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


Received: October 11, 2023.
Accepted: July 30, 2024.


Publisher’s Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors’ final approval; the final version of the manuscript will then appear in a regular issue of the journal.

All legal disclaimers that apply to the journal also pertain to this production process.
LP-118 is a novel B-cell lymphoma 2 / extra-large inhibitor that demonstrates efficacy in models of venetoclax-resistant chronic lymphocytic leukemia

Janani Ravikrishnan¹, Daisy Y Diaz-Rohena²*, Elizabeth Muhowski¹, Xiaokui Mo³, Tzung-Huei Lai¹, Shrilekha Misra¹, Charmelle D Williams², John Sanchez², Andrew Mitchell¹, Suresh Satpati⁴, Elizabeth Perry¹, Tierney Kaufman¹, Chaomei Liu², Arletta Lozanski¹, Gerard Lozanski¹, Kerry A Rogers¹, Adam S Kittai¹, Seema A Bhat¹, Mary C Collins⁵, Matthew S Davids⁶, Nitin Jain⁶, William G Wierda⁶, Rosa Lapalombella¹, John C Byrd⁷, Fenlai Tan⁸, Yi Chen⁸, Yu Chen⁸, Yue Shen⁸, Stephen P Anthony⁸, Jennifer A Woyach¹*, Deepa Sampath²#

¹ Division of Hematology, Department of Internal Medicine, The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA
² Division of Hematopoietic Biology and Malignancy, MD Anderson Cancer Center, Houston TX, USA
³ Center for Biostatistics, Department of Biomedical Informatics, The Ohio State University, Columbus, OH, USA
⁴ Department of Genomic Medicine, MD Anderson Cancer Center, Houston TX, USA
⁵ Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA
⁶ Department of Leukemia, MD Anderson Cancer Center, Houston TX, USA
⁷ Department of Internal Medicine, University of Cincinnati, Cincinnati, OH, USA
⁸ Newave Pharmaceutical Inc., Pleasanton, CA, USA

Corresponding Authors:
Jennifer A. Woyach, The Ohio State University. Wexner Medical Center, 410 W. 12th Avenue, Columbus, OH 43210. Phone: 614-685-5667; Fax: 614-293-7484; Email: Jennifer.Woyach@osumc.edu
Deepa Sampath, UT MD Anderson Cancer Center. So Campus Research Bldg 2 (2SCR2. 2225) 7435 Fannin St. Houston, TX 77054-1901. Phone: 713-498-9035; Email: DSampath@mdanderson.org

Running title: LP-118: A Promising Treatment for CLL

Declarations

Ethics approval and consent to participate
Studies and patient samples were used with approval and review by The Ohio State University IRB with written consent from patients. All experiments using animal models were carried out in accordance with the guidelines established by Ohio State University and the Institutional Animal Care and Use Committee.

Consent for publications
The authors consent to the publication of this manuscript.

Availability of data and materials
Any relevant and original data are available from the corresponding authors upon request.
Competing interests

JAW received research support from Verastem, Karyopharm, Morphosys, Schrodinger and has consulted for Pharmacyclics, Janssen, AstraZeneca, Arqule, Abbvie, Beigene, Loxo, Newave, and Genetech. DS is on the advisory board for Newave Pharma. KAR received research funding from Genentech, AbbVie, Janssen, and Novartis, consults for Genentech, AbbVie, AstraZeneca, Innate Pharma, Pharmacyclics, and Beigene, and received travel funding from Beigene. JAW and KAR are clinical scholars of the Leukemia and Lymphoma Society. ASK consulted for Abbvie, Beigene, Bristol-Myers Squibb, and Janssen. MSD reports receiving grant support, paid to his institution, and consulting fees from Ascentage Pharma, Astra-Zeneca, BMS, Genentech, MEI Pharma, Pharmacyclics, TG Therapeutics, and Verastem, grant support, paid to his institution from Surface Oncology, and consulting fees from AbbVie, Adaptive Biotechnologies, Aptitude Health, BeiGene, Celgene, Eli Lilly, Janssen, Merck, Research to Practice, and Takeda. JCB consulted for Acerta, AstraZeneca, Pharmacyclics, Astellas, Syndax, and Jazz Pharmaceuticals; stock ownership in Vincerx Pharmaceutics. SAB consulted for Pharmacyclics, Janssen, Beigene and Acerta/AstraZeneca. RL has membership on the Vincerx Pharma Inc Board of Directors or advisory committees. Yi C, SPA and Yu C are employed by and hold a position on the Board of Directors of Newave Pharmaceutical Inc, the maker of LP-118.; stock ownership in Newave Pharmaceutical Inc.; Yi C holds patents 10456397, 10377755, 10253029, 10239872, 10195200. YS and FT are employed by and hold a position on the Board of Directors of Lupeng Pharmaceutical Inc; stock ownership in Lupeng Pharmaceutical Inc.

Authors’ contributions

*JR and DYDR contributed equally as co-first authors.
#DS and JAW contributed equally as co-senior authors.

JR: Conceptualization, methodology, investigation, formal analysis, visualization, writing–original draft.

DYDR: Methodology, investigation, formal analysis, visualization, writing–review and editing.

EM: Methodology, investigation, formal analysis, writing–review and editing.

THL, XM, SM: Investigation, methodology, writing–review and editing.

CDW, JRS, AM, CL, AL: Investigation.

SS: Computational modelling.


GL: Resources, Investigation.

KAR, ASK, NJ, WGW: Resources, Writing–review and editing.

SAB: Resources.

MCC: Methodology.

MSD: Methodology, writing–review and editing.

RL: Writing–review and editing.

JCB: Supervision, Writing–review and editing.

FT, Yi C: Conceptualization, methodology, resources, funding acquisition, supervision, writing – review & editing.

Yu C, SPA: Conceptualization, methodology, resources, supervision, writing – review & editing.

YS: Conceptualization, methodology, supervision, writing – review & editing.

JAW, DS: Conceptualization, funding acquisition, methodology, resources, supervision, visualization, writing–original draft.
Acknowledgements
The authors would like to thank patients and their families and clinical and research personnel at The Ohio State University and MD Anderson Cancer Center. We acknowledge the Leukemia Tissue Bank shared resource from the Ohio State University Comprehensive Cancer Center (P30CA016058). LP-118 was kindly provided for all the studies by Newave Pharmaceutical Inc. The authors thank Dr. Jan A. Burger (MD Anderson Cancer Center) for providing the Nktert stromal cell line, and Dr. Natalia Baran (MD Anderson Cancer Center) and Dr. Marina Konopleva (Albert Einstein College of Medicine) for providing LOUCY, CCRF-CEM, PF-382, and MOLT4 cell lines.

