HMPL-760 is a highly potent and selective reversible BTK inhibitor, targeting BTK and BTK^{C481S} in B-cell malignancies

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INTRODUCTION

- Bruton's tyrosine kinase (BTK), a member of the Tec family, plays a crucial role in signaling through B-cell receptor (BCR). BTK inhibitors block BCR signals and inhibit B-cell activation and growth [1].
- First-generation BTK inhibitors, such as ibrutinib, covalently bind to a cysteine residue (C481) of BTK. Their most frequent acquired resistance is the development of a cysteine mutation such as C481S in the binding site [2]. Next generation BTK inhibitors such as LOXO-305 (pirtobrutinib) and ARQ 531 (nemtabrutinib) binding to BTK independent of C481, are being developed to overcome this resistance. LOXO-305 was approved by FDA for mantle cell lymphoma (MCL) in Jan 2023 [3].
- HMPL-760 is a next generation, highly potent, selective, reversible BTK inhibitor with potency against both wild-type and C481 mutated BTK. It's being developed in clinical trial (NCT 05190068).

METHODS

- **Kinase activity and selectivity:**

The Z'-LYTE™ kinase activity assays were used to determine the IC_{50} values of wild-type BTK (BTK^{WT}) and C481S mutant BTK (BTK^{C481S}) enzymes in HUTCHMED. The kinase selectivity of HMPL-760 at 3 μM was evaluated in the KinaseProfiler™ panel by Eurofins Cerep, including the activity against 413 kinases by radiometric protein kinase assays and HTRF® lipid and atypical kinase assays.

- **BTK reversibility**:

Test compounds were incubated with either recombinant human BTK or buffer alone followed by trypsin digestion. The concentration of intact compounds after incubation were detected by LC-MS/MS analysis. Reversibility rate was determined as the ratio of the concentration of intact compound incubated with BTK to that incubated with buffer alone. The reversible compound is expected to release as free compound from binding site after trypsin digestion and have a high reversibility rate.

- **Cellular BTK inhibition and cell growth inhibition:**

HEK-293 cell lines stably expressing BTK and BTK^{C481S} were generated using standard Lipofectamine™ transfection/selection methods. The inhibition on BTK phosphorylation (p-BTK) was evaluated with Phospho-BTK (Tyr223) HTRF assay or Western blot assay. Cell growth inhibition was evaluated by Cell Counting Kit-8 or CellTiter-Glo method.

- **Inhibition of human B cell activation:**

Anti-IgM-induced CD69 expression (marker for B-cell activation) on CD19^{+} B cells was evaluated by Flow Cytometry in human whole blood with HMPL-760 or other BTK inhibitor treatment.

- **In vivo anti-tumor efficacy:**

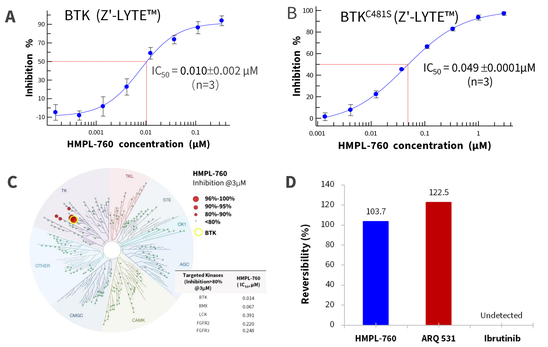
Human B-NHL xenograft models were established by subcutaneously inoculated the tumor cells into immuno-deficient mice. The tumor bearing mice were randomly assigned into different treatment groups (n=8~9/group), and tumor volume or tumor area and body weight were measured 2~3 times every week. Means and SD were plotted for each treatment group versus days of treatment.

- **In vivo target inhibition:**

After a single oral dosing of BTK inhibitors, tumor tissues from their tumor bearing mice were collected at various timepoints. p-BTK was determined by western blot analysis. The concentrations of BTK inhibitors were determined by LC-MS/MS.

RESULTS

Figure 1 : HMPL-760 is a potent, selective, reversible BTK inhibitor



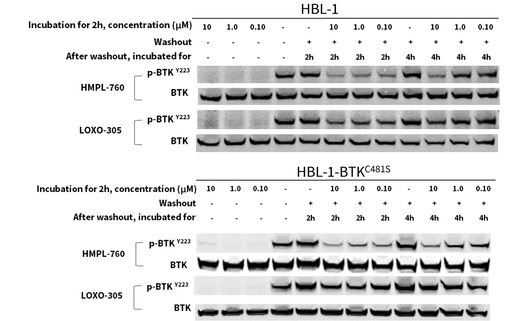
HMPL-760 strongly inhibited BTK kinase activities of BTK^{WT} and BTK^{C481S} (A and B). HMPL-760 demonstrated high selectivity in a panel containing 413 kinases (C). In the reversibility study, HMPL-760 showed fully recovery, while no recovery for ibrutinib (D).

