Supplemental Methods

Genomic DNA isolation

Genomic DNA isolation was performed using the QIAamp Blood DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Whaltam, MA, USA).

Illumina Next Generation Sequencing

The mutational profile of 160 CIN patients was determined using the Trusight Myeloid Sequencing Panel (Illumina, San Diego, CA, USA, Supplementary Table 3). The probe set targets 15 full genes (coding exons and splice sites) and 39 hotspot mutation regions across 568 amplicons of 250 bp in length for a 141 kb total genomic content. The probe pool was hybridized to 250ng of gDNA upstream and downstream of each region of interest. An extension-ligation reaction extended across the selected region followed by a ligation step. The resulting templates were amplified by PCR and two unique library specific indexes were incorporated. The resulting libraries were normalized to the same concentration using a fluorescence-based quantification procedure enabling pooling of libraries. Pooled DNA libraries were loaded onto the cBot System for cluster generation followed by 2x250 paired-end sequencing on a HiSeq2500 sequencer (both from Illumina).

Ion Torrent Next Generation Sequencing using Oncomine Myeloid™ Panel

The mutational profile of 25 CIN patients was investigated using the Oncomine Myeloid™ Panel (Thermo Fisher Scientific, Supplemental Table 4). The panel targets the complete exonic regions of 17 genes and exonic hotspot regions of 23 genes across 526 DNA amplicons with an average length of 279bp for a 113 kb total genomic content. Targets

were amplified starting from 50ng genomic DNA and using two DNA primer pools using highly multiplexed PCR amplification. The amplicons were partially digested with Fupa enzyme followed by ligation of unique barcode adapters for each library. The barcoded libraries were normalized to 100pM using the Equalizer Kit (Thermo Fisher Scientific). The normalized DNA libraries were diluted to optimized concentration. Each library was clonally amplified onto Ion Sphere Particles (ISP) by emulsion PCR with the Ion Chef System (Thermo Fisher Scientific) in line with the manufacturer's instructions. Enriched ISPs were loaded onto 530 chips using an Ion 510 & Ion 520 & Ion 530 Kit-Chef and sequencing was performed on an Ion S5 Prime Sequencer (Thermo Fisher Scientific).

Functional annotation of sequence variants

Synonymous variants and non-coding variants more than two bases from splice junctions were not retained in the study. Mutations with variant allele frequency (VAF) > 40% were excluded from the study as potential germline alterations after thorough examination of the information retrieved from germline population variant databases (PVDs) (dbSNP, 1000G, ExAc, ESP6500, gnomAD), as well as in-house healthy subjects' database. Germline DNA was not available for testing in this study. Variants not found in any of the PVDs at a population frequency ≥ 0.0014 threshold (reflecting the population incidence of myeloid disease and potentially rare variants that could be associated with myeloid malignancies) were labelled as candidate somatic mutations. These somatic variants were finally tagged as oncogenic based on the information retrieved from the literature (if previously known and reported as pathogenic variants) and from mutation databases (including the Catalogue of Somatic Mutations in Cancer (COSMIC),[1] cBioPortal platform (http://www.cbioportal.org), IARC *TP53* (http://p53.iarc.fr), ClinVar,[2] Clinical

Interpretations of Variants in Cancer (CIViC),[3] Ensembl,[4] MyCancerGenome[5]), on the expected effect on protein structure and function, involvement of conserved and functional domain, functional assay and *in silico* prediction, as previously described.[6] Moreover, manual curation of published parameters and datasets of criteria for identification of driver mutations associated with myeloid pathogenicity, where gene-, domain-, and site-specific mutation characteristics are taken into account, was performed in order to further discriminate between oncogenic variants and variants of unknown significance.[6-8] The analysis was limited to variants with VAF equal or greater than 2% and with a minimum coverage of 500 reads to avoid potential sequencing errors. The list of variants identified in the study is reported in Supplemental Table 5. VAF was calculated as the number of variant reads divided by the total reads. VAF values were not corrected for copy number variation. Aligned reads were manually curated for confirmation of the presence of the filtered-in variants within the Integrative Genomics Viewer (IGV) software (Broad Institute).[9]

References

1. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res.* 2017;45(D1):D777–783.
2. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* 2016;44(D1):D862–868.
3. Griffith M, Spies NC, Krysiak K, et al. CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nat Genet.* 2017;49(2):170-174.
4. Cunningham F, Achuthan P, Akanni W, et al. Ensembl 2019. *Nucleic Acids Research.*

2019;47(D1):D745–751.

5. Jain N, Mittendorf KF, Holt M, et al. The My Cancer Genome clinical trial data model and trial curation workflow. *J Am Med Inform Assoc.* 2020;27:1057–1066.
6. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood.* 2013;122(22):3616-3627.
7. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488–2498.
8. Lindsley RC, Saber W, Mar BG, et al. Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. N Engl J Med. 2017;376(6):536-547.
9. Robinson JT, Thorvaldsdóttir H, Winckler W, et al. Integrative genomics viewer. *Nat Biotechnol.* 2011;29(1):24–26.

