STIMULUS REPORT

blood advances

Genetic features and clinical outcomes of patients with isolated and comutated *DDX41*-mutated myeloid neoplasms

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Key Points

- Isolated and comutated *DDX41* myeloid neoplasms have different characteristics.
- *DDX41*-mutated AML has a relatively favorable outcome comparable to core binding factor AML.

DDX41 mutations (germline and somatic) are associated with late onset myelodysplastic syndromes/acute myeloid leukemia (MDS/AML). Myeloid neoplasms (MN) with germline predisposition was identified as a distinct category in the 2016 WHO classification revision, including MN with germline DDX41 mutation. We retrospectively analyzed the molecular findings and clinical characteristics of thirty-three DDX41-mutated (mDDX41) patients at our institution. We identified 14 distinct pathogenic DDX41 variants in 32 patients and 8 DDX41 variants of unknown significance (VUS) in 9 patients. Five (16%) patients had a second DDX41 somatic mutation p.R525H and 13 (40%) had at least one additional oncogenic co-mutation in other genes. The median age at the time of diagnosis was 66 years, with male predominance (72%) and the majority of patients had normal cytogenetics (91%). Two-year overall survival (OS) was 86% and 6 (21%) MDS/AML patients with relatively preserved hematopoietic function were observed without further intervention. In comparison to AML patients with prognostically more favorable subtypes [t(8;21), n=27 and inv(16), n=40], mDDX41 patients in our cohort showed similarly favorable OS. Our study highlights that mDDX41-MN patients often have an indolent course and mDDX41-AML has comparable OS to favorable-risk AML.

Introduction

The DEAD-box helicase 41 (*DDX41*) gene, located on chromosome 5q35, is presumed to be a tumor suppressor gene, encoding a DEAD-box type RNA helicase. *DDX41* is involved in pre-mRNA splicing, rRNA processing and innate immunity.[1-3] Unlike other germline predisposition syndromes, which typically present at an earlier age, *DDX41*-associated germline cases are characterized by late-onset development of myeloid neoplasms (MNs), and may occur as sporadic germline events.[1,2,4-12] *DDX41* mutations account for 0.5% to 5% of adult myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) and typically present as high-risk disease, with male predominance and variable history of preceding cytopenias.[1,2,13-16] Recent studies have reported that *DDX41*-related MN is associated with longer overall survival (OS) and response to lenalidomide.[10,14,17,18] In this study, we describe the clinical and genetic features and survival outcomes of patients with mutated *DDX41*.

Submitted 12 July 2021; accepted 13 September 2021; prepublished online on *Blood Advances* First Edition 13 October 2021; final version published online 19 January 2022. DOI 10.1182/bloodadvances.2021005738.

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Gene panel sequencing data are available by request to the corresponding author at alkhateeb.hassan@mayo.edu.

The full-text version of this article contains a data supplement.

Methods

This is a single-institution study encompassing the Mayo Clinic Cancer Center sites (Rochester, Florida, and Arizona). After institutional review board approval, we retrospectively screened for m*DDX41* cases from 4524 consecutive Mayo Clinic patient samples submitted for a 42-gene MN panel next-generation sequencing clinical assay in the Molecular Hematopathology Laboratory between July 2018 and December 2020. A chart review of m*DDX41* cases between January 2009 and April 2021 was conducted. Of 1404 consecutive patients with the diagnosis of AML, 27 (1.9%) patients with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* and 40 (2.8%) with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* were identified for survival comparison with m*DDX41* AML. All statistical analyses were performed using JMP Pro 14.1.0 Software (supplemental Methods).

Results and discussion

We identified 33 patients harboring *DDX41* genetic alterations, of which 32 (97%) had at least 1 pathogenic *DDX41* mutation and 1 (3%) had a *DDX41* variant of uncertain significance (VUS) (proven to be a germline variant). Of the 32 patients harboring pathogenic mutations, 5 (16%) had a second *DDX41* mutation (p.R525H), and 9 (27%) harbored a *DDX41* VUS (Figure 1; supplemental Table 1). The germline origin of *DDX41* variants was confirmed in 9 of 10 (90%) tested patients, among them at least 1 variant had variant allele frequency (VAF) $\geq$ 40%.

The median age at diagnosis was 66 years (range, 30-81 years), and 24 (72%) patients were men, consistent with late onset of *mDDX41* MN and male predominance previously reported.[8,14-16,19] All patients with AML were intermediate risk for the European LeukemiaNet (ELN), with a median marrow blast count of 29% (range, 20%-50%). The majority of MDSs were classified as excess blasts-2 (MDS-EB2; N = 13; 68%), similar to that previously reported in the literature.[15,16,19] Eleven (58%) and 4 (21%) patients with MDS were classified as intermediate risk and high risk by the revised International Prognostic Scoring System (IPSS-R), respectively.

