SUPPLEMENTAL METHODS

Intracellular Flow Cytometry

Lineage depleted (MACS) mouse BM and spleen cell suspensions were incubated with the biotinylated lineage-specific antibody cocktail (lineage cell depletion kit, Miltenyi), PeCy7-conjugated anti-Sca-1 and APC Alexa Fluor 750-conjugated c-Kit antibodies, and streptavidin conjugated-PE Texas Red (Invitrogen). Labeled cells were processed using the Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences) according to the manufacturer's instructions,. Afterwards, fixed/permeabilized cells were incubated with either the mouse anti-hnRNP A1 (4B10) antibody (Abcam, Cambridge, UK) or the isotype matched control (mIgG, Santa Cruz Biotechnology, Santa Cruz, CA), and a secondary goat F(ab') anti-mouse conjugated to Alexa Fluor 647 (Invitrogen). Analysis was performed using flow cytometry (LSRII; BD Biosciences) and data were analyzed using FlowJo FACS software (BD Biosciences).

Colony Forming Culture (CFC)/ replating assay and determination of Long Term Culture-Initiating Cell (LTC-IC) frequency.

Colony forming culture assays were performed with LSK from the bone marrow and/or spleen of 8 week-induced SCLtTA-BCR-ABL1 (dTg) mice and CML-CP and CML-BC CD34$^+$ progenitors plated in duplicate in 0.9% MethoCult supplemented with recombinant cytokines (LSK: recombinant mouse (rm) stem cell factor, rm IL-3, recombinant human (rh) IL-6 and rh Erythropoietin (Epo) (PeproTech, Rocky Hill, NJ); CML CD34$^+$ progenitors: H4435/Epo, Stem Cell Technologies) and either DMSO or chemical inhibitors. Colonies were scored 14 days later. For replating assays following exposure to ABT-263, contents of both plates were pooled, washed, and seeded at 10,000/ml in inhibitor free cytokine-supplemented MethoCult and number of colonies quantified after 14 days. Determination of LTC-IC frequency was performed as described[1]. Briefly, feeder/stromal layers were established by culturing bone marrow from wild type FVB/N mice in Myelocult M5300 medium supplemented with hydrocortisone sodium

hemisuccinate (Stem Cell Technologies). After reaching 70-80% confluency, feeder cells were irradiated (20 Gy), and dilutions (n=8, 3 to 6,500/well) of lineage-negative from the bone marrow of 8 week-induced SCLtTA-BCR-ABL1 mice, in replicates of 6, were added to the stromal layer and exposed to either DMSO (untreated) or a single dose of 1 μM ABT-263 for 7 days. Afterwards, medium was replaced weekly and dilutions were scored 4 weeks later. Presence of one cobblestone area was considered to be indicative of a dilution containing at least one LTC-IC[2]. The frequency of long term culture-initiating cells (LTC-IC) in untreated and ABT-263-treated cells was calculated with L-Calc software (Stem Cell Technologies).

Histology

Mice were euthanized and organs were removed and fixed in neutrally buffered 10% formalin at room temperature for at least 16 hours prior to being embedded in paraffin. Sectioned tissues were stained with hematoxylin and eosin and visualized with a Zeiss Axioskope 2 Plus (Zeiss, Guttingen, Germany). A 40X/0.65 lens was used for all tissues and images were taken with a QICLICK-F-M-12 CCD camera (QImaging) equipped with an RGB liquid crystal color filter module for capturing color images.

Lentiviral transduction

Pseudotyped lentiviruses were produced by transient calcium phosphate transfection (ProFection mammalian transfection System, Promega, Madison, WI) of 293T cells with the specific lentiviral construct (18μg/175cm2), the psPAX2 packaging construct (Addgene plasmid 12260, provided by Dr. D. Trono, Swiss Institutes of Technology EPFL, Lausanne, Switzerland; 9μg/175cm2) and the G-glycoprotein of vesicular stomatitis virus (VSV-G, 1.8μg/175cm2). Virus containing supernatant was concentrated with 40% PEG-8000 solution and centrifugation (30 min, 1500 x g at 4°C). Target cells ($1x10^6$/ml) were infected by spinoculation (1000 x g for 2 hr. at 25°C) with viral supernatants in polybrene-containing (4 mg/ml) complete medium and selected 48 hr. post-infection by green fluorescent protein (GFP)-mediated cell sorting.

