FT-6876, A Potent and Selective Inhibitor of CBP/p300 with Antitumor Activity in AR-Positive Breast Cancer

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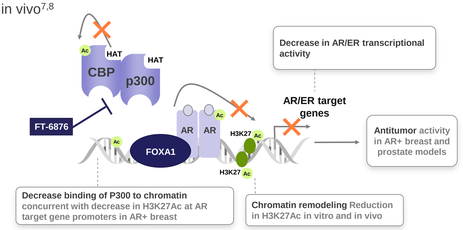
INTRODUCTION

CBP/p300 in AR+ Breast Cancer

- CREB binding protein, CBP, and E1A binding protein, p300, are closely related, multi-domain proteins that function as acetyltransferases and contain acetyl-lysine binding bromodomains[1]
- CBP/p300 function as transcriptional co-activators of numerous oncogenic transcription factors including the androgen receptor (AR)
  - CBP/p300 increases H3K27Ac relaxing chromatin and increasing transcriptional activity
  - CBP/p300 has been shown to acetylate AR stabilizing the receptor[2]
  - CBP/p300 interacts directly with AR at both the N and C-terminus of AR including with truncated forms of AR lacking the ligand-binding domain[3]

- A subset of triple negative breast cancers (TNBCs) express the AR which can substitute for the estrogen receptor to drive its oncogenic transcriptional signature
  - AR+ breast cancers have lower Ki67 expression and are less sensitive to chemotherapy than other types of TNBC[4]
  - AR antagonists have been tested clinically and have shown some early activity in AR+ breast cancer[5,6]
  - AR dependency of MDA-MB-453, a model of AR+ breast cancer has been demonstrated both in vitro and in vivo[7,8]

A CBP/p300 inhibitor is expected to reduce AR transcriptional activity in AR+ cancers and inhibit proliferation



CONCLUSIONS

- **FT-6876 is a potent, selective, bromodomain inhibitor of CBP/p300 that induces the rapid, reversible reduction of H3K27Ac**
- **FT-6876 impacts the transcriptional programs of the androgen receptor and estrogen receptor in AR+ TNBC**
- **FT-6876 has antiproliferative activity in AR+ breast cancer models in vitro and in vivo as well as AR+ prostate cancer models including AR-v7 positive**
- **A PK/PD relationship with FT-6876 was demonstrated with proximal and distal biomarkers, H3K27Ac and ER/AR genes respectively, of CBP/p300 activity which correlates with complete tumor growth inhibition at a well tolerated dose**
- **FT-6876 represents a means to study CBP/p300 biology in the context of its co-regulation of nuclear receptors such as AR in AR+ breast cancer and prostate cancer**

ABBREVIATIONS. AR, androgen receptor; CBP, CREB binding protein; BRD, bromodomain; TNBC, triple negative breast cancer.

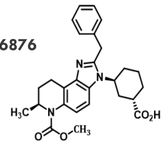
REFERENCES. 1. Marmostein et al. 2014; 2. Fu et al. 2003; 3. Fronsdal et al. 1998; 4. Lehmann et al. 2011; 5. Gucalp et al. 2013; 6. Traina et al. 2018; 7. Robinson et al. 2011; 8. Cochrane et al. 2014

RESULTS

Figure 1. FT-6876 is a Potent, Selective CBP/p300 Bromodomain Inhibitor

A.

FT-6876



B.

Bromodomain (TRF Assay)	FT-6876 IC50 (μM)
CBP	0.005
p300	0.002
BRD4	2.32

C.

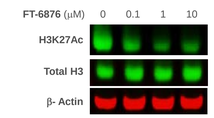
Bromodomain	FT-6876 IC50 (μM)
CBP*	0.06
BRD4*	5.7
TAF1-2*	8.7
ATAD2*	>10
BPTF*	>10
BRPF3*	>10
BRWD1*	>10
CECR2*	>10
SMARCA4*	>10
SP140*	>10
BRD9**	26.7
ASH1L**	>30
BRWD3**	>30

- FT-6876 is a pentacyclic carboxylic acid inhibitor of the bromodomains of CBP and p300 (**Figure 1A**)
- FT-6876 is potent against CBP/p300 and highly selective against BRD4 and other bromodomains representing the branches of the bromodomain tree
  * AlphaScreen™ & ** TRF assay (**Figure 1B & 1C**).

Figure 2. FT-6876 Reduces H3K27Ac in MDA-MB-453 Cells

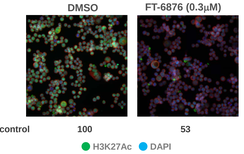
A.

Rapid Reduction of H3K27Ac After Exposure to FT-6876



B.

