LP-118 is a novel B-cell lymphoma 2 / extra-large inhibitor that demonstrates efficacy in models of venetoclax-resistant chronic lymphocytic leukemia

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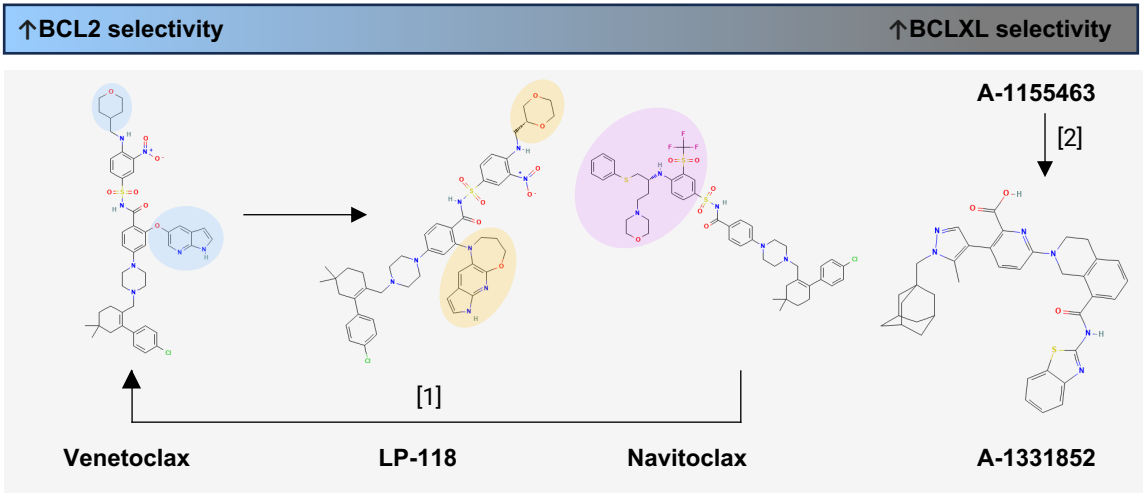
Supplemental Figure 1

A



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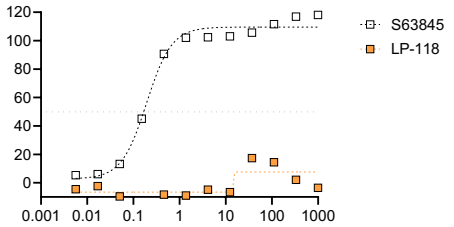

Supplemental Figure 1

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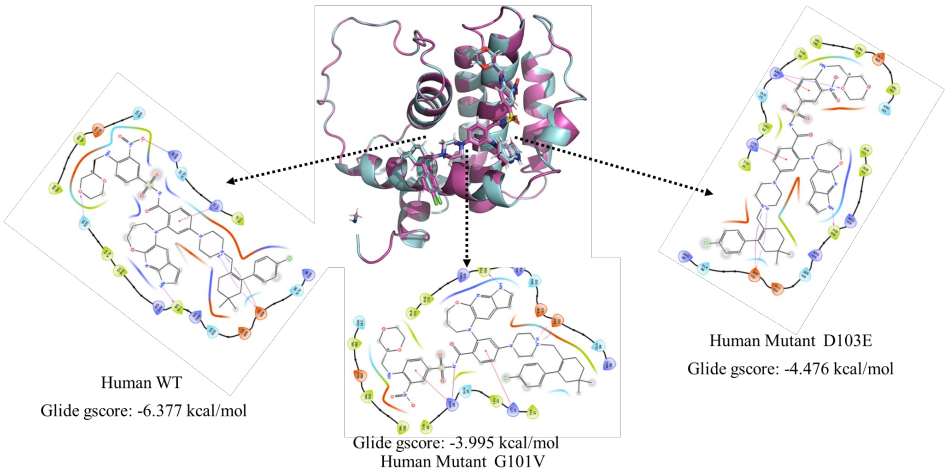

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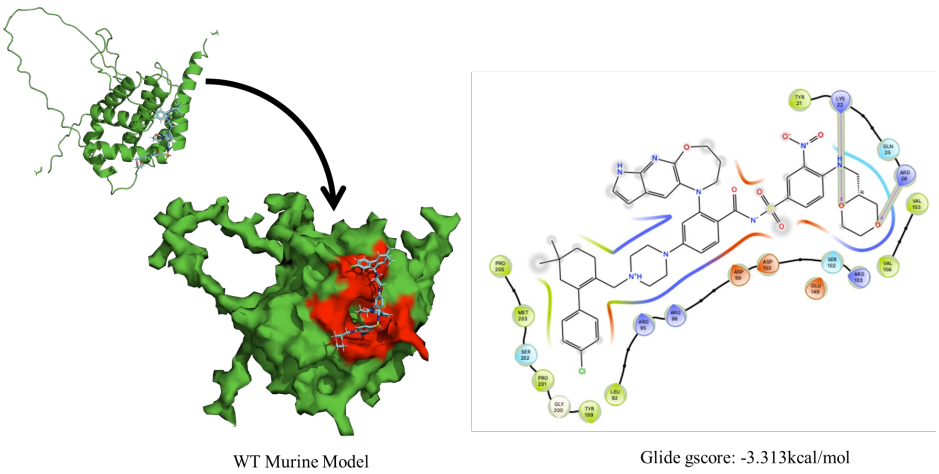

