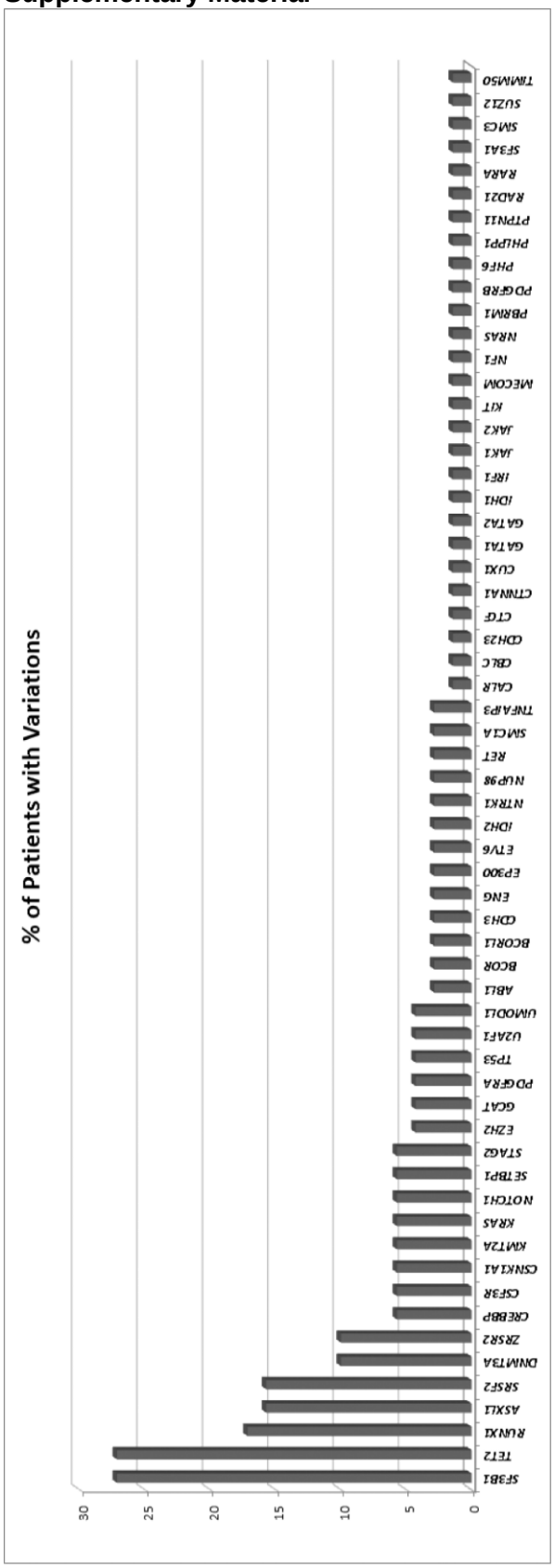
Bone marrow fibrosis in myelodysplastic syndromes: a prospective evaluation including mutational analysis

Supplementary Material



Supplemental Fig. 1. Frequency of gene mutations in 70 MDS patients.

Supplemental Table 1. Genes involved in MDS pathogenesis grouped into functional pathways.

