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641. CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Combined Therapy of Zanubrutinib and Zilovertamab in the Inhibition of Invasive Capability of Chronic Lymphocytic Leukemia CellsMd Kamrul Hasan, PhD¹, George F. Widhopf II, PhD², Thomas J. Kipps, MD³¹Center for Novel Therapeutics, Moores Cancer Center, University of California San Diego, La Jolla, CA²Center for Novel Therapeutics, School of Medicine, La Jolla, CA³Center for Novel Therapeutics, University of California, San Diego Moores Cancer Center, La Jolla, CA

Signaling pathways such as CXCR4/CXCL12 can regulate migration and trafficking of chronic lymphocytic leukemia (CLL) cells from the blood to lymphoid tissues, where they receive growth/survival signals from accessory cells within the lymphoid-tissue microenvironment. This requires extravasation of CLL cells that is contingent upon the leukemia-cells' ability to degrade the extracellular matrix via matrix metalloproteinases (MMP), such as MMP9, which is a type IV collagenase. We find that CLL cells that express high-levels of the receptor tyrosine kinase-like orphan receptor 1 (ROR1^{Hi}) express high-levels of MMP9 relative to that of CLL cells with low-level expression of ROR1 (ROR1^{Low}). However, short-term culture of ROR1^{Hi} CLL cells in serum-free medium causes attenuation in MMP9 expression, which can be counteracted by addition of exogenous Wnt5a, which is a non-canonical Wnt factor and ligand for ROR1 that is expressed at significantly higher levels in the plasma of patients with CLL than that of healthy adults. We found that stimulation of serum-starved CLL cells with Wnt5a enhance expression and release of MMP9 into the culture media. Such effects of Wnt5a could be inhibited by zilovertamab (also known as UC-961, or previously known as cirmtuzumab; 20 µg/ml), a humanized mAb highly-specific for ROR1 that can block CLL-cell ROR1-signaling (Choi et al., *Cell Stem Cell*, **22**:951, 2018), indicating that MMP9 may be regulated in part by Wnt5a-induced ROR1-signaling. To test this hypothesis, we used the CLL-cell derived cell line, MEC1, which expresses endogenous Wnt5a, but lacks expression of ROR1, and MEC1-Wnt5a^{-/-} cells in which we deleted functional genes encoding Wnt5a via CRISPR-Cas9. Stable transfection of MEC1 or MEC1-Wnt5a^{-/-} cells with an expression vector encoding ROR1 generated MEC1-ROR1 and MEC1-Wnt5a^{-/-}-ROR1 that each expressed high-levels of surface ROR1, as assessed via flow cytometry. We found that MEC1-ROR1 cells had significantly higher expression levels of MMP9 and greater capacity to invade Boyden -Chamber Matrigel than MEC1 cells or MEC1-Wnt5a^{-/-} or MEC1-Wnt5a^{-/-}-ROR1 cells. Noting the gene encoding MMP9 possesses a promoter that may be induced by activation of NF-κB, we examined for NF-κB-p65 and found MEC1-ROR1 cells had significantly higher activation of NF-κB-p65 than MEC1 cells or MEC1-Wnt5a^{-/-} or MEC1-Wnt5a^{-/-}-ROR1 cells. Similarly, serum-starved ROR1⁺ CLL cells also expressed activated NF-κB-p65 when treated with Wnt5a, an effect that also could be inhibited by zilovertamab. Consistent with this model, we found that an inhibitor (CAS 545380-34-5, 20 nM) of NF-κB also could block Wnt5a induced expression of MMP9 in ROR1⁺ CLL cells. We examined whether such effects also could be observed for zanubrutinib, a covalent inhibitor of BTK that recently was approved for treatment of patients with CLL. We found that zanubrutinib (0.5 µM) could not inhibit Wnt5a-induced activation of NF-κB-p65 or expression of MMP9 in ROR1⁺ CLL cells. We also use the Boyden -Chamber Matrigel Invasion Assay to study the response to CXCL12 without or with Wnt5a of primary CLL cells in vitro. We found that CLL could invade the Boyden -Chamber Matrigel in response to CXCL12 and the extent of such invasion was enhanced by Wnt5a. We also found that zanubrutinib (0.5 µM) could inhibit the CXCL12 induced capacity of CLL cells to invade Matrigel, however, this drug was unable to block Wnt5a enhanced invasiveness of CLL cells. Zilovertamab could inhibit Wnt5a enhanced invasiveness of CLL cells. Moreover, the combined treatment of zanubrutinib and zilovertamab had additive activity in inhibiting Matrigel invasiveness by CLL cells, supporting potential evaluation of the combined use of zilovertamab and zanubrutinib in the treatment of patients with CLL.

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