SUPPLEMENTARY APPENDIX

Progression in patients with low- and intermediate-1-risk del(5q) myelodysplastic syndromes is predicted by a limited subset of mutations

Christian Scharenberg,[1,2] Valentina Giai,[1] Andrea Pellagatti,[3] Leonie Saft,[4] Marios Dimitriou,[1] Monika Jansson,[1] Martin Jädersten,[1] Alf Grandien,[1] Iyadh Douagi,[1] Donna S. Neuberg,[5] Katarina LeBlanc,[1] Jacqueline Boultwood,[3] Mohsen Karimi,[1] Sten Eirik W. Jacobsen,[1,6] Petter S. Woll[1] and Eva Hellström-Lindberg[1]

[1]Department of Medicine, Center for Hematology and Regenerative Medicine, Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden; [2]Department of Medicine, Division of Hematology, Skaraborgs Hospital, Skövde, Sweden; [3]Bloodwise Molecular Haematology Unit, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, and NIHR Biomedical Research Centre, Oxford, UK; [4]Department of Pathology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [5]Department of Biostatistics and Computational Biology, Dana–Farber Cancer Institute, Boston, MA, USA and [6]Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

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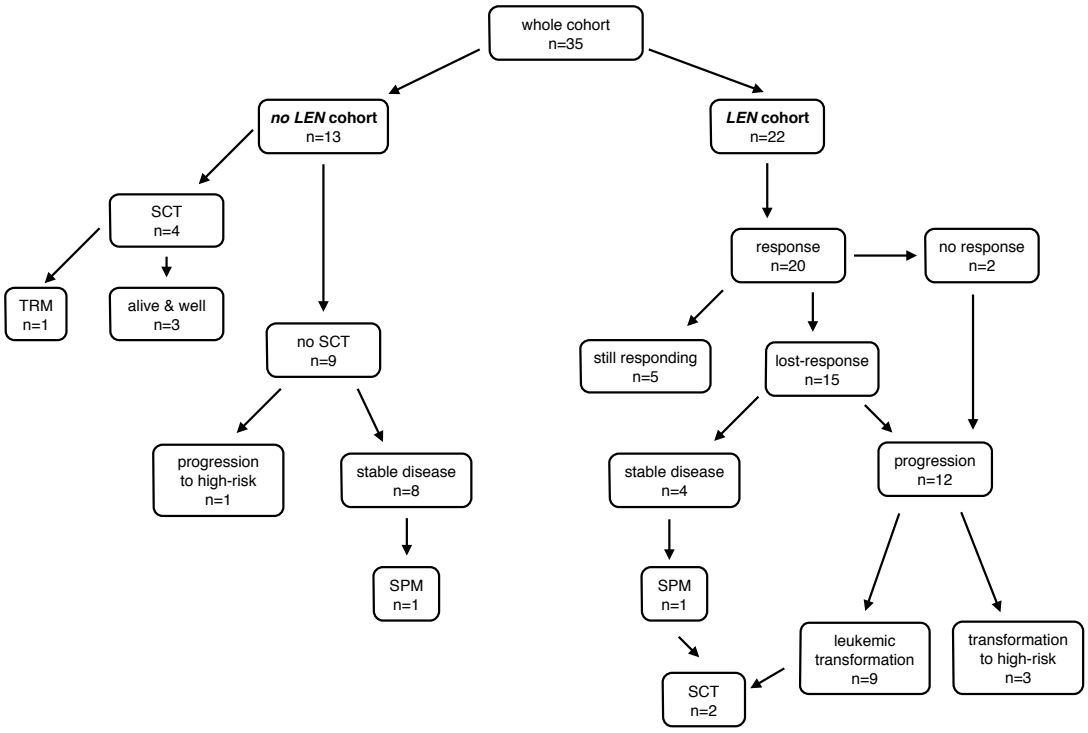
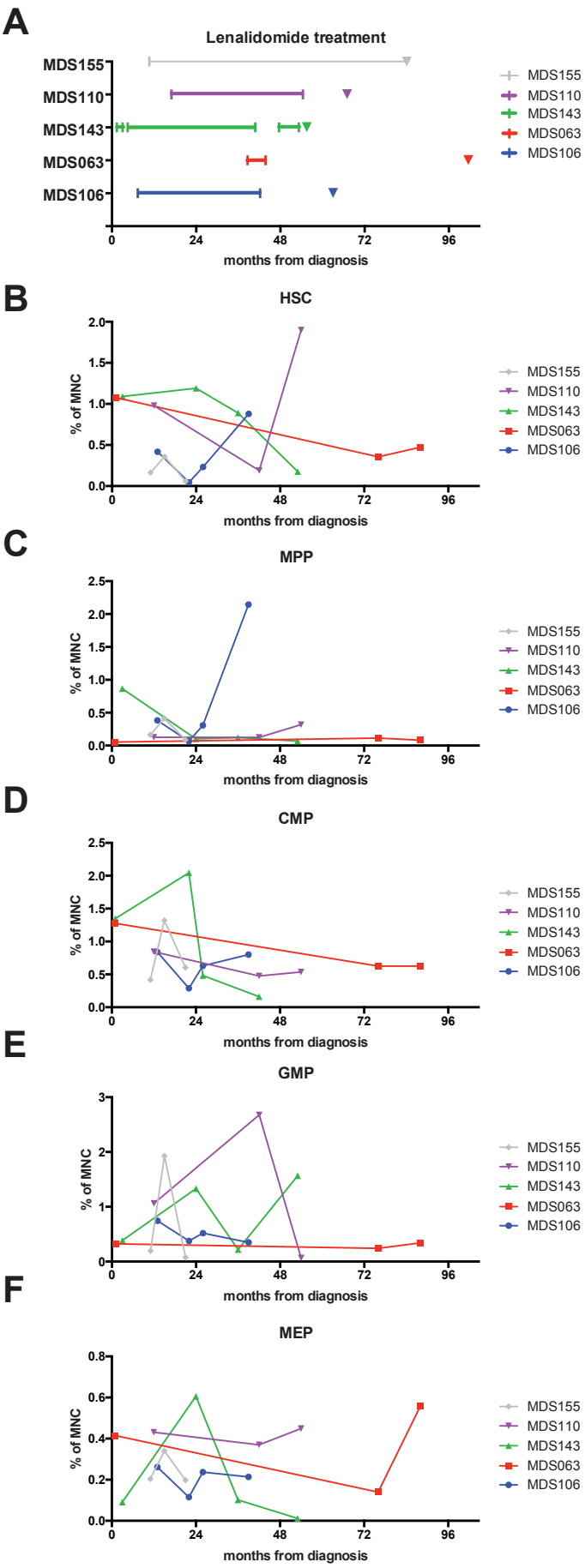
Correspondence: eva.hellstrom-lindberg@ki.se



