Received: 20 August 2023 | Accepted: 14 November 2023

DOI: 10.1111/bjh.19226

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

LETTER TO THE EDITOR

Pathogenic *DDX41* variants, possible response predictors to low-dose melphalan in hypo- and normocellular MDS and AML

Treating elderly patients with acute myeloid leukaemia (AML) and myelodysplastic syndromes with excess blasts (MDS-EB) is challenging, and the prognosis is poor.[1,2] Hypomethylating agents (HMAs) improve survival, compared to supportive care and conventional chemotherapy, to a median overall survival (OS) of about 12 months, although not statistically significant.[1,2] The combination of venetoclax with azacitidine is approved for AML patients unfit for intensive chemotherapy, as it improves the median OS compared to azacitidine monotherapy (14.7 and 9.6 months respectively).[3]

Data for patients with hypocellular bone marrow are lacking.[3] Hypocellular bone marrow (age-adjusted cellularity ≤25%) accounts for 10%–20% and 5%–10% of all MDS and AML patients respectively. Retrospective studies have demonstrated a survival benefit for patients with de novo hypo-/normocellular MDS versus hypercellular bone marrow, a benefit not confirmed for hypoplastic AML.[4]

Low-dose melphalan has been associated with an overall response rate (ORR) of 16.5%–40% in patients with hypocellular MDS-EB and AML.[5,6] In the present study, we evaluated the efficacy of low-dose melphalan on the clinical outcomes (ORR, complete remission [CR], haematological improvement [HI], transfusion reduction/independence and OS) of patients with hypo-/normocellular MDS-EB and AML without unfavourable cytogenetics, unfit for or refractory to intensive chemotherapy or failure to receive HMA treatment. We also investigated potential predictors of the melphalan response.

Data from 22 patients from 16 Norwegian hospitals with hypo-/normocellular (bone marrow cellularity 8%–41%; mean value 23%) MDS-EB ($N=6$) and AML ($N=16$) were collected retrospectively (Data S1). Baseline demographic characteristics are shown in Table 1 and Table S1. All included patients received oral melphalan 2 mg once daily. The recommended duration of the treatment was 8 weeks, but varied according to the physicians' choice (Figure 1). Low-dose melphalan was administered in an outpatient setting and were without side effects. Evaluation of response to treatment was performed according to the International Working Group (IWG) 2006 and the revised IWG 2018 haematological response criteria in MDS patients.[7,8] The responding patients remained without treatment after each cycle until relapse occurred (cytopenia and/or increase of blasts; Figure 1). Bone marrow samples were available for response evaluation in nine patients after C1 and in five patients after C2. Haematological values were available for all patients at C1–C4 (Figure 1; Figure S1A–C).

Seventeen of the 22 patients responded to low-dose melphalan after C1 (ORR = 77%; Figure 1, Table 1: grey colour). Eleven patients obtained marrow CR (mCR), seven after C1 and five after C2 (Figure 1, Table 1). Seventeen patients achieved HI; two had stable disease, while three patients (14%) had progressive disease (Figure 1). The responders after C1 had significantly improved survival compared to the non-responders, with a median OS of 25 months versus 4.7 months ($p=0.03$) respectively (Figure 2, Supplementary methods). Two patients went on to receive allogeneic haematopoietic stem cell transplantation (allo-HCT). One is still alive.

The 10 patients without available bone marrow in C1 were response evaluated by their haematological values, including peaks, at the start and end of each cycle (Figure S1A–C, Data S1). Among the responders, neutrophils and platelets increased to above 1×10^9/L and 50×10^9/L (one exception) respectively (Figure S1A–C).

During the period from the start of C1–C2 (which lasted for a median of 13.8 months [range 6.8–26.3]), the patients received no MDS/AML treatment (except melphalan; Figure 1). When relapse occurred (more than 10% blasts in the bone marrow), 13 patients received a second cycle (C2) with low-dose melphalan and 8 patients responded (ORR 62%). The relapses after C2 and C3 occurred earlier than after C1 (Figure 1).

Next-generation sequencing revealed causative variants in *DDX41* in 10 of the 20 patients (9 pathogenic and 1 likely pathogenic [LP]) in which the *DDX41* gene was sequenced (50%; Table 1).[9–11] *DDX41* was in Patient 2 considered LP since it has been found associated with MDS and somatic variants, indicating it is a disease-causative variant.[9,10] All patients carrying *DDX41* variants responded to low-dose melphalan treatment (Table 1; Patients 1–10), while none of the five non-responders had any *DDX41* variants (Table 1; white colour). Of the additional seven melphalan responders, two were not tested for *DDX41* (Table 1; Patients 11–12), four had no *DDX41* variants whereas Patient 17 had a somatic *DDX41* variant of uncertain significance (VUS) (Table 1; Patients 13–17).