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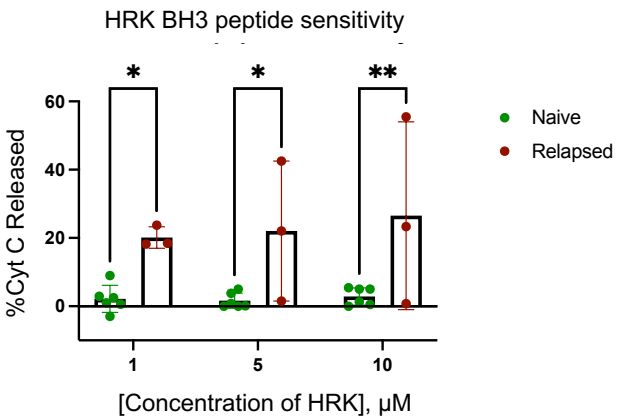
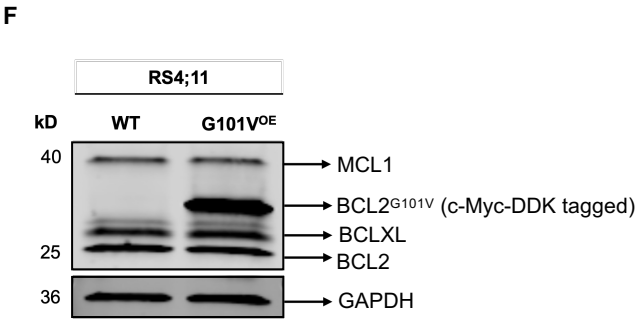
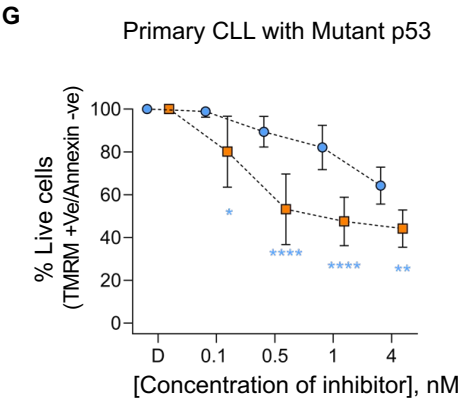
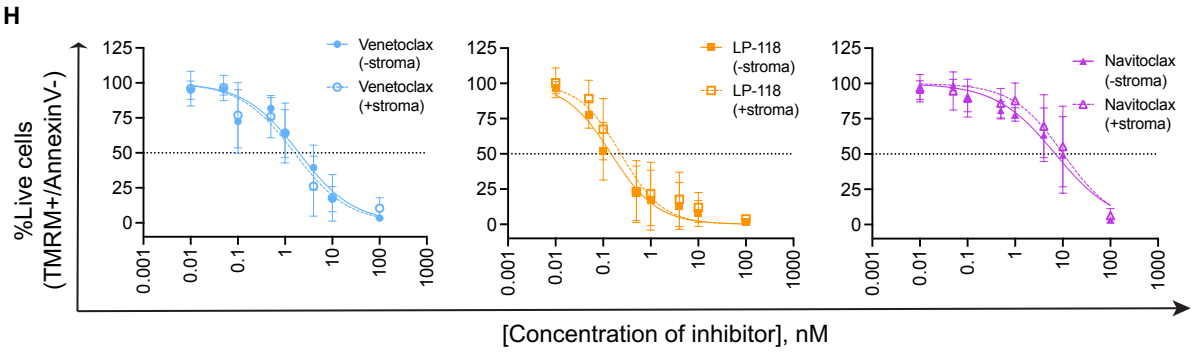
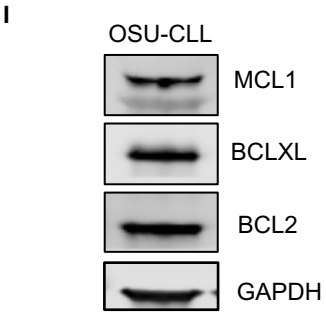


