blood neoplasia

TO THE EDITOR:

New leads to enhance tagraxofusp efficacy in pDC-AML

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Acute myeloid leukemia (AML), associated with an excess of plasmacytoid dendritic cells (pDC-AML), is a newly described entity of leukemia[1,2] that recently appeared in the World Health Organization hematolymphoid tumors 2022 classification as a separate entity within the histiocyte or dendritic cell neoplasia. pDC-AML have a clear male predominance (4:1 ratio), with a median age of 69 years at diagnosis, and presence of skin lesions (14%-55% of cases).[1,3] The pDC expansion in this pathology correlates with an unfavorable prognosis (median survival, 18.8 months vs 36.7 months for patients with AML without excess of pDC). pDC-AML have a distinct immune signature compared to blastic plasmacytoid dendritic cell neoplasm (BPDCN) with $CD56^{low}$ and $TdT^{high}/CD34^{high}$ expression.[1,2] CD123 was shown to be more strongly expressed on the pDC from pDC-AML than on blasts.[1,2]

No standard of care exists for pDC-AML and effective therapies are lacking. However, biologic similarities to AML and BPDCN suggest that CD123-targeted therapies could be evaluated for pDC-AML, such as tagraxofusp, a first-in-class CD123-directed therapy. Tagraxofusp was approved by the US Food and Drug Administration in 2018 for untreated or relapse/refractory BPDCN and approved in 2020 in Europe for first-line treatment of BPDCN under the name of ELZONRIS (tagraxofusp-erzs).[4]

The present study evaluates the cytotoxicity of tagraxofusp alone or in combination with different drugs to provide further evidence in support of the use of tagraxofusp in patients with pDC-AML.

Primary cells from 2 patients with pDC-AML were sequenced by single-cell RNA sequencing before and after 18 hours of treatment with tagraxofusp. A significant change in the population proportion was observed with a decrease of the pDC cluster for the tagraxofusp-treated cells (Figure 1A-B).[1] Hence, we evaluated tagraxofusp efficacy on different leukemic cell lines (supplemental Figure 1A), and then on primary cells from 7 patients with pDC-AML (supplemental Figure 1B-C). Flow cytometry analysis demonstrates that after tagraxofusp treatment (100 μg/mL) for 18 hours and 48 hours, pDC exhibited a significantly lower viability (57.2% ± 25.5% and 26.1% ± 30.2% respectively [n = 7]) as compared with blast (99.5% ± 1.2% and 71.2% ± 28.5%, respectively; Figure 1C-D). Elimination of pDC is probably favorable for pDC-AML treatment because they are known to induce tolerogenic orientation of the bone marrow microenvironment.[1,2,5,6] Blast component viability decreases significantly at the higher doses of tagraxofusp (21 and 100 μg/mL) and reached 83.3 ± 17.6% and 71.2% ± 28.5% respectively ($P = .0280$ and $P = .0204$, respectively) after 48 hours (Figure 1D).

Next, we evaluated if the differences in sensitivity to tagraxofusp between blasts and pDC could be explained by a difference in interleukin-3 receptor (IL-3R) expression. The CD123/CD131 expression was first measured on different leukemic cell lines (supplemental Figure 1E-F) and on cells from patients with pDC-AML (supplemental Figure 1G-H). Blasts cells from patients with pDC-AML exhibit a significantly lower expression of both CD123/CD131 as compared to pDC both at the genomic and

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Original data are available on request from the corresponding author, Fanny Angelot Delettre (fanny.delettre@efs.sante.fr).

The full-text version of this article contains a data supplement.