Funding
This work is supported in part by the Harry T Mangurian Jr. Foundation, D Warren Brown Foundation, and NCI (R01 CA177292 JAW/KR), and by the CLL Global Alliance (DS/JAW) and the Univ. of Texas MD Anderson CLL (DS) and T/NK (DS) Moonshots.
Abstract

Patients with chronic lymphocytic leukemia (CLL) respond well to initial treatment with the B-cell lymphoma 2 (BCL2) inhibitor venetoclax. Upon relapse, they often retain sensitivity to BCL2 targeting, but durability of response remains a concern. We hypothesize that targeting both BCL2 and B-cell lymphoma-extra large (BCLXL) will be a successful strategy to treat CLL, including for patients who relapse on venetoclax. To test this hypothesis, we conducted a pre-clinical investigation of LP-118, a highly potent inhibitor of BCL2 with moderate BCLXL inhibition to minimize platelet toxicity. This study demonstrated that LP-118 induces efficient BAK activation, cytochrome C release, and apoptosis in both venetoclax naïve and resistant CLL cells. Significantly, LP-118 is effective in cell lines expressing the BCL2 G101V mutation and in cells expressing BCLXL but lacking BCL2 dependence. Using an immunocompetent mouse model, Eμ-TCL1, LP-118 demonstrates low platelet toxicity, which hampered earlier BCLXL inhibitors. Finally, LP-118 in the RS4;11 and OSU-CLL xenograft models results in decreases in tumor burden and survival advantage, respectively. These results provide a mechanistic rationale for the evaluation of LP-118 for the treatment of venetoclax responsive and relapsed CLL.

Key words: Chronic Lymphocytic Leukemia, venetoclax resistance, BCL2 family, LP-118
Introduction
In chronic lymphocytic leukemia (CLL), up-regulation of the anti-apoptotic protein B-cell lymphoma 2 (BCL2) allows cells to evade apoptosis by sequestering pro-apoptotic proteins containing BCL2 homology 3 (BH3) domains. Venetoclax is a first-in-class BCL2 inhibitor approved for the treatment of CLL in front-line and relapsed settings. In combination with anti-CD20 monoclonal antibodies, venetoclax produces response rates of over 80% and durable remissions\(^1,2\). However, patients progressing on venetoclax often do so due to one or more resistance mechanisms\(^3-9\).

One recurring mechanism of venetoclax resistance in CLL is the acquisition of a G101V point mutation in BCL2 resulting in a conformational change in the binding pocket which reduces its binding affinity for venetoclax\(^5\). Our group and others have identified other novel point mutations in BCL2 in patients who relapsed with venetoclax therapy, including D103Y / E / V, F104L, L119V and duplication in R107_R110 \(^3,4,6\). Aberrations in the tumor suppressor gene TP53 are another marker of progression on venetoclax\(^9\). Lastly, resistance to venetoclax can arise from upregulation of other members of the BCL2 family, including myeloid cell leukemia-1 (MCL1) through copy number gain or gene upregulation\(^7,10\), and B-cell lymphoma-extra-large (BCLXL) by protein overexpression\(^11-13\). Patients resistant to venetoclax can develop multiple resistance mechanisms simultaneously due to clonal evolution\(^5,8\). These resistance mechanisms suggest that targeting multiple pro-survival BCL2 family members would be of clinical benefit.

Despite being attractive targets for venetoclax-resistant CLL, targeting MCL1 or BCLXL has had limited clinical utility due to on-target cardiac toxicity\(^14-16\) or platelet toxicity\(^17,18\), respectively, with the latter causing dose-limiting thrombocytopenia. However, a recent trial combining venetoclax with low dose navitoclax showed efficacy in patients with relapsed refractory acute lymphoblastic leukemia (ALL) without evidence of thrombocytopenia, suggesting that modulating BCLXL inhibition may allow successful therapeutic targeting\(^19\).

Here, we characterize and demonstrate the preclinical efficacy of a new selective BCL2 inhibitor with moderate BCLXL targeting, LP-118, designed to minimize platelet toxicity and bypass various venetoclax resistance mechanisms. Most notably, we show that LP-118 is more potent than venetoclax in various BCL2-dependent models and has preclinical efficacy in venetoclax-resistant CLL. Therefore, LP-118 has the potential to treat patients with CLL, including those with venetoclax relapsed disease, justifying the ongoing Phase I trial.

Methods
See supplemental methods for more details.

Drug treatments and cell lines
LP-118 was provided for all studies by Newave Pharmaceutical Inc. Navitoclax (#S1001), venetoclax (#S8048), A-1331852 (#S7801), and Z-VAD-FMK (#S7023) were purchased from Selleckchem. Cell lines RS4;11 (ATCC) and HS-5 cells (ATCC) were purchased.

Biochemical
BCL2 tagged with 6XHIS (BPS Bioscience) and BCLXL (R&D systems) were pre-incubated with compounds for 15 minutes. BAK BH3 peptide labeled with anti-GST antibodies labeled with TAMRA and Tb-cryptate (CisBio) were incubated for 1 hour and read on Analyst HT multimode plate reader (Molecular Devices).

**Computational Modelling**
Docking of LP-118 (PubChem, ID 146663563) on murine BCL2 (UniProt, P10417) or human BCL2 (UniProt, P10415) was modeled using SiteMap module of Schrodinger Maestro Suite. BCL2 mutations (G101V, D103E) were modeled using Mutagenesis in Pymol. Docked molecules were ranked based on GlideScore.