Supplemental Figure 1

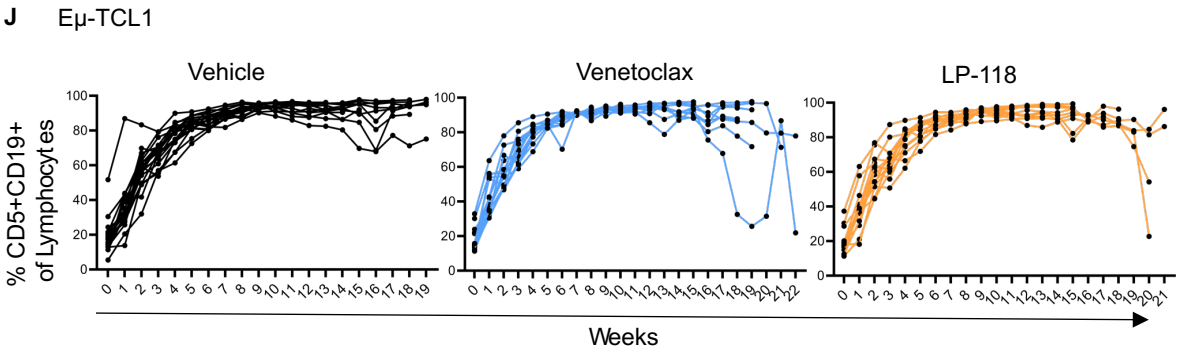


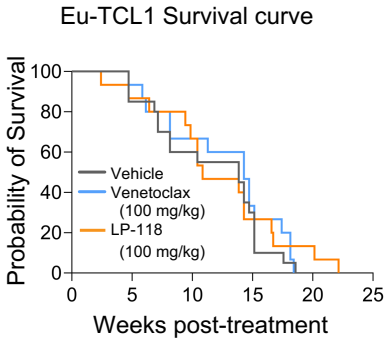


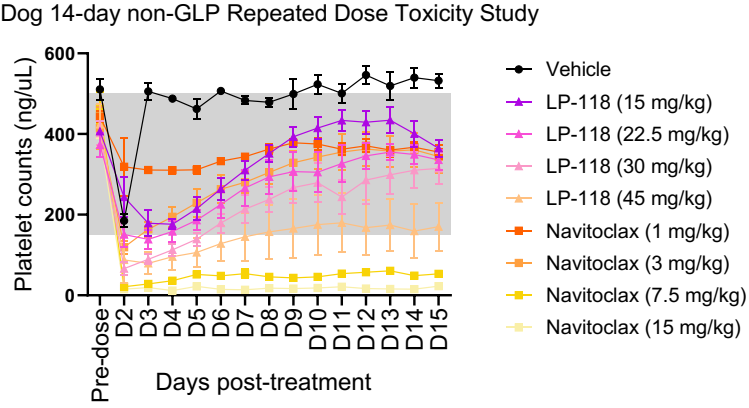


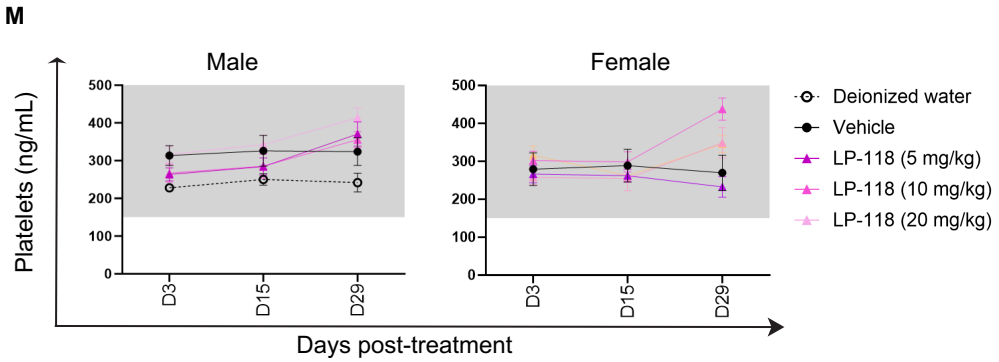


Supplemental Figure 1



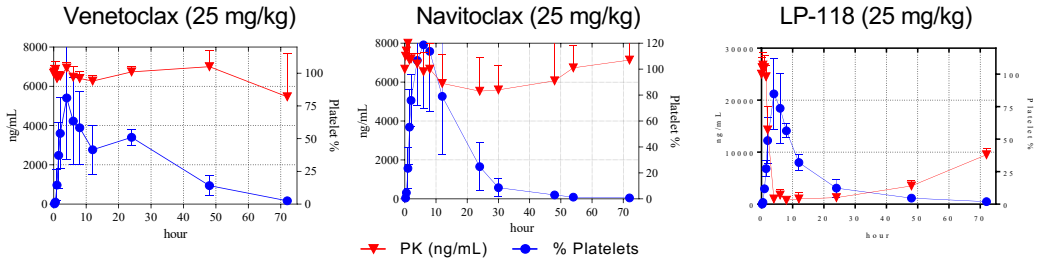






Supplemental Figure 1

N



	Target	AUC last (h*ng/mL)	Platelet Max Reduction	PO Dosage
Venetoclax	Selective Bcl-2	145,337	6%	25 mg/kg
Navitoclax	Dual Bcl-2/xL	295,242	97%	25 mg/kg
LP-118	Dual Bcl-2/xL	129,780	17%	25 mg/kg