Supplemental Table 1. Clinical and laboratory data of the CIN patients studied

UPN	Age (years)	Sex	Duration of disease* (months)	Duration of follow-up** (months)	WBC (x10^9/L)	Neutro (x10^9/L)	Lympho (x10^9/L)	Mono (x10^9/L)	Hb (g/dl)	MCV (fL)	Plts (x10^9/L)	Oncogenic Somatic Mutations	VAF (%)	Source	Outcome
1@	59	F	24	24	3.6	1.5	1.8	0.3	13.3	93.3	156	DNMT3A P633T	6.06	BM	S.D.
2@	51	M	24	8	3.3	0.7	2.3	0.2	12.7	71.9	159	IDH1 R132S/ DNMT3A R736H/DNMT3A Q248fs	20.9/24.55/ 17.3	BM	Transformation to MDS#
3	75	F	300	288	3.2	1.5	1.3	0.4	14.3	90.1	186	DNMT3A R882H	22.45	PB	S.D.
4	62	F	60	60	4.0	1.7	1.7	0.6	13.8	90.1	265	TET2 A295fs	25.42	PB	S.D.
5	69	M	12	8	3.3	1.1	1.8	0.3	14	91.5	150	ZRSR2 R295X	14.04	PB	S.D.
6	51	F	312	228	3.7	1.4	1.9	0.3	12.8	88.6	185	DNMT3A M852L	3.77	PB	S.D.
7@	73	F	132	108	2.5	0.5	1.5	0.5	14.3	89.1	168	TET2 H1382R/TET2 Q969X	8.67/13.94	PB	S.D.
8@	54	F	168	168	3.8	1.2	2.0	0.5	13.4	87.3	246	DNMT3A I780T	4.66	PB	S.D.
9	86	F	360	324	2.7	1.6	0.8	0.2	12.9	91.2	204	DNMT3A P904Q	2.4	PB	S.D.
10	83	F	396	276	3.3	1.7	1.1	0.4	11.3	66.9	150	DNMT3A R882H	17.99	PB	S.D.
11	82	F	216	204	3.0	1.3	1.2	0.3	13.3	87.0	184	SRSF2 P95R	27.6	PB	Transformation to CMML
12@	70	F	168	168	3.4	1.7	1.5	0.2	13.0	83.4	213	DNMT3A A741E	2.98	PB	S.D.
13	87	F	144	132	3.4	1.3	1.6	0.5	12.9	90.9	168	TET2 R1808fs	33.18	PB	S.D.
14	44	F	180	180	2.9	1.7	0.8	0.3	13.5	91.7	150	TET2 c.4182+1G>C	2.66	PB	S.D.
15@	55	F	60	48	3.8	1.5	1.8	0.4	13.9	87.0	263	TET2 Y234X	2.95	PB	S.D.
16@	68	M	96	84	3.5	1.7	1.3	0.4	13.6	88.2	240	IDH2 R140Q/SRSF2 P95R	23.19/25.5	PB	Transformation to MDS/MPN$
17	79	F	132	132	3.3	1.7	1.3	0.2	14.3	87.2	158	TET2 S509X	4.1	BM	S.D.
18	65	F	132	96	3.6	1.4	1.9	0.2	13.5	89.4	227	DNMT3A R882H	2.51	BM	S.D.
19	87	F	348	300	3.6	1.6	1.8	0.2	13.4	89.4	263	DNMT3A R366G	3.42	PB	S.D.
20@	44	F	324	324	3.3	1.2	1.6	0.3	12.5	88.9	241	DNMT3A P799A	2.76	PB	S.D.
21@	61	M	108	48	2.1	0.6	1.3	0.2	15.8	78.4	162	IDH1 R132S	12.75	PB	Transformation to AML&
22***	45	F	180	108	3.5	1.6	1.6	0.3	13.9	90.8	253	N.D.		PB	S.D.
23***	46	F	156	156	3.4	1.5	1.6	0.2	12.8	85.2	171	N.D.		PB	S.D.
24***	55	F	60	48	3.5	1.4	1.9	0.2	13.1	93.5	210	N.D.		PB	S.D.
25	51	F	132	96	3.9	1.3	2.0	0.5	12.3	94.3	232	N.D.		BM	S.D.
26***	67	F	300	132	2.4	1.1	1.1	0.2	12.6	90.2	236	N.D.		PB	S.D.
27***@	69	F	408	132	3.6	1.4	1.8	0.3	12.6	84.1	201	N.D.		PB	S.D.
28***	32	F	96	72	2.2	0.7	1.1	0.2	14.3	91.2	151	N.D.		BM	S.D.
29***	75	F	300	156	3.5	1.7	1.1	0.3	12.6	94.6	154	N.D.		PB	S.D.
30	62	M	168	156	3.8	1.4	2.0	0.4	13.5	92.7	210	N.D.		PB	S.D.
31***	28	F	216	132	3.0	1.5	1.3	0.2	12.0	81.5	303	N.D.		PB	S.D.
32	87	F	312	312	3.8	1.5	1.7	0.4	14.3	87.0	196	N.D.		PB	S.D.
33***	60	F	204	204	4.2	1.6	2.3	0.2	13.2	90.1	229	N.D.		PB	S.D.
34	68	F	60	24	4.2	1.7	2.1	0.3	13.9	92.7	225	N.D.		PB	S.D.
35***	89	F	396	324	2.7	1.0	1.3	0.2	13.3	87.0	200	N.D.		PB	S.D.
36***	55	F	204	192	3.8	1.7	1.3	0.6	12.1	82.4	271	N.D.		PB	S.D.