Twenty (60%) patients had an isolated *DDX41* mutation, whereas 13 (40%) had at least 1 additional comutation in other genes. Isolated m*DDX41* cases showed male predominance relative to their comutated counterparts (85% vs 54%; P = .05; Table 1). The median number of comutations in the 13 cases was 1 (range,1-3), and the most common comutations occurred in *DNMT3A* (N = 5, 38%), *ASXL1* (N = 4, 30%), *JAK2* (N = 3, 23%), and *EZH2* (N = 2, 15%; Figure 1; supplemental Tables 1 and 2). The incidence of *TP53* mutation was infrequent (3%), comparable to what was reported by Sébert et al[15] (6%) but lower than that reported by Quesada et al[16] (32%). Similarly, we observed a low incidence of splicing factor comutations (3%), in keeping with the report from Sébert et al.[10,16] Our cohort had fewer comutations (median of 0; range, 0-3) than what reported by recent studies, and interestingly, the comutation median VAF observed here was low 7% (range, 5%-52%).[15,16]

The most common pathogenic mutation type was the initiation codon substitution (start-loss) variant p.M1I (N = 10, 31%; Table 1; supplemental Table 1), previously described as the second most common germline *DDX41* variant in Whites and the most common in Swedish population.[8,14,15] Among isolated cases, 47% had p.M1I, whereas only 8% of comutated cases harbored p.M1I (P = .02). Twenty-one (65.6%) *DDX41* mutations clustered in the N-terminal domain (NTD), 4 (12.5%) in the DEAD-box domain, and 7 (22%) in the helicase-C domain (HCD). Mutations located in the HCD were more likely to have a concomitant *DDX41* VUS compared with NTD mutations (N = 6, 86% vs N = 2, 10%; P = .0001).

Sixteen patients (52%) had a family history (FH) of solid tumors and 12 (39%) had a FH of hematologic malignancies. Comutated cases were more likely to have FH of solid tumors (77% vs 33%; P = .02; Table 1).[20] However, this difference was not significant for hematologic or subgroups of solid malignancies. None of the HCD-mutated cases had FH of solid tumors, in comparison with 70% seen in NTD-mutated cases (P = .001), supporting the reported prevalence of germline mutations in the NTD.[8]

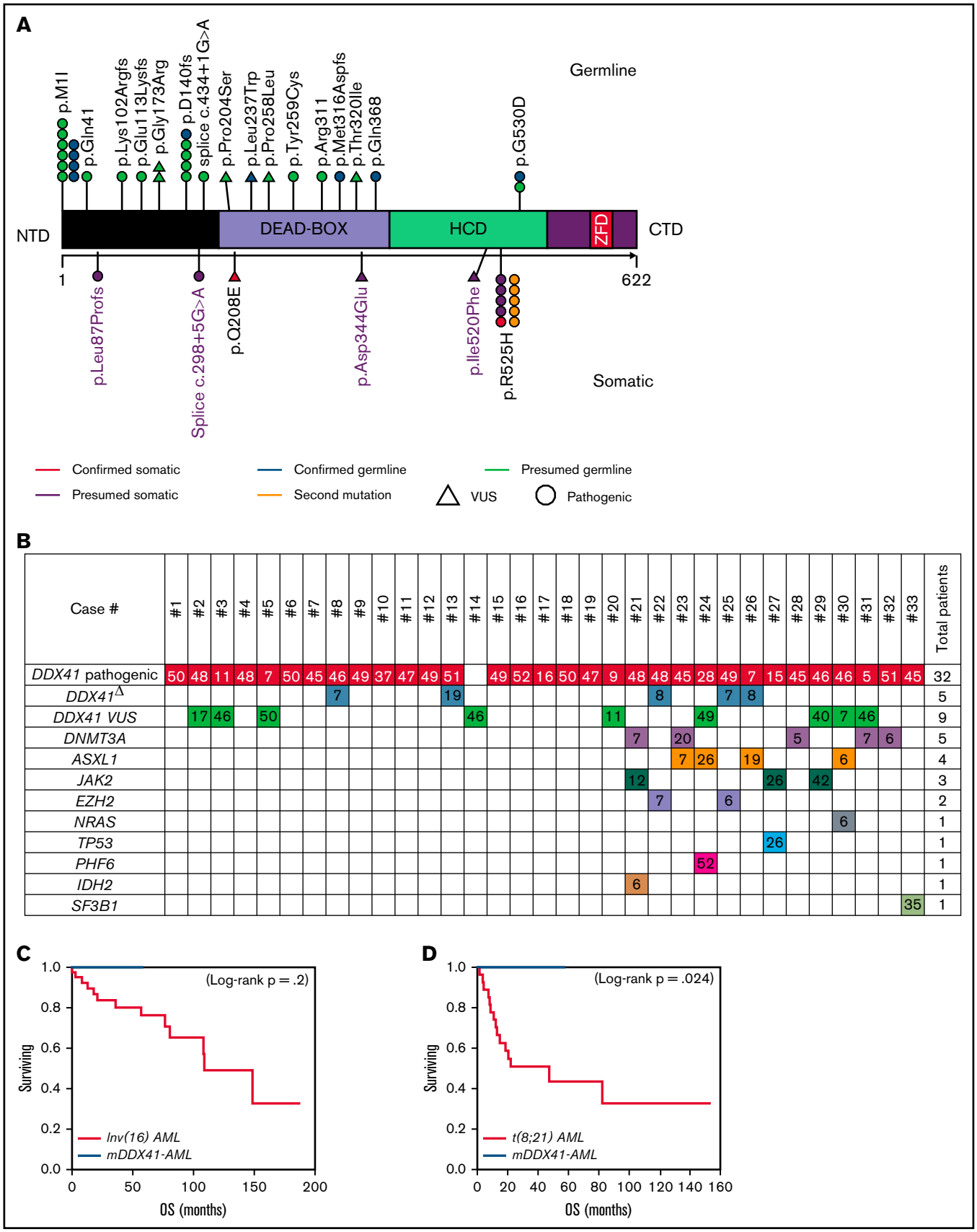
Cytogenetic results were available in 32 cases, and 29 (91%) showed a normal karyotype. Karyotypic abnormalities were thus infrequent (N = 3, 9%), consistent with previous reports.[15,16,19] Interestingly, all 3 cases with karyotypic abnormalities were comutated cases (P = .02).