	Sex	IGHV	Complex Karyotype?	del17p?	TP53 mutated?
Treatment naïve CLL					
1	F	M	no	no	no
2	M	M	no	no	no
3	M	M	no	no	no
4	M	M	no	no	no
5	F	U	no	no	no
6	M	unknown	no	no	no
7	M	M	no	no	no
8	M	M	no	no	no
9	M	U	no	no	no
10	M	unknown	yes	no	no
11	F	M	no	no	no
12	F	U	no	no	no
13	M	M	no	no	no
Treatment naïve CLL with altered TP53					
14	F	unknown	yes	del17p	no
15	M	unknown	no	del17p	no
16	M	unknown	yes	del17p	p53 mutated
17	M	M	no	del17p	p53 mutated
18	M	M	yes	no	p53 mutated
19	M	U	yes	del17p	no
20	M	U	yes	no	p53 mutated
21	M	U	yes	no	p53 mutated
22	F	M	no	del17p	no
Samples used for NK/T experiments					
23	F	unknown	no	no	no
24	M	M	yes	del17p	no
25	F	M	no	no	no
26	F	M	no	no	no
27	M	unknown	no	no	no

Supplemental Table 1. Characteristics from treatment naïve CLL patients. Sex = Female (F) or Male (M); IGHV status = mutated (M), unmutated (U) or unknown; complex karyotype = ≥3 or more abnormalities.

Supplemental Figure 1 Legend

A, Structures of venetoclax (ABT-199, PubChem ID: 49846579), LP-118 (NWP-0476, PubChem ID: 146663563), navitoclax (ABT-263, PubChem ID: 24978538) and A-1331852 (PubChem ID: 71565985). Inhibitors are ordered based on their decreasing selectivity to BCL2 and increasing selectivity to BCLXL. Atoms are color-coded as follows: carbon (black), oxygen (red), nitrogen (blue), hydrogen (gray), sulfur (yellow), chlorine (green). Structural optimization of navitoclax

(BCLXL/2 dual inhibitor) led to the discovery of venetoclax (highly selective BCL2 inhibitor) as previously reported [1]. Structural differences are highlighted in pink (navitoclax) and blue (venetoclax). Novel BCLXL/2 dual inhibitor LP-118 was synthesized from the scaffold of venetoclax. Structural differences are highlighted in blue (venetoclax) and yellow (LP-118). A-1131852 (highly selective BCLXL inhibitor) was re-engineered from A-1155463 (BCLXL inhibitor), using structure-based drug design as previously reported [2]. **B**, Representative result of LP-118, and S63842 (MCL1 inhibitor) binding to MCL1, determined by fluorescence energy transfer (TR-FRET) assay with human recombinant peptides of MCL-1 and BAK. **C,D** Docking of LP-118 in BCL2, showing wildtype (**C-left**), G101V (**C-middle**), and D103E (**C-right**) human BCL2, and murine BCL2 (**D**). Docked molecules shown represent the best binding poses, along their residue interaction diagram and ranking based on GlideScore. **E,** Cyt C release of unpaired CLL samples collected at baseline (naïve, n=6) or venetoclax-relapse (relapse, n=3), upon exposure to HRK BH3 peptide, by iBH3 profiling. Values were normalized to the corresponding DMSO control. 2-way ANOVA was performed. Plot displays mean ± SD. *p≤0.05; **p≤0.01, ****, p≤0.0001. **F,** Immunoblot of RS4;11 WT BCL2, and RS4;11 G101V^{OE} BCL2 cells. **G,** Primary CLL cells with TP53 aberrations were treated for 18 hours with increasing concentrations of venetoclax or LP-118 and stained with Annexin V and TMRM followed by flow cytometry analysis. Overall trend analysis is indicated by black stars and individual comparisons are indicated by blue stars. Plots display mean ± SD, n= 5. *p≤0.05; **p≤0.01, ****, p≤0.0001. **H,** Primary CLL cells (n=6) were co-cultured on HS-5 stromal cells for 24 hours (+stroma) or incubated in suspension for 2 hours (-stroma) prior to treatment with DMSO (negative control), venetoclax, LP-118 or navitoclax for 18 hours, and stained with Annexin V and TMRM followed by flow cytometry analysis. Plots display mean ± SD. **I,** MCL1, BCLXL and BCL2 protein levels by western blotting in OSU-CLL cells. GAPDH was used as loading control. **J,** Percent CD5+CD19+ Lymphocytes in Eμ-TCL1 mice treated with either vehicle, venetoclax, or LP-118. **K,** Survival curve of Eμ-TCL1 mice treated with either vehicle, venetoclax, or LP-118. Log-rank Mantel-Cox test was performed. **L,** Platelet counts from 14-day non-GLP repeated dose toxicity study in dogs. Treatment groups are vehicle (n=2), LP-118 (15 mg/kg, 22.5 mg/kg, 30 mg/kg, and 45 mg/kg, n=4 per group) and navitoclax (1 mg/kg, 3 mg/kg, 7.5 mg/kg, and 15 mg/kg; n=4 per group). Plot displays mean ± SEM. **M,** Platelet counts from 28-day GLP repeated dose toxicity study in male **(M-left)** and male **(M-right)** dogs. Treatment groups are deionized water (n=5), vehicle (n=5), and LP-118 (5 mg/kg, 10 mg/kg, and 20 mg/kg; n=5 per group). **N,** Dog PK and platelet toxicity study after single dose treatment with 25 mg/kg of venetoclax, navitoclax or LP-118 (n=3 per group). Graphs (top) show curves for PK in blue and platelet counts in red, and table (bottom) highlights key results. AUC = Area under curve, PO = oral administration.