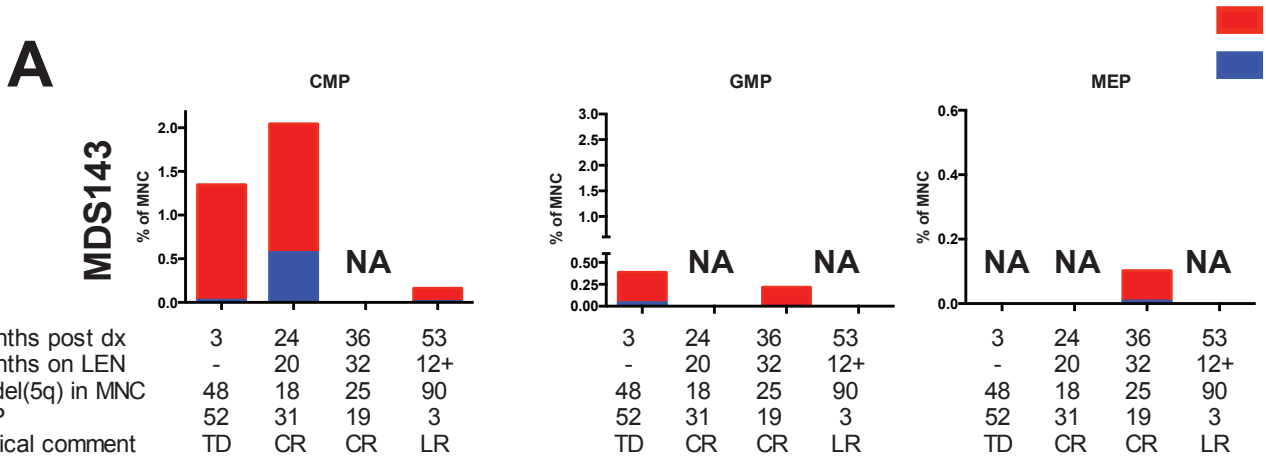
Supplementary Figure 1. A) Consort diagram of the cohort and description of the cohorts. *SPM = secondary primary malignancy.

