Supplementary Appendix

Clonal hematopoiesis in patients with stem cell mobilization failure: a nested case-control study

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Supplementary Methods

Histological subtypes

Histological subtypes were registered according to the WHO classification of 2008 or 2016. For analysis purposes, we defined general groups for lymphoma subtypes. Diffuse large B-cell lymphoma (DLBCL) or associated variants, transformed low-grade B-cell lymphoma, Burkitt lymphoma, high-grade B-cell lymphoma with rearrangement of MYC and BCL2 and/or BCL6 (previous double or triple hit DLBCL) and high-grade B-cell lymphoma with features between DLBCL and Burkitt lymphoma were summarized as high grade large B-cell lymphoma. Follicular lymphoma, mantle cell lymphoma, Hodgkin lymphoma and NK/T or T-cell lymphoma were classified separately. We included multiple myeloma, AL-amyloidosis, POEMS, extraosseous plasmacytoma and plasma cell leukemia in a subgroup of plasma cell dyscrasias. In case of transformation or more than one histological subtype (mixed or more than one diagnosis) of lymphoma, the histological subtype before mobilization was recorded.

R packages for analysis and visualization

Cox proportional hazards and competing risk regression analyses as well as visualization of survival were performed using R packages *survminer* (https://cran.r-project. org/web/packages/survminer/index.html), *survival* (http://cran.r-project. org/web/packages/survival/index.html), *cmprsk* (http://cran.r-project.org/web/packages/cmprsk/index.html) and *riskRegression* (https://cran.r-project.org/web/packages/riskRegression/index.html). The R package *compareGroups* (https://github.com/isubirana/compareGroups) was used for construction of tables with characteristics. All remaining figures were generated using *ggplot2* (https://github.com/tidyverse/ggplot2) and merged by *cowplot* (https://github.com/wilkelab/cowplot). For data manipulation and processing, we used *tidyr* (https://github.com/tidyverse/tidyr), *dplyr* (https://github.com/tidyverse/dplyr) and *reshape2* (https://github.com/cran/reshape2).

Supplementary Table 1. Overview of genes and regions in the sequencing panel.

Gene	Reference transcript	ENSEMBL reference transcript	Exon	Targeted codons/region
ASXL1	NM_015338	ENST00000375687	13 (partially)	exon 13
BRAF	NM_004333.4	ENST00000288602	15 (partially)	codon 600
CALR	NM_004343	ENST00000316448	9	exon 9
CBL	NM_005188	ENST00000264033	8-9	exon 8 and 9
CSF3R	NM_156039	ENST00000373103	14, 17	codon 618, 615 and exon 17
DNMT3A	NM_175629	ENST00000264709	2-23 (all coding exons)	all coding exons
ETNK1	NM_018638	ENST00000266517	3 (partially)	codon 243-244
EZH2	NM_004456	ENST00000320356	2-20 (all coding exons)	all coding exons
FLT3_835	NM_004119	ENST00000241453	20 (partially)	codon 835-842
IDH1	NM_005896	ENST00000415913	4 (partially)	codon 132
IDH2	NM_002168	ENST00000330062	4 (partially)	codon 140, 172
JAK2	NM_004972	ENST00000381652	12, 14 (partially)	codon 617 and exon 12
KIT	NM_000222	ENST00000288135	8 (partially), 17 (partially)	codon 816, 419
KRAS	NM_004985	ENST00000256078	2-3 (partially)	a.o. codon 12, 13, 61
MPL	NM_005373	ENST00000372470	10 (partially)	codon 515, 505
MYD88	NM_002468.4	ENST00000417037	4-5 (partially)	codon 265 and 232
NOTCH1	NM_017617.4	ENST00000277541	34 (partially)	codon 2514
NPM1	NM_002520	ENST00000517671	11 (partially)	codon 288-290
NRAS	NM_002524	ENST00000369535	2-3 (partially)	a.o. codon 12, 13, 61
RUNX1	NM_001754	ENST00000437180	2-9 (all coding exons)	all coding exons
SETBP1	NM_015559	ENST00000282030	4 (partially)	codon 850-910
SF3B1	NM_012433	ENST00000335508	13-16	codon 575-790
SRSF2	NM_003016	ENST00000392485	1 (partially)	codon 95, 96
TET2	NM_001127208	ENST00000380013	3-11 (all coding exons)	all coding exons
TP53	NM_000546	ENST00000269305	2-11 (all coding exons)	all coding exons
U2AF1	NM_006758	ENST00000291552	2, 6 (partially)	codon 34, 157
WT1	NM_024426	ENST00000332351	7, 9	exon 7 and 9
PPM1D	NM_003620	ENST00000305921	6	exon 6

Supplementary Table 2. Mutations indicative of clonal hematopoiesis detected in poor mobilizers and controls.

