# Supplementary document

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

### 1. Supplementary Methods

- Matching
- Flow cytometry
- Myeloid next-generation sequencing panel
- Variant calling
- Survival analyses

#### 2. Supplementary Tables

- Supplement Table 1A. Patient characteristics, cases
- Supplement Table 1B. Patient characteristics, controls
- Supplement Table 2. Patient characteristics at tMN diagnosis
- Supplement Table 3. Detailed characteristics of somatic mutations detected in leukapheresis sample
- Supplement Table 4. Odds Ratios for development of tMN for patients with one or more detectable mutation, or aberrant expression of CD7

#### 3. Supplementary Figures

- Supplement Figure 1. Gating strategy
- Supplement Figure 2. Quality of targeted next-generation sequencing for determination of variant calling.
- Supplement Figure 3. Numbers and characteristics of somatic mutations detected by NGS in cases and controls
- Supplement Figure 4. Figure 4. Survival of patients with tMN

# **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<sup>low</sup>/SSC<sup>low</sup> "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<sup>low</sup>CD45<sup>low</sup>CD14<sup>-</sup> population, we identified CD34<sup>+</sup>CD38<sup>-</sup> 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<sup>dim</sup> progenitor cells. The stem cell populations (SSC<sup>low</sup>CD45<sup>low</sup>CD34<sup>+</sup>CD38<sup>-</sup>) 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	СНОР	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	СНОР	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	СНОР	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	СНОР	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	СНОР	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 <sup>9</sup> (range)	3.0 (1.5 to 40.7)	3.3 (0.3 to 60.7)		
Neutrophil granulocytes, x10 <sup>9</sup> (range)	1.44 (0.3 to 25.8)	0.63 (0.1 to 5.8)		
Platelets, x10 <sup>9</sup> (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

D         Gene         V/F         gasesub         Depth         c.D/A         Protein Alt         Chromosome           1175         1175         13         Missense         C>T/A/S         8695         559A/S         Tyt22O(5)         17           1179         CALR         40.3         Frameshift         Not SNV         10791         1154_1155insTTGTC         Lys85Asnfs*7         19           2         F/2817         1.6         Missense         C>C         10765         1996C>C         Lys666Asn         22           1180         ZRSR2         0.8         Missense         C>C/G>A         5246         9867C         Leu329Mefs*7         X           1180         TP53         0.6         Missense         T>C/G>A         5227         743G>A         Arg248Cin         17           1182         ASXL1         1.1         Stop <c>A         5261         17481756de1GG         Tp583'f*1         22           1184         SF3B1         14.8         Missense         T&gt;C/G&gt;A         5621         1874G&gt;A         Arg255His         2           1186         TP53         1.4         Missense         T&gt;C/G&gt;A         6416         734G&gt;A         Gly465Asp         17</c>			-		_	-				
Cases         1177         7753         1.3         Missense         C>T/ArG         8995         659A-G         Tyt220Cys         17           1177         7753         1.0         Missense         C>CGAA         6765         7116>A         Met2371E         17           1179         CALR         40.3         Frameshift         Not SNV         10765         1998G>C         Lys858Asnfs*?         19           SF3B1         1.6         Missense         C>T/ArG         10866         108A-C         Lie32Walf1*?         X           ZRSR2         1.9         Frameshift         Not SNV         5267         984.987delTCT         Leu329Pro         X           1180         7P53         0.6         Missense         T>C/G>A         6927         743G>A         Arg248Gin         17           1184         S781         1.48         Missense         T>C/G>A         6521         1974G>A         Arg257His         20           1184         S7872         0.6         Missense         T>C/G>A         6416         7340>A         Arg273His         17           1186         7P53         1.4         Missense         T>C/G>A         6416         7340>A         Arg273His         17		ID	Gene	VAF	Туре	Basesub	Depth	c.DNA	Protein Alt	Chromosome