TABLE 1 Main biological features of patients included in the study.

Patient (*N*)	Gender	Age (Years)	Cellularity BM (%)	Blasts BM (%)	Diagnosis	Cytogenetics	Prognosis risk score
1	M	72	28	5	MDS-EB-1	Normal	INT (IPSS-R: 3.5)
2	F	76	18	20	AML	Normal	INT
3[a]	M	59	41	16	MDS-EB-2	-Y	High (IPSS-R: 5.5)
4	M	78	10	22	AML	Normal	INT
5	M	74	30	25	AML	Normal	INT
6	F	65	40	24	Secondary AML	Normal	INT
7[a]	F	66	10	10	MDS-EB-2	t(17;19)	Very high (IPSS-R: 7.5)
8	M	84	10	20	AML	Normal	INT
9	M	83	23	28	Secondary AML	-Y	UKN
10	F	87	40	19	MDS-EB-2	Normal	INT (IPSS-R: 4.5)
11	M	85	20	20	AML	Trisomy 8	INT
12	M	72	40	25	AML	Normal	INT
13	M	80	15	25	AML	Normal	INT
14	M	67	15	20	Secondary AML	+X	INT
15	M	77	40	12,5	MDS-EB-2	Normal	Very high (IPSS-R: 7.5)
16	M	62	8	11	MDS-EB-2	Normal	INT (IPSS-R: 4.5)
17	M	76	15	25	AML	Normal	INT
18	F	79	20	30	AML	Normal	INT
19	F	77	18	25	AML	Trisomy 11	INT
20	F	69	20	10	Secondary AML	Normal	INT
21	M	71	18	10	Secondary AML	-Y	Low 2,5
22	M	72	20	20	AML	Normal	INT

Note: Next-generation sequencing (NGS) panels: A, Twist panel; B, VariantPlex myeloid panel (ArcherDx); C, TruSight myeloid sequencing panel (Illumina). Diagnosis of acute myeloid leukaemia (AML) means de novo AML. Secondary AML (all were MDS-AML). MDS-EB-1/2, MDS with excess blast count types 1 or 2.

Responders to melphalan: .

Non-responders to melphalan: .

Abbreviations: BM, bone marrow; F, female; HI, haematological improvement; IPSS-R, international prognostic scoring system-revised; LP, likely pathogenic; M, male; mCR, marrow complete remission; *N*, number; NA, not applicable; ND, not done; RBC units, red blood cell units from C1 to 8 weeks before C2; VAF, variant allele frequency; V variant of uncertain significance.

[a]Relative with the same DDX41 mutation as the patient.

[b]None, no variants detected.

