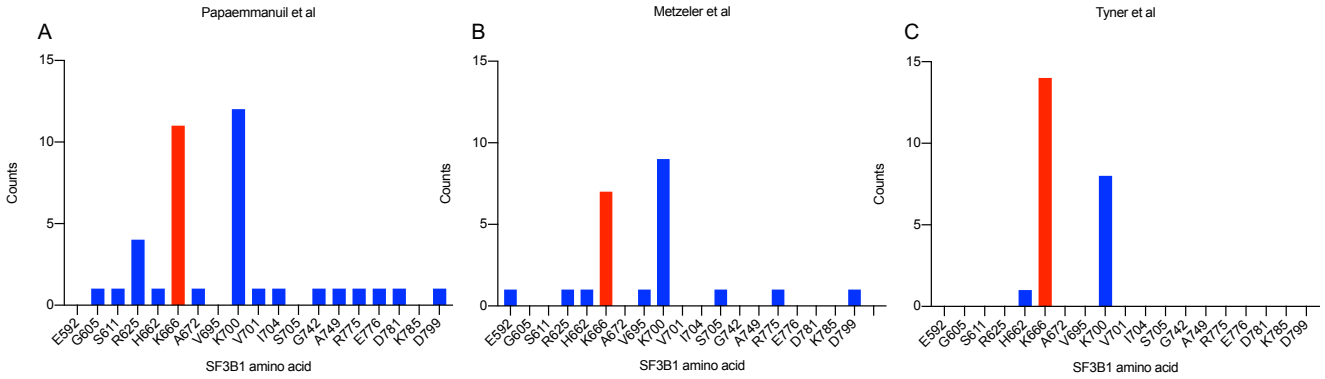
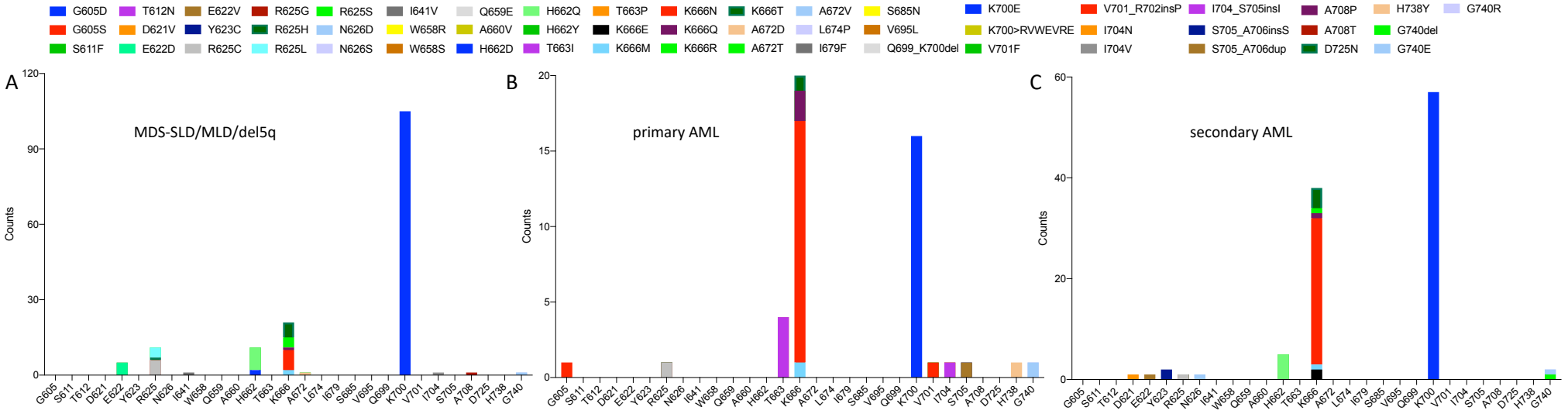
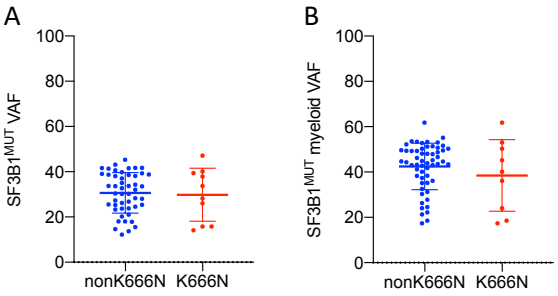


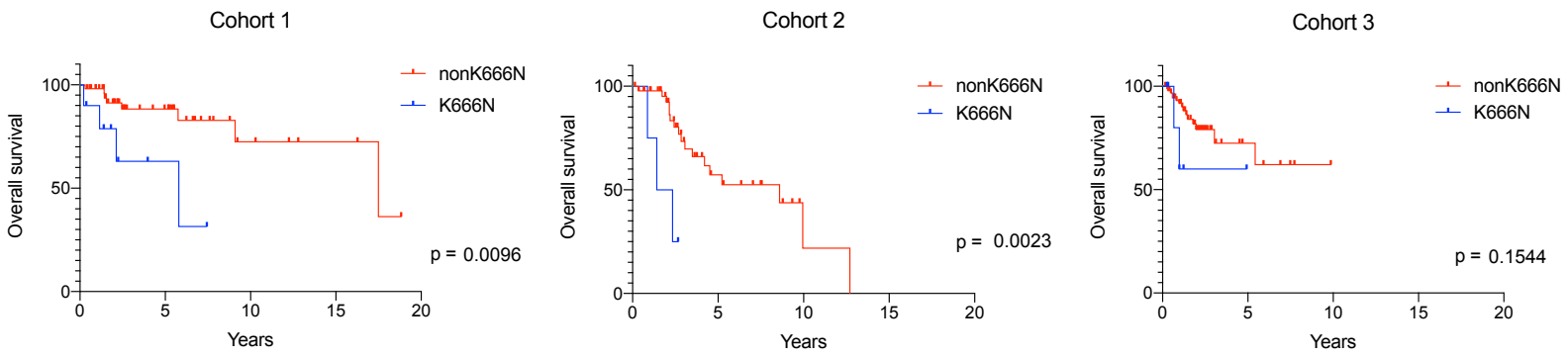
Supplemental Figure 1. Frequent mutations of K666 in SF3B1^{MUT} AML. Distributions of SF3B1 mutations in patients with AML are shown for the cohorts analyzed by A) Papaemmanuil et al, B) Metzeler et al, and C) Tyner et al. Mutations at K666 are shown in red.