	Poor mobilizers		Controls	
	# variants	# individuals	# variants	# individuals
DNMT3A	19	11	19	15
PPM1D	12	11	1	1
TET2	7	7	3	3
TP53	7	5	0	0
ASXL1	2	2	0	0
JAK2	0	0	2	2
RUNX1	0	0	2	2
U2AF1	1	1	0	0
SF3B1	1	1	0	0
ETNK1	0	0	1	1
EZH2	1	1	1	1

Supplementary Table 3. TP53 and PPM1D mutations detected in poor mobilizers.

ID	Gene	c.HGVS	VAF (%)	Sex	Age	CD34 yield*	Apheresis days	Mobilisation failure
1	TP53	659A>G	4.9	M	60+	<2	1	Group 1
	TP53	817C>T	5.9					
	PPM1D	1535del	3.9					
2	TP53	842A>G	1.8	M	60+	≥2	3	Group 3
3	TP53	524G>A	2.9	M	60+	≥2	2	Group 3
4	TP53	749C>T	1.6	M	60+	≥2	2	Group 3
5	TP53	715A>G	29	M	<60	NA	0	Group 2
	TP53	723del	1					
6	PPM1D	1423G>T	1.7	M	<60	≥2	1	Group 3
	PPM1D	1612_1613dup	15					
7	PPM1D	c.1469_1476del	1.7	F	<60	≥2	2	Group 3
8	PPM1D	c.1535del	1.3	M	<60	≥2	2	Group 3
9	PPM1D	c.1602del	1.2	M	<60	NA	0	Group 2
10	PPM1D	c.1535del	1.4	M	60+	<2	1	Group 1
11	PPM1D	c.1321C>A	1.3	M	60+	≥2	2	Group 3
12	PPM1D	c.1388del	2.6	F	60+	≥2	3	Group 3
13	PPM1D	c.1270_1273dup	1.1	M	<60	≥2	3	Group 3
14	PPM1D	c.1726_1727del	1.6	F	<60	≥2	2	Group 3
15	PPM1D	c.1469T>A	1.5	M	60+	NA	0	Group 2

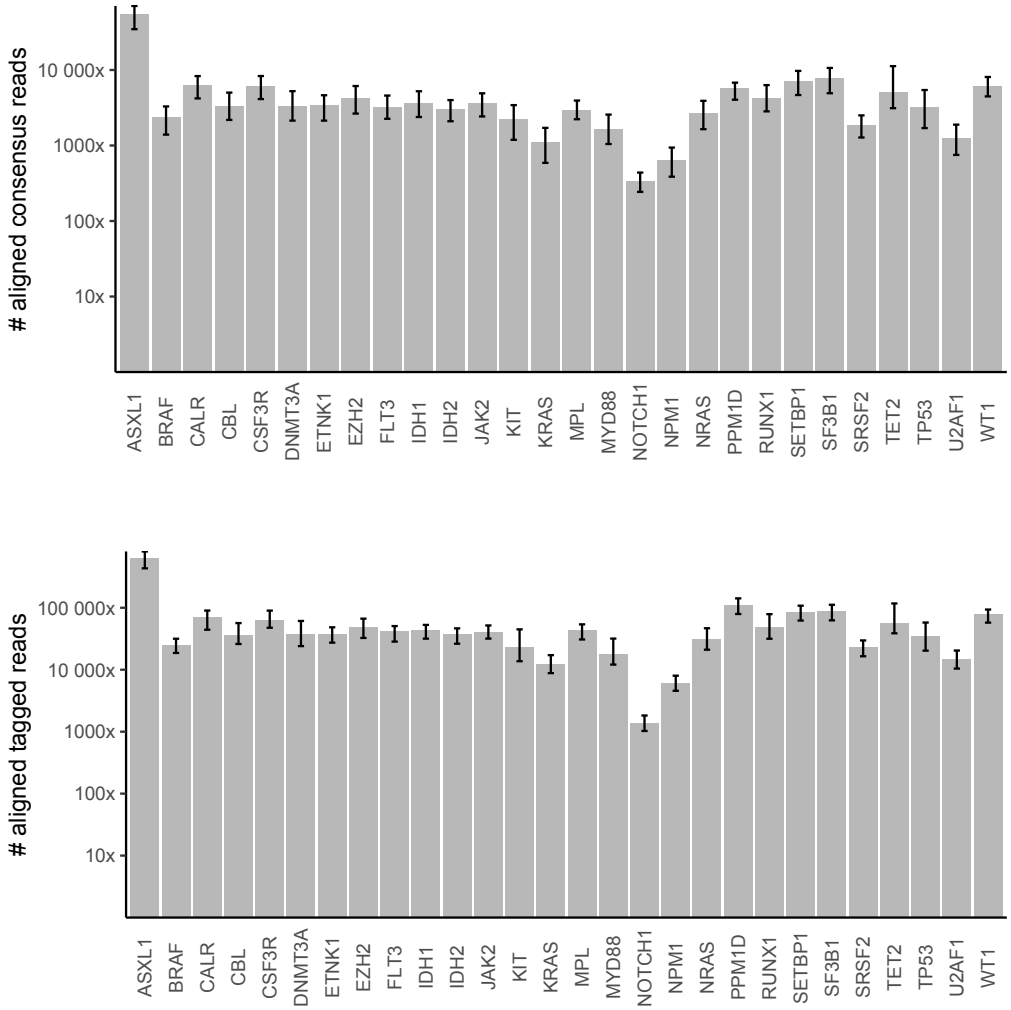
NA, apheresis was not initiated; VAF, variant allele frequency. *Number of CD34+ cells collected ($x10^9$/kg).

