Discovery of novel BAF inhibitors for the treatment of transcription factor-driven cancers

1224

Richard C. Centore, Luis M. M. Soares, Rishi G. Vaswani, Kana Ichikawa, Zhifang Li, Hong Fan, Jeremy Setser, David L. Lahr, Laura Zawadzke, Xueying Chen, Kimberly D. Barnash, Jordana Muwanguzi, , Neville Anthony, Gabriel J. Sandoval, Katharine Feldman, Ammar Adam, David Huang, Shawn Schiller, Kevin Wilson, Johannes Voigt, Martin Hentemann, David S. Millan, Ho Man Chan, Steven F. Bellon, Carl P. Decicco and Lan Xu

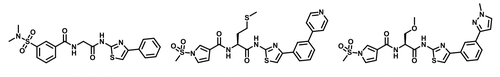
Foghorn Therapeutics, 500 Technology Square, Cambridge, MA 02139

FOGHORN THERAPEUTICS

Abstract

The BRG/Brahma-associated factors (BAF) family of chromatin remodeling complexes (also referred to as the mSWI/SNF complex) regulates the chromatin landscape of the genome. Through its ATP-dependent chromatin remodeling activity, BAF regulates the accessibility of gene-control elements, allowing for the binding of transcription factors. Thus, BAF is a major regulator of lineage- and disease-specific transcriptional programs. We have discovered and developed a novel series of compounds that potently and selectively inhibits the ATPase components of the BAF complex, SMARCA4 and SMARCA2 (also called BRG1 and BRM, respectively). Mutational, structural, and biochemical studies demonstrated that these SMARCA4/SMARCA2 inhibitors act through a unique allosteric mechanism. Pharmacologic inhibition of the BAF complex resulted in lineage-specific changes in chromatin accessibility in cancer cell lines from diverse origins. Phenotypic screening of cancer cell lines showed that uveal melanoma and hematological cancer cell lines were exquisitely sensitive to BAF inhibition. In the example of uveal melanoma, BAF inhibition (BAFi) resulted in the loss of accessibility at the binding sites of the SOX10 and MITF transcription factors, two essential proteins in supporting the proliferation and survival of uveal melanoma cells. Enhancer occupancy of SOX10 and MITF was reduced upon BAF inhibition, and subsequently, the melanocytic and pigmentation gene expression program regulated by these master transcription factors was suppressed. Finally, in a mouse xenograft model of uveal melanoma, BAF inhibition was well tolerated and resulted in dose-dependent tumor regression that correlated with pharmacodynamic modulation of BAF-target gene expression. These data provide the foundation for first-in-human studies of BAF ATPase inhibition as a novel therapeutic to treat uveal melanoma.

Discovery and characterization of novel SMARCA4/SMARCA2 ATPase inhibitors



B

Compound	IC_{50} (μM) SMARCA4 ATPase	SMARCA2 ATPase	CHD4 ATPase
FHT-185	6.04 ± 1.04 (n=6)	2.54 ± 0.45 (n=7)	> 400 (n=8)
FHT-1015	0.004 ± 0.001 (n=3)	0.005 ± 0.004 (n=3)	> 400 (n=2)
FHT-2344	0.026 ± 0.006 (n=5)	0.013 ± 0.003 (n=5)	> 200 (n=4)

C

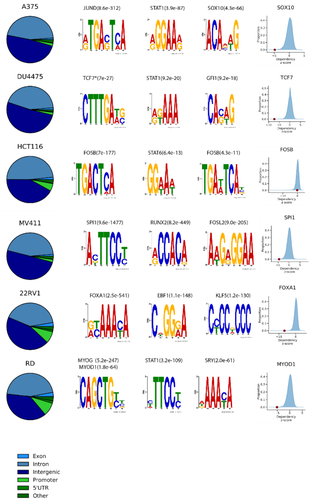


Figure 1. Discovery and characterization of a novel series of SMARCA4/SMARCA2 ATPase inhibitors with lineage-specific effects on chromatin accessibility profiles. A. Structures of inhibitors. **B.** Biochemical IC_{50} values demonstrating ATPase inhibition of SMARCA4 and SMARCA2, but not of the closely related ATPase, CHD4. **C.** SMARCA4/SMARCA2 ATPase inhibition elicits lineage-specific effects on chromatin accessibility. Pie charts representing annotation of ATAC-seq peaks that are reduced in the indicated cell lines following treatment with FHT-1015 (left). The bulk of regions with reduced accessibility following BAF inhibition are promoter-distal. Motif analysis identified lineage-specific transcription factor (TF) binding motifs at sites of reduced accessibility (middle). In most cases, these motifs correspond to master TFs with strong dependencies unique to that particular cell line's lineage.