37***@	66	F	348	108	3.6	1.7	1.7	0.2	12.9	90.9	263	N.D.	PB	S.D.
38***	41	F	132	132	3.7	1.7	1.6	0.4	12.5	94.1	171	N.D.	BM	S.D.
39	40	F	288	144	2.4	0.5	0.9	0.1	12.6	93.4	250	N.D.	PB	S.D.
40	50	F	192	168	3.4	1.4	1.7	0.3	12.7	89.5	271	N.D.	PB	S.D.
41@	57	F	228	108	3.3	1.5	1.6	0.3	13.6	92.7	263	N.D.	BM	S.D.
42	39	F	132	108	2.5	0.5	1.2	0.7	13.4	88.2	205	N.D.	PB	S.D.
43	86	F	360	324	3.4	1.6	1.1	0.4	12.5	91.2	204	N.D.	PB	S.D.
44@	52	F	240	240	3.7	1.5	1.7	0.3	13.4	88.0	231	N.D.	PB	S.D.
45	82	F	372	336	2.7	1.2	1.1	0.2	13.7	87.4	186	N.D.	PB	S.D.
46	29	F	180	144	3.7	0.6	2.4	0.4	11.8	85.8	288	N.D.	PB	S.D.
47@	62	F	192	168	3.4	1.5	1.3	0.4	12.1	89.9	329	N.D.	PB	S.D.
48	32	M	144	84	3.0	0.4	1.8	0.6	13.8	93.4	212	N.D.	BM	S.D.
49	51	M	144	96	3.1	1.0	1.7	0.2	15.4	92.9	170	N.D.	PB	S.D.
50	43	F	216	84	3.1	0.2	2.5	0.3	13.0	85.9	313	N.D.	BM	S.D.
51@	58	F	168	144	3.1	0.8	1.7	0.4	12.9	81.9	203	N.D.	PB	S.D.
52@	73	F	360	288	3.7	1.7	1.7	0.3	10.6	67.8	265	N.D.	PB	S.D.
53	40	F	216	132	3.0	1.5	1.1	0.3	12.5	87.5	186	N.D.	BM	S.D.
54	79	F	180	120	3.3	1.4	1.4	0.5	10.9	84.8	163	N.D.	PB	S.D.
55	46	F	264	132	3.2	1.4	1.4	0.2	12.9	87.2	150	N.D.	PB	S.D.
56@	25	M	180	84	2.9	0.2	1.7	0.6	14.2	94.0	239	N.D.	BM	S.D.
57	32	F	180	180	3.2	0.4	2.2	0.5	13.2	90.4	298	N.D.	BM	S.D.
58	82	F	300	228	3.0	1.4	1.2	0.3	13.1	89.6	259	N.D.	PB	S.D.
59	82	F	324	288	4.0	1.7	2.0	0.3	12.9	90.1	218	N.D.	PB	S.D.
60	75	F	348	228	3.7	1.4	1.7	0.4	13.1	89.0	246	N.D.	PB	S.D.
61	43	M	156	120	2.5	0.3	1.6	0.5	16.0	94.1	172	N.D.	PB	S.D.
62	47	M	240	156	3.1	0.4	2.1	0.5	15.1	90.7	267	N.D.	PB	S.D.
63	50	F	300	180	3.6	1.7	1.2	0.5	13.4	80.3	270	N.D.	PB	S.D.
64	44	F	192	180	3.5	1.7	1.6	0.1	13.6	85.9	184	N.D.	PB	S.D.
65	88	F	216	204	3.4	1.7	1.2	0.3	11.6	65.0	211	N.D.	PB	S.D.
66	57	F	264	144	3.5	1.7	1.5	0.2	14.0	91.3	243	N.D.	PB	S.D.
67	64	M	156	156	3.6	1.5	1.7	0.4	13.6	88.8	181	N.D.	PB	S.D.
68	80	F	228	180	5.7	1.7	3.3	0.5	13.0	87.0	266	N.D.	PB	S.D.
69	77	F	252	192	3.5	1.6	1.4	0.3	12.7	90.1	187	N.D.	PB	S.D.
70	73	F	168	168	3.4	1.6	1.4	0.4	12.8	89.0	218	N.D.	PB	S.D.
71	52	F	144	144	4.1	1.7	1.5	0.3	12.8	89.0	265	N.D.	PB	S.D.
72	61	F	144	144	4.6	1.6	2.5	0.2	13.1	94.3	177	N.D.	PB	S.D.
73@	55	F	288	276	3.9	1.7	1.8	0.3	11.2	97.2	185	N.D.	PB	S.D.
74@	70	F	192	180	3.7	1.7	1.6	0.3	13.6	89.3	243	N.D.	PB	S.D.
75	51	F	240	216	3.1	1.6	1.2	0.3	13.4	87.2	280	N.D.	PB	S.D.
76	61	M	144	144	4.6	1.7	2.2	0.3	15.4	90.5	195	N.D.	PB	S.D.
77	66	F	204	180	3.4	1.7	1.5	0.2	13.3	87.4	331	N.D.	PB	S.D.
78	62	F	192	156	3.7	1.7	1.7	0.3	12.3	84.5	165	N.D.	PB	S.D.
79	57	F	168	168	2.9	1.3	1.2	0.4	12.2	82.6	193	N.D.	PB	S.D.
80	87	M	96	96	2.6	0.4	2.0	0.1	13.0	87.0	162	N.D.	PB	Transformation to MDS#
81	69	F	120	120	4.0	1.7	2.0	0.3	13.6	95.7	150	N.D.	BM	S.D.

