Treatment with dual BRG1/BRM (SMARCA4/2) inhibitor FHD-286 ablates tumor-associated androgen response elements (AREs) in prostate cancer

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Abstract

The Androgen Receptor (AR) cistrome is critical in the development of prostate cell identity while its misregulation promotes prostate cancer development. The pioneer transcription factor Forkhead box A1 (FOXA1) has been shown to be essential for the recruitment of AR to androgen response elements (AREs) allowing for reprogramming of the AR cistrome resulting in prostate cell transformation. FHD-286 is a BRM/BRG1 dual ATPase inhibitor currently in clinical trials for AML. Here, we show that treatment of prostate cancer cell lines with FHD-286 results in ablation of FOXA1 mediated AREs at tumor-associated AR binding sites (T-ARBS). Dual ATPase treatment subsequently reduces the expression level of various oncogenic AR target genes, leading to a decrease in tumor cell viability. Both patient derived organoid and In vivo studies provide further validation by showing a decrease in tumor growth. Strikingly, inhibition of BAF complex activity bypasses AR resistance mechanisms commonly seen after castration and Enzalutamide treatment as cell lines containing AR-V7 splice variants and neuroendocrine organoids display sensitivity. Taken together, our data illustrates a novel mechanism of treating AR mediated prostate cancer though the inhibition of tumor associated AREs by treatment with FHD-286.

Prostate Cancer cell lines are sensitive to BAF ATPase inhibition

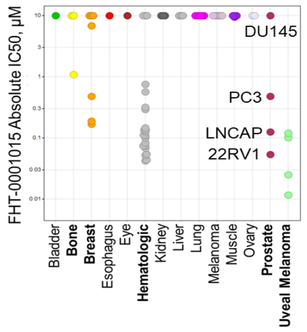
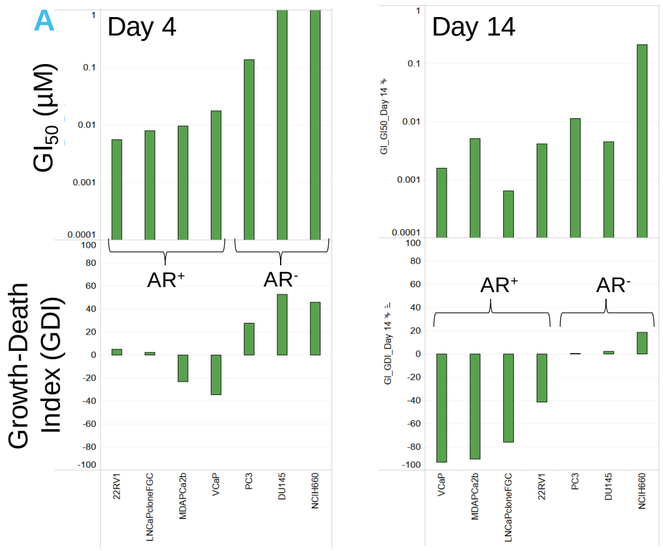


Figure 1. BAF ATPase inhibition reduces viability in Prostate Cancer cells. Various cancer lineages were treated with the dual ATPase inhibitor (FHT-0001015) for three days. Cell Titer Glo™ was used as a readout for viability and an absolute IC50 was calculated for each cell line. Highlighted are prostate cancer cells, which show a strong sensitivity to BAF inhibition.

Treatment with FHD-286 results in a cell death phenotype in AR+ Prostate Cancer cells



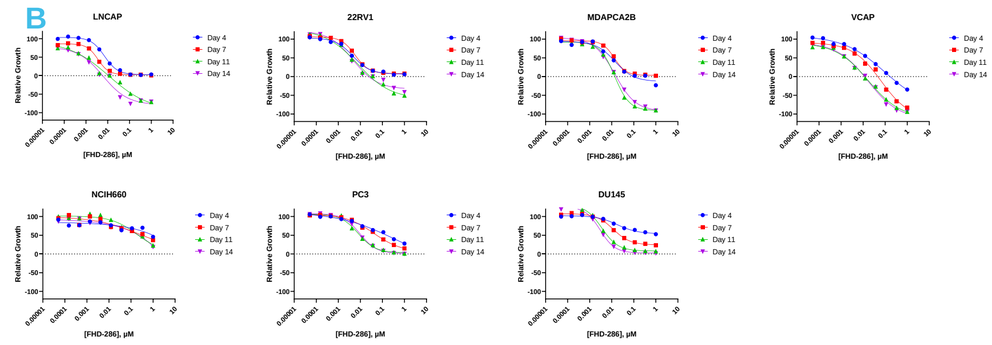
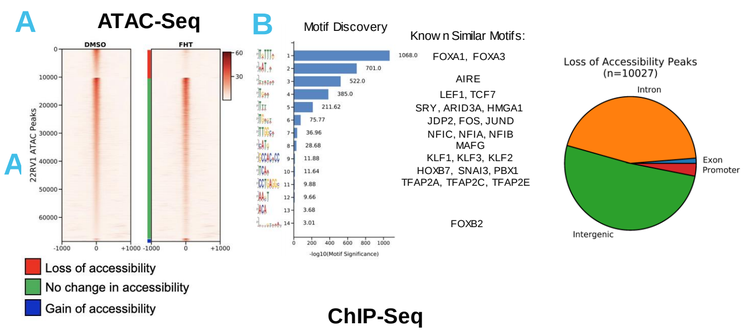


