Synergistic efficacy of the BRM/BRG1 ATPase inhibitor, FHD-286, and anti-PD-1 antibody in mouse syngeneic tumor models

Abstract #888

Kana Ichikawa, Ammar Adam, David L. Lahr, Hsin-Jung Wu, Lan Xu, Brandon Antonakos, Liv Johannessen, Steven F. Bellon, Ryan Kruger, Richard C. Centore, and Martin Hentemann
Foghorn Therapeutics, 500 Technology Square, Suite 700, Cambridge, MA, USA

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Abstract

The BAF family of chromatin remodeling complexes are critical regulators of chromatin accessibility and gene expression, and BRM and BRG1 (also known as SMARCA2 and SMARCA4), the catalytic subunits of BAF, provide the enzymatic activity required for chromatin remodeling activity. We have previously identified and characterized a series of novel dual inhibitors of the BRM/BRG1 ATPases, and FHD-286, a potent and selective BRM/BRG1 inhibitor, is currently under clinical investigation for the treatment of metastatic uveal melanoma and advanced hematological malignancies (NCT04879017 and NCT04891757). BAF chromatin remodeling complex activities are implicated in many immunologic responses, and previous studies have shown the involvement of PBAF in the regulation of antitumor immunity [1]. Given the recent reports correlating SMARCA4 deficiency and ICI response [2], we explored the combination of BRM/BRG1 ATPase inhibition and anti-PD-1 antibody in syngeneic mouse models from various lineages and with different sensitivities to checkpoint inhibition. The combination of FHD-286 and anti-PD-1 antibody provided synergistic efficacy and survival benefit compared to anti-PD-1 alone in A20, CT26, and the immunologically barren B16F10 melanoma model. FHD-286 increased MHCI expression on B16F10 cells, and increases in IFNγ and Th1-type chemokine levels were observed in immunocompetent mice following treatment, suggesting that combinatorial activity may result from effects on both the tumor and the immune system. FHD-286 has the potential to sensitize tumor to immune-checkpoint inhibition and represents a novel combination approach for cancer immunotherapy.

Combination of FHD-286 and αPD-1 provides significant synergistic efficacy and survival benefit compared to αPD-1 alone in 3 syngeneic models

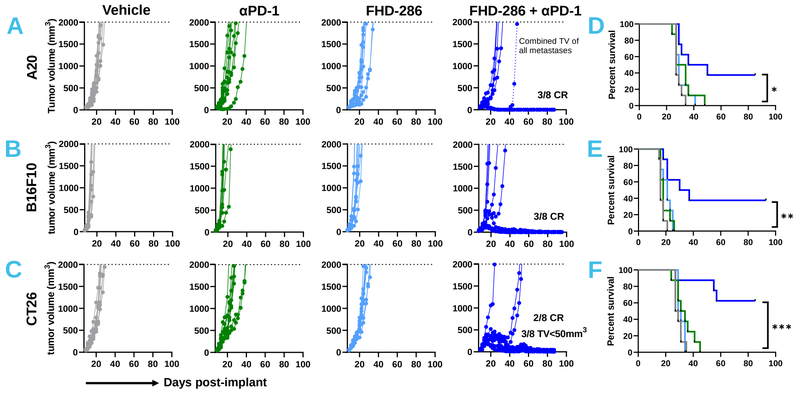

Figure 1. BAF ATPase inhibition synergizes with anti-PD-1 antibody in 3 syngeneic models of various lineages. (A-C) Tumor volumes of individual animals treated with vehicle controls, αPD-1 10mpk BIW, FHD-286 1.5mpk QD, or the combination of FHD-286 1.5mpk QD and αPD-1 10mpk BIW dosed continuously for up to 50 days, unless TV<10mm^3. **(D-F)** Kaplan-Meier survival curves of (**D**) A20, (**E**) B16F10, and (**F**) CT26 tumor-bearing animals. Statistical significance by Log-rank test, αPD-1 vs FHD-286 + αPD-1 (A20: p=0.03, B16F10: p=0.004, CT26: p=0.0009).

