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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Preclinical Study of DZD8586, a Non-Covalent LYN/BTK Dual Inhibitor with Excellent BBB Penetration, for the Treatment of B-Cell Non-Hodgkin Lymphoma (B-NHL)

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Introduction

Bruton's Tyrosine Kinase (BTK) inhibitors have been approved for the treatment of B-NHL, such as CLL and MCL. However, resistance to BTK inhibitors still inevitably occurred, and different types of mutations were identified post covalent and non-covalent BTK inhibitor treatment.

Although BTK inhibitors showed some activities in the treatment of relapsed or refractory non-germinal center B-cell (non-GCB) DLBCL, no drugs have been approved due to unsustainable tumor response. It is hypothesized that blockage of BTK pathway alone is not sufficient to achieve optimal anti-tumor efficacy in DLBCL.

Due to limited blood-brain barrier (BBB) penetration of the current therapies, treatment of central nervous system lymphoma (CNSL) remains a clinical challenge.

DZD8586 is a rationally designed, oral, non-covalent, LYN and BTK dual inhibitor with excellent BBB penetration as a potential treatment option for B-NHL. Here we report the preclinical activity of DZD8586 in lymphoma cell lines and animal models which supports its clinical development to address the above unmet medical needs.

Methods

The enzymatic activity of DZD8586 against multiple kinases was assessed at Eurofins panel with purified enzymes. TMD-8-IbruR cells carrying the C481S mutation were generated by stepwise induction of increasing concentrations of Ibrutinib. Cells carrying pirtobrutinib resistance mutations were obtained by transfection of different BTK mutations into RI-1 cells using lentivirus. Cell growth inhibition was determined by CellTiter-Glo. A pBTK enzyme-linked immunosorbent assay (ELISA) was used to evaluate the pharmacodynamic profile of DZD8586. Western blot was used to test the modulations of signaling pathways with DZD8586 treatment. Animal models were established by subcutaneous and intracranial inoculation of tumor cells, respectively, and animals were treated with DZD8586 or other BTK inhibitors. The anti-tumor efficacy was assessed by comparing the change in mean tumor volume between the different treatment groups and vehicle controls.

Results

Kinase panel profiling showed that DZD8586 potently inhibited LYN and BTK, with good selectivity against other kinases. In cell lines expressing C481X and pirtobrutinib resistance mutations, DZD8586 demonstrated concentration dependent anti-proliferative effects, with similar GI_{50}s among cells carrying different BTK mutations. In a panel of DLBCL cell lines, DZD8586 exhibited potent cell growth inhibition and induced cell death in a variety of cell lines, and its effect correlated with modulations of both LYN and BTK pathways. In both subcutaneous and CNS tumor models, DZD8586 exhibited profound tumor growth inhibition in a dose-dependent manner, and induced tumor regression at the doses of 50 mg/kg and above. There was a good correlation between plasma concentrations of DZD8586 and modulations of pBTK in whole blood and tumor tissue. DZD8586 has good cell permeability and is not significantly transported by P-gp and BCRP expressed at the BBB. The $K_{puu,CSF}$ of DZD8586 in rat and monkey were 1.2 and 1.3, respectively, suggesting its excellent BBB penetration in humans.

Conclusion

DZD8586 is a novel non-covalent LYN/BTK dual inhibitor, which could overcome resistance mutations to the approved covalent and non-covalent BTK inhibitors, and derive clinical benefit to patients with DLBCL and CNSL by blocking both BTK-dependent and BTK-independent signaling pathways with excellent BBB penetration.

