Modulation of SPI1 transcriptional program contributes to the preclinical anti-tumor activity of SMARCA4/SMARCA2 ATPase inhibitors in AML

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Gabriel J Sandoval , Katharine Feldman, Sal Topal, Ammar Adam, Hsin-Jung Wu, Darshan Sappal, Luis M. M. Soares, David L. Lahr, Lan Xu, Rishi G. Vaswani, Jordana Muwanguzi, Liyue Huang, Jessica Piel, Mike Collins, Ho Man Chan, Michael J. Thomenius, Steven F. Bellon, Ryan G. Kruger, Carl P. Decicco, Richard C. Centore and Martin Hentemann
Foghorn Therapeutics, 500 Technology Square, Cambridge, MA 02139

Abstract

SPI1 (PU.1) is an ETS family transcription factor that plays a critical role in hematopoietic development and differentiation. Regulation of SPI1 expression has also been implicated in Acute Myeloid Leukemia (AML) oncogenesis, though its mechanism is incompletely understood. Previously, we have identified and characterized a series of novel dual inhibitors of the SMARCA4/SMARCA2 ATPases (also referred to as BRG1/BRM), critical components of the BAF (mSWI/SNF) family of chromatin remodeling complexes; and FHD-286, a related SMARCA4/SMARCA2 ATPase inhibitor, is currently being explored clinically for the treatment of metastatic uveal melanoma and AML (NCT04879017 and NCT04891757). Here we show that AML cell lines are sensitive to BAF ATPase inhibition, resulting in both cell cycle arrest and apoptosis. We demonstrate that BAF ATPase inhibition primarily affects the SPI1 transcriptional profile by regulating SPI1 genomic occupancy at various enhancer elements, resulting in downregulation of key target genes. Using *in vivo* mouse models of AML, we demonstrate dose-dependent tumor growth inhibition and pharmacodynamic modulation of SPI1 target genes. Together, these data suggest that modulation of SPI1 function may, in part, provide a mechanistic basis for the clinical development of FHD-286 in AML.

High expression of SPI1 strongly correlates with SPI1 dependence in AML

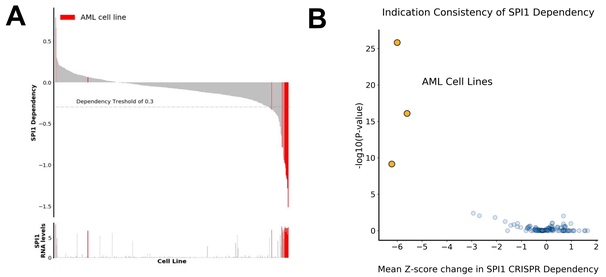
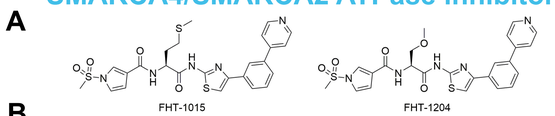


Figure 1. DepMap data shows that AML cell lines are sensitive to SPI1 loss. A. (Top) Cell lines in the DepMap database are ranked by SPI1 CRISPR dependency. (Bottom) mRNA levels of SPI1 across all cell lines in DepMap. AML cell lines are highlighted in red. **B.** Indication consistency of SPI1 dependency reveals that AML cell lines are particularly sensitive to SPI1 loss compared to other cancer indications.

Characterization of novel SMARCA4/SMARCA2 ATPase inhibitors



B

Compound	IC_{50} (μM)		
	SMARCA4 ATPase	SMARCA2 ATPase	CHD4 ATPase
FHT-1204	0.003 ± 0.001 (n=4)	0.004 ± 0.002 (n=4)	> 400 (n=4)
FHT-1015	0.004 ± 0.001 (n=3)	0.005 ± 0.004 (n=3)	> 400 (n=2)

Figure 2. Characterization of a novel series of SMARCA4/SMARCA2 ATPase inhibitors. A. Structures of inhibitors. **B.** Biochemical IC_{50} values demonstrating ATPase inhibition of SMARCA4 and SMARCA2, but not of the closely related ATPase, CHD4.

