A selective BCL-X_L PROTAC degrader achieves safe and potent antitumor activity

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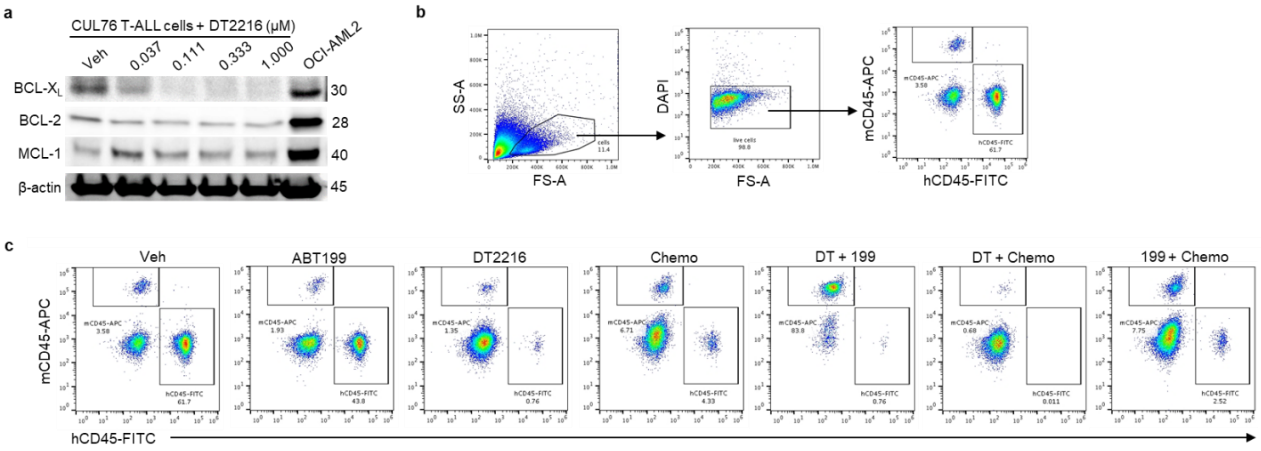
SUPPLEMENTARY FIGURE 1



Supplementary Figure 1. Analysis of T-ALL PDX cell engraftment by flow cytometry. a, A representative immunoblot analysis shows that DT2216 induced the degradation of BCL-X_L but not BCL-2 and MCL-1 in a dose-dependent manner in CUL76 PDX T-ALL cells. OCI-AML2 cells were used as control cells for the detection of BCL-X_L, BCL-2 and MCL-1.Similar results were obtained in one additional independent experiment. **b**. Gating strategy for the flow cytometric analysis of human $CD45^+$ CUL76 T-ALL cell engraftment. **c**, Flow cytometric analyses of human $CD45^+$ CUL76 T-ALL cell engraftment in blood from mice 4 weeks after various treatments as shown in Fig. 6f.

Supplementary Table 1. EC_{50} values of DT2216 and ABT263 in various tumor cell lines

Tumor Type	Cell line	Sensitive to			EC_{50} (µM)	
		A-1155463 (BCL-X_L)	ABT199 (BCL-2)	S63845 (MCL-1)	ABT263 (BCL-2/X_L)	DT2216
T-ALL	MOLT-4	++++	-	-	0.191	0.052
B-ALL	RS4;11	-	++++	-	0.028	0.23
Multiple Myeloma	EJM	-	-	+	>2	>2
	H929	-	-	++++	>2	>2
Small cell lung cancer	NCI-H146	++++	+	n.k.	0.030	0.160
Breast cancer	MDA-MB-231	+	-	-	0.707	0.229
Prostate cancer	PC3	-	-	-	>10	>10
Hepatic cancer	HepG2	-	-	-	>10	>10
Colon cancer	SW620	-	-	-	>10	>10
Renal cancer	786-0	n.k.	n.k.	n.k.	>10	>10

(++++): Highly sensitive (EC_{50} = <0.1 µM); (+): Moderately sensitive (0.1<EC_{50}<1 µM); (-): Insensitive (EC_{50} = >1 µM); n.k.: Not known

Supplementary Table 2. Mouse PK data following IV/IP injection and PO Administration with DT2216