Figure 2. BAF inhibition results in reduced proliferation or death in prostate cancer cells depending on AR status. A. Growth Inhibition (GI50) and Growth Death Index (GDI) of prostate cancer cell lines treated with the dual ATPase inhibitor for 4 and 14 day. Androgen Receptor (AR) positive cells (22rv1, LnCap clone FGC, MDAPCa2b, VCAP) displayed selective death over AR negative cells (PC3, DU145, NCHI660). B. Relative growth curves of AR+ and AR- lines treated with FHD-286 for 4, 7, 11 and 14 days. AR+ lines show a distinct cell death phenotype as viability falls below base levels at 11 and 14 days of treatment. Cell proliferation slows in AR- lines, but no cell death phenotype is observed.

BAF ATPase inhibition causes loss of chromatin accessibility at FOXA1 enhancers, reduces occupancy of AR at T-ARBs and down-regulates AR/FOXA1 target genes



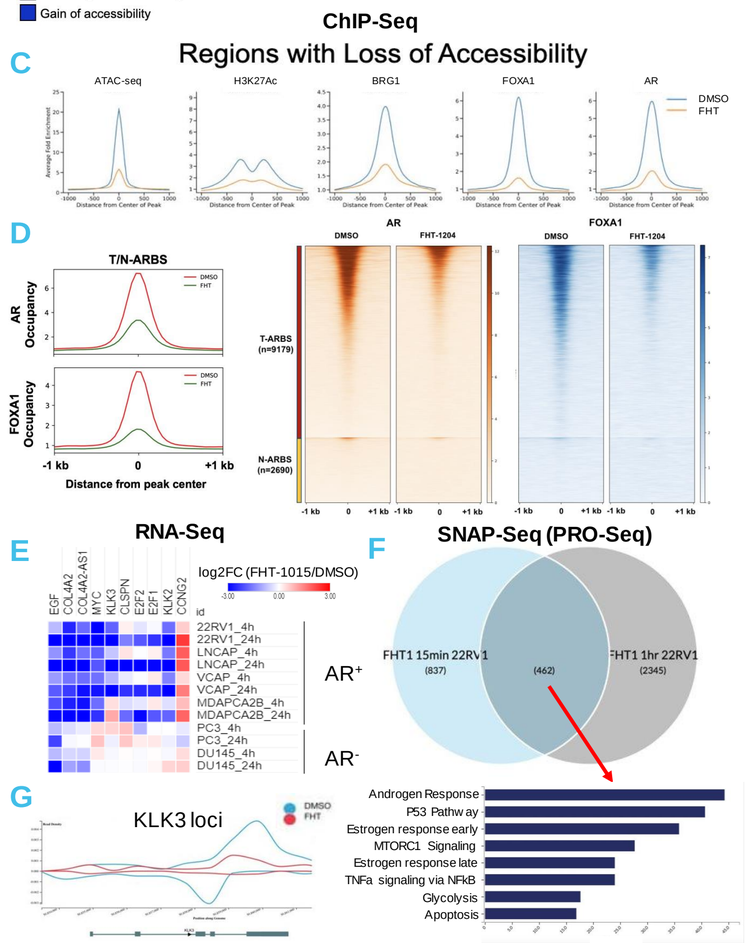


Figure 3. FHD-286 alters chromatin accessibility A. Tornado plots from ATAC-seq on AR+ 22rv1 cell lines treated with DMSO and FHT-1204. A loss of chromatin accessibility is seen with dual ATPase treatment. B. Motif discovery from ATAC-seq shows a loss of accessibility at FOXA1 motifs at enhancers (*right*). C. Chromatin Immunoprecipitation sequencing (ChIP-seq) of AR+ 22rv1 cell line treated with DMSO and FHD-286. This confirms that AR and FOXA1, can no longer occupy chromatin at regions of the genome that correspond to a loss of accessibility from ATAC-seq. D. AR and FOXA1 occupancy is diminished at T-ARBs (See Ref 1.). E. Relative expression of select genes via RNA-seq of AR+ and AR- cell lines treated with a dual ATPase inhibitor (FHT-0001015) for 4 and 24 hours shows downregulation of AR targets. F/G. Pro-seq analysis reveals that the AR gene signature is down-regulated as early as 15min-1hr after dual ATPase treatment, with KLK3 as an example loci.