Cases         1179         CALR         40.3         Frameshit         Not SNV         10791         1154_155insTrGC         Lys385Asris?         199           SF3B1         1.6         Missense         C>C         10765         1998G>C         Lys385Asris?         199           ZRSR2         0.8         Missense         C>C         10765         1998G>C         Lys385Asris?         199           ZRSR2         0.8         Missense         C>C/G>A         5246         986T>C         Leu329Metis?         XX           1180         TP53         0.6         Missense         T>C/G>A         5246         986T>C         Leu329Metis?         XX           1181         SRSL1         2.7         Frameshitt         Not SNV         10030         1748_1755deG         Trp633*fs*1         200           1184         SF381         14.8         Missense         C>C/G>A         6616         284C>A         Pro95His         177           ASXL1         1.1         Stop         C>T/A>G         8533         1471C>T         Gin491*         200           1185         SRSF2         0.5         Missense         T>C/G>A         6116         284C>A         Pro95His         177		1175	TP53	1.3	Missense	C>T/A>G	8695	659A>G	Tyr220Cys	17
CALR         40.3         Frameshif         Not SNV         10791         1154_1155insTTGTC         Lys868Asnfs*?         19           SF3B1         1.6         Missense         G>C         10765         19989C         Lys666Asn         2           HRAS         0.8         Missense         C>T/A>G         10866         106A>G         lie36Val         11           ZRSR2         1.9         Frameshif         Not SNV         5267         984 9987delTCTT         Leu329Metfs*?         XX           1180         TP53         0.6         Missense         T>C/G>A         8927         743G>A         Arg248Gin         17           1184         JSF3B1         141.8         Missense         T>C/G>A         5621         1874G>A         Arg248Gin         17           1184         SF3B1         141.8         Missense         T>C/G>A         5621         1874G>A         Arg248Gin         17           TF53         1.4         Missense         T>C/G>A         5621         1874G>A         Arg2273His         17           T186         TP53         1.4         Missense         T>C/G>A         10182         818G>A         Arg273His         17           1190         ASXL1		1177	TP53	1.0	Missense	T>C/G>A	6765	711G>A	Met237lle	17
Cases         SF381         1.6         Missense         G>C         10765         1998G>C         Lys666Asn         22           IRAAS         0.8         Missense         C7/CA         5267         984.987/delTCTT         Leu329Mefs*?         XX           ZRSR2         0.8         Missense         T>C/G>A         5246         986T>C         Leu329Pro         XX           1180         TP53         0.6         Missense         T>C/G>A         8927         743G>A         Ang2dGln         177           1182         ASXL1         2.7         Frameshit         Not SNV         10030         1748_1755delGG         Trp583*fs*1         200           1184         SF381         1.4.8         Missense         C>C/G>A         6016         284C>A         Pro95His         17           ASXL1         1.1         Stop         C>T/A>G         6016         284C>A         Pro95His         17           1185         SF326.1         Missense         T>C/G>A         10182         818G>A         Ang22His         20           1186         ASXL1         1.1         Missense         C>C/G>A         10182         818G>A         Ang27His         17           1190         ASXL1 <td rowspan="3"></td> <td>1179</td> <td>CALR</td> <td>40.3</td> <td>Frameshift</td> <td>Not SNV</td> <td>10791</td> <td>1154_1155insTTGTC</td> <td>Lys385Asnfs*?</td> <td>19</td>		1179	CALR	40.3	Frameshift	Not SNV	10791	1154_1155insTTGTC	Lys385Asnfs*?	19
HRAS         0.8         Missense         C>T/A>G         10866         106A>G         lie36Val         111           ZRSR2         1.9         Frameshit         Not SNV         5267         984 987delTCTT         Leu329Metfs??         XX           2RSR2         0.8         Missense         T>C/G>A         5246         986T>C         Leu329Pro         XX           1180 <i>TP53</i> 0.6         Missense         T>C/G>A         5247         743G>A         Arg249Gin         117           1182         ASXL1         2.7         Frameshit         Not SNV         10030         1748_1755delGG         Trp531*1         200           1184         S7387         1.4.8         Missense         T>C/G>A         6016         284C>A         Arg22His         27           1185         SRSF2         0.5         Missense         T>C/G>A         6116         734G>A         Gj24dsp         17           1186 <i>TP53</i> 1.4         Missense         T>C/G>A         61182         ASZL1         Gj44dsp         20           1190         ASXL1         30.8         Frameshit         Not SNV         9803         1934dupG         Gj466Trpfs12         200           <			SF3B1	1.6	Missense	G>C	10765	1998G>C	Lys666Asn	2
