The dual BRG1/BRM (SMARCA4/2) inhibitor FHD-286 induces functional differentiation and splicing defects in preclinical models of acute myeloid leukemia (AML)

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Abstract

The BRG1/BRM associated factor (BAF) complex is a chromatin remodeler critical for maintenance of cell viability in many cancers. This includes acute myeloid leukemia (AML), where BAF maintains the stem-like transcriptional state of blast cells. FHD-286 is a dual inhibitor of the BAF ATPase subunits BRG1 and BRM and is currently being investigated in relapsed or refractory AML and myelodysplastic syndrome. We have shown previously that FHD-286 induces the expression of the myeloid differentiation marker CD11b in preclinical models after treatment with sub-cytoreductive doses at long timepoints. Here we further characterized the differentiation phenotype induced by low-dose FHD-286 in AML cell lines through RNA-seq and a genome-wide CRISPR-Cas9 knockout screen. These datasets and mechanistic follow-up studies suggested AML cell lines treated with FHD-286 can functionally differentiate to gain the ability to produce superoxide anion and perform phagocytosis. We also found that FHD-286 treatment disrupts mRNA splicing and that this is a likely contributor to AML cell growth defects induced by BRG1/BRM inhibition. This study suggests multiple mechanisms by which FHD-286 is able to disrupt the stem-like transcriptional state of AML blasts to cause differentiation and ultimately cell death.

Background

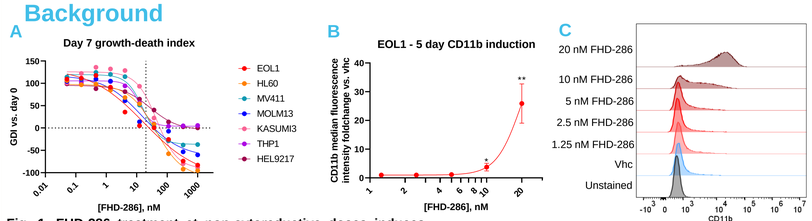

Fig. 1. FHD-286 treatment at non-cytoreductive doses induces expression of the myeloid maturation marker CD11b in multiple AML cell lines. A) Proliferation assay of AML cell lines treated with FHD-286 for 7 days. Growth-death index (GDI) calculated for day 7 cell counts compared to day 0 counts. GDI = 0 denotes cytostasis versus day 0; GDI < 0 denotes reduction in cell count versus day 0. Vertical line denotes highest dose examined in B-D (20 nM). **B)** Median fluorescence intensity of surface CD11b staining of EOL1 cells treated with FHD-286 doses up to 20 nM for 5 days. N = 4; mean +/- SD. One-sample *t*-test; $^{**}p < 0.01$, $^{*}p < 0.05$. **C)** Surface CD11b staining of EOL1 cells treated with FHD-286 doses up to 20 nM for 5 days. Representative experiment from B is shown. **D)** *ITGAM* (CD11b) RNA expression shown as log2 counts per million after 7 day treatment with up to 20 nM FHD-286. HL60 cells were also treated in parallel with vehicle (vhc) or 1 μM ATRA for 5 days. See also (1).

What is the nature of differentiation induced by sub-cytoreductive doses of FHD-286 in AML cells?

- What genes are modulated by FHD-286 treatment in addition to *ITGAM* (CD11b), especially at long treatment timepoints (i.e., 7 days)?
- Is differentiation induced by FHD-286 functional?

What genes/pathways modify the growth inhibitory activity of FHD-286 in AML cells?

- What are mechanisms of acquired resistance to FHD-286?
- Are there druggable targets that act in a synthetic lethal manner with FHD-286?

RNA-seq at extended FHD-286 treatment timepoints reveals upregulation of ROS and phagocytosis gene sets

