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

Bing Z. Carter,[1] Po Yee Mak,[1] Hong Mu,[1] Xiangmeng Wang,[1] Wenjing Tao,[1] Duncan H. Mak,[1] Elisha J. Dettman,[2] Michael Cardone,[2] Oleg Zernovak,[3] Takahiko Seki,[3] and Michael Andreeff[1]

[1]Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Eutropics, Cambridge, MA, USA and [3]Daiichi Sankyo Co. Ltd., Oncology Laboratories, R&D Division, 2-58, Hiromachi 1-Chrome, Shinagawa-ku, Tokyo, Japan

 doi:10.3324/haematol.2019.219261

Received: February 11, 2019.

Accepted: July 26, 2019.

Pre-published: August 1, 2019.

Correspondence: *BING Z. CARTER* - bicarter@mdanderson.org

MICHAEL ANDREEFF - mandreef@mdanderson.org

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.

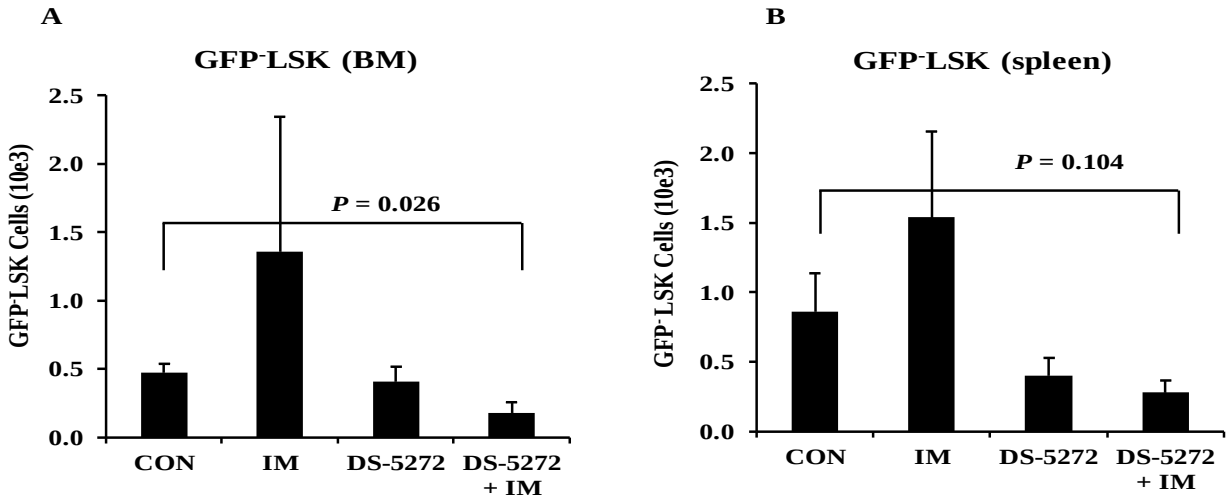
Mitochondrial Priming

Frozen bone marrow cells obtained from Tet-off and Tet-on transgenic Scl-tTa-*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.[5] To obtain sufficient cell numbers, we added Molm-13 cells to reach a 10^6 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 μM; others, 100 μM) for 2 h and 15 min. Total $CD45^+$ or $CD45^+$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).

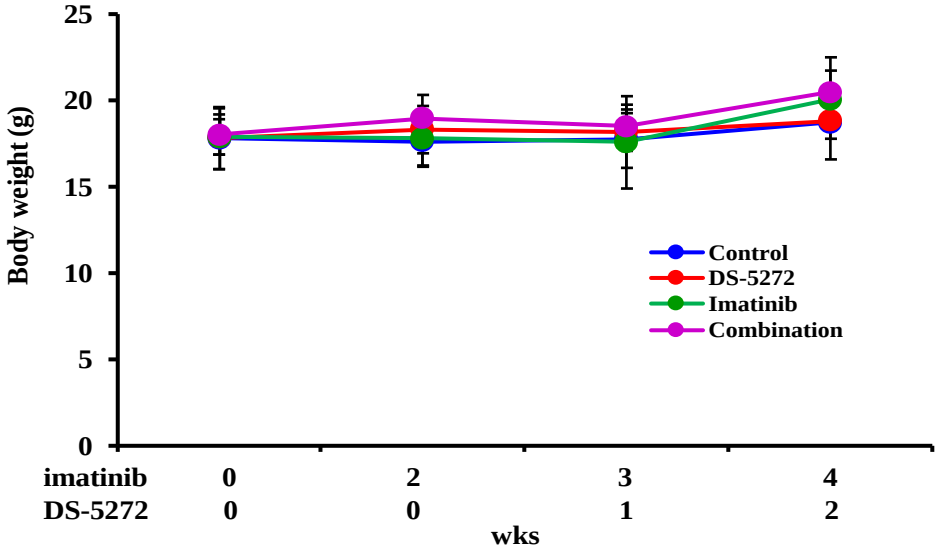
$$Priming = \frac{Dimethyl\ Sulfoxide\ MFI - Peptide\ MFI}{Dimethyl\ Sulfoxide\ MFI - Cyanide\ m - Chlorophenyl\ hydrazone\ \ MFI} \times 100\%$$

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



Supplemental Tables

Supplemental Table 1. Patient characteristics

CML-CP 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

Mouse		Human	
Name	**primers**	**Name**	**primers**
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
		BCR-ABL1	Hs03024541_ft

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

CD16, CD32	144Nd	93	DVS-Fluidigm
CD34	156Gd	MEC14.7	BioLegend
BAX	163Dy	5B7	BioLegend
p53	165Ho	184721	R&D Systems
p21	154Sm	CP74	Sigma
NOXA	168Er	114C307	Sigma
MDM2	173Yb	HDM2-323	Sigma

References

1. Carter BZ, Mak PY, Mu H, et al. Combined targeting of BCL-2 and BCR-ABL tyrosine kinase eradicates chronic myeloid leukemia stem cells. Sci Transl Med. 2016;8(355):355ra117.

2. Han L, Qiu P, Zeng Z, et al. Single-cell mass cytometry reveals intracellular survival/proliferative signaling in FLT3-ITD-mutated AML stem/progenitor cells. Cytometry A. 2015;87(4):346-356.

3. Zhou H, Mak PY, Mu H, et al. Combined inhibition of beta-catenin and Bcr-Abl synergistically targets tyrosine kinase inhibitor-resistant blast crisis chronic myeloid leukemia blasts and progenitors in vitro and in vivo. Leukemia. 2017.

4. Chen H, Lau MC, Wong MT, Newell EW, Poidinger M, Chen J. Cytofkit: A Bioconductor Package for an Integrated Mass Cytometry Data Analysis Pipeline. PLoS Comput Biol. 2016;12(9):e1005112.

5. Ishizawa J, Kojima K, McQueen T, et al. Mitochondrial Profiling of Acute Myeloid Leukemia in the Assessment of Response to Apoptosis Modulating Drugs. PLoS One. 2015;10(9):e0138377.