Combined inhibition of MDM2 and BCR-ABL1 tyrosine kinase targets chronic myeloid leukemia stem/progenitor cells in a murine model

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Supplemental Materials

Supplemental Methods

*Human cells*

Cells from newly diagnosed chronic phase CML patients (n = 5 for RT-PCR and n = 7 for Western blot, Supplemental Table 1) and normal bone marrow controls (n = 6 for RT-PCR and n = 5 for western blot) were collected in accordance with MD Anderson Cancer Center IRB-approved protocols. Mononuclear cells were isolated by density-gradient centrifugation using lymphocyte separation medium (Corning; Manassas, VA) and CD34+ cells were enriched using EasySep™ Human CD34 Positive Selection Kit II (Vancouver, BC, Canada) or Miltenyi Microbeads and autoMACS Separator (Miltenyi, Auburn, CA).

*CyTOF mass cytometry*

Mouse bone marrow cells were stained with a panel of metal-tagged antibodies for cell surface markers and intracellular proteins (Supplemental Table 3) and subjected to CyTOF analysis as previously described.1-3 Viable (cisplatin low) single cells were gated with FlowJo software (v10.2, FlowJo LLC) and exported as flow cytometry standard (FCS) data for subsequent analysis in Cytofkit.4 RPhenoGraph was used for unsupervised subset detection based on cell surface markers. t-SNE embedded FCS files were further analyzed in FlowJo and cell populations identified by RPhenoGraph were mimicked and gated on the t-SNE map for quantitation of intracellular marker expression. ArcSinh-transformed counts for the expression of each protein in the desired cell compartments were exported and visualized with heat maps.

*Mitochondrial Priming*
Frozen bone marrow cells obtained from Tet-off and Tet-on transgenic Scl-tTa-\textit{BCR-ABL1} mice were thawed. Viable cells were enumerated by Trypan blue exclusion and assessed for BCL-2 protein family function using the BH3 priming assay as previously described.\textsuperscript{5} To obtain sufficient cell numbers, we added Molm-13 cells to reach a 10\textsuperscript{6} cells/ml cell concentration. Cells were then stained with antibodies against mouse CD45, Lineage cocktail, SCA-1, and C-KIT or appropriate isotype-matched control antibodies (BD Biosciences, San Jose, CA). Because Molm-13 cells are of human origin, the antibodies do not stain these cells, so the mouse cells were gated as CD45-positive. Gating for positivity for each marker was based on isotype control staining. Cells were treated with various BH3 peptides (PUMA, 10 \(\mu\)M; others, 100 \(\mu\)M) for 2 h and 15 min. Total CD45\textsuperscript{+} or CD45\textsuperscript{+} LSK cells were gated and JC-1-red positivity was measured. Priming was calculated for each peptide using the following formula, with dimethyl sulfoxide as a negative control and carbonyl cyanide m-chlorophenylhydrazone as a positive control (both purchased from Sigma Aldrich, St. Louis, MO).

\[
\text{Priming} = \frac{\text{Dimethyl Sulfoxide MFI} - \text{Peptide MFI}}{\text{Dimethyl Sulfoxide MFI} - \text{Cyanide m - Chlorophenyl hydrazone MFI}} \\
\times 100\%
\]
Supplemental Figures

Supplemental Figure 1. Effects of combined activation of p53 by MDM2 inhibition and inhibition of BCR-ABL1 by imatinib *in vivo* on GFP-LSK cells in bone marrow (A) and spleen (B). Cells were collected at the end of treatments from bone marrow and spleen of each treatment group and the control (n = 5, 3, 4, and 4 for control, IM, DS-5272, DS-5272+IM; respectively). Numbers of GFP-LSK cells were determined by flow cytometry after cells were stained with a lineage cocktail and antibodies against SCA-1 and C-KIT (CD117). CON, control; IM, imatinib.

Supplemental Figure 2. Mouse body weight during treatments

![Body weight chart](image-url)
## Supplemental Tables

### Supplemental Table 1. Patient characteristics

| Patient no. | Blast% | Source | Cell population | Assay       |
|-------------|--------|--------|-----------------|-------------|
| 1           | 1      | BM     | Total           | RT-PCR      |
| 2           | 1      | BM     | Total           | RT-PCR      |
| 4           | 2      | BM     | Total           | RT-PCR      |
| 5           | 2      | BM     | Total           | RT-PCR      |
| 6           | 1      | BM     | Total           | RT-PCR      |
| 7           | 5      | BM     | CD34+           | Western blot|
| 8           | 2      | BM     | CD34+           | Western blot|
| 9           | 2      | BM     | CD34+           | Western blot|
| 10          | 2      | BM     | CD34+           | Western blot|
| 11          | 0      | BM     | CD34+           | Western blot|
| 12          | 2      | PB     | CD34+           | Western blot|
| 13          | 0      | BM     | CD34+           | Western blot|

**CP, chronic phase; BM, bone marrow; PB, peripheral blood.**

### Supplemental Table 2. Primer sets for PCR analysis

| Name | Mouse | Human |
|------|-------|-------|
| Abl1 | Mm00802029_m1 | ABL1 Hs01104728_m1 |
| Bax  | Mm00432051_m1 | BAX Hs00180269_m1 |
| Trp53| Mm01731287_m1 | TP53 Hs99999147_m1 |
| Mdm2 | Mm01233138_m1 | MDM2 Hs00242813_m1 |
| Cdkn1a| Mm04205640_g1 | CDKN1A Hs00355782_m1 |
| Pmaip1| Mm00451763_m1 | PMAIP1 Hs00560402_m1 |
| 18s  | Mm03928990_g1 | 18S Hs03928985_g1 |

### Supplemental Table 3. Antibody panel for CyTOF analysis

| Target | Label | Clone | Vendor           |
|--------|-------|-------|------------------|
| CD45   | 147Sm | 30-F11| DVS-Fluidigm     |
| CD4    | 145Nd | RM4-5 | DVS-Fluidigm     |
| CD11b  | 148Nd | M1/70 | DVS-Fluidigm     |
| IGM    | 151Eu | RMM-1 | DVS-Fluidigm     |
| CD3e   | 152Sm | 145-2C11| DVS-Fluidigm |
| TER119 | 162Dy | Ter-119| DVS-Fluidigm |
| LY6G/LY6C, GR-1 | 175Lu | RB6-8C5 | BioLegend |
| B220, CD45R | 176Yb | RA3-6B2 | Biolegend |
| LY6A/E, SCA-1 | 164Dy | D7 | DVS-Fluidigm |
| CD117, C-KIT | 166Er | 2B8 | DVS-Fluidigm |
| Antibody   | IS/AM | Cat. No. | Company     |
|-----------|-------|----------|-------------|
| CD16, CD32 |       | 144Nd    | DVS-Fluidigm |
| CD34      |       | 156Gd    | BioLegend   |
| BAX       |       | 163Dy    | BioLegend   |
| p53       |       | 165Ho    | R&D Systems |
| p21       |       | 154Sm    | Sigma       |
| NOXA      |       | 168Er    | Sigma       |
| MDM2      |       | 173Yb    | Sigma       |

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