bjh short report

Extended experience with a non-cytotoxic DNMT1-targeting regimen of decitabine to treat myeloid malignancies

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Summary

The nucleoside analogue decitabine can deplete the epigenetic regulator DNA methyltransferase 1 (DNMT1), an effect that occurs, and is saturated at, low concentrations/doses. A reason to pursue this molecular-targeted effect instead of the DNA damage/cytotoxicity produced with high concentrations/doses, is that non-cytotoxic DNMT1-depletion can cytoreduce even p53-null myeloid malignancies while sparing normal haematopoiesis. We thus identified minimum doses of decitabine (0·1–0·2 mg/kg) that deplete DNMT1 without off-target anti-metabolite effects/cytotoxicity, and then administered these well-tolerated doses frequently 1–2X/week to increase S-phase dependent DNMT1-depletion, and used a Myeloid Malignancy Registry to evaluate long-term outcomes in 69 patients treated this way. Consistent with the scientific rationale, treatment was well-tolerated and durable responses were produced (~40%) in genetically heterogeneous disease and the very elderly.

Keywords: myeloid neoplasms, decitabine, noncytotoxic DNMT1 depletion.

Nucleoside analogues are used to generate dose-dependent DNA damage in malignant cells that then triggers p53-dependent apoptosis (cytotoxicity). Normal dividing cells are unfortunately simultaneously destroyed. Therefore, to increase safety, nucleoside analogue doses are sometimes reduced, with an expected trade-off of less cytotoxicity-driven efficacy. Efficacy reduction with dose reduction may not apply, however, to the nucleoside analogue decitabine, because it has a molecular-targeted action of depleting DNA methyltransferase 1 (DNMT1) that occurs, and is saturated at, low decitabine concentrations (generally <1 μM). This molecular-targeted action can cytoreduce p53-null chemorefractory myeloid malignancies via terminal differentiation instead of apoptosis, simultaneously sparing normal haematopoiesis (Hu, *et al*, 2010; Hu *et al.*, 2011; Ng *et al.*, 2011; Negrotto *et al.*, 2012; Tsai *et al.*, 2012; Gu *et al.*, 2014; Saunthararajah *et al.*, 2015; Gu *et al.*, 2018; Velcheti *et al.*, 2018). Higher decitabine doses/concentrations (generally >1 μmol/l), by causing cytotoxicity, limit feasible exposure times for the S-phase-dependent DNMT1-depleting effect, and can further compromise treatment goals by destroying normal haematopoietic cells. The initial clinical development of decitabine, however, was motivated by conventional cytotoxic intent; thus the regimens approved for treatment of myelodysplastic syndromes (MDS) administer relatively high doses (20–45 mg/m^2/day) in pulses of 3–5 days every 4–6 weeks, intervals needed for recovery from cytotoxic side effects. To focus instead on a non-cytotoxic, DNMT1-targeting mode of action, we designed a decitabine regimen to avoid cytotoxicity and increase DNMT1 targeting, and describe here outcomes in 69 patients treated with this alternative decitabine regimen, 25 of whom were previously described with shorter follow-up [clinicaltrials.gov NCT00165996]) (Saunthararajah *et al.*, 2015).

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The Cleveland Clinic Institutional Review Board-approved Myeloid Malignancy Registry (16-020) and Sample Repository protocols (5024) collected demographic, laboratory, intervention, outcome and mutational data in myeloid malignancy patients after written informed consent, intended to facilitate better understanding of natural histories, treatment selections and treatment outcomes in these patients; 69 of 1694 patients in the Registry received the alternative decitabine regimen between August 2008 and September 2018. All 69 patients are described in this analysis, followed from diagnosis till death or loss-to-follow-up. All patient data were anonymized, and no patients were contacted to obtain any additional clinical or biological data for purposes of this report. The decitabine regimen-modified dose, schedule and route of administration were as follows: (i) Dose: 0·1–0·2 mg/kg (~3·5–5 mg/m^2), verified to deplete DNMT1 without cytotoxicity in several primate and human studies (a 75–90% reduction from the FDA-approved 20–45 mg/m^2/day dose) (Saunthararajah *et al.*, 2003; Olivieri *et al.*, 2011; Lavelle *et al.*, 2012; Saunthararajah *et al.*, 2015). The starting dose of 0·2 mg/kg was reduced to 0·15 mg/kg if infection risk was high [e.g. absolute neutrophil count (ANC) $<0.5 \times 10^9$/l]. (ii) Schedule: 1–2×/week, frequent and distributed, to increase S-phase-dependent DNMT1 depletion (contrasting with standard pulse-cycled administration for 3–5 days every 4–6 weeks). Frequency of administration was adjusted based on disease aggression and response: for example, 2×/week on consecutive days for myeloblasts >10%, or for lack of response to 1×/week administration. (iii) Route: subcutaneous, to avoid high peak concentrations (contrasting with approved regimens that infuse decitabine intravenously over 60 min). Neutropenia caused by treatment was managed by interruption until neutrophil count recovery then resumption at the same dose or with a dose lowered by 0·05 mg/kg. Routine anti-emetic prophylaxis was not required.

