LEUKEMIC STEM CELL DIFFERENTIATION VISIBLE AT SINGLE-CELL RESOLUTION IN ACUTE MYELOID LEUKEMIA PATIENTS TREATED WITH FHD-286, AN INHIBITOR OF BRG1/BRM (SMARCA4/2)

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Abstract

BAF chromatin remodeling complexes are critical to the regulation of cellular differentiation and are implicated in cancer as well as other diseases. FHD-286 is a first-in-class dual inhibitor of the BAF catalytic subunits BRG1 and BRM (SMARCA4/2) that is being developed for the treatment of advanced hematologic malignancies. We previously reported that exposure of acute myeloid leukemia (AML) cells to pharmacologically relevant concentrations of FHD-286 led to downregulation of stem cell markers and upregulation of the myeloid maturation marker CD11b (ITGAM). Morphologic features of differentiation were also observed, suggesting that BAF functions to maintain AML cells in an undifferentiated state, and that FHD-286 may inhibit AML cell growth by reducing their capacity for stem cell renewal. Using single-cell transcriptomic data, we further assessed the impact of FHD-286 on differentiation markers of tumor cells from clinical AML patients.

Single-cell RNA-sequencing (scRNA-seq) data from AML patients validates the preclinical observation that FHD-286 treatment induces changes in LSCs resembling differentiation towards a terminal myeloid cell state. This dataset further indicates that most patients undergo some degree of tumor cell differentiation after treatment, suggesting broad on-target activity of FHD-286.

Background

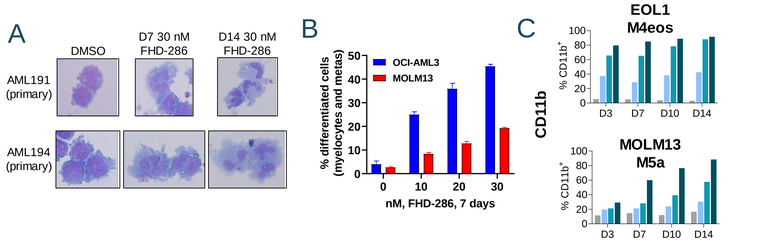

Figure 1. In patient-derived AML samples and cell lines, exposure to low concentrations of FHD-286 elicited differentiation-like responses. **A)** Representative light microscopy images of patient-derived AML cells treated with DMSO or 30 nM FHD-286 for 7 or 14 days and prepared with Wright's stain. **B)** AML cell lines OCI-AML3 and MOLM13 were exposed to DMSO or the indicated concentrations of FHD-286 for 7 days, and the percentages of morphologically differentiated cells were quantified by microscopy. **C)** Cells were exposed to either 0.1% DMSO or the indicated concentrations of FHD-286 for 14 days. Cells were split on days 3, 7 and 10 with drug replenishment. Cells were analyzed by flow cytometry on days 3, 7, 10 and 14 for myeloid maturation marker CD11b.

Methods

Dose Cohort	Patient ID	N Cells at Screening	N Cells on Treatment	Sampled Treatment Day
2.5mg	p1	1658	6828	23
5mg	p2	141	666	84
	p3	1086	410	27
	p4	696	861	28
	p5	1855	745	26
	p6	1232	1696	28
	p7	362	659	27
7.5mg	p8	1007	168	25
	p9	1239	2074	27
	p10	2514	1950	43
	p11	6230	1275	34
	p12	5369	1828	26
10mg	p13	2330	784	26

Figure 2. Bone marrow biopsies were sampled from 13 AML patients at screening (pre-treatment) and on treatment with FHD-286 for > 20 days in a phase I clinical trial. Single-cell transcriptomic profiling of bone marrow biopsies was performed using the 10x Genomics Chromium platform, following the manufacturer's guidelines for tissue preparation, library construction, and sequencing.