Overall, 2 (7%) patients died after a median follow-up of 20 months. Six (20%) patients (5 MDS and 1 AML) were observed because of stable blood indices with a median follow-up of 6.6 months (range, 1.5-32.6 months). Twenty-three (80%) patients received treatment with median time from diagnosis to treatment initiation of 0.7 months (range, 0-92 months). Overall response rate in patients with MDS/AML was 77%, and median time to response was 3.2 months (range, 0.9-20.5 months; supplemental Table 3). Fifteen (68%) patients achieved complete remission (CR), 2 (9%) patients had hematologic improvement, and 3 (23%) patients did not respond. Patients with AML had 100% CR when treated with induction chemotherapy or hypomethylating agents (HMA) plus venetoclax regimen, and median time to CR was 1 month (range, 0.86-4.1 months). Of the 9 (39%) patients who received second-line therapy, 6 (75%) achieved CR. Four (21%) of 19 patients with MDS progressed into AML with a median time to progression of 16 months (range, 1.3-27.6 months), and the 2-year progression-free survival rate for patients with MDS was 62%.

In m*DDX41* MDS/AML, the median OS was not reached, and the 2-year OS was 86% (95% confidence interval: 57%-97%). There was no statistically significant difference in OS between 2-year OS for isolated and comutated (P = .99) responders and nonresponders (90% vs 50%; P = .38) and treatment compared with the 2-year OS for the observation group (83% vs 100%; P = .52; supplemental Figure 1). Twelve (41%) patients with MDS/AML received hematopoietic stem cell transplantation (HSCT), and there was no difference in 2-year OS between patients who received HSCT vs no HSCT (87% vs 86%; P = 1.0; supplemental Figure 1).

All patients with m*DDX41* AML were alive at the end of follow-up without reaching median OS. Comparing the OS of m*DDX41* AML to the prognostically favorable group of core-binding factor AML, a statistically significant superior outcome was observed in m*DDX41*-AML compared with AML with t(8;21) (2-year OS, 100% vs 51%; P = .024; Figure 1D). In comparison with AML with inv(16), m*DDX41* AML showed a trend to better OS; however, statistical significance was not achieved (2-year OS, 100% vs 84%; P = .2), supporting at least noninferior clinical outcome (Figure 1C).

Our study reaffirms some previous observations and demonstrates several novel findings in patients with MN and *mDDX41*. Isolated



Case #	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30	#31	#32	#33	Total patients
DDX41 pathogenic	50	48	11	48	7	50	45	46	49	37	47	49	51		49	52	16	50	47	9	48	48	45	28	49	7	15	45	46	46	5	51	45	32
DDX41$^{\Delta}$								7					19									8			7	8								5
DDX41 VUS		17	46		50									46						11				49					40	7	46			9
DNMT3A																					7		20					5			7	6		5
ASXL1																							7	26		19				6				4
JAK2																					12						26		42					3
EZH2																						7			6									2
NRAS																														6				1
TP53																											26							1
PHF6																								52										1
IDH2																					6													1
SF3B1																																	35	1

Figure 1. Characteristics of patients with DDX41 mutation. (A) Representation of DDX41 variants detected, positioned on the DDX41 protein and its functional domains. (B) Patterns of the mutations identified in the cohort of 33 patients with DDX41 mutation. The number reported in the box represents the VAF of each mutation. (C-D) Kaplan-Meier survival curves in 10 patients with m*DDX41* AML compared with (C) 40 patients with inv 16 AML and (D) 27 patient with t(8;21) AML. CTD, C-terminal domain; ZFD, zinc finger domain; Δ, second mutation.