Cases         ZRSR2         1.9         Frameshift         Not SNV         5267         984_987delTCTT         Leu329Mefts??         XX           1180         TP53         0.6         Missense         T>C/G>A         5246         986T>C         Leu329Pro         XX           1181         TP53         0.6         Missense         T>C/G>A         8927         T43G>A         Arg248Gin         117           1182         ASXL1         2.7         Frameshift         Not SNV         10030         1748_17550elGG         Trp583*s*1         20           1184         SF3B7         14.8         Missense         T>C/G>A         6521         1874G>A         Arg625His         2           1186         SF3F2         0.5         Missense         T>C/G>A         6416         734G>A         Arg625His         17           1186         TF53         1.4         Missense         T>C/G>A         10182         818G>A         Arg727His         17           1180         NRAS         1.9         Missense         C>A/T>G         7762         284C>A         Pro95His         17           1190         ASXL1         1.2         Stop <c>T/A&gt;G         9874         2066C&gt;A         Ser689         <td< td=""><td></td><td>HRAS</td><td>0.8</td><td>Missense</td><td>C&gt;T/A&gt;G</td><td>10866</td><td>106A&gt;G</td><td>lle36Val</td><td>11</td></td<></c>			HRAS	0.8	Missense	C>T/A>G	10866	106A>G	lle36Val	11
Cases         ZRSR2         0.8         Missense         T>C/G>A         5246         986T>C         Leu329Pro         XX           1180         7753         0.6         Missense         T>C/G>A         8927         743G>A         Arg248Gin         117           1182         ASXL1         2.7         Frameshit         Not SNV         10030         1748_1755delGG         Tp583*fs*1         2.0           1184         SF381         14.8         Missense         T>C/G>A         5621         1874G>A         Arg25His         2.2           1185         SRSF2         0.5         Missense         T>C/G>A         5621         1874G>A         Arg25His         2.2           1186         TP53         1.4         Missense         T>C/G>A         6416         734G>A         Gly245Asp         1.7           1186         TP53         1.4         Missense         T>C/G>A         61182         818G>A         Arg273His         1.7           1190         ASXL1         1.0         RSrsense         C>C/G>A         9421         37G>C         Gly347g         1.1           1191         NAAS         1.9         Missense         C>C/G>A         9421         37G>C         Gly347g			ZRSR2	1.9	Frameshift	Not SNV	5267	984_987delTCTT	Leu329Metfs*?	X
Image: 180         TP53         0.6         Missense         T>C/G>A         8927         743G>A         Arg248Gin         17           1182         ASXL1         2.7         Frameshit         Not SNV         10030         1748_1755delGG         Trp583'ts*1         20           1184         SF38F         1.4.8         Missense         C/C/A         5621         1874G>A         Arg25His         2           1185         SRSF2         0.5         Missense         C/C/A         6616         284C>A         Pro95His         17           ASXL1         1.1         Stop         C/C/SA         6416         734G>A         Gly245Asp         17           1186         TP53         1.4         Missense         T>C/G>A         10182         818G>A         Arg273His         17           1186         TP53         1.4         Missense         C>C/G>A         10182         818G>A         Arg273His         17           1186         TP53         1.4         Missense         C>C/G>A         10182         818G>A         Arg273His         17           1190         ASXL1         1.9         Missense         C>C/G>A         762         284C>A         Pro95His         17			ZRSR2	0.8	Missense	T>C/G>A	5246	986T>C	Leu329Pro	X