Supplemental Methods

Cell culture

Primary CLL cells, RS4;11 (ATCC; Cat. #CRL-1873), HS-5 Cells (ATCC; Cat. #CRL-1873) were cultured in RPMI-1640 (ThermoFisher; Cat. #21875034) supplemented with 100 U/mL penicillin and streptomycin (ThermoFisher; Cat. #15140122) and 10% fetal bovine serum (Fisher Scientific; Cat.#16-140-071) and 2 mM Glutamine (ThermoFisher; Cat. #25030149). NKtert from Dr. Jan

Berger (MD Anderson Cancer Center, Houston, TX, USA) were cultured in Alpha-MEM supplemented with human serum (Valley Biomedical; #HP1022), hydrocortisone (Fisher Scientific; Cat. #AC352451000), fetal bovine serum (Fisher Scientific; Cat. #16-140-071). OSU-CLL cells were grown in RPMI-1640 (ThermoFisher; Cat. #21875034) supplemented with 100 U/mL penicillin and streptomycin (ThermoFisher; Cat. #15140122), and 15% fetal bovine serum and 2 mM Glutamine (ThermoFisher; Cat. #25030149). OSU-CLL from Dr. Erin Hertlein and Dr. John Byrd (University of Cincinnati, Cincinnati, OH, USA) cells were cultured in RPMI-1640 supplemented with 100 U/mL penicillin and streptomycin (ThermoFisher; Cat. #15140122), and 20% fetal bovine serum (Fisher Scientific; Cat. #16-140-071) and 2 mM Glutamine (ThermoFisher; Cat. #25030149). CCRF-CEM, LOUCY, SUPT11, PF382 and MOLT4 kindly provided by Dr. Natalia Baran (MD Anderson Cancer Center, Houston, TX, USA) were allowed to recover after thawing in RPMI-1640 supplemented with 2 nM glutamine plus 10% fetal bovine serum and grown in RPMI-1640 supplemented with 10% fetal bovine serum thereafter. All cells were grown at 37°C, with 5% CO2 and cells lines beyond passage 15 were not used.

Molecular docking studies of LP118 on BCL2 human & mouse models:

The initial structure of the LP118 molecule was downloaded from PubChem database and further processed in the Maestro module of Schrodinger suite. The ionization charges were assigned to the structure by keeping generate at most 32 stereoisomers per ligand. The geometry of molecule was optimized to a gradient of 0.001 kcal/mol using OPLS2005 force field. The molecule was processed for conformer generation in the LigPrep module of Schrodinger suite. Finally, a total of 128 conformers were generated which were used for further studies [3].

The three-dimensional structure of mouse Bcl2 protein receptor was obtained from the Uniprot (ID: P10417) [4]. The structure was prepared by adding the missing atoms and the hydrogens atoms, bond order assignment and charge assignment. Further, the heavy atoms were minimized with restrained minimization up to 0.3 Å RMSD. Finally, the prepared structure was used for further docking studies in Schrodinger Suite [3].

To detect Bcl2 protein binding sites we have used murine model and processed in SiteMap module of Schrodinger Maestro Suite [3]. The SiteMap is a proven algorithm that helps in identifying and ranking binding sites based on internal evaluation. Default settings and more restrictive approach with detectable shallow binding sites was used in this method. A total of 5 different sites were predicted. Out of these ‘site3’ was selected based on best ranked SiteScore ‘0.914’ and Volume ‘167’ value. The Site3 pocket residues were used for receptor grid box generation. Further, the docking was carried out using Glide module of Schrodinger suite in standard precision (SP) mode. Same approach was used to predict binding affinity of LP-118 to Human Bcl2 model and the same ‘site3’ was used by transferring ligand coordinates to the human Bcl2 model and mutations (G101V, D103E) were carried out on these using Mutagenesis in Pymol[5]. The residues around the 5 Å of a bound ligand was used for receptor grid generation. All the best binding poses, and residue interaction diagram are shown in **Figure S1-C,D**. The docked molecules were ranked based on GlideScore[6].

Expression of BCL2 Gly101Val in RS4;11

The Human BCL2 (NM_000633) tagged Open Reading Frame (ORF) clone (Origene; Cat. # RC204498) plasmid containing a G101V substitution was transferred from RC204498 vector backbone in pLenti-C-Myc-DDK-IRES-puro vector (Origene, Cat. #PS100069) using SfgI and Mlu cloning sites. The lentiviral particles were produced by transient co-transfection of 293T cells with the BCL2-G101V pLenti plasmid and lentiviral packaging plasmid mix (Sellecta, Cat. #CPCP-K2A). Supernatant containing lentiviral particles were harvested 48 hours after transfection followed by virus tittering (Origene, Cat. #TR30038). RS4;11 cells were transduced by spinoculation at estimated multiplicity of infection (MOI) of 5, 20 and 50 in presence of 8ug/mL of polybrene. Virally Transduced RS4;11 cells were selected at 0.5ug/mL of puromycin for about 3 weeks. Stable expression of BCL2 was confirmed using immunoblotting with anti-BCL2 (Sigma; Cat. #PRS3335), which recognized endogenous WT BCL2 (26 kDa) and c-Myc-DDK tagged BCL2 G101V (30 kDa), as shown in **Supplemental Figure 1F**.

