

Myelodysplastic Syndrome associated TET2 mutations affect NK cell function and genome methylation

Supplementary Information

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Supplementary Table 5. Mutations association in the whole cohort according to *TET2/IDH* mutations.

Supplementary Table 6. NGS at diagnosis in WMC and in NK/T sorted cells.

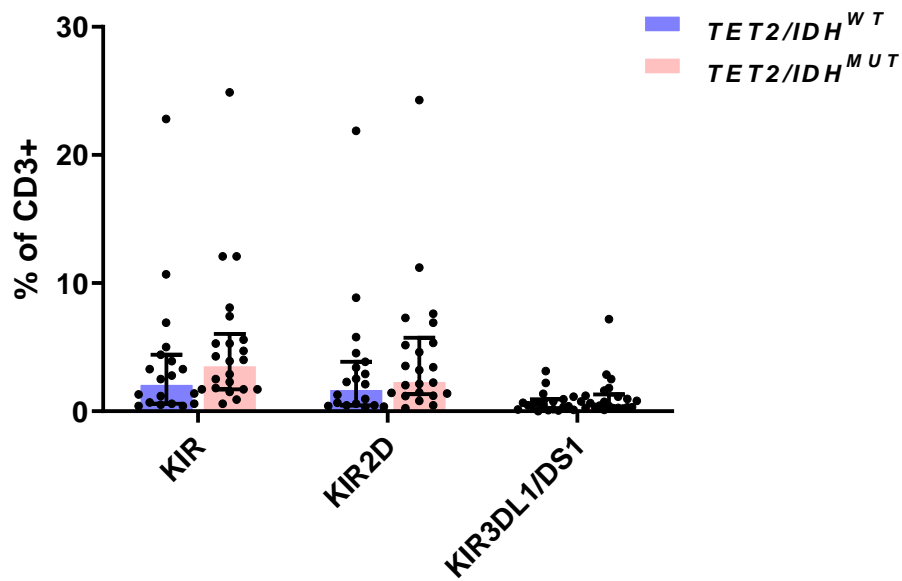
Supplementary Table 7. Patients' characteristics.

Supplementary Table 8. Patients of the Clinical Trial NCT02985190.

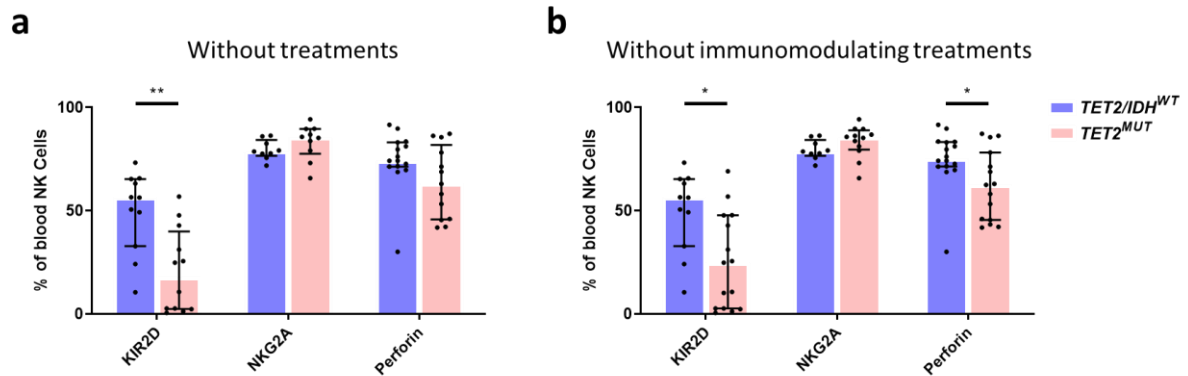
Supplementary Table 9. List of Antibodies used for Flow Cytometry.

Supplementary Table 10. List of primers used for the ChIP-qPCR assay.

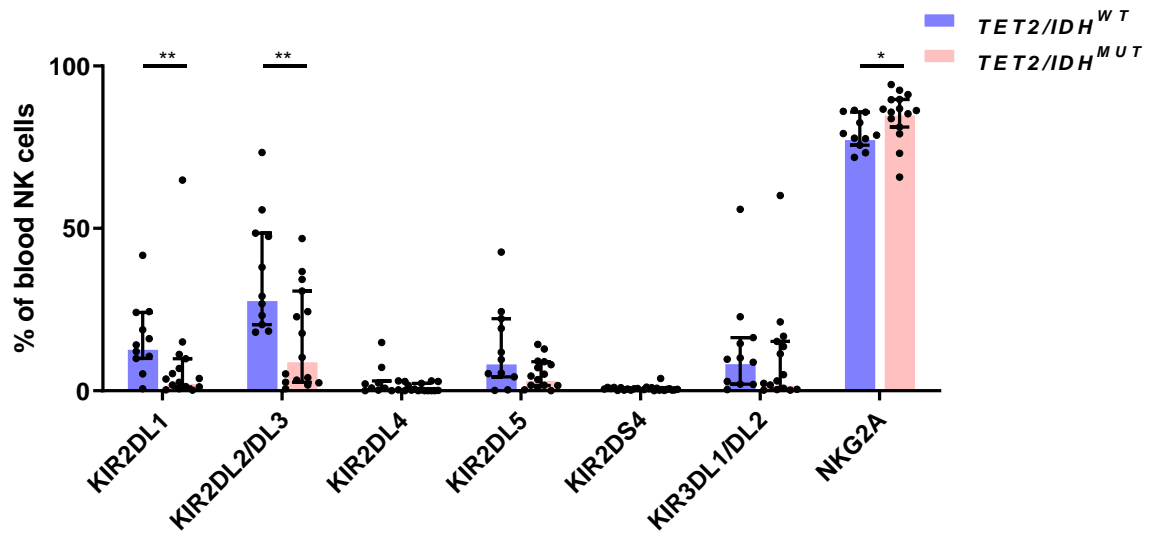
Supplementary Table 11. Specificities and probes used in the Multiplex RT-PCR analysis.



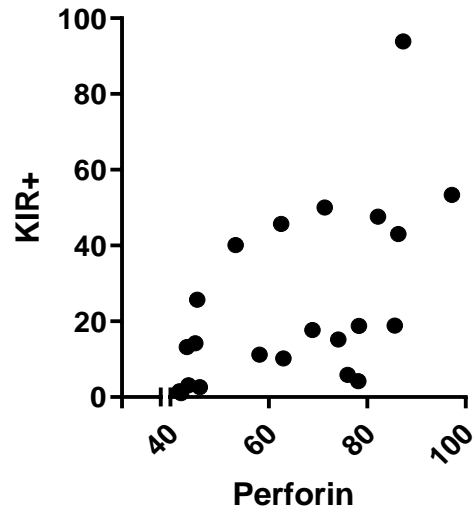
Supplementary Figure 1. KIR expression on CD3⁺ T cells in MDS/CMML patients according to the presence or absence of *TET2/IDH* mutations. Representation of the KIR, KIR2D and KIR3DL1/DS1 expression in blood CD3⁺ T cells of *TET2/IDH^{WT}* (n=19) and *TET2/IDH^{MUT}* (n=22). For all the analysis, median is represented, and error bars represents interquartile range. KIR = (KIR2D+ KIR3DL1/DS1-, KIR2D+ KIR3DL1/DS1+ and KIR2D- KIR3DL1/DS1+)