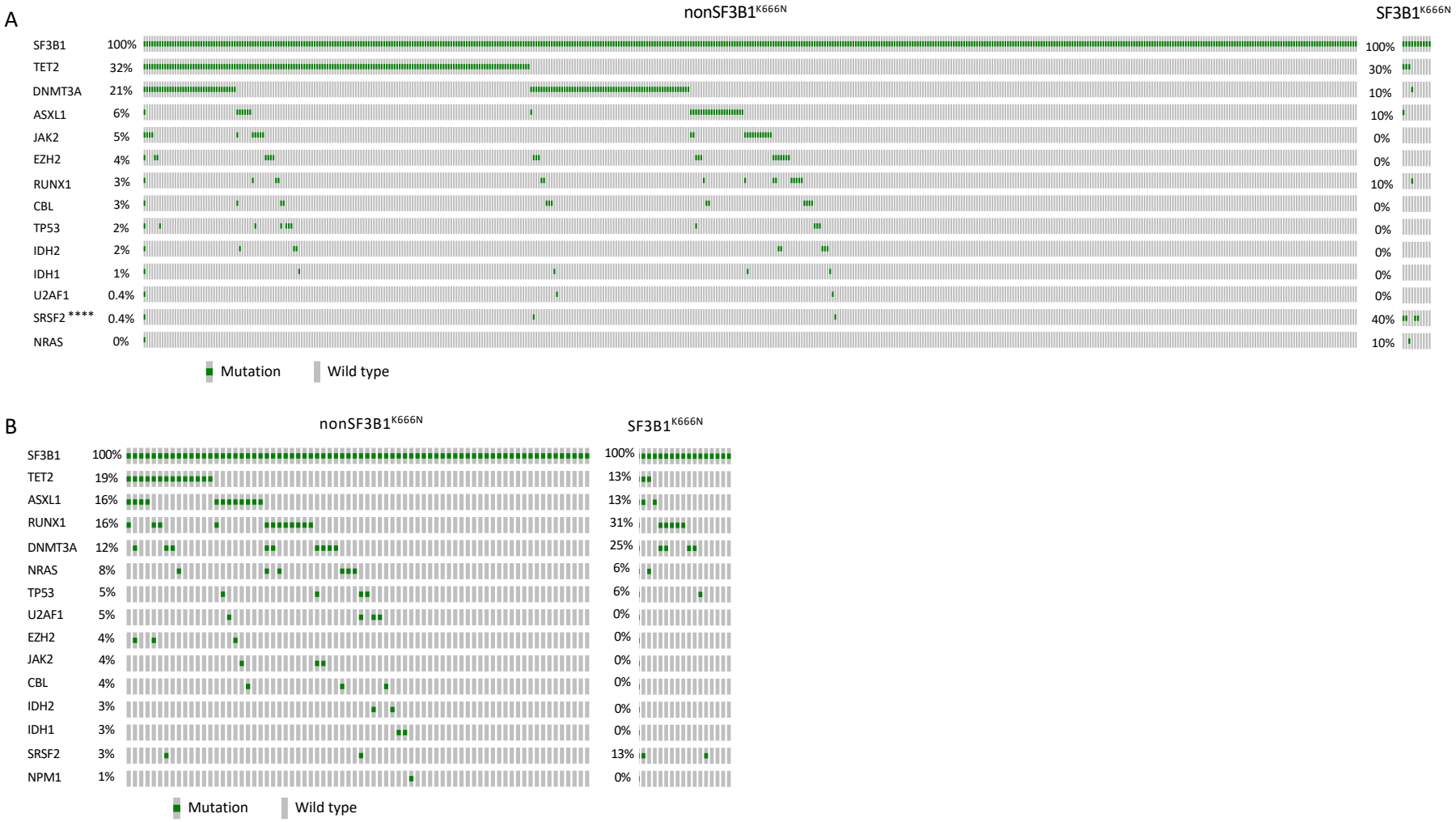
Supplemental Figure 2. SF3B1 mutation distribution in additional patient groups. A) MDS patients with 'non-ring-sideroblast' lower risk WHO classifications. The frequency of K666N was 5.1% (8/158), modestly but significantly higher than the 1.5% of MDS-RS (p-value = 0.011). For AML patients where the relevant clinical annotations were available, mutations were grouped according to B) primary/de novo AML or C) secondary AML.



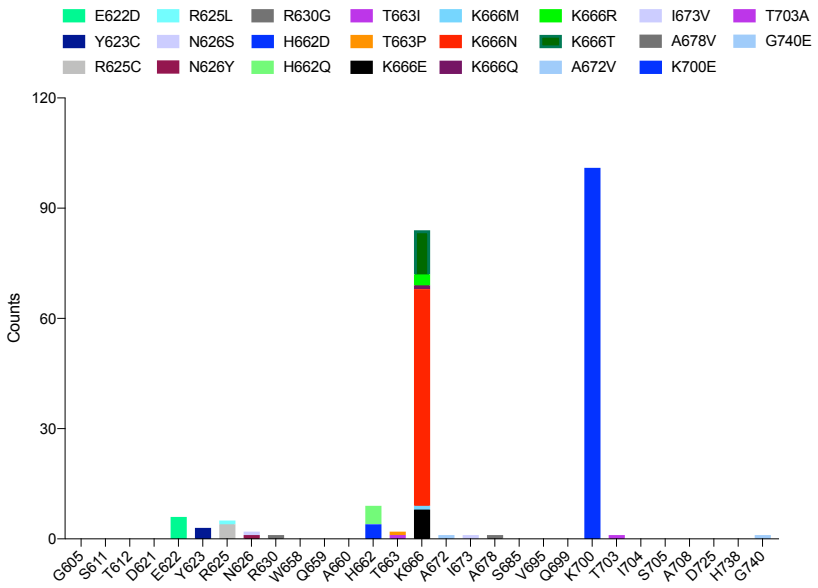
Supplemental Figure 3. Variant allele frequencies of SF3B1^{K666N} and other SF3B1 mutations in MDS. Both A) raw VAFs and B) lymphoid compartment-adjusted VAFs of myeloid cells show similar distribution in the two groups.



Supplemental Figure 4. Overall survival of individual SF3B1^{K666N} cohorts. Overall survival was significantly decreased for K666N patients in cohorts 1 (left) and 2 (center). For cohort 3 (right), follow up was shorter for all patients, and though there was a trend towards decreased survival for K666N, this was not statistically significant. P-values were calculated with Mantel-Cox tests.



Supplemental Figure 5. Genetic landscape of SF3B1^{MUT} MDS-RS and MDS-EB. A) In MDS-RS, the predominant co-mutations were in TET2 and DNMT3A. Though few SF3B1^{K666N} cases with co-mutation data were available, there was a notable, statistically significant enrichment in SRSF2 co-mutation. **** = p-value < 0.0001 for Fisher's exact test. B) In MDS-EB, there were more co-mutations of ASXL1, RUNX1, and NRAS than in MDS-RS.



Supplemental Figure 6. SF3B1 mutation distribution in CHIP. Among individuals with SF3B1^{MUT} clonal hematopoiesis of indeterminate potential (CHIP), K666N is a frequent hotspot.

Table S1. Cohort 1 of SF3B1-mutant MDS. World Health Organization (WHO) classification, white blood count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb), platelets (PLT), bone marrow blasts, ring sideroblasts (RS), cytogenetics, and Revised International Prognostic Scoring System (IPSS-R) at time of diagnosis are shown. NA = not available.