Male C57BL6 mice	Route of administration		
	IP	IV	PO
Formulation	5% DMSO/3% tween 80 in PBS		
Dose (mpk)	20	2	20
Co/C_{max} (ng/mL)	51900	54322	16.6
T_{max} (h)	2.0	NA	4.0
T1/2 (h)	6.3	6.1	ND
AUC0-t (ng.h/mL)	522484	77302	218
T_{last} (h)	24.0	24.0	8.0, 24.0
Cl (mL/min/kg)	NA	0.42	NA
Vss (L/kg)	NA	0.102	NA
F (%)	70.2	NA	0.028

Supplementary Table 3. Tissue distribution of DT2216 after i.p. injection in mouse (20 mpk)

Tissue	C_{max} (ng/mL or ng/g)	T_{max} (h)	AUC_{last} (ng.hr/mL)	T1/2 (h)	AUC ratio (tissue/plasma)
Plasma	24700	1.0	374852	15	1.0
Liver	31126	4.0	737677	ND	1.97
Kidney	6627	4.0	269685	ND	0.72
Brain	198	1.0	2505	ND	0.0067
Lung	1899	4.0	60475	ND	0.16
Small intestine	4848	1.0	116914	17	0.31
Large intestine	6867	4.0	209973	ND	0.56
Heart	1640	1.0	28032	15	0.72
Fat	12104	4.0	605636	ND	1.6
Skin	1523	24	78781	ND	0.21
Pancreas	21607	4.0	1197813	ND	3.2

T_{last} at 96 h for plasma and all of the tissues except the brain (T_{last} at 24 h)

Supplementary Table 4. Tumor pharmacokinetics of DT2216 in female CB-17 SCID-beige mice after a single 15 mpk i.p. injection

Parameters	
C_{max_T} (ng/g)	1329.8
T_{max_T} (h)	48.0
Cl/F (g/h/kg)	90.4
K_e (1/h)	0.011
$t_{1/2}$ (h)	64.2
AUC (h*µg/g)	166.1

[#]Values are presented as mean (n = 2 mice)

Abbreviations: C_{max_T} = maximum tumor concentration, T_{max_T} = time to C_{max}, Cl/F = clearance, K_e = elimination rate constant, $t_{1/2}$ = terminal elimination half-life and AUC= area under the tumor concentration-time curve

Supplementary Table 5. DT2216 sensitizes various cancer cells to chemotherapeutic agents

Cancer type	Cell line	Combination		Average of CI values at EC_{75} and EC_{90}	
		DT2216 +	ABT263 +	DT2216 + Chemo	ABT263 + Chemo
Breast	MDA-MB-231	Docetaxel	Docetaxel	0.142	1.38
Breast	MDA-MB-231	Doxorubicin	Doxorubicin	0.151	0.199
Breast	MDA-MB-231	Vincristine	Vincristine	<0.001	0.005
Prostate	PC-3	Docetaxel	Docetaxel	<0.001	<0.001
Prostate	PC-3	Doxorubicin	Doxorubicin	0.017	0.032
Prostate	PC-3	Vincristine	Vincristine	<0.001	0.006
Hepatic	HepG2	Docetaxel	Docetaxel	<0.001	<0.001
Hepatic	HepG2	Doxorubicin	Doxorubicin	<0.3	0.178
Hepatic	HepG2	Vincristine	Vincristine	0.001	0.001
Colon	SW620	Docetaxel	Docetaxel	<0.001	<0.001
Colon	SW620	Doxorubicin	Doxorubicin	0.325	0.386
Colon	SW620	Vincristine	Vincristine	<0.001	0.004
Renal	786-O (VHL null)	Docetaxel	Docetaxel	0.454	0.003

Combination Indices (CI): CI < 0.3 strong synergistic effect; CI < 1.0 synergistic effect; CI = 1.0 additive effect; CI > 1.0 Antagonistic effect