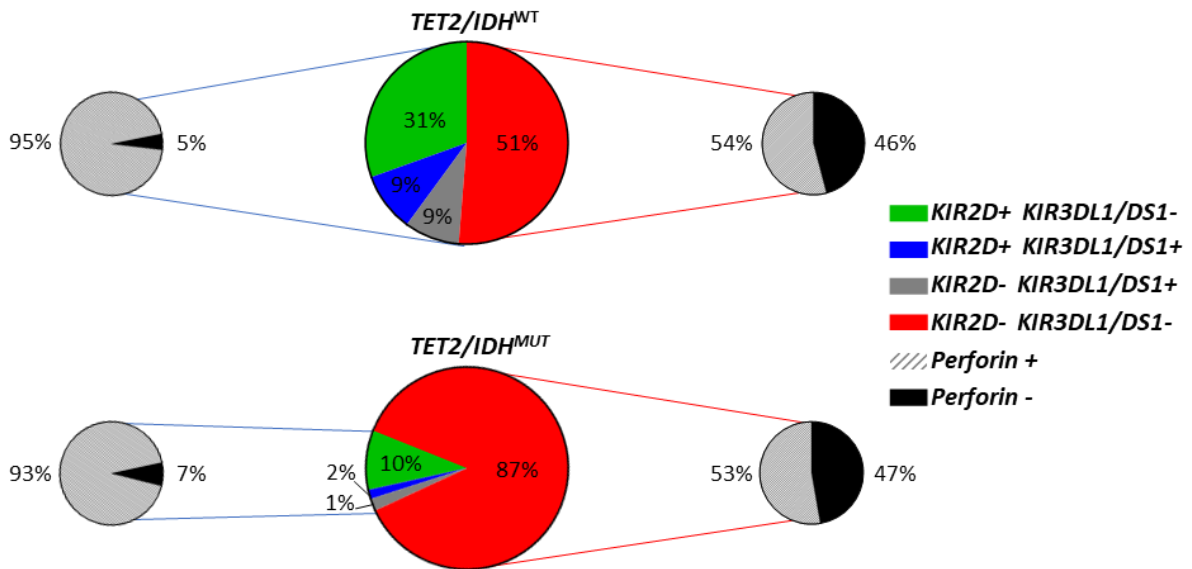
Supplementary Figure 2. Expression of KIR2D, NKG2A and Perforin in blood NK cells in absence of treatments. Expression of KIR2D, NKG2A and perforin in blood NK cells has been quantified by flow-cytometry. (a) markers expressions were analyzed in comparing $TET2/IDH^{WT}$ (n=11 for KIR2D, n=9 for NKG2A n=15 for perforin) and $TET2^{MUT}$ (n=13 for KIR2D, n=11 for NKG2A, n=13 for perforin) MDS patients in absence of any treatment. Statistics were calculated with the nonparametric Mann-Whitney test, two-sided, ** p=0.0036. (b) A similar analysis has been performed in $TET2/IDH^{WT}$ (n=11 for KIR2D, n=9 for NKG2A, n=16 for perforin) or $TET2^{MUT}$ (n=15, n=12 for NKG2A) MDS patients in absence of immunomodulating treatments. Data are presented as medians and interquartile ranges. Statistics were calculated with the two-sided nonparametric Mann-Whitney test, *p=0.0305, ** p=0.0092.



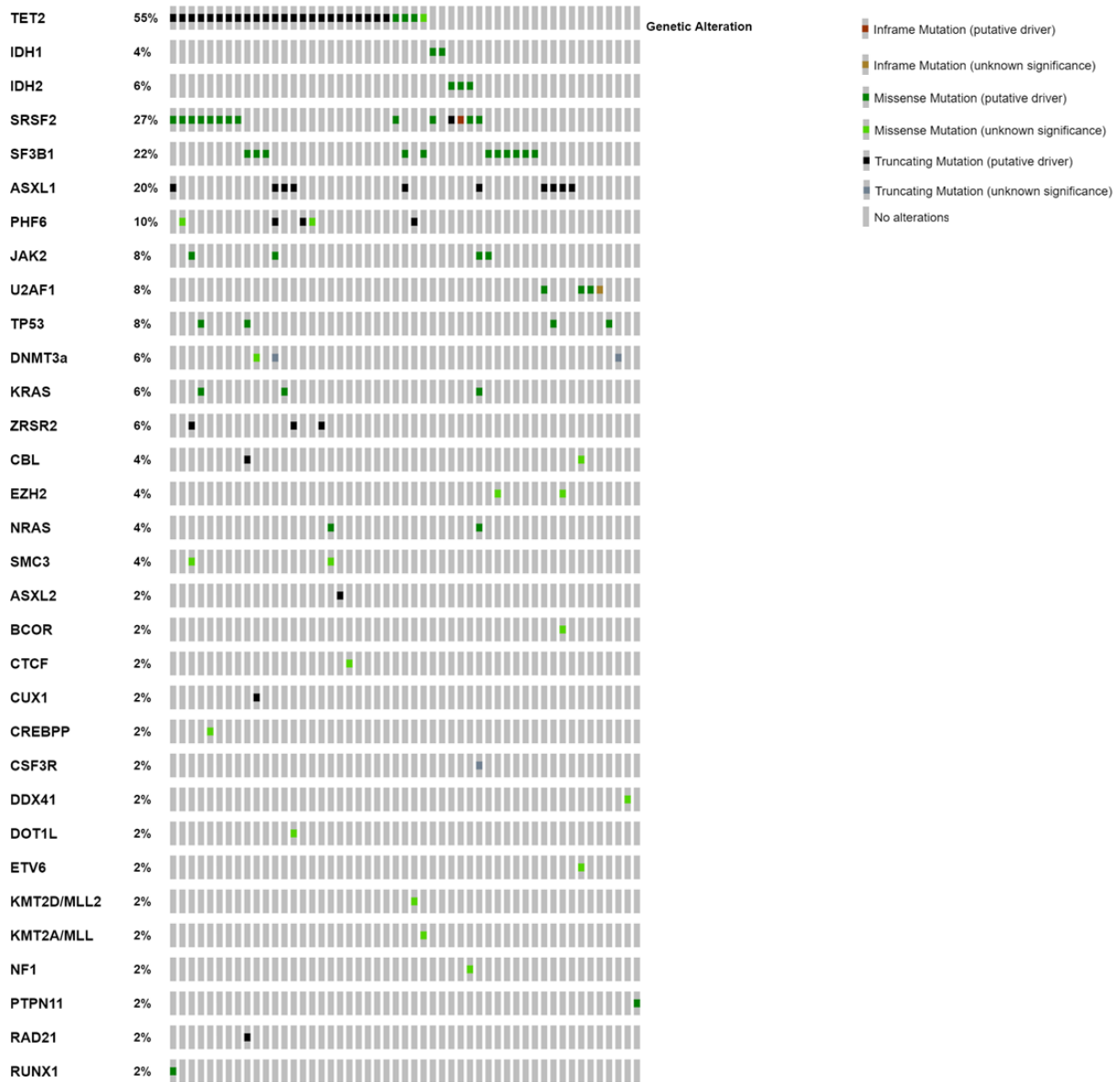
Supplementary Figure 3. Percentages of NK cells positive for various KIR molecules in *TET2/IDH*^{WT} and *TET2/IDH*^{MUT} MDS patients. *TET2/IDH*^{WT} (n= 11) are represented by blue bar and *TET2/IDH*^{MUT} (n=15) are represented by red bar. Data are presented as medians and interquartile ranges. Statistics were calculated with the two-sided nonparametric Mann-Whitney test. KIR2DL1: ** p=0.0092, KIR2DL2/L3: ** p=0.0077, NKG2A: * p=0.0246.