Non-cytotoxic DNMT1-depletion was confirmed by bone marrow γH2AX and DNMT1 measurements in an initial 25 patients treated (these results were previously published; Saunthararajah *et al.*, 2015). The 69 patients had MDS (n = 37, 54%), MDS/MPN (myeloproliferative neoplasms; n = 10, 14%), MPN (n = 12, 17%) and AML (acute myeloid leukaemia; n = 10, 15%) (Fig 1A, Table I; Table SI). Their median age was 69 years (range 45–89), 19 were female, and 50 were male (Table I; Table SI). Prior therapies (53/69, 77%) were 5-azacytidine (20/69, 29%), lenalidomide (14/69, 20%), erythropoietin (18/69, 26%), hydroxycarbamide (5/69, 7%), ruxolitinib (5/69, 7%), and cytarabine (3/69, 4%) (Fig 1A, Table I; Table SI). The side-effect was neutropenia (non-cytotoxic DNMT1 depletion skews myeloid differentiation away from granulocyte-monocyte progenitors and toward erythrocyte-megakaryocyte progenitors (Saunthararajah *et al.*, 2003; Milhem *et al.*, 2004) (Figure S1), complicated by fever/infection in 31 patients (45%), with one septic death. Blood count improvements meeting International Working Group (IWG) criteria (Cheson *et al.*, 2006) for response [haematologic improvement (HI)/complete remission (CR)] occurred in 30 patients (HI/CR 43%: HI 29%, CR 14%; Fig 1A, Tables I and II) and were durable [median response duration was 48 weeks (range 8–326); type of response and duration are summarized in Table II]. Patients not meeting criteria for HI or CR met IWG criteria for stable disease (SD; 30/69, 44%) or progressive disease (PD; 9/69, 13%) (Table SI). Responses (HI/CR) were seen in different histologic subtypes of myeloid malignancy [MDS 14/37 (38%), MDS/MPN 8/10 (80%), MPN 4/12 (33%), AML 4/10 (40%)], and in patients previously treated with 5-azacytidine (7/20, 35%), lenalidomide (5/14, 36%), or cytarabine (1/3, 33%; Fig 1A, Table I). Cytogenetics were normalized in 10/36 (28%) cases with abnormal karyotypes at baseline (Table I, Fig 1B) (chromosome Y deletion was considered a normal age-related change and not considered abnormal). Treatment decreased bone marrow myeloblasts, even in cases not meeting IWG criteria for response (Fig 1C). Patients with HI/CR had better overall survival (median 31 vs. 18 months; P = 0·036; Fig 1D). HI/CR was achieved in MDS/AML containing monosomy 7, trisomy 8, complex cytogenetic abnormalities and/or multiple mutations including in kinases (e.g. *JAK2*, *KRAS*, *FLT3*), epigenetic regulators (e.g. *TET2*, *DNMT3A*, *ASXL1*), splicing factors (e.g. *SF3B1*, *ZSRS2*, *SRSF2*), apoptosis regulators (e.g. *TP53*), transcription factors (e.g. *RUNX1*, *CEBPA*), and other genes (e.g. *NPM1*, *IDH1*, *IDH2*; Table I; Table SIII). Predictors of HI/CR were higher baseline neutrophils (median $3{\cdot}04 \times 10^9$/l vs. $1{\cdot}17 \times 10^9$/l; P = 0·002), higher baseline marrow cellularity (median 70%/ vs. 45%; P = 0·03) and higher baseline reticulocytes (56×10^9/L vs. 46×10^9/L; P = 0·03; Fig 1D, E) (Table SII, Fig 1E, F). Bone marrow myeloblasts, and other clinical phenomena such as Sweet's syndrome and/or granulocytic sarcomas, were improved by therapy even in patients with blood count changes that did not meet IWG criteria for response (HI/CR; Fig 1C). Such benefits, and improvements in blood counts even if these did not meet criteria for response (Figure S2), motivated extended treatment in some patients classified as non-responders: median treatment duration in patients with stable disease was 34 weeks (range 13–260), and 12 weeks (range 3–20) in patients with progressive disease.