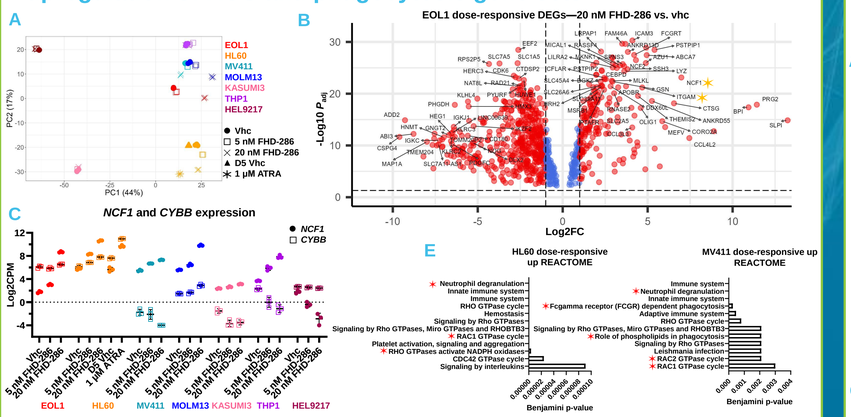

Fig. 2. Non-cytoreductive doses of FHD-286 upregulate gene sets related to ROS production and phagocytosis in multiple AML cell lines. A) PCA of RNA-seq of AML cell line samples treated with FHD-286 doses up to 20 nM for 7 days. HL60 cells were also treated in parallel with vhc or 1 μM ATRA for 5 days. **B)** Volcano plot of all genes in EOL1 which exhibited dose-responsive behavior between vhc, 5 nM, and 20 nM FHD-286 treatments. Log2 fold-changes and −log10 adjusted *p*-values are shown for the 20 nM FHD-286 condition versus vhc. **C)** *NCF1* and *CYBB* RNA expression shown as log2 counts per million. **D)** Diagram of NADPH oxidase subunits. See also (2). **E)** Dose-responsive differentially expressed up- and downregulated gene sets for each line were analyzed by DAVID. Top 12 significant REACTOME pathway results for HL60 and MV411 dose-responsive upregulated genes shown. Pathways suggesting functional myeloid differentiation are highlighted.

FHD-286 treatment of AML cells confers an enhanced ability to produce superoxide anion and perform phagocytosis

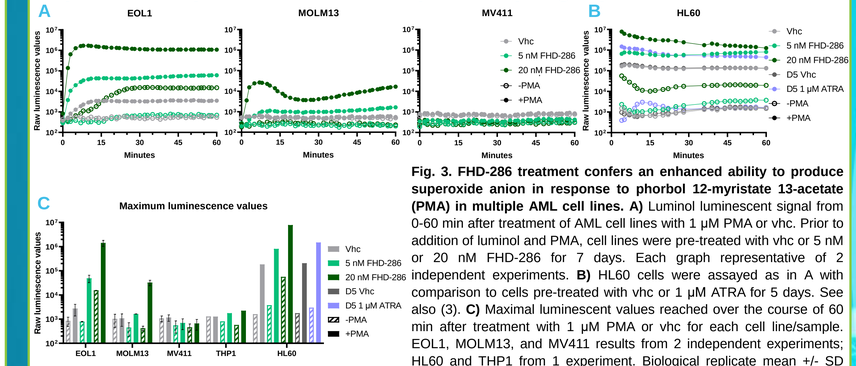

Fig. 3. FHD-286 treatment confers an enhanced ability to produce superoxide anion in response to phorbol 12-myristate 13-acetate (PMA) in multiple AML cell lines. A) Luminol luminescent signal from 0-60 min after treatment of AML cell lines with 1 μM PMA or vhc. Prior to addition of luminol and PMA, cell lines were pre-treated with vhc or 5 nM or 20 nM FHD-286 for 7 days. Each graph representative of 2 independent experiments. **B)** HL60 cells were assayed as in A with comparison to cells pre-treated with vhc or 1 μM ATRA for 5 days. See also (3). **C)** Maximal luminescent values reached over the course of 60 min after treatment with 1 μM PMA or vhc for each cell line/sample. EOL1, MOLM13, and MV411 results from 2 independent experiments; HL60 and THP1 from 1 experiment. Biological replicate mean +/- SD shown for EOL1, MOLM13, and MV411.

