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605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

ONO-7018, a First-in-Class MALT1 Inhibitor, Provides Novel Therapeutic Strategies for B Cell Malignancies: Overcoming BTK Inhibitor Acquired Resistance and Enhancing the Antitumor Effect of BTK Inhibitors

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Introduction: B cell receptor (BCR) signaling plays a crucial role in promoting survival and growth in B cell malignancies, and several compounds targeting the signaling pathways have been approved. Bruton's tyrosine kinase (BTK) is a key molecule in BCR signaling pathways and BTK inhibitors are one of the essential treatment options for chronic lymphocytic leukemia (CLL) and certain subtypes of B cell Non-Hodgkin Lymphoma. However, there are issues that acquired resistance mutations in the BTK kinase domain and gain of function mutation of its downstream molecules, which lead to BTK inhibitor resistance and limit its efficacy. For example, BTK C481S mutation is well known as a resistant mutation to covalent BTK inhibitors and several mutations of BTK (such as T474I and L528W mutation) have recently been reported in relapsed or refractory CLL patients with acquired resistance to pirtobrutinib, a non-covalent BTK inhibitor. In addition, there are unmet medical needs to improve the duration of response of current standard therapies including BTK inhibitors for B cell malignancies. Therefore, therapeutic strategies to overcome the BTK inhibitor resistance and novel combination therapies to enhance the efficacy of BTK inhibitors are required. Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is known as a key growth and survival regulator in BCR signaling and an emerging therapeutic target. ONO-7018 (formerly known as CTX-177), a potent and selective MALT1 inhibitor, demonstrated preclinical efficacy in several lymphoma models.
Aim: Firstly, to explore the potential of ONO-7018 to overcome BTK inhibitor acquired resistance, we tested the antitumor effects of ONO-7018 on cell lines harboring BTK inhibitor-resistant mutations. Secondly, we examined the combination anti-tumor efficacy of ONO-7018 and tirabrutinib, a second generation BTK inhibitor *in vitro* and *in vivo*.
Methods: Diffuse large B-cell lymphoma (DLBCL) cell lines harboring BTK inhibitor-resistant mutations (BTK T474I, C481S, or L528: PLCG2 R665W) were generated by continuous passage with BTK inhibitor or CRISPR-Cas9 gene editing followed by single cell cloning. In *in vivo* study, human DLBCL cell line, TMD8 cells were subcutaneously implanted in immune deficient mice. ONO-7018 and tirabrutinib were orally administered to the mice twice a day.
Results: Anti-proliferative activities of ONO-7018 on these BTK inhibitor resistant cell lines were evaluated *in vitro*. ONO-7018 inhibited the proliferation of these cells with comparable IC_{50} values to the parent cell line. Anti-proliferative activities of ONO-7018 alone or in combination with tirabrutinib on DLBCL and mantle cell lymphoma (MCL) cell lines were evaluated *in vitro*. ONO-7018 exhibited synergistic effects with tirabrutinib in both DLBCL and MCL cell lines. In addition, combination treatment of ONO-7018 with tirabrutinib strongly inhibited the expression of interferon regulatory factor 4, which is a downstream signal molecule in BCR signaling pathways, compared to their single treatment. We then evaluated the antitumor effects of ONO-7018 in combination with tirabrutinib in the xenograft model. Combination treatment of ONO-7018 with tirabrutinib showed dramatic antitumor effect with tumor regression. These results suggest that ONO-7018 could demonstrate antitumor effect on patients with B cell malignancies harboring BTK inhibitor acquired resistant mutations and synergistic antitumor effect with BTK inhibitors in clinic.
Conclusion: ONO-7018 would provide novel therapeutic strategies to overcome the BTK inhibitor acquired resistance and enhance the antitumor effect of BTK inhibitors in clinic. Phase 1 study of ONO-7018 (NCT05515406) is currently ongoing.

Disclosures Fujikawa: *Ono Pharmaceutical Co., Ltd.:* Current Employment. **Komichi:** *Ono Pharmaceutical Co., Ltd.:* Current Employment. **Ono:** *Ono Pharmaceutical Co., Ltd.:* Current Employment. **Yasuhiro:** *Ono Pharmaceutical Co., Ltd.:* Current

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