References and Acknowledgments

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Distinct CD11b+ Cell Population Emerges with FHD-286 Treatment in AML Patients

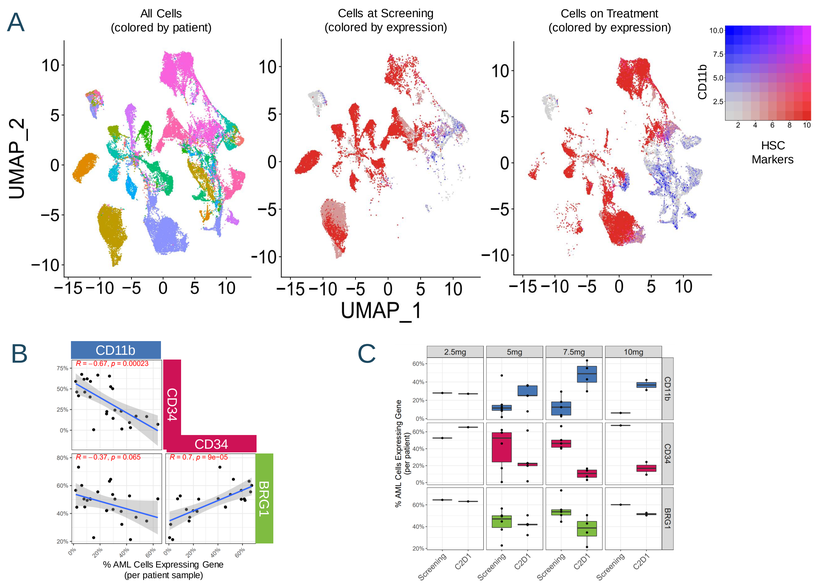

Figure 3. A) For 13 AML patients, leukemic cells were characterized by expression of hematopoietic stem cell (HSC) genes (PROM1, CD34, KIT/CD117, CD38) or mature myeloid gene CD11b/ITGAM. Cells clustered by cell type, patient, and treatment status. CD11b+ cells were most prevalent on treatment. **B)** Percentage of cells expressing CD11b was anti-correlated with CD34 expression in patient scRNA-seq samples, supporting their role in distinguishing cell subtypes. CD11b expression was also anti-correlated with FHD-286 target gene BRG1. **C)** BRG1 and CD34 showed a dose-dependent decrease in cell expression on treatment at Day 1 of Cycle 2, while CD11b showed a dose-dependent increase in expression.

Comparison of Leukemic Stem Cells (LSCs) to CD11b+ Cells Yields Transcriptional Signatures of Stemness and Differentiation

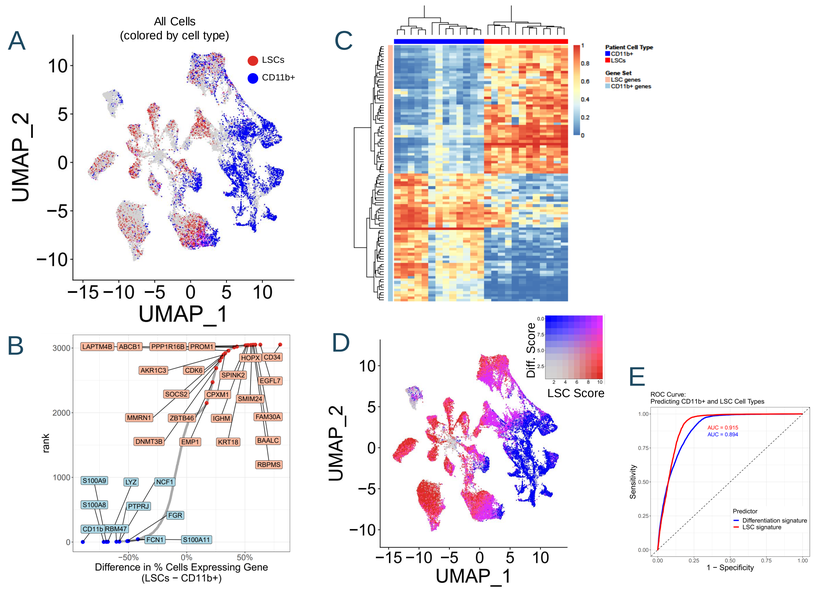

Figure 4. A) LSC markers (CD34+, CD123/IL3RA+, CD38-) identified putative LSCs, then compared to CD11b+ cells for transcriptional differences. **B)** 3,055 genes showed significant expression differences between LSC and CD11b+ cells. Genes with the greatest difference were enriched for known LSC and mature myeloid markers, respectively. **C)** Hierarchical clustering of expression for the top 50 LSC and CD11b-associated genes separated LSC and CD11b+ cell types across patients. **D)** LSC and differentiation signature scores were calculated as the log2 average expression of the top 50 differential genes for LSCs and CD11b+ cells. **E)** Signature scores were predictive of each cell type at single-cell level.

