Superior preclinical efficacy of BRG1/BRM inhibitor combined with BET inhibitor or decitabine against MECOM-rearranged AML

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

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Introduction

Although approximately 20,000 new cases of acute myeloid leukemia are identified every year, cases with a rearrangement involving chromosome 3q26.2, particularly inv(3)(q21q26.2) or a translocation t(3;3)(q21;q26.2) are rare, accounting for about 1% of newly diagnosed AML. Both 3q26.2 rearrangements (r) cause aberrant relocation of the distal GATA2 hematopoietic enhancer leading to activation of EVI1 expression from the MDS1-EVI1 complex (MECOM) locus. EVI1 is a transcriptional regulator that plays a role in proliferation, suppression of myeloid differentiation and maintenance of a stem cell-like phenotype. AML with 3q26.2-r, due to GATA2 haplo-insufficiency and increased activity of EVI1 demonstrate aggressive proliferation, are refractory to many currently available therapies and exhibit abysmal outcomes and poor overall survival. Therefore, there is a glaring unmet need to develop and test novel targeted therapies to repress EVI1 expression and improve survival of AML with 3q26.2-r and EVI1 overexpression.

BRG1 (SMARCA4) and BRM (SMARCA2) are the core ATPases within the three chromatin remodeling BAF (BRG1/BRM-associated factor) complexes (canonical, polybromo and non-canonical) that regulate gene transcription. Gene transcription in AML cells depends on the activity of BRG and BRM. FHD286 (Foghorn Therapeutics) is a small molecule, selective inhibitor of BRG1 and BRM- ATPase activity being evaluated for safety and clinical efficacy in early clinical trials in AML (NCT04891757). In a previous report, we demonstrated that FHD-286 treatment induced differentiation and lethality in AML cells with MLL1r or mtNPM1, concomitantly causing perturbed chromatin accessibility and repression of c-Myc, PU.1 and CDK4/6. In present studies, we determined that exposure to FHD-286 (10 to 100 nM) for 4 to 7 days induced CD11b and morphologic features of differentiation as well as loss of viability in AML cell lines and patient-derived (PD) AML cells with 3q26.2-r and EVI1 expression. FHD-286 caused whole-genome, concordant, up- or down-regulations in H3K27Ac ChIP-Seq peaks and RNA-Seq-determined mRNA expressions of specific loci, associated with significant reduction in the gene-sets of MYC TARGETS and depletion of EVI1. Immunoblot analysis showed that FHD-286 reduced EVI1 protein expression. Additionally, mass spectrometry on 3q26.2-r AML cells treated with FHD-286 also showed log2 fold-reductions in EVI1, c-Myc, CD44, c-KIT, CD93, CDK4 and PLK1. FHD-286 treatment also reduced EVI1 expression in phenotypically defined AML stem progenitor cells. Co-treatment with FHD-286 and decitabine, BET inhibitor (OTX015), or HAT inhibitor GNE-781 exerted synergistic in vitro lethality against AML cell lines and PD AML cells with 3q26.2-r, (Delta synergy scores > 5 by the ZIP method). Finally, in luciferase-transduced, patient-derived xenograft (PDX) models of AML cells with 3q26.2-r, we determined that co-treatment with FHD-286 and decitabine or OTX015, as compared to each drug alone or vehicle control, significantly reduced AML burden and improved the overall survival of the NSG 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 chromosome 3q26.2-r and EVI1 overexpression.

Results

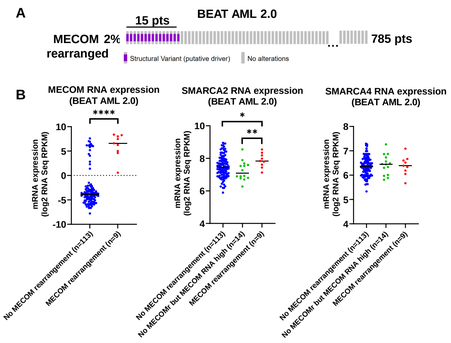


Figure 1. AML cells with 3q26.2 rearrangement express higher levels of MECOM (EVI1) and SMARCA2 RNA than AML cells without 3q26.2 rearrangement in the BEAT AML 2.0 patient dataset. A. Oncoprint of the 15 patients (2%) identified to possess a 3q26.2 rearrangement out of the 785 AML patients profiled and sequenced in the BEAT AML 2.0 study. **B**. Differential mRNA expression of MECOM (EVI1) and SMARCA2 in AML patients with 3q26.2 rearrangement (n=9) compared to patients without 3q26.2 rearrangement (n=113) and patients without 3q26.2 rearrangement but high (greater than 4 RKPM) expression of MECOM (n=14) from BEAT AML 2.0. *= p< 0.05; **= p< 0.01; ***= p< 0.001.