FHD-286 induced BRM/BRG1 inhibition transforms B16F10 tumor microenvironment

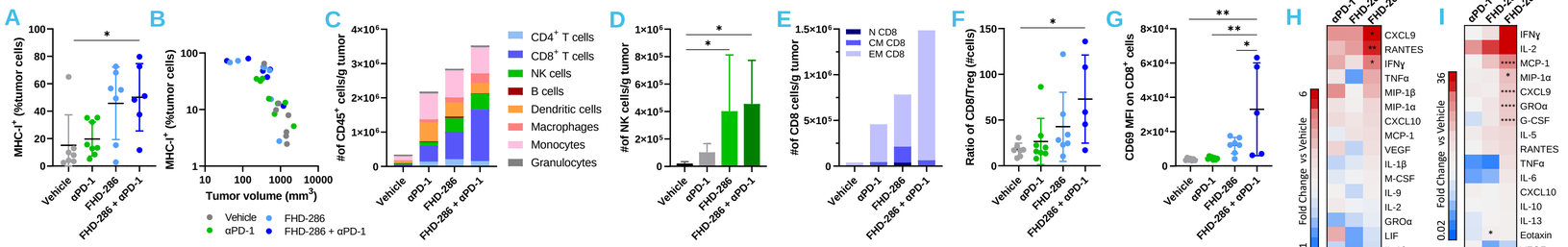

Figure 2. Changes in B16F10 tumor microenvironment (TME) induced by BRM/BRG1 ATPase inhibition. B16F10 tumor-bearing animals were dosed for 10 days (n=8 per group). Anti-PD-1, FHD-286, and FHD-286 + αPD-1 were dosed at 10 mpk BIW, 1.5 mpk QD, and 1.5 mpk QD/10 mpk BID, respectively, and samples were collected 4h post-final dose. Three animals were excluded from tumor analysis in the FHD-286 + αPD-1 combination group due to small tumor volume (TV = 0, 12, and 38 mm^3). Statistical significance by one-way ANOVA. (**A**) MHC-I expression on B16F10 tumor cells (p=0.04). (**B**) MHC-I expression on B16F10 tumor cells vs tumor volume. (**C**) Average number of CD45$^+$ cells per gram of B16F10 tumor. (**D**) Number of NK cells in the TME (p=0.04, p=0.02). (**E**) Number of CD8$^+$ cells and its subtypes in the TME. N=Naïve, CM=Central memory, EM=Effector memory. (**F**) Ratio of CD8$^+$ cells to FoxP3$^+$ Tregs in the TME (p=0.03). (**G**) Level of CD8$^+$ T cell activation, measured by CD69 MFI (Vehicle or αPD-1 vs FHD-286+αPD-1: p=0.0013; FHD-286 vs FHD-286+αPD-1: p=0.02). (**H-I**) Average fold change in cytokine and chemokine levels compared to the vehicle group (*p<0.05, **p<0.01, ***p<0.001, ****<0.0001) in (**H**) tumor and (**I**) plasma, measured by 32-plex Luminex panel. Cytokines with no detectable expression are excluded from the analysis.