Supplementary Table 4. Characteristics of TP53 and PPM1D mutant patients.

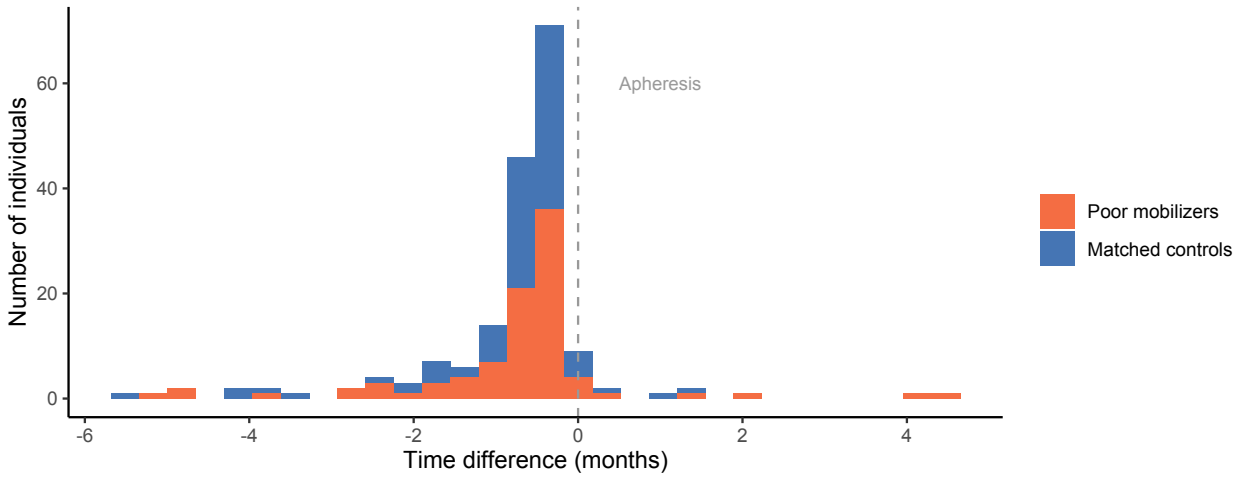
	Absence of clonal hematopoiesis	TP53 or PPM1D mutant	Other mutational spectra	P-value	N
	n=131	**n=16**	**n=32**		
Male sex - n (%)	97 (74.0%)	12 (75.0%)	20 (62.5%)	0.429	179
Age at apheresis (years) – median [IQR]	59.0 [50.0;63.0]	60.5 [55.0;64.2]	60.0 [53.0;64.0]	0.320	179
Major histological subtype - n (%)					179
Aggressive B-cell lymphoma	22 (16.8%)	8 (50.0%)	10 (31.2%)		
Follicular lymphoma	5 (3.82%)	1 (6.25%)	2 (6.25%)		
Hodgkin lymphoma	14 (10.7%)	1 (6.25%)	1 (3.12%)		
Mantle cell lymphoma	20 (15.3%)	0 (0.00%)	2 (6.25%)		
Other non-Hodgkin lymphoma	2 (1.53%)	1 (6.25%)	0 (0.00%)		
Plasma cell dyscrasia	64 (48.9%)	5 (31.2%)	15 (46.9%)		
T-cell lymphoma	4 (3.05%)	0 (0.00%)	2 (6.25%)		
Remission status at time of mobilisation - n (%)					179
CR	52 (40%)	6 (38%)	12 (38%)		
PR or VGPR	72 (55%)	9 (56%)	15 (47%)		
Stable or progressive disease	7 (5%)	1 (6%)	5 (16%)		
Bone marrow infiltration – n*	5	1	1		43
CD34 yield (10^6/kg) - median [IQR]	8.20 [5.44;11.5]	4.26 [2.15;7.58]	9.69 [6.10;11.6]	0.007	157
Number of apheresis days - median [IQR]	2.00 [1.00;2.50]	2.00 [1.00;2.00]	2.00 [1.00;2.00]	0.705	179
Allogeneic transplantation - n (%)	17 (13.0%)	2 (12.5%)	4 (12.5%)	1.000	179
Peripheral blood counts# - mean (SD)					
Hemoglobin level (g/dL)	11.9 (1.62)	10.8 (1.44)	11.8 (1.80)	0.047	179
Platelet count (x 10^9/L)	263 (108)	208 (78.8)	250 (98.9)	0.129	179
WBC (x 10^9/L)	6.32 (3.12)	5.91 (3.59)	7.19 (4.13)	0.345	179
ANC (x 10^9/L)	3.80 (2.08)	2.71 (1.74)	4.75 (2.79)	0.048	112