Generation of BCL2 KO OSU-CLL via CRISPR-Cas9

Ribonucleoproteins containing the Cas9 protein and synthetic chemically modified sgRNA targeting *BCL2* were electroporated into OSU-CLL cells. Editing efficiency was assessed upon recovery, 48 hours post electroporation. Single cells were isolated, by seeding at <1 cells/well into 96-well plates. To confirm BCL2 KO, monoclonal cell populations were screened by PCR and immunoblotting.

Patients and Lymphocyte Isolation

Studies and patient samples were used with approval and review by The Ohio State University IRB. Primary cells were isolated by Ficoll-Paque PLUS (Cytiva; Cat. #17144003) then B cells were isolated by RosetteSep Human B cell selection (Stem Cell; Cat. #15024) and T cells using EasySep human T cell Isolation kit (Stemcell; Cat. #17951) and NK cells using human NK cell isolation kit (Stem cell; Cat. #17955). Samples were either used for experiments or cryopreserved in liquid nitrogen until future use.

Apoptosis and MOMP Assay

For cytotoxicity assays, primary CLL cells were treated with DMSO or LP-118, venetoclax, or navitoclax. Cells were stained with TMRM (Thermo Fisher; T668) and Annexin V-FITC (Leinco; A432), or propidium iodide (PI) (Leinco; A432) and Annexin V-FITC to examine apoptosis on the Gallios flow cytometer (Beckman Coulter) flow cytometer.

Intracellular staining of active BAK

Primary CLL cells and RS4;11 cells (WT and BCL2 G101V^{OE}) were treated with DMSO, LP-118, or venetoclax. Cells were stained with near-IR live / dead staining (Thermo Fisher, #L10119, fixed (BD Biosciences, 554714), 0.25 ug of primary BAK (TC100) antibody (Enzo, BML-SA298-0050) and Dylight 488 conjugated goat anti-mouse secondary antibody (Thermo Fischer, 35502) and analyzed on a Gallios (Beckman Coulter) flow cytometer.

Cytochrome C release Assay

Primary CLL cells and RS4;11 cells (WT and BCL2 G101V^{OE}) were treated with DMSO, LP-118, or venetoclax. Cells were stained with near-IR live / dead staining (Thermo Fisher, #L10119, fixed (BD Biosciences, 554714). Cells were permeabilized with 0.001% Digitonin in MEB2 buffer, fixed with 4% formaldehyde and neutralized with N2 buffer. Cells were stained overnight with 0.025 µg FITC anti-cytochrome C antibody (Biolegend, Cat. # 612304) at 4ºC, and analyzed on a Cytoflex (Beckman) flow cytometer. Buffers were prepared as previously described by Letai Laboratory in the iBH3 profiling protocol.

Drug Sensitivity Assay

Cell lines were treated with DMSO, venetoclax, navitoclax, A-1331853, or LP-118 in black/clear bottom 96-well plates at 1 x 10^5 cells/mL with 100 uL final volume/well, for the specified timepoints. CellTiter-Glo® 2.0 assay (Promega) was performed per manufacturer's protocol to determine the viability of cells after treatment, based on ATP levels. For this, plates and CellTiter-Glo® Reagent were allowed to equilibrate at room temperature for 30 minutes, protected from light. 100 uL/well of CellTiter-Glo® Reagent were added, followed by 2 minutes orbital shake and 10 minutes incubation at room temperature, protected from light. Luminescence was measured on a Synergy H1 plate reader (BioTek).

CLL and NK.tert cco-culture with NK.Tert or HS-5

Studies and patient samples were used with approval and review by OSU IRB with written consent from patients. For CLL with stroma (+stroma) conditions, 5E4 5x10^4 cells/well (500 uL at 1x10^5 cells/mL in tissue culture treated 24-well plates) of NK.Tert or HS-5 cells were incubated for 24 hours. Media was removed and primary CLL cells were overlaid at 5x10^E5 cells/well to achieve a 1:10 ratio (500 uL at 1 x 10^6 cells/mL in 50% conditioned media for NK.Tert cocultures, in 50% conditioned media or fresh media for HS-5 co-cultures) for 24 hours. For CLL without stroma (-stroma) conditions, 500 uL at 1 x 10^6 cells/mL were seeded in tissue culture treated 24-well plates. Cells were treated with 100 uL of 6X compounds or DMSO (negative control) for 18 hours in co-cultures and suspensions.and treated for 18 hours.