Gene	Functional pathways	Gene	Functional pathways	Gene	Functional pathways
ASXL1	Chromatin modification	***ABL1***	Signalling	***CUX1***	Transcription
BCOR	Chromatin modification	***CBLC***	Signalling	***ETV6***	Transcription
BCORL1	Chromatin modification	***CDH3***	Signalling	***GATA1***	Transcription
CREBPP	Chromatin modification	***CSF3R***	Signalling	***GATA2***	Transcription
EP300	Chromatin modification	***CSNK1A1***	Signalling	***PHF6***	Transcription
EZH2	Chromatin modification	***CTNNA1***	Signalling	***RUNX1***	Transcription
KMT2A	Chromatin modification	***ENG***	Signalling	***TP53***	Transcription
MECOM	Chromatin modification	***IRF1***	Signalling	***UMODL1***	Transcription
PBRM1	Chromatin modification	***JAK1***	Signalling		
SETBP1	Chromatin modification	***JAK2***	Signalling		
SUZ12	Chromatin modification	***KIT***	Signalling		
		KRAS	Signalling		
SF3A1	RNA splicing	***NF1***	Signalling	***DNMT3A***	DNA methylation
SF3B1	RNA splicing	***NOTCH1***	Signalling	***IDH1***	DNA methylation
SRSF2	RNA splicing	***NRAS***	Signalling	***IDH2***	DNA methylation
UA2F1	RNA splicing	***NTRK1***	Signalling	***TET2***	DNA methylation
ZRSR2	RNA splicing	***PDGFRA***	Signalling		
		PDGFRB	Signalling		
CTCF	Cohesin complex	***PHLPP1***	Signalling	***CALR***	Others
RAD21	Cohesin complex	***PTPN11***	Signalling	***CDH23***	Others
SMC1A	Cohesin complex	***RARA***	Signalling	***GCAT***	Others
SMC3	Cohesin complex	***RET***	Signalling	***NUP98***	Others
STAG2	Cohesin complex	***TNFAIP3***	Signalling	***TIMM50***	Others

Supplemental Table 2. Comparative findings between myelodysplastic syndrome cases with or without relevant bone marrow fibrosis. PANEL A. Demographics and peripheral blood findings.

	MF-0/1	**MF-2/3**	p-value
No. of patients, (%)	*60* (77.9)	17 (22.1)	
DEMOGRAPHICS			
Age at diagnosis			
Median,(range), years	77 (70-82)	72 (64.5-80.5)	0.259
Gender			
Male, No. of patients, (%)	*38* (63.3)	14 (82.4)	0.240
Performance status (ECOG)			
No. of patients, (%)			
0-1	*47 (78.3)*	*12 (70.6)*	*0.840*
2	7 (10.0)	5 (29.4)	
3-4	7 (11.7)	0	
Transfusion dependence			
No. of patients, (%)	*15 (25.5)*	*10 (58.8)*	*0.017*
PERIPHERAL BLOOD			
WBC count (x 10^9/L)			
Median, (IQR)	3.9 (2.5-4.9)	3.5 (2.3-5.2)	0.968
Haemoglobin (g/L)			
Median, (IQR)	10.0 (8.9-11.3)	9.2 (7.1-10.3)	0.092
Platelet count (x 10^9/L)			
Median, (IQR)	121 (72-246)	156 (81-267)	0.925
Blast cells (%)			
Median, (range)	0 (0-0)	0 (0-0.5)	0.133
***WT1/GUS* ratio in PB** (x10^{-4})			
Median, (IQR)	*3* (1.0–13.0)	30 (8.0-85.5)	0.003
SERUM			
Ferritin (ng/mL)			
Median, (IQR)	309 (173-599)	578 (350-963)	0.025
LDH (U/L)			
Median, (IQR)	372 (326-465)	401 (287-514)	0.235
EPO (U/L)			
Median, (IQR)	58 (18-153)	134 (43.3-651.0)	0.067
Beta2-m (mg/L)			
Median, (IQR)	*3.0* (2.2-4.5)	3.2 (2.5-3.6)	0.978
sp53 (U/mL)			
Median, (IQR)	1.6 (0-3.4)	1.7 (1.1-2.9)	0.593
TNF alpha (pg/mL)			
Median, (IQR)	0 (0-0)	0 (0-0)	0.504
IL6 (pg/mL)			
Median, (IQR)	0 (0-6.9)	0 (0-0)	0.152
IL1 beta (pg/mL)			
Median, (IQR)	0 (0-0)	0 (0-0)	0.732
CXCL8 (pg/mL)			
Median, (IQR)	0 (0-20.1)	0 (0-8.7)	0.174
CXCL9 (pg/mL)			
Median, (IQR)	0 (0-164.2)	0 (0-0)	0.015
CXCL10 (pg/mL)			
Median, (IQR)	0 (0-137.8)	0 (0-0)	0.055

Supplemental Table 2. Comparative findings between myelodysplastic syndrome cases with or without relevant bone marrow fibrosis. PANEL B. Other parameters of interest. HR=High risk ratio. BMT= Blood and marrow transplantation.

