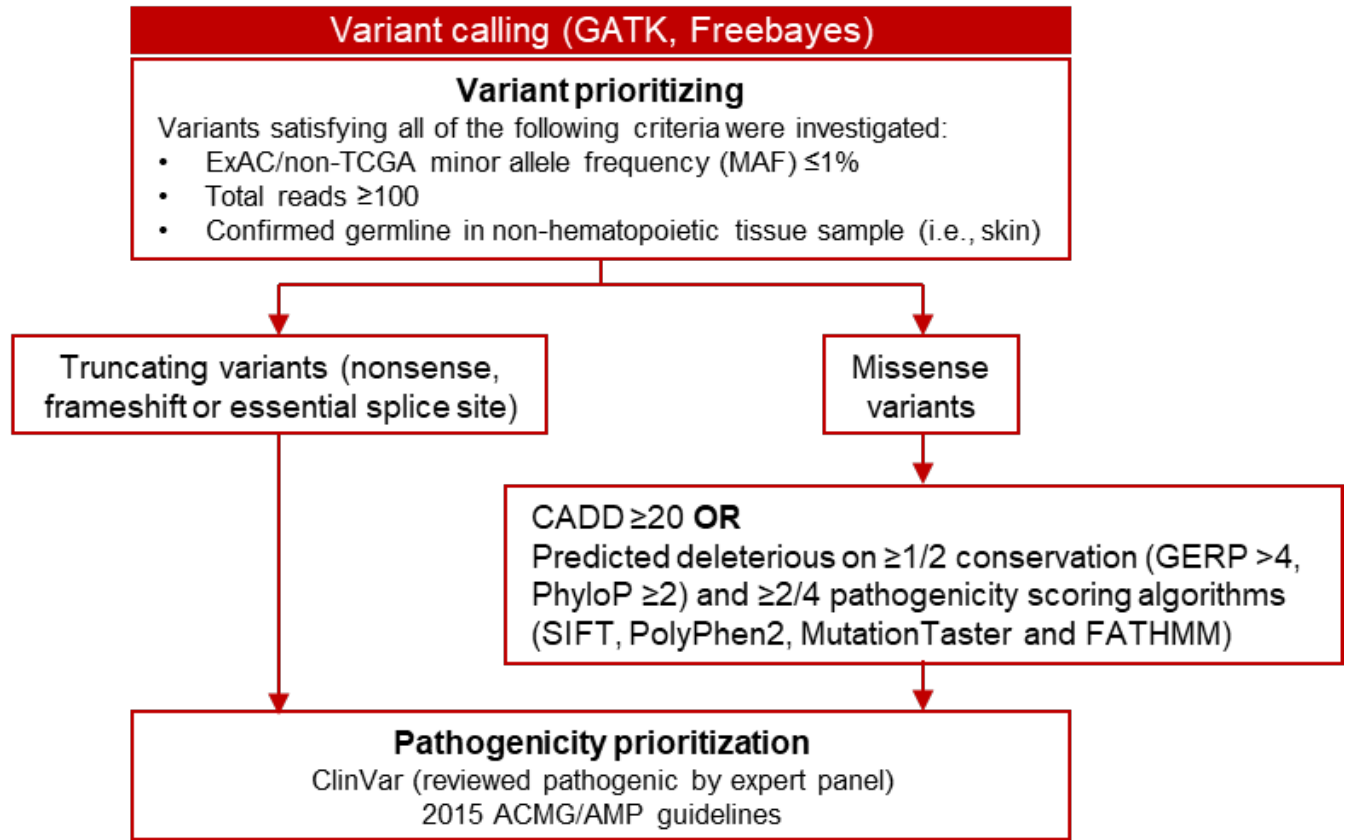
Pathogenic variants were prioritized using American College of Medical Genetics and Genomics (ACMG)/ Association for Molecular Pathology (AMP) guidelines[2].