Thus, a decitabine regimen designed and previously demonstrated to be non-cytotoxic yet DNMT1-depleting in non-human primates and humans (Saunthararajah *et al.*, 2003; Olivieri *et al.*, 2011; Lavelle *et al.*, 2012; Saunthararajah *et al.*, 2015) produced durable responses (follow-up was for up to 6·5 years) in myeloid malignancies across the clinicopathologic spectrum of disease — that is, in patients with MDS, MDS/MPN overlap, MPN or AML and in disease containing diverse genetic abnormalities — consistent with scientific data implicating DNMT1 as a mutation-agnostic target that operates in a final common pathway of myeloid transformation (Hu *et al.*, 2010; Hu *et al.*, 2011; Ng *et al.*, 2011; Negrotto *et al.*, 2012; Gu *et al.*, 2014; Saunthararajah

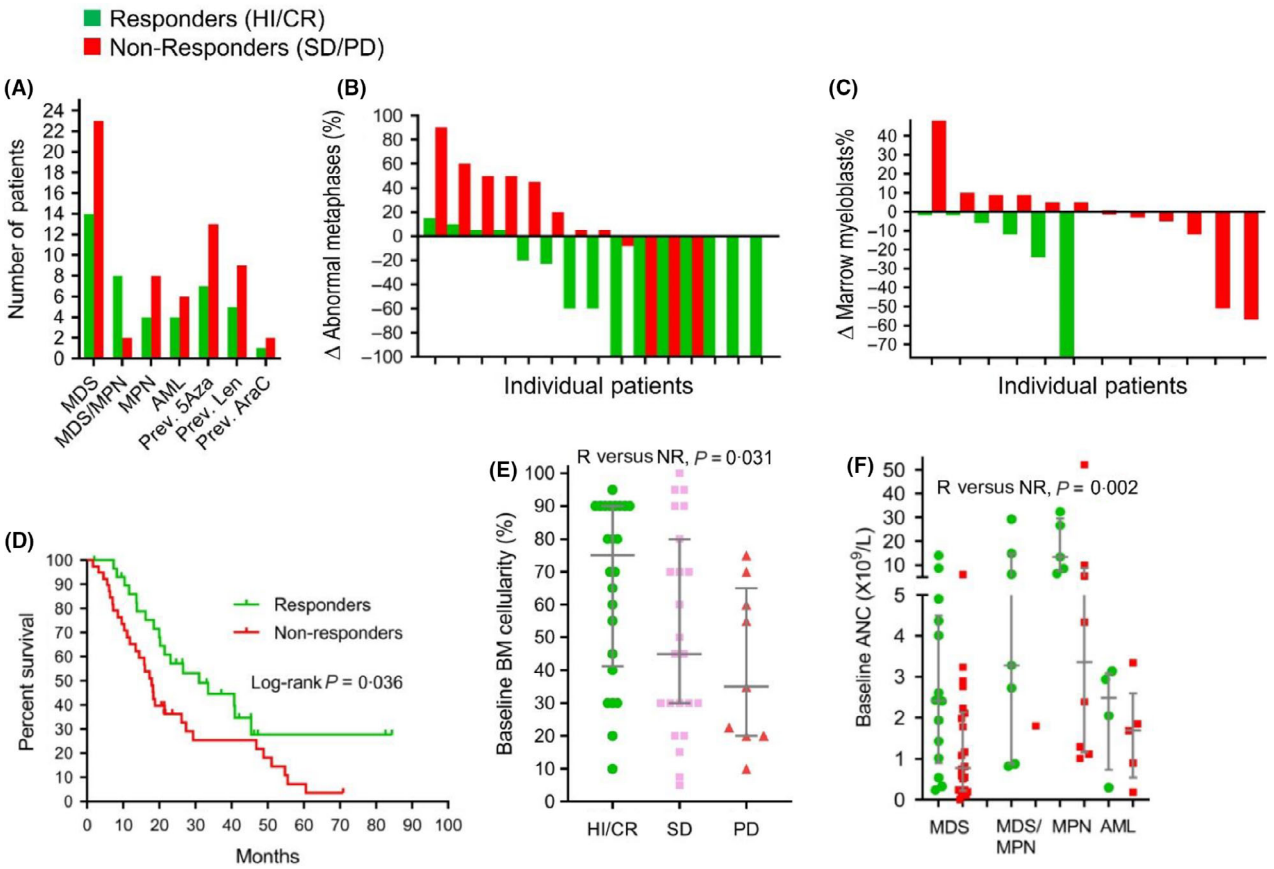

Fig 1. (A) Response (haematologic improvement or complete remission [HI/CR]) *versus* non-response [stable disease or progressive disease (SD/PD)] in patients grouped by myeloid malignancy sub-type and previous 5-azacytidine (5Aza), lenalidomide or cytarabine (AraC) therapy. (B) Change in abnormal metaphases on therapy [(abnormal metaphases on treatment − pretreatment)/pretreatment ×100]. Only cases with both on-treatment and pretreatment karyotype analyses shown. (C) Change in the bone marrow myeloblast percentage on therapy (best response myeloblasts% on-treatment − pretreatment myeloblasts%). Only cases with pretreatment (with this decitabine regimen) myeloblasts ≥5% together with on-treatment bone marrow myeloblast data available shown. (D) Overall survival, stratified by responders and non-responders. (E) Pretreatment bone marrow cellularity. Lines, Median ± interquartile range (IQR); *P*-value, Wilcoxon test, two-sided, HI/CR *versus* SD/PD. (F) Pretreatment absolute neutrophil counts (ANC;×10^9/l) by response *versus* non-response within each disease subtype. Lines, Median ± IQR; *P*-value, Wilcoxon test, two-sided, HI/CR *versus* SD/PD [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2015; Gu *et al.*, 2018; Velcheti *et al.