**Cytotoxicity Assays**
Studies and patient samples were used with approval and review by OSU IRB with written consent from patients. BH3 profiling was performed as previously described by Letai et al\textsuperscript{20} and run on CytoFLEX LX (Beckman Coulter). Primary CLL cells were treated with the indicated compounds and for the indicated timepoints, in suspension or co-cultures. For cytotoxicity assays, primary CLL cells were stained with Annexin V-FITC (Leinco), and TMRM (Thermo Fisher) or propidium iodide (PI) (Leinco) to examine apoptosis on the Gallios flow cytometer (Beckman Coulter). Primary CLL cells and RS4;11 cells were treated and stained with near-IR live / dead staining (Thermo Fisher, fixed (BD Biosciences), 0.25 ug of primary BAK (TC100) antibody (Enzo, Cat. #BML-SA298-0050) and Dylight 488 conjugated goat anti-mouse secondary antibody (Thermo Fischer, Cat. #35502) and analyzed on Gallios (Beckman Coulter) flow cytometer.

**Mouse Models**
Experiments using animal models were done with the guidelines established by Ohio State University IACUC. Adoptive transfer of CD5+CD19+ cells from spleens of an Eμ-TCL1 mouse into C57Bl6 mice. At 10% of CLL cells in peripheral blood, mice were dosed daily via oral gavage. Female SCID mice (CB17/Icr-Prkdcscid/IcoIcrCrl, Charles River) were engrafted with 1x10^7 RS4;11 cells, injected subcutaneously on the right flank. At 100-150 mm^3 tumor volume, mice were drugged orally for 28 days. Tumors were measured twice a week then weekly after Day 63. NOG-F and NOG-M mice (Taconic, Albany, NY, USA) were engrafted with 1x10^7 OSU-CLL cells intravenously. After 4 days, mice were treated once a day via oral gavage and tracked for overall survival. Early removal criteria are described in supplemental methods.

**Statistical Analysis**
Experiments with continuous variables were analyzed using mixed-effects model, accounting for observational dependencies across treatment conditions, group comparisons, and trend tests IC\textsubscript{50}s were obtained through nonlinear mixed-effects modeling. Skewed data such as cell counts were normalized by log transformation. Other data was analyzed following the scales demonstrated on the figures (subtracting DMSO or standardizing over DMSO). Survival data were analyzed by logrank test. Data were analyzed in SAS 9.4 (SAS Institute, Cary, NC).
Results

LP-118, a second generation novel BCL2 inhibitor with rationally designed BCLXL inhibition

LP-118 is a BCL2 potent inhibitor with moderate BCLXL inhibition, rationally designed to provide improved antitumor efficacy relative to navitoclax while sparing platelets to reduce the risk of thrombocytopenia in the clinical setting. This was achieved through rational structure-based design using the platelet sparing structure of venetoclax as the starting scaffold (Figure 1A, Supplemental Figure 1A). We tested whether LP-118 is a genuine BH3 mimetic for BCL2 and BCLXL. For such classification, compounds must occupy the BH3 binding pocket of anti-apoptotic proteins and inhibit their interaction with pro-apoptotic proteins, such as pore-forming protein BAK. A cell-free Fluorescence Resonance Energy Transfer (FRET) competitive assay was performed to evaluate the ability of LP-118 to displace BAK from BCL2, BCLXL, or MCL1. This in vitro biochemical study with LP-118 shows strong targeting of BCL2 (Figure 1B) with IC₅₀ of 0.25 nM, compared to 0.34 nM for venetoclax and 0.75 nM for navitoclax. The BCLXL affinity of LP-118 (Figure 1C) is in-between navitoclax and venetoclax (IC₅₀ = 3.76 nM, 0.9 nM and 34 nM, respectively; LP-118 vs navitoclax p<0.0001; LP-118 vs venetoclax p<0.0001). LP-118 does not inhibit MCL1 (IC₅₀ > 1 μM, Supplemental Figure 1B), denoting selectivity for BCL2 and BCLXL. Furthermore, LP-118 has the highest percentage of inhibition of a recombinant BCL2 G101V peptide (IC₅₀ = 1.6 nM, 6-fold shift relative to WT, Figure 1D), compared to venetoclax (IC₅₀ = 19.5 nM, p<0.0001, 57-fold) and navitoclax (IC₅₀ = 33.2 nM, p<0.0001, 44-fold). Computational modeling predicts docking of LP-118 at the BH3 binding groove of human BCL2 (Supplemental Figure 1C). GlideScores, an empirical measure of the free energy of binding, revealed a decreasing order of binding affinity for LP-118 with human BCL2 WT (-6.377 kcal/mol), D103E (-4.476 kcal/mol), and G101V (-3.995 kcal/mol), followed by murine WT BCL2 (-3.313 kcal/mol) (Supplemental Figure 1C-D).

Three CLL patient samples were collected at the point of clinical relapse during venetoclax treatment. Importantly, using an ion torrent panel of selected genes, we did not detect BCL2 mutations in these patients prior to venetoclax therapy or at the point of relapse on venetoclax (Table 1). This suggests the existence of alternative resistance mechanisms in these samples. To determine the functional dependence of these venetoclax-resistant cells on BCL2-family proteins for survival, we performed intracellular BH3 profiling (iBH3, Figure 1E)²⁰⁻²². Initially, in six treatment naïve CLL cells (Figure 1F, Supplemental Table 1) we observe dose-dependent sensitivity for BH3-only activator BIM (p<0.0001) and BH3-only sensitizer BAD (p<0.0001) indicating priming to undergo apoptosis upon liberation from their anti-apoptotic partner, BCL2, as has been previously established²³. Unpaired venetoclax resistant CLL cells (Figure 1G) show apoptotic priming, denoted by sensitivity to BIM peptide treatment (p<0.0001), but lack sensitivity to BAD (p=0.0850), consistent with resistance to BCL2 inhibition. There was no cytochrome C (Cyt C) release upon exposure to the MS-1 peptide in our cohort (p=0.3643). Statistical support for dose-dependent sensitivity to HRK was not established within this limited cohort of venetoclax relapsed CLL (p=0.3643). However, unlike treatment naïve CLL cells, which uniformly lacked HRK sensitivity, two out of three venetoclax resistant CLL samples displayed significant Cyt C release upon HRK treatment (Supplemental Figure 1E, HRK 1 μM, 5 μM and 10 μM p=0.0303, 0.0152 and 0.0059, respectively). Our findings suggest that venetoclax relapsed CLL cells may employ multiple BCL2-family anti-apoptotic proteins to resist apoptosis. While BCL2 or MCL1 selective BH3 sensitizers (BAD and MS-1) may be
ineffective, responses to a BCLXL sensitizer (HRK) are heterogenous. Given the retained sensitivity to BIM, the apoptotic threshold may be overcome by simultaneously inhibiting various survival proteins.

