Supplementary document

Sørensen *et al.*, Clonal Hematopoiesis Predicts Development of Therapy-related Myeloid Neoplasms post-Autologous Stem Cell Transplantation

Supplementary Methods

Matching
All controls had as long, or longer follow-up than their respective case with the exception of 2 patients who had 18 and 337 days longer latency than their respective case. In these 2 instances, it was deemed to have negligible influence as both controls had more than 10 years of total follow-up after ASCT before the date of censoring. For 9 patients it was not possible to match age within a frame of 5 years (primarily due to follow-up time having priority). For two cases, the age at ASCT was 29 years, and due to a limited number of patients in this age group, an older control was chosen. For the 7 remaining patients, 2 of the controls chosen were more than 5 years younger than their respective case at ASCT. One patient, 1206, did not have available data from the ASCT treatment, and was matched with a control that received BEAM conditioning.

Flow cytometry
In the gating strategy used (Supplemental Figure 2), live cells were identified in a forward scatter (FSC) versus side scatter (SSC) plot after which doublet exclusion was performed using a FSC height versus area plot. In a SSC versus CD45 expression plot we defined a $CD45^{low}/SSC^{low}$ "blast gate". As monocytes may be located in close proximity to the "blast gate", we used CD14 to exclude potential monocyte contamination. Within the $SSC^{low}CD45^{low}CD14^{-}$ population, we identified $CD34^{+}CD38^{-}$ stem cells based on nucleated erythroid cells as internal negative control. This gating strategy was applied to avoid contamination of the stem cell gate by inclusion of $CD38^{dim}$ progenitor cells. The stem cell populations ($SSC^{low}CD45^{low}CD34^{+}CD38^{-}$) were evaluated for aberrant positive expression of CLEC12A, CD7, CD19 and CD123 in order to assess the presence of potential LSCs.

Myeloid next-generation sequencing panel
The individual genes were sorted in different categories based on their function within the cell as reported in the Genetics Home Reference by the National Institute of Health (11).

Variant calling
Cutoff values for variant calling were determined based on analysis of data from .vcf files from all NGS analyses performed (Supplemental Figure 2). Most observations were located at frequencies < 0.5%, indicating a high number of false positive variants within this range of frequencies, primarily located at frequencies lower than 0.2% (Supplemental Figure 2D). Of all observations with VAF under 0.5%, just 1% of observations were located at an allele frequency $\geq$ 0.3%, and thus, this was chosen as the cutoff VAF in this study. It remains possible that false positive observations are present within the 1% low frequency mutations located at allele frequencies $\geq$ 0.3%, but we expect that nearly all false positive observations have been excluded using this cutoff combined with the read depth cutoff.
The VAF cutoff resulted in exclusion of 1 mutation (*ZRSR2*) that had a VAF 0.1%, but sufficient read depth to be included otherwise. After exclusion, the mutation reported with the lowest VAF in this study had a VAF of 0.5%.

Survival
Survival analysis was performed using the Kaplan-Meier Methods, and hazard ratios were calculated via Cox-Regression analysis. Survival was defined as until death from any cause after being diagnosed with tMN or censoring.

Supplementary Tables

Supplemental Table 1A.