Both AR+ and neuroendocrine prostate organoid models are sensitive to FHD-286

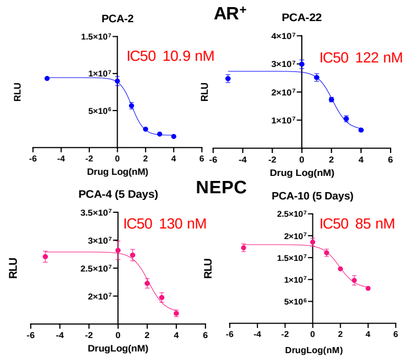
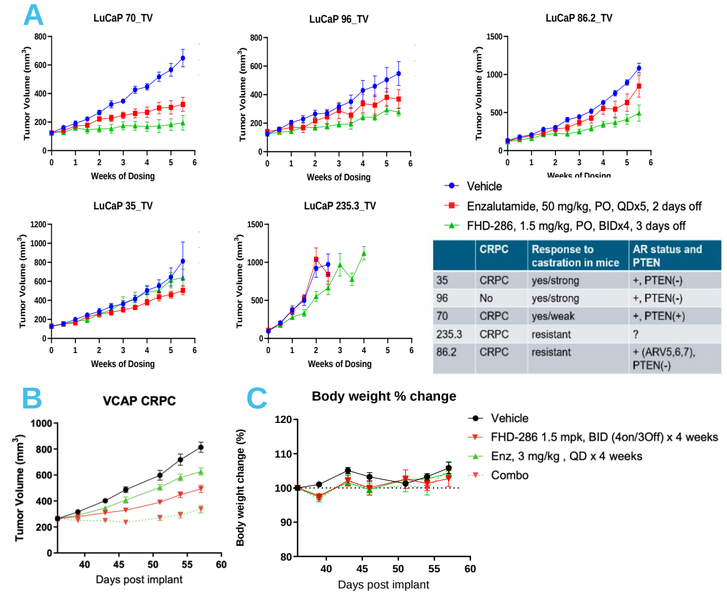


Figure 4. AR+ and NEPC organoid models show at least partial sensitivity to dual BAF ATPase inhibition. Both AR-positive (PCA-2, PCA-22: *top*) and NEPC (PCA-4, PCA-10: *bottom*) prostate organoid models were treated with FHD-286 at various doses to establish an IC50 for each model based on luminescence readout. IC50s for each line are highlighted in red for each model.

FHD-286 causes tumor growth inhibition in AR+ prostate in vivo cancer models



	CRPC	Response to castration in mice	AR status and PTEN
35	CRPC	yes/strong	+, PTEN(-)
96	No	yes/strong	+, PTEN(-)
70	CRPC	yes/weak	+, PTEN(+)
235.3	CRPC	resistant	?
86.2	CRPC	resistant	+ (ARV5,6,7), PTEN(-)

Figure 5: FHD-286 treatment results in tumor growth inhibition. A. Volume of tumors (nm^3) from AR+ Patient Derived Xenograft (PDX) models treated with vehicle, Enzalutamide and FHD-286 for 6 weeks. 4 out of 5 lines are castration resistant prostate cancer (CRPC). Treatment with FHD-286 lead to decreased tumor growth in most PDX models. B. Volume of tumors from AR+ VCaP tumors treated with vehicle, Enzalutamide, FHD-286 or Combo for 4 weeks. Treatment with Enzalutamide, FHD-286 and combination of both drugs resulted in reduced tumor growth in a dose dependent manner. Notably, the combination of Enzalutamide and FHD-286 resulted in larger reduction of tumor volume than either drug alone. C. Body weight of corresponding mice from Fig B.

Conclusions

- Prostate Cancer cells are sensitive to treatment with a BAF dual ATPase inhibitor and AR+ models display a cell death phenotype.
- Dual ATPase treatment ablates FOXA1 and AR occupation at tumor-associated AR binding sites, resulting in the down-regulation of AR target genes.
- Treatment with FHD-286 inhibits tumor growth in both organoid and in vivo models.

References

1. Pomerantz M.M. et al. The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. *Nat Genet* **47**, 1346–1351 (2015). https://doi.org/10.1038/ng.3419