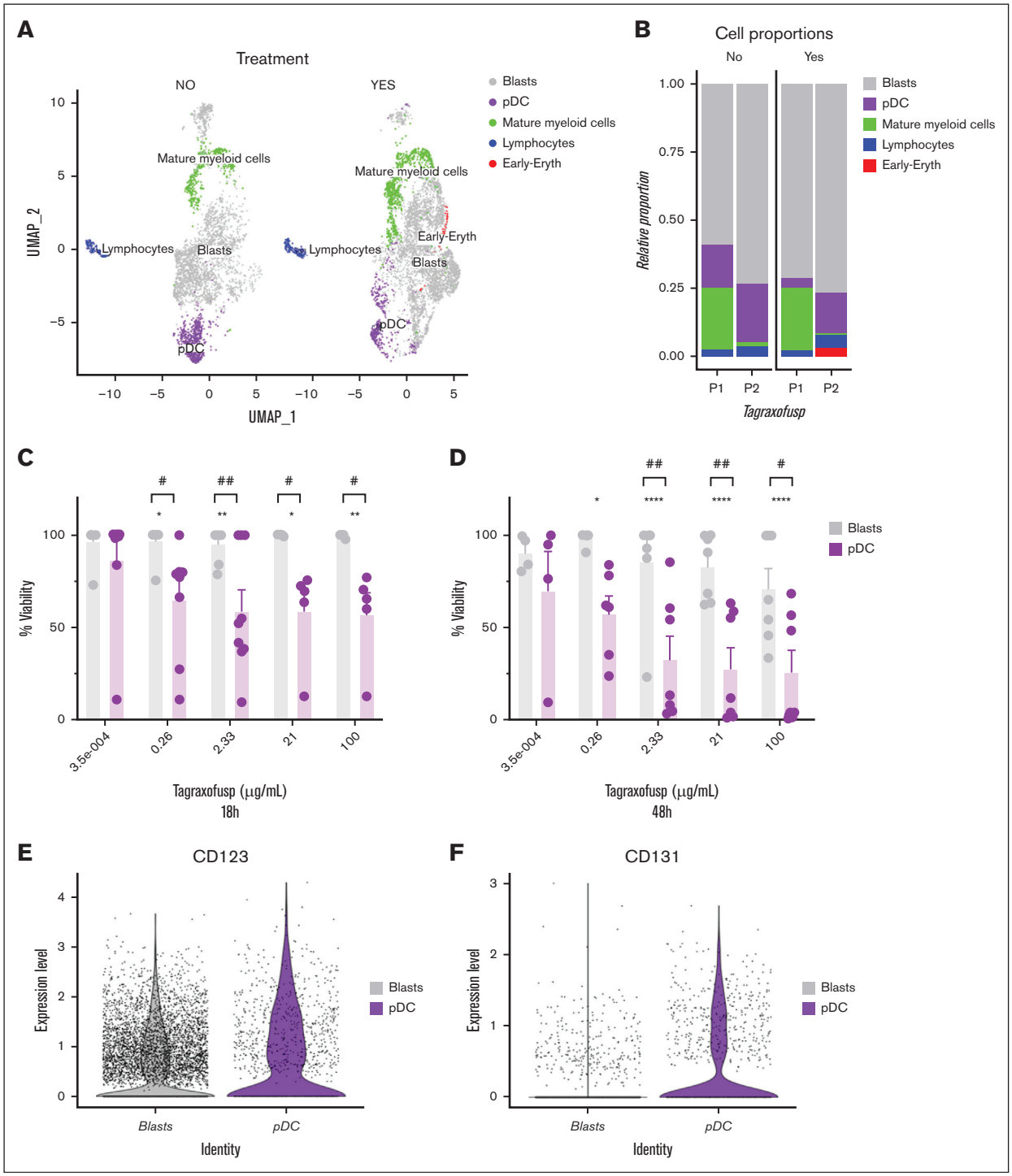

Figure 1. In vitro efficacy of tagraxofusp on primary pDC-AML cells. (A) Peripheral blood or bone marrow cells were obtained from 10 patients with pDC-AML from the national network of BPDCN (The ROMI network), which collects data and cells from cases diagnosed in France since 2004 (authorization number #DC-2008-713). Primary pDC-AML cells were incubated at 3×10^5 cells per mL in media with or without tagraxofusp for 18 hours and analyzed by single-cell RNA sequencing (scRNA-seq; n = 2). Single-cell libraries were prepared using the 10x Genomics Chromium 3' scRNA-seq according to manufacturer's protocol. On average, 5000 cells were loaded on each channel, resulting in recovery of an average of 1200 cells per sample. (B) Representative histograms of the proportion of primary cells before and after 18 hours of tagraxofusp treatment, analyzed by scRNA-seq. (C-D) Viability of blasts and pDC was evaluated by flow cytometry (FC) using Trucount tubes (BD Biosciences) to obtain absolute number of cells in each experimental condition. Cells were labeled with different monoclonal antibodies, anti-human CD45 APC (clone 2D1, BD Biosciences), anti-human CD123 PeCy7 (clone 6H6, Sony Biotechnology), anti-human CD34 PE (clone 581, Beckman Coulter, Brea, CA) and 7-AAD (Sony Biotechnology) after 18 hours (C) (n = 7) and 48 hours (D) (n = 7) of tagraxofusp treatment at different concentrations (3.5×10^{-4} to 