Resistance studies demonstrate on-target activity of FHT-1015

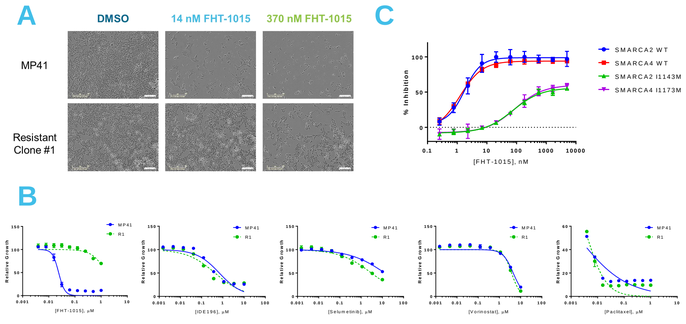


Figure 2. SMARCA4 I1143M mutation confers resistance to FHT-1015. A. Images of parental MP41 cells or MP41-derived cells that emerged after prolonged exposure to FHT-1015. Whole exome sequencing identified the I1173M mutation as unique to the resistant cells (not shown). **B.** Resistant cells tolerate growth in the presence of FHT-1015, but maintain sensitivity to other agents. **C.** The SMARCA4 I1143M or the corresponding SMARCA2 I1173M mutation conferred resistance to enzymatic inhibition by FHT-1015.

Uveal melanoma cell lines are exquisitely sensitive to SMARCA4/SMARCA2 ATPase inhibition

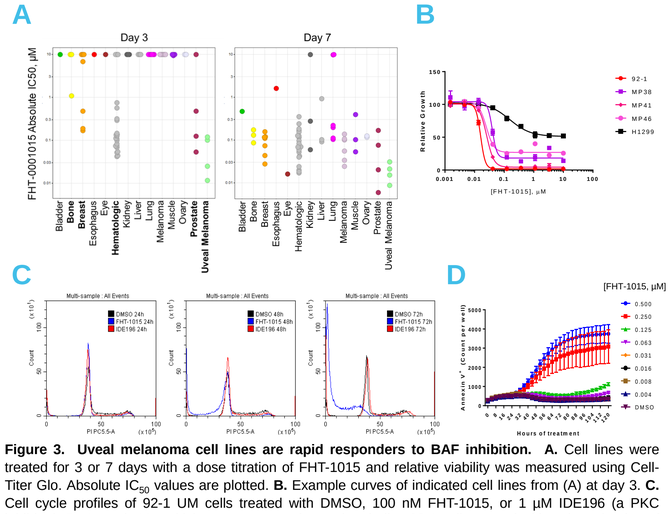


Figure 3. Uveal melanoma cell lines are rapid responders to BAF inhibition. A. Cell lines were treated for 3 or 7 days with a dose titration of FHT-1015 and relative viability was measured using Cell-Titer Glo. Absolute IC_{50} values are plotted. **B.** Example curves of indicated cell lines from (A) at day 3. **C.** Cell cycle profiles of 92-1 UM cells treated with DMSO, 100 nM FHT-1015, or 1 μM IDE196 (a PKC inhibitor). **D.** Annexin V positivity was measured over time in live 92-1 cells treated with a dose titration of FHT-1015.

SMARCA4/SMARCA2 inhibition alters the enhancer accessibility of critical disease-associated transcription factors

