Abstract # 2122

The dual BRM/BRG1 (SMARCA2/4) inhibitor FHD-286 induces differentiation in preclinical models of acute myeloid leukemia (AML)

*Mike Collins[1], *Astrid Thomsen[1], Victoria Amaral[1], Ashley Gartin[1], Gabriel J. Sandoval[1], Ammar Adam[1], Sarah Reilly[1], Laure Delestre[2], Virginie Penard-Lacronique[2], Warren Fiskus[3], Kapil N. Bhalla[3], Stephane De Botton[2], Samuel Agresta[1], Jessica Piel[1], Murphy Hentemann[1]

*Co-first authors; [1]Foghorn Therapeutics, Cambridge, MA; [2]Institut Gustave Roussy, Villejuif, France; [3]The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

The BAF (BRM/BRG1-associated factors) complex (also known as mSWI/SNF) is a critical regulator of the chromatin landscape of the genome. By controlling chromatin accessibility, BAF regulates lineage-specific transcriptional programs, including those important for AML blast cell growth and survival. FHD-286 is a highly potent inhibitor of the BAF catalytic subunits BRM and BRG1 (SMARCA2/4), which is being developed for the treatment of relapsed/refractory AML and MDS. FHD-286 has demonstrated broad efficacy in ex vivo treatment of AML patient-derived samples from diverse genetic backgrounds, including those with difficult to treat mutational profiles, such as mtNPM1, FLT3 ITD, and Inv(3) with EVI1 overexpression. Interestingly, while higher concentrations (≥90 nM) of FHD-286 predominantly induced cytoreduction, lower concentrations (≤30 nM) induced differentiation-like responses. To investigate this differentiation effect, we performed immunophenotyping of cell lines and primary AML samples following prolonged exposure to FHD-286. Extended treatment (7+ days) with pharmacologically relevant concentrations (5-20 nM) of FHD-286 led to time- and dose-dependent upregulation of the myeloid maturation marker CD11b. CD11b+ cells expressed lower levels of the proliferation and survival proteins Ki67 and BCL2, as well as BRG1 protein, implying that immature blasts are characterized by high levels of BRG1. These results suggest that BAF functions to drive transcriptional programs required to maintain AML cells in an undifferentiated state, and that FHD-286 may inhibit AML cell growth by overcoming this differentiation block. We have also demonstrated combination benefit with standard of care cytotoxic agents in multiple AML cell lines in vitro, and significant survival benefit in vivo. Taken together, these findings suggest that FHD-286 is able to target blast progenitor populations that are heavily BRM/BRG1-dependent, and that combination with standard of care agents can achieve profound, mutationally agnostic antitumor activity in AML.

Background

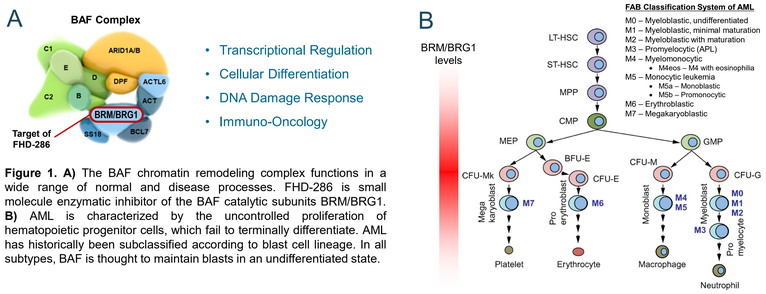

Figure 1. A) The BAF chromatin remodeling complex functions in a wide range of normal and disease processes. FHD-286 is small molecule enzymatic inhibitor of the BAF catalytic subunits BRM/BRG1. **B)** AML is characterized by the uncontrolled proliferation of hematopoietic progenitor cells, which fail to terminally differentiate. AML has historically been subclassified according to blast cell lineage. In all subtypes, BAF is thought to maintain blasts in an undifferentiated state.