Patient cohort not treated with LEN ('no LEN cohort'). Of thirteen patients (13/35, 37%) who did not receive LEN, only one patient (1/13, 8%) progressed to refractory anemia with excess blasts (RAEB-1) and no patient developed AML, eight patients (8/13, 62%) remained in stable phase, four of which received EPO-treatment. In this group, the median observation time from start of diagnosis was 31 months (range 0.5-187). The remaining four patients underwent allogeneic stem cell transplantation (SCT) during stable phase (INT-1 risk) at 45 to 72 years of age. Three of the nine non-transplanted patients have died (disease progression n=1, unrelated causes n=2). Of the four transplanted patients one died of transplantation-related mortality (TRM) while the other three patients are currently alive and well 30, 37 and 63 months post transplantation.

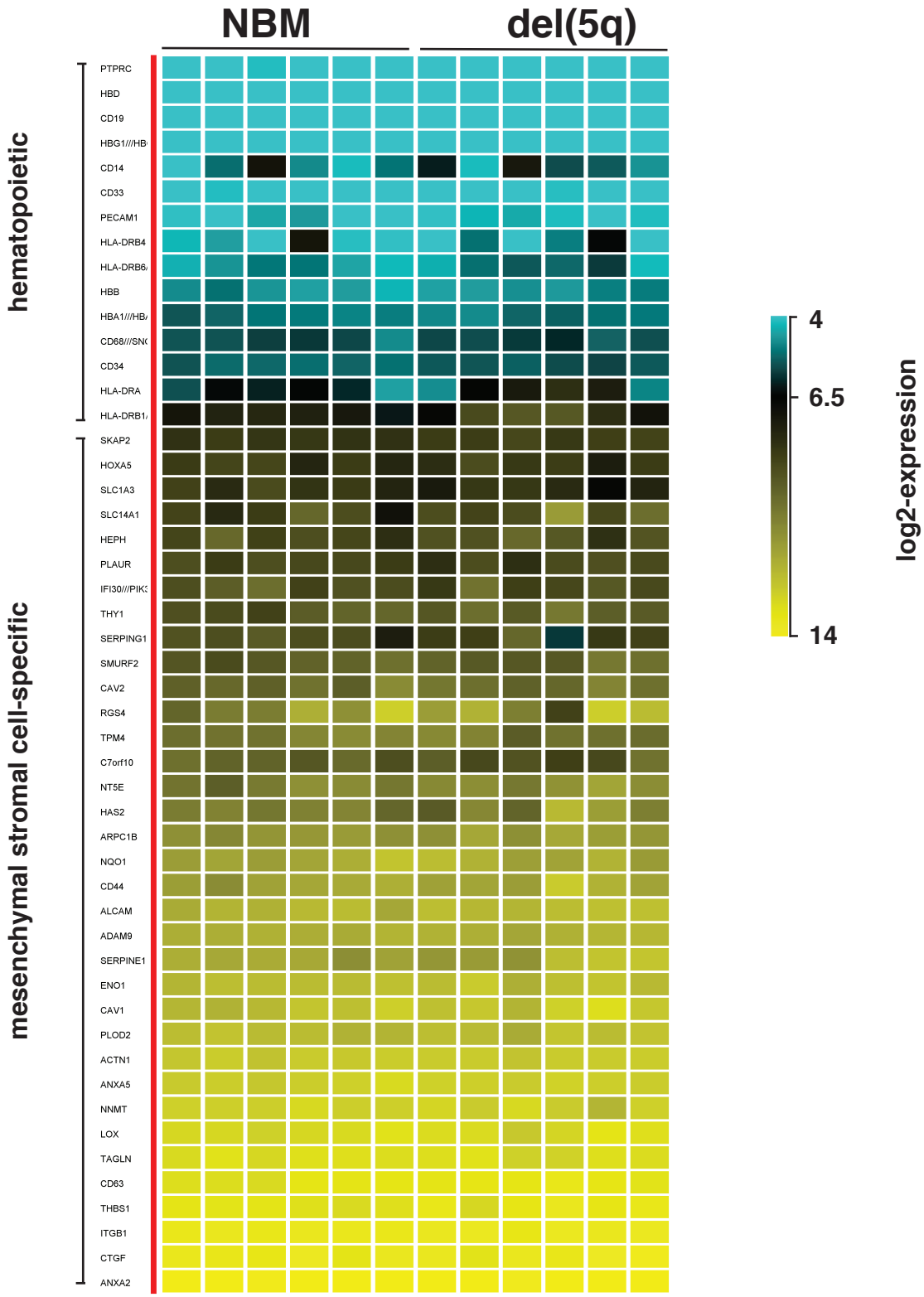
Patient cohort treated with LEN ('LEN cohort'). 22 patients (63%), who were transfusion dependent and either refractory to EPO or unlikely to respond based on high serum-EPO levels and transfusion intensity (Hellström-Lindberg, E., 1995. *British journal of haematology*, 89(1), pp.67–71) received LEN treatment. The median time from diagnosis to start of LEN was 20 months (range 0.5-82), the median duration of LEN treatment was 24 months (range 1-96), and the median observation time from start of LEN treatment was 55 months (range 19-129). Twenty of 22 patients (90%) showed at least a