UPN	WHO	SF3B1	VAF	Source	Nonlymph %	Nonlymph VAF	WBC	ANC	Hb	PLT	Blasts	RS	Cytogenetics	IPSS-R
101	MDS-RS-SLD	E783K	18.0	PB	59.5	30.3	2.1	1.1	10	287	<=2	>15	46XX	2
102	MDS-RS-SLD	D781G	23.8	PB	51	46.6	4.1	2.5	9.5	371	<=2	5	46XY	2
103	MDS-RS-MLD	D781E	NA	PB	89	NA	2	0.8	8.1	124	2	<5	46XY	2
104	MDS-RS-SLD	K700E	21.0	PB	54.9	38.3	2.5	1.1	11.1	251	<=2	>15	46XY	1
105	MDS-RS-MLD	K700E	40.6	BM	91	44.6	5.8	4.2	9.5	218	<=2	>15	46XY	2
106	MDS-RS-MLD	K700E	41.5	PB	86.6	47.9	2.3	1.1	9.1	303	<=2	>15	46XX	2
107	MDS with isolated del(5q)	K700E	39.3	BM	91	43.2	6.7	4.9	9.9	185	<=2	NA	46,XX,del(5)(q13q33)[7]/46,XX[13]	2
108	MDS-MLD	K700E	NA	80	NA	NA	6.4	3.9	10.2	226	<=2	0	46,XY,del(20)(q?11.2)[2]/46,XY[18]	1
109	MDS-EB-2	K700E	41.7	PB	79	52.8	5.2	3.4	9.2	196	19	NA	46,XX,add(2)(q31),der(3)t(3;8)(q21;p21),der(8)t(2;8)(q?31;p21)[20]	7
110	MDS-RS-MLD	K700E	32.0	PB	66.7	48.0	3.7	1.9	8.6	149	<=2	50	46,XX,del(20)(q11.2)[20]	2
111	MDS-RS-SLD	K700E	39.8	PB	74.6	53.4	4.9	2.7	9.6	400	<=2	>15	46XX	2
112	MDS-RS-MLD	K700E	38.8	BM	77	50.3	3.7	1.8	9.9	332	<=2	>15%	46,XX,inv(3)(q21q26.2)[20]	4
113	MDS-RS-MLD	K700E	34.3	PB	71.3	48.1	NA	NA	NA	NA	NA	40	46XX	NA
114	MDS-RS-SLD	K700E	33.5	PB	75	44.7	4.4	2.3	9.1	391	<=2	30	46XX	2
115	MDS-RS-SLD	K700E	38.0	PB	71.1	53.5	6.3	3.8	9.1	373	<=2	>15	47,XX,+8[13]/46,XX[7]	3
116	MDS-RS-MLD	K700E	26.8	PB	69.7	38.4	3.4	1.3	9.5	384	<=2	40	46XX	2
117	MDS-RS-MLD	K700E	33.7	PB	80.7	41.8	4.5	2.5	9.1	220	<=2	50	46XX	2
118	MDS-RS-MLD	K700E	15.5	PB	63.1	24.5	4.2	1.8	9.4	177	<=2	50	46XX	2
119	MDS-RS-MLD	K700E	43.1	BM	85	50.7	NA	NA	NA	NA	<=2	50	del(20q)	NA
120	MDS-RS-SLD	K700E	NA	PB	73.4	NA	5.2	3.2	10.8	239	<=2	>15	46XY	1
121	MDS-RS-SLD	K700E	18.0	PB	64.7	27.8	7.3	4.4	10.7	244	<=2	>15	46XY	1
122	MDS-RS-SLD	K700E	33.6	PB	64.4	52.2	7.3	3.5	11.9	221	<=2	>15	46XY	1
123	MDS-RS-MLD	K700E	17.9	PB	55.1	32.5	3.5	1.5	10.7	322	<=2	5	46XX	1
124	MDS-RS-SLD	K700E	23.3	PB	62.4	37.4	5.1	2.1	10.4	315	<=2	15	46XY	1
125	MDS-RS-MLD	K700E	25.3	BM	49	51.6	5.8	3.5	6.3	65	<=2	>15	46XX	3
126	MDS-RS-MLD	K700E	38.5	PB	90	42.8	3.1	1	9.2	370	<=2	>15	47,XX,+8[19]/46,XX[1]	3
127	MDS-RS-SLD	K700E	23.7	PB	55.2	43.0	4.5	1.9	12.2	283	<=2	NA	46XY	1
128	MDS-EB-1	K700E	12.2	BM	55	22.2	2.8	1.5	12.3	233	5	0	46,XX,der(3)t(3;5)(q12;q31),der(5)del(5)(q13q31)t(3;5)(q12;q31)[17]/46,XX[3]	3
129	MDS-RS-SLD	K700E	41.4	PB	83.6	49.5	4.6	2.806	9.8	306	<=2	20	46XX	2
130	MDS-RS-MLD	K700E	13.7	BM	52	26.3	2.1	1.3	9.4	90	<=2	20	46XY	2.5
131	MDS-RS-MLD	K700E	30.4	PB	59.2	51.4	3.6	1.8	9.7	218	<=2	20	46XX	2
132	MDS-RS-MLD	K666T	25.4	PB	NA	NA	2.3	1.6	7.3	83	<=2	>15	46XY	3
133	MDS-RS-SLD	K666R	37.0	PB	74.9	49.4	9.9	5.8	9.8	355	<=2	>15	46XY	2
134	MDS-RS-MLD	K666R	NA	BM	92	NA	1.7	1.1	9	41	<=2	>15	46XY	3
135	MDS-RS-MLD	K666N	26.0	PB	64.9	40.1	4.9	2.2	10.2	139	<=2	5	46XY	1
136	MDS-RS-MLD	K666N	14.1	BM	81	17.4	3.7	2.2	14.2	81	<=2	5	46,XY,del(20)(q11.2q13.1)[10]/46,XY[10]	1.5
137	MDS-RS-MLD	K666N	33.7	BM	67	50.3	2	0.5	7.7	65	4	6	46XY	4.5
138	MDS-RS-MLD	K666N	37.9	PB	84	45.2	9.4	5.9	8.2	122	2	>15	46XY	2.5
139	MDS-EB-2	K666N	40.1	BM	65	61.8	4.7	2.4	11.4	34	15	10	46,XY,del(20)(q11.2q13.3)[20]	5
140	MDS-RS-MLD	K666N	47.1	BM	NA	NA	2.1	1.3	7.3	26	<=2	>15	46XX	3.5
141	MDS-MLD	K666N	39.4	PB	74.3	53.0	3.9	1.4	10.1	91	3	0	46XX	2.5
142	MDS-EB-1	K666N	15.8	BM	66	24.0	3.9	0.7	11.8	103	5	0	46XY	3.5
143	MDS-EB-1	K666N	28.2	PB	78	36.1	1.7	0.8	6.7	56	5	NA	46XY, ider(20) (q10)del(20)(q11.2q13.3) [5] 47,idem,+der(2) (q10)del(20)(q11.2q13.3) [10] 46XY [5]	8
144	MDS-RS-MLD	K666N	15.8	PB	85.2	18.5	3.8	2.6	12.2	114	<=2	5	NA	NA
145	MDS-RS-MLD	K666M	41.7	PB	84.2	49.6	4.5	2.7	11.3	52	<=2	>15	46XY	1.5
146	MDS-EB-1	T663I	39.0	PB	79.2	49.3	7.8	4.7	8.2	477	5	11	46XY	4
147	MDS-RS-SLD	H662Q	28.0	PB	79.8	35.1	8.9	6.4	9.8	332	<=2	45	46XX	2
148	MDS-RS-MLD	H662Q	25.5	PB	49.9	51.2	3.1	1.1	9.1	188	<=2	15	46XX	2
149	MDS-RS-MLD	H662Q	27.3	PB	59.1	46.2	3.2	0.8	7.7	318	<=2	15	46XX	2.5
150	MDS-RS-MLD	W658S	45.3	BM	92	49.3	1.9	0.3	10.5	175	<=2	>15	46XY	1.5
151	MDS-RS-MLD	N626D	41.6	PB	75.5	55.1	2.8	1.6	8.3	143	2	30	46XY	2
152	MDS-RS-MLD	R625L	24.5	PB	57.1	43.0	4.9	2.7	7.3	196	<=2	5	46XY	2.5
153	MDS-RS-SLD	R625L	30.2	PB	69.7	43.4	NA	NA	NA	NA	<=2	>15	46,XY,+8	NA
154	MDS-RS-SLD	R625G	38.7	PB	86.1	44.9	4.3	2.3	11.1	215	<=2	>15	46XY	1
155	MDS with isolated del(5q)	R625C	14.6	PB	68.5	21.3	2.5	1.4	9.6	180	<=2	0	46,XX,del(5)(q15q33)[7]/46,XX[14]	2
156	MDS-SLD	R625C	30.2	PB	68.7	43.9	4.1	1.3	9.1	118	<=2	NA	46XY	2
157	MDS-RS-MLD	R625C	26.2	PB	60.2	43.5	6.1	3.3	10.3	319	<=2	>15	46XY	1
158	MDS-RS-MLD	R625C	32.7	BM	93	35.2	3.9	2	9	177	<=2	>15	46XX	2
159	MDS-RS-MLD	Y623C	20.2	PB	64.6	31.2	1.2	0.7	12.1	95	4	5	46,XX,del(2)(q11.2q21),del(3)(p21),-7,del(7)(q22),inv(9)(p12q13),add(20)(q13.1),add(21)(q22),+mar[2]/46,XX[18]	6
160	NA	E622D	33.7	PB	63.2	53.3	9.7	5.9	9.7	639	NA	NA	NA	NA
161	MDS-RS-MLD	E622D	38.1	PB	86	44.3	5.3	2.9	11.4	475	<=2	>15	46XY	1
162	MDS-RS-MLD	E622D	28.3	PB	58	48.7	5.4	2.29	8.7	333	2	5	46XY	2
163	MDS-RS-MLD	E622D	35.0	BM	85	41.2	6.2	3.5	8.4	446	<=2	10	46XY	2