Caspase Inhibition Assay

Primary CLL cells from three patients with CLL were treated in triplicate with DMSO, LP-118 1 nM, LP-118 4 nM, or venetoclax 4 nM, with or without Z-VAD-FMK in 96-well plates at 1 x 10^5 cells/mL, for 24 hours. Cells were stained with BV421 anti-CD19 antibody (BD Biosciences, Cat. #562440) to evaluate B/CLL compartment by cytochrome C release assay, as described above. Viability was evaluated by CellTiter-Glo 2.0 assay (Promega).

Immunoblotting of BCL2 family proteins

To assess levels of anti-apoptotic proteins in cell lines, protein lysates were prepared by sonication in 1x RIPA Lysis buffer (0.5M Tris-HCl, pH 7.4, 1.5M NaCl, 2.5% deoxycholic acid, 10% NP-40, 10mM EDTA). Samples were diluted with 4–12% precast polyacrylamide gels Hand cast tris–glycine-SDS gels were used for protein electrophoresis in tris–glycine running buffer (25 mM tris

pH 8.5, 190 mM glycine, 0.1% SDS) using the Mini-PROTEAN® Tetra cell electrophoresis system (Bio-Rad). Samples were diluted with 4x Laemmli buffer (Bio-Rad; #161-0747) with 10% 2-mercaptoethanol and water, then heated to 95 °C for 5 min prior to loading on 4-12% polyacrylamide gels. Proteins were transferred to nitrocellulose membranes using the Trans-Blot Turbo Transfer System (Bio-Rad) according to the manual. Membranes were incubated in Intercept® (PBS) Blocking Buffer (LI-COR; #927-70001) 1 hour prior, probed overnight with anti-BCL2 (Sigma; #PRS3335), anti-BCXL (Cell Signalling; #2764S), anti-MCL-1 (Cell Signalling; #5453S), or anti-GAPDH (Sigma; Cat #CB1001) used for loading control, and incubated 1 hour in 680RD anti-mouse or 800CW anti-rabbit secondary antibodies. Images were captured on LI-COR Odyssey CLx Imaging System.

Biochemical

6XHIS-tagged BCL2 (BPS Bioscience; Cat. #50272) and BCLXL (R&D systems; Cat. #827-BC-050) were preincubated with LP-118, venetoclax, navitoclax at 12 different concentrations in triplicate for 15 minutes. After incubation, TAMRA-labeled BAK BH3 peptide (5 -TAMRA – GQVGRQLAIIGDDINR, custom synthesized) and Tb-cryptate-labeled anti-GST antibodies (CisBio; Cat. #61H12TLA) were added and incubated for 1 hour at room temperature. Plate was read on Analyst HT multi-mode plate reader (Molecular Devices) with donor excitation of 330-80 nm and emission of 460nm-10nm. Acceptor/Donor ratio was calculated as a measure of BCL2:BAK, BCLXL:BAK, or MCL1:BAK BH3 interaction. IC50 was calculated according to the dose response curve (%-inh) = $(A/D_{DMSOcontrol}-A/D_{Test\ article})/(A/D_{DMSOcontrol}-A/D_{no\ enzyme\ control})\times 100\%$.

Platelet Toxicity Assay

Plasma was isolated from whole ACD-A-stabilized human blood (AllCells, A lameda CA), incubated with Tyrode buffer supplemented with 1 uM PGE1 incubated at room temperature for 10 minutes. Buffer was removed and platelets were re-suspended in Tyrode buffer supplemented with 1 uM PGE1 and incubated for 10 minutes at room temperature. Platelets were spun down and resuspended at 2x10^8 cells per mL in Tyrode buffer (Boston Bioproducts; Cat. # PY-921) with 1 uM PGE1(Santa Cruz Bio; Cat. #sc-201223), 0.2U/mL apyrase (Sigma; Cat. #A6237) and 10% NHS. 2 mLs of platelets were dispensed and incubated with LP-118, venetoclax, or navitoclax at concentrations up to 25 uM for 72 hours at room temperature in shaker. At 72 hours platelets were resuspended and 200 uL were used in 96 well plate with the addition of 40 uL of MTS with PMS (Promega; Cat. #G5421) for 1 hour at 37C. Absorbance was read at 490nM on Multi-mode plate reader Spectramax (Molecular Devices).

Eμ-TCL-1 Mouse Model

All experiments using animal models were performed after approval and in accordance with guidelines set by The Ohio State University and the Institutional Animal Care and Use Committee.

Adoptive transfer of CD5+CD19+ cells isolated from the spleen of an Eμ-*TCL1* mouse into 50 C57Bl6 mice and used it to generate the Eμ-TCL1 spontaneous mouse model of CLL. We monitored peripheral blood of mice for CD5+/CD19+ cells weekly. We waited until peripheral blood of mice had greater than 10% of CLL cells (CD5+/CD19+) then enrolled mice in one of four

treatment groups: vehicle (n=20), 50 mg/kg venetoclax (n=15), or 50 mg/kg LP-118 (n=15). Venetoclax was dissolved in vehicle consisting of 10% ethanol, 30% PEG 400, 60% Phosal 50 PG. LP-118 was dissolved in vehicle consisting of 2.5% Ethanol, 10% PEG 400, 20% Kolliphor HS 15, and 67.5% water. Mice were dosed daily via oral gavage at 50 mg/kg venetoclax or LP-118 or vehicle controls until reaching early removal criteria. Weekly bleed was used to monitor CD19+/CD5+ CLL cells, CD4+, CD8+ T cells, and NK1.1+/NKp46+ NK cells. Complete Blood Counts (CBC) was performed weekly using Element HT5 Heska.