Table 1: HMPL-760 demonstrates strong inhibition on BTK phosphorylation, B-cell activation and growth

Inhibition of BTK phosphorylation, IC_{50} (μM, mean±SD, n=3)				
	HMPL-760	Ibrutinib	ARQ 531	LOXO-305
HEK-293-BTK^{WT}	0.006±0.002	0.006±0.002	0.074±0.044	0.008±0.004
HEK-293-BTK^{C481S}	0.006±0.003	0.194±0.150	0.163±0.050	0.016±0.002
Inhibition of B-NHL cell growth, GI_{50} (μM)				
	HMPL-760	Ibrutinib	ARQ 531	LOXO-305
TMD-8 (DLBCL)	0.0041	0.001	0.085	0.0079
OCI-LY10 (DLBCL)	0.0028	0.0012	0.143	0.019
REC-1 (MCL)	0.0015	0.00044	0.104	0.0047
HBL-1 (DLBCL)	0.014	0.0081	0.571	0.021
HBL-1-BTK^{C481S}	0.046	3.855	0.471	0.161
Inhibition of human whole blood B-cell activation, IC_{50}/IC_{90} (μM)				
B-cell activation	0.006/0.016	ND	0.286/0.678	0.004/0.013

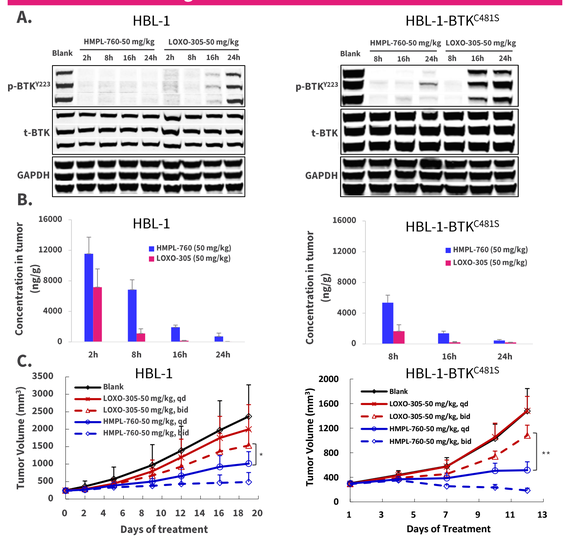
HMPL-760 potently blocked p-BTK in both HEK-293-BTK^{WT} and HEK-293-BTK^{C481S} cells, CD69 expression induced by anti-IgM in human whole blood and B-NHL cells growth. HMPL-760 showed ≥ 10-fold inhibitory potency than ARQ 531 in both BTK-WT and BTK-C481S cells, and ~3-fold higher inhibitory potency than LOXO-305 in BTK-C481S cells respectively.

Figure 2: HMPL-760 maintains a longer duration for p-BTK inhibition after compound washout



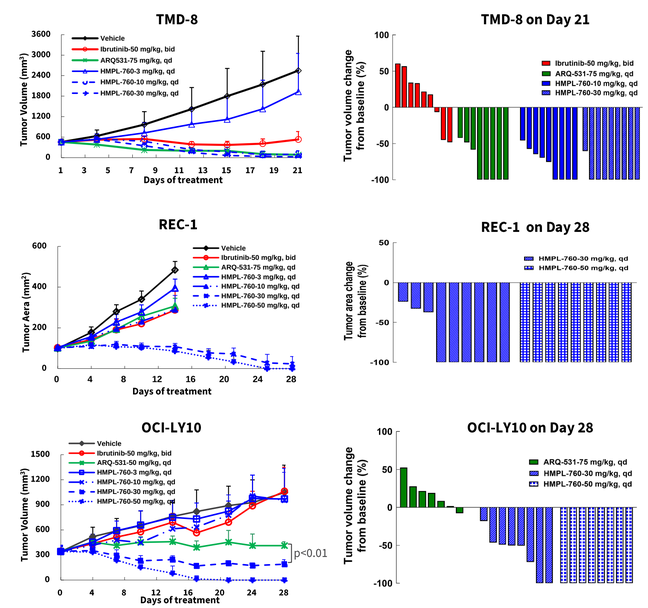
HMPL-760 maintained a longer duration for p-BTK inhibition than LOXO-305 in both BTK wild type (HBL-1) and BTK mutant (HBL-1-BTK^{C481S}) cell lines after compound washout.

Figure 3: HMPL-760 exhibits stronger efficacy in both HBL-1 and HBL-1-BTK^{C481S} xenograft model



In a head-to-head comparison study, HMPL-760 demonstrated better anti-tumor efficacy than LOXO-305 at the same doses in both HBL-1 and HBL-1-BTK^{C481S} xenograft models, respectively (C, *p<0.05; **p<0.01). The observations were consistent with that HMPL-760 displayed a longer inhibition time of p-BTK (A), and higher drug exposures in tumor tissues (B) than LOXO-305 did at same dose.

Figure 4: Oral treatment of HMPL-760 displays robust antitumor efficacy in multiple human B-NHL cell xenograft models



HMPL-760 oral treatment showed a dose-dependent anti-tumor activity in human B-NHL xenograft models (TMD-8, REC-1, OCI-LY10). HMPL-760 (≥10 mg/kg) resulted in tumor complete regression (CR) in some individual animals.

CONCLUSIONS

- **HMPL-760 is a reversible, selective, highly potent, BTK inhibitor targeting both BTK^{WT} and BTK^{C481S}.**
- **The preclinical studies support clinical evaluation of HMPL-760 in B-cell lymphoma.**

REFERENCES

[1] Nat Chem Biol. 2011;7(1):4-5.
[2] N Engl J Med. 2014 ;370(24):2286-94.
[3] https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-pirtobrutinib-relapsed-or-refractory-mantle-cell-lymphoma.