Image: 182         ASX.1         2.7         Frameshift         Not SNV         10030         1748_17556elGG         Tp583*fs*1         20           1184         SF3B1         14.8         Missense         T>C/G>A         5621         1874G>A         Arg625His         22           1185         SRSF2         0.5         Missense         T>C/G>A         6016         224C>A         Pro55His         17           ASXL1         1.1         Stop         C>T/A>G         8533         1471C>T         Gin491*         200           7P53         1.4         Missense         T>C/G>A         6416         734G>A         Giy245Asp         17           1186         TP53         1.4         Missense         T>C/G>A         10182         818G>A         Arg273His         17           1190         ASXL1         30.8         Frameshift         Not SNV         9003         1934dupG         Giy646Trpfs*12         200           SRSF2         34.8         Missense         C>/T>G         762         284C>A         Pro95His         17           1191         NRAS         1.9         Missense         C>T/A>G         9750         1534C>T         Gin512*         20           1197 <td>1180</td> <td>TP53</td> <td>0.6</td> <td>Missense</td> <td>T&gt;C/G&gt;A</td> <td>8927</td> <td>743G&gt;A</td> <td>Arg248GIn</td> <td>17</td>		1180	TP53	0.6	Missense	T>C/G>A	8927	743G>A	Arg248GIn	17
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         Pr095His         117           ASXL1         1.11         Stop         C>T/A>G         6633         1471C>T         Gln491*         20           7P53         1.4         Missense         T>C/G>A         6416         734G>A         Gly245Asp         17           1186         TP53         1.4         Missense         C>A/T>G         284C>A         Arg273His         17           1190         ASXL1         30.8         Frameshift         Not SNV         9803         1934dupG         Gly646Tpfs*12         200           SRSF2         34.8         Missense         C>A/T>G         7762         284C>A         Pr095His         17           1191         NRAS         1.9         Missense         C>C/G>A         6906         428T>C         Vali617Phe         9           1192         ASXL1         1.2         Stop         C>A/T>G         9874         2066C>A         Ser689*         200           1197         TP53         1.0 <th></th> <td>1182</td> <td>ASXL1</td> <td>2.7</td> <td>Frameshift</td> <td>Not SNV</td> <td>10030</td> <td>1748_1755delGG</td> <td>Trp583*fs*1</td> <td>20</td>		1182	ASXL1	2.7	Frameshift	Not SNV	10030	1748_1755delGG	Trp583*fs*1	20
Image: constraint of the series of		1184	SF3B1	14.8	Missense	T>C/G>A	5621	1874G>A	Arg625His	2
ASXL1         1.1         Stop         C>T/A>G         8533         1471C>T         Gin491*         200           TP53         1.4         Missense         T>C/G>A         6416         734G>A         Gly245Asp         117           1186         TP53         1.4         Missense         T>C/G>A         10182         818G>A         Arg273His         117           1190         ASXL1         30.8         Frameshift         Not SNV         9803         1934dupG         Gly646Trpfs*12         220           SRSF2         34.8         Missense         C>A/T>G         7762         284C>A         Pro95His         117           1191         NRAS         1.9         Missense         C>A/T>G         7762         284C>A         Pro95His         117           1191         ASXL1         1.2         Stop         C-T/A>G         7762         284C>A         Pro95His         117           1193         ASXL1         1.2         Stop         C-T/A>G         9874         2066C>A         Ser689*         200           1194         ASXL1         12.1         Stop         C>A/T>G         9874         2066C>A         Ser689*         200           1190         BASL1 <th></th> <td>1185</td> <td>SRSF2</td> <td>0.5</td> <td>Missense</td> <td>C&gt;A/T&gt;G</td> <td>6016</td> <td>284C&gt;A</td> <td>Pro95His</td> <td>17</td>		1185	SRSF2	0.5	Missense	C>A/T>G	6016	284C>A	Pro95His	17
