Pre-clinical efficacy of targeting BAF complexes through inhibition of the dual ATPases BRG1 and BRM by FHD-286 in cellular models of AML of diverse genetic background

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Introduction

DNA in eukaryotic cells is packaged in nucleosomes and higher order chromatin structures, making it relatively inaccessible to transcriptional machinery. ATP-dependent chromatin-modifying and remodeling complexes allow transcriptional machinery, composed of transcription factors (TFs) and co-factors, to gain access and modulate transcription. Chromatin remodeling complexes, e.g., BAF complex, contain one ATPase, BRG1 (SMARCA4) or BRM (SMARCA2), and several other factors. The BAF (BRG1/BRM-associated factor) complex is essential for lineage specific gene expression by TFs and for hematopoiesis. Expression and dependency on BRG1/BRM have also been documented for AML cells. Mutations in BRG1 and other BAF complex components are common and mechanistically involved in various cancer types. Recently, cancer cells with mutation and reduced expression of BRG1 were shown to be dependent for survival on the BRM activity of the BAF complex. BRM depletion was shown to selectively inhibit in vitro and in vivo growth of BRG1 mutant cancer cells. BRG1 and BRM have high protein sequence homology, and both contain the core catalytic ATPase domain that drives chromatin remodeling. BRG1 and BRM also contain a bromodomain, which is not a dependency in cancer cells. Small molecule inhibitors of dual BRM and BRG ATPase activity have been developed, which repress BRG1/BRM-dependent gene-expression and growth of cancer cells. FHD286 (Foghorn Therapeutics) is a highly potent, selective, small molecule, oral, catalytic enzyme activity inhibitor of BRM and BRG1. FHD286 is active against AML cells. Evaluation of the CRISPR-dependency screen map (DepMap) showed greater dependency of numerous AML cell lines on SMARCA4 expression. FHD-286 is currently being evaluated in AML for clinical efficacy in early clinical trials. However, it is unclear which of the genetically characterized AML sub-types, including those associated with poor clinical outcome, would be susceptible or resistant to FHD286. Gene expression signature (GES) of FHD286 activity also needs to be elucidated and tested. In the present studies, we interrogated the in vitro and in vivo efficacy of FHD-286 in inducing differentiation and loss of viability, as well as their molecular correlates in AML cell lines and patient-derived (PD) AML cells. Exposure to FHD-286 overcame differentiation block and significantly induced CD11b expression and morphologic features of differentiation in AML cell lines with MLL-r, mtNPM1 and chromosome 3q26 lesions (with EVI1 overexpression). This was followed by a loss of viability of the differentiated AML cells. A similar exposure to FHD-286 induced loss of viability in PD AML cells. Following treatment FHD-286, RNA-Seq analysis of MOLM13 cells demonstrated significant reduction in the normalized enrichment scores for expressions of gene-sets of targets of MYC, mTORC1, E2F, Interferon-gamma, IL6-JAK-STAT3, inflammatory response and oxidative phosphorylation genes. QPCR analyses determined significant reduction in mRNA expression of MYC, SPI1 and BCL2 genes. Western analyses showed that treatment with FHD-286 significantly increased p21, p27, PU.1 and CD11b expressions, while reducing expressions of c-Myc and BCL2. Based on these observations, and clinical efficacy of the combination of venetoclax and decitabine/azacytidine, we determined in vitro lethal activity of co-treatment with FHD-286 and venetoclax or decitabine against AML cell lines and PD AML cells. Notably, co-treatment with FHD-286 and venetoclax or decitabine exerted synergistic lethality against AML cell lines and PD AML cells, especially those expressing MLL-r, mtNPM1 or EVI1. Based on the known efficacy of the Menin inhibitor SNDX-50469 in AML with MLL-r or mtNPM1, we also found that co-treatment with FHD-286 and SNDX-50469 was synergistically lethal against AML cell lines and PD AML cells with MLL-r or mtNPM1. Since treatment with BET (bromodomain and extraterminal) protein inhibitor also inhibits c-Myc and BCL2 expression and was shown to be lethally active in AML cells with EVI1 overexpression, or with MLL-r or mtNPM1, we also found that co-treatment with FHD-286 and BET protein inhibitor OTX015 exerted synergistic lethality against AML cell lines and PD AML cells with chromosome 3q26 lesions and EVI1 overexpression, or with MLL-r or mtNPM1. Finally, in a luciferase-transduced, patient-derived xenograft (PDX) model of AML cells with MLL-AF9 and FLT3, KMT2C/2D and NOTCH2 mutations, we determined that treatment with FHD-286 administered orally alone was significantly effective in reducing AML burden and improving overall survival of the mice. Additionally, co-treatment with FHD-286 and venetoclax or decitabine or OTX015, as compared to each drug alone or vehicle control, significantly reduced the AML burden and improved median and overall survival of the immune-depleted mice, without inducing significant toxicity. Taken together, these findings highlight the promise of FHD-286 treatment alone and in rational combinations in exerting significant anti-AML efficacy against cellular models of AML, especially those with MLL-r, mtNPM1 or chromosome 3q26 lesions and EVI1 overexpression.