Statistical analysis

Clinical and pathologic data were available from medical records. Comparisons were made using the chi-square or Fisher`s exact tests for categorical variables, and the Kruskal-Wallis test for continuous variables. Analyses of treatment outcomes used accepted definitions of complete remission (CR) and overall survival (OS)[5,6]. Survival estimates were calculated using the Kaplan-Meier method, and differences between curves were assessed using the log-rank test. Disease-related index (DRI) groups were assigned based on the CIBMTR refined criteria[7]. Multivariable logistic regression was used to integrate known clinical and genetic risk factors and potential confounders for analysis of graft-versus-host disease (GVHD). Staging of acute and chronic graft-versus-host disease (GVHD) was performed using standard algorithms[8,9]. All analyses were performed using JMP software v.14.0.0 (SAS Inc. Cary, NC), with p≤ 0.05 considered statistically significant. Graphs were created using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA).

Supplementary Results

Table S1. List of pathogenic/likely pathogenic (P/LP) germline variants in our inherited myeloid malignancy cohort

Patient nu.	Center	Gene	P/LP variant
1	UofC	*RUNX1*	p.S388X
5	UofC	*NBN*	p.K219Nfs*16
12	UofC	*RUNX1*	p.R204X
13	UofC	*TERT*	p.M970V
15	UofC	*BRCA1*	del exons 13-15
16	UofC	*DDX41*	p.D140fs
20	UofC	*CHEK2*	p.I200T
21	UofC	*CHEK2*	p.S428F
22	UofC	*DDX41*	p.P78Qfs*3
23	UofC	*DDX41*	p.P78Qfs*3
24	UofC	*RTEL1*	p.R1264H
25	UofC	*DDX41*	p.M1?
26	UofC	*CHEK2*	p.T367Mfs*15
27	UofC	*CHEK2*	p.I200T
28	UofC	*DDX41*	p.A500Cfs*9
29	UofC	*CHEK2*	p.I200T
30	UofC	*ATM*	c.2921+1G>A, p.?
31	UofC	*DDX41*	p.R164W
32	UofC	*TP53*	p.R181H
37	UofC	*CHEK2*	p.I200T
38	UofC	*DDX41*	p.L574R*fs143
39	UofC	*CHEK2*	p.I200T
40	UofC	*BRCA1*	c.3109insAA
47	UofC	*BRCA1*	c.187delAG
51	UofC	*TERC*	n.357_365 del
51	UofC	*TERT*	p.A202T
52	UofC	*BRCA2*	p.S1982Rfs*22
52	UofC	*APC*	p.I1307K
54	UofC	*BRCA1*	c.5503_*1416del
55	UofC	*CHEK2*	p.Y72*
56	UofC	*CHEK2*	p.I200T
58	UofC	*DDX41*	p.Q41*
59	UofC	*DDX41*	p.A492Gfs*17
64	UofC	*ATM*	p.V2716A
65	UofC	*TP53*	exon1 del
65	UofC	*MLH1*	p.V612del
71	UofC	*SBDS*	p.Lys62* and c.258+2T>C (p.?)
72	UofC	*BLM*	p.C237Afs*12
81	UofC	*PALB2*	p.R170Ifs*14
84	UofC	*GATA2*	c.872-46_1017+692del, p.?
85	UofC	*USB1*	p.G69Dfs*46)
87	UofC	*CHEK2*	p.S428F
91	UofC	*DDX41*	p.M1?
92	UofC	*DDX41*	p.P258L
95	UofC	*FANCE*	p.R371W
100	UofC	*DDX41*	p.K108Sfs*3
101	UofC	*CHEK2*	p.I200T
123	UofC	*RUNX2*	p.E72Qfs*102
123	UofC	*NF2*	p.W60Gfs*63