Table 1. Characteristics and hematologic features of patients with isolated and comutated *DDX41* mutation

Variable	Isolated	Comutated	*P*
No. of patients, (%)	20 (60)	13 (40)	
Age, y, median (range), at diagnosis	65 (30-81)	66 (50-76)	.767
Sex (male), n (%)	17 (85)	7 (54)	.0496*
Hemoglobin, g/dL, median (range)	11.2 (7.5-15.6)	10.05 (6.6-14)	.1988
Leukocytes, 10^9/L, median (range)	2.15 (1-4.4)	2.4 (1.6-8.5)	.1239
Thrombocytes, 10^9/L, median (range)	87 (28-241)	94 (63-571)	.1443
ANC, median (range)	0.925 (0.16-3.73)	1.005 (0.65-4.78)	.2058
MCV median (range)	104.3 (85.2-114.8)	105.6 (90-115)	.7151
RDW, median (range)	14.2 (12.4-23.4)	15.05 (12.5-21.3)	.2824
BM blasts, median (range)	13 (1-45)	12 (0-50)	.6056
BM blasts (AML only), median (range)	34 (20-45)	25.5 (21-50)	.91
Number of comutations, median (range)	0	1 (1-3)	
DDX41 VAF %	48 (7-52)	45 (5-51)	.1656
DDX41 mutations > 1	2 (10)	3 (23)	.306
Pathogenic mutation type			
Missense	5 (26)	3 (23)	.8354
Nonsense	1 (5)	2 (15)	.3347
Frameshift	4 (21)	5 (38)	.2820
Splice site mutation	0	2 (15)	.0774
Start-loss variant	9 (47)	1 (7)	.0174*
Diagnosis			
MDS	13 (65)	6 (46)	.2845
AML	6 (30)	4 (30)	.96
MPN	0	2 (15)	
Carrier	1 (5)	0	
CCUS	0	1 (7)	
Abnormal cytogenetics	0	3 (25)	.0188*
Family history			
Solid or hematologic malignancies	11 (61)	12 (92)	.0501
Solid tumors	6 (33)	10 (77)	.0166*
Hematologic malignancies	8 (44)	4 (30)	.4405
Gastrointestinal malignancies	3 (15)	3 (23)	.6040
Genitourinary malignancies	2 (10)	4 (30)	.1496
Lung cancer	1 (5)	3 (23)	.1345
Breast cancer	2 (10)	2 (15)	.6832
Any personal history			
Hematologic malignancies	0	1 (7)	.2078
Solid tumors	1 (5)	3 (23)	.1200
***DDX41* VUS**			
Yes	5 (25)	4 (30)	.7161
No	15 (75)	9 (70)	

ANC, absolute neutrophil count; BM, bone marrow; CCUS, clonal cytopenia of undetermined significance; MCV, mean corpuscular volume; MPN, myeloproliferative neoplasms; RDW, red cell distribution width.
*Statistically significant.

DDX41 mutations were associated with male predominance (85%), the start-loss p.M1I mutation (47%), normal cytogenetics (100%), and less frequent association with a FH of solid tumors (33%) compared with their comutated counterpart. Patients with m*DDX41* MN have a low incidence of *TP53* and splicing factor gene comutations. Despite the categorization of all patients with m*DDX41* AML intermediate risk for ELN, we found they fit favorable-risk AML in our study cohort.[21-23] Finally, some m*DDX41* MN cases can be

observed for a long time if they have preserved hematopoiesis, and therapeutic intervention could be delayed. We acknowledge that our study is limited by the retrospective nature and small sample size.

Authorship

Contribution: A.N., A.A.-K., H.B.A., and D.V. designed the study, interpreted the data, and wrote the manuscript; A.N. collected the data and conducted the statistical analysis; M.V.S., J.M.F., T.B., A.T., M.R.L., M.M.P., N.G, A.A.M., L.S., H.B.A., and A.A.-K., cared for the patients and provided patient information; R.H., P.N., D.J., and D.V. performed the next-generation sequencing; P.G. performed cytogenetic analysis; and all authors critically reviewed and approved the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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