*, 2018). Likewise, a broad spectrum of activity has been observed with standard intravenously infused regimens of decitabine [reviewed in Saunthararajah (2013)]. We postulate that target validity and avoidance of cytotoxicity enabled the sustained disease control/transfusion freedom observed in several patients treated with the present regimen, some of whom were >80 years old. Reported links by others between some mutations, for example, in *TET2*, and response to decitabine could be via higher neutrophils (*TET2* mutations are also linked with high white cell counts) that in turn enable on-time, regular exposures to this S-phase-dependent therapeutic [reviewed in Saunthararajah *et al.* (2015)] — the main side-effect of DNMT1-depletion is a shift in myeloid differentiation toward erythroid-megakaryocyte progenitors and away from granulocyte-monocyte progenitors, thereby depressing neutrophil counts, a shift that is more likely to interrupt or delay treatment exposures if neutrophil counts are low to begin with. Another fundamental is that haematologic response (HI/CR) requires not just suppression of malignant clones but marrow capacity to regenerate and support functional haematopoiesis — low baseline marrow cellularity that suggests compromise to such capacity is therefore linked with lack of haematologic response, even though malignant clones may be suppressed by therapy as shown by decreasing bone marrow myeloblasts and fewer abnormal metaphases (Saunthararajah, 2013).

Other "low-dose" decitabine regimens have been evaluated, although some are more accurately described as "low-frequency" since they administer potentially cytotoxic doses less often (e.g., 20 mg/m^2/day for three instead of five consecutive days every four weeks) — the overall response rate (HI/CR) with subcutaneous decitabine 20 mg/m^2/day for three days every four weeks was 23% (n = 65) and with

Table I. Pretreatment characteristics of patients with blood count changes meeting International Working Group criteria for response (HI/CR).