AML cell lines are sensitive to BAF ATPase inhibition

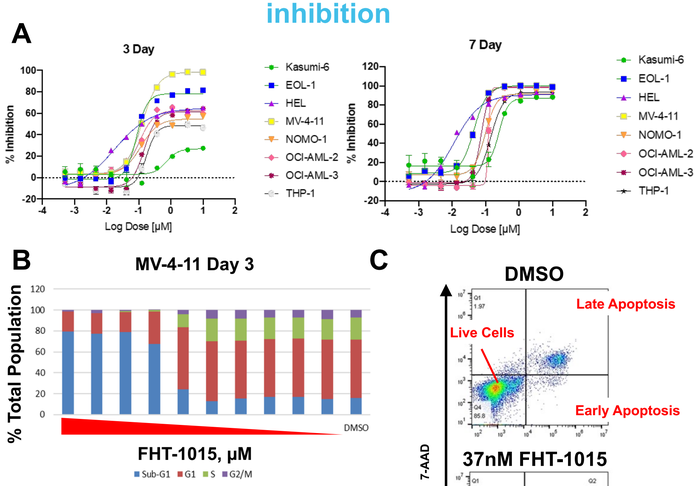


Figure 3. BAF inhibition results in a decrease of cell proliferation via a slow down in cell cycle and initiation of apoptosis in AML cells. A. AML Cell lines were treated for 3 or 7 days with a dose titration of FHT-1015 and relative viability was measured using Cell-Titer Glo. **B.** Cell cycle profile of MV-4-11 cells treated with a dose titration of FHT-1015 for 3 days. **C.** Apoptosis measured via Annexin V/7-AAD flow cytometry assay on MV-4-11 cells 3 days after treatment of 37nM FHT-1015 or DMSO control.

ATACseq in THP1 cells reveal that SPI1 regulatory elements are BAF dependent

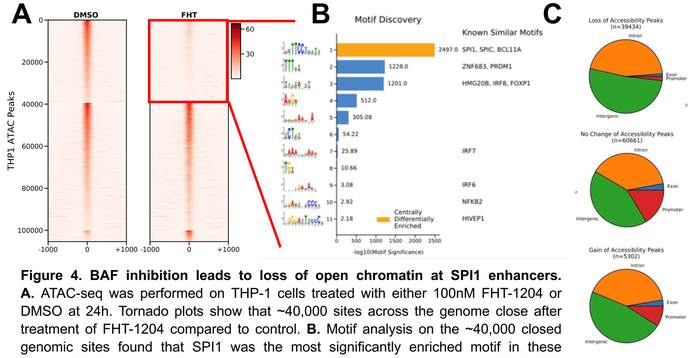


Figure 4. BAF inhibition leads to loss of open chromatin at SPI1 enhancers. A. ATAC-seq was performed on THP-1 cells treated with either 100nM FHT-1204 or DMSO at 24h. Tornado plots show that ~40,000 sites across the genome close after treatment of FHT-1204 compared to control. **B.** Motif analysis on the ~40,000 closed genomic sites found that SPI1 was the most significantly enriched motif in these regions. **C.** Comparison of the genomic location of open chromatin based on FHT-1204 treatment. Note that promoter regions make up less than 5% of peaks at sites that lose accessibility after BAF inhibition.

RNA-seq/SNAP-seq demonstrate direct regulation of SPI1 regulatory elements by BAF

