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Permalink

https://escholarship.org/uc/item/8n54x3fj

Journal

Blood, 144(Supplement 1)

ISSN

0006-4971

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Publication Date

2024-11-05

DOI

10.1182/blood-2024-210584

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Blood 144 (2024) 744

The 66th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL - MOLECULAR AND GENETIC

Expression of ROR1 and ROR2 in Hairy Cell Leukemia Cells Enhances Constitutive Activation of ERK1/2 and Cancer Stemness

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Hairy cell leukemia (HCL) is a malignancy of B-cells that co-express CD19, CD20, CD11c, CD25, CD103, CD123, and CD200 (but not CD5, CD10, CD21, or CD23) and that harbor a V600E BRAF mutation, which promotes constitutive activation of ERK1/2. HCL variant (HCL-V) is a rare subtype of HCL that does not express CD25 and typically lacks BRAF V600E. We examined both types of HCL for expression of ROR1 and ROR2, which are developmentally-restricted receptors for Wnt5a. We generated monoclonal antibodies (mAbs) that each are highly specific for human ROR1 (zilovertamab) or ROR2 (6E6) and that respectively can block Wnt5a-induced ROR1- or ROR2-dependent signaling. We examined HCL cells from 17 pts (13 male, 4 female). Fifteen had classic HCL (cHCL) and 2 pts had HCL-V. We found that the HCL cells of all pts expressed ROR1 *and* ROR2; a finding validated by analysis of anti-ROR1 or anti-ROR2 immune precipitates of HCL-cell lysates by iTRAQ mass spectrometry. We assessed the numbers of ROR1 or ROR2 on HCL cells by measuring the molecules of equivalent soluble fluorochrome (MESF) using fluorochrome-labeled microbeads. The numbers of ROR1 (median = 3.7×10^3 molecules per cell, n=17, range $1.4\text{-}4.8 \times 10^3$) or ROR2 (median = 3.1×10^3 molecules per cell, n=17, range $0.6\text{-}11.1 \times 10^3$) on cHCL or HCL-V were less than the median ROR1 levels on chronic lymphocytic leukemia (CLL) cells, for which 5.8×10^3 ROR1 molecules per cell was the threshold that defined high-level expression of ROR1 that was associated with adverse prognosis (*Cancer Stem Cell* 22:951, 2018). Nonetheless, the median combined number of ROR1 and ROR2 molecules per HCL cell among pts with HCL was 6.8×10^3 (n=17, range 3.6-12.5). 13 of the 17 patient samples had combined numbers of ROR1 and ROR2 that exceeded the threshold for defining high-expression ROR1, which associated with a significantly shorter median time from diagnosis until initial therapy and overall survival in pts with CLL (*Blood* 128:2931, 2016). To evaluate for ROR1- and ROR2-signaling in cHCL we performed transcriptome analyses of negatively-selected leukemia cells before and after culture without or with Wn5a, zilovertamab, and/or 6E6. Gene-set enrichment analyses (GSEA) comparing the cHCL cells of two pts before and after 36-hour culture in serum-free media without exogenous Wnt5a demonstrated significant time-dependent attenuation in the expression of stemness genes, which included gene targets of the Yamanaka factors Oct4, Sox2, and c-Myc, along with genes induced by activation of ERK1/2, despite each case harboring the activating BRAF V600E mutation (FDR q value <0.001). Addition of exogenous Wnt5a upon initiation of culture significantly enhanced expression of such genes. However, treatment of HCL cells with either zilovertamab or 6E6 significantly inhibited Wnt5a-induced expression of target genes induced by Oct4, Sox2, and c-Myc, along with genes induced by activation of ERK1/2 (FDR q value <0.001). Co-treatment with zilovertamab and 6E6 also repressed expression of such gene sets (FDR q value <0.001). Our studies demonstrate that ROR1 and ROR2 are expressed in cHCL and HCL-V. In cHCL cells that harbor BRAF V600E, we found that Wnt5a ROR1/2-dependent signaling can enhance ERK1/2 activation significantly above that induced by the BRAF V600E mutation alone. In addition, we found Wnt5a can induce ROR1/2-dependent activation of targets of Oct4, Sox2, and c-Myc and cancer stemness genes signatures found expressed by embryonic stem cells. We speculate that strategies that target ROR1 and/or ROR2, or that block ROR1/2-dependent signaling, may reverse HCL cancer stemness *in vivo* and mitigate the risk of tumor dormancy and disease-relapse that may be seen years after apparent successful therapy.

Disclosures Kipps: *Abbvie/Janssen/Pharmacyclics/Genentech:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Oncternal Therapeutics:* Current equity holder in private company; *Lymphoma and Leukemia Society:* Research Funding.

https://doi.org/10.1182/blood-2024-210584

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