100 µg/mL) (* or $^{\#}P > .01$; ** or $^{\#\#}P < .01$; **** or $P < .0001$). * Indicates comparison between blasts and pDC and # indicates comparison between pDC and untreated cells. (E-F) Violin plot representing CD123 and CD131 expression ($P < .0001$; LFC, −0.738)

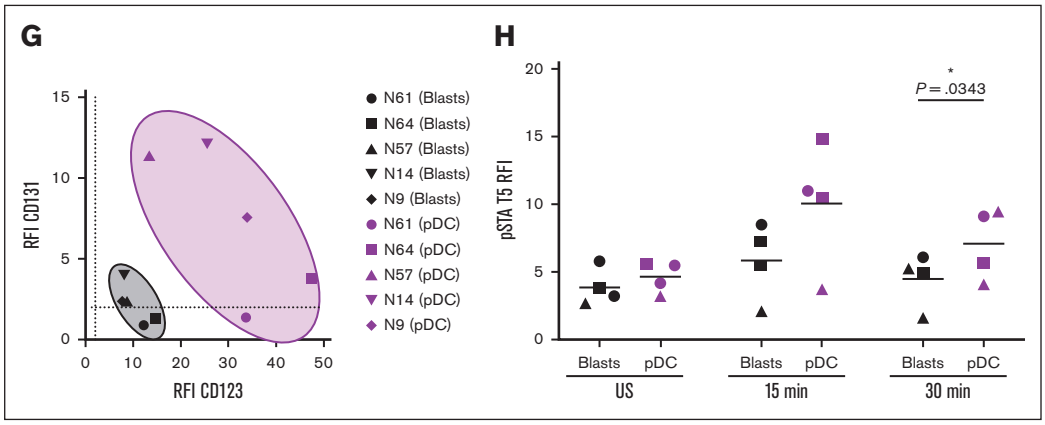

Figure 1 (continued) on pDC and blasts from primary pDC-AML cells (n = 2) (G) Dual relative fluorescence intensity (RFI) expression of CD123 and CD131 on blasts ($CD34^+$ $CD123^{low}$) and pDC ($CD34^+$ $CD123^{high}$) components measured by FC with the RFI, on primary cells from patients with pDC-AML (n = 5; ***$P < .001$). (H) Primary pDC-AML cells were treated with tagraxofusp for 15 and 30 minutes, then fixed with BD Cytofix Buffer. Then cells were permeabilized with BD Phosflow Perm Buffer, labeled with pSTAT5 Alexa Fluor 647 antibody (BD Phosflow clone 47/Stat5(pY694)) or its isotype (BD immunoglobulin G1 kappa Alexa Fluor 647, clone MOPC-21) and washed with BD Pharmingen Stain Buffer according to supplier recommendations. STAT5 phosphorylation as compared with unstimulated cells (USs). UMAP, Uniform Manifold Approximation and Projection; LFC, Log Fold Change

protein level in primary pDC-AML cells (Figure 1E-F). Dual expression analysis of both CD123/CD131 revealed that blasts and pDC cluster separately and far apart with blasts exhibiting a lower expression of both markers compared to pDC (Figure 1G).