82[@]	44	F	24	24	3.4	1.7	1.2	0.4	12.4	88.3	163	N.D.	PB	S.D.
83[@]	66	F	24	24	3.0	1.7	1.3	0.4	13.4	88.8	244	N.D.	PB	S.D.
84	34	F	96	36	2.3	0.3	1.2	0.6	12.7	90.8	311	N.D.	PB	S.D.
85[@]	64	F	36	24	4.2	1.7	2.2	0.2	12.7	84.5	311	N.D.	PB	S.D.
86[@]	57	F	72	48	3.7	1.6	1.8	0.3	14.8	90.0	153	N.D.	PB	S.D.
87	29	F	96	24	3.0	0.6	2.0	0.3	12.8	85.9	177	N.D.	PB	S.D.
88	60	M	168	144	2.5	0.6	1.5	0.3	15.6	90.1	200	N.D.	BM	S.D.
89	72	F	204	180	4.2	1.7	2.0	0.4	13.5	87.5	298	N.D.	BM	S.D.
90	82	F	300	276	3.4	1.3	1.5	0.5	12.5	95.5	171	N.D.	PB	S.D.
91	38	F	192	180	4.4	1.5	2.2	0.5	14.1	85.2	319	N.D.	PB	S.D.
92	38	F	216	204	4.5	1.4	2.5	0.5	12.6	85.0	277	N.D.	PB	S.D.
93	60	F	168	156	3.7	1.7	1.6	0.4	12.8	93.9	176	N.D.	PB	S.D.
94	81	F	300	216	2.8	1.7	1.5	0.5	12.9	90.7	228	N.D.	PB	S.D.
95	62	F	264	252	3.4	1.2	1.5	0.7	12.5	88.5	208	N.D.	PB	S.D.
96	23	F	180	48	3.2	0.7	1.8	0.6	12.4	86.7	322	N.D.	PB	S.D.
97	56	M	144	36	3.3	1.7	1.1	0.5	15.1	87.5	180	N.D.	PB	S.D.
98	51	F	276	60	3.3	1.7	1.3	0.4	13.6	92.1	202	N.D.	PB	S.D.
99	60	F	348	336	2.1	0.3	1.1	0.4	12.3	86.6	316	N.D.	PB	S.D.
100	34	F	36	24	3.7	1.7	1.7	0.3	12.6	87.9	248	N.D.	PB	S.D.
101	37	M	48	36	2.7	0.8	1.5	0.4	15.1	91.1	197	N.D.	PB	S.D.
102	62	F	348	180	3.6	1.7	1.1	0.4	13.7	92.0	247	N.D.	PB	S.D.
103	70	F	192	168	3.3	1.6	1.3	0.3	14.4	89.5	261	N.D.	PB	S.D.
104	55	F	168	168	3.4	1.7	1.4	0.3	13.4	89.6	336	N.D.	PB	S.D.
105	66	F	168	60	3.7	1.6	1.8	0.4	12.7	86.8	195	N.D.	PB	S.D.
106	60	F	216	168	3.5	1.7	1.3	0.3	13.7	90.9	332	N.D.	PB	S.D.
107	61	F	228	144	3.3	1.7	1.3	0.2	12.7	94.0	261	N.D.	PB	S.D.
108	68	F	144	132	4.7	1.7	2.1	0.4	12.5	84.9	233	N.D.	PB	S.D.
109	66	F	168	156	3.7	1.7	1.6	0.4	13.4	90.9	167	N.D.	PB	S.D.
110[@]	56	F	216	192	3.0	1.1	1.5	0.2	13.1	87.9	231	N.D.	PB	S.D.
111[@]	62	F	132	132	3.4	1.5	1.4	0.3	12.9	91.7	168	N.D.	PB	S.D.
112[@]	23	M	96	72	3.2	1.2	1.9	0.2	12.9	85.5	168	N.D.	PB	S.D.
113[@]	35	F	108	36	2.0	1.0	0.7	0.2	12.9	66.9	168	N.D.	PB	S.D.
114	44	M	24	24	3.0	1.5	1.2	0.3	12.9	97.3	168	N.D.	PB	S.D.
115	24	M	24	24	3.5	1.7	1.4	0.3	14.2	87.7	272	N.D.	PB	S.D.
116	42	F	156	48	3.3	1.6	1.2	0.5	13.2	82.9	229	N.D.	PB	S.D.
117[@]	19	M	24	24	2.2	0.7	1.1	0.4	13.2	85.2	229	N.D.	PB	S.D.
118[@]	47	M	36	36	3.9	1.7	2.0	0.2	13.2	92.2	229	N.D.	PB	S.D.
119	52	F	24	24	3.0	1.0	1.6	0.4	12.6	82.1	263	N.D.	PB	S.D.
120	23	F	36	36	3.5	1.4	1.9	0.2	13.9	83.8	263	N.D.	PB	S.D.
121	64	F	84	60	4.6	1.3	2.8	0.4	13.9	91.9	263	N.D.	PB	S.D.
122	34	F	132	108	2.9	1.2	1.3	0.3	13.9	87.1	263	N.D.	PB	S.D.
123	30	M	24	24	3.7	0.8	2.0	0.5	13.9	88.4	263	N.D.	PB	S.D.
124	66	F	24	24	4.8	1.7	2.5	0.5	13.9	89.5	263	N.D.	PB	S.D.
125	46	F	36	24	3.9	1.7	1.8	0.3	13.9	89.1	263	N.D.	PB	S.D.
126	61	F	24	24	3.2	1.7	1.1	0.3	13.3	88.6	262	N.D.	PB	S.D.
127	77	F	300	168	4.1	1.3	2.3	0.3	12.6	81.9	256	N.D.	PB	S.D.