141	UofC	CHEK2	p.S428F
148	UofC	FANCA	p.L845P and c.3624C>T, p.(=)
150	UofC	CHEK2	p.S428F
151	UofC	CHEK2	p.T367Mfs*15
156	UofC	CHEK2	p.I200T
167	UofC	DDX41	deletion of exons 12-17
171	UofC	CHEK2	p.I200T
172	UofC	CHEK2	p.I200T
173	UofC	DDX41	p.D140Gfs*2
174	UofC	GATA2	p.A286V
176	UofC	APC	p.I1307K
179	UofC	CHEK2	p.I200T
191	UofC	CHEK2	p.T367Mfs*15
193	UofC	TP53	p.V216M
196	UofC	BRCA2	p.Q1089Sfs*10
207	UofC	MUTYH	c.939+3A>C, p.?
215	UofC	CHEK2	p.I200T
219	UofC	CHEK2	p.T476M
220	UofC	ATM	c.7630-2A>C, p.?
222	UofC	BRCA2	p.K585fs*3
224	UofC	DDX41	p.M1?
226	UofC	BRCA2	p.R2892Tfs*14
240	UofC	MSH6	p.Q835T
254	UofC	PALB2	p.R686Gfs*23
255	UofC	BRCA2	p.F1546Lfs*22
256	UofC	CHEK2	p.I200T
258	UofC	FANCA	p.D944Gfs*5 and p.E288*
259	UofC	BRCA1	p.Q1777Pfs*74
262	UofC	DDX41	p.R164W
263	UofC	ATR	c.2634-1G>A, p.?
264	UofC	NBN	p.N419Lfs*5
266	UofC	CDKN2A	p.A4_P11dup
267	UofC	DDX41	p.L452fs
268	Adelaide	TP53	p.E271K
274	Adelaide	MPL	p.W398C
284	Adelaide	MUTYH	p.Y176C
312	Adelaide	WRAP53	c.1164+1G>A
315	Adelaide	DDX41	p.D140fs
318	Adelaide	DDX41	p.Y516C
330	Adelaide	DDX41	c.435-2_435-1delAGinsCA
336	Adelaide	NBN	p.K219fs
347	Adelaide	PALB2	p.R170fs*14
349	Adelaide	GATA2	p.T354M
350	Adelaide	CHEK2	p.T367Mfs*15
354	Adelaide	BLM	p.R899*
355	Adelaide	BRCA1	p.Q1777Pfs*74
359	Adelaide	ATR	p.K1273N
360	Adelaide	SBDS	c.258+2T>C
362	Adelaide	RUNX1	Loss of whole gene
363	Adelaide	TERT	p.R971H
368	Adelaide	TP53	p.R196L
374	Adelaide	PTPN11	p.M504T
381	Adelaide	DDX41	p.M1?
384	Adelaide	DDX41	p.D140fs

385	Adelaide	*GATA2*	c.1017+2T>G
390	Adelaide	*DDX41*	p.M1?
396	Adelaide	*DDX41*	p.D140Gfs*2
397	Adelaide	*DDX41*	p.M1?
398	Adelaide	*NBN*	p.K219fs
413	Adelaide	*FANCB*	p.R409W homozygous
419	Adelaide	*NF1*	p.F894C
419	Adelaide	*TERT*	p.V56L
420	Adelaide	*SBDS*	p.K62*
421	Adelaide	*MSH6*	p.T1284fs
425	Adelaide	*BRCA1*	p.E1257fs
427	Adelaide	*DDX41*	c.644+5G>C p.(?)
430	Adelaide	*DDX41*	p.T529fs
437	Adelaide	*RUNX1*	p.R169I
438	Adelaide	*RUNX1*	p.A324Lfs*7
443	UPenn	*DDX41*	p.Q41*
444	UPenn	*DDX41*	p.M1?
445	UPenn	*DDX41*	p.L452Cfs*9
448	UPenn	*DDX41*	p.M1?
472	UofC	*CHEK2*	p.I200T