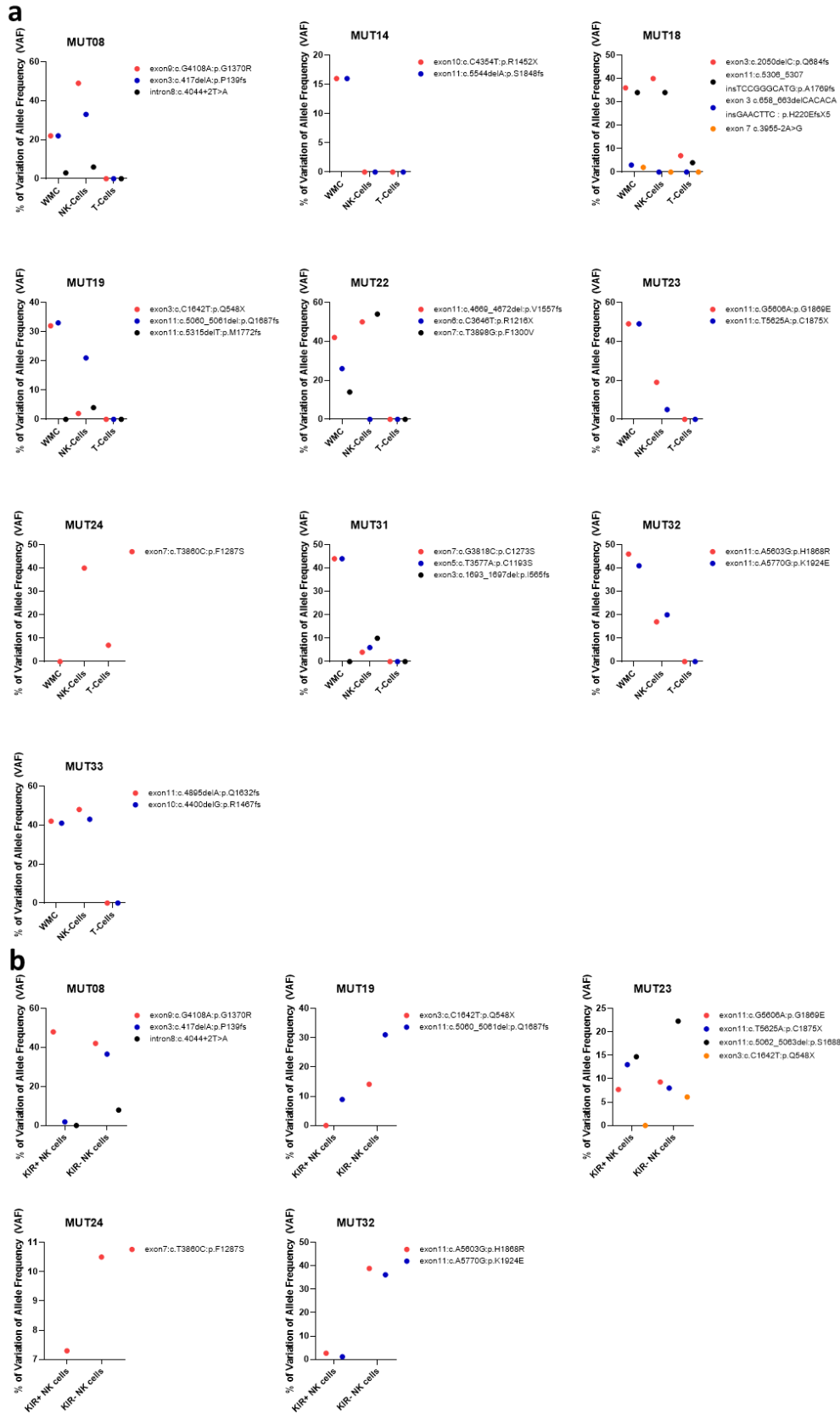
Supplementary Figure 4. KIR and Perforin correlation in MDS/CMML patients with *TET2/IDH* mutations. Correlation between the % of KIR (KIR2D+ KIR3DL1/DS1-, KIR2D+ KIR3DL1/DS1+ and KIR2D- KIR3DL1/DS1+) expressing NK cells and the % of Perforin expressing NK cells in *TET2/IDH*^{MUT} MDS/CMML patients (n=22). The nonparametric Spearman's rank-order correlation, two-sided, has been used, $r = 0.651$ $p = 0.001$.



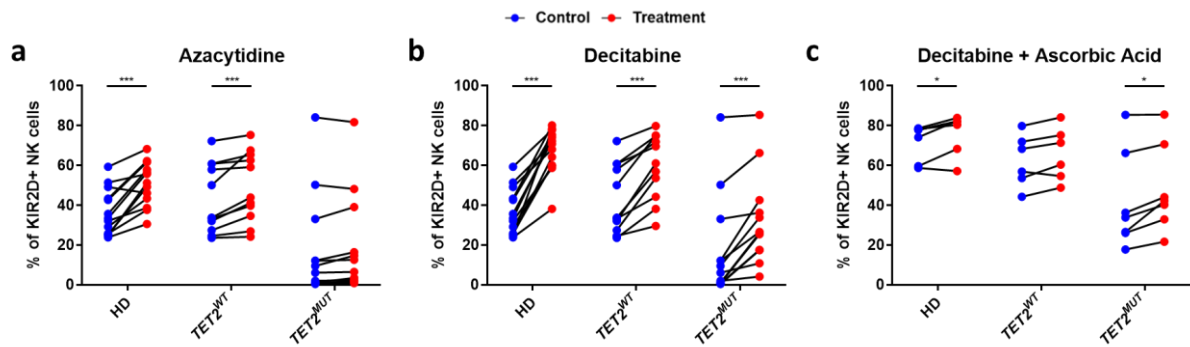
Supplementary Figure 5. Fraction of Perforin positive NK cells according to KIR expression. Central pie charts represent the expression of KIR molecules on blood NK cells in *TET2/IDH^{WT}* (n=19) and *TET2/IDH^{MUT}* (n=22). Lateral pie charts represent the percentage of Perforin positive and negative cells in KIR+ (left) and KIR- (right) NK cells. KIR (KIR2D+ KIR3DL1/DS1-, KIR2D+ KIR3DL1/DS1+ and KIR2D- KIR3DL1/DS1+)



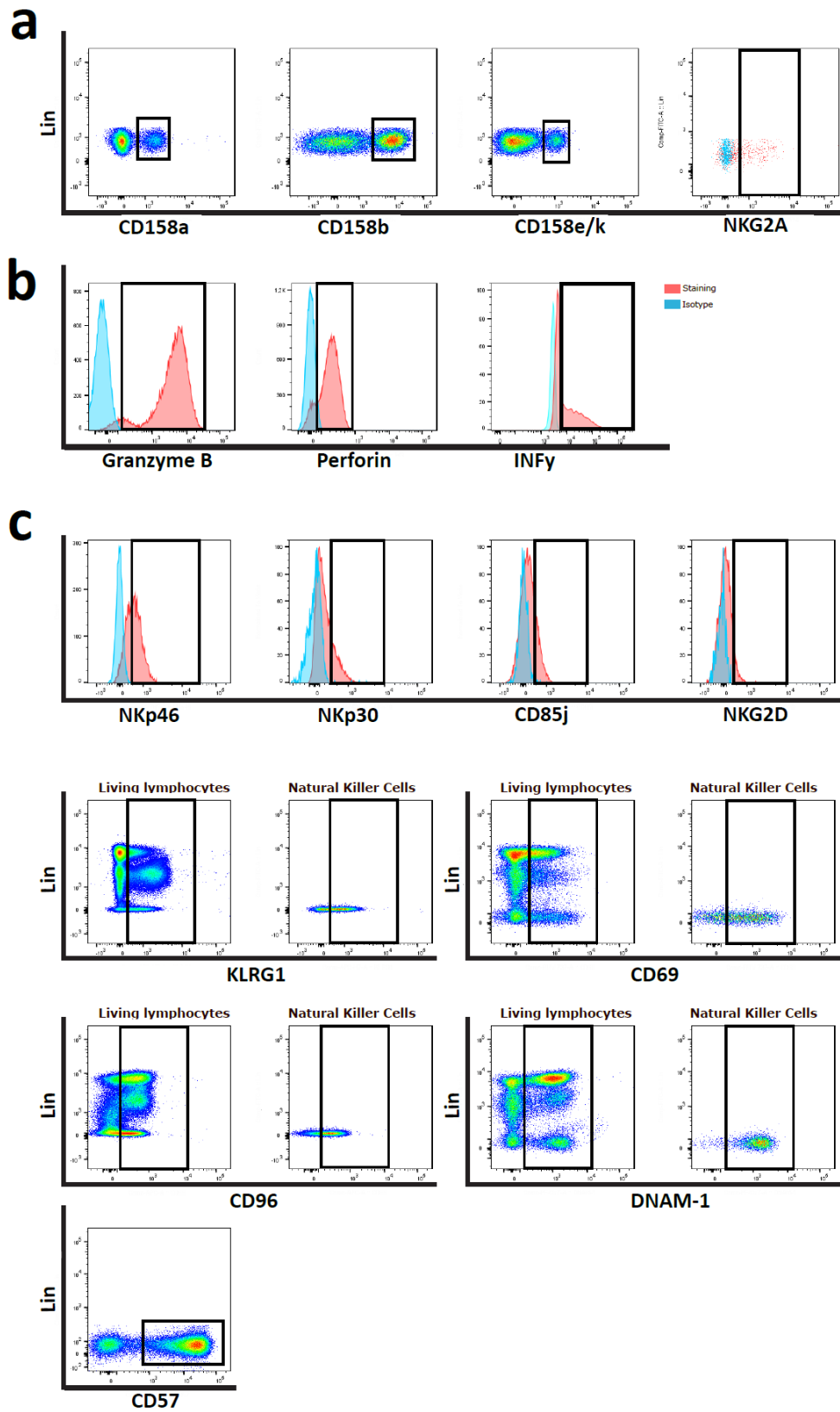
Supplementary Figure 6. Mutational landscape in the MDS/CMML cohort. Mutational Landscape of the 51 patients carrying mutation in at least one of the 80 genes analyzed by NGS.