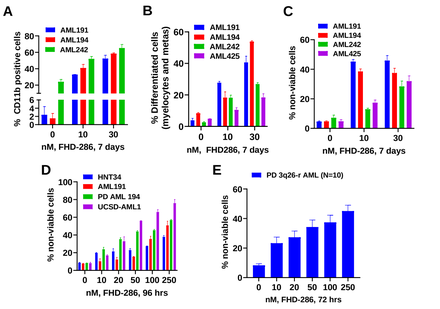


Figure 2. Treatment with FHD-286 overcomes differentiation block and significantly induced CD11b expression and morphologic features of differentiation in AML cell lines with 3q26.2 rearrangement. A-C. CD11b expression, morphologic features of differentiation and % of non-viable cells following 7 days of FHD-286 treatment. **D**. HNT34, AML191, AML194 and UCSD-AML1 cells were treated with FHD-286 for 96 hours and the % non-viable cells were determined by flow cytometry. **E**. Patient-derived (PD) 3q26.2-r AML samples were treated with the indicated concentrations of FHD-286 for 72 hours. Following this, cells were stained with TO-PRO-3 iodide and the % non-viable cells were determined by flow cytometry.

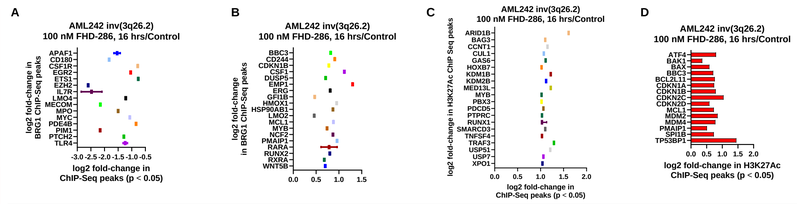


Figure 3. Treatment with FHD-286 depletes BRG1 occupancy on chromatin while increasing H3K27Ac occupancy on loci involved in differentiation and loss of cell viability. A-B. AML242 cells were treated with 100 nM of FHD-286 for 16 hours. ChIP-Seq analysis was conducted with anti-BRG1 antibody. Log2 fold-decline or log2 fold-increase of BRG1 binding at selected AML relevant loci. **C-D**. AML242 cells were treated with 100 nM of FHD-286 for 16 hours. ChIP-Seq analysis was conducted with anti-H3K27Ac antibody. Panel **D** shows the log2 fold-increase in H3K27Ac occupancy on loci involved in differentiation and loss of viability.

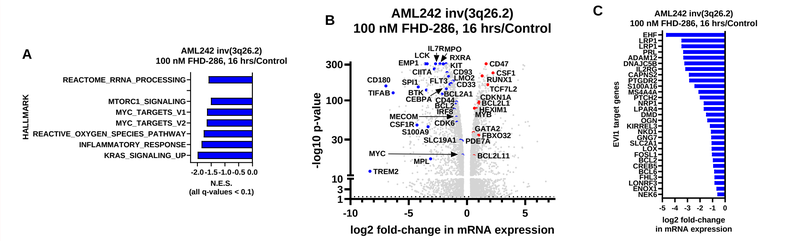


Figure 4. Treatment with FHD-286 alters chromatin accessibility and mRNA expression in MECOM-rearranged AML cells with reduction in the enrichment scores for MYC, mTORC1, as well as of inflammatory response gene sets. A-C. AML242 cells were treated with 100 nM of FHD-286 for 16 hours as biologic replicates. Total RNA was isolated and utilized for RNA-Seq analysis. **A**. Gene set enrichment analysis of FHD-286-treated AML242 cells compared to HALLMARK and REACTOME pathways. Normalized enrichment scores are shown. All q-values are < 0.1. **B**. Volcano plot (log2 fold-change versus –log10 p-value) of RNA-Seq determined mRNA expression changes in FHD-286-treated AML242 cells. **C**. Log2 fold-change in mRNA expression of significantly altered (p < 0.05) EVI1 target genes in FHD-286 treated AML191 cells compared to DMSO-treated control cells.