Cases         TP53         1.4         Missense         T>C/G>A         6416         734G>A         Gly245Asp         17           1186         TP53         1.4         Missense         T>C/G>A         1012         818G>A         Arg273His         117           1190         ASXL1         30.8         Frameshit         Not SNV         9803         1934dupG         Gly646Trpfs*12         200           SRSF2         34.8         Missense         C>AT>G         7762         2284C>A         Pro95His         17           1191         NRAS         1.9         Missense         C>C         9421         37G>C         Gly13Arg         1           1192         ASXL1         1.2         Stop         C>T/A>G         7550         1534C>T         Gli512*         200           1197         TP53         1.0         Missense         T>C/G>A         6960         428T>C         Vali47Phe         99         200           1197         TP53         1.0         Missense         T>C/G>A         6960         428T>C         Vali43Aba         17           1198         ASXL1         12.1         Stop         C>AT>G         9874         2066C>A         Ser689*         200			ASXL1	1.1	Stop	C>T/A>G	8533	1471C>T	Gln491*	20
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         11           JAK2         0.7         Missense         C>C         9421         37G>C         Gly13Arg         11           JAK2         0.7         Missense         C>C/G>F         9026         1849G>T         Val617Phe         99           1192         ASXL1         1.2         Stop         C>T/A>G         7550         1534C>T         Glin512*         200           1198         ASXL1         12.1         Stop         C>A/T>G         9874         2066C>A         Ser689*         200           1199         BRAF         0.9         Missense         C>T/A>G         9571         1696C>T         Arg368Cys         7           1201         ASXL1			TP53	1.4	Missense	T>C/G>A	6416	734G>A	Gly245Asp	17
Cases         1190         ASXL1         30.8         Frameshift         Not SNV         9803         1934dupG         Gly646Trpfs*12         200           SRSF2         34.8         Missense         C>A/T>G         7762         284C>A         Pro95His         177           1191         NRAS         1.9         Missense         G>C         9421         37G>C         Gly13Arg         1           1191         JAK2         0.7         Missense         G>C         9421         37G>C         Gly13Arg         1           1192         ASXL1         1.2         Stop         C>T/A>G         7550         11534C>T         Glin512*         200           1197         TP53         1.0         Missense         T>C/G>A         6960         428T>C         Val143Ala         177           1198         ASXL1         12.1         Stop         C>A/T>G         9874         2066C>A         Ser689*         200           EZH2         10.8         Frameshift         Not SNV         8633         1123         1124insGA         Hie375Argfs*50         77           1199         BRAF         0.9         Missense         C>T/A>G         9430         2237C>T         Ala746Val <td< td=""><th></th><td>1186</td><td>TP53</td><td>1.4</td><td>Missense</td><td>T&gt;C/G&gt;A</td><td>10182</td><td>818G&gt;A</td><td>Arg273His</td><td>17</td></td<>		1186	TP53	1.4	Missense	T>C/G>A	10182	818G>A	Arg273His	17
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           1491         NRAS         0.7         Missense         A>C/G>T         9026         1849G>T         Val617Phe         99           1192         ASXL1         1.2         Stop         C>T/A>G         7550         1534C>T         Glin512*         200           1197         TP53         1.0         Missense         T>C/G>A         6960         428T>C         Val617Phe         99           1197         TP53         1.0         Missense         T>C/G>A         6960         428T>C         Val617Phe         200           1197         TP53         1.0         Missense         C>T/A>G         9874         2006C>A         Ser689*         200           EZH2         10.8         Frameshift         Not SNV         8633         1123 1124insGA         Ile375Argfs*50         7           1199         BRAF         0.9         Missense         C>T/A>G         9571         1696C>T         Arg566Cys         7           1199 </td <th>0</th> <td>1190</td> <td>ASXL1</td> <td>30.8</td> <td>Frameshift</td> <td>Not SNV</td> <td>9803</td> <td>1934dupG</td> <td>Gly646Trpfs*12</td> <td>20</td>	0	1190	ASXL1	30.8	Frameshift	Not SNV	9803	1934dupG	Gly646Trpfs*12	20
