SUPPLEMENTAL INFORMATION

Materials and Methods

Patient and cord blood samples

CML samples were obtained from newly diagnosed, TKI-naïve chronic phase CML patients (n=12). Healthy cord blood samples (n = 8) were purchased from St. Louis Cord Blood Bank (St. Louis, MO). Mononuclear cells (MNCs) from CML or cord blood samples were separated by density gradient centrifugation on Ficoll-Hypaque (Nycomed, Zürich, Switzerland). $CD34^+$ cells isolated from MNCs using an autoMACS system (Miltenyi Biotec, San Diego, CA) were assessed for purity >90% with a Guava easyCyte HT Flow Cytometer (Millipore, Billerica, MA). Informed consent was obtained from all sample donors in accordance with the Declaration of Helsinki, and the studies were approved by the University of Utah Institutional Review Board. Baseline characteristics of CML patients are summarized in Supplemental Table S1.

Cell preparation and fluorescence-activated cell sorting

Cryopreserved $CD34^+$ cells from CML patients or fresh $CD34^+$ cord blood cells were cultured overnight in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% BIT 9500 (StemCell Technologies) supplemented with cytokines (StemSpan™ CC100 containing rhFlt3L, rhSCF, rhIL-3 and rhIL-6; StemCell Technologies) at 37°C prior to experiments. $CD34^+$ cells were simultaneously stained with anti-CD38-allophycocyanin (APC) (Clone HIT2; Thermo Fisher Scientific, Waltham, MA) and anti-CD34-fluorescein isothiocyanate (FITC) (Clone 4H11; Thermo Fisher Scientific) antibodies. Cells were sorted on BD FACSAria II (BD Biosciences, San Jose, CA) into $CD34^+CD38^-$ cells (enriched for stem cells) and $CD34^+CD38^+$ progenitor cells.

Pharmacologic inhibitors

Imatinib was a gift of Novartis. Karyopharm Therapeutics provided selinexor (KPT-330).

Clonogenic assays

$CD34^+$ cells were cultured overnight with cytokines followed by sorting into $CD34^+38^+$ and $CD34^+38^-$ cell populations and plated at 2.2 x 10^3 cells/mL in Methocult H4230 (StemCell Technologies) with cytokines ± 50 nM selinexor, 2.5 μM imatinib, or combination of both. The selinexor concentration was selected based on our previous report that showed minimal toxicity against normal $CD34^+$ cells at this concentration (1). Colony forming unit granulocyte-macrophage (CFU-GM) colonies were counted after 14 days. Colony counts are summarized in Supplemental Table S3.

Apoptosis assays

$CD34^+38^+$ or $CD34^+38^-$ cells from CML patients (n=3) or CB (n=3) were cultured for 72 hours ± selinexor and/or imatinib at the indicated concentrations. Apoptosis was measured by annexin V-APC and 7-aminoactinomycin D (BD Biosciences, San Jose, CA) on a BD LSRFortessa. Data analysis was performed using FlowJo software (Tree Star, Ashland, OR).

Long term culture-initiating cell (LTC-IC) assays

LTC-IC assays were performed as described (2, 3). Briefly, $1x10^4$ viable $CD34^+$ cells from CML patients (n=6) were cultured ± selinexor (50 nM) and/or imatinib (2.5 μM) for 72 hours, followed by plating in MyeloCult H5100 (Stem Cell Technologies), using irradiated (80Gy) M2-10B4 cells as feeder layers. Following 5-6 weeks of culture with weekly half medium changes, cells were trypsinized and plated in clonogenic assays in Methocult H4435 Enriched (Stem Cell Technologies). CFU-GM colonies were scored and harvested after 14-18 days, and genotyped for BCR-ABL1 by fluorescence in situ hybridization (FISH) with Vysis LS1 BCR/ABL Dual Color Dual Fusion probes according to the manufacturer's instructions (Abbott Molecular Inc., Des Plaines, IL). LTC-IC colony counts are summarized in Supplemental Table S3.