To evaluate the effect of BCL2 mutations such as G101V on the survival dependence of cells on BCL2 family proteins, we conducted iBH3 profiling (Figure 1H) on RS4;11 cells overexpressing (OE) WT or G101V\(^{OE}\) (Supplemental Figure 1F). WT cells are highly primed and BCL2-dependent, as indicated by Cyt C release after incubation with BIM and BAD peptide. Mutant G101V\(^{OE}\) cells show decreased priming and reduced sensitivity to BAD (G101V\(^{OE}\) vs WT, at 1, 5, 10 µM: BIM p<0.0001, 0.1786, 0.7106; BAD p<0.0001, 0.01, 0.707). The moderate sensitivity of RS4;11 cells to the BCLXL selective peptide XXA1_Y4eK was not affected by the presence of G101V mutations (G101V\(^{OE}\) vs WT, at 1, 5, 10 µM: XXA1_Y4eK p=0.09, 0.1786, 0.8799), and mutant cells remained insensitive to MCL1 inhibition by MS-1 peptide. This suggests that BCL2 and BCLXL remain excellent targets for G101V mutant cells. Together, these data indicate that LP-118 is a potent BCL2 inhibitor, and can target BCLXL and G101V mutated BCL2 with higher potency than venetoclax. We predict that LP-118 could be useful in the CLL population of patients regardless of previous venetoclax treatment.

LP-118 is cytotoxic to CLL cells in vitro and leads to mitochondrial cytochrome C release and apoptosis

To mechanistically understand how LP-118 affects apoptosis in CLL cells, we performed BAK activation, Cyt C release, and viability assays. Exposure of treatment naïve CLL cells to LP-118 for 8 hours activates BAK proteins in the mitochondria at higher levels than venetoclax treated cells (Figure 2A). Subsequently, at 12 hours, it releases more Cyt C, a marker of mitochondrial outer membrane permeabilization (Figure 2B-middle). To assess whether LP-118 is cytotoxic to primary CLL cells in vitro, we treated cells for 18 hours and performed TMRM and Annexin V staining. LP-118 significantly induces more apoptosis (IC\(_{50}\) = 0.056 nM) compared to venetoclax (IC\(_{50}\) = 3.8 nM) and navitoclax treatment (IC\(_{50}\) = 10.09 nM) in treatment naïve CLL samples (Figure 2B-right) and was also cytotoxic to samples with TP53 mutations (Supplemental Figure 1G). To determine whether LP-118 is more potent in cells resistant to venetoclax, we took samples from patients with CLL who clinically progressed with venetoclax. Venetoclax resistant samples treated with LP-118 show significant increases in BAK transformation (Figure 2A, 2C-left) and more Cyt C release (Figure 2C-middle) than venetoclax, at matched concentrations. We demonstrate that LP-118 (IC\(_{50}\) = 0.34 nM) is superior to venetoclax (IC\(_{50}\) = 15.96 nM) and navitoclax (IC\(_{50}\) = 4.13 nM) at inducing apoptosis of venetoclax relapsed CLL, in vitro, by TMRM and Annexin V staining (Figure 2C-right). Lastly, since caspase activation marks a late phase in mitochondrial apoptosis, we sought to determine if pan-caspase inhibition by Z-VAD-FMK could impede the cytotoxic effects of LP-118 on CLL cells. As expected, inhibition of caspases does not affect Cyt C release induced by LP-118, reflecting that Cyt C release occurs upstream of caspase activation. Notably, pan-caspase inhibition protects primary CLL cells from the cytotoxic effects of 4 nM LP-118 or venetoclax, as observed at 24 hours by CellTiter-Glo (Figure 2D,2E). Taken together, these findings strongly suggest that LP-118 effectively kills CLL cells via mitochondrial apoptosis.
The stromal microenvironment provides a survival advantage to CLL cells by means of anti-apoptotic and pro-survival signals including an increase in BCLXL. Therefore, we wanted to investigate how LP-118, venetoclax and navitoclax would perform in the presence of stroma. To mimic the bone marrow niche, we cocultured patient-derived CLL cells on monolayers of human bone marrow derived cell lines, HS-5 or NK.Tert, for 24 hours. Exposure to HS-5 stroma does not alter the sensitivity of CLL cells to venetoclax, navitoclax nor LP-118 (Supplemental Figure 1H). Notably, CLL cells cocultured with NK.Tert cells exhibit a downward shift in their sensitivity to venetoclax, from IC$_{50}$ of 0.908 nM without stromal protection to 1.196 nM in cocultures, and for navitoclax, from 5.90 nM to 15.97 nM (Figure 3A). LP-118 was potent against CLL cells in suspension and in stromal co-cultures, with IC$_{50}$ s of 0.081 nM and 0.075 nM, respectively. As observed by immunoblot, NK.Tert stroma tended to increase the levels of BCL2 and BCLXL in CLL cells (Figure 3B). Together, these data indicate that LP-118 is cytotoxic to CLL cells that have not received prior treatment, as well as highly pretreated venetoclax-resistant samples, and it has the potential to overcome stromal protection driven by BCLXL upregulation.

**LP-118 induces apoptosis in cell lines with the BCL2 G101V mutation or overexpression of BCLXL**

We sought to determine whether LP-118 is effective in the presence of the venetoclax-resistant BCL2 G101V mutation. To model this in an isogenic system, BCL2 G101V was overexpressed (OE) in RS4;11 cells. A caveat of this model is that it expresses the G101V mutant construct in addition to endogenous WT BCL2, as confirmed by immunoblot (Supplemental Figure 1F). Compared to venetoclax, LP-118 produces significantly higher increases in active BAK at 4 hours in WT and G101V$^{OE}$ cells (Figure 4A-left). At 15 hours, LP-118 treatment also significantly releases more Cyt C from WT RS4;11 cells compared to venetoclax. Cells with a BCL2 G101V$^{OE}$ mutation are resistant to venetoclax; however, they release Cyt C following LP-118 treatment (Figure 4A-middle). Consistently, at 72 hours, TMRM/AnnexinV staining shows that RS4;11 with WT BCL2 or G101V$^{OE}$ mutant BCL2 are more sensitive to LP-118 (IC$_{50}$ = 1.93 nM and 15.67 nM, respectively) than venetoclax (IC$_{50}$ = 3.58 nM and 1324 nM, respectively) (Figure 4A-right). Together, these data demonstrate that LP-118 effectively induces apoptosis in cells harboring a G101V mutation.