Results

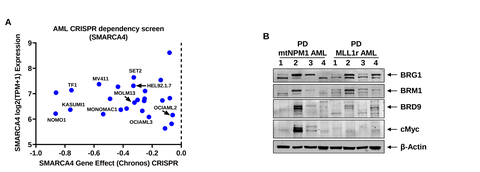

Figure 1. SMARCA4 is a dependency in AML cells. A. CRISPR-dependency (Chronos) screening scores from DepMap show increased dependency of AML cells on SMARCA4. The lower the score, the greater the dependency of the AML cell line on SMARCA4. **B.** Immunoblot analysis of BRG1 (SMARCA4), BRM (SMARCA2), BRD9 and c-Myc. The expression levels of β-Actin in the cell lysates served as the loading control.

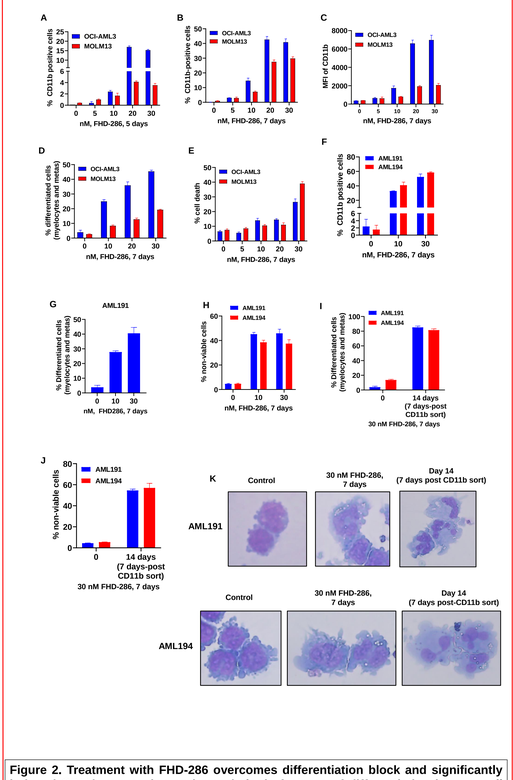

Figure 2. Treatment with FHD-286 overcomes differentiation block and significantly induced CD11b expression and morphologic features of differentiation in AML cell lines with MLL-r, mtNPM1 and chromosome 3q26 lesions (with EVI1 overexpression). A-E. MOLM13 and OCI-AML3 cells were treated with the indicated concentrations of FHD-286 for 5-7 days. At the end of treatment, cells were assessed for CD11b expression, morphologic features of differentiation and loss of cell viability. **F-H**. AML191 and AML194 (3q26 lesion with EVI1 overexpression) cells were treated with the indicated concentrations of FHD-286 for 7 days. At the end of treatment, cells were assessed for CD11b expression, morphologic features of differentiation and loss of cell viability. **I-J**. AML191 and AML194 cells were treated with 30 nM of FHD-286 for 7 days. Then, CD11b-positive cells were sorted by flow cytometry and cultured for an additional 7 days. At the end of 14 days, cells were assessed for CD11b expression. morphologic features of differentiation and loss of cell viability. **K**. Representative light microscopy images of AML191 and AML194 treated with 30 nM of FHD-286 for 7 and 14 days.

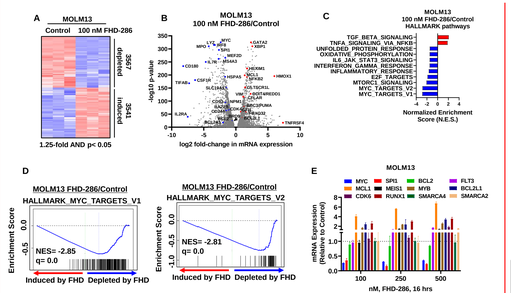

Figure 3. RNA-Seq analysis of FHD286-treated MOLM13 cells demonstrates significant reduction in the normalized enrichment scores for MYC, mTORC1, E2F, Interferon-gamma, IL6-JAK-STAT3, as well as of inflammatory response and oxidative phosphorylation genes. A. MOLM13 cells were treated with the indicated concentration of FHD-286 for 16 hours. Total RNA was isolated and RNA Seq analysis was conducted. Heat map indicates the number of mRNAs induced or depleted at a 1.25 fold change and p < 0.05. **B**. Volcano plot (log2 fold-change versus –log10 p-value) of RNA-Seq determined mRNA expression changes in FHD-286 treated MOLM13 cells. **C**. Gene set enrichment analysis of FHD-286 treated MOLM13 cells compared to HALLMARK pathways. Normalized enrichment scores are shown. All q-values are < 0.1. **D**. Enrichment plot of FHD-286 treated MOLM13 cells compared to HALLMARK MYC TARGETS V1 and HALLMARK MYC TARGETS V2. **E**. qPCR analysis of MOLM13 cells treated with the indicated concentrations of FHD-286 for 16 hrs. Expression was normalized to GAPDH and is presented relative to the untreated cells.

