SUPPLEMENTARY APPENDIX

Targeting BCL2 with venetoclax is a promising therapeutic strategy for "double-protein-expression" lymphoma with *MYC* and *BCL2* rearrangements

Akiko Uchida,[1*] Yasushi Isobe,[1*] Junko Asano,[1] Yu Uemura,[1] Masahiro Hoshikawa,[2] Masayuki Takagi[2] and Ikuo Miura[1]

[1]Division of Hematology and Oncology, Department of Internal Medicine, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa and [2]Department of Pathology, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa, Japan

**AU and YI contributed equally to this work.*

 doi:10.3324/haematol.2018.204958

Received: August 21, 2018.

Accepted: November 23, 2018.

Pre-published: December 6, 2018.

Correspondence: *YASUSHI ISOBE* - yisobe@marianna-u.ac.jp

Supplementary documents

Supplementary Methods

Antibodies

Immunohistochemistry was performed using following primary antibodies: 124 (Agilent DAKO, Santa Clara, CA, USA) for BCL2; Y37 (Abcam, Cambridge, UK) for MCL1; C34C5 (Cell Signaling Technology, Danvers, MA, USA) for BIM; E63 (Abcam) for BAX, and Y60 (Abcam) for MYC.

Western blot analysis was performed using following primary antibodies: H12 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for BCL6; A-7 (Santa Cruz Biotechnology) for BRD4; D84C12 (Cell Signaling Technology) for MYC; C-2 (Santa Cruz Biotechnology) for BCL2; 5H2 (Cell Signaling Technology) for phosphorylated BCL2 at serine 70; Y37 (Abcam) for MCL1; E18 (Abcam) for BCL-xL; C34C5 (Cell Signaling Technology) for BIM; D4E4 (Cell Signaling Technology) for BAK; BAD antibody (Cell Signaling Technology); E63 (Abcam) for BAX; D8L7U (Cell Signaling Technology) for NOXA; 23 (Santa Cruz Biotechnology) for protein phosphatase 2A (PP2A) B56α; H5D12 (Santa Cruz Biotechnology) for PP2A B56δ; and AC74 (Sigma-Aldrich, St. Louis, MO, USA) for β-actin.