Next, we investigated whether LP-118 can target cells resistant to venetoclax due to BCLXL dependence. Given that OSU-CLL cells express both BCL2 and BCLXL (Supplemental Figure 1I), we generated OSU-CLL BCL2 knockout (KO) cells to model this feature. As assessed by CellTiter-Glo at 72 hours, venetoclax is unable to clear BCL2 KO cells, reaching a plateau at 50% killing above 200 nM. In contrast, LP-118 is effective at eliminating BCL2 KO cells with an IC$_{50}$ of 29.5 nM, versus 0.26 nM for WT cells (Figure 4B). To further characterize the relative potency of LP-118 in cells with increasing dependency on BCLXL, we used a panel of five lymphoid cell lines and determined their relative dependencies on BCL2 and BCLXL based on their sensitivity to venetoclax, navitoclax and A-1331852 (BCLXL inhibiting tool compound) at 24 hours of exposure, by CellTiter-Glo. The order of increasing BCLXL dependency is: RS4;11, LOUCY, CCRF-CEM, PF-382, and MOLT4 (Figure 4C). LP-118 (IC$_{50}$ = 0.05 nM) has sub-nanomolar potency in RS4;11, surpassing the activity of venetoclax (IC$_{50}$ = 4.7 nM) and navitoclax (IC$_{50}$ = 42.1 nM), whereas A-1331852 is not effective at killing these BCL2-
dependent cells. Notably, LOUCY is moderately sensitive to selective inhibitors of either anti-apoptotic protein (venetoclax IC50 = 125.1 nM and A-1331852 IC50 = 130.3 nM), and dual inhibitors are most potent at overcoming the apoptosis blockade. LP-118 (IC50 = 5.5 nM) surpasses the activity of navitoclax (IC50 = 47.4 nM) in this dual BCL2 / BCLXL dependent cell line. Lastly, LP-118 retained efficacy to clear BCLXL dependent cells resistant to venetoclax. Consistent with its low BCLXL binding affinity, LP-118 was less potent than navitoclax and A-1331852 in BCLXL dependent cell lines (CCRF-CEM IC50s= 979.9 nM, 491.9 nM and 13.9 nM, respectively; PF-382 IC50s= 812 nM, 378.7 nM and 46.61 nM, respectively; and MOLT4 IC50s= 626.8 nM, 290.7 nM and 5.5 nM, respectively). Our results indicate that LP-118 is successful in targeting two resistance mechanisms to venetoclax: BCL2 G101V mutations and increased dependence on BCLXL.

**LP-118 has limited platelet toxicity in vitro and in vivo**

We investigated whether LP-118 is toxic to human platelets, performing an MTS assay on normal donor platelets. The IC50 of LP-118 was determined to be 6360 nM, which is in-between that of venetoclax at 25000 nM and navitoclax at 170 nM (**Figure 5A**). Although venetoclax is clinically effective in treating patients with CLL, the well-established Eμ-TCL1 adoptive transfer CLL mouse model is partially sensitive to single-agent venetoclax for short durations24, due to high dependence on MCL125. Although this model cannot be used to investigate the efficacy of LP-118, due to lack of BCLXL dependence, we utilized this model which mimics the CLL microenvironment to evaluate T cell, NK cell, and platelet toxicity in vivo. Wild-type C57BL/6NTac mice engrafted with Eμ-TCL1 donor splenocytes were treated once daily via oral gavage with either vehicle (n=20), LP-118 (50 mg/kg, n=15), or venetoclax (50mg/kg, n=15). As expected, all mice had similar peripheral CD5+CD19+ CLL cells throughout the course of treatment, and similar survival across groups (**Supplemental Figure 1J-K**). After 6 weeks of LP-118 treatment, there were significant decreases in CD4+ (p=0.0434), CD8+ (p<0.0001), and NK cells (p<0.001) compared to vehicle treated mice, similar to venetoclax-treated mice (**Figure 5 B-D**) suggesting that the effects of LP-118 on non-B cell populations may be comparable to venetoclax and therefore clinically manageable. We found that LP-118 does not decrease platelet counts in vivo in these Eμ-TCL1 engrafted mice (**Figure 5E**). Furthermore, platelet toxicity of LP-118 was preclinically evaluated in dogs, which platelet counts normally range between (150-500) x10^9/L. In a 14-day repeat-dose non-Good Laboratory Practice (GPL) toxicity study, platelet toxicity was induced with concentrations above 3 mg/kg for navitoclax and above 30 mg/kg for LP-118 (**Supplemental Figure 1L**) which corresponds to 10-fold reduced platelet toxicity for LP-118 in vivo. No thrombocytopenia was observed for LP-118 doses within 5 mg/kg to 20 mg/kg in a 28-day repeat-dose non-GLP toxicity study (**Supplemental Figure 1M**). Lastly, a 25 mg/kg single dose study revealed the maximum concentration of LP-118 in plasma (mean = 8 µg/mL) was reached 10 hours after treatment (**Supplemental Figure 1N**). A notable difference between venetoclax and LP-118 pharmacokinetics was seen at 30 hours post-treatment where only venetoclax persisted in plasma, suggesting faster clearance for LP-118. In this study, the maximum reduction in platelets was 6%, 17% and 97% for venetoclax, LP-118 and navitoclax, respectively. Therefore, LP-118 is an ideal candidate for clinical evaluation due to its minimal platelet targeting and has similarities to venetoclax in its toxicity profile.
Xenograft mouse models depict superiority of LP-118 compared to venetoclax in vivo