Activation of JAK/STAT pathway is one of the downstream signaling pathways of IL-3R and requires the entire CD123/CD131 complex[7] to be triggered. We observed a lower phosphorylation of STAT5 on blasts as compared to pDC cells after treatment with tagraxofusp, with a significant difference observed after 30 minutes (4.5 ± 2 for blasts and 7.1 ± 3 for pDC, respectively; Figure 1H). Overall, blasts exhibit a lower expression of both CD123/CD131 as compared to pDC as well as decreased STAT5 phosphorylation after tagraxofusp treatment. As the blast components have a lower sensitivity to tagraxofusp compared to pDC, we hypothesize that low expression of CD123/CD131 subunit of the IL-3R may explain the lower sensitivity of blasts to single agent tagraxofusp as compared with pDC.

We further explore the efficacy of tagraxofusp in combination with other molecules (ie, venetoclax, 5-azacytidine, and carfilzomib), to investigate potential combination that could more effectively target blast component. Carfilzomib has been shown to be beneficial in BPDCN, so it could be effective for pDC-AML.[8,9] Combinations were tested on BPDCN and AML cell lines (supplemental Figure 2) and further on pDC-AML primary cells. For pDC-AML primary cells, $CD45^+$ cells viability decreased when tagraxofusp was combined with venetoclax (Figure 2A). The viability of both pDC and blasts cells decreased significantly when tagraxofusp was combined with venetoclax as compared to tagraxofusp alone (from 52.1% ± 30% to 19.2% ± 18% for pDC and from 82.6% ± 18% to 52.5% ± 13% for blasts; Figure 2B-C). JAK/STAT activation through IL-3 treatment in $CD34^+$ cells increase BCL-2 and decrease BAX expression, which may explain the lake of synergistic effect when tagraxofusp is combined with venetoclax, compared with venetoclax alone.[10,11] In addition, single-cell RNA sequencing analysis revealed higher *BCL-2* expression in blasts as compared with pDC (Figure 2D). These data were further confirmed by flow cytometry on primary cells. In all 10 patients, pDC exhibit a significantly lower BCL-2 relative fluorescence intensity than blasts (6.07 ± 3 and 9.75 ± 4, respectively; Figure 2E). This difference of expression in BCL-2 between pDC and blast cells may explain the higher sensitivity of blasts to venetoclax as compared to pDC in pDC-AML.

Transcriptomic analysis revealed 2 genes commonly upregulated between pDC and blasts, *AHRR* and *PLCG2* (Figure 2F). *AHRR* was shown to be a putative suppressor gene in several cancers with reduced tumor growth observed in vitro for breast and lung when silenced.[12] Upregulation of *AHRR* in blasts and pDC could thus enhance cancer suppressive action triggered by tagraxofusp treatment. To date, there are no described *AHRR* inhibitors but its tumor suppressive activity makes it an attractive biological target for the development of new therapies. In contrast, *PLCG2* codes for transmembrane signaling enzyme which is known to be involved in tumorogenesis in some hematologic malignancies. However, Bruton tyrosine kinase (BTK) inhibitors, such as ibrutinib, can block *PLCG2* signaling through the inhibition of BTK. Upregulation of *PLCG2* in both blasts and pDCs could thus render them sensitive to BTK inhibitors, adding a new therapeutic option to treat patients with pDC-AML.[13] We also used pathway analysis to reveal potential molecular drivers of interest for combining tagraxofusp (Figure 2G). A significant enrichment of interferon (IFN) and MAPK signaling was observed in the blast component (P adjusted $< .05$). Higher level of IFN-γ signaling scores in cells from patients with AML compared to healthy stem cells was described and correlates with venetoclax resistance in cells from patients with primary AML.[14] IFN pathway is known to be involved in JAK-STAT pathways, and thus the use of ruxolitinib (JAK 1 and JAK2 inhibitor) could be a possible combination strategy that is already tested on myeloproliferative neoplasms.[15] Overexpression of MAPK pathway has also been shown to play an important role in the pathogenesis and progression of breast and other cancers.[16] One treatment (CI-1040) has already been tested in patients with advanced malignant tumors, is well tolerated, and induces antitumor activity.[16]