1191         NRAS         1.9         Missense         G>C         9421         37G>C         Giy13Arg         1           JAK2         0.7         Missense         A>C/G>T         9026         1849G>T         Val617Phe         99           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*         200           EZH2         10.8         Frameshift <not snv<="" td="">         8633         1123         1124insGA         Ile375Argfs*50         7           EZH2         11.8         Missense         C&gt;T/A&gt;G         9571         1696C&gt;T         Arg566Cys         7           1199         BRAF         0.9         Missense         C&gt;T/A&gt;G         8303         1781A&gt;G         Asp594Giy         7           1201         ASXL1         47.8         Missense         C&gt;T/A&gt;G         8303         1781A&gt;G         Asp594Giy         7           1201         ASXL1<th>Cases</th><td></td><td>SRSF2</td><td>34.8</td><td>Missense</td><td>C&gt;A/T&gt;G</td><td>7762</td><td>284C&gt;A</td><td>Pro95His</td><td>17</td></not>	Cases		SRSF2	34.8	Missense	C>A/T>G	7762	284C>A	Pro95His	17
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         GIn512*         200           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*         200           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         6374         673-2A>G         n/a         17           1201		1191	NRAS	1.9	Missense	G>C	9421	37G>C	Gly13Arg	1
1192         ASXL1         1.2         Stop         C>T/A>G         7550         1534C>T         Gin512*         200           1197         7P53         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*         200           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           W71         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         200           1207         TP53         4.9         Splicesite         C>T/A>G         9492         2237C>T         Ala746val         201			JAK2	0.7	Missense	A>C/G>T	9026	1849G>T	Val617Phe	9
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*         200           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         Arg544_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         6374         673-2A>G         n/a         17           1207         TP53         4.9         Splicesite         C>T/A>G         6374         673-2A>G         n/a         17           2RSR2         1.1         Missense         C>C/A>G         8109         464C>A         Thr155Asn         17           2RSR2         9.2		1192	ASXL1	1.2	Stop	C>T/A>G	7550	1534C>T	Gln512*	20
1198         ASXL1         12.1         Stop         C>AT>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         200           1207         TP53         4.9         Splicesite         C>T/A>G         6374         673-2A>G         n/a         17           ZRSR2         1.1         Missense         C>A/A>G         8109         464C>A         Thr155Asn         17           ZRSR2         99.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447_Arg448dup         X           ZRSR2         99.2		1197	TP53	1.0	Missense	T>C/G>A	6960	428T>C	Val143Ala	17
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           2RSR2         1.1         Missense         C>C/G>A         4959         560G>A         Cys187Tyr         X           1245         TP53         0.6         Missense         C>A/T>G         8109         464C>A         Thr155Asn         17           2RSR2         9.9.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447_Arg448dup         X           2RSR2         9		1198	ASXL1	12.1	Stop	C>A/T>G	9874	2066C>A	Ser689*	20
EXAMPLE         Initial Missense         C>T/A>G         9571         1100C>T         MarchageCys         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           2RSR2         1.1         Missense         C>C/G>A         4959         560G>A         Cys187Tyr         X           1245         TP53         0.6         Missense         C>A/T>G         8109         464C>A         Thr155Asn         17           2RSR2         99.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447 Arg448dup         X           2RSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           2RSR2         99.2 <t< td=""><th></th><td></td><td>EZH2</td><td>10.8</td><td>Frameshift</td><td>Not SNV</td><td>8633</td><td>1123 1124insGA</td><td>lle375Arafs*50</td><td>7</td></t<>			EZH2	10.8	Frameshift	Not SNV	8633	1123 1124insGA	lle375Arafs*50	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         200           1207         TP53         4.9         Splicesite         C>T/A>G         6374         673-2A>G         n/a         117           2ZSR2         1.1         Missense         C>T/A>G         6374         673-2A>G         n/a         117           ZRSR2         1.1         Missense         C>A/T>G         8109         464C>A         Thr155Asn         117           ZRSR2         99.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447_Arg448dup         X           ZRSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           ZRSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           CBL         0.5         Missens			EZH2	11.8	Missense	C>T/A>G	9571	1696C>T	Ara566Cvs	7