We wanted to study the efficacy of LP-118 in vivo, in cells harboring WT or G101V BCL2. Immunodeficient CD.17 SCID mice were engrafted with RS4;11 cells with WT or G101V\textsuperscript{OE} BCL2. After tumors reached an average size of 113 to 116 mm\textsuperscript{3}, WT RS4;11 mice were treated orally once daily with vehicle (n=7), LP-118 (6.25 mg/kg, n=7), or venetoclax (6.25 mg/kg, n=7) for 28 days. Mice were removed from study when tumors reached an endpoint volume of 2000 mm\textsuperscript{3}, or at 77 days, whichever occurred first. Upon stopping treatment, tumors from LP-118 treated mice did not show increased volume, while venetoclax treated mice relapsed and exhibited increased tumor growth (Figure 6A). Mice treated with LP-118 had a significant survival advantage of 10 days over vehicle and venetoclax treated mice (venetoclax p=0.0005; vehicle p=0.0005) (Figure 6B). RS4;11 BCL2 G101V\textsuperscript{OE} cells were engrafted in mice and treated with vehicle, venetoclax (100 mg/kg, q.d., n=7) or LP-118 (50, 100 and 150 mg/kg, q.d., n=7) after reaching a tumor size of 131 to 134 mm\textsuperscript{3}. Contrary to venetoclax 100 mg/kg, mice treated with LP-118 at all doses exhibited a decrease in tumor growth (Figure 6C). All doses of LP-118 also resulted in a significant overall survival of mice versus venetoclax (50 mg/kg p=0.0104, 100 mg/kg p= 0.0012, 150 mg/kg p=0.0005) (Figure 6D). Taken together, both studies show that, at the same doses, LP-118 is more potent than venetoclax in reducing tumor burden and prolonging survival of mouse models of leukemia harboring either WT or G101V BCL2.

Next, we evaluated whether LP-118 is potent in a CLL cell line xenograft. Immunodeficient NOG mice were engrafted with OSU-CLL cells via tail vein injection. Four days post-engraftment, we initiated daily treatment with vehicle (n=8), LP-118 (50 mg/kg, n=12), or venetoclax (50 mg/kg, n=8). Splenic involvement with leukemia was confirmed by flow cytometry at the end point, determined by hindlimb paralysis\textsuperscript{26}. Compared to vehicle, LP-118 and venetoclax significantly improved the survival of mice (p=0.0002) (Figure 6E). LP-118 and venetoclax treated mice also maintained similar platelet counts throughout the course of treatment (Figure 6F). The data presented here indicates that LP-118 increases survival and decreases tumor growth in two leukemia xenograft models and can target cells harboring G101V mutant BCL2 in vivo.

Discussion

We characterized LP-118, a novel, highly potent, orally bioavailable, selective BCL2 inhibitor that also targets BCLXL moderately and can potentially overcome several venetoclax resistance mechanisms. LP-118 retains high binding affinity to WT BCL2 and is a more potent inhibitor of G101V mutant BCL2 than venetoclax. It shows improved platelet sparing capability than navitoclax in human platelets ex vivo and Eμ-TCL1 mice in vivo.

There are multiple mechanisms of venetoclax resistance that have been well characterized. We showed that LP-118 is highly potent at inducing mitochondrial apoptosis in both treatment naïve and venetoclax relapsed CLL cells. BH3 profiling revealed that venetoclax relapsed CLL may lose sensitivity to BAD peptide, which binds most tightly to BCL2. The observation that BCLXL targeting peptide HRK initiated apoptosis in some venetoclax-relapsed cells, but not naïve CLL suggests increased dependence on BCLXL at relapse. We additionally showed that LP-118 can induce apoptosis of venetoclax-resistant cell lines with high dependence on BCLXL, or with G101V BCL2 mutations. Additional studies are required to assess its potency against

11
other BCL2 mutations, such as D103E which makes BCL2 more structurally similar to BCLXL. In vivo, LP-118 improves the survival of RS4;11 WT, RS4;11 G101V BCL2 and OSU-CLL xenografts. These results highlight the preclinical efficacy and success of LP-118 and warrant clinical evaluation to determine its potential to treat frontline or venetoclax-relapsed CLL.

A recent study described the few treatment options for patients who progress on venetoclax, and the limited and relatively ineffective therapeutic interventions for patients refractory to, both, BCL2 and BTK inhibitors. Inhibitors of multiple BH3 proteins are attractive to overcome venetoclax resistance and must be carefully designed to limit on-target toxicities. For example, targeting MCL1 amplification and overexpression, venetoclax resistance factors in lymphoid malignancies, will require strategies to circumvent cardiac side effects such as CDK9 inhibition. Clinical evaluation of first generation BCL2 / BCLXL inhibitors like navitoclax found on-target platelet toxicity leading to concentration-dependent thrombocytopenia that limit the clinical utility of these inhibitors. LP-118 has low platelet toxicity, similar to venetoclax in vitro and in vivo, in Eμ-TCL1 mice and dogs. Therefore, we predict LP-118 to have clinically manageable side effects.

Dependencies on various antiapoptotic proteins of the BCL2 family have been identified in a wide range of hematological malignancies and solid tumors; therefore, LP-118 may also be of clinical interest in other cancers. Venetoclax is FDA approved for both CLL and acute myeloid leukemia (AML) and is currently under investigation for other non-Hodgkin lymphomas, T cell lymphoma, ALL, and solid tumors. Moreover, in some solid tumors such as breast cancer and colon cancer, BCLXL is highly associated with resistance to therapy. Therefore, the high potency and broader activity the BCL2 / BCLXL inhibitor LP-118 may have many areas of potential impact, surpassing more selective BH3 mimetics. These areas include ALL and venetoclax resistant T-cell leukemia, as evidenced by the efficacy shown in RS4;11 and MOLT4 cells, respectively, among other cell lines.

While the preclinical data are encouraging, clinical trials will be required to determine whether the preclinical advantages seen with LP-118 compared to venetoclax are relevant in patients. Pharmacokinetics and platelet toxicity must be monitored carefully. These preclinical data support the ongoing Phase I evaluation of LP-118 in CLL, as well as other hematologic malignancies.
References

Table 1. Clinical history and targeted sequencing panel of CLL primary patient samples pre- and post-venetoclax relapse. M = Male, IGHV = immunoglobulin heavy chain variable region genes, UM = Unmutated IGHV, BCL2 = B-cell lymphoma 2, BTK = Bruton tyrosine kinase, TP53 = tumor protein 53, PLCY2 = phospholipase C gamma 2.
Figure Legends