hematologic improvement to LEN according to the international guidelines (Cheson, B.D. et al., 2006. *Blood*, 108(2), pp.419–425), with a median response duration of 24 months (range 11-94). At the time of last follow-up, 5 patients (5/22, 23%) (MDS237, MDS239, MDS218, MDS489, and MDS538) were still responding 48, 37, 24, 20 and 19 months after start of LEN-treatment with a treatment duration of 1 to 24 months; two of these patients (MDS237 and MDS239) were treated for 11 and 1 months, respectively, and showed a continuous response despite being off LEN for more than two years. Amongst 17 patients who either lost their response to LEN or failed to respond upfront, twelve (12/17, 71%) progressed to higher-risk MDS (n=3) or leukemia (n=9; AML (n=8), ALL (n=1)) after a median of 76 months (range 31-184) post diagnosis, and 54 months (range 18-128) from start of LEN-treatment. Of the two patients who failed to respond to LEN, one patient (MDS063) discontinued LEN after 4 weeks due to poor compliance and no response, and developed AML 102 months after diagnosis, and 62 months after start of LEN. In the other non-responder (MDS224), LEN was discontinued after 3 months and the patient has currently stable disease receiving regular transfusions. Two patients underwent SCT, one was still responding to LEN but had developed breast cancer, the other patient was transplanted after transformation to AML and achieving complete remission after standard induction chemotherapy.