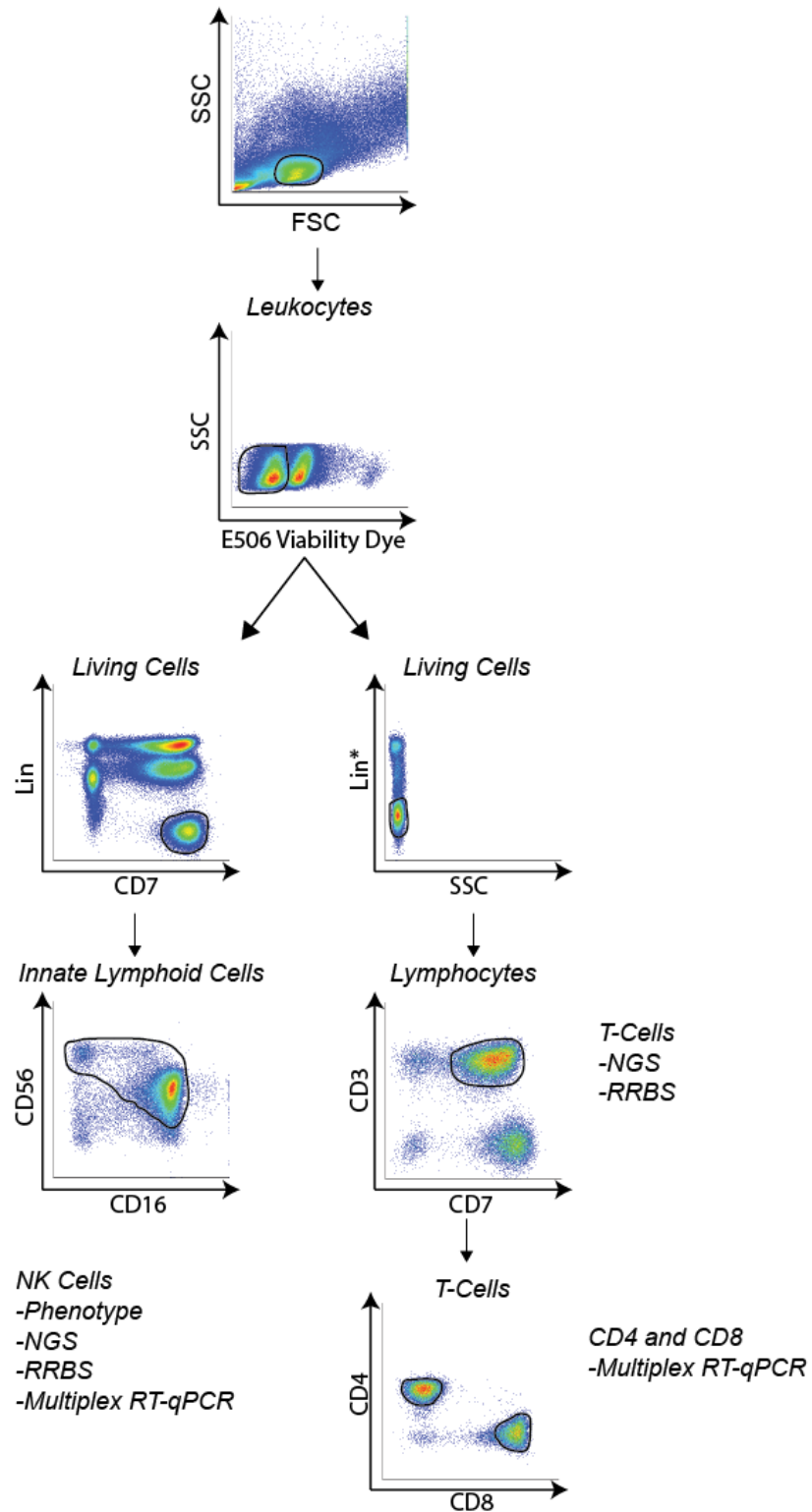
Supplementary Figure 7. Percentage of VAF in the WMC, sorted NK/T cells and KIR+ and KIR- NK cells at diagnosis. The percentage of Variant Allele Frequency (VAF) in the WMC and in sorted NK/T cells (a) or in KIR+ and KIR- NK cells (b) at diagnosis. WMC, White mononuclear cell.



Supplementary Figure 8. Percentages of KIR2D+ NK cells in healthy donors, TET2^{WT} and TET2^{MUT} MDS patients after *in vitro* treatment. NK cells from healthy donors (n=13, n=7 for Decitabine + Acid Ascorbic), TET2^{WT} (n=11, n=6 for Decitabine + Acid Ascorbic), and TET2^{MUT} (n=12, n=7 for Decitabine + Acid Ascorbic) MDS patients were treated 5 days with (a) Azacytidine (0.5 μ M), (b) Decitabine (0.5 μ M), and (c) Decitabine supplemented with Ascorbic Acid (125 μ M). Blue dots show the basal KIR2D expression in presence of DMSO for the test with Azacytidine or Decitabine alone, or in presence of Decitabine alone for the test with ascorbic acid. Red dots show KIR2D expression after the different treatments. Non-parametric two-sided Wilcoxon paired-test has been performed. Azacytidine treatment: ***p=0.005 for HD, and *** p=0.001 for TET2^{WT}. Decitabine treatment: *** p=0.0002 for HD, *** p=0.001 for TET2^{WT} and *** p=0.005 TET2^{MUT}. For Decitabine + Ascorbic Acid treatment: * p=0.0313 and * p=0.0156 for HD and TET2^{MUT} respectively.



Supplementary Figure 9. NK cells phenotyping. Gating strategy of KIR phenotyping (a), Perforin/Granzyme B/IFN- γ (b) and general phenotype (c) of NK cells (Fig 1, 2C, 3B, 5D, 6).



Supplementary Figure 10. Flow Chart. Flow cytometry chart deciphering the main gating strategy used to phenotype NK cells (Fig. 1, 2C, 3B, 5D, 6), or to sort NK and T cells (Fig 2A, 2B, 2D, 3F-H, 4, 5A-C).

Proximal promoter

TATA BOX -64 -58

KIR2DL1prom1 Primer F1 (-147 to -127) KIR2DL1prom2 Primer R1 (+43 to +63)

KIR2DL1prom2 Primer F2 (-63 to -43) KIR2DL1prom2 Primer R2 (+87 to +107)

TTGCTCTGTCGCCCAGGCTGGACTGCAGTGACACAATCTCAGCTCACTGCAACTTCTGCCTCCCAGGTTT
AAGCGATTCTCATGCCTCAGCCTCTTGTAGTAGCTGGCGTTACAGGTGCCTGCCACCAGGCACGGCTAAT
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TCCCATGATGTGGTCAACATGTAACCTGCATGGGCAGGGCGCCAATAAACATCCTGTGCGCTGCTGAGCT
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AGGAGAAGGTTGGC

Supplementary Figure 11. Analysis of KIR2DL1 proximal promoter and localization of primer used for CHIP and luciferase vector construction.

Supplementary Table 1. Circulating NK cells phenotyping.