ID	Indication for ASCT	Age at ASCT	Gender	Priming	Induction	Conditioning	Relapse	Cytotoxic treatment post-ASCT	Time to tMN (years)	Age at tMN	tMN Diagnosis	Cytogenetics	Treatment Intention	Survival (days)	Allo-SCT
1174	MM	66	Male	Cyklophosphamid	VRd	HD-Melphalan	Yes	Yes	3.4	69	MDS	Complex	Palliative	105	No
1175	TBCL	68	Male	R-CHOP	R-CHOP	BEAM	No	No	0.8	69	MDS	-7/del7q	Curative	1940*	Yes
1176	MM	56	Male	Cyklophosphamid	Cy-Dex	HD-Melphalan	Yes	No	11.0	67	MDS	Complex	Palliative	266	No
1177	MM	58	Male	Cyklophosphamid	ACVDL	HD-Melphalan	Yes	Yes	7.4	65	AML	nd.	Palliative	2	No
1178	TBCL	68	Male	CHOP	R-DHAP	BEAM	No	No	0.7	69	MDS	Normal	Palliative	2784*	No
1179	MCL	65	Male	R-araC	R-araC	BEAM	No	No	6.1	71	MPN	Other	Palliative	1218*	No
1180	Hodgkin	66	Male	araC	DHAP	BEAM	No	No	4.2	70	MDS	Complex	Palliative	168	No
1181	MCL	54	Male	R-araC	R-maxi-CHOP + R-araC	BEAM	No	No	1.9	56	AML	Complex	Curative	312	No
1182	MM	57	Male	Cyklophosphamid	Cyklophosphamid	HD-Melphalan	Yes		1.1	58	MDS	Normal	*ND*	227	No
1183	PTCL	29	Female	CHOEP	CHOEP	BEAM	Yes	No	2.8	32	MDS	Normal	Palliative	62	No
1184	MM	60	Male	Cyklophosphamid	VCD	HD-Melphalan	No	No	0.9	61	MDS	Other	Palliative	853*	No
1185	TBCL	69	Male	Rituximab	R-CHOP + methotrexat	TBI-Cyclophosphamide	Yes	Yes	1.1	70	MDS	-7/del7q	Azaciditin	614	No
1186	PTCL	50	Male	DHAP	DHAP	BEAM	Yes	Yes	7.7	58	MDS	Complex	Azaciditin	136	No
1187	MCL	63	Male	Rituximab	R-maxi-CHOP + R-araC	R-BEAM	No	No	3.5	66	AML	Complex	Azaciditin	149	No
1188	TBCL	58	Male	MIME	R-DHAP	TBI-Cyclophosphamide	No	No	0.2	58	MDS	nd.	Palliative	27	No
1189	MCL	64	Male	Rituximab	R-maxi-CHOP + R-araC	BEAM	Yes	Yes	8.3	72	AML	Complex	Azaciditin	565	No
1190	MM	69	Male	Cyclophosphamid	VCD	HD-Melphalan	No	No	3.6	73	AML	Normal	Azaciditin	25	No
1191	MCL	61	Male	Rituximab	R-maxi-CHOP + R-araC	BEAM	Yes	No	1.5	63	MDS	-7/del7q	Curative	908*	Yes
1192	MCL	68	Male	Rituximab	R-maxi-CHOP + R-araC	BEAM	Yes	Yes	4.3	72	MDS	Normal	Palliative	429*	No
1193	DLBCL	68	Female	R-CHOP	R-DHAP	BEAM	Yes	Yes	3.2	71	AML	Complex	Azaciditin	57	No
1194	DLBCL	58	Female	CHOP	R-MIME	BEAM	No	Yes	12.1	70	MDS	Other	Azaciditin	623	No
1195	TCL	59	Male	CHOEP	CHOEP	BEAM	No	No	1.4	60	MDS	Normal	Palliative	924	No
1196	TBCL	40	Female	CHOP	MIME	TBI-Cyclophosphamide	Yes	No	3.6	44	MDS	Other	Curative	494	Yes
1197	DLBCL	55	Male	MIME	MIME	TBI-Cyclophosphamide	No	No	6.7	62	MDS	Complex	Curative	565	No
1198	TCL	37	Female	Cyclophosphamid	CHOEP	BEAM	Yes	Yes	0.7	38	AML	Other	Palliative	58	No
1199	TCL	42	Male	CHOP	CHOP + metotrexat	TBI-Cyclophosphamide	No	No	8.6	51	AML	Complex	Curative	34	No
1200	DLBCL	44	Male	MIME	MIME	BEAM	No	No	5.2	49	MDS	Complex	Curative	328	No
1201	Hodgkin	35	Male	MIME	MIME	TBI-Cyclophosphamide	No	No	9.9	45	MDS	Complex	Curative	176	No
1202	MM	53	Female	Cyclophosphamid	VAD	HD-Melphalan	Yes	Yes	10.9	64	AML	nd.	Palliative	8	No
1204	MM	63	Female	Cyclophosphamid	Cy-Dex	HD-Melphalan	Yes	Yes	7.6	71	MDS	nd.	Palliative	2268*	No
1205	TBCL	33	Male	Mab-Thera CHOP	R-ICE	BEAM	No	No	2.3	35	AML	-7/del7q	Curative	84	No
1206	Seminoma	29	Male	*ND*	*ND*	*ND*	No	No	18.1	47	MDS	Other	Curative	141	No
1207	MCL	54	Male	R-CHOP	R-maxi-CHOP + R-araC	BEAM	No	*ND*	3.6	58	MDS	Complex	Palliative	8	No
1208	MM	59	Male	Cyclophosphamid	VAD	HD-Melphalan	Yes	Yes	2.7	62	MDS	-5/del5q	*ND*	661	*ND*
1209	ALL	54	Male	Cyclophosphamid	CHOP	TBI-Cyclophosphamide	No	No	15.3	69	MDS	Other	Palliative	438	No
1245	MCL	48	Male	AraC	R-maxi-CHOP + R-araC	BEAM	Yes	Yes	5.7	54	MDS	-7/del7q	Curative	914	Yes

Supplemental Table 1A. ***Patient characteristics, cases***. ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; DLBCL: Diffuse Largecell B-Cell Lymphoma; MCL: Mantlecell Lymphoma; MDS: Myelodysplastic Syndrome MM: Multiple Myeloma; MPN: Myeloproliferative Neoplasm; PTCL: Peripheral T-Cell Lymphoma; TBCL: Transformed B-Cell Lymphoma; TCL: T-Cell Lymphoma.
* Alive at study end.

Supplemental Table 1B.

