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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Dual Targeting of ROR1 and BTK Augments the Anti-Lymphoma Activity in Mantle Cell Lymphoma

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Background

Mantle cell lymphoma (MCL) is a rare and aggressive subtype of non-Hodgkin lymphoma. Development of BTK inhibitors (BTKis) and *CAR-T cells* revolutionized the treatment paradigm for the patients with *MCL*. However, this aggressive B-cell lymphoma frequently relapses leading to poor survival. Therefore, novel therapies are in urgent need to overcome the therapeutic resistance.

ROR1 is normally expressed during embryonic development but is usually absent or expressed at low levels in adult tissues. However, re-expression of ROR1 was found in various types of cancer, especially in cancer stem/tumor-initiating cells. ROR1 persistently activates oncogenic signaling pathways in the BCR/BTK-independent manner. Accordingly, we hypothesize that dual targeting of ROR1 and BTK may augment the therapeutic efficacy, and thus combination of monoclonal antibody zilovertamab (cirmtuzumab) targeting ROR1, and BTKis will overcome the therapeutic resistance and effectively kill the relapsed/refractory MCL cells.

Methods

A panel of MCL cell lines and primary patient samples were used in this study. ROR1 expression levels in MCL cell lines and primary MCL samples were determined by flow cytometry and western blotting. For establishment of patient-derived organoid (PDO), the patient samples or MCL cell from PDX tumors were resuspended in culture medium containing cytokine cocktails. The cell aggregates within 50% Matrigel were gently transferred into multiple-well plates as needed. The established PDOs were applied for drug sensitivity assays. In addition, a patient-derived xenograft (PDX) mouse model was also used for evaluating single agent and combination efficacy of BTKis and zilovertamab (Zilo). Cell viability assays were performed using CellTiter routine or 3D cell viability assay kit (Promega).

Results

We hypothesize dual targeting of both BTK and ROR1 signaling pathways will augment cytotoxic effect on the MCL cells including major proliferative and minor ROR1 $^{+}$CD117 $^{+}$ population, whose survival is not dependent on BTK, in tumor mass. To test the hypothesis, we first analyzed the ROR1 expression in MCL cell lines, primary and PDX MCL cells using flow cytometry and immunoblotting analysis. Our results indicated that in comparison with normal PBMC, ROR1 expression were detected in all examined MCL cell lines including JeKo-1, Mino, Z-138, Granta519, JVM2, SP49, Maver-1 and Rec-1. To test whether p53 status relates to sensitivity of MCL cells to BTKi and Zilo, we examined cytotoxic effect of combination of BTKi and Zilo on MCL cell lines (1) harboring mutated *TP53* (T *P53* ^{Mut}): JeKo-1, JeKo-R, Mino, and Mino-R and (2) harboring wild-type *TP53* (*TP53* ^{WT}): Z-138 and Maver-1, and PDO as well as PDX tumors derived from biopsies bearing either *TP53* ^{WT} or *TP53* ^{Mut}. Treatment with single BTKi at 2-10 μM (Ibrutinib, zanubrutinib, acalabrutinib or pirtobrutinib) significantly induced cytotoxicity in the *TP53* ^{Mut}MCL cells, and the cell death was remarkedly increased when BTKi was combined with Zilo at 25-50 μg/ml. In contrast, in MCL cell lines with *TP53* ^{WT}, BTKi-Zilo combination did not significantly result in increase of cytotoxic effect compared to single agents. These results suggest that the genetic status of *TP53* is important for the augmented cytotoxic effect of BTKi-Zilo combination on MCL cells. To further evaluate potentiated effect of BTKi-Zilo combination, we performed *ex vivo* viability assay using PDO platform. Typically, PDOs were treated with either single agent or combined regimens. Consistent with the data in MCL cell lines, BTKi-Zilo combination resulted in potentiated cytotoxic activity in the PDO models derived from naïve

and relapsed patients harboring *TP53* ^{Mut}. Furthermore, BTKi-Zilo combination displayed synergistic effect in a PDX model derived from a patient harboring *TP53* ^{Mut}.

Conclusion

Dual targeting of BTK and ROR1 signaling pathways augmented efficacy selectively in preclinical MCL models with *TP53* ^{Mut}. These data provide insights to develop tailored therapeutics to improve patient outcome for patients with TP53 mutation.

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