Data are presented as mean (SD), median [IQR] or n (%), as appropriate. ANC: absolute neutrophil count; CR: complete remission; IQR: interquartile range; PR: partial remission; SD: standard deviation; VGPR: very good partial response; WBC: white blood cell count. #Peripheral blood levels were recorded before start of the chemomobilisation regimen. *For lymphoma patients with stable or progressive disease or partial remission.

Supplementary Figure 1. Coverage across all samples in the sequenced cohort of poor mobilizers and controls (n=179).

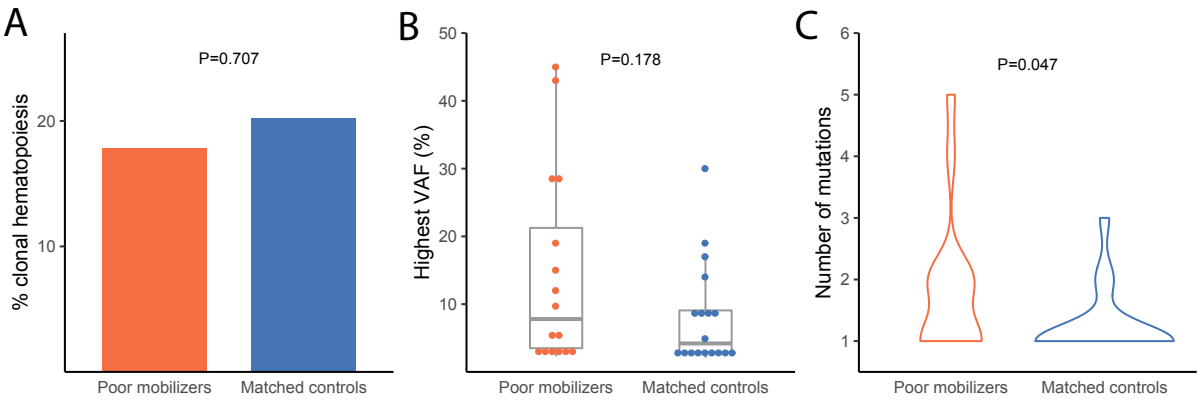


Supplementary Figure 2. Time differences between (planned) date of apheresis and DNA sample collection.



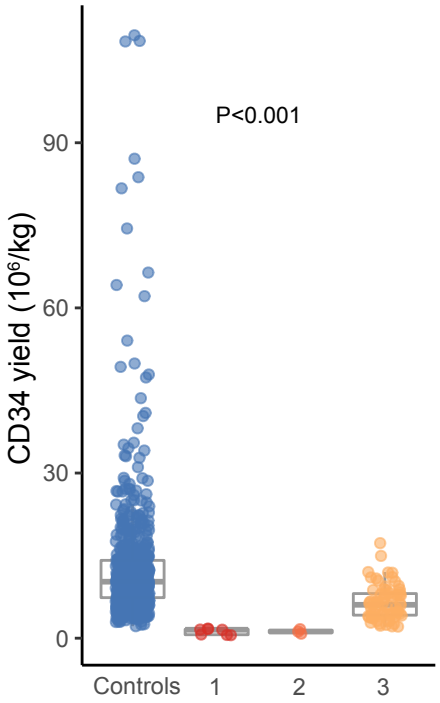
Supplementary Figure 3. Spectrum of CHIP (VAF ≥2%) detected in poor mobilizers and controls