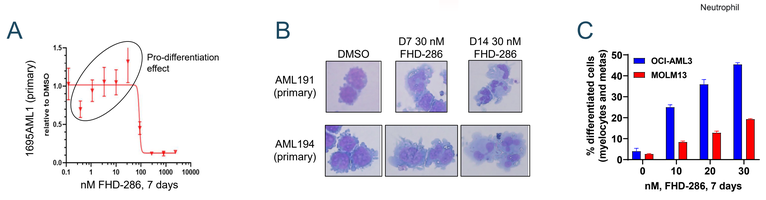

Figure 2. A) In several patient-derived AML samples, exposure to low concentrations of FHD-286 elicited differentiation-like responses. **B)** Representative light microscopy images of patient-derived AML cells treated with DMSO or 30 nM FHD-286 for 7 or 14 days and prepared with Wright's stain. **C)** 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.

References

Centore, R.C., et al. 2020. *Trends Genet.* 36(12):936–950.

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Key results

- Pharmacologically relevant concentrations of FHD-286 induce dose- and time-dependent upregulation of myeloid maturation marker CD11b in AML cells of diverse lineages *in vitro*.
- Increased expression of CD11b corresponded with a sharp reduction in cell proliferation and concomitant decreases in markers associated with more aggressive, immature blast phenotypes (Ki67, BRG1, and BCL2).
- Marked upregulation of CD11b in AML CDX models *in vivo* at well-tolerated, efficacious dose levels of FHD-286.
- Strong combination benefit observed with FHD-286 and cytarabine or decitabine in multiple AML cell lines *in vitro*.
- FHD-286 combination with cytarabine demonstrates significant survival benefit in MV411 CDX model

FHD-286 exposure upregulates the myeloid maturation marker CD11b in AML cells from diverse lineages

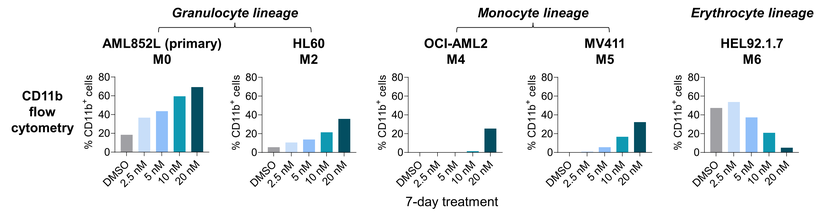

Figure 3. Cells were exposed to either 0.1% DMSO or the indicated concentrations of FHD-286 for 7 days. Following treatment, CD11b expression was assessed by flow cytometry. Note: In the DMSO condition, all cell lines had low levels of CD11b positivity with the exception of the M6 line HEL92.1.7. Since CD11b expression is lost during erythropoiesis, the observed decrease upon FHD-286 exposure may be a result of erythrocytic differentiation in this cell line.

Prolonged exposure to FHD-286 achieves more complete CD11b upregulation, and downregulation of blast proliferation and survival markers

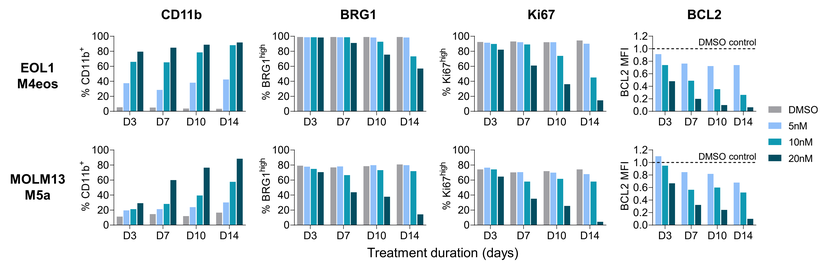

Figure 4. Cells were exposed to either 0.1% DMSO or the indicated concentrations of FHD-286 for 14 days. Cells were split on days 3, 7 and 10 with drug replenishment. Cells were analyzed by flow cytometry on days 3, 7, 10 and 14 for the indicated markers. Values for BCL2 are mean fluorescence intensity (MFI) relative to DMSO control.