Table S2. Demographic characteristics of 472 patients

|	Germline *DDX41* (n= 35)	Germline *CHEK2* (n= 23)	Other IMM (n= 63)	No IMM (n= 351)	p value
Age at diagnosis, years, median (range)	67 (51 – 84)	62 (39 – 82)	61 (19 – 80)	66 (18 – 90)	0.002
Female, n (%)	10 (28.6)	13 (56.5)	27 (42.9)	158 (45)	0.16
Diagnosis, n (%)					
AML	23 (65.7)	13 (56.5)	31 (49.2)	175 (49.9)	
MDS	10 (28.5)	4 (17.4)	27 (42.8)	139 (39.6)	0.005
MDS/MPN	1 (2.9)	1 (4.4)	3 (4.8)	24 (6.8)	
MPN	1 (2.9)	5 (21.7)	2 (3.2)	13 (3.7)	
Therapy-related MN, n (%)	4 (11.4)	6 (26.1)	26 (41.3)	132 (37.6)	0.01
First degree relative with any cancer, n (%)	16/19 (84.2)	21/22 (95.4)	33/38 (86.8)	131/187 (70)	0.10
First degree relative with hematologic cancer, n (%)	10/19 (52.6)	9/22 (40.9)	6/38 (15.8)	36/187 (19.2)	0.001

AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; MN, myeloid neoplasm; MPN, myeloproliferative neoplasm; IMM, inherited myeloid malignancy

Figure S2. Deleterious germline variants in patients with inherited myeloid malignancies
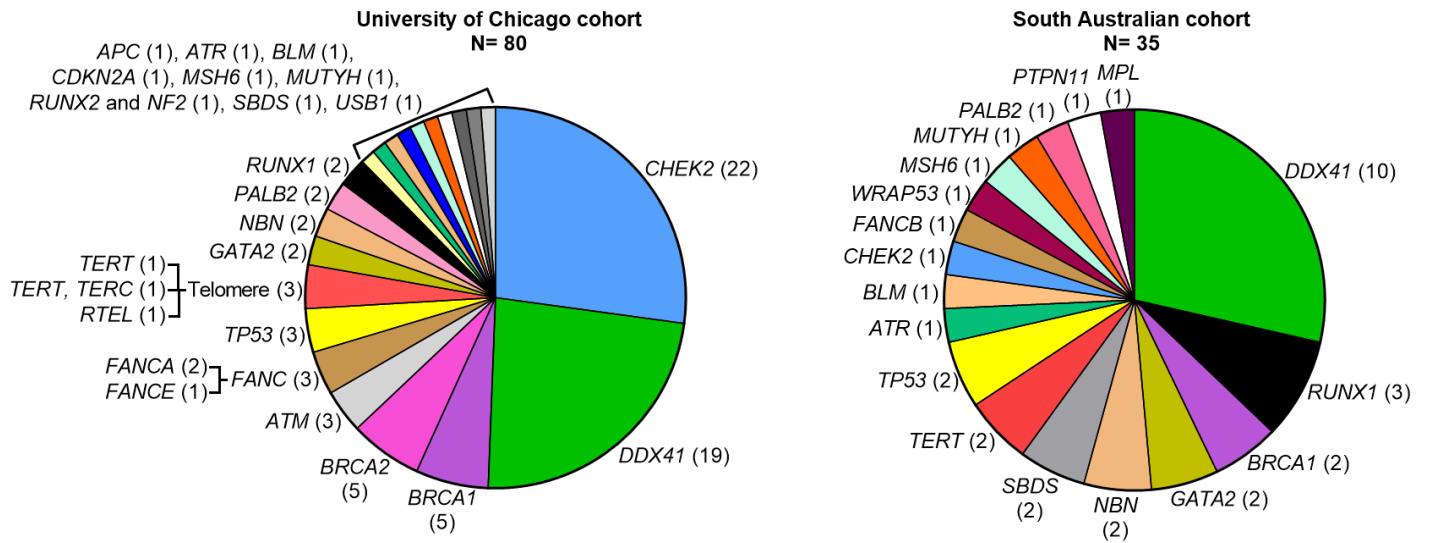

Distribution of pathogenic and likely pathogenic germline variants in University of Chicago and South Australian cohorts.