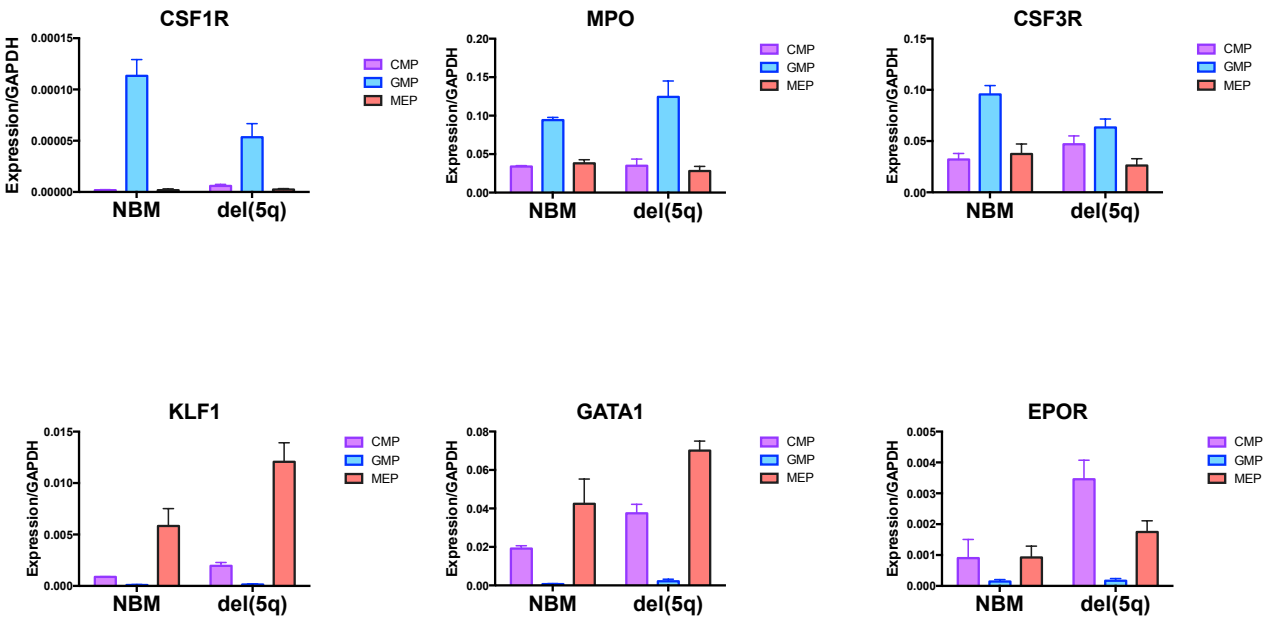
Supplementary figure 2 (related to Figure 4). Kinetic changes in HSPC subsets within the same patient over time during treatment and disease progression. A) shows the scheme for lenalidomide treatment. Triangle indicates time point of progression. B-F) Stem- and progenitor cell percentages in total BM for patients who initially responded to lenalidomide (n=4) or not (n=1). All patients shown eventually progressed to leukemia.



Supplementary figure 3 (related to Figure 4). Clonal size in the lin-CD34+ CD38 progenitor compartment.
Abbreviations: TTP, time to progression; MNC, mono-nuclear cells; TD, transfusion-dependent; CR, complete response; LR, loss of response; PR, partial response; NR, no response



Supplementary Figure 4 (related to Figure 5). Heatmap of raw expression values of MSC-related genes in MSC samples. Expression of several hematopoietic genes are shown as a control. The left six lanes show the 5q- cases and the right six the healthy controls.
The color bar indicates log2 expression values.



Supplementary Figure 5. Mean (SEM) expression of myeloid and erythroid transcripts within CMPs, GMPs and MEPs from normal BM (n=6) and del(5q) MDS (n=8).

Supplementary Table 1. Genes studied using 2 haloplex gene panels.
note that the 74 gene panel includes all the genes in the 42 gene panel.

haloplex-42
ASXL1
BCOR
CBL
CEBPA
CSF3R
DNMT3A
EPOR
ETV6
EZH2
FLT3
GATA1
GATA2
GATA3
IDH1
IDH2
JAK2
KDM6A
KIT
KRAS
MLL
MPL
NPM1
NRAS
PDS5B
PRPF40B
RAD21
RUNX1
SF1
SF3A1
SF3B1
SH2B3
SMC1A
SMC3
SRSF2
STAG1
STAG2
TET2
TP53
U2AF1
U2AF2
WT1
ZRSR2

haloplex-74	
APC	NOTCH1
ASXL1	NPM1
ATRX	NRAS
BAP1	NXF1
BCOR	PDGFRB
BCORL1	PDS5B
BRAF	PHF6
CBL	PRPF40B
CEBPA	PTEN
CREBBP	PTPN11
CSF3R	RAD21
CTCF	RB1
CTNNA1	RIT1
CUX1	RPS14
DIS3	RUNX1
DNMT3A	SETBP1
ELANE	SF1
EP300	SF3A1
EPOR	SF3B1
ETV6	SH2B3
EZH2	SMC1A
FAM5C	SMC3
FLT3	SRSF2
GATA1	STAG1
GATA2	STAG2
GATA3	STAT5B
GNAS	TET2
HNRNPK	TP53
IDH1	U2AF1
IDH2	U2AF2
IKZF1	WT1
IRF1	ZRSR2
JAK2	
JAK3	
KDM6A	
KIT	
KRAS	
LUC7L2	
MLL	
MPL	
MYC	
NF1	

Supplementary Table2. Individual patient samples analyzed and mutations detected.