Total RNA isolation and real-time PCR

Total RNA was isolated by acid guanidinium-phenol/chlorophorm-based extraction (Trizol reagent, Invitrogen, Carlsbad, CA). RNA samples were DNAse-treated (Turbo DNAse, Applied Biosystems, Carlsbad, CA) and first-strand synthesis was achieved with random hexamer primers and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen). Expression of Bcl-x, Bcl-2, and Mcl-1 was measured as a percentage of L19 expression using a SYBR Green Assay (Power SYBR Green PCR Master Mix, Applied Biosystems). All reactions were performed in duplicate. Sequences of primers are provided in table 1.

Western blot analysis

Total proteins were obtained by lysing cells in either RIPA (150 mM NaCl, 1% NP-40, 0.1% SDS, 50 mM Tris [pH 8.0]) supplemented with complete protease and PhosSTOP inhibitor cocktails (Roche, Manheim, Germany) (cell lines) or Laemmli (primary cells) buffer, whereas cytoplasmic and mitochondrial subcellular fractions were isolated as described[3, 4]. Lysates containing 1-5x10^4 FACS-purified hematopoietic stem cell-enriched ($CD34^+$/$CD38^-$) and progenitor (e.g., CMPs and GMPs) cells from CML patients were heat-denatured and fractionated onto a 4-15% SDS-PAGE (BioRad Mini Protean), overnight transferred onto nitrocellulose membrane, and immunoblotted using conventional immunoblot technique. Chemiluminescent detection was performed with Amersham ECL Prime Western blotting reagent (GE Healthcare Life Sciences, Piscataway, NJ). Immunoblotting was performed with the following primary antibodies: anti-BAD, anti-Bcl-x and anti-Grb2 (BD Transduction, San Diego, CA); anti-Actin (C-11), anti-Abl (24-11), anti-c-MYC (N-262), anti-I2PP2A (E-15) (SET), and anti-Bcl-2 (N19) (Santa Cruz Biotechnology, Santa Cruz, CA); anti-Mcl-1 (Rockland Immunochemical, Gilbertsville, PA); anti-Phosphotyrosine (4G10) (Millipore, Billerica, MA); anti-Phospho-Akt (Ser473) (D9E), anti-Akt, and anti-Phospho-CrkI (Y207) (Cell Signaling, Danvers, MA); anti-hnRNP A1 (Sigma, St. Louis, MO), and anti-hnRNP K (3C2) (Abcam, Cambridge,

UK); The anti-hnRNP E2 was a kind gift of Dr. S.A. Liebhaber, University of Pennsylvania School of Medicine, Philadelphia PA).

REFERENCE TO SUPPLEMENTAL METHODS

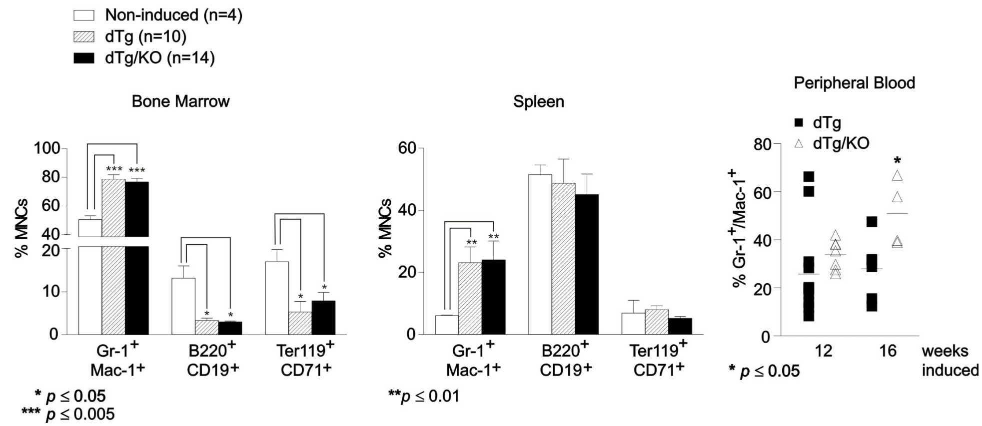
1. van Os RP, Dethmers-Ausema B, de Haan G. In vitro assays for cobblestone area-forming cells, LTC-IC, and CFU-C. In: Bunting KD (ed) Methods in Molecular Biology, vol. 430, 2008, pp 143-157.