NGS							Melphalan response		
Panel	Tissue	GENE	cDNA	Protein	VAF %	Assessment		Type	RBC Units
A	BM	***DDX41***	c.1574G>A	p.R525H	4	Pathogenic	Yes	HI	0
		DDX41	c.1301del	p.P434fs	48	Pathogenic			
		KMT2A	c.1961C>T	p.P654L	48	VUS			
A	BM	***DDX41***	c.773C>T	p.P258L	49	LP	Yes	mCR	0
		ANKRD26	c.4078A>G	p.I1360V	50	VUS		C1, C2	
A	BM	***DDX41***	c.1574G>A	p.R525H	8	Pathogenic	Yes	mCR	0
		DDX41	c.992_994del	p.K331del	49	Pathogenic		C1	
A	BM	***DDX41***	c.415_418dup	p.D140fs	51	Pathogenic	Yes	mCR	4
		NOTCH1	c.2917G>A	p.A973T	50	VUS		C2	
A	BM	***DDX41***	c.232_233insAA	p.P78fs	50	Pathogenic	Yes	mCR	0
								C2	
A	BM	***DDX41***	c.1574G>A	p.R525H	7	Pathogenic	Yes	mCR	0
		DDX41	c.1098+1G>A	p.?	45	Pathogenic		C2	
		CUX1	c.1405C>T	p.R469W	47	VUS			
A	BM	***DDX41***	c.992_994del	p.K331del	49	Pathogenic	Yes	HI	0
		ASXL1	c.3977C>T	p.P1326L	52	VUS			
		TET2	c.323A>T	p.Q108L	48	VUS			
B	Colon mucosa	***DDX41***	c.922_994del	p.K331del	54	Pathogenic			
ND	BM	NA					Yes	HI	1
B	Skin	***DDX41***	c.1098+1G>A		51	Pathogenic			
A	BM	***DDX41***	c.415_418dup	p.D140fs	49	Pathogenic	Yes	HI	1
		GNAS	c.49G>A	p.D17N	55	VUS			
		DDX41	c.1574G>A	p.R525H	6	Pathogenic			
A	BM	***DDX41***	c.1574G>A	p.R525H	8	Pathogenic	Yes	mCR	0
		DDX41	c.415_418dup	p.D140fs	52	Pathogenic		C1	
		GNAS	c.939C>A	p.S313R	48	VUS			
		MPL	c.1183A>G	p.N395D	51	VUS			
		NOTCH1	c.3338C>T	p.A1113V	54	VUS			
B	Buccal mucosa	***DDX41***	c.415_418dup	p.D140fs	47	Pathogenic			
C	BM	*DNMT3A*	c.856-1G>C	p.L1285T	9	Pathogenic	Yes	HI	0
		BCORL1	c.3854A>C		99	VUS			
C	NA	NA					Yes	HI	0
ND	BM	NA					Yes	mCR	0
B	Lipoma	None[b]						C1	
A	BM	*NRAS*	c.181C>A	p.Q61K	26	Pathogenic	Yes	mCR	0
		NRAS	c.35G>A	p.G12D	19	Pathogenic		C1	
		CBL	c.1111T>G	p.Y371D	5	Pathogenic			
A	BM	None[b]					Yes	mCR	13
								C2	
A	BM	*BCOR*	c.4240C>T	p.Q1414*	7	Pathogenic	Yes	mCR	0
		RUNX1	c.427_430del	p.E143*	8	Pathogenic		C1	
		SRSF2	c.284C>T	p.P95L	24	Pathogenic			
		IDH1	c.756C>A	p.D252E	51	VUS			
		GNAS	c.2507G>A	p.S836N	50	VUS			
B	BM	*DNMT3A*	c.994G>A	p.G332R	4	Pathogenic	Yes	mCR	3
		DDX41	c.662T>G	p.M221R	3	VUS		C1	
A	BM	*IDH2*	c.515G>A	p.R172K	13	Pathogenic	No	PD	0
		GATA2	c.1017+559G>C	p.?	48	VUS			
A	BM	*DNMT3A*	c.2645G>A	p.R882H	27	Pathogenic	No	SD	34
		IDH2	c.515G>A	p.R172K	21	Pathogenic			
		BCORL1	c.4096C>T	p.Q1366*	20	Pathogenic			
A	BM	*CSF3R*	c.2556C>G	p.I852M	50	VUS	No	PD	3
		SF3B1	c.440T>C	p.M147T	53	VUS			
		PPM1D	c.1422dup	p.E475*	16	Pathogenic			
A	BM	*TET2*	c.5157_5170del	p.K1720fs	32	Pathogenic	No	PD	25
		U2AF1	c.101C>T	p.S34F	31	Pathogenic			
A	BM	*TP53*	c.659A>G	p.Y220C	7	Pathogenic	No	SD	20