Sample ID	at Dx or before any treatment	Time after diagnosis (months)	Treatment	months on LEN	clin status at sampling	Clinical outcome	Gene	Mutation	Variant allele ratio
MDS038	yes	0	none		TD		**TP53**	p.Y163C	0.20
MDS038	no	19	LEN	3	CHR		**TP53**	p.Y163C	0.21
MDS038	no	28	LEN	11	CHR		**TP53**	p.Y163C	0.14
MDS038	no	47	none		RAEB-2	**AML**	**TP53**	p.Y163C	0.23
MDS063	yes	18	none		TD		**MLL**	p.E3013Q	0.48
							PTPN11	p.E69V	0.33
MDS063	no	88	none		progression	**AML**	**MLL**	p.E3013Q	0.50
							PTPN11	p.E69V	0.39
							TP53	p.C176Y	0.01
MDS094	no	57	LEN	16	CHR		**TET2**	p.R1383G	0.19
							TP53	p.C275F	0.15
MDS094	no	118	none		progression	**RAEB-2 & ovarial Ca**	**KDM6A**	p.R393X	0.05
							TP53	p.C275F	0.11
MDS106	no	13	LEN	5	CCyR		**no mutation**		
MDS106	no	45	none		progression	**AML**	**TP53**	p.Q100fs	0.01
MDS124	yes	1	none		TD		**No Mutation**		
MDS124	no	44	HSCT		post SCT	**SCT**	**No Mutation**		
MDS143	yes	3	none		TD		**No Mutation**		
MDS143	no	52	none		progression	**AML**	**TP53**	p.L265P	0.32
							TP53	p.R116Q	0.07
MDS155	yes	11	None		TD		**No Mutation**		
MDS155	no	72	LEN	61	CCyR	**ALL**	**JAK2**	p.V617F	0.03
							TP53	p.L194R	0.06
							TP53	p.H179R	0.03
							TP53	p.C176S	0.03
MDS175	yes	8	none		TD		**ASXL1**	p.G645fs	0.33
							IDH2	p.R140Q	0.43
MDS175	no	31	LEN	21	progression	**AML**	**TET2**	p.P1367L	0.11
							TP53	p.I255N	0.29
MDS185	yes	114	none		TD		**DNMT3A**	p.S669fs	0.09
MDS185	no	155	none		post SCT	**SCT**	**No Mutation**		
MDS218	yes	9	none		TD		**ASXL1**	p.G645fs	0.06
MDS218	no	50	EPO		TD	**still responding**	**ASXL1**	p.G645fs	0.03
							CSF3R	p.T781fs	0.27
							CUX1	p.N579fs	0.31
MDS224	yes	20	none		TD		**DNMT3A**	p.F354fs	0.37
MDS224	no	48	none		TD	**SD**	**DNMT3A**	p.F165fs	0.33
MDS237	yes	48	none		TD		**No Mutation**		
MDS237	no	95	none		CCyR	**still responding**	**No Mutation**		
MDS335	yes	0	none		TD		**No Mutation**		
MDS335	no	19	EPO		CHR	**SD**	**No Mutation**		
MDS019	yes	47	none		TD		**DNMT3A**	N516fs	0.52
							RUNX1	K83Q	0.02
MDS019	no	92	LEN	24	CHR		**DNMT3A**	N516fs	0.52
							RUNX1	K83Q	0.61
MDS019	no	106	none		AML	**AML & SCT**	**DNMT3A**	N516fs	0.50
							RUNX1	K83Q	0.99
MDS110	yes	12	none		TD		**SF3B1**	p.K700E	0.18
							TET2	p.K1439fs	0.10
MDS110	no	66	none		progression	**AML**	**NRAS**	p.G12V	0.43
							SF3B1	p.K700E	0.44
MDS075	no	70	**LEN**	14	CHR	**SD**	**DNMT3A**	p.R729Q	0.08
MDS075	no	184	**none**		progression	**AML**	**DNMT3A**	p.R899Afs	0.24
							MYD88	p.L273P	0.06
							TET2	p.H1325fs	0.23
							TP53	p.R273C	0.29
							BCOR	p.A1496P	0.05
MDS032	no	86	**LEN**	46	CHR	**breast ca & SCT**	**EZH2**	p.V568fs	0.95
							ASXL1	p.A946fs	0.45
MDS076	no	42	**LEN**	26	PHR	**SD**	**No Mutation**		
MDS096	no	61	**LEN**	14	PHR	**RAEB-1**	**DNMT3A**	p.R882H	0.35
							RUNX1	p.S303X	0.27
							SRSF2	p.P95L	0.61
							TET2	p.R1262Q	0.37
MDS107	no	100	**LEN**	67	CHR	**RAEB-1**	**RUNX1**	p.P200fs	0.40
							TET2	p.S1107X	0.48
							TET2	p.R1261H	0.44
							ASXL1	p.R634fs	0.32
							ZRSR2	p.E246X	0.91
MDS117	no	9	**LEN**	7	CCyR	**SD**	**ASXL1**	p.Q780X	0.09
MDS153	yes	1	**none**		SD	**SD & SPM**	**No Mutation**		
MDS207	yes	132	**EPO**		SD	**RAEB-1**	**EZH2**	p.R34X	0.06
							TP53	p.R141H	0.80
MDS233	yes	0	none		SD	**SD**	**ASXL1**	p.R634fs	0.06
							SETBP1	p.D868N	0.15
MDS239	yes	0	none		SD	**still responding**	**KDM6A**	p.H1357L	0.47
MDS240	yes	0	none		SD	**SD**	**No Mutation**		
MDS355	yes	0	none		SD	**SD**	**No Mutation**		
MDS359	yes	0	none		SD	**SCT**	**JAK2**	p.V617F	0.06
							TP53	p.R267W	0.22
MDS432	yes	0	none		SD	**SD**	**SF3B1**	p.K700E	0.11
MDS445	yes	0	none		TD	**SD**	**TET2**	p.L431X	0.35
MDS489	yes	0	none		SD	**still responding**	**DNMT3A**	p.R736C	0.29
MDS499	yes	0	none		TD	**SCT**	**DNMT3A**	p.731_732del	0.40
MDS526	yes	0	none		TD	**SD**	**DNMT3A**	p.I655T	0.12
							SF3B1	p.K700E	0.30
MDS538	yes	0	none		TD	**still responding**	**No mutation**		
MDS555	yes	0	none		SD	**SD**	**BCOR**	p.Q231fs	0.03
							TP53	p.P278A	0.22
							TP53	p.V104A	0.18