BRM/BRG1 inhibition sensitizes B16F10 cells to immune response

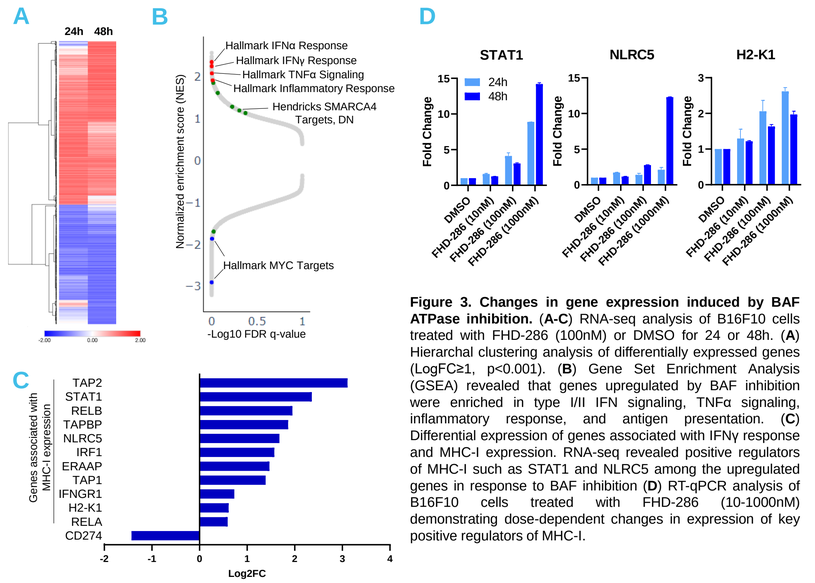

Figure 3. Changes in gene expression induced by BAF ATPase inhibition. (**A-C**) RNA-seq analysis of B16F10 cells treated with FHD-286 (100nM) or DMSO for 24 or 48h. (**A**) Hierarchal clustering analysis of differentially expressed genes (LogFC≥1, p<0.001). (**B**) Gene Set Enrichment Analysis (GSEA) revealed that genes upregulated by BAF inhibition were enriched in type I/II IFN signaling, TNFα signaling, inflammatory response, and antigen presentation. (**C**) Differential expression of genes associated with IFNγ response and MHC-I expression. RNA-seq revealed positive regulators of MHC-I such as STAT1 and NLRC5 among the upregulated genes in response to BAF inhibition (**D**) RT-qPCR analysis of B16F10 cells treated with FHD-286 (10-1000nM) demonstrating dose-dependent changes in expression of key positive regulators of MHC-I.

1. Pan D, et al. A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing. Science. 2018; 359:770-775.
2. Zhou M et al. Emerging role of SWI/SNF complex deficiency as a target of immune checkpoint blockade in human cancers. Oncogenesis. 2021; 10.

FHD-286 induced BRM/BRG1 inhibition enhances antigen presentation

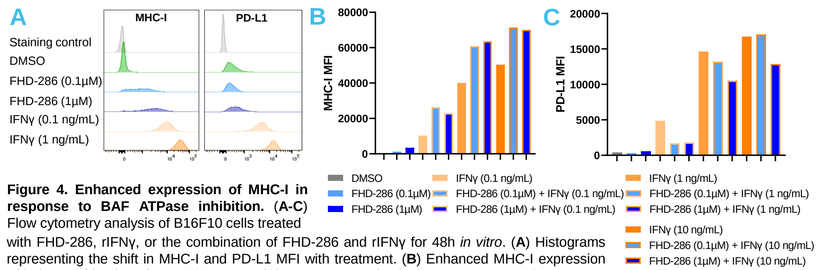

Figure 4. Enhanced expression of MHC-I in response to BAF ATPase inhibition. (**A-C**) Flow cytometry analysis of B16F10 cells treated with FHD-286, rIFNγ, or the combination of FHD-286 and rIFNγ for 48h *in vitro*. (**A**) Histograms representing the shift in MHC-I and PD-L1 MFI with treatment. (**B**) Enhanced MHC-I expression with the combination of FHD-286 + IFNγ. (**C**) PD-L1 expression in response to FHD-286 + IFNγ.

Conclusions

- Dual BRM/BRG1 ATPase inhibitor FHD-286 synergizes with αPD-1 antibody to promote tumor regression and animal survival in 3 unique syngeneic models.
- FHD-286 increases immune cell trafficking and infiltration into the TME. The combination with αPD-1 antibody enhances the activation of CD8$^+$ T cells and the expression of pro-inflammatory cytokines, transforming the TME.
- FHD-286 induced BAF inhibition enhances antigen presentation and sensitizes B16F10 cells to immune response.
- FHD-286 has the potential to sensitize tumor to immune-checkpoint inhibition and represents a novel combination approach for cancer immunotherapy.