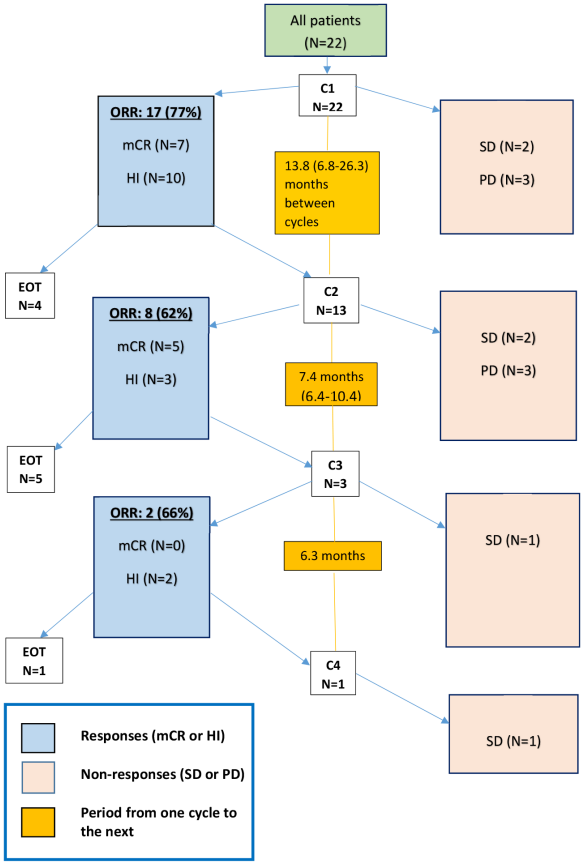

FIGURE 1 Response and survival in patients treated with low-dose melphalan. Blue boxes show melphalan responders, patients with mCR or HI. Beige boxes show non-responders, patients with SD and PD. Orange boxes represent periods from the start of each treatment cycle to the start of the next (time to relapse). Duration of treatment with melphalan in: C1, median 9.7 (5–22) weeks; C2, median 11.5 (2.1–31.1) weeks; C3, median 12.1 (7.5–15.1) weeks; C4, unknown period. C1, cycle 1; C2, cycle 2; C3, cycle 3; C4, cycle 4; EOT, end of treatment; HI, haematological improvement; mCR, marrow complete response; *N*, number; ORR, overall response rate; PD, progressive disease; SD, stable disease.

Patients 3 and 7 had siblings with MDS and the same *DDX41* variant respectively. For Patients 7 and 10, the *DDX41* variants were found in non-haematological tissues, confirming the germline origin (Table 1). Five (Patients 1, 3, 6, 9 and 10) of the 10 patients with *DDX41* variants (50%) also had the somatic hotspot variant p.R525H (Table 1).[12,13]

The responders with pathogenic/LP *DDX41* variants had improved survival compared to the responders without *DDX41*, with a median OS of 25 months versus 15.1 months ($p = 0.07$) (Figure 3).

Germline *DDX41* variants appear to be the most common predisposing syndrome for MDS and AML (incidence 2%–5%), characterized by late onset, male gender predominance, normal cytogenetics, hypocellular bone marrow and a high response rate to intensive chemotherapy and HMA.[9,10,13] The prognosis of our patients is not comparable in general with that of myeloid neoplasm patients with *DDX41* variants, since we included older patients with MDS-EB and AML refractory to HMA or unfit for or refractory to intensive chemotherapy. In fact, our study included patients without any other treatment options.

The 17 responders demonstrated important improvement in blood counts, with a neutrophil count above critical values for infections and a platelet count sufficient to prevent bleeding. This, together with the increase in haemoglobin and the low transfusion dependence in the responders, is likely to have an impact on the patients' quality of life (Table 1).

When we take into account that low-dose melphalan was given to patients with no other treatment options, both the high response rate, tolerability and long treatment-free periods strengthen the need to find predictors indicating response to this relatively simple but effective treatment. It may also, for some patients, serve as a bridge to allo-HCT.

The 100% response rate to low-dose melphalan in patients with hypo-/normocellular MDS-EB and AML with *DDX41* variants was unexpected. A possible explanation might be a potential immunological cross-talk between melphalan and

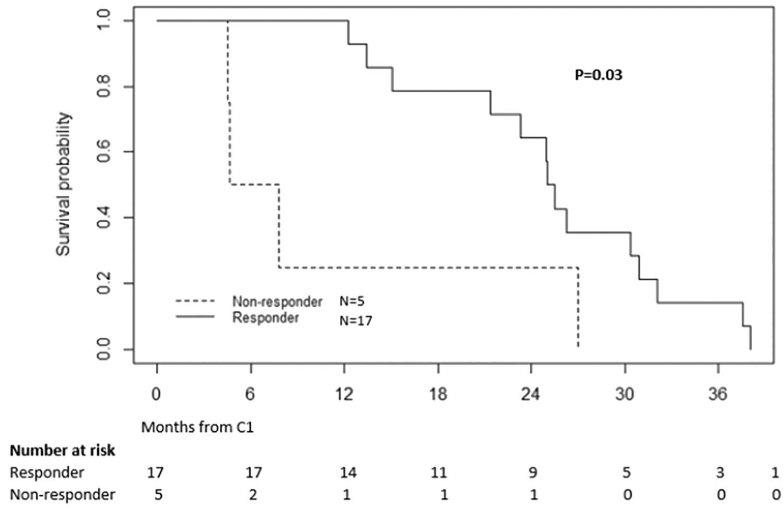

FIGURE 2 The impact of low-dose melphalan on survival. Responders are shown with a *whole line*, and non-responders with a *stippled line*. Median overall survival (OS): 25 months versus 4.7 months ($p = 0.03$).