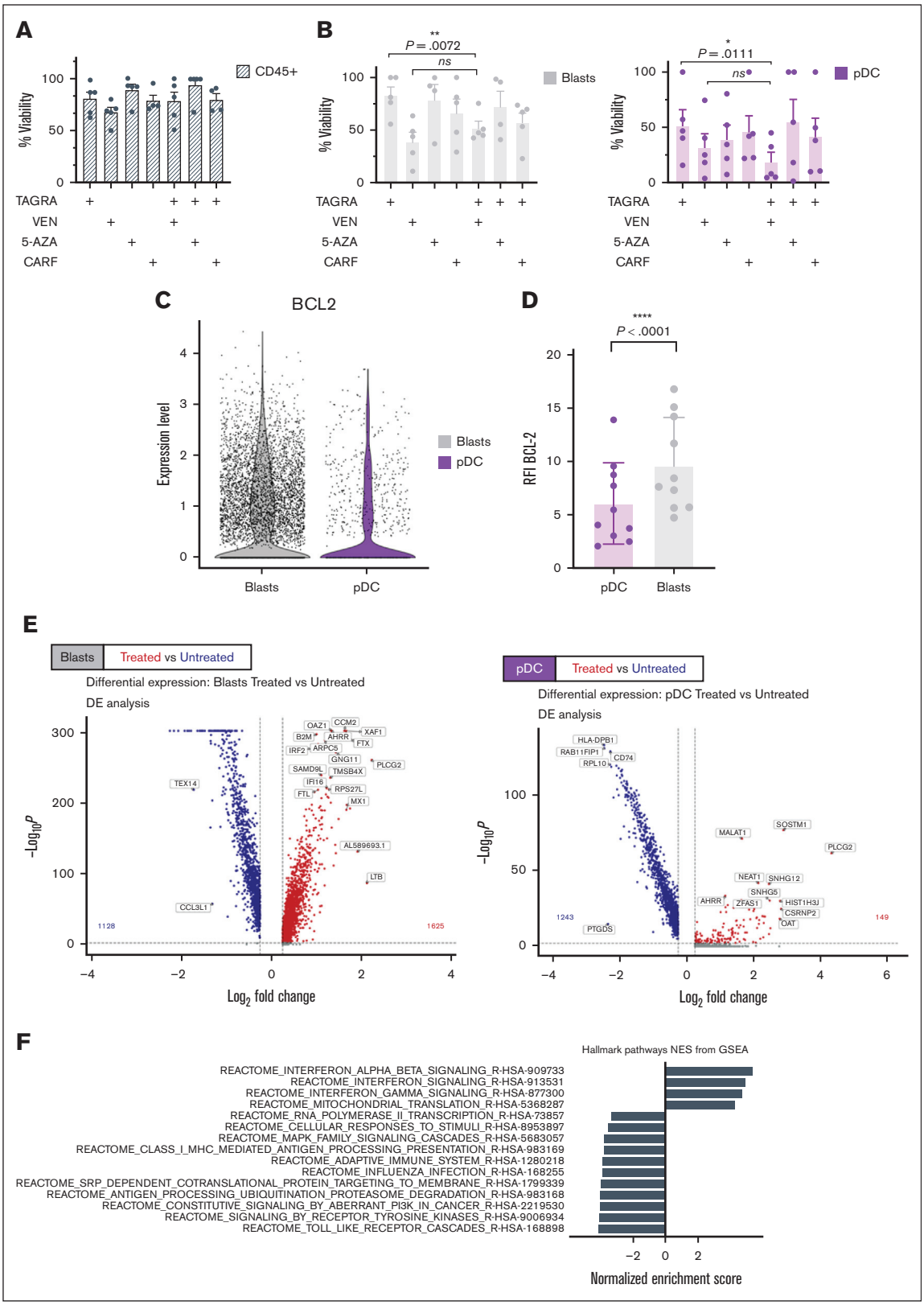

Figure 2. In vitro efficacy of tagraxofusp (TAGRA) as a single agent or in combination with venetoclax (VEN), 5-azacytidine (5-AZA), or carfilzomib (CARF) in primary pDC-AML cells. (A) Primary cells from pDC-AML 5 patients were treated with TAGRA (2.33 μg/mL) as a single agent or in combination with VEN (0.003 μM), 5-AZA (4 μM), or CARF (55.6 nM) for 18 hours. (B) Percentage viability of pDC (left) or blasts (right) component of primary pDC-AML cells treated by TAGRA (2.33 μg/mL) as a single agent or in combination during the 18 hours with VEN (0.003 μM), 5-AZA (4 μM), or CARF (55.6 nM; n = 5). (C) BCL-2 expression level on blasts and pDC from one

Figure 2 (continued) primary pDC-AML cells ($P < .0001$; LFC, 0.367) analyzed by scRNA-seq. (D) Blasts and pDC from 10 primary pDC-AML cells were labeled with a PE anti–BCL-2 antibody (clone 100, Sony Biotechnology, San José, CA) or Alexa Fluor 647 anti–BCL-2 antibody (clone Bcl-2/100; BD Biosciences, Le Pont de Claix, France) using an intracellular staining kit (InstraStain kit, Agilent Dako, Santa Clara, CA) according to the manufacturer's instructions, and BCL-2 expression was assessed by FC ($P < .0001$). (E) Volcano plot of differential gene expression comparing untreated and treated cells from 2 primary pDC-AML cells on blasts (left) and pDC (right). The x-axis represents the fold change as transformed by $\log_2$ (untreated/treated); thus, red dots mark upregulated proteins, blue dots mark downregulated proteins at $P < .001$ and a fold change cutoff 0.25. (F) Ingenuity pathway analysis identification of dysregulated pathways based on DEGs of blasts from primary pDC-AML cells treated vs untreated (P adjusted $< .05$). All statistical analyses in this study