Patient-derived xenografts

For the first PDX experiment, viable 1×10^7 CML $CD34^+$ cells were cultured in the absence or presence of selinexor (50 nM) and/or imatinib (2.5 μM) for 72 hours. Cultured cells from each indicated treatment were transplanted via tail vein injections into 10-week-old sub-lethally irradiated (2Gy) female NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice (The Jackson Laboratory) (5 mice per treatment arm). The mice were sacrificed at 12 weeks and assessed for human engraftment in the bone marrow by immunophenotyping using anti-mouse CD45-FITC (Clone 30-F11, Tonbo Biosciences, San Diego, CA) and anti-human CD45-APC (Clone HI30, Tonbo Biosciences). FACS-sorted human $CD45^+$ cells were genotyped for *BCR-ABL1* by FISH, as above. The second, third, and fourth PDX experiments were performed with 1.1×10^7, 3.78×10^6, 4×10^6 $CD34^+$ cells from different CML patients, as described above. Body weights of mice and splenic weights at the time of harvest from the four PDX experiments are summarized in Supplemental Figure S2.In PDX 1, one mouse in the imatinib group and two mice in the combination group died of unknown causes between weeks 2 and 3. In PDX 2 and 3, no mice died, and in PDX 4, one selinexor mouse and all five imatinib mice died by week 4. All dead mice were excluded from further analysis. In PDX 3, one untreated and one combination mouse, did not yield sufficient human cells for scoring by FISH.

Meta-analysis of CML microarray data and prediction of CRM1 cargo proteins

Gene expression information of primitive $CD34^+CD38^-$ stem cells or quiescent (G0) cells in CML vs normal hematopoiesis were available in 5 published microarray datasets from Stemformatics (https://www.stemformatics.org) (4-8). Each probe was mapped to a gene, and multiple probes for each gene in each dataset were sorted for statistical significance according to t-tests with the Satterthwaite and unequal variance assumptions. Differentially expressed genes between CML and normal stem cells were ranked and identified as candidate genes if concordant ≥2-fold changes or ≤2-fold changes in gene expression were observed in at least 3 out of 5 datasets, each with $p<0.05$. Visual representation of the intersections of differentially expressed genes

identified in the various datasets were made with the UpSet (9) module of the Intervene (10) Shiny App web application, with the first portion of the last name of the first author listed for each study (references 4-8). Proteins encoded by these candidate genes were cross-referenced against putative CRM1 cargos for the presence of nuclear export signals (NES) predicted by the LocNES online tool (11). The top scoring up- or down-regulated candidate genes/potential CRM1 cargo proteins in CML stem cells are summarized in Supplemental Table S2.

To determine whether genes with putative NES were enriched within the genes with differential expression between LSCs and HSCs, we performed a genome-wide benchmark of the LocNES tool for randomly selected 100 RefSeq genes and also for genes with high expression in HSCs without regard for LSC expression. This was done by identifying genes whose highest expressed probe was in the 95th percentile of expression or higher in at least three of the five studies. This identified 619 genes, and from these we randomly chose 100 genes for an additional comparison of LocNES calling. Of note, these latter genes were generally also highly expressed in LSCs. We utilized the LocNES recommended cut-off of 0.1 for assessing NES on each of the genes, comparing the overall frequencies of NES being called in each gene set by Fisher's Exact test.

Supplemental Figures

Figure S1. Example annexin v plots from patient samples P02 and P03; to go with Figure 1C.

Figure S2. Body weights of mice and splenic weights at the time of harvest from the four PDX experiments. Sub-lethally irradiated female NOD scid gamma (NSG) mice (5 mice per treatment arm) injected with $CD34^+$ cells from four different CML patients were treated ex vivo with each single inhibitor or the combination for 72 hours. At 12 weeks, mice were sacrificed and assessed for engraftment. Mice were weighed periodically (A; averages shown) and spleen weight assessed at time of harvest (B). In the fourth PDX, mice transplanted with imatinib-treated cells

died between days 28 and 30 of an unknown cause; autopsy failed to reveal abnormalities and the %Ph^+ cells are shown in panel (C).

* indicates $p<0.05$ when compared to the untreated group.

Supplemental Tables

Table S1. Baseline clinical characteristics of CML patients (sample donors) in the study.

Table S2. CFU-GM colony counts from clonogenic assays and LTC-IC assays.

Table S3. Top scoring differentially expressed genes in LSCs and their putative nuclear export signals (from meta-analysis of published CML microarray datasets)

Table S4. 100 random genes in the genome and their putative nuclear export signals

Table S5. 100 random genes highly expressed in both HSCs and LSCs and their putative nuclear export signals

References

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