Supplementary Information

Supplementary Methods and Materials

Bone marrow samples of the patient and a normal donor

The patient's BM sample was obtained after written informed consent was obtained. This study was approved by the Institutional Review Board of University of Minnesota in accordance with Declaration of Helsinki. A normal donor's BM sample was purchased (Lonza, Basel, Switzerland). All reported studies were conducted using fresh BM samples.

Next Generation Sequencing (NGS)

NGS analysis was performed using a CLIA-validated 70-gene assay custom designed for hematologic malignancies as described previously (**Supplementary Table S1**).[16] Variant allele fraction (VAF) refers to the proportion of mutant/variant alleles relative the proportion of reference (wildtype) alleles in the DNA sequencing data.

To assess the gene variants for clinical significance, we used the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/). The *ASXL1 Glu222Asp* variant is absent from normal human variation databases, it has not been characterized in the literature; it does not occur in a well-characterized functional domain; the three main in silico algorithms used by our laboratory (SIFT, Provean, Polyphen 2) provide conflicting predictions of pathogenic vs. benign impact. Based on these features, we have clinically interpreted the variant as uncertain. Missense variation is less common (vs. frameshift or nonsense loss of functions) in hematologic neoplasms. Thus, we believe the *ASXL1* variant is unlikely to significantly impact disease pathogenesis.

Flow cytometry-based cell sorting

A total of 100 million cells of the patient's BM sample were stained with fixable viability dye E fluor 450 (e-Bioscience), blocked with Fc block (BD pharmingen, San Diego, CA), and stained with surface markers (BD biosciences, San Jose, CA) (**Supplementary Table S2)**. Cells were sorted into CD34+ progenitors, myeloid, and lymphoid fractions using BD FACS Aria II (BD Biosciences, San Jose, CA) **(Supplementary Figure S1).**

Colony-formation assay (CFA)

12,500 patient BM cells were plated in semi-solid methylcellulose-based media (MethoCult H4435, StemCell Technologies, Vancouver, Canada) in the presence of midostaurin (400nM), ruxolitinib (400nM), or DMSO vehicle. Colony numbers were scored after a 7-day culture. Colonies are defined as a cluster of at least 10 cells.

Intracellular signaling assay by mass cytometry

BM samples were incubated for 30 minutes at 37^{o}C in a medium of RPMI 1640 (Gibco, Billings, MT) with 10% fetal bovine serum and 1% penicillin/streptomycin, termed RPMI-10. Cells were washed in phosphate buffered saline (PBS), treated with cisplatin (Fluidigm, San Francisco, CA), blocked with Human Fc block (BD Pharmingen, San Diego, CA), and stained for surface markers. Stained cells were fixed in 1.6% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA), permeabilized in methanol, and stored at -80^{o}C. Intracellular staining was done followed by intercalation and analyzed with a CyTOF mass cytometer (DVS Sciences, Ontario, Canada). All antibodies for mass cytometry were from Fluidigm (**Supplementary Table S3**).

Proliferation Assay by flow cytometry

The patient's fresh BM cells were labeled with CellTrace Violet Cell Proliferation Dye (Invitrogen). Cells were washed and then treated with DMSO, thrombopoietin (TPO) 10ng/ml, stem cell factor (SCF) 10ng/ml, ruxolitinib 400nM, midostaurin 400nM in the media (RPMI with 10% fetal bovine serum). Approximately 1.5 million cells per 1ml per condition were incubated in a 24-well plate with flat bottom for 7 days at 37^{o}C and harvested. Cells were stained with Fixable Near-IR Dead Cell Dye (Invitrogen, Carlsbad, CA), then with fluorochrome-conjugated antibodies **(Supplementary Table S2).**