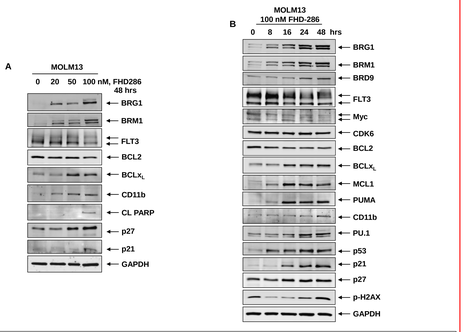

Figure 4. Treatment with FHD-286 significantly increased p21, p27, PU.1 and CD11b expressions, while reducing expressions of c-Myc and BCL2 in AML cells. **A-B**. MOLM13 cells were treated with FHD-286 at the doses and times indicated. At the end of treatment, total cell lysates were prepared and immunoblot analyses were conducted. The expression levels of GAPDH served as the loading control.

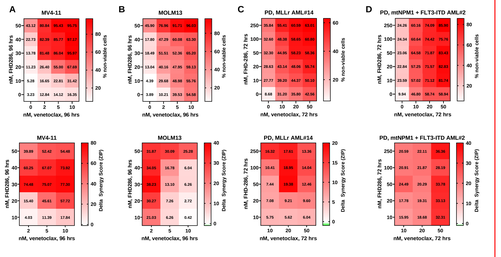

Figure 5. Co-treatment with FHD-286 and venetoclax exerts synergistic *in vitro* lethality in cultured and patient-derived AML cells expressing MLL-r or mtNPM1 with or without mtFLT3. **A-B**. MV4-11 and MOLM13 cells were treated with the indicated concentrations of FHD-286 and/or venetoclax for 96 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination. **C-D**. Patient-derived AML cells with MLL rearrangement or mtNPM1 with FLT3 alteration were treated with the indicated concentrations of FHD-286 and/or venetoclax for 72 hours. The % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination.

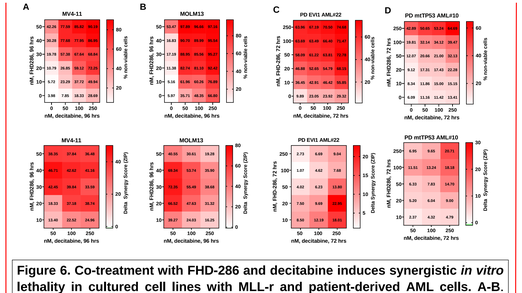

Figure 6. Co-treatment with FHD-286 and decitabine induces synergistic *in vitro* lethality in cultured cell lines with MLL-r and patient-derived AML cells. A-B. MOLM13 and MV4-11 cells were treated with the indicated concentrations of FHD-286 and/or decitabine for 96 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination. **C-D**. Patient-derived AML cells with inv(3) and EVI1 overexpression or MLL-r were treated with the indicated concentrations of FHD-286 and/or decitabine for 72 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination.

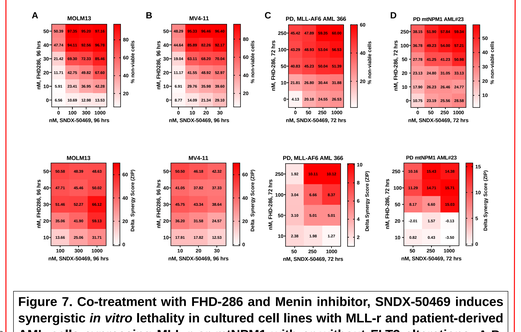

Figure 7. Co-treatment with FHD-286 and Menin inhibitor, SNDX-50469 induces synergistic *in vitro* lethality in cultured cell lines with MLL-r and patient-derived AML cells expressing MLL-r or mtNPM1 with or without FLT3 alterations. A-B. MOLM13 and MV4-11 cells were treated with the indicated concentrations of FHD-286 and/or SNDX-50469 for 96 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination. **C-D**. Primary AML cells with MLL-r or mtNPM1 were treated with the indicated concentrations of FHD-286 and/or SNDX-50469 for 72 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination.