ID	Indication for ASCT	Age at ASCT	Gender	Priming	Induction	Conditioning	Relapse	Cytotoxic treatment post-ASCT	Follow-up (years)
1210	MM	66	Male	Cyclophosphamid	Vd	HD-Melphalan	Yes	Yes	4.3
1211	TBCL	64	Male	R-CHOP	R-CHOP	BEAM	No	No	6.2
1212	MM	58	Male	Cyclophosphamid	Cy-Dex	HD-Melphalan	Yes	No	10.1
1213	MM	60	Male	Cyclophosphamid	ACVDL	HD-Melphalan	Yes	Yes	8.4
1214	DLBCL	64	Male	R-DHAP	R-DHAP	BEAM	No	No	8.4
1215	MCL	63	Male	R-maxi-CHOP + RaraC	R-araC	BEAM	No	No	9.4
1216	Hodgkin	66	Male	DHAP	DHAP	BEAM	No	No	10.0
1217	MCL	58	Male	R-araC	R-maxi-CHOP + R-araC	BEAM	Yes	Yes	5.6
1218	MM	58	Male	Cyclophosphamid	Cy-Dex	HD-Melphalan	Yes	Yes	5.3
1219	PTCL	35	Female	CHOEP	CHOEP	BEAM	No	No	12.0
1220	PTCL	53	Male	CHOEP	CHOEP	BEAM	Yes	Yes	12.7
1221	MCL	60	Male	Rituximab	AraC	BEAM	No	No	12.1
1222	TBCL	57	Male	Rituximab	CHOEP	TBI-Cyclophosphamide	Yes	Yes	4.6
1223	MCL	59	Male	Rituximab	R-maxi-CHOP + R-araC	BEAM	No	No	13.1
1224	MM	60	Male	Cyclophosphamid	VAD	HD-Melphalan	Yes	Yes	13.6
1225	MCL	63	Male	Rituximab	R-maxi-CHOP + R-araC	BEAM	No	No	4.4
1226	MCL	67	Male	Rituximab	R-maxi-CHOP + R-araC	BEAM	No	No	5.7
1227	DLBCL	56	Female	Cyclophosphamid	DHAP	BEAM	No	No	4.5
1228	DLBCL	57	Female	R-MIME	R-MIME	BEAM	Yes	Yes	12.0
1229	TBCL	50	Female	Rituximab	R-CHOP	TBI-Cyclophosphamide	No	No	13.1
1230	TBCL	55	Male	Rituximab	CHOP	TBI-Cyclophosphamide	Yes	Yes	10.1
1231	PTCL	37	Female	CHOP	CHOP + ICE	BEAM	Yes	Yes	2.2
1232	Hodgkin	37	Male	MIME	MIME	TBI-Cyclophosphamide	No	No	16.7
1233	DLBCL	52	Male	Cyclophosphamid	MIME	BEAM	No	Yes	15.6
1234	MM	58	Male	Cyclophosphamid	VAD	HD-Melphalan	Yes	Yes	12.9
1235	MM	62	Male	Cyclophosphamid	VCD	HD-Melphalan	No	No	3.1
1236	TBCL	67	Male	R-CHOP	R-CHOP-14 + 2R	TBI-Cyclophosphamide	No	No	3.8
1237	DLBCL	58	Female	MabThera + MIME	R-MIME	BEAM	Yes	Yes	2.9
1238	MM	63	Female	Cyclophosphamid	CyDex	HD-Melphalan	Yes	Yes	14.6
1239	MCL	62	Male	R-araC	R-maxi-CHOP + R-araC	BEAM	Yes	Yes	8.7
1240	MCL	60	Male	MabThera	R-maxi-CHOP + R-araC	BEAM	Yes	Yes	3.8
1241	Hodgkin	35	Male	MIME	MIME	BEAM	No	No	16.4
1242	MM	65	Male	Cyclophosphamid	CyDex	HD-Melphalan	Yes	Yes	9.6
1243	MCL	48	Male	R-araC	R-maxi-CHOP + R-araC	BEAM	No	No	11.8
1244	Burkitt	56	Male	CHOP	LBLx4 (CHOP + Mtx)	TBI-Cyclophosphamide	No	Yes	14.9
1246	MCL	50	Male	R-araC	R-maxi-CHOP + R-araC	BEAM	No	No	14.6

Supplemental Table 1B. ***Patient characteristics, controls***. DLBCL: Diffuse Largecell B-Cell Lymphoma; MCL: Mantlecell Lymphoma; MM: Multiple Myeloma; PTCL: Peripheral T-Cell Lymphoma; TBCL: Transformed B-Cell Lymphoma

Supplemental Table 2.