Table S2. Cohort 2 of SF3B1-mutant MDS. World Health Organization (WHO) classification, white blood count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb), platelets (PLT), bone marrow blasts, ring sideroblasts (RS), cytogenetics, and Revised International Prognostic Scoring System (IPSS-R) at time of diagnosis are shown. NA = not available.

UPN	WHO	SF3B1	WBC	ANC	Hb	PLT	Blasts	RS	Cytogenetics	IPSS-R
201	MDS-RS-SLD	A744Y	9	5.5	10.9	436	<=2	35	46XX	1
202	MDS-RS-MLD	A744P	11.2	8.3	6.5	157	<=2	>15	46XY	2.5
203	MDS-MLD	G740E	NA	NA	NA	NA	<=2	<5	NA	NA
204	MDS-RS-MLD	G740E	3.2	0.85	10.9	211	<=2	10	46XX	1
205	MDS-RS-MLD	K700E	5.5	3.025	8.9	144	<=2	>15	46XY	2
206	MDS-RS-MLD	K700E	3.4	1.87	9.1	218	<=2	50	46XX	2
207	MDS-RS-SLD	K700E	7.4	4.07	10.7	149	<=2	>10	46XX	1
208	MDS-RS-MLD	K700E	NA	NA	NA	NA	<=2	>15	NA	NA
209	MDS-RS-SLD	K700E	6.6	3.5	7.2	425	<=2	22	45X,-Y[18],XY[2]	1.5
210	MDS with isolated del(5q)	K700E	6.1	3.355	10.9	180	<=2	>15	46,XX,del(5)(q22q35)[12]/46,XX[8]	1
211	MDS with isolated del(5q)	K700E	NA	NA	NA	NA	<=2	>15	46,XY,del(5)(q22q35)[11]/46,XY[9]	NA
212	MDS-RS-MLD	K700E	4.8	2.64	8.7	319	4	>15	46XX	3
213	MDS-EB-1	K700E	9.6	5.28	9	98	7	NA	46XY	4.5
214	MDS-RS-MLD	K700E	3.8	2.09	7.9	102	<=2	50	46,XY,t(11;12)(q23;q13)[20]	3
215	MDS-MLD	K700E	3.9	2.145	12.4	90	3	<5	46,XY,inv(3)(q21q26.2)[10]/46,XY[10]	3.5
216	MDS with isolated del(5q)	K700E	6.4	3.52	8.6	473	<=2	>15	46,XX,del(5)(q13q33)[20]	2
217	MDS-RS-MLD	K700E	2.2	1.21	5.9	93	<=2	35	46XX,del(20)(q11.2q13.1)[20]	3
218	MDS-RS-SLD	K700E	7.5	4.7	8	406	<=2	>15	46,XX	2
219	MDS-EB-1	K700E	1.6	0.48	7.97	96	6	<5	46,XY,del(11)(q13q23)[11]/46-48,XY,idem,del(20)(q11.2q13.1),+mar[cp8]/46,XY[1]	6.5
220	MDS-RS-MLD	K700E	4.4	2.1	8.4	268	<=2	>15	46XX	2
221	MDS-RS	K700E	6.4	3.2	9.7	459	<=2	70	46XY	1
222	MDS-EB1	K700E	3	1.2	9.7	86	5	NA	46,XX,del(5)(q31q33)[11]/46,XX[9]	3.5
223	MDS-RS-MLD	K700E	7.3	3.4	9.2	489	<=2	50	46XY	2
224	MDS-RS-MLD	K700E	3.7	1.5	9.2	225	<=2	50	46XY	2
225	MDS-RS-MLD	K666T	11.7	10.2	6.8	5	<=2	5	45,X,-Y[3]/46,XY[17]	2.5
226	MDS-SLD	K666T	NA	NA	10.1	NA	<=2	NA	46XY	NA
227	MDS-SLD	K666T	NA	NA	NA	NA	<=2	NA	46XX	NA
228	MDS-RS-MLD	K666R	4.73	2.7	8.5	489	<=2	23	46XX	2
229	MDS-RS-SLD	K666R	6.7	4.1	10.9	288	<=2	0	46XY	1
230	MDS-RS-MLD	K666R	2.83	1.5	9.5	227	<=2	>50	46XY	1
231	MDS-EB-1	K666N	4.1	2	9.4	107	5	>15	44~47,XY,add(1)(p23),-4,add(4)(q31),del(5)(q31q35),del(6)(q15q21),-7,+8,+11,add(11)(q23),der(11)add(11)(p15)add(11)(q23),-18,add(21)(q22),+1~2mar[cp15]/46,XY[5]	7
232	MDS-EB-2	K666N	3.4	1.3	8.6	167	15	0	45,XX,t(3;3)(q21;q26.2),-7[15]/46,XX[5]	7
233	MDS-MLD	K666N	5.6	2.1	9	92	4	0	46XY	3.5
234	MDS-EB-2	K666N	10.2	2.7	11.5	77	15	<5	46XY	4.5
235	MDS-RS-MLD	K666M	8.1	5	8.5	83	<=2	>10	46XY	1.5
236	MDS-RS-SLD	H662Y	5.5	3.2	8.8	303	<=2	>15	46XY	2
237	MDS-RS-SLD	H662Y	7.9	4.345	11.5	295	<=2	40	46XY	1