**Figure S3. Schematic representation of deleterious germline variants observed in *DDX41* and *CHEK2***
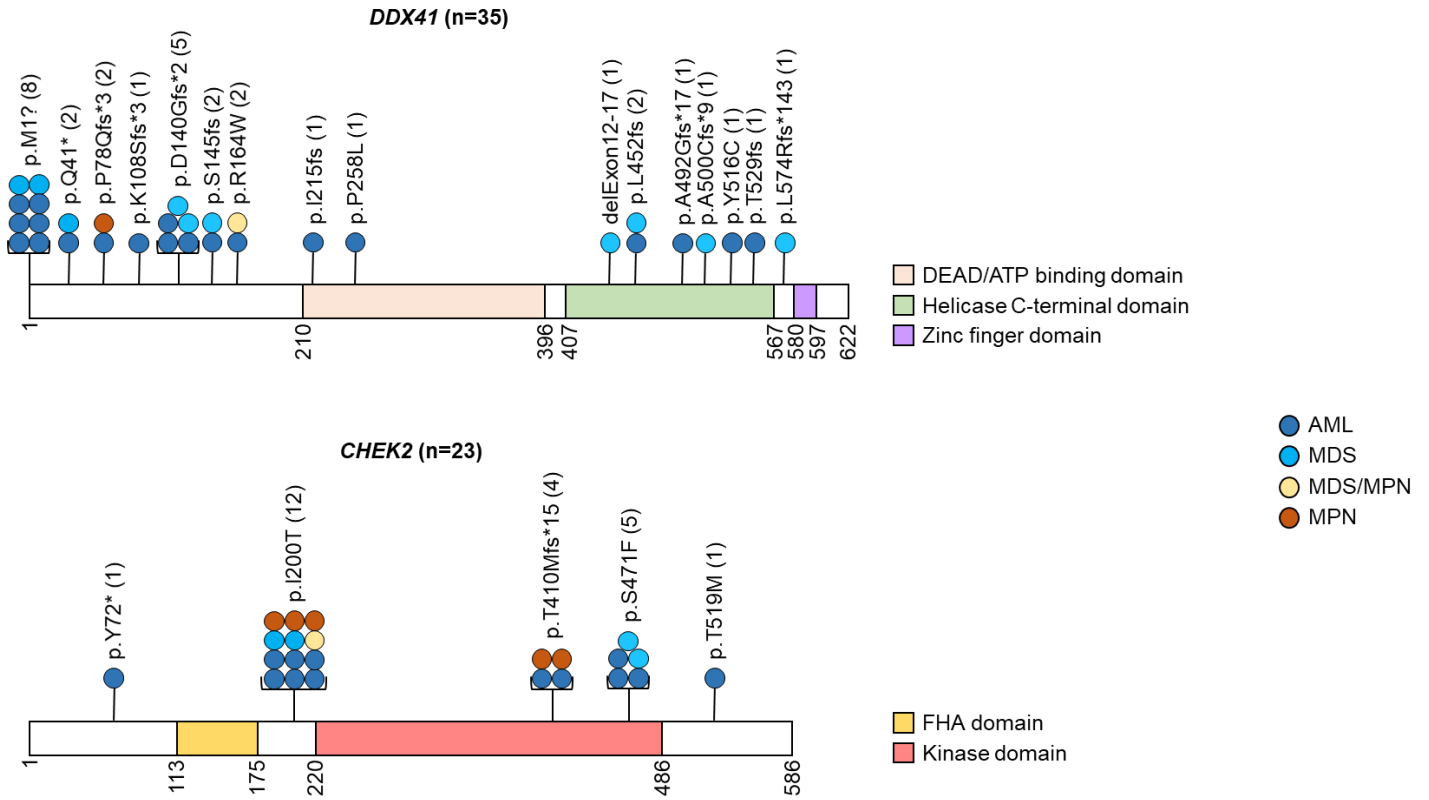
Representation of pathogenic/likely pathogenic germline variants in DDX41 and CHEK2, with their recognized functional domains.