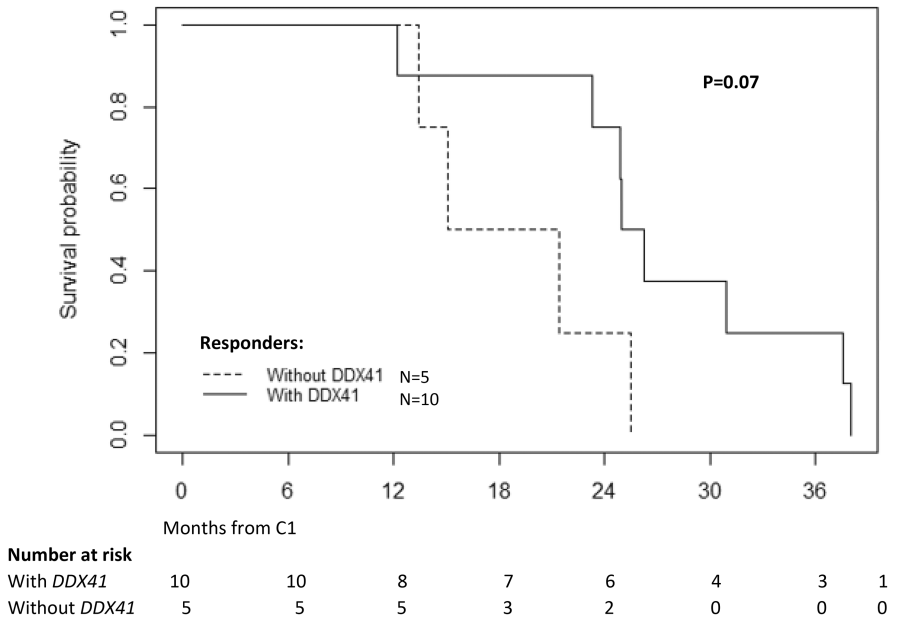

FIGURE 3 The impact of pathogenic *DDX41* on survival in the responding group. Responders with pathogenic/LP *DDX41* ($N = 10$) are shown with a *whole line*, and responders without pathogenic/LP *DDX41* ($N = 5$) with a *stippled line*. Median overall survival (OS): 25 months versus 15.1 months ($p = 0.07$).

DDX41, both of which have been shown to have immunomodulatory effects.[14,15]

The association between response to melphalan and the presence of pathogenic/LP *DDX41* variants is striking. If further studies can verify this excellent effect of low-dose melphalan in patients with pathogenic *DDX41* variants and hypo-/normocellular AML and MDS-EB, *DDX41* variants might be a novel predictor of melphalan response in similar patients.

Otto Emil Nyquist[1]
Jakob Dalgaard[2]
Signe Spetalen[3]
Synne Torkildsen[4]
Hege Frøen[4]
Eivind Galteland[4]
Ole Klungsøyr[5]
Astrid Bergrem[6]
Camilla Vo[4]
Hjalmar Sørbø[7]
Birgitte Eiken[8]
Hedda Lerdal[9]
Ann-Karin Solvang[6]
Hilde Jensvoll[10]
Tatjana Pandzic[11,12]
Panagiotis Baliakas[11,12]
Ingunn Dybedal[4,13]

[1]*Cancer and Hematology Center, Vestfold Hospital Trust, Tønsberg, Norway*
[2]*Medical Department, Drammen Hospital, Vestre Viken Trust, Drammen, Norway*
[3]*Department of Pathology, and Institute of Clinical Medicine, The Medical Faculty, Oslo University Hospital, University of Oslo, Oslo, Norway*
[4]*Department of Hematology, Oslo University Hospital, Rikshospitalet, Oslo, Norway*
[5]*Oslo Centre of Biostatistics and Epidemiology Department for Research and Education, Division of Mental Health and Addiction Oslo University Hospital, Oslo, Norway*
[6]*Department of Hematology, Lovisenberg Hospital, Oslo, Norway*
[7]*Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway*
[8]*Consultant Department of Hematology, Central Hospital Østfold Kalnes, Grålum, Norway*
[9]*Medical Department, Sorlandet Hospital HF, Kristiansand, Norway*
[10]*Hematological Department, University Hospital of North Norway, Tromsø, Norway*
[11]*Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*
[12]*Department of Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden*
[13]*Clinical Research Unit, Pharmacological Department, Oslo University Hospital, Oslo, Norway*

Correspondence
Otto Emil Nyquist, Cancer and Hematology Center, Vestfold Hospital Trust, Tønsberg, Norway.
Email: emil.nyquist@siv.no

ORCID

Otto Emil Nyquist https://orcid.org/0009-0005-2776-3686
Panagiotis Baliakas https://orcid.org/0000-0002-5634-7156
Ingunn Dybedal https://orcid.org/0000-0002-3229-3069

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.