Age/Sex	WHO classification 2016	Previous treatments	Gene mutations (selected)	Pretreatment blast %	On-treatment blast %	Pretreatment cytogenetics	On-treatment cytogenetics	Haematologic response (IWG*)
Responders								
69/M	PMF	Pred, IVIg,Thal	*JAK2*	1	1	ND	Normal	HI
86/M	MDS-SLD	None	ND	0	1	Normal	ND	CR
79/F	MDS-MLD	Rom	None	1	1	del(20)	del(20)	HI
73/M	CMML2	Len	*JAK2*	12	0	del(20)	Normal	CR
64/M	Post-PV MF	Ima, Rux	*JAK2*	3	2	Complex	Complex	HI
66/M	MDS-RS-MLD	Len, 5-aza	*SF3B1*	2	1	−Y	−Y	HI
65/M	MDS-MLD	Epo	None	3	0	Complex	Normal	CR
67/F	MDS-EB1	Vcr, Dox, Mel	None	6	4	Complex	Normal	HI
58/M	CMML1	None	*TET2, ZRSR2*	2	0	Normal	+1,t(1;15)	CR
86/M	PMF	Dan	Not Done	0	ND	ND	ND	HI
66/M	CMML1	None	*KRAS, SRSF2, TET2*	4	0	+mar[2/20]	Normal	CR
61/M	MDS-EB2	Len	*FLT3*	2	0	Complex	Normal	CR
83/F	MDS-EB1	Epo	*BCOR, CEBPA, DNMT3A, RUNX1, SRSF2, TET2*	9	3	Normal	Normal	HI (marrow CR)
69/M	CMML1	None	*BCOR, CBL, PRPF8, RUNX1, SRSF2, TET2*	2	1	Normal	Normal	HI
89/M	sAML	Cyt, 5-aza	*SF3B1*	11	ND	-Y	-Y	HI
69/M	PMF	5-aza	None	5	3	del(20)	del(20)	HI
67/M	MDS-MLD	None	*TET2*	2	ND	del(13)	ND	HI
58/M	MDS-U	5-aza	*ASXL1, IDH1, RUNX1, ZRSR2*	0	2	del(11)	del(11)	HI
86/M	MDS-MLD	Epo, Eltrom	ND	1	ND	del(20)	ND	CR
52/F	MDS-RS-SLD	Epo, G-CSF	None	2	4	Normal	Normal	CR
78/F	MDS-MLD	Pred	*NRAS, TP53*	1	1	Complex	Normal	CR
69/M	CMML1	Len, Epo	*BCOR, ETV6, EZH2, U2AF1*	2	4	Normal	Normal	HI
77/F	aCML	Rux, Hyd	*SETBP1, SF3B1, SRSF2*	0	ND	Normal	Not Done	HI
70/F	sAML	Lef	*IDH2, NPM1, SRSF2, TET2*	80	3	del(4)	Normal	HI (marrow CR)
81/M	CMML1	Niv	*CUX1, SRSF2, TET2*	1	ND	Normal	Normal	HI
68/M	sAML	None	*JAK2*	26	2	−7	−7	HI (marrow CR)
71/M	MDS-MLD	ESA, G-CSF, Len, 5-aza,	*IDH1, NPM1, PHF6, U2AF1*	0	ND	Complex	ND	HI
61/M	CMML2	5-aza, Epo	*ASXL1, IDH2, JAK2, RUNX1, STAG2*	10	ND	Normal	ND	CR
81/F	MDS-MLD	Rom	None	2	0	del(20)	del(20)	HI
75/M	sAML	5-aza	ND	1	ND	Normal	ND	HI

MDS, myelodysplastic syndrome; MDS-U, MDS unclassified; MDS-SLD, MDS with single lineage dysplasia; MDS-MLD, MDS with multilineage dysplasia; MDS-EB1, MDS with with excess blasts (blasts make up 5–9% of the cells in the bone marrow, or 2–4% of the cells in the blood); MDS-EB2, MDS with with excess blasts (blasts make up 10–19% of the cells in the bone marrow, or 5–19% of the cells in the blood); MDS-RS-SLD, MDS with ring sideroblasts with single lineage dysplasia; MDS-RS-MLD, MDS with ring sideroblasts with multilineage dysplasia; CMML, chronic myelomonocytic leukemia; PMF, primary myelofibrosis; Post-PV MF, post–polycythaemia vera myelofibrosis; aCML, atypical chronic myeloid leukemia; sAML, secondary acute myeloid leukemia; 5-aza, azacitidine; Len, lenalidomide; Mel, melphalan; Rom, romiplostim; Rux, ruxolitinib; Ima, imatinib; Hyd, hydroxycarbamide; Cyt, cytarabine; Dan, danazol; Eltrom, eltrombopag; Lef, leflunomide; Niv, nivolumab; Epo, erythropoietin or darbepoietin; Vcr, vincristine; Dox, doxorubicin; Pred, prednisone; IVIg, Intravenous immunoglobulin; G-CSF, granulocyte-colony stimulating factor; Thal, thalidomide. M, male; F, female; ND, not done; HI, haematologic improvement; CR, complete remission; WHO, World Health Organization.

*International Working Group (IWG) 2006 Criteria for Response in MDS Clinical Trial.