128	60	F	204	204	3.8	1.7	1.0	0.1	12.6	93.9	206	N.D.	PB	S.D.
129[@]	57	F	72	24	3.8	1.7	1.9	0.2	12.7	97.7	211	N.D.	PB	S.D.
130	75	F	312	288	3.0	1.7	1.1	0.2	12.9	83.0	176	N.D.	PB	S.D.
131[@]	35	M	24	24	3.1	1.5	1.1	0.3	15.0	83.3	177	N.D.	PB	S.D.
132	79	M	216	204	3.8	1.7	1.6	0.4	15.5	92.0	214	N.D.	PB	S.D.
133	70	F	360	240	3.6	1.7	1.6	0.3	12.5	92.2	287	N.D.	PB	S.D.
134	46	M	192	180	5.2	1.7	3.2	0.3	14.8	90.6	273	N.D.	PB	S.D.
135	78	M	252	180	3.8	1.7	1.7	0.2	15.0	87.0	192	N.D.	PB	S.D.
136	54	F	228	180	3.0	1.5	1.0	0.3	13.4	87.6	160	N.D.	PB	S.D.
137	78	F	300	276	4.5	1.7	2.3	0.4	12.9	90.8	238	N.D.	PB	S.D.
138	59	F	300	264	3.5	1.7	1.4	0.3	13.2	93.7	150	N.D.	PB	S.D.
139	57	M	228	156	4.5	1.6	2.3	0.3	14.3	95.0	203	N.D.	PB	S.D.
140	88	F	228	228	3.4	1.7	1.6	0.2	14.0	87.3	181	N.D.	PB	S.D.
141[@]	59	F	168	72	3.7	1.3	2.0	0.3	13.6	89.4	228	N.D.	PB	S.D.
142[@]	59	F	96	72	4.8	1.7	2.4	0.4	13.8	88.8	260	N.D.	PB	S.D.
143	64	F	132	120	3.6	1.4	1.9	0.3	13.5	94.2	294	N.D.	PB	S.D.
144	81	M	204	144	3.5	0.9	2.2	0.2	16.2	94.6	260	N.D.	PB	S.D.
145	68	F	300	288	3.3	1.6	1.4	0.2	12.8	84.3	221	N.D.	PB	S.D.
146	50	F	156	144	3.0	1.4	1.2	0.3	12.8	89.0	182	N.D.	PB	S.D.
147	59	F	216	156	3.6	1.7	1.3	0.3	12.9	88.3	274	N.D.	PB	S.D.
148	75	F	144	132	4.4	1.0	2.8	0.4	13.4	91.0	205	N.D.	PB	S.D.
149	83	F	108	108	3.6	1.6	1.8	0.2	13.4	93.2	263	N.D.	PB	S.D.
150	85	F	156	156	3.8	1.7	1.3	0.6	13.4	72.8	271	N.D.	PB	S.D.
151	57	F	120	72	3.8	1.7	1.3	0.6	13.4	86.5	271	N.D.	PB	S.D.
152	30	F	108	108	3.8	1.7	1.3	0.6	13.4	86.8	271	N.D.	PB	S.D.
153	35	F	180	108	3.8	1.7	1.3	0.6	13.4	85.3	271	N.D.	PB	S.D.
154	75	F	120	72	3.8	1.7	1.3	0.6	13.4	86.1	271	N.D.	PB	S.D.
155	48	M	180	156	3.8	1.7	1.6	0.3	15.9	85.2	252	N.D.	PB	S.D.
156	89	F	144	120	3.6	1.7	1.4	0.3	12.5	94.8	220	N.D.	PB	S.D.
157	48	M	132	108	3.0	1.6	1.1	0.3	14.6	88.9	217	N.D.	PB	S.D.
158	61	F	132	120	3.4	1.7	1.3	0.3	12.5	93.9	177	N.D.	PB	S.D.
159	69	M	84	48	4.7	1.7	2.2	0.4	14.0	90.5	188	N.D.	PB	S.D.
160	64	F	132	132	3.4	1.7	1.3	0.3	12.5	92.0	177	N.D.	PB	S.D.
161	64	F	228	156	4.0	1.7	2.1	0.1	12.5	94.8	226	N.D.	PB	S.D.
162	61	F	84	84	4.0	1.7	2.1	0.1	12.4	90.0	226	N.D.	PB	S.D.
163	47	F	48	48	4.0	1.7	2.1	0.1	12.6	73.4	226	N.D.	PB	S.D.
164	45	F	60	36	4.0	1.7	2.1	0.1	12.2	88.9	226	N.D.	PB	S.D.
165	28	F	84	84	4.0	1.7	2.1	0.1	12.6	89.9	226	N.D.	PB	S.D.
166	46	F	192	12	4.0	1.7	2.1	0.1	12.5	92.0	226	N.D.	PB	S.D.
167	43	F	36	24	3.7	1.7	1.6	0.4	12.6	89.5	253	N.D.	PB	S.D.
168	68	F	144	144	2.8	1.5	1.1	0.2	13.4	89.0	159	N.D.	PB	S.D.
169	58	F	24	12	3.5	1.5	1.6	0.3	12.5	86.0	270	N.D.	PB	S.D.
170	58	F	108	12	3.1	1.7	1.1	0.3	13.3	94.5	150	N.D.	PB	S.D.
171[@]	63	M	372	108	3.4	0.7	1.5	0.4	14.2	82.0	187	N.D.	PB	S.D.
172	67	F	12	12	2.5	0.4	1.5	0.5	14.3	93.7	192	N.D.	PB	S.D.
173	52	F	180	12	2.4	1.4	0.8	0.2	12.2	84.7	150	N.D.	PB	S.D.