	HD (n=23)	MDS/CMML (n=33)	p=	<i>TET2/IDH</i> ^{WT} (n=13)	<i>TET2/IDH</i> ^{MUT} (n=19)	p=
	Median [range] %	Median [range] %		Median [range] %	Median [range] %	
NK cells						
DNAM-1	96.2 [37.8-99.8]	90.5 [14.7-99.2]	0.0109	94.4 [14.7-99.2]	87.8 [33.7-98.8]	
NKG2D	65.4 [33.5-85.8]	61.4 [29-90.5]		62.9 [39.9-87.4]	59.9 [29-90.5]	
NKp30	78.6 [33.6-95.5]	79.5 [31.6-99.5]		81.1 [62-99]	74.5 [31.6-99.5]	
NKp46	33.2 [9.04-65.5]	37 [11.2-93.5]	0.0483	39.2 [15.6-93.5]	34.7 [11.2-90.3]	
CD85j	24.9 [5.56-76.5]	25.6 [11.7-76.5]		23.4 [11.7-56.9]	29.3 [13.7-76.5]	
CD96	5.51 [0.95-21.8]	11 [2.8-42.9]	0.0006	9.06 [5.67-42.9]	11.2 [2.8-41.4]	
KIR2D	54.8 [33.3-76.8]	31.2 [0.58-77.7]	0.0034	56.5 [10.5-77.7]	11.9 [0.58-69.1]	0.0005
CD57	63.2 [28.1-81]	60.1 [4.87-95.1]		63.1 [18.8-95.1]	58.9[4.87-85.6]	
KLRG1	21.9 [9.2-73.3]	29 [2.56-96.5]		26 [6.7-76]	38.5 [2.56-96.5]	
CD69	20.9 [8.69-81.7]	24.8 [3.85-82.7]		47.7 [4.01-82.7]	23.6 [3.85-73.5]	
CD56^{Dim} NK cells						
DNAM-1	96.1 [39.3-99.8]	87.9 [13.9-99.2]	0.0038	94.5 [13.9-99.2]	86.6 [33.7-98.7]	
NKG2D	66 [33.8-86.7]	61.3 [29.1-89.9]		62.3 [39.9-87.3]	60.6 [29.1-89.9]	
NKp30	68 [33.8-96.3]	79.4 [29.6-99.8]		83.6 [59.9-99]	73.7 [31-99.8]	
NKp46	28.5 [8.07-56.6]	35.1 [10.5-93.1]	0.0493	38.4 [10.7-93.1]	34.4 [10.5-89.9]	
CD85j	21.4 [5.72-75]	26.1 [11-76.9]		23.4 [11-57.1]	28.8 [14-76.9]	
CD96	4.32 [0.65-23.2]	10.9 [2.66-42]	0.0001	8.77 [4.53-37.5]	11.5 [2.66-41.6]	
KIR2D	60.8 [37-80]	35.1 [0.63-82.3]	0.0009	60 [10.7-82.3]	17.9 [0.63-71.2]	0.0004
CD57	67 [37.2-84.5]	64.1 [5.76-86.5]		69.6 [20.1-95.9]	60.7 [5.76-86.5]	
KLRG1	30.1 [9.54-79.2]	35.1 [2.31-98.3]		27.5 [6.85-80.1]	41.7 [2.31-98.3]	
CD69	20 [8.88-84.9]	25.4 [3.8-83.1]		49.6 [3.8-83.1]	23.8 [4.13-76]	
CD56^{Bright} NK cells						
DNAM-1	97.5 [8.26-99.9]	90.4 [21.2-99.6]	0.0037	92.3 [21.2-99.6]	89 [33.7-96.6]	
NKG2D	64.6 [19.9-96.8]	83.1 [19.7-97.7]	0.0046	88.9 [58.4-95.1]	79.2 [19.7-97.7]	
NKp30	63 [20.3-93.8]	81.2 [29-98.5]	0.0029	79 [45.3-98.5]	84.4 [29-97.1]	
NKp46	62.1 [10.7-85.5]	64.5 [34-99.1]		63.9 [39.7-98.5]	64.5 [34-99.1]	
CD85j	24.7 [2.33-82.1]	25.7 [12.1-68.1]		22.4 [13.8-64]	31 [12.1-68.1]	
CD96	11 [3.28-29.6]	17.4 [2.48-54.5]	0.0182	33.5 [14.7-54.5]	15.2 [2.48-42.6]	0.0045
KIR2D	6.27 [3.07-17.8]	6.55 [0-40.9]		14.1 [3.34-40.9]	1.76 [0-26.2]	0.0035
CD57	3.51 [0.21-38.1]	3.8 [0.18-83.1]		3.64 [1.52-83.1]	4.58 [0.18-47.7]	
KLRG1	7.2 [2.33-26.8]	15.8 [3.16-78.3]	0.0012	10.2 [4.97-42.1]	20.4 [3.16-78.3]	
CD69	5.43 [1.72-35.1]	19.7 [2.34-78.5]	<0.0001	17.9 [6.05-78.5]	22.3 [2.34-66.1]	

Unpaired statistics analysis has been realized by using nonparametric Mann Whitney test two sided.

Supplementary Table 2. Specific KIR Locus phenotyping and NKG2A in circulating NK cells.

	<i>TET2/IDH^{WT}</i> (n=11)	<i>TET2/IDH^{MUT}</i> (n=15)	p=
	Median [range] %	Median [range] %	
KIR2DL1	14.1 [0.65-41.7]	3.63 [0.2-64.9]	0.0092
KIR2DL2/DL3	29.1 [18-73.4]	10.3 [0.62-46.9]	0.0077
KIR3DL1/DL2	9.71 [0.34-55.9]	3.08 [0.18-60.1]	
NKG2A	78.7 [71.9-86.4]	86.3 [65.8-9.3]	0.0246

Unpaired statistics analysis has been realized by using nonparametric Mann Whitney test two sided.

Supplementary Table 3. KIR Locus genotyping on 22 MDS/CMML patients

Identifiant	2DL1	2DL2	2DL3	2DL4	2DL5	3DL1	3DL2	3DL3	2DS1	2DS2	2DS3	2DS4	1D (2DS4v)	2DS5	3DS1	Genotype KIR
MUT01	+	-	+	+	-	+	+	+	-	-	-	-	+	-	-	AA
WT03	+	+	+	+	-	+	+	+	-	+	-	-	+	-	-	AB
WT04	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	AB
WT05	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	AB
MUT07	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	AB
WT08	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	AB
MUT08	+	+	+	+	-	+	+	+	-	+	-	-	+	-	-	AB
MUT14	+	-	+	+	-	+	+	+	-	-	-	+	+	-	-	AA
MUT15	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	AB
MUT16	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	AB
WT13	+	-	+	+	-	+	+	+	-	-	-	-	+	-	-	AA
WT14	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	AB
WT15	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	AB
WT17	+	+	+	+	-	+	+	+	-	+	-	-	+	-	-	AB
WT18	+	-	+	+	-	+	+	+	-	-	-	+	+	-	-	AA
MUT23	+	-	+	+	+	+	+	+	+	-	-	-	+	+	+	AB
MUT24	+	-	+	+	-	+	+	+	-	-	-	+	+	-	-	AA
WT22	+	-	+	+	-	+	+	+	-	-	-	+	+	-	-	AA
WT24	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	AB
MUT27	+	+	+	+	-	+	+	+	-	+	-	-	+	-	-	AB
MUT30	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	AB
WT29	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	AB

Supplementary Table 4. List of 80 genes for targeted Next-Generation Sequencing.

N°	Gene	Reference	N°	Gene	Reference
1	<i>ASXL1</i>	NM_015338	41	<i>KMT2A/MLL</i>	NM_001197104
2	<i>ASXL2</i>	NM_018263	42	<i>KMT2D/MLL2</i>	NM_003482
3	<i>ATM</i>	NM_000051	43	<i>KRAS</i>	NM_033360
4	<i>ATRX</i>	NM_000489	44	<i>LIG4</i>	NM_002312.3
5	<i>BCOR</i>	NM_017745	45	<i>MDM4</i>	NM_002393
6	<i>BCORL1</i>	NM_021946	46	<i>MECOM</i>	NM_001105078
7	<i>BRAF</i>	NM_004333	47	<i>MPL</i>	NM_005373
8	<i>BRCA1</i>	NM_007294	48	<i>MYC</i>	NM_002467
9	<i>BRCA2</i>	NM_000059	49	<i>NF1</i>	NM_001042492
10	<i>BRCC3</i>	NM_024332	50	<i>NPM1</i>	NM_002520
11	<i>CALR</i>	NM_004343	51	<i>NRAS</i>	NM_002524
12	<i>CBL</i>	NM_005188	52	<i>PARN</i>	NM_002582
13	<i>CDAN1</i>	NM_138477	53	<i>PHF6</i>	NM_001015877
14	<i>CEBPA</i>	NM_004364	54	<i>PPM1D</i>	NM_003620
15	<i>CHEK2</i>	NM_007194.3	55	<i>PRPF8</i>	NM_006445
16	<i>CREBBP</i>	NM_004380	56	<i>PTEN</i>	NM_00314
17	<i>CSF3R</i>	NM_156039	57	<i>PTPN11</i>	NM_002834
18	<i>CTC1</i>	NM_02599	58	<i>RAD21</i>	NM_006265
19	<i>CTCF</i>	NM_006565	59	<i>RIT1</i>	NM_006912
20	<i>CUX1</i>	NM_001913	60	<i>RPL11</i>	NM_000975
21	<i>DDX41</i>	NM_016222	61	<i>RPL5</i>	NM_000969
22	<i>DKC1</i>	NM_0001363.2	62	<i>RTEL1</i>	NM_032957
23	<i>DNAJC21</i>	NM_001012339	63	<i>RUNX1</i>	NM_001754
24	<i>DNMT3A</i>	NM_022552	64	<i>SAMD9</i>	NM_017654
25	<i>EP300</i>	NM_001429	65	<i>SAMD9L</i>	NM_152703
26	<i>ERCC6L2</i>	NM_001010895	66	<i>SBDS</i>	NM_016038
27	<i>ETNK1</i>	NM_018638	67	<i>SETBP1</i>	NM_015559
28	<i>ETV6</i>	NM_001987	68	<i>SF1</i>	NM_004630
29	<i>EZH2</i>	NM_004456	69	<i>SF3B1</i>	NM_012433
30	<i>FLT3</i>	NM_004119	70	<i>SMC1A</i>	NM_006306
31	<i>GATA2</i>	NM_032638	71	<i>SMC3</i>	NM_005445
32	<i>GSKIP</i>	NM_001271904	72	<i>SRP72</i>	NM_006947
33	<i>HRAS</i>	NM_005343	73	<i>SRSF2</i>	NM_003016
34	<i>IDH1</i>	NM_005896	74	<i>STAG2</i>	NM_001042749
35	<i>IDH2</i>	NM_002168	75	<i>TET2</i>	NM_001127208
36	<i>IRF1</i>	NM_002198	76	<i>TP53</i>	NM_001126112
37	<i>JAK2</i>	NM_004972	77	<i>U2AF1</i>	NM_006758
38	<i>KDM5A</i>	NM_001042603	78	<i>U2AF2</i>	NM_007279
39	<i>KDM6A</i>	NM_021140	79	<i>WT1</i>	NM_024426
40	<i>KIT</i>	NM_000222	80	<i>ZRSR2</i>	NM_005089

