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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Characterization and Preclinical Evaluation of AS-1763, an Oral, Potent and Selective Noncovalent BTK Inhibitor, in Chronic Lymphocytic Leukemia

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Background: Covalent Bruton's tyrosine kinase (BTK) inhibitors (cBTKi) have transformed treatment landscape of chronic lymphocytic leukemia (CLL). These inhibitors bind to C481 residue in the kinase domain of BTK. This is also the site for the most common mutations rendering cells resistant to cBTKi. To circumvent this limitation of the cBTKi, non-covalent BTKi (ncBTKi) such as pirtobrutinib have been developed. Recently, non-C481 BTK mutations have been reported in patients with CLL at the time of disease progression during pirtobrutinib treatment. These observations underscore the need for ncBTKi that can target C481 as well as non-C481 mutations of BTK. AS-1763 is a potent, highly selective, orally available, and ncBTKi, equipotent against both wild-type and C481S-mutated BTK when tested in biochemical assays. In vivo, AS-1763 demonstrated significant antitumor effects in OCI-LY10 tumor xenograft models harboring wild-type or C481S mutant BTK (Kawahata et al. J Med Chem 64:14129, 2021).
Study Design and Methods: In the present project, first we used cell free assay systems to evaluate selectivity and potency of AS-1763 by a kinome-wide profiling and inhibitory effect of AS-1763 in enzyme assays using recombinant mutant BTK. Second, we examined dose- and time-dependent inhibition of mutant BTK that is expressed in HEK293 cell line. Third, we tested biological, biochemical, and molecular impact of AS-1763 in primary CLL cells. Fourth, we determined sensitivity of CLL cells to AS-1763 when combined with other targeted agents.
Results: AS-1763 showed a highly selective profile for BTK in a panel of 291 kinase assays with >260-fold selectivity except 3 Tec family kinases (BMX, ITK, and TEC). We have generated a total of 17 recombinant BTK mutant proteins (C481, T474, L528 variants and other BTK mutants; Table 1) reported in the literature or predicted by single nucleotide change in the codon, and established assay methods to measure inhibitory potency of BTKi for those BTK mutants (Table 1). AS-1763 showed potent inhibitory activities for those BTK mutants while inhibitory potencies of other cBTKi and ncBTKi were diminished against some BTK mutants such as T474 and/or L528 mutations. AS-1763 exhibited dose-dependent and slow-off rate inhibitions of BTK autophosphorylation (pY223) in HEK293 cells transfected with various BTK mutants. Furthermore, the observed inhibitory effects of AS-1763 on the BTK autophosphorylation (pY223) in HEK293 cells were continued up to 24 h after washing out of AS-1763. In primary CLL samples, AS-1763, pirtobrutinib or ibrutinib induced a modest apoptosis. AS-1763 effectively inhibited the BCR signaling pathway in a dose dependent manner as evidenced by downregulation of pY223-BTK expression which was also observed in cBTKi and ncBTKi relapsed/refractory CLL samples. In vitro incubations with AS-1763 inhibited CLL cell spontaneous migration, decreased CCL3/CCL4 levels in culture supernatant and was accompanied with the inhibition of intracellular calcium release and B-cell activation, as measured by surface CD86 expression. Besides, AS-1763 incubation for 24 hours was shown to modulate the expression of BCL-2 family proteins with the downregulation of MCL-1 and BCL-xl. Interestingly, in vitro treatment of CLL patient samples with AS-1763 demonstrated a notable elevation in cellular ROS and mitochondrial superoxide levels starting at 1 μM, concurrently impacting SOD1 expression in CLL patient samples. Evaluation of drug interaction models utilizing Compusyn and Synergy Finder applications predominantly indicated additive effects between AS-1763 with BCL-2 inhibitor, venetoclax as well as p53 activator APR-246. Consistent with this data, AS-1763 and venetoclax combinations showed high-rates of apoptosis in samples that were relapsed/refractory to cBTKi and ncBTKi.
Conclusions: AS-1763 is a selective ncBTKi that inhibits both wild-type and mutant BTKs listed in Table 1. In CLL cells, AS-1763 was effective in inhibiting BCR pathway signaling and sensitized cells to other agents such as venetoclax. Based on these

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encouraging data we have initiated a clinical trial to test AS-1763 in patients with CLL and other B cell malignancies who have failed or are intolerant to at least two prior lines of systemic therapy, including cBTKi (NCT05602363 Clinical Trials.gov).

Disclosures Fujiwara: *Carna Biosciences, Inc:* Current Employment. **Hatakeyama:** *Carna Biosciences, Inc:* Current Employment. **Asami:** *Carna Biosciences, Inc:* Current Employment, Patents & Royalties. **Ohmoto:** *Carna Biosciences, Inc:* Current Employment. **Miyamoto:** *CarnaBio USA, Inc.:* Current Employment. **Nishioka:** *Carna Biosciences, Inc:* Current Employment. **Arimura:** *Carna Biosciences, Inc:* Current Employment, Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees. **Sawa:** *Carna Biosciences, Inc:* Current Employment, Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties. **Jain:** *Aprea Therapeutics:* Research Funding; *CareDX:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses; *Fate Therapeutics:* Research Funding; *Ipsen:* Consultancy, Honoraria, Other: TRAVEL, ACCOMMODATIONS, EXPENSES; *Precision Biosciences:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Genentech:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Beigene:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses; *Medisix:* Research Funding; *ADC Therapeutics:* Research Funding; *Janssen:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses; *AstraZeneca:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *BMS:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Kite/Gilead:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Adaptive Biotechnologies:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Servier:* Research Funding; *Incyte:* Research Funding; *Cellectis:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Pfizer:* Research Funding; *TG Therapeutics:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses; *MEI Pharma:* Consultancy, Honoraria, Other: TRAVEL, ACCOMMODATIONS, EXPENSES; *AbbVie:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding; *Mingsight:* Research Funding; *Takeda:* Research Funding; *Loxo Oncology:* Research Funding; *Novalgen:* Research Funding; *Dialectic Therapeutics:* Research Funding; *Newave:* Research Funding; *TransThera Sciences:* Research Funding; *Pharmacyclics:* Consultancy, Honoraria, Other: Travel, Accommodations, Expenses, Research Funding. **Gandhi:** *Pharmacyclics:* Research Funding; *Clear Creek Bio:* Consultancy, Research Funding; *AbbVie:* Research Funding; *LOXO:* Research Funding; *Dava Oncology:* Honoraria; *Sunesis:* Honoraria, Research Funding.

Table 1. List of recombinant BTK mutant proteins used in this study.

Mutation	Enzymatic activity
WT	Yes
C481S	Yes
T316A	Yes
V416L	No
A428D	No
M437R	Yes (very weak)
T474I	Yes
T474S	Yes
T474M	Yes
T474L	Yes
T474M/C481S	Yes
T474I/C481S	Yes
T474M/C481T	Yes
L528W	No
L528M*	Yes
L528V*	Yes
L528S	No
L528F*	No

*Possible mutants predicted by single nucleotide change in the codon for L528.

Figure 1

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