174	31	F	132	12	1.9	0.3	1.1	0.4	12.6	75.9	233	N.D.	PB	S.D.
175	35	M	12	12	4.0	1.5	2.0	0.4	15.2	93.9	159	N.D.	PB	S.D.
176	66	F	372	228	3.5	1.7	1.4	0.4	12.3	86.7	210	N.D.	PB	S.D.
177	27	M	12	8	2.9	0.8	1.3	0.6	15.1	84.8	178	N.D.	PB	S.D.
178	40	M	12	8	2.6	0.3	1.6	0.6	14.5	87.7	280	N.D.	PB	S.D.
179	58	F	60	12	3.3	1.4	1.5	0.2	13.9	87.3	182	N.D.	BM	S.D.
180	41	M	84	12	3.3	1.7	1.4	0.1	14.4	92.4	150	N.D.	PB	S.D.
181	31	F	132	24	3.4	0.9	1.7	0.5	14.5	87.3	225	N.D.	PB	S.D.
182	54	F	60	24	4.2	1.4	2.6	0.2	11.2	67.0	241	N.D.	PB	S.D.
183	33	M	24	24	3.7	1.5	1.7	0.4	11.8	79.8	205	N.D.	PB	S.D.
184	61	M	24	8	3.6	1.7	1.5	0.3	13.9	91.1	156	N.D.	PB	S.D.
185	31	M	192	132	2.5	0.8	1.3	0.4	13.3	86	170	N.D.	PB	S.D.

Abbreviations: CIN, chronic idiopathic neutropenia; UPN, unique patient number; WBC, white blood cells; Neutro, neutrophils; Lympho, lymphocytes; Mono, monocytes; Hb, hemoglobin; MCV, mean corpuscular volume; Plts, platelets; VAF, Variant Allele Frequency; N.D., not detected; S.D., stable disease; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MPN,myeloproliferative neoplasm.

* Time from the identification of neutropenia until the last follow-up or disease transformation.

** Time from the diagnosis of CIN in our Department and inclusion in the study until the last follow-up or disease transformation.

*** Patients detected with variants having a VAF within the germline range and following our variant pipeline analysis were subsequently removed and not included in the study.

[@] In these patients, follow-up analysis by next generation sequencing profiling has been performed.

[#] MDS with multilineage dysplasia according to the World Health Organization classification.

[$] Unclassifiable MDS/MPN according to the World Health Organization classification.

[&] Acute myelomonocytic leukemia according to the World Health Organization classification.

Supplemental Table 2. List of 195 genes analyzed (NGS panel) to exclude congenital neutropenia in the Severe Chronic Neutropenia International Registry reference laboratory, Tubingen, Germany.