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         117           2RSR2         1.1         Missense         C>T/A>G         6474         673-2A>G         n/a         117           2RSR2         1.1         Missense         C>T/A>G         8109         464C>A         Cys187Tyr         X           1245         TP53         0.6         Missense         C>A/T>G         8109         464C>A         Thr155Asn         17           2RSR2         99.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447_Arg448dup         X           2RSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           1213         EZH2         49.8         INDEL         Not SNV         10016         552_554delTGA         Arg420Gln         111           TP53         0.5 <th></th> <td></td> <td>WT1</td> <td>9.5</td> <td>INDEL</td> <td>Not SNV</td> <td>10323</td> <td>1301 1302insAACACA</td> <td>Ara434 Ser435insThrHis</td> <td>11</td>			WT1	9.5	INDEL	Not SNV	10323	1301 1302insAACACA	Ara434 Ser435insThrHis	11
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           2RSR2         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           1245         TP53         0.6         Missense         C>A/T>G         8109         464C>A         Thr155Asn         17           2RSR2         99.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447_Arg448dup         X           2RSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           2RSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           2Controls         1216         TP53         0.5         Missense         T>C/G>A         9469         824G>A         Cys275Tyr         17           12		1199	BRAF	0.9	Missense	C>T/A>G	8303	1781A>G	Asp594Glv	7
1207         TP53         4.9         Splicesite         C>T/A>G         6374         673-2A>G         n/a         17           1207         TP53         4.9         Splicesite         C>T/A>G         6374         673-2A>G         n/a         17           1245         TP53         0.6         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           2RSR2         99.2         INDEL         Not SNV         10016         552_554delTGA         Asp189del         7           2RSR2         99.2         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         T>C/G>A         9469         824G>A         Cys275Tyr         17           1223         CSF3R         49.4		1201	ASXI 1	47.8	Missense	C>T/A>G	9492	2237C>T	Ala746Val	20
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           ZRSR2         99.2         INDEL         Not SNV         4540         1338_1343dupGAGCCG         Ser447_Arg448dup         X           ZRSR2         99.2         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           Controls         1216         TP53         1.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1223         CSF3R         49.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1230         SETRP1		1207	TP53	4.9	Splicesite	C>T/A>G	6374	673-2A>G	n/a	17
1245         TP53         0.6         Missense         C>A/T>G         8109         464C>A         Thr155Asn         17           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           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           Controls         1216         TP53         1.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1223         CSF3R         49.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1230         SETRP1         51.7         Missense         T>C/G>A         9435         2343G>A         Vel11150         18 <th></th> <td></td> <td>ZRSR2</td> <td>1.1</td> <td>Missense</td> <td>T&gt;C/G&gt;A</td> <td>4959</td> <td>560G&gt;A</td> <td>Cvs187Tvr</td> <td>x</td>			ZRSR2	1.1	Missense	T>C/G>A	4959	560G>A	Cvs187Tvr	x
Interview         Interview <t< td=""><th></th><td>1245</td><td>TP53</td><td>0.6</td><td>Missense</td><td>C&gt;A/T&gt;G</td><td>8109</td><td>464C&gt;A</td><td>Thr155Asn</td><td>17</td></t<>		1245	TP53	0.6	Missense	C>A/T>G	8109	464C>A	Thr155Asn	17
Image: Second		-	ZRSR2	99.2	INDEL	Not SNV	4540	1338 1343dupGAGCCG	Ser447 Arg448dup	x
Controls         1216         TP53         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           1223         CSF3R         49.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1230         SETERP1         51.7         Missense         T>C/G>A         9435         2343G>A         Val11150         18		1213	E7H2	49.8	INDEL	Not SNV	10016	552 554delTGA	Asp189del	7