CD11b⁺ cells have reduced BRG1 (SMARCA4) and Ki67 protein levels, and proliferate more slowly than CD11b⁻ cells

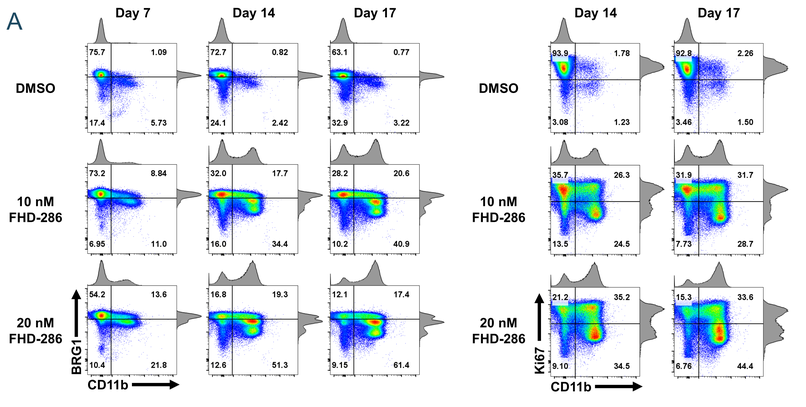

Figure 5. A) HL60 Cells were exposed to either DMSO or the indicated concentrations of FHD-286 for 17 days, and analyzed by flow cytometry on days 7, 14 and 17 (Ki67 days 14 and 17 only). Equal numbers of events were analyzed for all samples. Shown are 2-parameter pseudocolor plots with CD11b MFI on the x-axis and BRG1 or Ki67 MFI on the y-axis. Values represent the percentage of cells within each quadrant. Fluorescent intensity distributions are shown as adjunct histograms. **B)** HL60 cells were exposed to DMSO or FHD-286 for 7 days, and CD11b$^{+/-}$ cells were separated with magnetic beads. Treatment was continued for 10 more days to observe effects on proliferation rates. **C)** Viable cell densities of HL60 cells exposed to DMSO or the indicated concentrations of FHD-286 for 17 days, with CD11b$^{+/-}$ isolation on day 7.

FHD-286 treatment upregulates CD11b in AML cell line xenograft models *in vivo*

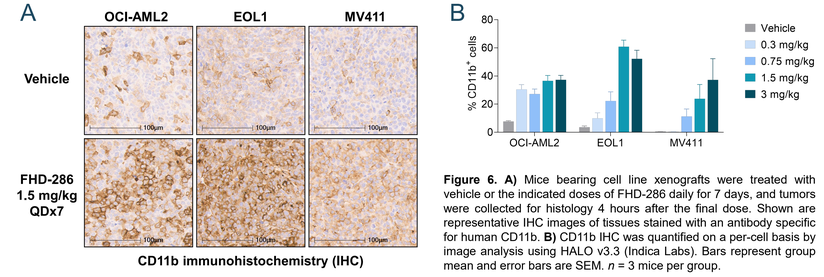

Figure 6. A) Mice bearing cell line xenografts were treated with vehicle or the indicated doses of FHD-286 daily for 7 days, and tumors were collected for histology 4 hours after the final dose. Shown are representative IHC images of tissues stained with an antibody specific for human CD11b. **B)** CD11b IHC was quantified on a per-cell basis by image analysis using HALO v3.3 (Indica Labs). Bars represent group mean and error bars are SEM. *n* = 3 mice per group.