To compare with recent literature, we additionally performed analyses restricting our variant calls to mutations with VAF ≥2%, corresponding to proposed definition of "clonal hematopoiesis of indeterminate potential" (CHIP)[1]. A total of 51 mutations in 34 individuals (16 poor mobilizers and 18 matched controls) were detected ≥2% VAF. A) Prevalence of CHIP in 90 poor mobilizers and 89 matched controls. B) Distribution in highest variant allele frequency (VAF) for poor mobilizers (orange) and matched controls (blue) carrying CHIP. Boxplots indicate median, first and third quartiles, with whiskers extending to 1.5x interquartile range. C) Violin plot displaying the distribution in number of detected mutations in poor mobilizers (orange) and controls (blue) carrying CHIP.

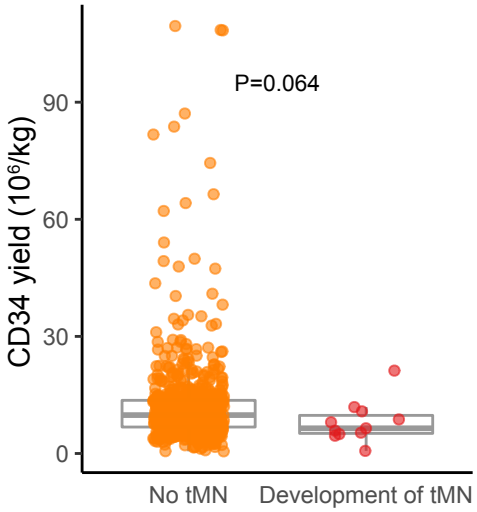


1. Steensma DP, Bejar R, Jaiswal S et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9-16.

Supplementary Figure 4. CD34 yield for controls and failure subgroups.
Controls and failure subgroups 1, 2 and 3 are shown. Leukapheresis was not initiated in 14 respectively 11 individuals from failure subgroups 1 and 2 based on the expected CD34+ collection yield. These data are missing from the analysis.

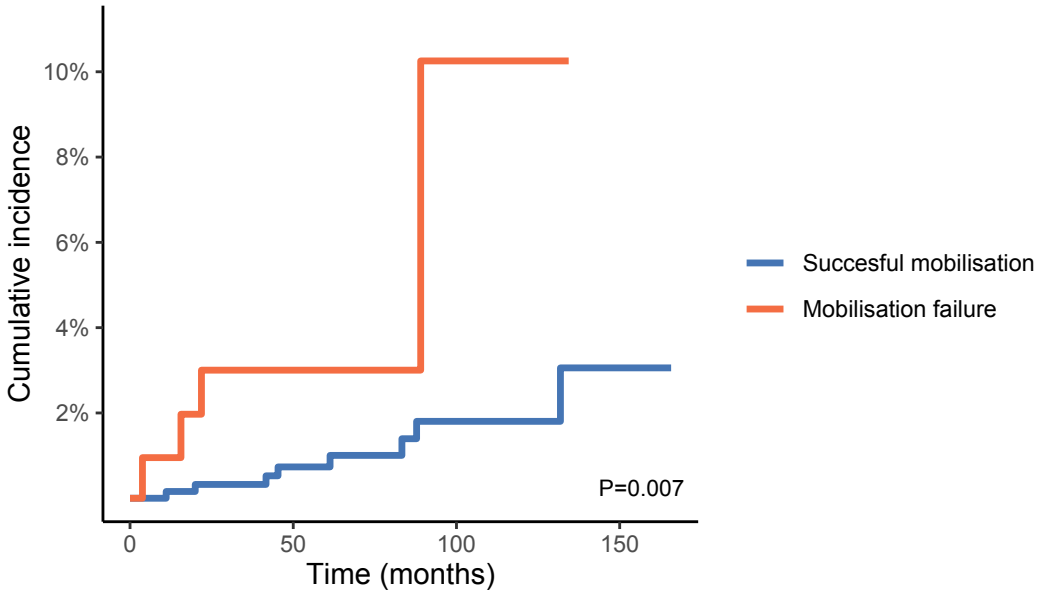


Supplementary Figure 5. CD34 yield for individuals with and without subsequent development of t-MN.



Supplementary Figure 6. Cumulative incidence of t-MN development with allogeneic transplant and death as competing risk.

Cumulative incidence curves were constructed using the Aalen-Johansen estimator, with death and allogeneic transplant as the competing risk. P-values from Gray's test were reported.



Supplementary Figure 7. Overall survival for three groups of mobilization failure and controls.