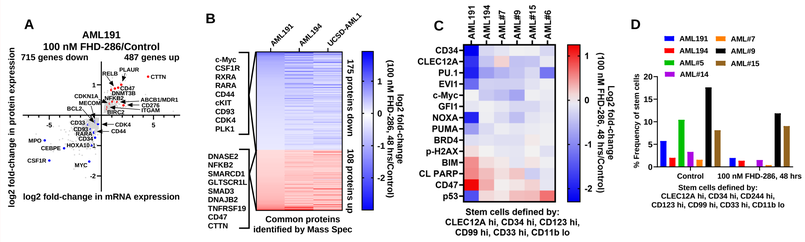


Figure 5. Treatment with FHD-286 depleted EVI1, c-Myc, CD44, and CDK4 expression in bulk AML cells and EVI1, c-Myc and PU.1 in phenotypically defined AML stem/progenitor cells with MECOM rearrangement. A.. Scatterplot of log2 fold-changes of selected, concordant mRNA and protein expression alterations in FHD-286-treated over DMSO-treated control AML191 cells. **B**. Heat map of log2 fold-change (100 nM FHD-286, 48 hrs/DMSO-treated Control) of common overlapping protein expressions determined by mass spectrometry in FHD-286-treated AML191, AML194 and UCSD-AML1 cells. **C**. Patient-derived MECOM-r AML samples (n=6) were treated with 100 nM of FHD-286 for 48 hours. CyTOF analyses were conducted utilizing cocktails of rare metal element tagged antibodies. The heat map shows the fold change (FHD-286 treated over DMSO-treated control) of depleted and induced proteins in phenotypically defined AML stem/progenitor cells (CLEC12A hi, CD34 hi, CD123 hi, CD99 hi, CD33 hi and CD11b low). **D**. Percent stem cell frequency of DMSO-treated control and FHD-286-treated patient-derived MECOM-rearranged AML cells.

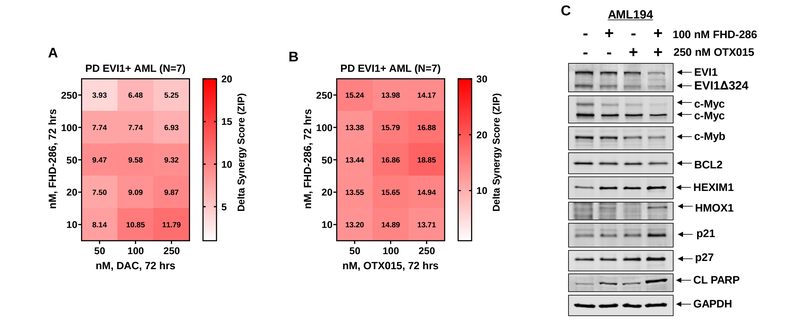


Figure 6. Treatment with FHD-286-based combinations exerted synergistic *in vitro* lethality in cultured and patient-derived AML cells expressing MECOM-r. A-B. PD, MECOM-rearranged AML cell samples were treated with the indicated concentrations of FHD-286 and decitabine (DAC) or OTX015 or DMSO control for 72 hours. The % To-Pro-3 iodide-positive, non-viable cells were determined by flow cytometry. Delta synergy scores were determined by the ZIP method within the SynergyFinder web application. Synergy scores >1.0 indicate a synergistic interaction. **C**. AML194 cells were treated with 100 nM of FHD-286 and/or 250 nM of OTX015 or DMSO control for 48 hours. Following this, total cell lysates were prepared and immunoblot analyses were conducted. The expression levels of GAPDH in the cell lysates served as the loading control.

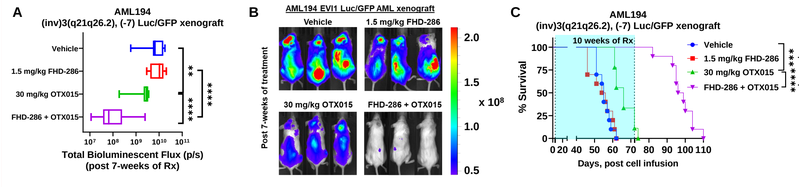


Figure 7. Treatment with FHD-286-based combinations reduced leukemia burden and significantly improved survival of NSG mice bearing MECOM-rearranged AML PDX models. A. Total photon counts [flux] (determined by bioluminescent imaging) in NSG mice bearing luciferized AML194 (inv)3(q21q26.2), (-7) xenografts and treated for 7 weeks with vehicle, FHD-286 and/or OTX015 at the indicated doses. **B**. Representative bioluminescent images of mice from panel **A**. **C**. Kaplan-Meier survival plot of NSG mice engrafted with luciferized AML194 (inv)3(q21q26.2), (-7) cells and treated with vehicle, 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 10 weeks. Significance was determined by a Mantel-Cox log-rank test. *** = p<0.005; **** = p<0.001.

Conclusions

1. Treatment with FHD-286 overcame the differentiation block and significantly induced CD11b expression and morphologic features of differentiation in AML cell lines with 3q26.2 rearrangement.

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 KRAS signaling genes.

3. Treatment with FHD-286 depleted EVI1, c-Myc, and PU.1 in phenotypically defined AML stem/progenitor cells with MECOM-r.

4. Compared to treatment with FHD-286, or OTX015 alone or vehicle control, co-treatment with FHD-286 and OTX015 reduced AML burden and significantly improved survival of mice bearing MECOM-r AML PDX cells.

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 MECOM rearrangement.