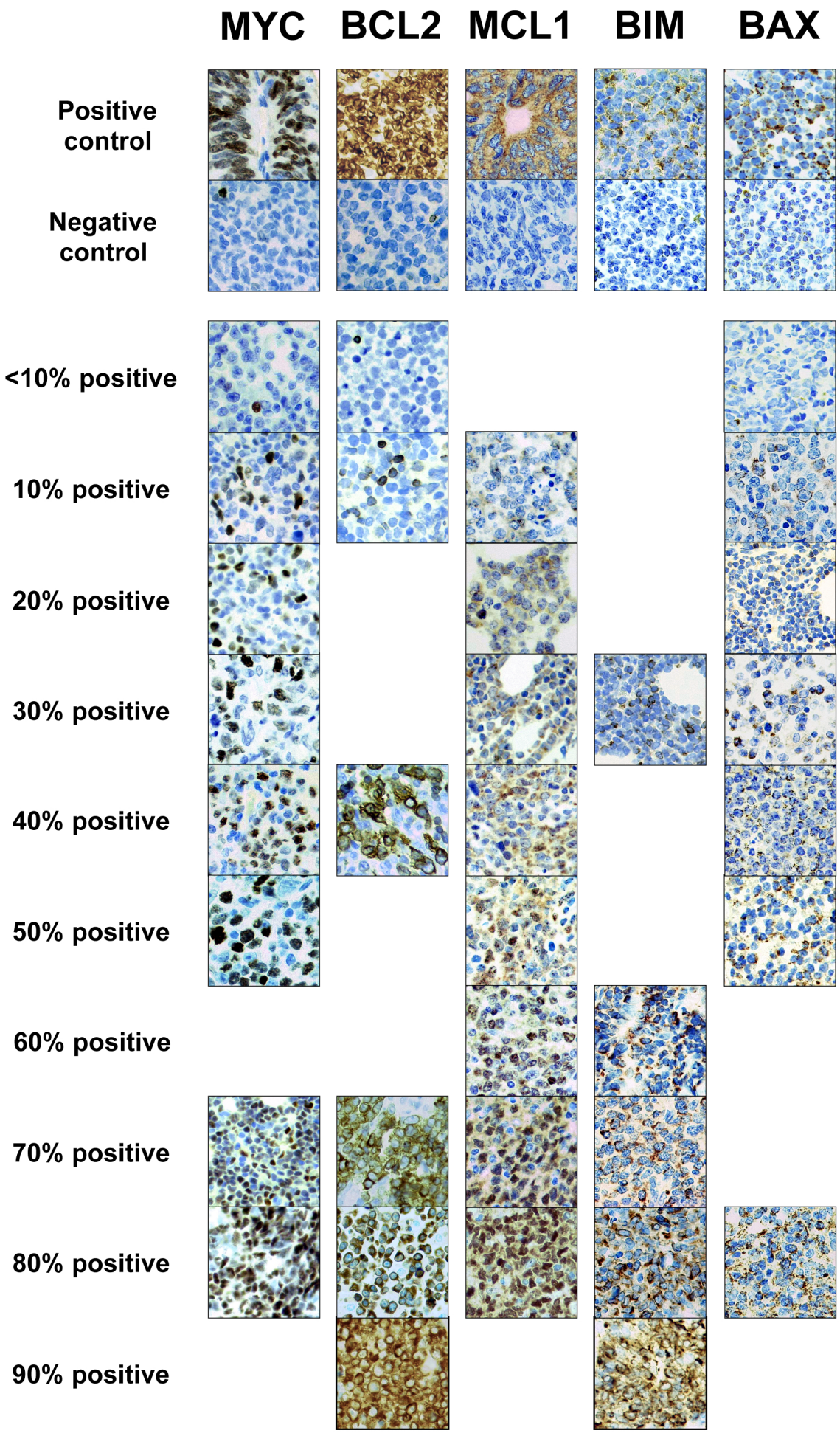
Immunoprecipitation was performed using following antibodies: control rabbit IgG (Agilent DAKO); EPR17509 (Abcam) for BCL2; and C34C5 (Cell Signaling Technology) for BIM.