Supplementary Table 6. Suppliers for various compounds and enzymes

Compound name	Suppliers	Catalog #
ABT263 (Navitoclax)	Selleckchem, Houston, TX,USA	S1001
ABT-199 (Venetoclax)	Selleckchem, Houston, TX,USA	S8048
S63845	Selleckchem, Houston, TX,USA	S8383
MG132	Selleckchem, Houston, TX, USA	S2619
Docetaxel	Selleckchem, Houston, TX,USA	S1148
Doxorubicin	Selleckchem, Houston, TX,USA	S1208
Vincristine	Selleckchem, Houston, TX,USA	S1241
Dexamethasone	Selleckchem, Houston, TX,USA	S1322
L-Asparaginase	KRS Global Biotechnology, Boca Raton, FL, USA	_
Hexadimethrine bromide (Polybrene)	Sigma-Aldrich, St. Louis, MO, USA	H9268
Polyethylene glycol 400	Hampton Research, Aliso Viejo, CA, USA	HR2-603
Polyethylene glycol 300	Sigma-Aldrich, St. Louis, MO, USA	202371
PHOSAL 50 PG	American Lecithin Company, Oxford, CT, USA	368315-3130003/020
MIGLYOL® 810 N	IOI Oleochemical, Hamburg, Germany	_
Polysorbate 80	Spectrum Chemical, New Brunswick, NJ, USA	P0138
Prostaglandin E1 (PGE1)	Santa Cruz Biotechnology, Dallas, TX, USA	sc-201223A
Apyrase from potatoes	Sigma-Aldrich, St. Louis, MO, USA	A6410-100UN
DAPI	Thermo Fisher Scientific, Waltham, MA, USA	D1306

Supplementary Table 7. Antibodies for immunoblotting, immunoprecipitation and flow cytometry

Antibody	Clone	Antibody isotype	Catalog #	Concentration
VHL[1]	_	Rabbit IgG Polyclonal	68547S	1:1000
BCL-X_L[1]	_	Rabbit IgG Polyclonal	2762S	1:2000
MCL-1[1]	D35A5	Rabbit IgG Monoclonal	5453S	1:1000
BCL-2[1]	50E3	Rabbit IgG Monoclonal	2870S	1:500
BCL-W[1]	31H4	Rabbit IgG Monoclonal	2724S	1:500
Caspase-3[1]	_	Rabbit IgG Polyclonal	9662S	1:1000
Cleaved Caspase-3[1]	Asp175	Rabbit IgG Polyclonal	9661S	1:1000
PARP1[1]	46D11	Rabbit IgG Monoclonal	9532S	1:1000
Cleaved PARP1[1]	Asp214	Rabbit IgG Polyclonal	9541S	1:1000
BAX[1]	_	Rabbit IgG Polyclonal	2772S	1:1000
BAK[1]	D4E4	Rabbit IgG Monoclonal	12105S	1:1000
BIM[1]	C34C5	Rabbit IgG Monoclonal	2933S	1:1000
PUMA[1]	D30C10	Rabbit IgG Monoclonal	12450S	1:1000
HA tag[1]	C29F4	Rabbit IgG Monoclonal	14031S	1:1000
Flag tag[1]	_	Rabbit IgG Polyclonal	2044S	1:1000
β-actin[1]	13E5	Rabbit IgG Monoclonal	4970L	1:5000
β-actin[2]	C4	Mouse IgG Monoclonal	8691001	1:10000
β-tubulin[1]	_	Rabbit IgG Polyclonal	2146S	1:5000
BCL-X_L[3]	7B2.5	Mouse IgG Monoclonal	sc-56021	_
Normal mouse IgG[3]	_	_	sc-2025	_
Secondary antibody[1]		Anti-rabbit IgG, HRP	7074S	1:3000
Secondary antibody[1]		Anti-mouse IgG, HRP	7076S	1:3000
Secondary antibody[4]		Anti-Rabbit IgG, Fc fragment specific, HRP	111-035-046	1:10000
mCD45-APC[5]	30-F11	Rat IgG2b, κ, APC	103111	1:250
hCD45-FITC[5]	H130	Mouse IgG1, κ, FITC	304038	1:250

Footnotes: [1]Cell Signaling Technology, Danvers, MA, USA; [2]MP Biomedicals, Santa Ana, CA, USA; [3]Santa Cruz Biotechnology, Dallas, TX, USA; [4]Jackson ImmunoResearch Inc., West Grove, PA, USA; and [5]Biolegend, San Diego, CA, USA

Supplementary Table 8. Source parameters for DT2216 and the internal standard (IS)

S. No.	Analyte	Mass transition (m/z)	Cone voltage (V)	Collision energy (V)
1	DT2216	771.57 > 202.14	50	42
2	Amiodarone (IS)	645.92 > 201.12	90	34