	MF-0/1	**MF-2/3**	p-value
No. of patients, (%)	*60* (77.9)	17 (22.1)	
BONE MARROW ASPIRATION			
Blast cells at diagnosis (%)			
Median, (IQR)	2.0 (0.6-4.8)	1.6 (0.9-4.2)	0.681
Red cell progenitors (%)			
Median, (IQR)	35 (23.6-45.2)	24.2 (15.4-32.5)	0.050
BONE MARROW BIOPSY			
Cellularity (%)			
Median, (IQR)	55 (40-75)	80 (60-92.5)	0.006
p53 IHC score			
Median, (IQR))	1 (0-3)	2 (1.5-8.5)	0.027
Strong p53 reactivity (>1%)			
No. of patients, (% out of 76)	7 (11.9)	4 (26.7)	0.217
ALIP			
No. of patients, (%)	15 (25.0)	11 (64.7)	0.004
SCHANZ'S CYTOGENETIC STRATA			
No. of patients, (%)			
Very good/good	43 (71.7)	12 (70.6)	0.458
Intermediate	7 (11.7)	5 (29.4)	
Poor/very poor	10 (16.7)	0	
IPSS-R CATEGORY			
No. of patients, (%)			
Very good/good	36 (60.0)	12 (70.6)	0.617
Intermediate	11 (18.3)	2 (11.8)	
Poor/very poor	13 (21.7)	3 (17.6)	
FUNCTIONAL PATHWAYS			
No. of patients, (%)	*56 (80.0)*	*14 (20.0)*	
Splicing	*27 (48.2)*	*9 (64.3)*	*0.374*
Signalling	*26 (46.4)*	*8 (57.1)*	*0.557*
Chromatin modification	*19 (33.9)*	*7 (50.0)*	*0.356*
Transcription regulation	*16 (28.6)*	*6 (42.9)*	*0.344*
Methylation	*20 (35.7)*	*7 (50.7)*	*0.368*
Cohesin complex	*3 (5.4)*	*5 (35.7)*	*0.006*
Bejar's HR score	*17 (30.4)*	*9 (64.3)*	*0.029*
THERAPY			
No. of patients, (%)			
Azacitidine	15 (25.5)	5 (29.4)	0.758
Intensive chemotherapy	7 (11.7)	3 (17.6)	0.683
BMT	6 (10.0)	2 (11.8)	0.834

Supplemental Table 3. Main patient characteristics at diagnosis. PANEL A. Demographics and peripheral blood findings.

DEMOGRAPHICS	
Age at diagnosis	
Median,(range), years	76 (31-88)
Gender	
Male, No. of patients, (%)	52 (67.5)
Performance status (ECOG)	
No. of patients, (%)	
0-1	59 (76.6)
2	11 (14.3)
3-4	7 (9.1)
Transfusion dependence	
No. of patients, (%)	27 (35.1)
PERIPHERAL BLOOD	
WBC count (x 10^9/L)	
Median, (range)	3.9 (0.9-15.4)
IQR	*2.5-4.9*
Haemoglobin (g/L)	
Median, (range)	9.9 (5.9-16.1)
IQR	8.6-13.0
Platelet count (x 10^9/L)	
Median, (range)	122 (9-1067)
IQR	72-247
Blast cells (%)	
Median, (range)	0 (0-5)
IQR	0-0
WT1/GUS ratio in PB (x10^{-3})	
Median, (range)	0.5 (0-654.5)
IQR	1.0-31.0
SERUM	
Ferritin (ng/mL)	
Median, (range)	375 (27-1495)
IQR	189-633
LDH (U/L)	
Median, (range)	377 (120-922)
IQR	319-473
EPO (U/L)	
Median, (range)	59 (7-1536)
IQR	21-175
Beta2-m (mg/L)	
Median, (range)	3.1 (1.3-12.7)
IQR	2.2-4.0
sp53 (U/mL)	
Median, (range)	1.70 (0-6.25)
IQR	0-3.05
TNF alpha (pg/mL)	
Median, (range)	0 (0-195.6)
IQR	0-0
IL6 (pg/mL)	
Median, (range)	0 (0-111.1)
IQR	0-7.6
IL1 beta (**(**pg/mL)	
Median, (range)	0 (0-160.3)
IQR	0-0
CXCL8 (**(**pg/mL)	
Median, (range)	0 (0-339.7)
IQR	0-19.4
CXCL9 (pg/mL)	
Median, (range)	0 (0-2204.2)
IQR	0-341.0
CXCL10 (**(**pg/mL)	
Median, (range)	0 (0-893.3)
IQR	0-125.8