Characteristics	**tMDS (n=25)**	**tAML (n=10)**
Age, years (range)	61.6 (31.7 to 72.2)	64.6 (35.3 to 72.5)
Hemoglobin, mmol/l (range)	6.4 (5.1 to 9.5)	5.4 (4.3 to 6.4)
Total Leukocytes, $x10^9$ (range)	3.0 (1.5 to 40.7)	3.3 (0.3 to 60.7)
Neutrophil granulocytes, $x10^9$ (range)	1.44 (0.3 to 25.8)	0.63 (0.1 to 5.8)
Platelets, $x10^9$ (range)	41 (7 to 346)	25 (6 to 322)
Bone marrow blasts at diagnosis, % (range)	0 (0 to 15)	30 (20 to 80)
Karyotype (n)		
Complex	8	5
-7/del7q	4	1
-5/-5delq	1	0
Normal	5	1
Other	5	1
Not done	2	2
Treated with curative intent (n)	8	3
Allogeneic transplantation (n)	4	0

Supplemental Table 2. Patient characteristics at tMN diagnosis.

Supplemental Table 3

	ID	Gene	VAF	Type	Basesub	Depth	c.DNA	Protein Alt	Chromosome
Cases	1175	*TP53*	1.3	Missense	C>T/A>G	8695	659A>G	Tyr220Cys	17
	1177	*TP53*	1.0	Missense	T>C/G>A	6765	711G>A	Met237Ile	17
	1179	*CALR*	40.3	Frameshift	Not SNV	10791	1154_1155insTTGTC	Lys385Asnfs*?	19
		SF3B1	1.6	Missense	G>C	10765	1998G>C	Lys666Asn	2
		HRAS	0.8	Missense	C>T/A>G	10866	106A>G	Ile36Val	11
		ZRSR2	1.9	Frameshift	Not SNV	5267	984_987delTCTT	Leu329Metfs*?	X
		ZRSR2	0.8	Missense	T>C/G>A	5246	986T>C	Leu329Pro	X
	1180	*TP53*	0.6	Missense	T>C/G>A	8927	743G>A	Arg248Gln	17
	1182	*ASXL1*	2.7	Frameshift	Not SNV	10030	1748_1755delGG	Trp583*fs*1	20
	1184	*SF3B1*	14.8	Missense	T>C/G>A	5621	1874G>A	Arg625His	2
	1185	*SRSF2*	0.5	Missense	C>A/T>G	6016	284C>A	Pro95His	17
		ASXL1	1.1	Stop	C>T/A>G	8533	1471C>T	Gln491*	20
		TP53	1.4	Missense	T>C/G>A	6416	734G>A	Gly245Asp	17
	1186	*TP53*	1.4	Missense	T>C/G>A	10182	818G>A	Arg273His	17
	1190	*ASXL1*	30.8	Frameshift	Not SNV	9803	1934dupG	Gly646Trpfs*12	20
		SRSF2	34.8	Missense	C>A/T>G	7762	284C>A	Pro95His	17
	1191	*NRAS*	1.9	Missense	G>C	9421	37G>C	Gly13Arg	1
		JAK2	0.7	Missense	A>C/G>T	9026	1849G>T	Val617Phe	9
	1192	*ASXL1*	1.2	Stop	C>T/A>G	7550	1534C>T	Gln512*	20
	1197	*TP53*	1.0	Missense	T>C/G>A	6960	428T>C	Val143Ala	17
	1198	*ASXL1*	12.1	Stop	C>A/T>G	9874	2066C>A	Ser689*	20
		EZH2	10.8	Frameshift	Not SNV	8633	1123_1124insGA	Ile375Argfs*50	7
		EZH2	11.8	Missense	C>T/A>G	9571	1696C>T	Arg566Cys	7
		WT1	9.5	INDEL	Not SNV	10323	1301_1302insAACACA	Arg434_Ser435insThrHis	11
	1199	*BRAF*	0.9	Missense	C>T/A>G	8303	1781A>G	Asp594Gly	7
	1201	*ASXL1*	47.8	Missense	C>T/A>G	9492	2237C>T	Ala746Val	20
	1207	*TP53*	4.9	Splicesite	C>T/A>G	6374	673-2A>G	n/a	17
		ZRSR2	1.1	Missense	T>C/G>A	4959	560G>A	Cys187Tyr	X
	1245	*TP53*	0.6	Missense	C>A/T>G	8109	464C>A	Thr155Asn	17
		ZRSR2	99.2	INDEL	Not SNV	4540	1338_1343dupGAGCCG	Ser447_Arg448dup	X
Controls	1213	*EZH2*	49.8	INDEL	Not SNV	10016	552_554delTGA	Asp189del	7
		CBL	0.5	Missense	T>C/G>A	9820	1259G>A	Arg420Gln	11
		TP53	0.5	Missense	C>A/T>G	7027	752T>G	Ile251Ser	17
	1216	*TP53*	1.4	Missense	T>C/G>A	9469	824G>A	Cys275Tyr	17
	1223	*CSF3R*	49.4	Missense	C>A/T>G	8365	1142C>A	Ala381Asp	1
	1230	*SETBP1*	51.7	Missense	T>C/G>A	9435	3343G>A	Val1115Ile	18
	1235	*CBL*	30.5	Missense	T>C/G>A	9307	1244G>A	Gly415Asp	11

Supplemental Table 3. Detailed characteristics of somatic mutations detected in leukapheresis samples.

