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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Novel CD20 Mutations As a Mechanism of Resistance to CD20-CD3 Targeted Therapies in Non-Hodgkin's Lymphoma

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Non-Hodgkin's Lymphoma (NHL) is one of the most common blood cancer types in the world. CD20 is a B cell restricted lineage marker that is a well-established target for the treatment of NHL and anti-CD20 agents are included as in standard of care regimens. Given the success of these targeted therapies, strategies to further enhance therapeutic activity are being explored, including the CD20-CD3 bispecific molecule, mosunetuzumab, which is approved for treatment of relapsed and refractory Follicular Lymphoma in adults who have received >2 lines of treatment. Mosunetuzumab acts by redirecting CD3+ T cells to engage and eliminate CD20-expressing B cells. As with other targeted therapies, mutations and loss of target expression have been described following mosunetuzumab therapy [1]. Here we describe the impact of novel mutations identified in patient biopsy samples from the mosunetuzumab monotherapy trial, GO29781 that were located within the extracellular and transmembrane domains of CD20 on protein expression, localization and T-cell mediated killing.

NHL cell lines, SU-DHL-16 and MAVER-1, were engineered using CRISPR-Cas9 system to create CD20 knock out (KO) cell lines. Wild-type (WT) or mutant CD20 were exogenously re-expressed in the CD20 KO cell lines and in the CD20 negative acute lymphoblastic leukemia cell line, REH, to create isogenic cell lines for further analysis. Complete CD20 loss was observed in cells expressing CD20 P160fs (frameshift) and Q187* (truncating) mutations. CD20 missense mutations located in the extracellular domain (C167G, K175E) expressed CD20 protein levels comparable to CD20 WT cells and membrane localization was confirmed by flow cytometry and immunofluorescence microscopy. To test the functional effect of CD20 mutations, *in vitro* co-culture cell killing assays were carried out using healthy donor CD8+ T cells, isogenic cell lines (3:1 effector to tumor cell ratio) and a proof-of-concept CD20-CD3 bispecific antibody. Differences in T-cell mediated killing were observed across mutation types. CD20 WT cell lines demonstrated 95-99% cell killing following 48 hour treatment while the CD20 KO, frameshift and truncating mutations all showed negligible T-cell mediated killing (~10-17% cell death). Cells expressing the missense mutations in the extracellular domain showed resistance to T-cell mediated killing (~30-45% cell death) compared to CD20 WT cells. T-cell activation was assessed by CD69 (early) and CD25 (late) surface markers. In all co-cultures, the level of CD69+ T cells was equivalent, while the CD69+CD25+ CD8 T cells were only significantly increased in the CD20 WT co-cultures, consistent with the high level of T-cell mediating killing.

We have confirmed that these mutations can play a role in the development of resistance through both loss of target protein expression and interference within the anti-CD20 binding site. These studies help to characterize mechanisms of resistance to mosunetuzumab that could be extended to other anti-CD20 targeted agents.

[1] Schuster et al. *Journal of Clinical Oncology* 2022, 40 (16), 7526.

Disclosures Maximov: *Genentech:* Current Employment; *F Hoffmann-La Roche Ltd:* Current equity holder in publicly-traded company. **Bolen:** *Genentech, Inc.:* Current Employment; *F Hoffmann-La Roche Ltd:* Current equity holder in publicly-traded company. **Polson:** *F Hoffmann-La Roche Ltd:* Current equity holder in publicly-traded company; *Genentech:* Current Employment. **Penuel:** *Genentech, Inc. / F. Hoffman-La Roche Ltd:* Current Employment, Current holder of *stock options* in a privately-held company. **Lasater:** *Genentech:* Current Employment; *F Hoffmann-La Roche Ltd:* Current equity holder in publicly-traded company.

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