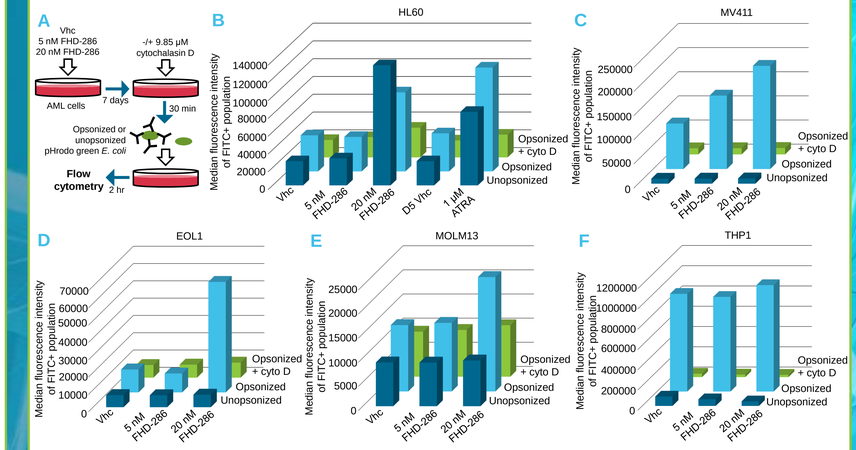

Fig. 4. FHD-286 treatment confers an enhanced ability to phagocytose opsonized *E. coli* in multiple AML cell lines. A) Cells were pre-treated with vhc or 5 nM or 20 nM FHD-286 for 7 days. On day 7 FHD-286 was removed and cells were treated with vhc or 9.85 μM cytochalasin D (cyto D) for 30 min to inhibit phagocytosis and macropinocytosis. Either opsonized or unopsonized pHrodo green-labeled, heat-killed *E. coli* were spiked into the culture at 250 bacteria/cell for 2 hr, and then cells were harvested for flow cytometry. **B-F)** Median fluorescence intensity of live, FITC+ cells shown for each treatment condition. N = 1. In B, comparison is shown for HL60 cells treated in parallel with vhc or 1 μM ATRA for 5 days. See also (4).

Genome-wide CRISPR screening reveals an FHD-286 interaction with other epigenetic modifiers and mRNA regulation/splicing pathways

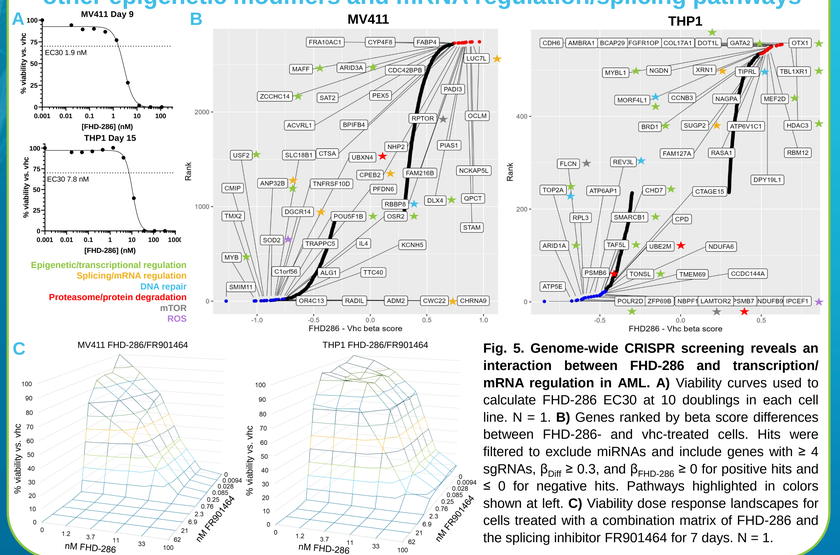

Fig. 5. Genome-wide CRISPR screening reveals an interaction between FHD-286 and transcription/mRNA regulation in AML. A) Viability curves used to calculate FHD-286 EC30 at 10 doublings in each cell line. N = 1. **B)** Genes ranked by beta score differences between FHD-286- and vhc-treated cells. Hits were filtered to exclude miRNAs and include genes with ≥ 4 sgRNAs, $\beta_{Diff} \geq 0.3$, and $\beta_{FHD\text{-}286} \geq 0$ for positive hits and ≤ 0 for negative hits. Pathways highlighted in colors shown at left. **C)** Viability dose response landscapes for cells treated with a combination matrix of FHD-286 and the splicing inhibitor FRF901464 for 7 days. N = 1.