Supplemental Table 4

	ID	Gene	VAF	Type	Basesub	Depth	c.DNA	Protein Alt
Cases	1174	*DNMT3A*	1.0	Missense	T>C/G>A	9986	2578T>C	Trp860Arg
		DNMT3A	1.7	Missense	C>T/A>G	9007	1858C>T	Pro620Ser
		DNMT3A	0.7	Missense	T>C/G>A	9614	2114T>C	Ile705Thr
	1175	*DNMT3A*	14.1	Frameshift	Not SNV	8298	1688_1689delTG	Val563Glyfs*14
		DNMT3A	0.4	Stop	C>A/T>G	10390	1988C>A	Ser663*
	1177	*DNMT3A*	1.8	Stop	T>C/G>A	6836	938G>A	Trp313*
		DNMT3A	2.5	Splice_donor	A>C/G>T	8631	1279+1G>T	
	1179	*TET2*	1.2	Splice_donor	C>A/T>G	11255	3954+2T>G	
	1180	*DNMT3A*	1.3	Missense	T>C/G>A	9069	1541G>A	Cys514Tyr
		DNMT3A	0.4	Frameshift	Not SNV	9960	1269_1270delGC	Glu423Aspfs*21
		DNMT3A	0.9	INDEL	Not SNV	9772	1729_1737delAAGGAAGAC	Lys577_Asp579del
		DNMT3A	0.4	Frameshift	Not SNV	10126	1012delG	Val338Trpfs*7
		DNMT3A	0.7	Missense	C>T/A>G	10171	2477A>G	Lys826Arg
	1181	*DNMT3A*	0.7	Splice_donor	T>C/G>A	9013	2478+1G>A	
	1182	*DNMT3A*	1.3	Missense	C>T/A>G	9931	2644C>T	Arg882Cys
		TET2	3.6	Missense	T>C/G>A	11188	3785G>A	Arg1262Gln
	1185	*TET2*	0.4	Stop	C>T/A>G	9748	2710C>T	Gln904*
		TET2	1.3	Missense	C>T/A>G	8839	55A>G	Ile19Val
	1188	*DNMT3A*	1.1	Missense	G>C	8705	2507G>C	Arg836Thr
	1189	*DNMT3A*	1.0	Splice_donor	G>C	7366	1015-1G>C	
	1190	*TET2*	1.1	Splice_donor	T>C/G>A	9133	4044+1G>A	
		TET2	2.7	Frameshift	Not SNV	9026	4248_4249insA	Val1417Serfs*9
		TET2	0.8	Splice_donor	C>A/T>G	9103	3954+2T>G	
	1191	*DNMT3A*	20.3	Splice_donor	T>C/G>A	9223	1474+1G>A	
		DNMT3A	0.5	Splice_donor	G>C	8857	2478+1G>C	
	1192	*DNMT3A*	1.2	Missense	T>C/G>A	7691	2578T>C	Trp860Arg
	1193	*DNMT3A*	0.5	Splice_donor	C>T/A>G	7445	1555-2A>G	
		TET2	0.4	Stop	C>T/A>G	8904	2227C>T	Gln743*
	1194	*DNMT3A*	47.5	Missense	T>C/G>A	7299	1811G>A	Arg604Gln
	1197	*DNMT3A*	0.6	INDEL	Not SNV	9398	2105_2110delATCTGG	Asp702_Leu703del
	1200	*TET2*	7.4	Stop	C>T/A>G	8273	4528C>T	Gln1510*
		DNMT3A	1.0	Missense	C>T/A>G	7456	2644C>T	Arg882Cys
	1207	*DNMT3A*	0.8	Missense	T>C/G>A	8274	1676G>A	Cys559Tyr