Controls         1216         TP53         0.5         Missense         C>A/T>G         7027         752T>G         Ile251Ser         17           1223         CSF3R         49.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1230         SETRP1         51.7         Missense         T>C/G>A         9435         2343G>A         Viol111150         18		-	CBI	0.5	Missense	T>C/G>A	9820	1259G>A	Arg420Gln	11
Controls         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         SETERP1         51.7         Missense         T>C/G>A         9435         3343G>A         Value         18			TP53	0.5	Missense	C>A/T>G	7027	7527>G	lle251Ser	17
1223         CSF3R         49.4         Missense         C>A/T>G         8365         1142C>A         Ala381Asp         1           1230         SETRP1         51.7         Missense         T>C/G>A         9435         3343G>A         Val1116lia         18	Controls	1216	TP53	1.4	Missense	T>C/G>4	9460	824G>A	Cvs275Tvr	17
1230 SETERD1 51.7 Missonso TxC/C>A 0435 2343C>A \/ol1115llo 18	00111010	1223	CSE3P	40 /	Missense	C>A/T>G	8365	1142054	Δla381Δen	1
		1230	SETRD1	51 7	Missoneo	T>C/G>A	0/35	33/13/254	\/al1115  a	19
1235 CB/ 30.5 Missense T>C/G>A 9307 1244G>A Glv415Asp 11		1235	CBI	30.5	Missense	T>C/G>A	9307	1244G>A	Glv415Asn	11

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

## Supplemental Table 4

	ID	Gene	VAF	Туре	Basesub	Depth	c.DNA	Protein Alt
	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	lle705Thr
	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	Framesnin		10126	1012delG	Val338Trpts"7
	1181	DNMT3A	0.7	Splige deper		0012	2477426	LysozoArg
	1182	DNMT3A	1.2	Missonas	120/G2A	9013	2470+1G-A	Arg 992010
		TET2	3.6	Missense	T>C/G>A	11188	3785G>A	Arg1262Gin
	1185	TET2	0.4	Stop	C>T/A>G	9748	2710C>T	Gln904*
		TET2	1.3	Missense	C>T/A>G	8839	55A>G	lle19Val
Cases	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	
	44.04	TET2	0.4	Stop	C>T/A>G	8904	2227C>T	Gln743*
	1194	DNMT3A	47.5	Missense	T>C/G>A	7299	1811G>A	Arg604GIn
	1200	DNMT3A	0.6		Not SNV	9398	2105_2110delATCTGG	Asp702_Leu703del
	1200	TET2	1.4	Stop	C>T/A>G	8273	45280>1	Gin1510"
	1207	DNMT3A	1.0	Missense		7456	26440>1	Argoozeys
	1208	DNMT2A	1.0	Stop	C>A/T>G	8441	10/0G-A	Cys559 Tyr
	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
	1210	DNMT3A	1.2	Missense	A>T/A>T	9407	2083A>T	lle695Phe
	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	lle1873Thr
		TET2	0.6	Frameshift	Not SNV	8298	4153_4154delTT	Leu1385Alafs*15
		TET2	0.6	Frameshift	Not SNV	10160	530delC	Pro177GInfs*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
	1220	TET2	0.3	Missense	T>C/G>A	10446	3455G>A	Gly1152Glu
	1220	1E12 TET2	8.0	Frameshift		9938	5448dupA	HIS 1817 I INTS "5
		1E12 TET2	5.5	Stop	NOL SINV	9181	4230del1	Glu 14 TAIgis 37
			0.8	Frameshift	Not SNV	8854	2265dupT	Glu756*fs*1
	1221	DNMT3A	0.0	Missense	C>T/A>G	9822	1642A>G	Met548Val
	1223	TET2	3.1	Splice donor	C>T/A>G	7592	3594+4A>G	
Controlo		DNMT3A	0.6	Frameshift	Not SNV	8259	1066 1073delCAGGCCAC	Gln356Valfs*34
Controls	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
	1020	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
	1025	DNMT3A	0.9	Splice_donor	A>C/G>T	9038	2597+1G>T	
	1230	TET2	0.4	Missense	T>G/C>A	8439	4118C>A	Ala1373Glu
	1230	1E12 TET2	0.5	Stop	INUL SINV	7054	4/99_4000INSATGAG	
	1240		2.0	Missoneo		7544	2007021	Dha290 Car
	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.

	Mutatio	n status	Odds Ratio (CI 95%)			
	Mut+	Mut-	Crude	Age-adjusted		
Cases (n=36)	17	19		E 0 (1 0 to 10 1)		
Controls (n=36)	5	31	5.5 (1.7 to 17.5)	5.9 (1.0 10 19.1)		
	CD7 Exp	pression	Odds Ratio (CI 95%)			
	CD7+	CD7-				
Cases (n=32)	12	20				
Controls (n=36)	3	33	0.0 (1.6 to 26.2)			

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<sup>+</sup>CD38<sup>-</sup> 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<sup>low</sup> cells (C). Within the CD45<sup>low</sup> population, CD14<sup>-</sup> (D) CD34<sup>+</sup>CD38<sup>-</sup> cells were selected (E) and displayed in a SSC-A vs. CD7 plot for further gating of their CD7 expression (F). CD7<sup>+</sup> 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<sup>low</sup>SSC<sup>low</sup>CD14<sup>-</sup>CD34<sup>+</sup>CD38<sup>-</sup> 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. 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.