ABCD4	*COG4*	*FIP1L1*	*IGHM*	*MECOM*	*PCCB*	*RFXAP*	*RPS26*	*SRP19*	*TONSL*
ACP5	*CSF3R*	*FMD3*	*IGLL1*	*MMAA*	*PGM3*	*RINT1*	*RPS27*	*SRP54*	*TP53*
ADA2	*CXCR2*	*FOXP3*	*IL21R*	*MMAB*	*PIK3CD*	*RMRP*	*RPS28*	*SRPRA*	*TSR2*
AGA	*CXCR4*	*FUT8*	*IL7R*	*MMACHC*	*PIK3R1*	*RNF113A*	*RPS29*	*STAT1*	*UBA1*
ANAPC1	*DIPK1A*	*FXN*	*IRAK4*	*MMUT*	*PML*	*RPGR*	*RPS7*	*STAT3*	*UBE2A*
AOPEP	*DLK1*	*G6PC3*	*IRF2BP2*	*MPLKIP*	*PNP*	*RPL11*	*SAMD9L*	*STAT5B*	*USB1*
AP3B1	*DNAJC21*	*GATA1*	*ITCH*	*MRTFA*	*POLG*	*RPL15*	*SARM1*	*STK4*	*VPS13B*
AP3D1	*DNASE1L1*	*GATA2*	*JAGN1*	*MSN*	*POSTN*	*RPL18*	*SBDS*	*STX11*	*VPS45*
BCOR	*DOP1A*	*GFI1*	*KRAS*	*MST1*	*PRDX1*	*RPL26*	*SCN1A*	*TARS1*	*VPS4A*
BLNK	*ELF1*	*GINS1*	*LAMTOR2*	*MTRR*	*PRF1*	*RPL27*	*SDS*	*TA2*	*WAS*
BRCA1	*EIF2AK3*	*GSS*	*LARS2*	*MVK*	*PRKAR1A*	*RPL31*	*SEC61A1*	*TBL1XR1*	*WDR1*
BTK	*ELANE*	*GTF2E2*	*LBR*	*MYSM1*	*PRKCD*	*RPL35*	*SF3B1*	*TCF3*	*WIPF1*
CASP10	*ERCC2*	*GTF2H5*	*LMBRD1*	*NABP1*	*RAB27A*	*RPL35A*	*SH2D1A*	*TCIRG1*	*XIAP*
CCDC107	*ERCC3*	*HAX1*	*LOXL3*	*NAIP*	*RAC2*	*RPL5*	*SLC25A42*	*TCN2*	*ZBTB16*
CD40	*ETV6*	*HCFC1*	*LRBA*	*NFKB2*	*RAG1*	*RPS10*	*SLC35A1*	*TDP2*	*ZNF276*
CD40LG	*FAS*	*HPS3*	*LRRC8A*	*NPM1*	*RARA*	*RPS15A*	*SLC37A4*	*TERC*	*ZNF627*
CD79A	*FASLG*	*HTRA2*	*LYST*	*NRAS*	*RASGRP1*	*RPS17*	*SLC46A1*	*TERT*	
CD79B	*FBXL4*	*HYOU1*	*MAD2L2*	*NUMA1*	*RECQL4*	*RPS19*	*SLX4*	*TET2*	
CIITA	*FDX2*	*ICOS*	*MARS1*	*PACS2*	*RFX5*	*RPS20*	*SMARCAL1*	*TFR2*	
CLP3	*FIBP*	*IFNG*	*MDM4*	*PCCA*	*RFXANK*	*RPS24*	*SMARCD2*	*TFRC*	

Supplemental Table 3. List of 54 genes analyzed by Illumina TruSight Myeloid Sequencing Panel

ABL1	*CALR*	*CSF3R*	*FLT3*	*IDH2*	*KRAS*	*NRAS*	*RUNX1*	*STAG2*
ASXL1	*CBL*	*CUX1*	*GATA1*	*IKZF1*	*MLL*	*PDGFRA*	*SETBP1*	*TET2*
ATRX	*CBLB*	*DNMT3A*	*GATA2*	*JAK2*	*MPL*	*PHF6*	*SF3B1*	*TP53*
BCOR	*CBLC*	*ETV6*	*GNAS*	*JAK3*	*MYD88*	*PTEN*	*SMC1A*	*U2AF1*
BCORL1	*CDKN2A*	*EZH2*	*HRAS*	*KDM6A*	*NOTCH1*	*PTPN11*	*SMC3*	*WT1*
BRAF	*CEBPA*	*FBXW7*	*IDH1*	*KIT*	*NPM1*	*RAD21*	*SRSF2*	*ZRSR2*

Supplemental Table 4. List of 40 genes analyzed by Ion Torrent Oncomine Myeloid™ Panel

ABL1	*CEBPA*	*GATA2*	*KIT*	*NRAS*	*SETBP1*	*TP53*
ASXL1	*CSF3R*	*HRAS*	*KRAS*	*PHF6*	*SF3B1*	*U2AF1*
BCOR	*DNMT3A*	*IDH1*	*MPL*	*PRPF8*	*SRSF2*	*WT1*
BRAF	*ETV6*	*IDH2*	*MYD88*	*PTPN11*	*STAG2*	*ZRSR2*
CALR	*EZH2*	*IKZF1*	*NF1*	*RB1*	*SH2B3*	
CBL	*FLT3*	*JAK2*	*NPM1*	*RUNX1*	*TET2*	

Supplemental Table 5. Clonal mutations identified by next generation sequencing in study participants*