Supplemental Table 3. Main patient characteristics at diagnosis. PANEL B. Bone marrow, classification, IPSS-R and OS.

BONE MARROW ASPIRATION	
Blast cells at diagnosis (%)	
Median, (range)	2.0 (0-19)
IQR	0.6-4.9
Red cell progenitors (%)	
Median, (range)	33 (7.8-64.0)
IQR	23.2-41.5
SCHANZ'S CYTOGENETIC STRATA	
No. of patients, (%)	
Very good	4 (5.2)
Good	51 (66.2)
Intermediate	12 (15.6)
Poor	5 (6.5)
Very poor	5 (6.5)
BONE MARROW BIOPSY	
Cellularity (%)	
Median, (range)	*60 (1-95)*
IQR	*40-80*
Fibrosis grade (European consensus)	
No. of patients, (%)	
MF - 0	29 (37.7)
MF - 1	31 (40.3)
MF - 2	15 (19.5)
MF - 3	2 (2.6)
Fibrosis pattern	
No. of patients, (%)	
Absent	28 (36.4)
Focal	21 (27.3)
Diffuse	28 (36.4)
ALIP	
No. of patients, (%)	26 (33.8%)
p53 IHC score	
Median, (range)	1 (0-460)
IQR	1-4
Strong p53 IHC reactivity (>1%)	
No. of patients, (% out of 76)	11 (14.5)
WHO CLASSIFICATION CATEGORY	
No. of patients, (%)	
RCUD	2 (2.6)
RARS	4 (5.2)
RCMD	40 (51.9)
RAEB-1	15 (19.5)
RAEB-2	7 (9.1)
5q-	7 (9.1)
MDS-U	2 (2.6)
IPSS-R CATEGORY	
No. of patients, (%)	
Very good	15 (19.5)
Good	33 (42.9)
Intermediate	13 (16.9)
Poor	10 (13.0)
Very poor	6 (7.8)
OVERALL SURVIVAL	
MF-0	
No. of patients, (%)	*29 (37.7)*
Median (years), CI95%	*4.0 (1.92-6.09)*
MF-1	
No. of patients, (%)	*31 (41.3)*
Median (years), CI95%	*3.9 (0.29-7.46)*
MF-2/3	
No. of patients, (%)	*17 (22.1)*
Median (years), CI95%	*1.2 (0-3.29)*

Supplemental Table 4. WHO subtype distribution in cohesin complex mutated and non-mutated cases.

RCUD, Refractory cytopenia with unilineage dysplasia; RARS, Refractory anemia with ring sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RAEB-1, Refractory anemia with excess blasts type 1; RAEB-2, Refractory anemia with excess blasts type 2; 5q-, MDS with isolated del(5q); MDS-U, MDS-unclassified.

	RCUD	**RARS**	**RCMD**	**RAEB-1**	**RAEB-2**	**5q-**	**MDS-U**	Total
Cohesin complex non-mutated	2	3	32	11	6	6	2	62
Cohesin complex mutated	0	0	4 (11.1%)	3 (21.4%)	0	1 (14.3%)	0	8
Total	2	3	36	14	6	7	2	70

Supplemental Table 5. IPSS-R strata distribution in cohesin complex mutated and non-mutated cases.

	Very good	**Good**	**Intermediate**	**Poor**	**Very poor**	Total
Cohesin complex non-mutated	13	28	8	7	6	62
Cohesin complex mutated	1 (47.8%)	3 (9.7%)	3 (27.3%)	1 (14.3%)	0	8
Total	14	31	11	8	7	70