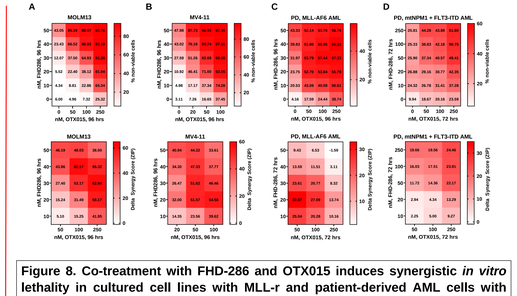

Figure 8. Co-treatment with FHD-286 and OTX015 induces synergistic *in vitro* lethality in cultured cell lines with MLL-r and patient-derived AML cells with MLL-r or expressing mtNPM1 with or without FLT3 alterations. A-B. MOLM13 and MV4-11 cells were treated with the indicated concentrations of FHD-286 and/or OTX015 for 96 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination. **C-D**. Primary AML cells with MLL-r or mtNPM1 were treated with the indicated concentrations of FHD-286 and/or OTX015 for 72 hours. At the end of treatment, the % non-viable cells were determined by staining with To-Pro-3 iodide and flow cytometry analysis. Delta synergy scores were determined by the ZIP method. Synergy scores >1.0 indicate a synergistic interaction of the two agents in the combination.

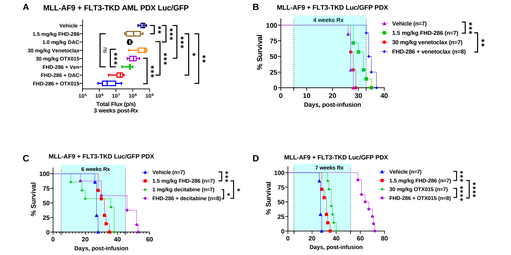

Figure 9. Treatment with FHD-286-based combinations reduced leukemia burden and significantly improved survival of NSG mice bearing MLL-r plus FLT3-TKD AML xenografts. A. Total photon counts [flux] (determined by bioluminescent imaging) in NSG mice engrafted with luciferized MLL-AF9 + FLT3-TKD AML PDX cells and treated for 3 weeks with FHD-286 and/or venetoclax, decitabine or OTX015 at the indicated doses. **B**. Kaplan-Meier survival plot of NSG mice engrafted with luciferized MLL-AF9 + FLT3-TKD AML PDX cells and treated with 1.5 mg/kg of FHD-286 (daily x 5 days, P.O.) and/or 30 mg/kg of venetoclax (daily x 5 days, P.O.) for 4 weeks. Significance was calculated by a Mantel-Cox log-rank test. **C**. Kaplan-Meier survival plot of NSG mice engrafted with luciferized MLL-AF9 + FLT3-TKD AML PDX cells and treated with 1.5 mg/kg of FHD-286 (daily x 5 days, P.O.) and/or 1 mg/kg of DAC (Days 1-5 days only, IP) for 6 weeks. Significance was calculated by a Mantel-Cox log-rank test. **D**. Kaplan-Meier survival plot of NSG mice engrafted with luciferized MLL-AF9 + FLT3-TKD AML PDX cells and treated with 1.5 mg/kg of FHD-286 (daily x 5 days, P.O.) and/or 30 mg/kg of OTX015 (daily x 5 days, P.O.) for 7 weeks. Significance was calculated by a Mantel-Cox log-rank test.

Conclusions

1. Treatment with FHD-286 for up to 7 days overcame differentiation block and significantly induced CD11b expression and morphologic features of differentiation in AML cells with MLL-r, mtNPM1 and chromosome 3q26 lesions (with EVI1 overexpression).

2. RNA-Seq analysis of AML cells treated with FHD-286 demonstrated significant reduction in the normalized enrichment scores for expressions of gene-sets of targets of MYC, mTORC1, E2F, Interferon-gamma, IL6-JAK-STAT3, inflammatory response and oxidative phosphorylation genes.

3. Compared to treatment with FHD-286 or venetoclax or vehicle control, co-treatment with FHD-286 and venetoclax exerted superior in vivo anti-AML efficacy without any host toxicity in a PDX model of MLL-r AML.

4. Compared to treatment with FHD-286, decitabine (DAC), OTX015, or vehicle control, co-treatment with FHD-286 and OTX015 or DAC exerted superior in vivo anti-AML efficacy without host toxicity in a PDX model of MLL-r AML.

5. These preclinical findings highlight the promise of FHD-286 treatment alone and in rational combinations in exerting significant anti-AML efficacy against cellular models of AML, especially those with MLL-r, mtNPM1 or chromosome 3q26 lesions and EVI1 overexpression.