Stemness and Differentiation Signatures Indicate Dose-dependent Differentiation Response to FHD-286 Across Patient Cells

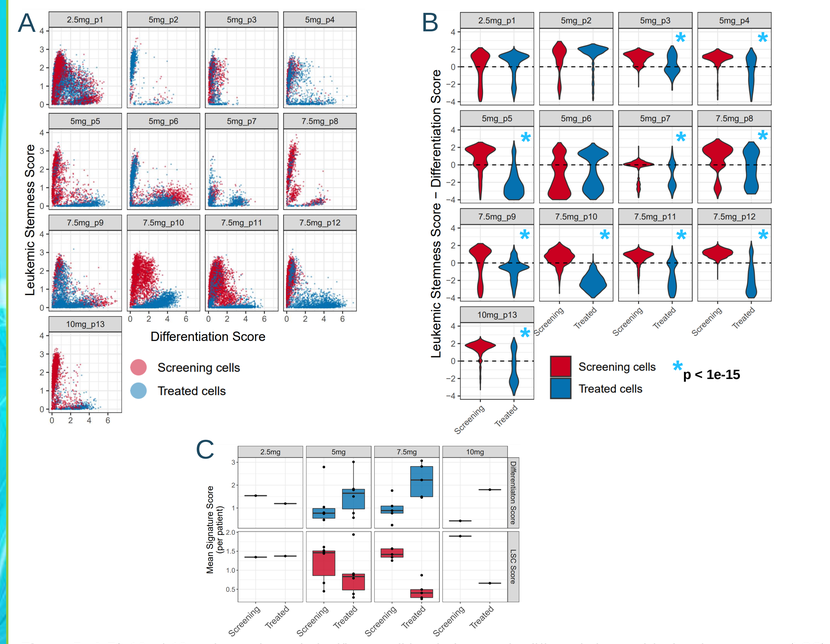

Figure 5. A-B) 10 of 13 patients showed significant cell-level changes in differentiation and leukemic stemness (LSC) scores on treatment, suggesting a broad treatment-induced shift toward a differentiated state (p-values from Wilcoxon test). **C)** Averaging LSC and differentiation scores across cells per patient showed a dose-dependent response resembling CD34 and CD11b in **Figure 3C.**

Patient-derived Stemness and Differentiation Signatures Capture Differentiation Response in AML Cell Line Models

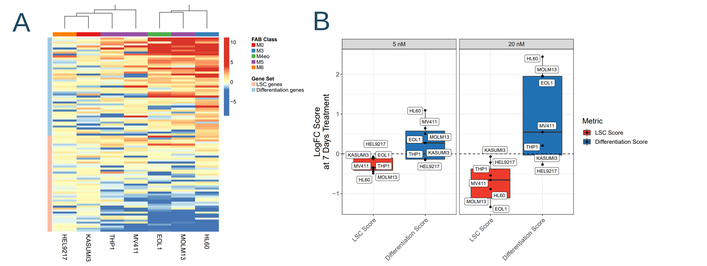

Figure 6. A) 8 AML cell lines were treated with 20nM FHD-286 in vitro for 7 days and a majority showed downregulation of LSC genes and upregulation of differentiation genes. **B)** Changes in LSC and differentiation scores were dose-dependent.

Conclusions

- AML patients treated with FHD-286 showed dose-dependent reduction of cells expressing leukemic stem cell (LSC) genes and increased cells expressing myeloid differentiation genes by scRNA-seq
- Patient-derived LSC and differentiation gene signatures effectively quantified stemness and treatment response in all patients evaluated, and translated to AML cell line models treated with FHD-286 in vitro
- On-treatment changes to LSC and differentiation signatures in AML patient cells suggest FHD-286 has broad on-target clinical activity and induces differentiation of LSCs, consistent with preclinical observations