	1208	*DNMT3A*	1.1	Stop	C>A/T>G	8441	2049C>A	Tyr683*
	1209	*DNMT3A*	0.7	Stop	C>A/T>G	8813	1988C>A	Ser663*
		DNMT3A	0.9	Stop	C>T/A>G	7674	958C>T	Arg320*
	1245	*DNMT3A*	0.6	Missense	T>G/C>A	8906	1049T>G	Phe350Cys
Controls	1210	*DNMT3A*	1.2	Missense	A>T/A>T	9407	2083A>T	Ile695Phe
	1214	*DNMT3A*	1.0	Missense	T>C/G>A	8579	2251T>C	Phe751Leu
	1216	*TET2*	1.7	Missense	T>C/G>A	9266	5618T>C	Ile1873Thr
		TET2	0.6	Frameshift	Not SNV	8298	4153_4154delTT	Leu1385Alafs*15
		TET2	0.6	Frameshift	Not SNV	10160	530delC	Pro177Glnfs*6
	1217	*DNMT3A*	2.7	Missense	C>T/A>G	8667	920C>T	Pro307Leu
	1218	*DNMT3A*	6.3	Frameshift	Not SNV	9873	102delCinsTAA	Glu36Argfs*37
		TET2	0.3	Missense	T>C/G>A	10446	3455G>A	Gly1152Glu
	1220	*TET2*	8.0	Frameshift	Not SNV	9938	5448dupA	His1817Thrfs*5
		TET2	5.5	Frameshift	Not SNV	9181	4230delT	Glu1411Argfs*37
		TET2	1.1	Stop	C>T/A>G	9761	2185C>T	Gln729*
		DNMT3A	0.8	Frameshift	Not SNV	8854	2265dupT	Glu756*fs*1
	1221	*DNMT3A*	0.7	Missense	C>T/A>G	9822	1642A>G	Met548Val
	1223	*TET2*	3.1	Splice_donor	C>T/A>G	7592	3594+4A>G	
		DNMT3A	0.6	Frameshift	Not SNV	8259	1066_1073delCAGGCCAC	Gln356Valfs*34
	1224	*TET2*	1.8	Missense	C>A/T>G	10657	3633T>G	Cys1211Trp
	1226	*DNMT3A*	1.0	Splice_donor	G>C	8052	1015-1G>C	
		DNMT3A	0.7	Frameshift	Not SNV	8896	1439dupT	Tyr481Valfs*11
	1228	*TET2*	10.3	Splice_donor	C>T/A>G	9570	3500+4A>G	
	1229	*DNMT3A*	2.1	Missense	C>A/T>G	9537	2069T>G	Val690Gly
		DNMT3A	0.3	Missense	C>T/A>G	9424	1903C>T	Arg635Trp
	1230	*DNMT3A*	2.0	Missense	T>C/G>A	8563	2645G>A	Arg882His
		DNMT3A	0.9	Splice_donor	A>C/G>T	9038	2597+1G>T	
	1235	*TET2*	0.4	Missense	T>G/C>A	8439	4118C>A	Ala1373Glu
	1238	*TET2*	0.5	Frameshift	Not SNV	10486	4799_4800insATGAG	Ser1601*fs*1
	1239	*TET2*	0.7	Stop	C>T/A>G	7851	2887C>T	Gln963*
	1240	*DNMT3A*	2.0	Missense	T>C/G>A	7544	869T>C	Phe290Ser
	1241	*TET2*	0.4	Missense	T>C/G>A	11728	3782G>A	Arg1261His
	1242	*DNMT3A*	0.5	Stop	C>T/A>G	9519	691C>T	Gln231*
		DNMT3A	0.9	Frameshift	Not SNV	7998	1505_1506delTT	Val502Aspfs*43