238	MDS-RS-SLD	H662Q	8.09	4.4495	10	259	<=2	>15	46XY	1
239	MDS-RS	H662Q	NA	NA	8.9	NA	<=2	NA	46XY	NA
240	MDS-RS-MLD	H662D	3.9	2.18	8.1	121	3	30	46XY	3
241	MDS-MLD	I641V	3.1	1.4	12.4	107	<=2	0	del20q	1
242	MDS-RS-SLD	R625L	8.4	4.9	9.1	352	<=2	5-15	45,X,-Y[15]/46,XY[5]	1
243	MDS-RS-MLD	R625C	3.8	2.3	10.4	259	<=2	>!5	46XX	1
244	MDS-MLD	R625C	NA	NA	NA	NA	<=2	NA	46XY	NA
245	MDS-RS-SLD	R625C	5.2	2.6	10.9	226	<=2	>15	46XY	1
246	MDS with isolated del(5q)	R625C	6.2	4.5	8.2	61	<=2	0	46,XX,del(5)(q13q33)[10]/46,XX[10]	2.5
247	MDS-RS-SLD	E622D	11.1	5.4	10	430	<=2	>15	46XY	1
248	MDS-RS-MLD	E622D	NA	NA	NA	NA	<=2	50	46XY	NA
249	MDS-EB-1	E592K	5.7	4.1	9.1	40	7	0	46XX	5
250	MDS-MLD	E592K	2.3	1.4	9.2	79	3	0	46XX	3.5

Table S3. Cohort 3 of SF3B1-mutant MDS. World Health Organization (WHO) classification, white blood count (WBC), hemoglobin (Hb), platelets (PLT), and Revised International Prognostic Scoring System (IPSS-R) risk category at time of diagnosis are shown.

UPN	WHO	SF3B1	WBC	Hb	PLT	IPSS-R
301	MDS-MLD	D907Y	2.4	7.7	23	Intermediate
302	MDS-MLD	I787S	4.3	10.5	83	Intermediate
303	MDS-MLD	G742D	1.6	7.4	29	Very high
304	MDS-MLD-RS	G742D	3.6	7.2	358	High
305	MDS-SLD-RS	G740E	5.9	8.3	95	Intermediate
306	MDS-RS-SLD	G740E	4.7	5.8	381	Intermediate
307	MDS-EB-2	I704N	8.2	7.5	70	High
308	MDS-MLD-RS	K700E	5.2	6.9	14	High
309	MDS-MLD	K700E	9.4	6.5	21	Very high
310	MDS-EB-2	K700E	3.5	4.6	21	Very high
311	MDS-EB-1	K700E	2.8	7.4	23	High
312	MDS-SLD-RS	K700E	2.4	8.7	26	Low
313	MDS-MLD	K700E	4.4	5.8	30	Intermediate
314	MDS-MLD	K700E	2.8	8	39	Low
315	MDS-MLD	K700E	7.7	7.7	48	Very high
316	MDS-EB1	K700E	6.8	5.8	49	Intermediate
317	MDS-EB-2	K700E	6.0	9.5	50	High
318	MDS-EB-1	K700E	7.1	5	50	High
319	MDS-MLD	K700E	1.8	7.6	52	High
320	MDS-MLD	K700E	4.2	6.2	52	Intermediate
321	MDS-MLD	K700E	1.5	6.9	55	High
322	MDS-MLD	K700E	2.2	7.3	63	Low
323	MDS-MLD	K700E	5.4	11.1	65	Very low
324	MDS-MLD	K700E	2.2	4.6	67	Low
325	MDS-MLD-RS	K700E	9.0	6.4	69	Intermediate
326	MDS-MLD	K700E	3.4	4.7	70	Low
327	MDS-MLD-RS	K700E	1.6	7.1	70	High
328	MDS-EB-2	K700E	2.7	5.2	75	Very high
329	MDS-MLD-RS	K700E	1.4	8	80	Low
330	MDS-MLD-RS	K700E	2.4	6.1	94	Low
331	MDS-EB2	K700E	2.9	6.6	99	Very high
332	MDS-EB1	K700E	2.22	8.8	104	Intermediate
333	MDS-MLD	K700E/K666N	2.7	6.5	108	Low
334	MDS-MLD	K700E	3.4	8.6	110	Low
335	MDS-MLD	K700E	5.6	9.2	114	Low
336	MDS-SLD-RS	K700E	3.2	9.4	115	Intermediate
337	MDS-MLD	K700E	2.1	10.4	118	Low
338	MDS-MLD-RS	K700E	1.6	6.6	122	Low
339	MDS-EB-2	K700E	2.9	5.1	130	High
340	MDS-RS-MLD	K700E	3.0	8.6	130	Low
341	MDS-MLD-RS	K700E	3.1	7.3	135	Low
342	MDS-SLD-RS	K700E	2.1	7.3	136	Intermediate
343	MDS-SLD-RS	K700E	3.2	6.6	139	Low
344	MDS-RS-MLD	K700E	7.0	8.5	140	Low
345	MDS-RS-SLD	K700E	3.3	9	146	Low
346	MDS-RS-SLD	K700E	4.0	5.7	149	Intermediate
347	MDS-RS-SLD	K700E	12.7	9.1	150	Low
348	MDS-MLD-RS	K700E	1.5	6	155	High