Table II. Type and duration of response in patients meeting International Working Group criteria for haematologic response (HI/CR)

Case	Haematologic response (IWG)	Haematological improvement							CR Duration (w)
		Baseline cytopenias	Erythroid response	Duration (w)	Platelet response	Duration (w)	Neutrophil response	Duration (w)	
1	HI	E, P	+	89					
2	CR	P			+	30			9
3	HI	E, P	+	321	+	326			
4	CR	E, P, N	+	36	+	147	+	160	52
5	HI	P			+	74			
6	HI	E	+	28					
7	CR	E, P, N	+	49	+	42	+	42	40
8	HI	E, P	+	62	+	145			
9	CR	N					+	12	12
10	HI	E, P			+	66			
11	CR	E	+	133					133
12	CR	E, P	+	43	+	178			36
13	HI	P			+	11			
14	HI	E, P	+	41					
15	HI	E, N	+	53			+	14	
16	HI	E, P	+	47					
17	HI	E, P, N			+	31	+	15	
18	HI	E	+	30					
19	CR	P			+	44			18
20	CR	E	+	89					86
21	CR	E, P	+	41	+	35			28
22	HI	E, P	+	30	+	53			
23	HI	E, P	+	24					
24	HI	E, P	+	28	+	14			
25	HI	E, P			+	8			
26	HI	E, P			+	8			
27	HI	E, P, N			+	20			
28	CR	E, P	+	79	+	58			10
29	HI	P, N					+	155	
30	HI	E, P	+	48	+	51			
Response duration, median (range) weeks			47 (24–321)		44 (8–326)		15 (12–160)		32 (9–133)

Baseline cytopenias: E = haemoglobin <110 g/l or transfusion dependent; P = platelets $<100 \times 10^9$/l; N = neutrophils $<1 \times 10^9$/l. Erythroid response: Hgb increase by ≥15 g/l for >8 weeks or transfusion reduction per IWG criteria. Platelet response: increase from <20 to $>20 \times 10^9$/l and by at least 100%, or increase of $\geq 30 \times 10^9$/l if baseline is $>20 \times 10^9$/l, for >8 weeks. Neutrophil response: at least 100% increase and an absolute increase $>0{\cdot}5 \times 10^9$/l for >8 weeks. IWG, International Working Group; CR, complete remission; w, weeks; +, response achieved.

decitabine 20 mg/m^2/day infused over 1 h for three days every four weeks was 70% (n = 73), with both trials treating previously untreated low/intermediate-risk MDS patients (Table SIV). By contrast, the response rate here was ~40%, even though approximately 50% of the patients had disease that was relapsed/refractory to 5-azacytidine, lenalidomide and/or cytarabine, and approximately 25% of them had pre-treatment bone marrow myeloblasts of ≥10% (Table SIV). There remains a need to address mechanisms of resistance to decitabine (and 5-azacytidine), and in addition, a need for separate measures to simultaneously support regeneration by functional haematopoiesis, since in this mostly elderly patient population such capacity is diminished by age, disease and previous cytotoxic treatments.

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Author contribution

All authors contributed to the writing and review of the manuscript. HA collected and analyzed clinical and molecular data; RZM analyzed data; TK and CMK performed and analyzed DNA sequencing data and collected clinical data; BJP, JD, AK, VV, HEC and AL participated in patient management and edited the manuscript; TR analyzed data; JPM collected and analyzed clinical and molecular data and contributed to study design; YS conceptualized and

designed the overall research and analyzed clinical and molecular data.

Conflict of Interest

YS is a Board member and consultant for, and has equity and royalty rights with, EpiDestiny.

Supporting Information

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

Table SI. Characteristics of patients with blood count changes not meeting IWG criteria for response.

Table SII. Baseline characteristics of responders *versus* non-responders (patients meeting *versus* not meeting IWG criteria for response).

Table SIII. Genes sequenced (targeted sequencing panel).

Table SIV. Summary of results of clinical trials evaluating decitabine to treat myelodysplastic syndrome (MDS).

Fig S1. Serial blood counts (first 52 weeks shown only) in all patients by International Working Group (IWG) response *versus* non-response (haemoglobin in g/l, platelet in 10^9/l and absolute neutrophil count (ANC) in 10^9/l).

Fig S2. Serial blood counts in patients not meeting International Working Group (IWG) criteria for response but with long-term stability or small improvements on therapy.

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