Supplementary Tables

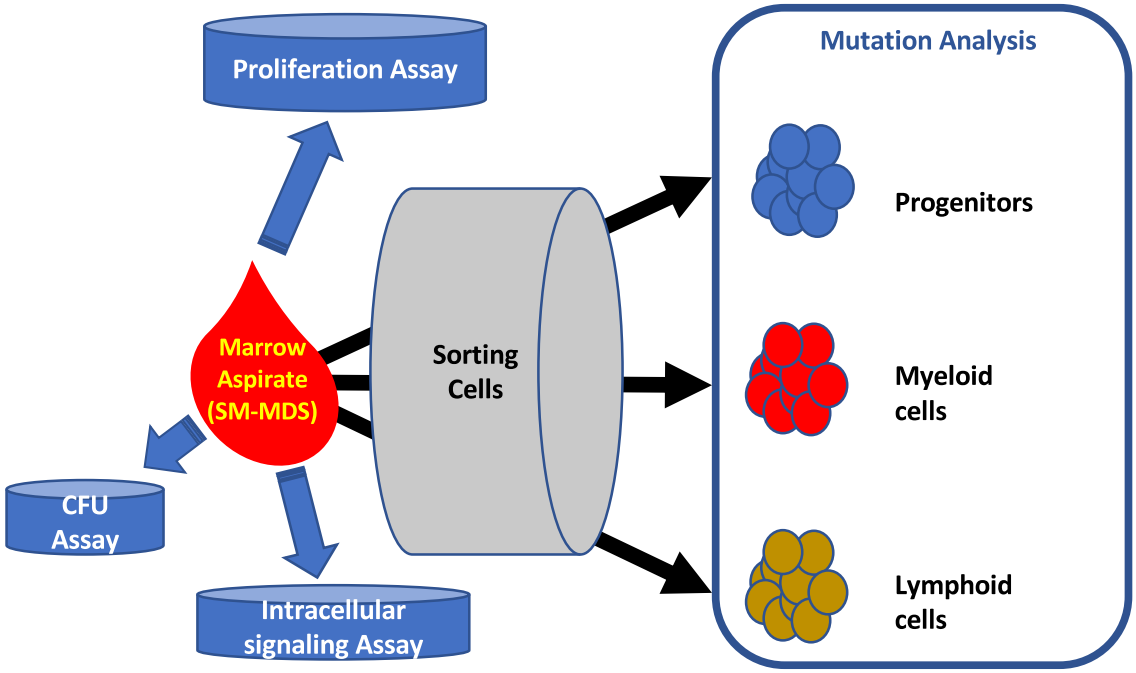
Supplementary Table S1: Mutation analysis in CD34+ and myeloid cells

Supplementary Table S2: Antibodies for flow cytometry

Supplementary Table S3: Antibodies for mass cytometry

Supplementary Figure. Overall Study Scheme and gating of the primary bone marrow sample. A. Overall study scheme. The bone marrow sample was obtained one year after midostaurin therapy. SM-AHN bone marrow material mononuclear cells were sorted for cell-lineage-specific mutational analysis, and plated in proliferation assays and colony forming assays, and processed for intracellular signaling assays. **B.** Gating scheme for flow cytometry analysis.