Figure 1. LP-118, a novel inhibitor targeting BCL2 and BCLXL.
A, Chemical Structure of LP-118. Key structural differences with its precursor, venetoclax, are highlighted in yellow. B, Testing of LP-118, venetoclax, and navitoclax in time-resolved fluorescence energy transfer (TR-FRET) assay with human recombinant peptide. BCL2 (n=3, independent experiments) C, BCLXL, (n=3, independent experiments) D, BCL2 G101V mutant (n=3, independent experiments). E, Diagram of BH3-only peptides (pro-apoptotic proteins) and their binding interactions with anti-apoptotic protein targets20,22. F, iBH3 profiling in treatment naïve primary CLL patient samples (n=6) showing cytochrome C (Cyt C) release. + Control is 25 uM alamethicin (black) and - Control is 0.01 nM PUMA 2A (purple). G, iBH3 profiling in venetoclax relapsed primary CLL patient samples (n=3). + Control is 25 uM alamethicin and – Control is 0.01 nM PUMA 2A (purple). H, iBH3 profiling in RS4;11 WT BCL2 cells and RS4;11 cells with a WT BCL2 and overexpressing (OE) BCL2 G101V mutation. Data included from two independent experiments with three replicates. -control is 100 nM PUMA2A and +control is 25 uM alamethicin. For A-G, plots display mean ± SD, and for H, mean from each independent experiment is shown. Statistical analysis was performed and analyzed using the linear mixed effect model (*p <0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Figure 2. LP-118 is cytotoxic to treatment naïve and venetoclax relapsed CLL cells.
A, Representative flow cytometry analysis of treatment naïve CLL cells or venetoclax relapsed / refractory (Ven R/R) CLL cells with in-vitro treatment of DMSO, 4 nM venetoclax or 4 nM LP-118 for 8 hours and assayed for BAK activation. B-left, Quantification of relative BAK activation in treatment naïve CLL cells after treatment with 0.1 nM to 4 nM venetoclax or LP-118 for 8 hours. Plots display, n= 8. ***p<0.001, **** p ≤0.0001, mixed effect model. B-middle, Treatment naïve primary CLL cells treated for 12 hours with concentrations of venetoclax or LP-118 ranging between 0.1nM to 10 nM and assayed for cytochrome C release. Plots display mean ± SD, n= 4. *** p<0.001, mixed effect model. B-right, Treatment naïve Primary CLL cells were treated with increasing concentrations of venetoclax or LP-118 and stained with Annexin V and TMRM followed by flow cytometry analysis. Plots display mean ± SD, n= 7. *p≤0.05, **, p≤0.01 *** p≤0.0001. C-left, Quantification of relative BAK activation in relapse/refractory CLL cells after treatment with 0.1 nM to 10 nM venetoclax or LP-118 for 8 hours. Plots display mean ± SD, n= 5. *p≤0.05; **p≤0.01, ***, p≤0.001, mixed effect model. C-middle, Venetoclax relapsed primary CLL cells treated for 15 hours with concentrations of venetoclax or LP-118 ranging between 0.1 nM to 10 nM and assayed for cytochrome C release. Plots display mean ± SEM, n= 3; **** p<0.0001, mixed effect model. C-right, Venetoclax relapsed primary CLL cells were treated with increasing concentrations of venetoclax or LP-118 and stained with Annexin V and TMRM followed by flow cytometry analysis. Plots display mean ± SD, n= 4. *p≤0.05, ** p≤0.01, ****p≤0.0001, mixed effect model. Overall trend analysis is indicated by black stars and individual comparisons are indicated by purple or blue stars. D-E, Primary CLL cells were treated for 24 hours with DMSO, LP-118 1 nM, LP-118 4 nM, or venetoclax 4 nM, alone (-) or in presence of caspase inhibitor Z-VAD-FMK (+) and assayed for cytochrome C (Cyt C) release (D) and viability by CellTiter-Glo assay (E). Plots display mean ± SD, n=3 samples treated in triplicate. ****p<0.0001, mixed effect model.

Figure 3. LP-118 induces apoptosis in CLL cells supported by NK-tert stroma.
A, Representative flow of n=6 primary CLL samples cultured with or without NKtert stroma with in-vitro treatment of venetoclax, LP-118, or navitoclax. IC$_{50}$ calculated by Nonlinear Regression. Plots display mean ± SD. B-D, Western blot, and quantification of BCL2 and BCLXL expression normalized to GAPDH, in Primary CLL patient samples cultured with or without NKtert stroma (n=5).

**Figure 4.** LP-118 targets RS4;11 cells with BCL2 G101V mutation and cells lines without strong BCL2 dependency. RS4;11 cells with WT BCL2 or overexpressing G101V BCL2 (G101V$^{OE}$) were treated with increasing concentrations of venetoclax or LP-118. A-left, Cells were treated for 4 hours and stained with BAK antibody used to determine active Bak conformation and analyzed on the flow cytometer. Plots display mean ± SD, n= 3 independent experiments. *p≤0.05; *** p≤0.001, ****, p≤0.0001, mixed effect model. Comparisons between the two treatment groups with WT BCL2 are in blue and between the two treatment groups with BCL2 G101V mutation are in grey. A-middle, RS4;11 cells were collected at 15 hours and stained with cytochrome C and analyzed on flow cytometer. Plots display mean ± SD, n=3 independent experiments. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, mixed effect model. Comparisons between the two treatment groups with WT BCL2 are in blue and between the two treatment groups with BCL2 G101V mutation are in grey. A-right, Cells were collected at 72 hours and stained with Annexin V-FITC/TMRM for flow cytometry. Plots display mean ± SD, n= 3 independent experiments. ** p<0.01, **** p<0.0001, mixed effect model. Overall trend analysis is indicated by black stars and individual comparisons are indicated for comparisons between the two treatment groups with WT BCL2 in blue and between the two treatment groups with BCL2 G101V mutation in grey. D, OSU-CLL WT and BCL2 KO cell lines were treated with venetoclax, A-1331852, navitoclax, or LP-118 for 72 hours, followed by CellTiter-Glo viability assay. Plots display mean ± SD, n=3 independent experiments in triplicate. *p≤0.05, ** p≤0.01, mixed effect model. E, RS4;11 (n=2), LOUCY (n=3), CCRF-CEM (n=3), PF-382 (n=3), and MOLT4 (n=3) cell lines were treated with venetoclax, A-1331852, navitoclax, or LP-118 for 24 hours, followed by CellTiter-Glo viability assay. Plots display mean ± SD from the indicated numbers of independent experiments, in triplicate.