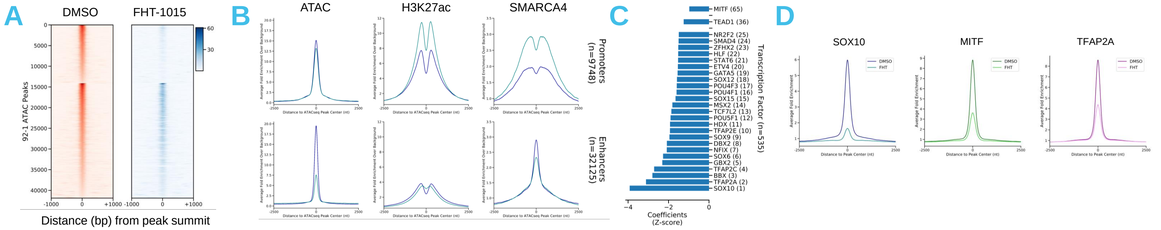


Figure 4. BAFi alters enhancer accessibility of UM master TFs. A. ATAC-seq profiles of 92-1 cells treated with DMSO or FHT-1015. **B.** ATAC-seq and H3K27ac or SMARCA4 ChIP-seq peaks were annotated as promoter- or enhancer-associated, and effects on global enrichment following FHT-1015 treatment are shown. **C.** SOX10 and TFAP2A binding motifs are most enriched at sites of reduced chromatin accessibility following BAFi. **D.** BAFi causes loss of enhancer occupancy of SOX10, MITF, and TFAP2A transcription factors, as measured by ChIP-seq.

SMARCA4/SMARCA2 inhibition disrupts the SOX10-MITF transcriptional axis in uveal melanoma

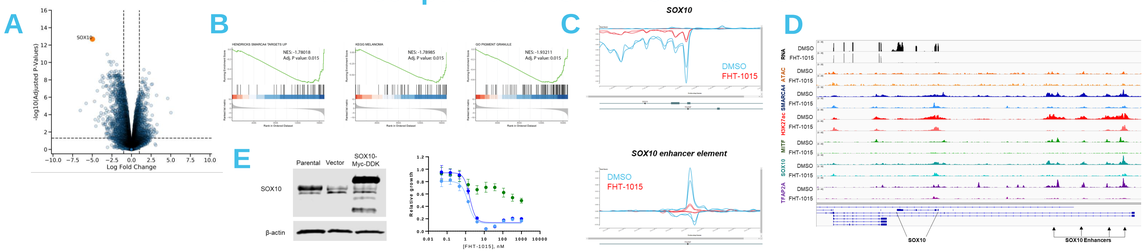


Figure 5. BAFi disrupts the SOX10-MITF transcriptional axis. A. Differential gene expression as measured by RNA-seq in 92-1 cells treated with DMSO or FHT-1015. **B.** GSEA identified SMARCA4 targets, melanoma, and pigmentation gene sets as enriched among genes down-regulated by FHT-1015 treatment. **C.** Nascent transcripts at the *SOX10* gene body and enhancer are down-regulated within 1 hour of FHT-1015 treatment in MP46 UM cells. **D.** Genome browser view of the *SOX10* locus, showing the loss of accessibility, SMARCA4, and TF occupancy at the SOX10 enhancers following FHT-1015 treatment in 92-1 cells. **E.** Forced expression of *SOX10* from a BAF-independent promoter can rescue the growth inhibition phenotype elicited by FHT-1015.

SMARCA4/SMARCA2 ATPase inhibition causes UM tumor regression

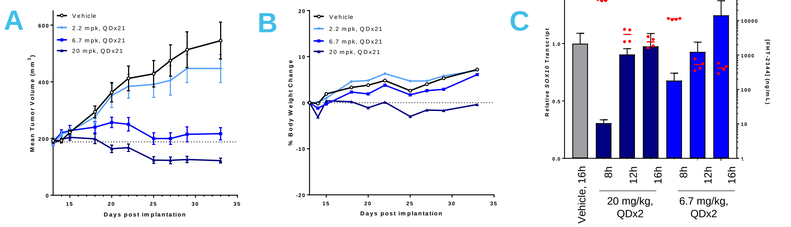


Figure 6. BAFi inhibits UM tumor growth *in vivo*. A. Dose-dependent of efficacy of FHT-2344 in a 92-1 UM xenograft model. **B.** Percent body weight change of animals following dosing with FHT-2344. **C.** Dose- and exposure-dependent suppression of *SOX10* transcription was observed 8 hours post-dose of FHT-2344.

Conclusions

- We have identified a novel series of potent and selective inhibitors of the SMARCA4 and SMARCA2 ATPases
- BAF ATPase inhibition affects the proliferation of many cancer cell types, and elicits rapid effects on UM, hematological cancer, and other cell lines
- SMARCA4/SMARCA2 ATPase inhibition affects lineage-specific enhancer accessibility, and causes reduced occupancy of master disease-associated TFs in UM.
- In UM, BAFi inhibits the master TF, SOX10, and its downstream transcriptional program, impacting cell survival and lineage specification.
- Foghorn tool compound elicited tumor regression at tolerated doses, demonstrating therapeutic utility in UM and perhaps other TF-driven cancers.