



The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

605. MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Targeting Covalent and Non-Covalent Btki-Resistant CLL Using the Dual Irreversible/Reversible 4th Generation BTK Inhibitor LP-168

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Background: Treatment of chronic lymphocytic leukemia (CLL) has been transformed with targeted therapies including inhibitors of Bruton's tyrosine kinase (BTKi). Currently three covalent BTK inhibitors (cBTKi) are approved for CLL, but most patients eventually relapse, commonly through acquisition of the C481S BTK mutation (Woyach *et al.* 2014). Pirtobrutinib and nemtabrutinib are non-covalent BTK inhibitors (ncBTKi) developed to target and inhibit C481S mutant BTK. However novel secondary site mutations in BTK, namely T474I and L528W, have been found to confer resistance to both cBTKi and ncBTKi (Wang *et al.* 2022). Regardless of these mutations, BCR signaling remains intact, suggesting that inhibition of BTK maintains its therapeutic importance. LP-168 is a novel ultra-selective 4th generation BTKi with an active warhead capable of covalent interaction with WT BTK or non-covalent binding when a BTK C481 mutation is present.

Methods: To determine the target selectivity of LP-168 we screened the compound against 468 kinases in the scanMAX Kinase Assay. For validation of covalent binding, dialysis against WT BTK was performed. Primary CLL B cells were isolated by negative selection and treated with LP-168 for all experiments. BCR signaling alterations were assessed via immunoblot to observe changes in target protein phosphorylation. Changes in CLL migration towards CXCL12 or CXCL13 were interrogated by transwell migration. Cytotoxicity towards primary CLL cells was measured via Annexin V/PI flow cytometry with and without HS-5 stromal cell support. Experiments using TMD8 cells were performed following CRISPR modification to insert WT, C481S, or T474I BTK. *In vivo* survival studies were performed using both the E μ -TCL1 and E μ -MTCP1 mouse engraftment models.

Results: LP-168 demonstrated selectivity towards BTK with roughly 700-fold selectivity for BTK vs its next off-target. In an enzymatic assay, LP-168 displayed nanomolar potency towards both WT BTK (IC₅₀=0.11 nM) and C481S BTK (IC₅₀=1.0 nM). Following 2-hour drugging, LP-168 inhibited BCR signaling in primary CLL B cells at increasing concentrations, demonstrated by reduced phosphorylation of BTK (92%, *p*<0.0001, *n*=8) and its immediate downstream target PLC γ 2 (41%, *p*=0.0013, *n*=8). Migration capacity of primary CLL cells towards CXCL12 and CXCL14 was also found to decrease after 2-hours of treatment with LP-168 (52%, *p*<0.001, *n*=10 and 51%, *p*<0.001, *n*=10, respectively). After 48 hours of exposure, LP-168 was able to induce cytotoxicity of primary CLL cells alone and in co-culture with HS-5 stromal cells in a dose-dependent manner, with similar or improved efficacy to other BTKi. LP-168 decreased CLL cell production of CCL3 and CCL4 chemokines (CCL3: 93%, *p*<0.001, *n*=8; CCL4: 93%, *p*<0.001, *n*=8). LP-168 also demonstrated modest cytotoxicity towards TMD8 cells harboring either C481S BTK (24%, *p*=0.0008, *n*=3) or T474I BTK (13%, *p*=0.0690, *n*=3), as well as decreased production of CCL3 (C481S: 95%, *p*<0.0001, *n*=3; T474I: 99%, *p*<0.0001, *n*=3) and CCL4 (C481S: 97%, *p*<0.0001, *n*=3; T474I: 98%, *p*<0.0001, *n*=3) (Figure 1A). Further, we observed inhibition of downstream BCR signaling in primary CLL B cells harboring C481S via immunoblotting

and cytokine production. Finally, to evaluate the *in vivo* efficacy of LP-168 we tested this compound in both the E μ -TCL1 and E μ -MTCP1 models. We treated mice daily via oral gavage and found that 50 mg/kg of LP-168 significantly improved survival in the E μ -TCL1 model when compared to vehicle (median 51 vs 44 days; $p=0.0018$) or ibrutinib at 50 mg/kg (median 51 vs 45 days; $p=0.0098$) and the E μ -MTCP1 model when compared with vehicle (median 122 vs 62 days; $p<0.0001$) or ibrutinib at 50 mg/kg (median 122 vs 96 days; $p=0.0162$) (Figure 1B).

Conclusions: Collectively, our data demonstrate that LP-168 is a potent and selective inhibitor of BTK with activity against CLL, even in the presence of mutations that mediate resistance to cBTKi and ncBTKi. These data support the continued preclinical and clinical investigation of LP-168, which is currently being studied in the phase 1 setting of CLL (NCT04775745) and NHL (NCT04993690).

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Figure 1

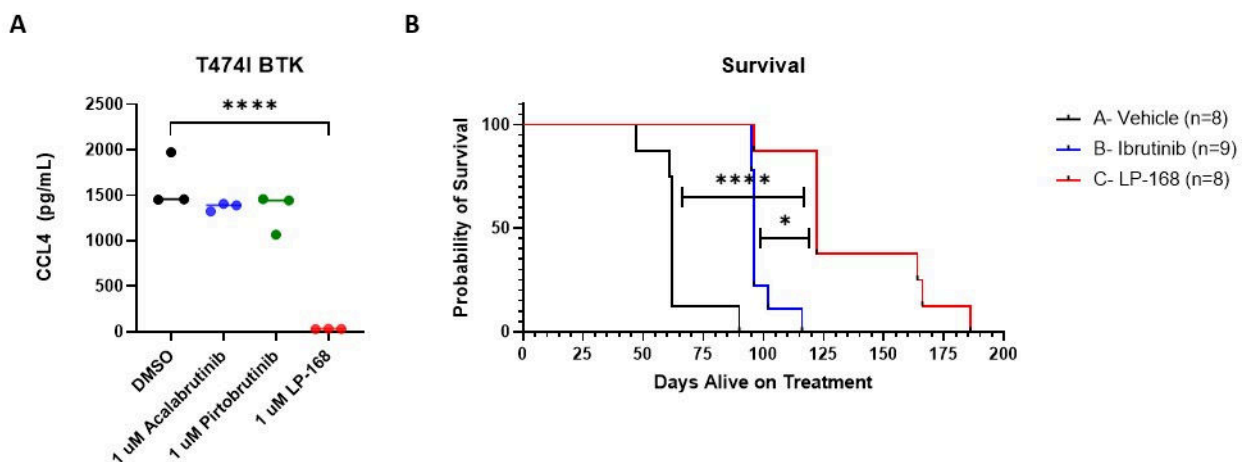


Figure 1

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