Disclosures Bai: *Dizal Pharmaceutical:* Current Employment. **Wu:** *Dizal Pharmaceutical:* Current Employment. **Hu:** *Dizal Pharmaceutical:* Current Employment. **Han:** *Dizal Pharmaceutical:* Current Employment. **Liu:** *Dizal Pharmaceutical:* Current Em-

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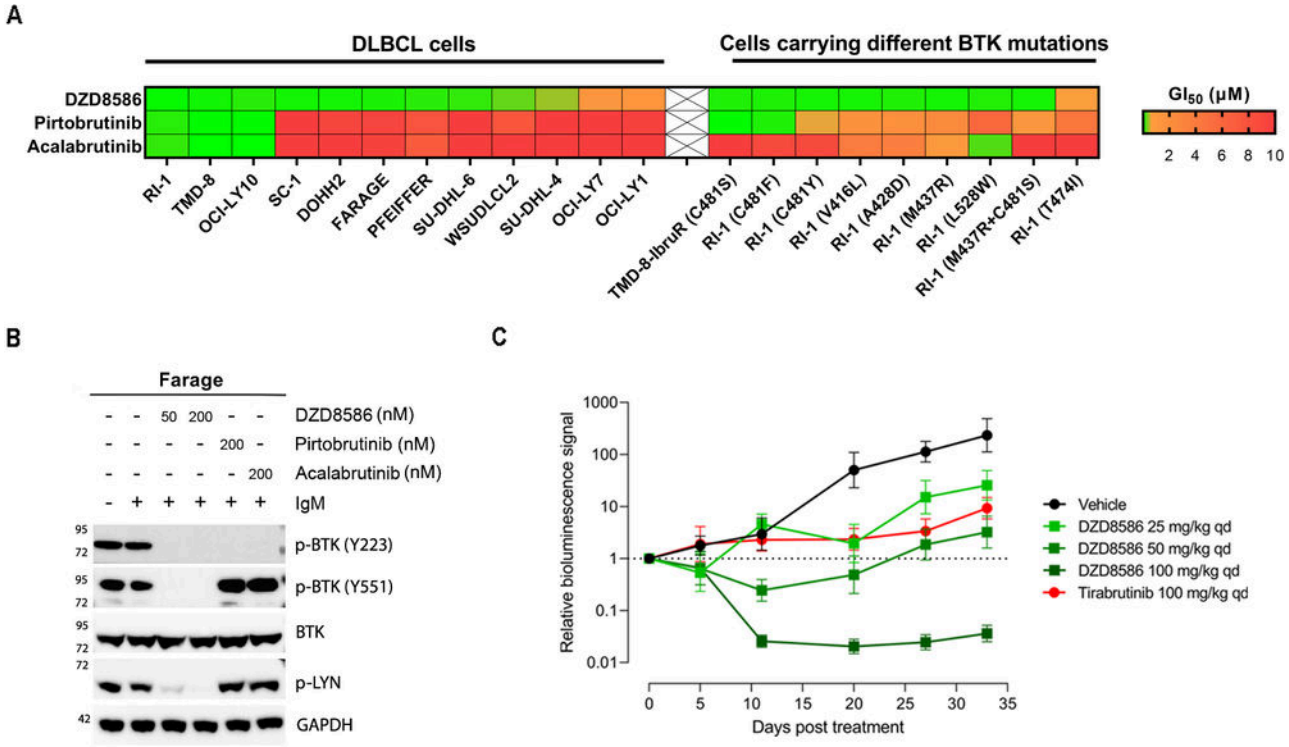

Figure 1. Anti-tumor activity of DZD8586 in cell lines and in animal models with brain tumors. (A) Anti-proliferative effect of DZD8586, pirtobrutinib and acalabrutinib in a panel of DLBCL cell lines and RI-1 cells transfected with different BTK mutations. **(B)** DZD8586 blocked both BTK-dependent and BTK-independent signaling pathways in FARAGE cells (DLBCL). **(C)** Anti-tumor activity of DZD8586 in TMD-8-Luci (DLBCL) brain tumor model. Statistically significant difference was calculated by using two-way ANOVA (*: $p < 0.05$, **: $p < 0.01$, ****: $p < 0.0001$). qd: once daily.

Figure 1

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