RS4; 11 and OSUCLL Mouse Model

Female SCID mice (Fox Chase SCID®, CB17/Icr-*Prkdcscid*/IcoIcrCrl, Charles River) at 8 weeks old with a body weight range of 15.5 to 20.8 g were engrafted 1E7 cells (0.1 mL volume) RS4;11 with WT RS4;11 cells resuspended in PBS with 50% Matrigel™ (BD Biosciences). Cells were injected subcutaneously in the right flank and monitored until reaching the target range of 100-150 mm^3. Mice were randomized into groups: vehicle (n=7), venetoclax 6.25 mg/kg (n=7), LP-118 6.25 mg/kg (n=7). Mice were drugged orally (p.o.) with either vehicle (2.5% ethanol, 10% PEG400, 20% Kolliphor HS 15, 67.5% water), venetoclax at 6.25 mg/kg once a day for 28 days (q.d. x 28); or group 3) LP-118 at 6.25 mg/kg once a day for 28 days (q.d. x 28). Tumor volumes were measured twice a week then weekly after Day 63 and individual animals exited the study when they reached an endpoint tumor volume of 2000 mm^3 or 77 days, whichever came first.

OSUCLL Xengograft model: NOG-F and NOG-M mice (Taconic, Albany, NY, USA) were engrafted with 1E7 OSUCLL cells via tail vein injection. After 4 days mice were treated with vehicle, venetoclax 50 mg/kg and LP-118 50 mg/kg once a day via oral gavage in the same formulation as described for the Eμ-*TCL1* mice and tracked for overall survival until mice reached early removal criteria with hind limb paralysis.

Dog Platelet Toxicity and Pharmacokinetics Studies

a. **Fourteen-day repeat-dose non-GLP platelet toxicity study for LP-118 vs navitoclax.** Thirty-four Beagle dogs were randomly assigned into 9 groups. Animals in group 1 were given vehicle control. Animals in group 2, 3, 4, and 5 were given LP-118 （NW-4-76） at doses of 15, 22.5, 30 and 45 mg/kg. Animals in group 6, 7, 8, and 9 were given navitoclax at doses of 1, 3, 7.5 and 15 mg/kg. There were 2 dogs/sex in each group except vehicle control group. Compounds were administrated once daily for 14 consecutive days via oral gavage. Approximately 5 mL of blood sample was collected through jugular vein on the day-1 (pre-dose), day-2 to day-15, for hematology analysis including platelets analysis.
b. **Twenty-eight-day repeat-dose GLP toxicity study of LP-118.** Fifty healthy Beagle dogs (common grade), with 25 males and 25 females were selected and randomly assigned into 5 groups (5 animals in each group) using a simple randomization procedure based on their body weights and sex. The body weight range of males was 7.2 to 9.3 kg and females, 5.7 to 8.15 kg. Animals in group 1 were given deionized water and animal in group 2 were given vehicle control (2.5% Ethanol, 37.5% PEG400, and 60% Phosal 50PG). Animals in group 3, 4 and 5 were given LP-118 (NWP-4-76) at doses of 5, 10 and 20 mg/kg, respectively. Compound solutions were administrated once daily for 28 consecutive days

via oral gavage. Approximately 5 mL of blood sample was collected through jugular vein on day-3, day-15, and day-29 for hematology analysis including platelets analysis.

c. **Single-dose GPL platelet toxicity and pharmacokinetics study of LP-118 vs ABT-263 and ABT-199:** Male Beagle dogs (Beijing Marshall Biotechnology Co. LTD) were fasted overnight and fed at 30 min before drug dosing. Vehicle (60% Phosal 50 PG+30% PEG400+10% EtOH), LP-118, ABT-263 or ABT-199 was orally administered at the dose of 25 mg/kg. Approximately 1.5 mL of blood sample was collected via cephalic vein puncture in K2EDTA tubes at different time points. One aliquot (~1.0 mL) was transferred for hematology analysis including platelets analysis and another aliquot (~0.5 mL) was put on wet ice and centrifuged to obtain plasma (2000 g, 4℃, 5 min) within 15 minutes post sampling. The concentrations of LP-118, ABT-263 and ABT-199 in plasma samples were determined by LC-MS/MS method and followed by a non-compartmental pharmacokinetic analysis using the Phoenix WinNonlin software. The linear/log trapezoidal rule was applied to obtaining the PK parameters.

Supplemental References

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Availability of data and materials

Any relevant and original data are available from the corresponding authors upon request.