349	MDS-MLD	K700E	5.8	6.1	157	Intermediate
350	MDS-SLD-RS	K700E	4.08	6	159	Low
351	MDS-SLD-RS	K700E	3.0	7	169	Low
352	MDS-MLD	K700E	6.7	7.6	170	Low
353	MDS-SLD-RS	K700E	2.9	7.4	173	Intermediate
354	MDS-MLD	K700E	2.2	5.3	188	Low
355	MDS-MLD	K700E	2.9	9.2	189	Low
356	MDS-RS-MLD	K700E	3.5	4.8	196	Intermediate
357	MDS-MLD-RS	K700E	3.2	8.7	218	Low
358	MDS-EB-1	K700E	1.9	9.4	219	Intermediate
359	MDS-SLD-RS	K700E	3.4	9.6	233	Low
360	MDS-RS-SLD	K700E	4.6	8.2	235	Low
361	MDS-RS-SLD	K700E	5.4	8.5	242	Intermediate
362	MDS-RS-SLD	K700E	5.7	9	244	Very low
363	MDS-SLD-RS	K700E	4.3	11.1	251	Very low
364	MDS-MLD	K700E	2.4	7.8	262	Low
365	MDS-MLD	K700E	4.7	7.1	278	Low
366	MDS-EB2	K700E	2.4	9.4	301	Very high
367	MDS-RS-SLD	K700E	3.6	7.4	307	Low
368	MDS-RS-MLD	K700E	3.1	7.4	332	Intermediate
369	MDS-MLD-RS	K700E	4.3	6.9	349	Low
370	MDS-MLD	K700E	3.4	6.3	352	Intermediate
371	MDS-MLD	K700E	3.9	5.3	357	Low
372	MDS-RS-SLD	K700E	3.2	7.3	365	Intermediate
373	MDS-MLD	K700E	2.7	4	378	Low
374	MDS-RS-SLD	K700E	5.8	8.1	421	Low
375	MDS-MLD-RS	K666T	8.5	10.6	48	Low
376	MDS-EB1	K666T	6.4	7	52	High
377	MDS-MLD	K666T	0.9	4.8	73	Intermediate
378	MDS-RS-SLD	K666R	18.2	7.2	164	Intermediate
379	MDS-EB1	K666Q	5.4	8.3	276	Intermediate
380	MDS-EB-2	K666N	1.9	6.5	10	Very high
381	MDS-EB-1	K666N	1.8	9.4	36	High
382	MDS-EB1	K666N	6.4	9.2	78	High
383	MDS-EB1	K666N	1.8	10.5	87	Intermediate
384	MDS-EB2	K666N	7.7	10	90	Intermediate
385	MDS-MLD	K666N	1.3	6.3	140	Low
386	MDS-MLD	K666M	3.6	7.9	94	Intermediate
387	MDS-MLD-RS	K666M	3.78	5.3	367	Intermediate
388	MDS-EB2	K666E	1.7	7.4	41	Very high
389	MDS-EB-2	K666E	1.8	12.6	171	Intermediate
390	MDS-MLD	H662Q	2.2	5.9	105	Very high
391	MDS-EB-1	H662Q	2.1	6.7	350	High
392	MDS-MLD	H662D	1.8	7.7	156	Low
393	MDS-SLD-RS	H662D	2.1	8	190	Low
394	MDS-MLD	R625L	3.4	8.4	211	Low
395	MDS-MLD	R625H	3.6	9.6	77	Low
396	MDS-MLD-RS	R625G	3.38	6.9	244	Low
397	MDS-MLD-RS	R625C	3.9	4.1	166	Low
398	MDS-MLD-RS	R625C	3.4	7.1	295	Low
399	MDS-MLD	R625C	2.6	8.1	306	Low
400	MDS-RS-SLD	R625C	6.4	7.3	340	Intermediate
401	MDS-MLD	E592K	2.3	7.8	22	Intermediate

Table S4. Clonal architecture of MDS cases with SF3B1 K666N and SRSF2 mutations. Variant allele frequencies (VAFs) and non-lymphoid-adjusted VAFs show that both SF3B1 and SRSF2 mutations are of high abundance and likely present in the same cells.

UPN	WHO	Gene	Variant	VAF	Nonlymph VAF
135	MDS-RS-MLD	ASXL1	P1377Sfs*3	33	51
		SETBP1	G870S	10	15
		SF3B1	K666N	26	40
		SRSF2	P95L	24	37
		TET2	S585*	6	9
		TET2	N582Kfs*53	12	18
137	MDS-RS-MLD	BCOR	K839fs	21.98	33
		SF3B1	K666N	33.7	50
		SRSF2	P95H	24.52	37
		STAG2	L864*	35.4	53

Supplemental references

1. Woll PS, Kjällquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. *Cancer cell*. 2014;25(6):794–808.

2. Montalban-Bravo G, Takahashi K, Patel K, et al. Impact of the number of mutations in survival and response outcomes to hypomethylating agents in patients with myelodysplastic syndromes or myelodysplastic/myeloproliferative neoplasms. *Oncotarget*. 2018;9(11):9714–9727.

3. Gentien D, Kosmider O, Nguyen-Khac F, et al. A common alternative splicing signature is associated with SF3B1 mutations in malignancies from different cell lineages. *Leukemia*. 2014;28(6):1355.

4. Kim T, Tyndel M, Kim H, et al. The clonal origins of leukemic progression of myelodysplasia. *Leukemia*. 2017;31(9):1928–1935.

5. Ali A, Penneroux J, Bello R, et al. Granulomonocytic progenitors are key target cells of azacytidine in higher risk myelodysplastic syndromes and acute myeloid leukemia. *Leukemia*. 2018;32(8):1856–1860.

6. Martin R, Acha P, Ganster C, et al. Targeted deep sequencing of CD34+ cells from peripheral blood can reproduce bone marrow molecular profile in myelodysplastic syndromes. *American journal of hematology*. 2018;93(6):E152–E154.

7. Rouault-Pierre K, Mian S, Goulard M, et al. Preclinical modeling of myelodysplastic syndromes. *Leukemia*. 2017;31(12):2702.

8. Mizuta S, Yamane N, Komai T, et al. Evaluation of SF3B1 Mutation Screening by High-Resolution Melting Analysis and its Clinical Utility for Myelodysplastic Syndrome with Ring Sideroblasts at the Point of Diagnosis. *Laboratory medicine*. 2018;

9. Li B, Liu J, Jia Y, et al. Clinical features and biological implications of different U2AF1 mutation types in myelodysplastic syndromes. *Genes, Chromosomes and Cancer*. 2018;57(2):80–88.

10. Martín I, Such E, Navarro B, et al. Prognostic impact of gene mutations in myelodysplastic syndromes with ring sideroblasts. *Blood Cancer Journal*. 2017;7(12):630.