UPN	Chromosome	Position	Reference	Alternate	Gene	Function	Nucleotide change	Aminoacid change	VAF (%)
1	2	25466806	G	T	DNMT3A	nonsynonymous SNV	c.C1897A	p.P633T	6.06
2	2	209113113	G	T	IDH1	nonsynonymous SNV	c.C394A	p.R132S	20.9
2	2	25463286	C	T	DNMT3A	nonsynonymous SNV	c.G2207A	p.R736H	24.55
2	2	25471004	GGTCAGTGGGCTGCT	G	DNMT3A	frameshift deletion	c.743_756delAGCAGCCCACTGAC	p.Q248fs	17.3
3	2	25457242	C	T	DNMT3A	nonsynonymous SNV	c.G2645A	p.R882H	22.45
4	4	106155982	GCC	G	TET2	frameshift deletion	c.884_885delCC	p.A295fs	25.42
5	X	15838385	C	T	ZRSR2	stopgain SNV	c.C883T	p.R295X	14.04
6	2	25458619	T	A	DNMT3A	nonsynonymous SNV	c.A2554T	p.M852L	3.77
7	4	106158004	C	T	TET2	stopgain SNV	c.C2905T	p.Q969X	13.94
7	4	106190867	A	G	TET2	nonsynonymous SNV	c.A4145G	p.H1382R	8.67
8	2	25462068	A	G	DNMT3A	nonsynonymous SNV	c.T2339C	p.I780T	4.66
9	2	25457176	G	T	DNMT3A	nonsynonymous SNV	c.C2711A	p.P904Q	2.4
10	2	25457242	C	T	DNMT3A	nonsynonymous SNV	c.G2645A	p.R882H	17.99
11	17	74732959	G	C	SRSF2	nonsynonymous SNV	c.C284G	p.P95R	27.6
12	2	25463271	G	T	DNMT3A	nonsynonymous SNV	c.C2222A	p.A741E	2.98
13	4	106197089	A	-	TET2	frameshift deletion	c.5422delA	p.R1808fs	33.18
14	4	106190905	G	C	TET2	splicing	c.4182+1G>C		2.66
15	4	106155801	T	G	TET2	stopgain SNV	c.T702G	p.Y234X	2.95
16	15	90631934	C	T	IDH2	nonsynonymous SNV	c.G419A	p.R140Q	23.19
16	17	74732959	G	C	SRSF2	nonsynonymous SNV	c.C284G	p.P95R	25.5
17	4	106156625	C	G	TET2	stopgain SNV	c.C1526G	p.S509X	4.1
18	2	25457242	C	T	DNMT3A	nonsynonymous SNV	c.G2645A	p.R882H	2.51
19	2	25469946	G	C	DNMT3A	nonsynonymous SNV	c.C1096G	p.R366G	3.42
20	2	25462012	G	C	DNMT3A	nonsynonymous SNV	c.C2395G	p.P799A	2.76
21	2	209113113	G	T	IDH1	nonsynonymous SNV	c.C394A	p.R132S	12.75

* Each mutation in each individual was identified by chromosome, position, nucleotide sequence of reference and alternate, associated gene, function, nucleotide and amino acid change and the percentage of variant allele frequency.

Abbreviations: UPN, unique patient number; VAF, Variant Allele Frequency.

Supplemental Table 6. Missense mutations identified by next generation sequencing in this study were categorized according to whether they are known either in clonal hematopoiesis or malignancy literature or they are novel.

UPN	Gene	Nucleotide change	Aminoacid change	Clonal Hematopoiesis	Malignancy	Novel
1	DNMT3A	c.C1897A	p.P633T	No	Yes[1]	No
2	IDH1	c.C394A	p.R132S	Yes[2,3]	Yes[1,4]	No
2	DNMT3A	c.G2207A	p.R736H	Yes[5,6]	Yes[1,4]	No
3	DNMT3A	c.G2645A	p.R882H	Yes[2,3]	Yes[1,4]	No
6	DNMT3A	c.A2554T	p.M852L	No	Yes[1]	No
7	TET2	c.A4145G	p.H1382R	No	Yes[4]	No
8	DNMT3A	c.T2339C	p.I780T	Yes[5,6]	Yes[4]	No
9	DNMT3A	c.C2711A	p.P904Q	Yes[6]	Yes[4]	No
10	DNMT3A	c.G2645A	p.R882H	Yes[2.3]	Yes[1,4]	No
11	SRSF2	c.C284G	p.P95R	Yes[2.3]	Yes[1,4]	No
12	DNMT3A	c.C2222A	p.A741E	Yes[5]	No	No
16	IDH2	c.G419A	p.R140Q	Yes[2.3]	Yes[1,4]	No
16	SRSF2	c.C284G	p.P95R	Yes[2.3]	Yes[1,4]	No
18	DNMT3A	c.G2645A	p.R882H	Yes[2.3]	Yes[1,4]	No
19	DNMT3A	c.C1096G	p.R366G	Yes[2]	No	No
20	DNMT3A	c.C2395G	p.P799A	No	Yes[4]	No
21	IDH1	c.C394A	p.R132S	Yes[2.3]	Yes[1,4]	No

[1] http://www.cbioportal.org/

[2] Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014;371(26):2488–2498.

[3] Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med.* 2014;371(26):2477–2787.

[4] Catalogue of Somatic Mutations in Cancer, COSMIC (Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res.* 2017;45(D1):D777–783).

[5] Young AL, Tong RS, Birmann BM, Druley TE. Clonal hematopoiesis and risk of acute myeloid leukemia. *Haematologica*. 2019; 104(12): 2410–2417.

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