were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA). Comparisons between 2 groups were assessed by Student's t tests. A $P < .05$ was considered statistically significant. GSEA, Gene Set Enrichment Analysis; NES, Normalized Enrichment Score; ns, nonsignificant; DEG, Differential Gene Expression; LFC, Log Flow Change.

With its poor prognosis, the treatment of pDC-AML is challenging. The presence of leukemic blasts associated with excess pDC, which express high levels of CD123, makes tagraxofusp a potentially attractive therapeutic. As a single agent, tagraxofusp was able to effectively eliminate pDCs, whereas blasts were less sensitive, probably in relation to a lower expression of CD123 and CD131 on blast cells. But the combination of tagraxofusp and venetoclax enhances tagraxofusp efficacy on both populations, indicating an effective combination strategy to eradicate both blasts and pDCs in patients with PDC-AML. Overall, this study provides further elucidation of pDC-AML pathophysiology and supports the use of tagraxofusp in combination with other drugs for patients with pDC-AML.

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Contribution: M.B. and I.B. performed the experiments; M.B., I.B., and M.P. analyzed data; M.P. and S.B. provided help with flow cytometry analysis; M.B. and M.P. performed statistical analysis; X.R. analyzed the single-cell experiment data; X.R., S.B., M.F., F.R., F.G.-O., and F.A.D. provided guidance and expertise in their respective areas of study; M.B., M.P., and F.A.D. wrote the manuscript; C.B., M.F., F.G.-O., and O.A. commented on the manuscript; F.G.-O. and F.A.D supervised the research; and all authors provided input and edited and approved the final version of the manuscript.

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