Supplementary Table S1. The list of clinical samples

UPN	diagnosis	specimens	FISH		immunohistochemistry						
			MYC split	*IGH-BCL2*	CD10	BCL6	MYC	BCL2	MCL1	BIM	BAX
1	DH-HGBL (ALL-like)	bone marrow	+	+	+	+	70%	90%	20% (Cy)	60%	30%
2	DH-HGBL (DLBCL)	ileum	+	+	+	+	<10%	90%	10% (Cy)	80%	20%
3	DH-HGBL (ALL-like)	bone marrow	+	+	+	+	70%	90%	60% (Cy)	70%	<10%
4	DH-HGBL (ALL-like)	bone marrow	+	+	+	+	70%	80%	30% (Cy)	70%	10%
5	DH-HGBL (DLBCL)	bone marrow	+	+	+	+	70%	90%	50% (Cy)	30%	20%
6	DH-HGBL (DLBCL)	bone marrow	+	+	+	+	80%	90%	60% (Cy)	90%	<10%
7	DH-HGBL (DLBCL)	lymph node	+	+	+	+	80%	90%	60% (Cy)	60%	20%
8	DH-HGBL (DLBCL)	mediastinal tumor	+	+	+	+	80%	90%	30% (Cy)	80%	20%
9	GCB-DLBCL	lymph node	+	−	+	+	40%	90%	70% (Cy+N)	70%	30%
10	GCB-DLBCL	nasal tumor	−	−	+	+	50%	90%	50% (Cy+N)	70%	10%
11	GCB-DLBCL	gastric tumor	−	−	+	+	40%	70%	60% (Cy)	60%	10%
12	GCB-DLBCL	lymph node	+	−	+	+	80%	90%	80% (Cy)	80%	<10%
13	GCB-DLBCL	lymph node	−	+	+	+	80%	90%	80% (Cy)	90%	<10%
14	GCB-DLBCL	lymph node	−	+	+	+	20%	90%	40% (Cy)	80%	<10%
15	GCB-DLBCL	nasal tumor	−	−	+	+	10%	70%	60% (Cy)	90%	<10%
16	GCB-DLBCL	lymph node	−	−	+	+	30%	<10%	60% (Cy+N)	80%	<10%
17	GCB-DLBCL	lymph node	−	−	+	+	20%	90%	50% (Cy+N)	70%	50%
18	GCB-DLBCL	lymph node	−	−	+	+	40%	<10%	60% (Cy)	70%	<10%
19	GCB-DLBCL	adrenal tumor	−	−	+	+	<10%	80%	60% (Cy+N)	80%	80%
20	GCB-DLBCL	parotid tumor	−	−	+	+	<10%	<10%	80% (Cy+N)	80%	30%
21	GCB-DLBCL	lymph node	+	−	+	+	80%	<10%	80% (Cy)	90%	40%
22	GCB-DLBCL	tonsil	−	−	+	+	30%	<10%	70% (Cy)	90%	<10%
23	GCB-DLBCL	lymph node	−	+	+	+	20%	80%	80% (Cy+N)	80%	20%
24	GCB-DLBCL	parotid tumor	−	−	+	+	40%	<10%	40% (Cy+N)	70%	10%
25	GCB-DLBCL	sinonasal tumor	−	−	+	+	20%	90%	70% (Cy)	70%	<10%
26	GCB-DLBCL*	lymph node	−	+	−	+	10%	40%	70% (Cy)	30%	20%
27	GCB-DLBCL	subcutaneous tumor	−	−	+	+	50%	10%	60% (Cy)	70%	20%

Abbreviations: FISH, fluorescence *in situ* hybridization; DH-HGBL, high grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements; ALL, acute lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; GCB-DLBCL, germinal center B-cell-like DLBCL; Cy, cytoplasmic staining; N, nuclear staining

* UPN26 was diagnosed as GCB-DLBCL because the lymphoma cells were negative for MUM1.


MYC
BCL2
MCL1
BIM
BAX
Positive control
Negative control
<10% positive
10% positive
20% positive
30% positive
40% positive
50% positive
60% positive
70% positive
80% positive
90% positive

Supplementary Figure S1.

Immunohistochemistry for MYC, BCL2, MCL1, BIM and BAX on clinical samples.

The percentages of positive cells were shown by following clinical samples. Fractions less than 10% were truncated. **MYC**: <10% positive (UPN19); 10% positive (UPN26); 20% positive (UPN14); 30% positive (UPN16); 40% positive (UPN9); 50% positive (UPN10); 70% positive (UPN3); and 80% positive (UPN8) cases. **BCL2**: <10% positive (UPN18); 10% (UPN10); 40% positive (UPN26); 70% positive (UPN11); 80% positive (UPN19); and 90% positive (UPN6) cases. **MCL1**: 10% positive (UPN2); UPN1 for 20%; 30% positive (UPN4); 40% positive (UPN14); 50% positive (UPN10); 60% positive (UPN19); 70% positive (UPN9); and 80% positive (UPN23) cases. **BIM**: 30% positive (UPN5); 60% positive (UPN11); 70% positive (UPN9); and 80% positive (UPN14) cases. **BAX**: <10% positive (UPN6); 10% positive (UPN10); 20% positive (UPN5); 30% positive (UPN20); 40% positive (UPN21); 50% positive (UPN17); and 80% positive (UPN19) cases. Colonic carcinoma samples were used as positive controls for MYC and MCL1. Follicular lymphoma samples were used as positive and negative controls for BCL2 and MYC, respectively. Cell blocks obtained from Burkitt cell line Daudi (purchased from the JCRB cell bank) were used as positive controls for BIM and BAX. Tonsillar germinal center B and mantle zone B cells obtained from UPN22 were used as negative controls for BCL2 and BIM/BAX, respectively.