SUPPLEMENTARY APPENDIX

Phase I trial of plerixafor combined with decitabine in newly diagnosed older patients with acute myeloid leukemia

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Supplement 1

Cell Isolation and Culture. Primary human AML cells were obtained after informed consent with WCMC IRB approval. Mononuclear cells were isolated from the samples using Ficoll-Plaque (Pharmacia Biotech, Piscataway, NY) density gradient separation. Cells were cryopreserved in CryoStor™ CS-10 (StemCell Technologies).

Flow Cytometry/Immunophenotype. Primary cells were thawed and stained with the following surface antibody panels: (1) CD31-Fluorescein isothiocyanate (FITC) (clone WM59; BD Pharmingen), CD123-Peridinin-chlorophyll cyanin-5.5 (PerCP-Cy5.5) (clone 7G3; BD Pharmingen); CD45-allophycocyanin-Hilite®.7-BD (APC-H7) (clone 2D1; BD), CD184 (CXCR4)-allophycocyanin (APC) (clone 12G5; biolegend), CD90 (Thy1)-Alexa-fluor®700 (AxF700) (clone5E10;biolegend), CD38-phycoerythin cyanin-5 (PECy5) (clone HIT2; BD); CD202b (Tie2)-phycoerythin (PE) (clone 33.1; biolegend); CD34-phycoerythrin cyanin-7 (PECy7) (clone 8G12; BD) and DAPI (4',6-diamidino-2-phenylindole). (2) CD33-FITC (clone P67.6; BD), CD25-PerCP-Cy5.5 (M-A251; BD Pharmingen), CD45-APC-H7 (clone 2D1; BD), CD133/2-APC (clone 293C3; MACS Miltenyi biotec); CD19-AxF700 (clone HIB19; biolegend), CD34-PE-Cy5 (clone 581; BD), TIM3-PE (clone 344823; R&D Systems), CD117-PE-Cy7 (clone 104D2, biolegend), CD56 V450 (Clone B159; BD Horizon), HLA-DR-V500 (clone G46-6; BD Horizon). (3) CD123-PerCP-Cy5.5 (clone 7G3; BD Pharmingen); CD45-APC-H7 (clone 2D1; BD), CD34-PE-Cy5 (clone 581; BD), CD99-PE (Clone TÜ12; BD Pharmingen), CD38-PE-Cy7 (clone HB7; BD) and DAPI. **Cell cycle:** Cells were fixed (BD Cytofix/Cytoperm Buffer), permeabilized (Perm/wash buffer) and stained with CD45-APC-H7 (BD), CD34-APC (clone 8G12; BD), CD38-PE-Cy7 (clone HB7; BD), CD184-PE (clone 1D9; BD Pharmingen), Ki-67-FITC (BD), and DAPI. Cells were evaluated either in a BD LSR-II or BD LSR-Fortessa.

Instruments were evaluated prior every run with the same BD™ Cytometer Setup and Tracking (CST) beads. Data was analyzed using FlowJo Software. Statistical analyses and graphs were performed using GraphPad Prism software (GraphPad Software, San Diego, CA).

Mutational profiling

A research next-generation sequencing (NGS) analysis was also performed using a customized 29-gene myeloid malignancy targeted amplicon enrichment panel (RainDance Technologies, Billerica, MA), including *ASXL1, BCOR, BRAF, CBL, DNMT3A, ETV6, EZH2, FLT3-TKD, GATA1, GATA2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NPM1, NRAS, PHF6, PTPN11, RUNX1, SF1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1*, and *ZRSR2*. Amplicons were sequenced using an Illumina MiSeq (v3 chemistry) with 251-bp paired end reads. Quality- and adapter-trimmed reads passing stringent quality control were aligned to human reference genome (GRCh37) plus decoy sequences (hs37d5) using the BWA-MEM algorithm.(1) A median average coverage depth of 2755x (1549x - 4148x) was achieved across all samples with a median of >98% (95.2%-99.0%) of targeted bases achieving >1000x depth. Single nucleotide variants (SNVs) and small insertion-deletions (indels) were identified using the VarDict algorithm(2), excluding both primer and low complexity regions. Single nucleotide polymorphisms (SNPs) with no known clinical impact of global minor allele frequency (MAF) >0.25% (dbSNP132) were filtered from subsequent analyses. Variant annotation was performed using SnpEff 4.1(3)

References

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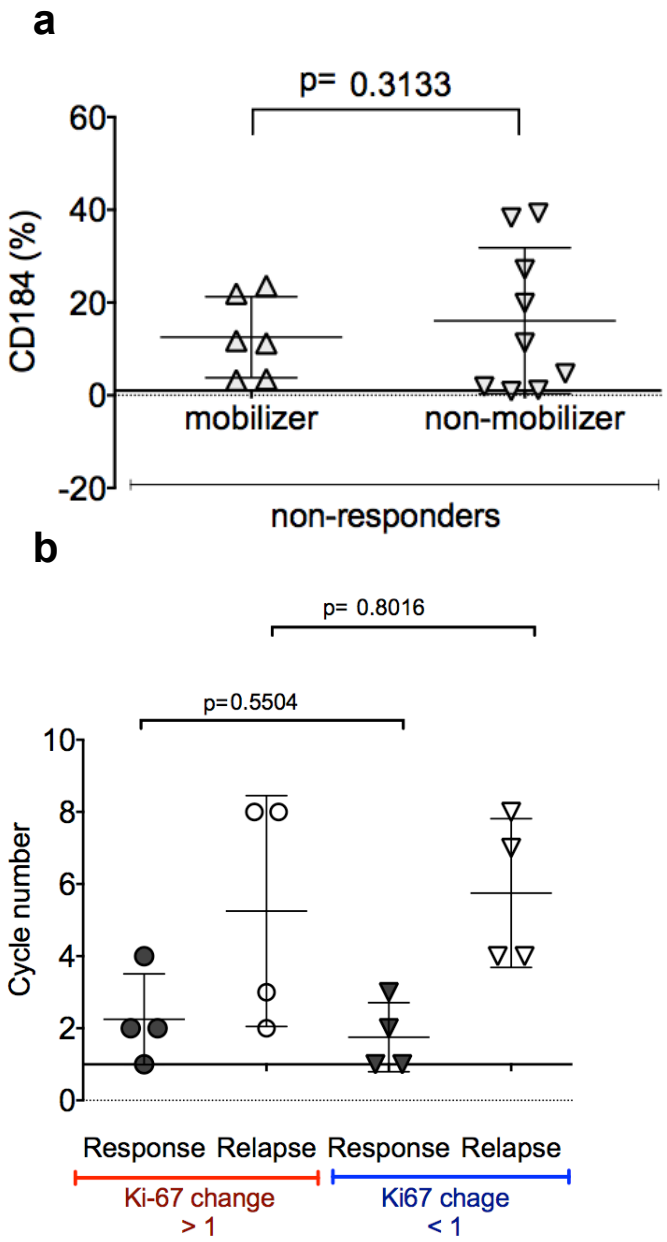

Supplemental figure 1. CXCR4 expression has no impact on mobilization within the non-responding group and increased cycling of stem/progenitor cells does not impact the timing of relapse. (a) Scatter plot for the expression of CXCR4 at diagnosis comparing mobilizers and non-mobilizers within the clinical non-responders group. **(b)** Scatter plot showing the cycle number in which a response or relapse was noted, separated based on change in Ki-67 expression. Each symbol represents a patient, horizontal bar represents the mean cycle number, error bars represent the S.E.M.