Figure S4. Chronic GVHD-free survival after allogeneic HSCT in patients with versus without P/LP germline *DDX41* variants
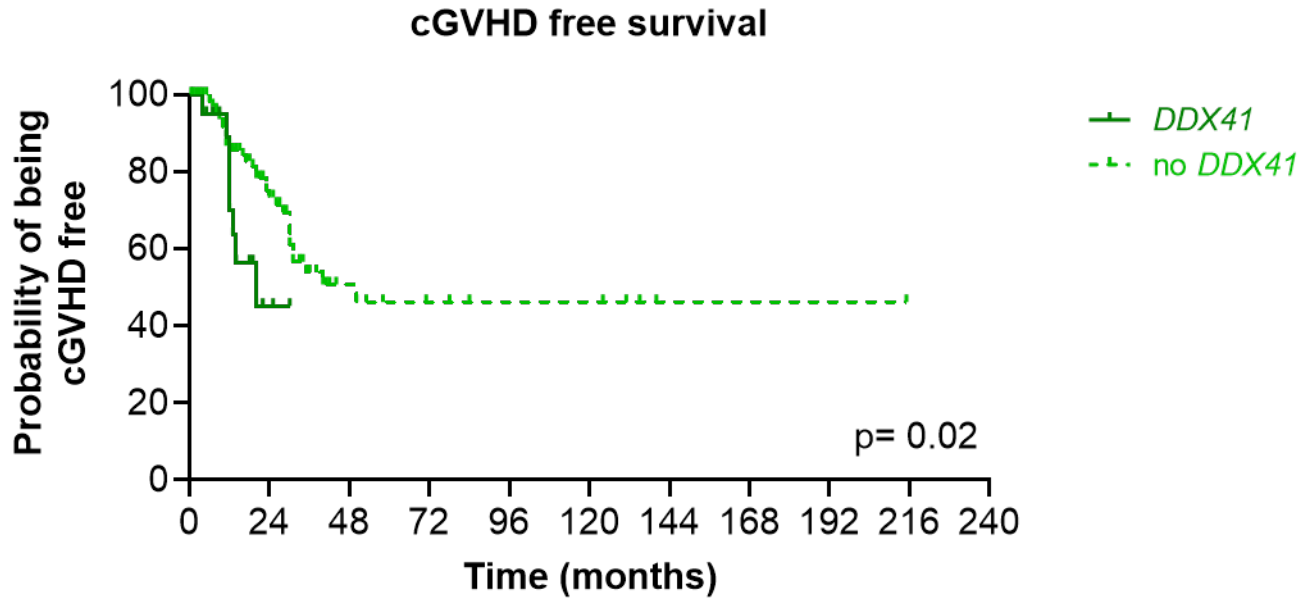
Kaplan-Meier curve showing the probability of chronic graft-versus-host disease (cGVHD) free survival in transplanted myeloid neoplasm patients stratified based on germline P/LP *DDX41* status.