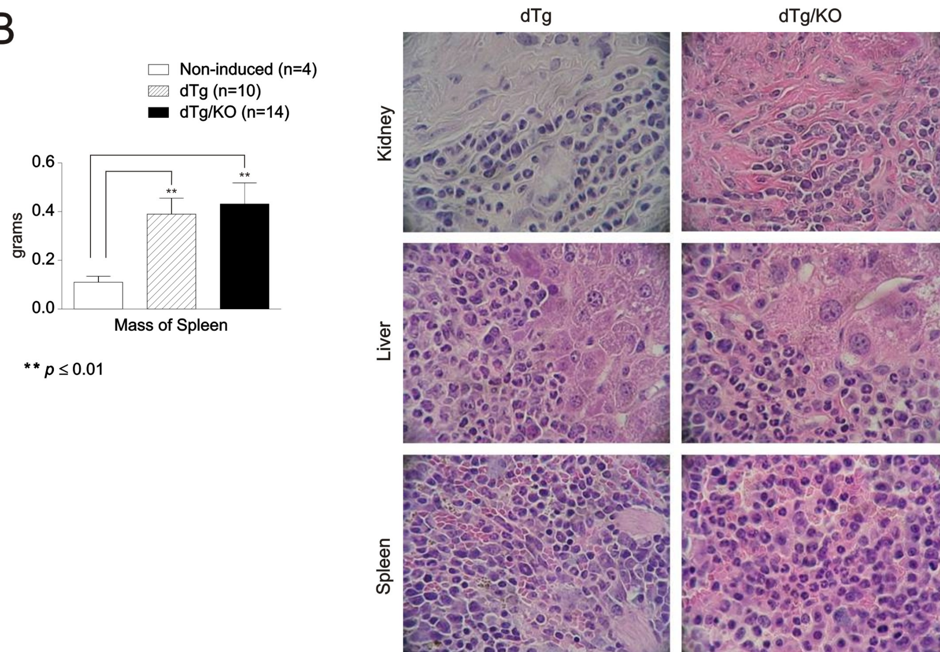
2. Breems DA, Blokland EAW, Neben S, Ploemacher RE. FREQUENCY-ANALYSIS OF HUMAN PRIMITIVE HEMATOPOIETIC STEM-CELL SUBSETS USING A COBBLESTONE AREA FORMING CELL ASSAY. Leukemia 1994; 8(7): 1095-1104.

3. Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, Liu SJ et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. Cancer Cell 2005; 8(5): 355-368.

4. Keeshan K, Cotter TG, McKenna SL. High Bcr-Abl expression prevents the translocation of Bax and Bad to the mitochondrion. Leukemia 2002; 16(9): 1725-1734.

SUPPLEMENTAL FIGURE





Supplemental Figure 1. ***Deletion of Bcl-xL does not affect CML development in vivo. (A)*** Flow cytometric analysis shows percentage (mean ± SEM) of Gr-1$^+$/Mac-1$^+$, B220$^+$/CD19$^+$ and Ter119$^+$/CD71$^+$ cells in the bone marrow (*left*) and spleen (*middle*) of non-induced, and leukemic dTg and dTg/KO mice. Percentage of Gr-1$^+$/Mac-1$^+$ in MNCs from the peripheral blood of 12 and 16 week-induced dTg (squares) and dTg/KO (triangles) mice is shown (*right*). ***(B)*** (*left*) Graph shows spleen weights (mean ± SEM) of non-induced (n=4), leukemic dTg (n=10) and dTg/KO (n=14) animals. (*right*) H&E-stained sections of kidney, liver, and spleen of leukemic dTg (left) and dTg/KO (right) mice. Original magnifications x 40X.