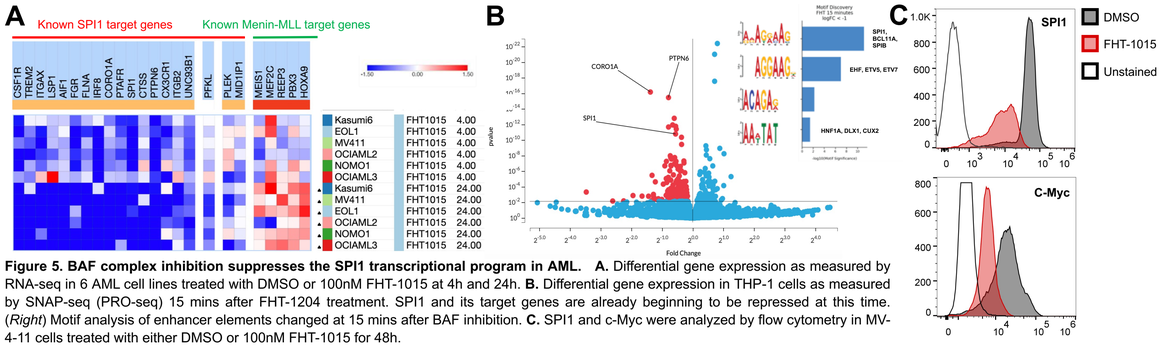


Figure 5. BAF complex inhibition suppresses the SPI1 transcriptional program in AML. A. Differential gene expression as measured by RNA-seq in 6 AML cell lines treated with DMSO or 100nM FHT-1015 at 4h and 24h. **B.** Differential gene expression in THP-1 cells as measured by SNAP-seq (PRO-seq) 15 mins after FHT-1204 treatment. SPI1 and its target genes are already beginning to be repressed at this time. (*Right*) Motif analysis of enhancer elements changed at 15 mins after BAF inhibition. **C.** SPI1 and c-Myc were analyzed by flow cytometry in MV-4-11 cells treated with either DMSO or 100nM FHT-1015 for 48h.

BAF inhibition results in a substantial loss of SPI1 protein levels across the genome

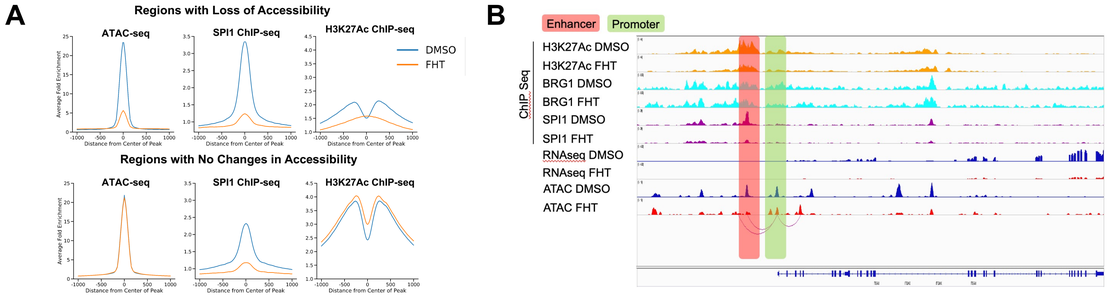


Figure 6. BAF inhibition disrupts SPI1 genomic placement. A. SPI1 and H3K27Ac levels are greatly reduced at regions of the genome that have a loss in accessibility in THP-1 cells treated for 24h with 100nM FHT-1204. **B.** An overview of the changes induced by BAF inhibition at the SPI1 target gene *ITGAX* (CD11c) loci. Loss of SPI1 at an enhancer results in closed chromatin, and a loss of both H3K27Ac and *ITGAX* gene expression.

SMARCA4/SMARCA2 ATPase inhibition causes tumor growth inhibition at a well tolerated dose

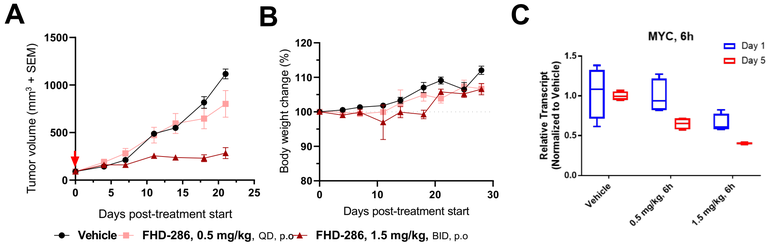


Figure 7. BAF dual ATPase treatment inhibits AML tumor growth *in vivo*. A. Dose-dependent of efficacy of FHD-286 in a MV-4-11 AML xenograft model. **B.** Percent body weight change of animals following dosing with FHD-286. **C.** Dose- and time-dependent suppression of *MYC* transcription was observed 6 hours post-treatment.

Conclusions

- We have identified a series of novel potent and selective inhibitors of the SMARCA4 and SMARCA2 ATPases
- Dual BAF ATPase inhibition affects SPI1 levels and its downstream transcriptional program, impacting cell proliferation and cellular survival
- SMARCA4/SMARCA2 ATPase inhibition affects enhancer accessibility and causes reduced occupancy of SPI1 at enhancer elements in AML
- Treatment with BAF inhibitor in an AML xenograft model elicited tumor regression at tolerated doses, demonstrating therapeutic utility in AML
- We believe that BAF inhibition affects lineage-specific transcriptional programs beyond SPI1 and can have broad implications across a range of malignancies including non-SPI1 driven AML subtypes