Figure S5. Clonal evolution of relapse after allogeneic hematopoietic stem cell transplant in patients with P/LP germline *DDX41* variants
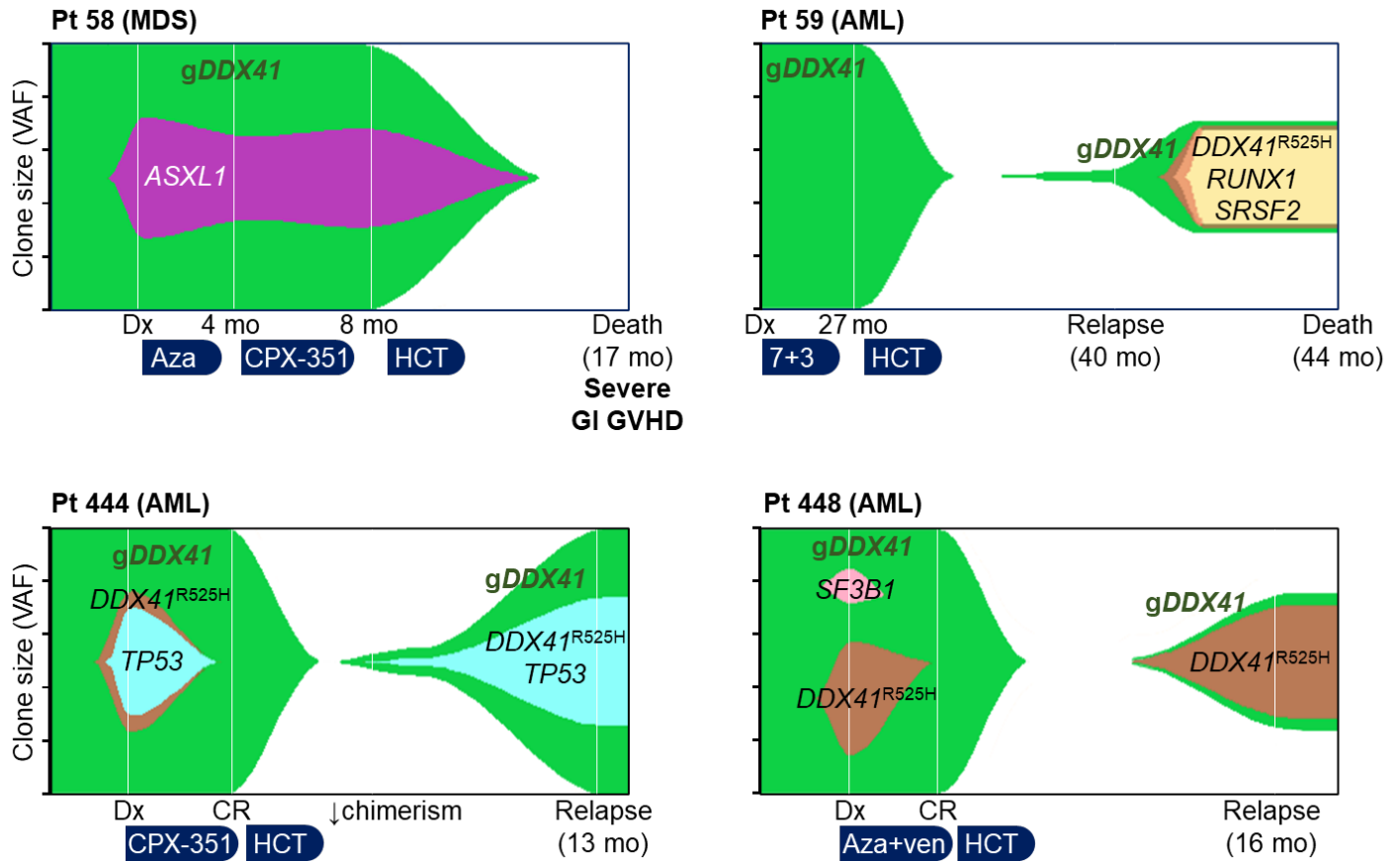
We analyzed data on four patients with pathogenic/likely pathogenic germline *DDX41* variants who underwent serial genetic assessments of their disease pre- and posttransplant. At the time of relapse after HSCT, the reduction in chimerism correlated with the re-emergence of germline *DDX41* variant. The subclonal somatic mutations (e.g., *ASXL1, DDX41* R525H) expanded subsequently with morphologic relapse. Pt 58 died as a result of severe GI GVHD. Patients 444 and 448 received post-transplant cyclophosphamide for GVHD prophylaxis. 7+3, 7-days of cytarabine plus 3-days of daunorubicin chemotherapy; Aza+ven, azacitidine + venetoclax; CR, complete remission; Dx, diagnosis; GI, gastrointestinal; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplant; VAF, variant allelic frequency

References

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