TD = transfusion-dependent

CHR = complete hematologic response

CCyR = complete cytogenetic response

PHR = partial hematologic response

SD = stable disease

Supplementary Table3. Clinical details of samples used for surveillance of HSPC subsets by flow cytometry.

Patient ID	months post diagnosis	sex	WHO	Karyotype	IPSS	Hb	WBC	ANC	BM blasts	transfusion	%del(5q) of MNC by FISH	treatment lenalidomide	treatment response
MDS063	1	Female	5q-	46,XX,del(5)(q13q33) [9], 44-45,XX,del(5)(q13q33) [3], 46,XX [13], 44-45,XX [2]	Low	95	9.1	6.1	4	no	44	pre	NA
MDS063	76	Female	5q	46,XX,del(5)(q13q33)[11]/45,XX,-5[3]/45,XX,del(5)(q13q33),-5[1]/46,XX[11]	Low	116	7.6	4.3	4	yes	78	NA	NR
MDS063	88	Female	5q	46,XX,del(5)(q13q33)[16]/46,XX,idem,del(17)(p?13)[9]	Low	93	8.1	4.1	5	yes	59	NA	NR
MDS106	13	Male	5q-	NA	Low	127	4.9	3	4.5	yes	0	on (5 mo)	CCyR
MDS106	22	Male	5q-	47,XY,+21[4]/47,XY,del(5)(q13q33),+21[2]/46,XY[21]	Int-1	126	11	8.6	5.5	no	9	on (15 mo)	CHR
MDS106	26	Male	5q-	NA	Int-1	130	3.4	1.7	0	no	7	on (19 mo)	CHR
MDS106	39	Male	5q	47,XY,+21[3]/47,XY,del(5)(q13q33),+21[12]/46,XY[9]	Low	103	4.1	2.2	3	no	53	on (31 mo)	PR
MDS110	12	Female	5q	46,XX,del(5q)/9[25]	Int-1	89	3.5	1.6	2	no	45	pre	TD
MDS110	42	Female	5q	46,XX,del(5)(q13q33)[20]/46,XX[5]	Low	110	4.9	2.3	2	no	43	on (25 mo)	CHR
MDS110	54	Female	5q	46,Xxdel(5)(q13q33)[20]/46,XX[5]	Low	107	6.9	3.8	3.5	yes	36	on (37 mo)	LR
MDS143	3	Male	5q	46 XY, del(5)(q13q33) [14], 46 XY [13]	Low	89	3.3	2	5	yes	48	pre	TD
MDS143	24	Male	5q	NA	Low	148	23	8	3	no	18	on (20 mo)	CHR
MDS143	36	Male	5q	46,XY,del(5)(q13q31)[6]/46,XY[19]	Low	127	3.2	1.8	3	no	25	on (32 mo)	CHR
MDS143	53	Male	5q	46,XY,del(5)(q12q33)[11]745~47,XY,del(5)(q12q33),del((7)(q22),-11,-17,-18,-20,-21,+3-7mar[cp8]/46,XY[8]	Int-1	90	3.5	1.8	9.5	yes	90	post (12 mo)	LR
MDS155	11	Female	5q-	46,XX,del(5)(q13q33 [24]/46,XX [4]	Low	114	3.4	2.3	1.5	yes	62	pre	TD
MDS155	15	Female	5q-	NA	Low	146	4.7	2.9	4.5	no	0	on (4 mo)	CCyR
MDS155	21	Female	5q-	NA	Low	129	3.3	1.3	3.5	no	21	on (11 mo)	CHR

Abbreviations:
TD = transfusion-dependent
CHR = complete hematologic response
PR = partial response
LR = loss of response
CCyR = complete cytogenetic response

Supplementary Table 4. Comparison of TP53 analysis by IHC against targeted sequencing and deep sequencing

PAT ID	months post dx	% TP53 by deep seq	TP53+ by IHC	type of TP53 by targeted sequencing	% TP53 by targeted seq
MDS143	3	NA	0	no mut	0
MDS143	12	0	0	no material available	
MDS143	24	NA	0		
MDS143	41	NA	2		
MDS143	53	NA	8	L265P and R116Q	32 and 7
MDS143	55	NA	25		
MDS094	20	NA	1	no material available	
MDS094	60	NA	3	C275F	15
MDS094	72	29	5.5	no material available	
MDS094	91	NA	5		
MDS094	103	NA	15		
MDS094	115	NA	11		
MDS094	118	NA	10	C275F	11
MDS175	0	NA	4	NA	NA
MDS175	9	NA	4	other mut	0
MDS175	10	NA	11	NA	NA
MDS175	14	0	4	NA	NA
MDS175	32	NA	50	I255N	29
MDS106	13	NA	0	no mut	0
MDS106	26	NA	0	no material available	
MDS106	39	0	NA		
MDS106	45	NA	2	Q100fs	1
MDS063	0	NA	0	no material available	
MDS063	18	NA	NA	other mut	0
MDS063	22	NA	0	no material available	
MDS063	34	0	0		
MDS063	76	NA	5		
MDS063	88	NA	7	C176Y	1
MDS075	34	NA	0	no material available	
MDS075	70	0	0		
MDS075	184	NA	10	R273C	29
MDS038	0	20	1	no material available	
MDS038	3	NA	2		
MDS038	4	NA	7		
MDS038	19	21	10		
MDS038	28	14	1		
MDS038	47	23	10		
MDS038	55	NA	10.5		
MDS155	21	NA	10	no material available	
MDS155	29	NA	10		
MDS155	57	NA	12	L194R, H179R and C176S	6, 3 and 3
MDS359	0	NA	6	R267W	22
MDS207	111	NA	5	NA	
MDS207	132	NA	<5	R131W	80
MDS207	172	NA	20	NA	
MDS555	10	NA	NA	P278A and V104A	22 and 18