Supplementary Table 5. Mutations association in the whole cohort according to *TET2/IDH* mutations.

	All patients (n = 63)	<i>TET2</i> mutation profile			p=
		<i>IDH1/2^{MUT}</i> patients (n = 28)	<i>TET2^{MUT}</i> patients (n = 28)	<i>TET2/IDH</i> ^{WT} patients (n = 30)	
ASXL1	10	0	5	5	NS
ASXL2	1	0	1	0	NS
ATM	0	0	0	0	NA
ATRX	0	0	0	0	NA
BCOR	1	0	0	1	NS
BCORL1	0	0	0	0	NA
BRAF	0	0	0	0	NA
BRCA1	0	0	0	0	NA
BRCA2	0	0	0	0	NA
BRCC3	1	0	1	0	NS
CALR	0	0	0	0	NA
CBL	2	0	1	1	NS
CDAN1	0	0	0	0	NA
CEBPA	0	0	0	0	NA
CHEK2	0	0	0	0	NA
CREBBP	1	0	1	0	NS
CSF3R	1	0	0	1	NS
CTC1	0	0	0	0	NA
CTCF	1	0	1	0	NS
CUX1	2	0	2	0	NS
DDX41	1	0	0	1	NS
DKC1	0	0	0	0	NA
DNAJC21	0	0	0	0	NA
DNMT3A	3	0	2	1	NS
DOT1L	1	0	1	0	NS
EP300	0	0	0	0	NA
ERCC6L2	0	0	0	0	NA
ETNK1	0	0	0	0	NA
ETV6	1	0	0	1	NS
EZH2	2	0	0	2	NS
FLT3	0	0	0	0	NA
GATA2	0	0	0	0	NA
GSKIP	0	0	0	0	NA
HRAS	0	0	0	0	NA
IDH1	2	2	0	0	NS
IDH2	3	3	0	0	NS
IRF1	0	0	0	0	NA
JAK2	4	0	2	2	NS
KDM5A	0	0	0	0	NA
KDM6A	0	0	0	0	NA
KIT	0	0	0	0	NA
KMT2A/MLL	1	0	1	0	NS
KMT2D/MLL	1	0	1	0	NS
KRAS	3	0	2	1	NS
LIG4	0	0	0	0	NA
MDM4	0	0	0	0	NA
MECOM	0	0	0	0	NA
MPL	0	0	0	0	NA
MYC	0	0	0	0	NA
NF1	1	1	0	0	NA
NPM1	0	0	0	0	NA
NRAS	2	0	1	1	NS
PARN	0	0	0	0	NA
PHF6	5	0	5	0	0,0214
PPM1D	0	0	0	0	NA
PRPF8	0	0	0	0	NA
PTEN	0	0	0	0	NA
PTPN11	1	0	0	1	NS
RAD21	1	0	1	0	NS
RIT1	0	0	0	0	NA
RPL11	0	0	0	0	NA
RPL5	0	0	0	0	NA
RTEL1	0	0	0	0	NA
RUNX1	1	0	1	0	ns
SAMD9	0	0	0	0	NA
SAMD9L	0	0	0	0	NA
SBDS	0	0	0	0	NA
SETBP1	0	0	0	0	NA
SF1	0	0	0	0	NA
SF3B1	11	0	5	6	NS
SMC1A	0	0	0	0	NA
SMC3	2	0	2	0	NS
SRP72	0	0	0	0	NA
SRSF2	13	4	8	1	0,0107
STAG2	0	0	0	0	NA
TET2	28	0	28	0	<0,0001
TP53	4	0	2	2	NS
U2AF1	4	0	0	4	NS
U2AF2	0	0	0	0	NA
WT1	0	0	0	0	NA
ZRSR2	3	0	3	0	ns

Fisher's exact test has been realized NS: Not Significant NA: Not Applicable

Supplementary Table 6. NGS at diagnosis in WMC and in NK/T sorted cells.

	VAF White Mononuclear Cells	VAF NK Cells			VAF T Cells	%KIR2D+ NK cells
		Whole	KIR-*	KIR+*		
MUT22		Blood 28/02/2017	Blood 31/10/2017			0,60%
TET2:NM_001127208:exon11:c.4669_4672delp.V1557fs	42	50	NA	NA	-	
SRSF2:NM_001195427:exon1:c.C284T:p.P95L	42	45	NA	NA	-	
TET2:NM_001127208:exon6:c.C3646T:p.R1216X	26	-	NA	NA	-	
TET2:NM_001127208:exon7:c.T3898G:p.F1300V	14	54	NA	NA	-	
ASXL2:NM_018263:exon12:c.1966_1976delp.T656fs	-	2	NA	NA	-	
NRAS:NM_002524:exon2:c.G35A:p.G12D	-	15	NA	NA	-	
RIT1:NM_006912:exon4:c.G229C:p.A77P	-	7	NA	NA	-	
MUT33		Bone Marrow 19/04/2017	Blood 09/01/2018			2,70%
PHF6:NM_001015877:exon2:c.65_66insTA:p.S22fs	91	93	NA	NA	-	
TET2:NM_001127208:exon11:c.4895delA:p.Q1632fs	42	48	NA	NA	-	
TET2:NM_001127208:exon10:c.4400delG:p.R1467fs	41	43	NA	NA	-	
MUT08		Blood 18/07/2017	Blood 18/07/2017			10,20%
TET2:NM_001127208:exon9:c.G4108A:p.G1370R	22	49	42	48	-	
TET2:NM_001127208:exon3:c.A17delA:p.P139fs	22	33	37	2	-	
SF3B1:NM_012433:exon15:c.A2098G:p.K700E	22	31	33	4	-	
TET2:NM_001127208:intron8:c.4044+2T>A	3	6	8	-	-	
MUT18		Bone Marrow 24/03/2010	Blood 06/03/2018			10,70%
TET2:NM_001127208:exon3:c.2050delC:p.Q684fs	36	40	NA	NA	7	
TET2:NM_001127208:exon11:c.5306_5307insTCCGGGCATG:p.A1769fs	34	34	NA	NA	4	
TET2:NM_001127208:exon 3 c.658_663delCACACAinsGAACTTC : p.H220Efs	3	-	NA	NA	-	
TET2:NM_001127208:exon 7 c.3955-2A>G	2	-	NA	NA	-	
KRAS:NM_033360:exon2:c.G34A:p.G12S	-	12	NA	NA	-	
MUT24		Bone Marrow 23/06/16	Bone Marrow 23/06/2016			37,20%
ASXL1:NM_015338:exon12:c.1927dupG:p.G642fs	3	6	6	3	-	
TET2:NM_001127208:exon7:c.T3860C:p.F1287S	2	11	11	7	-	
SF3B1:NM_012433:exon14:c.C1984T:p.H662Y	31	19	20	12	-	
MUT23		Bone Marrow 08/12/16	Bone Marrow 08/12/2016			45,90%
TET2:NM_001127208:exon11:c.G5606A:p.G1869E	49	19	9	8	-	
TET2:NM_001127208:exon11:c.T5625A:p.C1875X	49	5	8	13	-	
TET2:NM_001127208:exon11:c.5062_5063delp.S1688fs	-	-	22	15	-	
TET2:NM_001127208:exon3:c.C1642T:p.Q548X	-	-	6	-	-	
SRSF2:NM_003016:exon1:c.C284T:p.P95L	49	14	29	23	-	
MUT32		12/08/2016	Blood 28/12/2017			47,90%
TET2:NM_001127208:exon11:c.A5603G:p.H1868R	46	17	39	3	-	
TET2:NM_001127208:exon11:c.A5770G:p.K1924E	41	20	36	1	-	
SF3B1:NM_012433:exon15:c.A2098G:p.K700E	44	20	34	-	-	
MUT19		Blood 08/03/17	Blood 11/01/2018			56,90%
TET2:NM_001127208:exon3:c.C1642T:p.Q548X	32	2	14	-	-	
TET2:NM_001127208:exon11:c.5060_5061delp.Q1687fs	33	21	31	9	-	
SRSF2:NM_003016:exon1:c.C284A:p.P95H	33	20	29	23	-	
TET2:NM_001127208:exon11:c.5315delT:p.M1772fs	-	4	-	-	-	
MUT31		Bone Marrow 14/12/17	Blood 10/03/2016			69,10%
TET2:NM_001127208:exon7:c.G3818C:p.C1273S	34	4	NA	NA	-	
TET2:NM_001127208:exon5:c.T3577A:p.C1193S	38	6	NA	NA	-	
SRSF2:NM_003016:exon1:c.C284A:p.P95H	27	4	NA	NA	-	
CTCF:NM_006565:exon5:c.C1024T:p.R342C	-	-	NA	NA	-	
TET2:NM_001127208:exon3:c.1693_1697delp.I565fs	-	10	NA	NA	-	
MUT14		Blood 22/11/16	Blood 19/12/2017			92,90%
TET2:NM_001127208:exon10:c.C4354T:p.R1452X	16	-	NA	NA	-	
TET2:NM_001127208:exon11:c.5544delA:p.S1848fs	16	-	NA	NA	-	
PHF6:NM_001015877:exon7:c.T724A:p.C242S	10	-	NA	NA	-	