FHD-286 treatment induces splicing defects in AML cells likely via the exonization of Alu elements

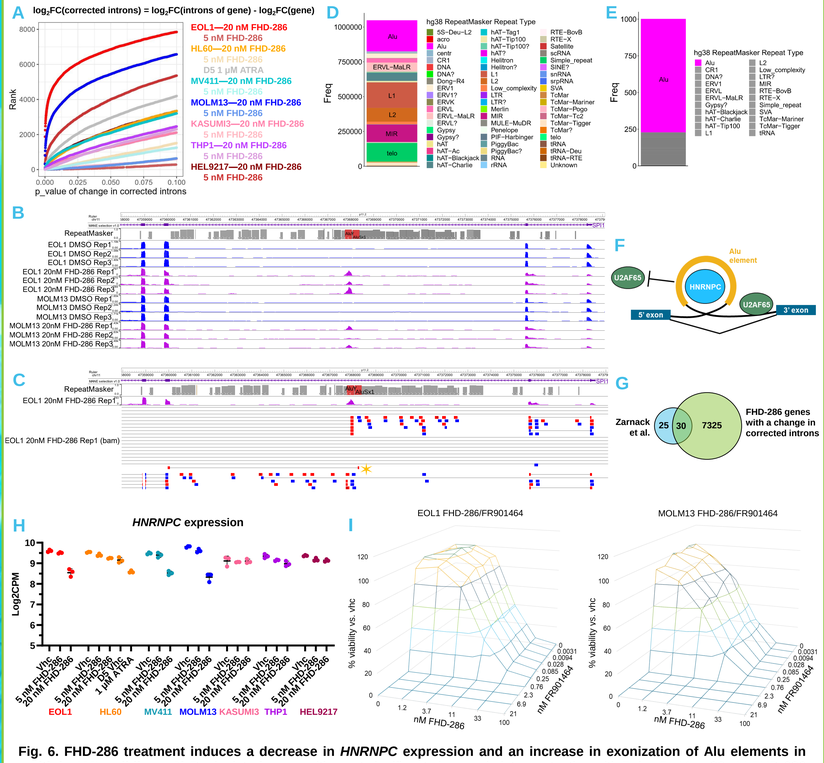

Fig. 6. FHD-286 treatment induces a decrease in *HNRNPC* expression and an increase in exonization of Alu elements in multiple AML cell lines. A) Rank plot of genes with most significant changes in intron retention with 7 day 5 nM or 20 nM FHD-286 treatment after correction for changes in gene expression. **B)** Expression of intronic Alu elements (red) in *SPI1* following 7 day 20 nM FHD-286 treatment in EOL1 and MOLM13. **C)** RNA-seq read mapping showing AluSx1 element highlighted in B is incorporated into the *SPI1* mRNA (see read highlighted by yellow star). **D)** Frequency of different repeat types within the human genome. **E)** Overrepresentation of Alu repeats relative to D in the top 1000 corrected introns with largest effect size in EOL1 treated with 20 nM FHD-286. **F)** Model of HNRNPC protection of Alu elements through competition for binding with splicing factor U2AF65. Loss of HNRNPC deprotects Alu elements, inducing their exonization. See also (5). **G)** All genes with *padj* ≤ 0.05 for corrected intron retention in any FHD-286-treated AML cell line were compared with genes from (5) which were verified by RT-qPCR to undergo differential splicing in response to HNRNPC knockdown. **H)** *HNRNPC* RNA expression shown as log2 counts per million after 7 day treatment with up to 20 nM FHD-286. HL60 cells were also treated in parallel with vhc or 1 μM ATRA for 5 days. **I)** Viability dose response landscapes for cells treated with a combination matrix of FHD-286 and the splicing inhibitor FRF901464 for 7 days. N = 1.

Key results

- RNA-seq conducted at long treatment timepoints with sub-cytoreductive doses of FHD-286 revealed upregulation of pathways related to ROS production and phagocytosis in multiple AML cell lines.
- Subunits of the granulocyte NADPH oxidase were among the most highly upregulated genes even in cell lines that did not significantly upregulate *ITGAM*.
- FHD-286 treatment (7 days) conferred an enhanced ability to produce superoxide anion in response to phorbol 12-myristate 13-acetate in multiple AML cell lines.
- FHD-286 treatment (7 days) conferred an enhanced ability to phagocytose opsonized *E. coli* in multiple AML cell lines.
- A genome-wide CRISPR-Cas9 drug modifier screen in two AML cell lines revealed pathways related to epigenetic modifiers, mRNA regulation, and splicing as potential synthetic lethal targets with FHD-286.
- FHD-286 downregulated *HNRNPC* and induced exonization at intronic Alu elements in multiple AML cell lines, suggesting a mechanism by which treatment disrupts mRNA splicing.

References & acknowledgments

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