Supplemental Table 4. Detailed characteristics of excluded mutation.

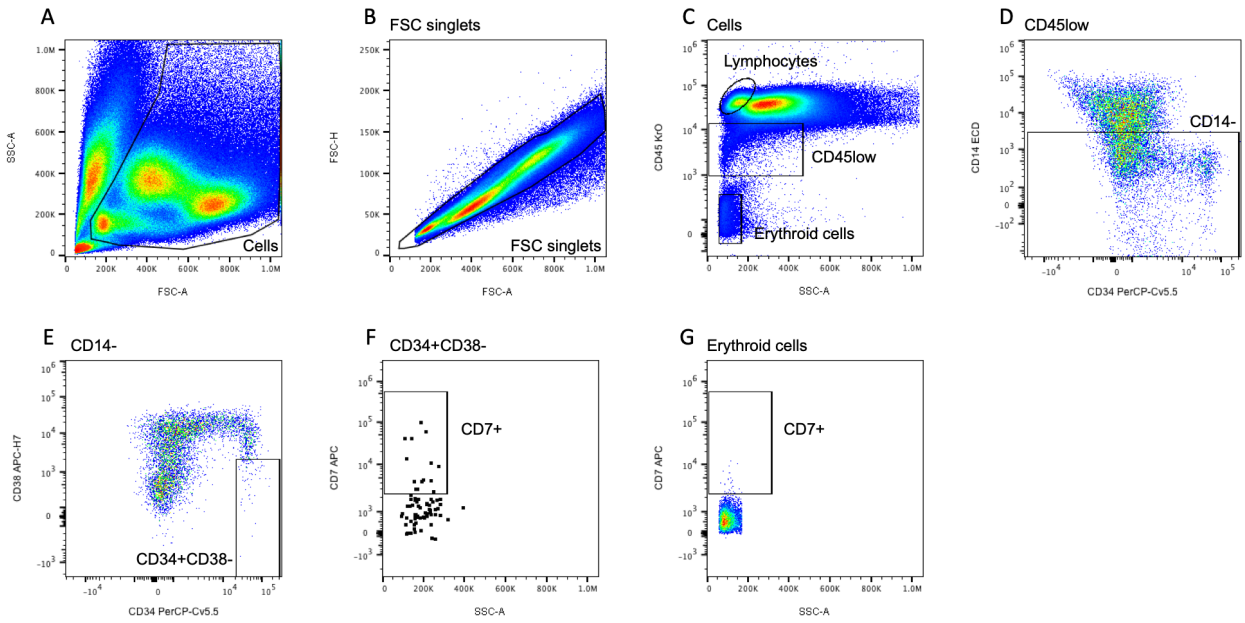
Supplemental Table 5

	Mutation status		Odds Ratio (CI 95%)	
	Mut+	**Mut-**	**Crude**	**Age-adjusted**
Cases (n=36)	17	19	5.5 (1.7 to 17.5)	5.9 (1.8 to 19.1)
Controls (n=36)	5	31		
	CD7 Expression		**Odds Ratio (CI 95%)**	
	CD7+	**CD7-**		
Cases (n=32)	12	20	6.6 (1.6 to 26.2)	
Controls (n=36)	3	33		

Supplemental Table 5. ***Odds Ratios for development of tMN for patients with one or more detectable mutations, or aberrant expression of CD7.***

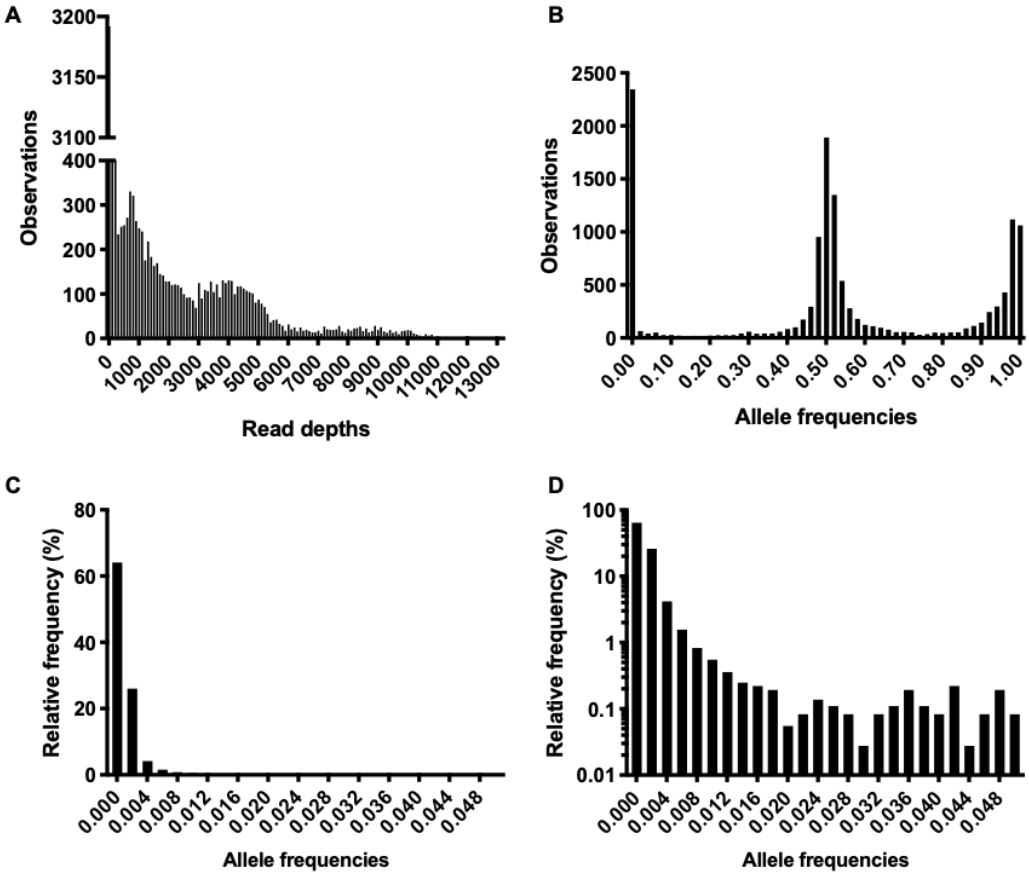
Supplementary Figures

Supplemental Figure 1.