FHD-286 sensitizes AML cells to standard of care cytotoxic agents

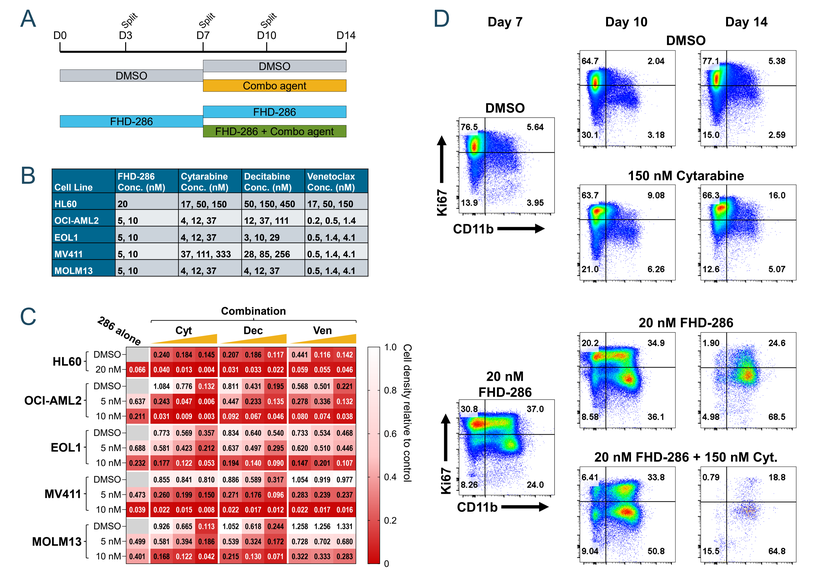

Figure 7. A) Combination treatment design. To observe effects on cell growth, all treatment conditions were split at the same ratio as the DMSO control condition. **B)** Cell lines and drug concentrations tested in combination experiments. Combination agent concentrations were chosen based on experimentally derived IC_{50} values. **C)** Heatmap of cell densities relative to DMSO control on day 14. The cytarabine and decitabine combinations were generally more effective than the venetoclax combination. **D)** HL60 cells were treated as in **A**, and analyzed by flow cytometry on days 7, 10 and 14. Equal volumes were analyzed for all samples, in order to observe effects on cell density. Shown are 2-parameter pseudocolor plots with CD11b MFI on the x-axis and Ki67 MFI on the y-axis. Values represent the percentage of cells within each quadrant. Similar results were obtained in other cell lines, and with decitabine combination.

Enhanced efficacy and significant survival benefit with FHD-286 + cytarabine combination *in vivo*

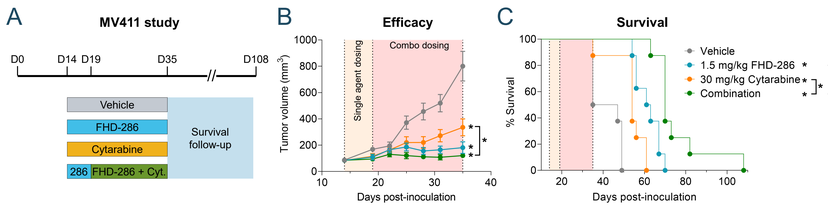

Figure 8. A) Study design. Mice were inoculated with MV411 cells on day 0 and randomized (n = 8/group) for treatment on day 14. Mice were treated 5 days on, 2 days off for a period of 21 days. For the combination group, mice received 5 days of FHD-286 prior to the addition of cytarabine. **B)** Compared with the vehicle control group, all treatment groups had significantly reduced tumor burden on day 35 ($p > 0.0001$), as assessed by one-way ANOVA followed by Tukey's multiple comparisons test. FHD-286 monotherapy and FHD-286 + cytarabine demonstrated better tumor control (86.6% and 95% TGI, respectively) than cytarabine monotherapy (64.8% TGI). However, only the combination was statistically superior to cytarabine monotherapy ($p = 0.0003$). **C)** All treatment groups had statistically improved survival compared to vehicle ($p > 0.0001$), as assessed by log-rank (Mantel-Cox) test. Median survival times for vehicle control, single-agent FHD-286 and cytarabine, and the combination were 41, 62, 54, and 70 days post- inoculation, respectively. The combination had a significantly greater effect on median survival time compared with cytarabine alone ($p < 0.0001$) and FHD-286–alone ($p = 0.0022$).