



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604. MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Analysis of Tagraxofusp Activity in AML-Pdc As a Single Agent and in Combination with BCL2 Inhibitors

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Acute myeloid leukemia with increased plasmacytoid dendritic cells (AML-PDC) is a new leukemia recently clarified in the classification of hematological diseases (WHO 2022). AML-PDC presents with leukemic CD34+ blasts associated with an excess of pDCs, both of which are characterized by the same mutational profile. Xiao *et al.* (Blood 2021) also demonstrated that certain leukemic blasts upregulate a pDC transcriptional program, enabling these cells to differentiate into pDCs. Blasts and pDCs express the alpha-subunit of the interleukin-3 receptor, known as CD123, at different levels. Tagraxofusp, a first-in-class CD123-directed therapy, is a recombinant fusion protein consisting of human interleukin-3 conjugated to a truncated diphtheria toxin payload. Tagraxofusp is approved for the treatment of BPDCN (US/EU), a disease where pDCs are critical factors in disease development.

Given the elevated number of pDCs expressing high levels of CD123 and the lack of therapies available to treat AML-PDC, we evaluated tagraxofusp activity on AML-PDC models.

We first evaluated tagraxofusp on primary cells from AML-PDC patients and characterized cytotoxicity by flow cytometry (FC) on both pDCs and blasts. We observed a decrease of pDC viability both in a time and concentration dependent manner, with viability reaching $32.8\% \pm 33.4\%$ after 48 h of treatment at $2.33 \mu\text{g/mL}$.

We next evaluated sensitivity at different levels of IL-3R expression. We observed a significantly lower level of CD123 expression from AML-PDC patient blasts by FC than pDCs. This was similarly observed for CD131 expression, with a RFI of 2.2 ± 1.2 on blasts compared to 7.3 ± 4.7 on pDCs. The CD131 result was confirmed at the genomic level with scRNAseq analysis on two patients with a significantly lower expression of *CSF2RB* (coding for CD131) in the blasts compared to pDCs.

Dimerization of CD123 and CD131 upon IL-3 binding activates downstream signaling pathways (such as the JAK/STAT pathway) and internalization of tagraxofusp. Given this and our findings related to CD131, we next evaluated phosphorylation of STAT5 as an IL-3 binding surrogate to IL-3R. We observed significantly lower STAT5 phosphorylation on blasts compared to the pDCs after 30 minutes of stimulation with tagraxofusp. Overall, blasts present a lower CD123 and CD131 expression compared to pDCs as well as a lower STAT5 phosphorylation after stimulation by tagraxofusp. Of note, pDCs from two patients presenting a low CD131 RFI also exhibited a lower sensitivity to tagraxofusp compared to pDCs from other patients. The low expression of these IL-3R components could explain the low sensitivity of blasts to tagraxofusp compared to pDCs in some patient samples.

We further combined tagraxofusp with other available therapies used in AML (ie, BCL-2 inhibitors, proteasome inhibitors, or hypomethylating agents) to determine the optimum combination needed to eliminate both pDCs and blasts. A significant reduction in blast viability was observed when tagraxofusp was combined with venetoclax. Combination of tagraxofusp with venetoclax showed an increased reduction in blast viability compared to tagraxofusp alone, with a viability of $52.5\% \pm 13.1\%$ and $82.6\% \pm 18.4\%$, respectively. Also, scRNAseq analysis showed a higher expression level of BCL-2, the target of venetoclax, in blasts compared to the pDCs; this could explain the differential venetoclax sensitivity between blasts and pDCs in AML-PDC.

The treatment of AML-PDC is challenging, and the presence of CD123 over expressing leukemic blasts associated with excess pDCs, makes tagraxofusp an attractive treatment option in this setting. Tagraxofusp was able to effectively eliminate pDCs and blasts to a lesser extent as a single agent. High expression of BCL-2 in blasts supports the consideration of tagraxofusp

in combination with venetoclax as an effective combination therapy to eradicate both blasts and pDCs in AML-PDC patient samples.

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