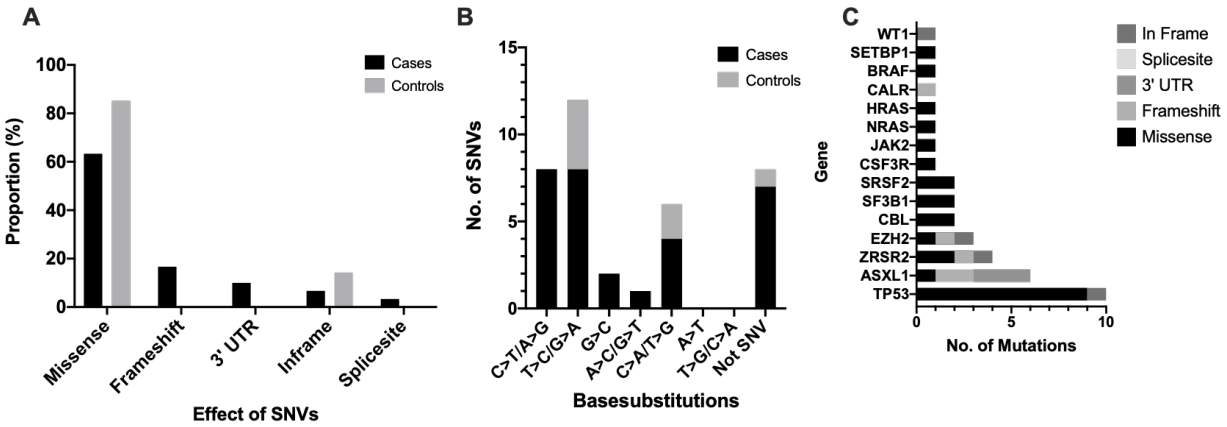
Supplemental Figure 1. Gating strategy. Representative gating strategy for CD7 expression analysis on the $CD34^{+}CD38^{-}$ stem cells (ID1175 used as example). Initially, intact cells were gated in a forward scatter (FSC-A) vs. side scatter (SSC-A) plot (**A**), and singlets (B) were depicted within a SSC-A and CD45 expression plot for identification of lymphocytes and $CD45^{low}$ cells (C). Within the $CD45^{low}$ population, $CD14^{-}$ (D) $CD34^{+}CD38^{-}$ cells were selected (E) and displayed in a SSC-A vs. CD7 plot for further gating of their CD7 expression (F). $CD7^{+}$ lymphocytes were gated within the lymphocyte population (G) and erythroid cells were used as internal negative control for CD7 positivity. The CD7+ stem cells were back gated into the $CD45^{low}SSC^{low}CD14^{-}CD34^{+}CD38^{-}$ population, as shown in Figure 3 in the manuscript

Supplemental Figure 2.



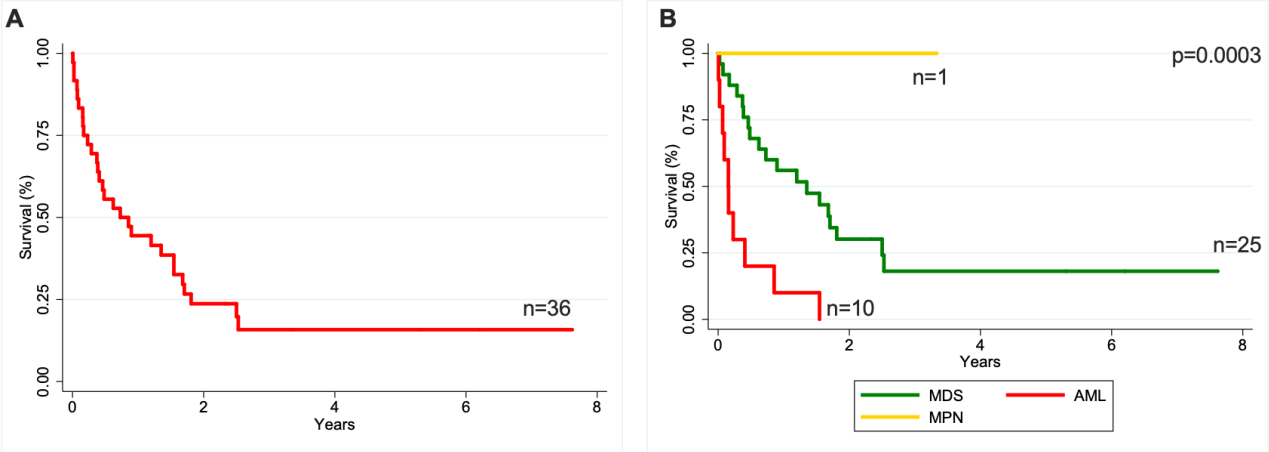
Supplemental Figure 2. Quality of targeted next-generation sequencing for determination of variant calling. (A) Number of observations per read depth; (B) Number of observations by allele frequency; (C) Relative frequency of observations by allele frequency; (D) Relative frequency of observations on logarithmic scale by allele frequency.

Supplemental Figure 3.



Supplemental Figure 3. Numbers and characteristics of somatic mutations detected by NGS in cases and controls. (A) Proportion of SNVs sorted by predicted effect on gene-expression; (B) Distribution of individual basesubstitutions; (C) Predicted effect of SNVs detected in each gene.
3' UTR, Three prime untranslated region; NGS, Next-Generation Sequencing; SNV, single-nucleotide variant.
Cases, Black; Controls, Grey.

Supplemental Figure 4



Supplemental Figure 4. Survival of patients with tMN. (A) Overall survival for the entire tMN cohort; (B) Overall survival among tAML (red), tMDS (green) and tMPN (yellow). P-value for Log-Rank test.