11. Duncavage EJ, Jacoby MA, Chang G, et al. Mutation Clearance after Transplantation for Myelodysplastic Syndrome. *New England Journal of Medicine*. 2018;379(11):1028–1041.

12. Montes P, Kerick M, Bernal M, et al. Genomic loss of HLA alleles may affect the clinical outcome in low-risk myelodysplastic syndrome patients. *Oncotarget*. 2018;9(97):36929–36944.

13. Ribeiro A, Coucelo M, Tenreiro R, et al. Clonal shifts in MDS - from SF3B1 to EZH2. *Leukemia & lymphoma*. 2018;1–4.

14. Stosch JM, Heumüller A, Niemöller C, et al. Gene mutations and clonal architecture in myelodysplastic syndromes and changes upon progression to acute myeloid leukaemia and under treatment. *British Journal of Haematology*. 2018;182(6):830–842.

15. Ali A, Huang Y, Pinheiro R, et al. Severely impaired terminal erythroid differentiation as an independent prognostic marker in myelodysplastic syndromes. *Blood Advances*. 2018;2(12):1393–1402.

16. Taskesen E, Havermans M, Lom K van, et al. Two splice-factor mutant leukemia subgroups uncovered at the boundaries of MDS and AML using combined gene expression and DNA-methylation profiling. *Blood*. 2014;123(21):3327–3335.

17. Xu Y, Li Y, Xu Q, et al. Implications of mutational spectrum in myelodysplastic syndromes based on targeted next-generation sequencing. *Oncotarget*. 2017;8(47):82475–82490.

18. Zhang S-J, Rampal R, Manshouri T, et al. Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. *Blood*. 2012;119(19):4480–4485.

19. Matsuda K, Ishida F, Ito T, et al. Spliceosome-related gene mutations in myelodysplastic syndrome can be used as stable markers for monitoring minimal residual disease during follow-up. *Leukemia Research*. 2012;36(11):1393–1397.

20. Je E, Yoo N, Kim Y, Kim M, Lee S. Mutational analysis of splicing machinery genes SF3B1, U2AF1 and SRSF2 in myelodysplasia and other common tumors. *International Journal of Cancer*. 2013;133(1):260–265.

21. Yang J, Qian J, Yao D, et al. SF3B1 mutation is a rare event in Chinese patients with acute and chronic myeloid leukemia. *Clinical Biochemistry*. 2013;46(7–8):701–703.

22. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506(7488):328.

23. Network C. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *The New England journal of medicine*. 2013;368(22):2059–74.

24. Wong TN, Miller CA, Jotte MR, et al. Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. *Nature Communications*. 2018;9(1):455.

25. Scharenberg C, Giai V, Pellagatti A, et al. Progression in patients with low- and intermediate-1-risk del(5q) myelodysplastic syndromes is predicted by a limited subset of mutations. *Haematologica*. 2016;102(3):498–508.

26. Thol F, Klesse S, Köhler L, et al. Acute myeloid leukemia derived from lympho-myeloid clonal hematopoiesis. *Leukemia*. 2017;31(6):1286–1295.

27. Hirsch P, Zhang Y, Tang R, et al. Genetic hierarchy and temporal variegation in the clonal history of acute myeloid leukaemia. *Nature Communications*. 2016;7(1):12475.

28. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128(5):686–98.

29. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *The New England Journal of Medicine*. 2016;374(23):2209–2221.

30. Madan V, Shyamsunder P, Han L, et al. Comprehensive mutational analysis of primary and relapse acute promyelocytic leukemia. *Leukemia*. 2016;30(8):1672.

31. Hou H-A, Liu C-Y, Kuo Y-Y, et al. Splicing factor mutations predict poor prognosis in patients with de novo acute myeloid leukemia. *Oncotarget*. 2016;7(8):9084–9101.

32. Garg M, Nagata Y, Kanojia D, et al. Profiling of somatic mutations in acute myeloid leukemia with FLT3-ITD at diagnosis and relapse. *Blood*. 2015;126(22):2491–2501.

33. Hsu J, Reilly A, Hayes BJ, et al. Reprogramming identifies functionally distinct stages of clonal evolution in myelodysplastic syndromes. *Blood*. 2019;134(2):186–198.

34. Singhal D, Wee L, Kutyna MM, et al. The mutational burden of therapy-related myeloid neoplasms is similar to primary myelodysplastic syndrome but has a distinctive distribution. *Leukemia*. 2019;

35. Lin C, Hou H, Chou W, et al. SF3B1 mutations in patients with myelodysplastic syndromes: The mutation is stable during disease evolution. *American Journal of Hematology*. 2014;89(8):E109–E115.

36. Visconte V, Tabarroki A, Gerace CJ, et al. Screening for SF3B1 mutations is a useful tool to differentiate between acquired clonal and non-clonal sideroblastic anemia. *Leukemia & Lymphoma*. 2014;56(6):1888–1890.

37. Makishima H, Yoshida K, Nguyen N, et al. Somatic SETBP1 mutations in myeloid malignancies. *Nature Genetics*. 2013;45(8):942–946.

38. Yang J, Qian J, Lin J, et al. Development of a High-Resolution Melting Analysis for the Detection of theSF3B1Mutations. *Genetic Testing and Molecular Biomarkers*. 2013;17(4):342–347.

39. Donaires F, Martelli F, Alves-Paiva R de, et al. Splicing factor SF3B1 mutations and ring sideroblasts in myelodysplastic syndromes: a Brazilian cohort screening study. *Revista Brasileira de Hematologia e Hemoterapia*. 2016;38(4):320–324.

40. Bartels S, Schipper E, Hasemeier B, Kreipe H, Lehmann U. Routine clinical mutation profiling using next generation sequencing and a customized gene panel improves diagnostic precision in myeloid neoplasms. *Oncotarget*. 2016;7(21):30084–93.

41. Ohba R, Furuyama K, Yoshida K, et al. Clinical and genetic characteristics of congenital sideroblastic anemia: comparison with myelodysplastic syndrome with ring sideroblast (MDS-RS). *Annals of Hematology*. 2013;92(1):1–9.

42. Nikpour M, Scharenberg C, Liu A, et al. The transporter ABCB7 is a mediator of the phenotype of acquired refractory anemia with ring sideroblasts. *Leukemia*. 2013;27(4):889.

43. Traina F, Visconte V, Elson P, et al. Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. *Leukemia*. 2014;28(1):78.

44. Martín I, Such E, Navarro B, et al. Negative impact on clinical outcome of the mutational co-occurrence of SF3B1 and DNMT3A in refractory anemia with ring sideroblasts (RARS). *Leukemia & Lymphoma*. 2016;58(7):1–8.

45. Seo J, Lee K-O, Kim S-H, et al. Clinical significance of SF3B1 mutations in Korean patients with myelodysplastic syndromes and myelodysplasia/myeloproliferative neoplasms with ring sideroblasts. *Annals of Hematology*. 2014;93(4):603–608.