**Figure 5.** Effects of LP-118 on other immune cell types. A, Human platelets were isolated and treated with venetoclax, LP-118 or navitoclax for 72 hours. Inhibition of platelets was determined by MTS assay. Plots display mean ± SD, n= 3 healthy donors. ****p<0.001, **** p≤0.0001, mixed effect model. B-D, Blood from Eμ-TCL1 mice were stained for CD4+ T cells (D) CD8+ (E) or NKp1.1 (F) at week 1 and week 6 post-treatment and analyzed via flow cytometry to identify the subsets of immune cells. Plots display mean ± SD. *p≤0.05; ** p≤0.01, mixed effect model. E, Blood from Eμ-TCL1 mice was also used and analyzed for platelet count using Complete Blood Count (CBC) machine. Plots display mean ± SD, *p<0.05; **p≤0.01, **** p≤0.0001, mixed effect model.

**Figure 6.** LP-118 decreases tumor size in RS4;11 xenograft and improves survival in OSU-CLL xenograft models. A, NOG mice were engrafted with RS4;11 WT BCL2 cells and monitored for tumor volume. Data were cubic root transformed to minimize variance. Mixed effect model was used to compare tumor sizes among groups (*, 0.05 ≥ P > 0.01; **, 0.01 ≥ P ≥0.001; ***, P < 0.001). B, Survival curve of NOG mice engrafted with RS4;11 WT BCL2 and treated with vehicle, venetoclax (6.25 mg/kg), or LP-118 (6.25 mg/kg). Log-rank Mantel-Cox
test was performed. C, NOG mice were engrafted with RS4;11 cells overexpressing G101V mutant BCL2 (G101V\text{OE}), treated with vehicle, venetoclax (100 mg/kg), or LP-118 (50, 100 or 150 mg/kg), and monitored for tumor volume. Data were cubic root transformed to minimize variance. Mixed-effect model was used to compare tumor sizes among groups (\(*, 0.05 \geq P > 0.01; **, 0.01 \geq P \geq 0.001; ***, P < 0.001\)). D, Survival curve of NOG mice engrafted with RS4;11 G101V\text{OE} BCL2 and treated with vehicle, venetoclax (100 mg/kg), or LP-118 (50, 100 or 150 mg/kg). Gehan-Breslow-Wilcoxon test was performed (\(*, 0.05 \geq P > 0.01; **, 0.01 \geq P \geq 0.001; ***, P < 0.001\)). E, NOG mice were engrafted with OSU-CLL and monitored for survival until the endpoint of hind-limb paralysis. Log-rank Mantel-Cox test was performed. F, CBC of OSU-CLL engrafted NOG mice were used to determine platelet counts. Mixed-effect model was used to compare among groups (\(*, 0.05 \geq P > 0.01; **, 0.01 \geq P \geq 0.001; ***, P < 0.001\)). Ven, venetoclax; LP, LP-118.
Supplemental Figure 1

A

↑BCL2 selectivity  ↑BCLXL selectivity

Venetoclax  LP-118  Navitoclax  A-1331852

B

![Graph showing concentration vs. response](image)

- S63845
- LP-118

[1] [2]
Supplemental Figure 1

**C**

Human WT
Glide gscore: -6.377 kcal/mol

Human Mutant D105E
Glide gscore: -4.476 kcal/mol

Human Mutant G101V
Glide gscore: -3.995 kcal/mol

**D**

WT Murine Model
Glide gscore: -3.313 kcal/mol

**E**

HRK BH3 peptide sensitivity

<table>
<thead>
<tr>
<th>Concentration of HRK, μM</th>
<th>%Cyt C Released</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naive</td>
</tr>
<tr>
<td></td>
<td>Relapsed</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* and ** indicate significant differences.
Supplemental Figure 1

F

<table>
<thead>
<tr>
<th>kD</th>
<th>WT</th>
<th>G101V&lt;sup&gt;OE&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- MCL1
- BCL2<sup>G101V</sup> (c-Myc-DDK tagged)
- BCLXL
- BCL2
- GAPDH

G

Primary CLL with Mutant p53

![Graph showing concentration of inhibitor vs. % Live cells](image)

H

- Venetoclax (-stroma)
- Venetoclax (+stroma)
- LP-118 (-stroma)
- LP-118 (+stroma)
- Navitoclax (-stroma)
- Navitoclax (+stroma)

![Graph showing concentration of inhibitor vs. % Live cells](image)

I

OSU-CLL

- MCL1
- BCLXL
- BCL2
- GAPDH
### Supplemental Figure 1

<table>
<thead>
<tr>
<th></th>
<th>Target</th>
<th>AUC last (h*ng/mL)</th>
<th>Platelet Max Reduction</th>
<th>PO Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venetoclax</td>
<td>Selective Bcl-2</td>
<td>145,337</td>
<td>6%</td>
<td>25 mg/kg</td>
</tr>
<tr>
<td>Navitoclax</td>
<td>Dual Bcl-2/xL</td>
<td>295,242</td>
<td>97%</td>
<td>25 mg/kg</td>
</tr>
<tr>
<td>LP-118</td>
<td>Dual Bcl-2/xL</td>
<td>129,780</td>
<td>17%</td>
<td>25 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>IGHV</td>
<td>Complex Karyotype?</td>
<td>del17p?</td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
<td>------</td>
<td>-------------------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>U</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>unknown</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>U</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>unknown</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>U</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>unknown</td>
<td>yes</td>
<td>del17p</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>unknown</td>
<td>no</td>
<td>del17p</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>unknown</td>
<td>yes</td>
<td>del17p</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>M</td>
<td>no</td>
<td>del17p</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>M</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>U</td>
<td>yes</td>
<td>del17p</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>U</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>U</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>M</td>
<td>no</td>
<td>del17p</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>unknown</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>M</td>
<td>yes</td>
<td>del17p</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>M</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>unknown</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

**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: 146665563), 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 G101VŒ 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 SfgiI 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\textsuperscript{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 4^{\circ}C, 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 co-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<sub>DMSOcontrol</sub>-A/D<sub>Test article</sub>)/(A/D<sub>DMSOcontrol</sub>-A/D<sub>no enzyme control</sub>)×100%.

**Platelet Toxicity Assay**

Plasma was isolated from whole ACD-A-stabilized human blood (AllCells, Alameda 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 2x10⁸ 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-TCL1 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®, CB17/1cr-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 mm³. 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 mm³ 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**


**Availability of data and materials**

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