*KIR = KIR2D / KIR3DL1 / KIR3DL2

WMC, Whole mononuclear cell; VAF variant allele frequency

Supplementary Table 7. Patients' characteristics.

Variables	All patients (n = 63)	Non <i>TET2/IDH</i> mutated patients (n = 30)	<i>TET2</i> mutated patients (n = 28)	p=	<i>IDH</i> mutated patients (n = 5)
Age at MDS/CMML diagnostic	70 [63-75]	71 [62.75-75]	70 [51-86]	ns	73 [59-84.5]
% women	38	40	36	ns	40
Peripheral blood					
Hb at diagnosis (g/dL)	11.45 [9.35-13.5]	10.15 [9.05-11.88]	13.3 [10.3-14.45]	0.0032	11.2 [9.1-13.4]
Platelets at diagnosis (G/L)	129.5 [89.25-286.8]	124.5 [89.5-369.5]	115 [85-223.5]	ns	155 [108-325.5]
WBC at diagnosis (G/L)	4.8 [3.9-7.4]	4.65 [3.9-7.0]	5 [3.8-7.6]	ns	5.05 [3.6-7.55]
ANC at diagnosis (G/L)	2.7 [1.8-4.2]	2.8 [2.2-4.675]	2.2 [0.6-3.95]	ns	1.8 [1.25-4.75]
Lymphocyte count at diagnosis (G/L)	1.250 [0.9-1.9]	1.2 [0.9-1.7]	1.5 [1.125-2.45]	ns	0.4 [0.2-1.3]
Peripheral blast % at diagnosis (%)	0 [0-0]	0 [0-0]	0 [0-0]	ns	0 [0-0]
Bone Marrow					
SLD/MLD*	25	17	6	ns	2
RS-SLD/RS-MLD*	10	5	5	ns	0
EB1*	8	4	4	ns	0
EB2*	1	1	0	ns	0
CMML*	17	2	12	0.0018	3
Other MDS/MPN	0	0	0	N.A	0
5q.*	2	1	1	ns	0
Unknown	0	0	0	N.A	0
Medullary blasts (%)	3 [2.0-4.0]	3 [2.0-4.0]	3 [1.0-4.0]	ns	3 [2-3.5]
IPSS-R					
Low	52	24	23	ns	5
High	11	6	5	ns	0
Treatments	14	5	7	ns	2
Prednisone	4	1	1	ns	2
Lenalidomid	4	3	1	ns	0
Rigosertib	1	0	1	N.A	0
Eltrombopag	1	0	1	N.A	0
Darbepoietin	4	1	3	ns	0

*SLD = MDS with single-lineage dysplasia; MLD = MDS with multilineage dysplasia; RS-SLD = MDS with ring sideroblasts and with single-lineage dysplasia; RS-MLD = MDS with ring sideroblasts and with multilineage dysplasia); MPN = Myeloproliferative Neoplasm; del5q = MDS with isolated del(5q); EB1 = MDS with excess blasts (5 to 9 percent blasts in the bone marrow or 2 to 4 percent blasts in the blood); EB2 = MDS with excess blasts (10 to 19 percent blasts in the bone marrow or 5 to 19 percent blasts in the blood); CMML = Chronic Myelomonocytic Leukemia.

Supplementary Table 8. Patients of the Clinical Trial NCT02985190

Code	Patient number (in Mekinian et al. Leukemia 2022)	Age	Sex	WHO 2016 classification*	Karyotype	Mutations (Targeted NGS)	IPSS-R
MUT34	#24	73	F	MDS-U	45,X,-Y[19]/46,XY[5]	<i>TET2, ASXL1, CBL, JAK2, PHF6</i>	2.0
MUT35	#14	72	M	MDS-EB2	46,xy[20]	<i>TET2, DNMT3A, TP53</i>	9.0
MUT36	#18	86	F	CMML-1	46,XY,del(20)(q11q13)[1]/46,XY[1]	<i>TET2, NRAS, KRAS, CBL</i>	2.0
WT31	#21	77	F	CMML-2	46,XXdel(5)(q13q33)	<i>SF3B1, NRAS, RUNX1, SETBP1</i>	4.5
WT32	#29	76	M	MDS-MLD	46,XY,del(13)(q13q21)[3]/46,XY[17]	<i>UBA1</i>	2.0
WT33	#26	68	M	MDS-MLD	46,X,inv(Y)(p11q12)c[20]	<i>UBA1</i>	4.5
WT34	#16	69	M	MDS-MLD	46,XX,del(3)(p21)[3]/46,XX[20]	<i>UBA1</i>	1.0
WT35	#22	82	M	MDS-MLD	92,XXXX[3] / 46,XX[17]	<i>UBA1</i>	3
WT36	#11	82	M	MDS-MLD	45,X,-Y[20]	<i>DNMT3A</i>	2.5
WT37	#16	69	M	MDS-MLD	46,XX,del(3)(p21)[3]/46,XX[20]	<i>UBA1</i>	1.0

*SLD = MDS with single-lineage dysplasia; MLD = MDS with multilineage dysplasia; RS-SLD = MDS with ring sideroblasts and with single-lineage dysplasia; RS-MLD = MDS with ring sideroblasts and with multilineage dysplasia; del5q = MDS with isolated del(5q); EB1 = MDS with excess blasts (5 to 9 percent blasts in the bone marrow or 2 to 4 percent blasts in the blood); EB2 = MDS with excess blasts (10 to 19 percent blasts in the bone marrow or 5 to 19 percent blasts in the blood). CMML-1 = Chronic Myelomonocytic Leukemia (5 to 9 percent blasts in the bone marrow or 2 to 4 percent blasts in the blood). CMML-2 = Chronic Myelomonocytic Leukemia (10 to 19 percent blasts in the bone marrow or 5 to 19 percent blasts in the blood).

Supplementary Table 9. List of Antibodies used for Flow Cytometry.