46. Xu L, Gu Z-HH, Li Y, et al. Genomic landscape of CD34+ hematopoietic cells in myelodysplastic syndrome and gene mutation profiles as prognostic markers. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(23):8589–94.

47. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2011;118(24):6239–6246.

48. Visconte V, Makishima H, Jankowska A, et al. SF3B1, a splicing factor is frequently mutated in refractory anemia with ring sideroblasts. *Leukemia*. 2011;26(3):leu2011232.

49. Patnaik MM, Lasho TL, Hodnefield JM, et al. SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. *Blood*. 2012;119(2):569–572.

50. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *The New England journal of medicine*. 2011;365(15):1384–95.

51. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64–9.

52. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616–27; quiz 3699.

53. Chesnais V, Renneville A, Toma A, et al. Effect of lenalidomide treatment on clonal architecture of myelodysplastic syndromes without 5q deletion. *Blood*. 2016;127(6):749–60.

54. Lindsley R, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–76.

55. Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. *Nature*. 2018;1–6.

56. Dunlap JB, Leonard J, Rosenberg M, et al. The combination of NPM1, DNMT3A, and IDH1/2 mutations leads to inferior overall survival in AML. *American journal of hematology*. 2019;94(8):913–920.

57. Pellagatti A, Armstrong RN, Steeples V, et al. Impact of spliceosome mutations on RNA splicing in myelodysplasia: dysregulated genes/pathways and clinical associations. *Blood*. 2018;132(12):1225–1240.

58. Ohgami RS, Ma L, Merker JD, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. *Modern Pathology*. 2015;28(5):706.

59. Gröschel S, Sanders MA, Hoogenboezem R, et al. Mutational spectrum of myeloid malignancies with inv(3)/t(3;3) reveals a predominant involvement of RAS/RTK signaling pathways. *Blood*. 2015;125(1):133–9.

60. Cho Y-UU, Jang S, Seo E-JJ, et al. Preferential occurrence of spliceosome mutations in acute myeloid leukemia with preceding myelodysplastic syndrome and/or myelodysplasia morphology. *Leukemia & lymphoma*. 2015;56(8):2301–8.

61. Mian SA, Smith AE, Kulasekararaj AG, et al. Spliceosome mutations exhibit specific associations with epigenetic modifiers and proto-oncogenes mutated in myelodysplastic syndrome. *Haematologica*. 2013;98(7):1058–1066.

62. Makishima H, Visconte V, Sakaguchi H, et al. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood*. 2012;119(14):3203–10.

63. Eisfeld A-KK, Kohlschmidt J, Mrózek K, et al. Mutational Landscape and Gene Expression Patterns in Adult Acute Myeloid Leukemias with Monosomy 7 as a Sole Abnormality. *Cancer research*. 2017;77(1):207–218.

64. Mossner M, Jann J-CC, Wittig J, et al. Mutational hierarchies in myelodysplastic syndromes dynamically adapt and evolve upon therapy response and failure. *Blood*. 2016;128(9):1246–59.

65. Silva-Coelho P da, Kroeze LI, Yoshida K, et al. Clonal evolution in myelodysplastic syndromes. *Nature Communications*. 2017;8:15099.

66. Mortera-Blanco T, Dimitriou M, Woll PS, et al. SF3B1-initiating mutations in MDS-RSs target lymphomyeloid hematopoietic stem cells. *Blood*. 2017;130(7):881–890.

67. Yoshimi A, Lin K-T, Wiseman DH, et al. Coordinated alterations in RNA splicing and epigenetic regulation drive leukaemogenesis. *Nature*. 2019;1–5.

68. Jaiswal S, Fontanillas P, Flannick J, et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *The New England Journal of Medicine*. 2014;371(26):.

69. Genovese G, Kähler AK, Handsaker RE, et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *New England Journal of Medicine*. 2014;371(26):2477–2487.

70. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *New Engl J Medicine*. 2017;377(2):111–121.

71. Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nature Medicine*. 2018;24(7):1015–1023.

72. Frick M, Chan W, Arends CM, et al. Role of Donor Clonal Hematopoiesis in Allogeneic Hematopoietic Stem-Cell Transplantation. *J Clin Oncol*. 2019;37(5):375–385.

73. Dorsheimer L, Assmus B, Rasper T, et al. Hematopoietic alterations in chronic heart failure patients by somatic mutations leading to clonal hematopoiesis. *Haematologica*. 2019;haematol.2019.224402.

74. Coombs CC, Gillis NK, Tan X, et al. Identification of clonal hematopoiesis mutations in solid tumor patients undergoing unpaired next-generation sequencing assays. *Clinical Cancer Research*. 2018;24(23):clincanres.1201.2018.

75. Ptashkin RN, Mandelker DL, Coombs CC, et al. Prevalence of Clonal Hematopoiesis Mutations in Tumor-Only Clinical Genomic Profiling of Solid Tumors. *JAMA Oncology*. 2018;

76. Arends C, Galan-Sousa J, Hoyer K, et al. Hematopoietic lineage distribution and evolutionary dynamics of clonal hematopoiesis. *Leukemia*. 2018;32(9):1908–1919.

77. Coombs CC, Zehir A, Devlin SM, et al. Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell stem cell*. 2017;21(3):374-382.e4.

78. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood*. 2017;130(6):742–752.

79. Acuna-Hidalgo R, Sengul H, Steehouwer M, et al. Ultra-sensitive Sequencing Identifies High Prevalence of Clonal Hematopoiesis-Associated Mutations throughout Adult Life. *Am J Hum Genetics*. 2017;101(1):50–64.

80. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal Hematopoiesis Associated With Adverse Outcomes After Autologous Stem-Cell Transplantation for Lymphoma. *J Clin Oncol*. 2017;35(14):JCO.2016.71.671.

81. McKerrell T, Park N, Moreno T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell reports*. 2015;10(8):1239–1245.

82. Razavi P, Li BT, Brown DN, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat Med*. 2019;25(12):1928–1937.

83. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nature Medicine*. 2014;20(12):1472.

84. Abelson S, Collord G, Ng SW, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. *Nature*. 2018;1.

85. Young AL, Tong RS, Birmann BM, Druley TE. Clonal haematopoiesis and risk of acute myeloid leukemia. *Haematologica*. 2019;haematol.2018.215269.

86. Hansen JW, Pedersen DA, Larsen LA, et al. Clonal hematopoiesis in elderly twins: concordance, discordance and mortality. *Blood*. 2019;

87. Fabre MA, McKerrell T, Zwiebel M, et al. Concordance for clonal hematopoiesis is limited in elderly twins. *Blood*. 2019;

88. Boucai L, Falcone J, Ukena J, et al. Radioactive Iodine–Related Clonal Hematopoiesis in Thyroid Cancer Is Common and Associated With Decreased Survival. *J Clin Endocrinol Metabolism*. 2018;103(11):4216–4223.