A



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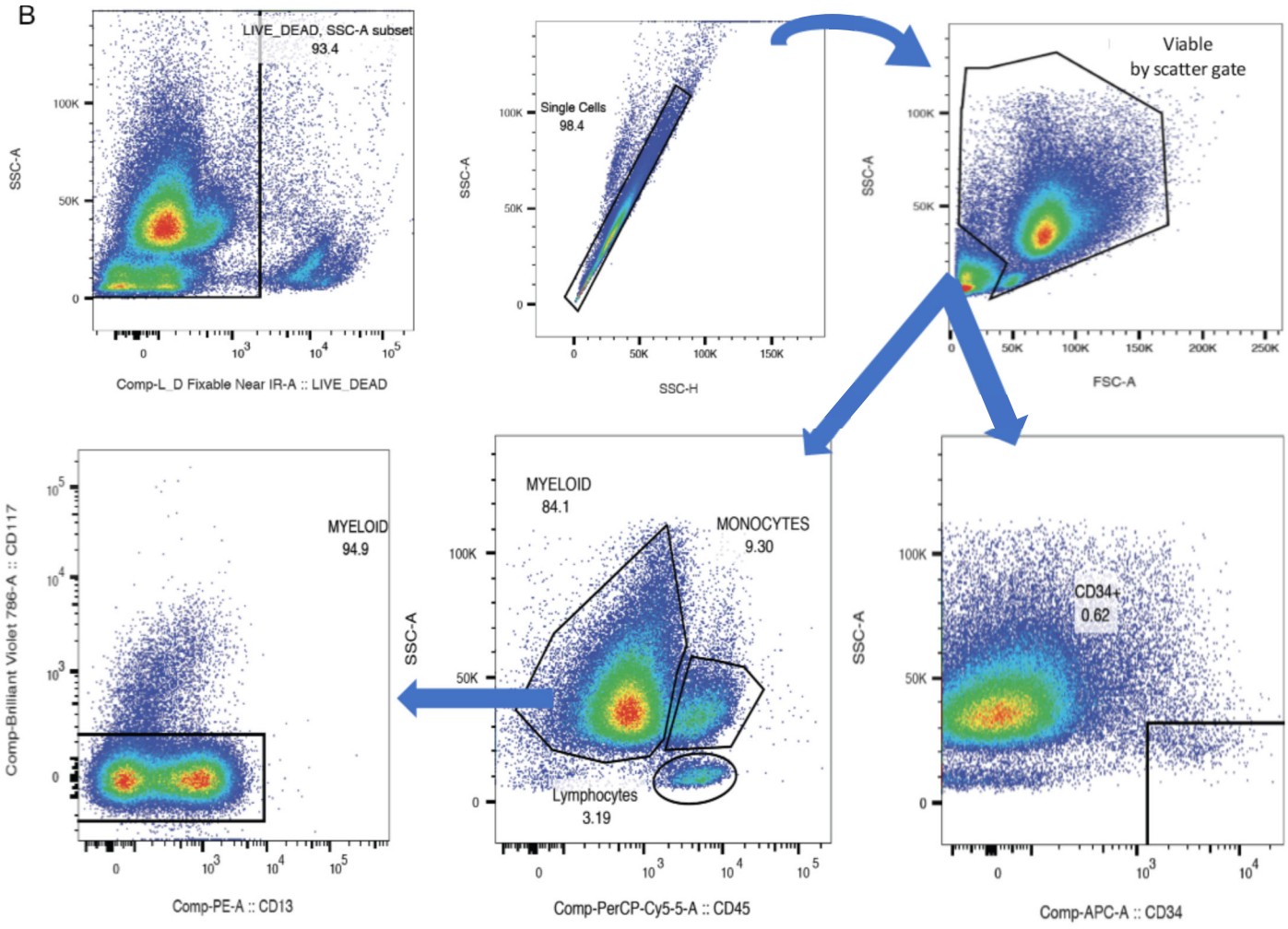

Supplementary Figure

Mutation analysis

Genes	Alteration
ABL1	No
ALK	No
ASXL1	Yes
BCOR	No
CALR	No
CBL	Yes
CEBPA	No
CSF3R	No
DNMT3A	No
ETV6	No
EZH2	No
FLT3	No
GATA1	No
GATA2	No
HRAS	No
IDH1	No
IDH2	No
JAK1	No
JAK2	No
JAK3	No
KIT	Yes
KRAS	No
KMT2A	No
MPL	No
NF1	No
NPM1	No
NRAS	No
PDGFRA	No
PDGFRB	No
PHF6	No
PTPN11	No
RARA	No
RUNX1	No
SETBP1	No
SF1	No
SF3A1	No
SF3B1	No
SH2B3	No
SRSF2	No
TET2	Yes
TP53	No
U2AF2	No
WT1	No
ZRSR2	No

Antibodies for flow cytometry

Antibody	Conjugated Fluorophore
Anti CD117	BV786
Anti CD25	PE-Cy7
Anti CD45	Per-Cp-Cy-5.5
Anti CD34	APC
Anti CD13	PE
Anti CD38	FITC
Anti CD3	PE-CF594

Antibodies for mass cytometry

Antibody	Conjugated Isotope
CD45	89Y
CD117(c-Kit)	143Nd
CD38	144Nd
CD2	151Eu
CD13	160Gd
CD34	163Dy
CD25 (IL-2R)	169Tm
pSHP2(Y580)	141Pr
Caspase 3 (cleaved)	142Nd
P STAT5	147Sm
P4E-BP1	149Sm
pAKT(S473)	152Sm
P STAT1(Y701)	153Sm
p-p38(T180/Y182)	156Gd
Cyclin A	158Gd
pMAPAPK II	159Tb
CyclinB1	164Dy
β-Catenin	165Ho
pNF-kB p65 [S529]	166Er
Ki-67	168Er
pERK 1/2 [T202/Y204]	171Yb
pSTAT4 [Y693]	174Yb
pHistone H3 [S28]	175Lu
c-Myc	176Yb

Supplementary Table