Target	Conjugation	Clone	Supplier	Reference
CD3	FITC	OKT3	Biolegend	317306
CD3	V450	UCHT1	BD	560365
CD3	BB700	SK7	BD	566575
CD4	FITC	OKT4	Biolegend	317408
CD4	APC-H7	RPA-T4	BD	560158
CD5	FITC	UCHT2	Biolegend	300606
CD7	PE-CF594	M-T701	BD	562541
CD7	APC	CD7-6B7	Miltenyi	130-105-841
CD8	BV785	SK1	Biolegend	344740
CD14	FITC	M5E2	BD	555397
CD16	BUV395	3G8	BD	563785
CD16	APC-H7	3G8	BD	560715
CD16	BV711	3G8	BD	563127
CD19	FITC	HIB19	Biolegend	302206
CD33	PE	WM53	BD	555450
CD56	AF700	B159	BD	557919
CD56	PE-Cy7	B159	BD	557747
CD56	BV421	HCD56	Biolegend	318328
CD57	BV605	QA17A04	Biolegend	393304
CD69	BUV737	FN50	BD	564439
CD85j	PE-Cy5	GHI/75	BD	551054
CD96	APC	NK92.39	Biolegend	338410
CD107a	APC-H7	H4A3	BD	561343
DNAM-1	PE-Vio770	REA1040	Miltenyi	130-099-966
KLRG1	APC-Vio770	REA260	Miltenyi	130-103-642
NKG2A	BV786	131411	BD	747917
NKG2D	BV650	1D11	BD	563408
NKp30	BV421	p30-15	BD	563385
NKp46	BV786	9-E2	BD	563329
CD127	PE-Cy7	A019D5	Biolegend	351320
IFN- γ	AF488	B27	BD	557718
CD158a (KIR2DL1)	APC	REA284	Miltenyi	130-103-935
CD158b (KIR2DL2/DL3)	BB700	CH-L	BD	746236
CD158d (KIR2DL4)	PE	REA768	Miltenyi	130-112-465
CD158e/k (KIR3DL1/DL2)	PE	5.133	Miltenyi	130-095-205
CD158e/k (KIR3DL1/DL2)	APC-Vio770	REA970	Miltenyi	130-116-181
CD158e1/e2 (KIR3DL1/DS1)	PerCP-Vio700	REA168	Miltenyi	130-104-837
CD158f (KIR2DL5)	PE-Vio770	REA955	Miltenyi	130-115-841
CD158i (KIR2DS4)	VioBlue	REA860	Miltenyi	130-114-622
KIR2D (all KIR2D)	PE	NKVFS1	Miltenyi	130-092-688
KIR2D (all KIR2D)	APC	NKVFS1	Miltenyi	130-092-687

Supplementary Table 10. List of primers used for the ChIP-qPCR assay

ID	Sequence
CRE Forward	GGGGAGGGCAGTTTCCGTC
CRE Reverse	GGTCATGGCGTCTCCTCTGC
KIR2DL1 Forward	GGCCTCATGCAAGGTAGAAA
KIR2DL1 reverse	CCCTCCCTCTATTGCTTTCC
KIR2DL2/DL3 Forward	CTCTTGAGCGAGCACCCAC
KIR2DL1prom2 Reverse	CACCAACACACACCATGCTG

Supplemental Table 11. Specificities and primers used in the Multiplex RT-PCR analysis.

Target Gene	Reference	Target Gene	Reference
<i>TET2</i>	Hs00325999_m1	<i>IKZF3</i>	Hs05037772_s1
<i>IDH1</i>	Hs00271858_m1	<i>ZBTB16</i>	Hs00232313_m1
<i>IDH2</i>	Hs00158033_m1	<i>IRF2</i>	Hs01082884_m1
<i>CXCL10</i>	Hs00171042_m1	<i>JUN</i>	Hs01103582_s1
<i>RUNX1</i>	Hs01021970_m1	<i>FOS</i>	Hs99999140_m1
<i>MYC</i>	Hs00153408_m1	<i>DUSP5</i>	Hs00244839_m1
<i>DNMT3A</i>	Hs01027166_m1	<i>EOMES</i>	Hs00172872_m1
<i>TET1</i>	Hs00286756_m1	<i>NFIL3</i>	Hs00705412_s1
<i>TET3</i>	Hs00379125_m1	<i>PRDM1</i>	Hs00153357_m1
<i>PRF1</i>	Hs00169473_m1	<i>STAT3</i>	Hs00374280_m1
<i>GZMA</i>	Hs00989184_m1	<i>STAT4</i>	Hs01028017_m1
<i>GZMB</i>	Hs00188051_m1	<i>STAT5B</i>	Hs00560026_m1
<i>GZMK</i>	Hs00157878_m1	<i>STAT6</i>	Hs00598625_m1
<i>GZMH</i>	Hs00277212_m1	<i>ID2</i>	Hs04187239_m1
<i>INFG</i>	Hs00989291_m1	<i>ID3</i>	Hs00171409_m1
<i>TNF</i>	Hs00174128_m1	<i>BCL6</i>	Hs00153368_m1
<i>BCL2</i>	Hs00608023_m1	<i>ZEB1</i>	Hs01566410_m1
<i>KIR2DL1</i>	Hs04961778_gH	<i>ZEB2</i>	Hs00207691_m1
<i>KIR2DL2</i>	Hs04961776_uH	<i>BCL6</i>	Hs00158218_m1
<i>KIR2DL3</i>	Hs04961777_gH	<i>LEF1</i>	Hs01547250_m1
<i>KIR2DL4</i>	Hs00427106_m1	<i>TCF7</i>	Hs00175273_m1
<i>KIR3DL1</i>	Hs01592437_m1	<i>TNFRSF18</i>	Hs00188346_m1
<i>KIR3DL2</i>	Hs00601497_gH	<i>EZH2</i>	Hs00544830_m1
<i>KIR3DL3</i>	Hs00601067_m1	<i>MYB</i>	Hs00920556_m1
<i>CXCR4</i>	Hs00976734_m1	<i>GAPDH</i>	Hs99999905_m1
<i>ACKR3</i>	Hs00664172_s1	<i>ACTB</i>	Hs99999903_m1
<i>ITGA4</i>	Hs00168433_m1	<i>RPL27</i>	Hs03044961_g1
<i>KLRC1</i>	Hs00970273_g1	<i>NCAM1</i>	Hs00941830_m1
<i>CD96</i>	Hs00976975_m1	<i>FCGR3B</i>	Hs04334165_m1
<i>CD69</i>	Hs00934033_m1	<i>CD3E</i>	Hs01062241_m1
<i>KLRG1</i>	Hs00195153_m1	<i>CD4</i>	Hs01058407_m1
<i>CXCR3</i>	Hs01847760_s1	<i>CD8B</i>	Hs00174762_m1
<i>CCR2</i>	Hs00704702_s1	<i>CD33</i>	Hs00233544_m1
<i>NCR2</i>	Hs00183113_m1	<i>CD5</i>	Hs00204397_m1
<i>NCR3</i>	Hs00394809_m1	<i>CD7</i>	Hs00196191_m1
<i>NCR1</i>	Hs00183118_m1	<i>SPI1</i>	Hs02786711_m1
<i>TIGIT</i>	Hs00545087_m1	<i>NT5E</i>	Hs00159686_m1
<i>CD226</i>	Hs00170832_m1	<i>ENTPD1</i>	Hs00969556_m1
<i>IL2RA</i>	Hs00907777_m1	<i>CTLA4</i>	Hs00175480_m1
<i>IL7R</i>	Hs00902334_m1	<i>HAVCR2</i>	Hs00958618_m1
<i>IL15RA</i>	Hs00542602_g1	<i>PDCD1</i>	Hs01550088_m1
<i>AHR</i>	Hs00169233_m1	<i>LAG3</i>	Hs00958444_g1
<i>CYP11A1</i>	Hs01054796_g1	<i>CD244</i>	Hs00175569_m1
<i>NQO1</i>	Hs01045993_g1	<i>FNI</i>	Hs01549976_m1
<i>TBX21</i>	Hs00203436_m1	<i>CD44</i>	Hs01075864_m1
<i>FOXO1</i>	Hs00231106_m1	<i>ITGA4</i>	Hs00168433_m1
<i>FOXO3</i>	Hs00818121_m1	<i>ITGB1</i>	Hs01127536_m1
<i>ELF4</i>	Hs01086126_m1	<i>SELL</i>	Hs00174151_m1