Uniform Reduction of H3K27Ac in Cells After Exposure to FT-6876



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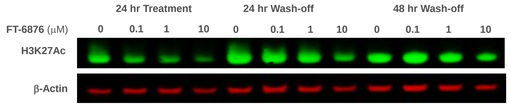
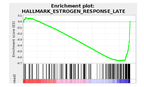


- FT-6876 induces the rapid reduction of H3K27Ac in MDA-MB-453 cells demonstrated by western blot (1 hr) (**Figure 2A**) and confirmed by high content analysis (24 hr) (**Figure 2B**)
- The reduction is reversible, returning to baseline following removal of the compound (**Figure 2C**)

Figure 3. FT-6876 Modulates ER and AR Target Genes in MDA-MB-453 Cells

A.

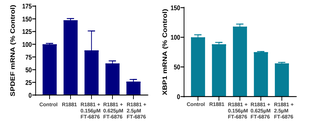
Gene Set Enrichment 24 hr	Rank
HALLMARK_MYC_TARGETS_V1	1
HALLMARK_E2F_TARGETS	2
HALLMARK_MYC_TARGETS_V2	3
HALLMARK_ESTROGEN_RESPONSE_LATE	4
HALLMARK_ESTROGEN_RESPONSE_EARLY	5
HALLMARK_ANDROGEN_RESPONSE	14



NES=-1.84: FDR<0.001

NES=-2.1: FDR<0.001

B.



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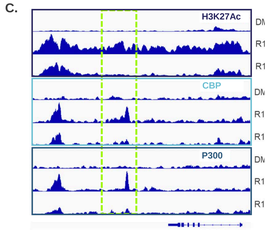
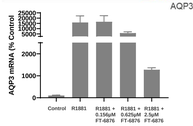


D.



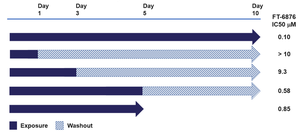
- Gene set enrichment analysis (GSEA) of RNAseq data from MDA-MB-453 cells exposed to 2 μM FT-6876 for 24 hr (NES= Normalized enrichment scores; FDR= False discovery rate) (**Figure 3A**)
- mRNA expression of AR (SPDEF) and ER (XBP1) target genes after exposure to AR agonist R1881 ± FT-6876 (**Figure 3B**)
- Reduced CBP and p300 binding upstream of the ER regulated gene, AQP3 is concurrent with reduction in H3K27Ac at this location denoted by the green dotted box (**Figure 3C**) and associated with a reduction of AQP3 mRNA levels (**Figure 3D**)

Figure 4. FT-6876 has Anti-proliferative Activity in AR+ Breast and Prostate Cancer Cell Lines

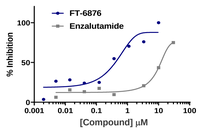
A.

Cell Line	AR Status	FT-6876 IC50 (μM)
MDA-MB-453	WT	0.197 ± 0.087
LnCaP	T878A/WT	0.5 ± 0.50
VCaP	WT	0.5 ± 0.50
22RV1	H875Y/H875Y AR-v7+	0.6 ± 0.036
PC-3	AR negative	1.8 ± 0.017
DU-145	AR negative	5.4 ± 0.011
BPH1	AR negative	5.1 ± 0.012

B. Time-dependent Activity of FT-6876 on the Proliferation of MDA-MB-453 Cells



C. MDA-MB-453 Colony Formation Assay



	FT-6876	Enzalutamide
IC50	0.2621	11.66

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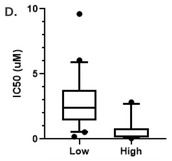
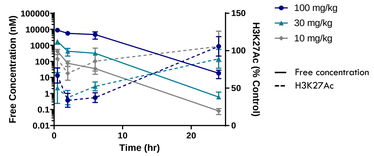


- FT-6876 inhibits proliferation of AR+ breast cancer cell line MDA-MB-453. FT-6876 also potently inhibits the proliferation of AR+ prostate cancer models including the AR-v7 expressing 22Rv1 cells (**Figure 4A**)
- Time-dependent growth inhibition is demonstrated in MDA-MB-453 cells with maximal inhibition observed after 10 days continuous exposure; 5 days of exposure defines the minimal period to achieve sustained growth inhibition (**Figure 4B**)
- FT-6876 is more than 40 times as potent as enzalutamide against MDA-MB-453 cells and preferentially active against breast cancer cell lines expressing high levels of AR (**Figure 4C & 4D**)

Figure 5. FT-6876 Induces Pharmacodynamic and Anti-tumor Effects in MDA-MB-453 Xenografts

A.



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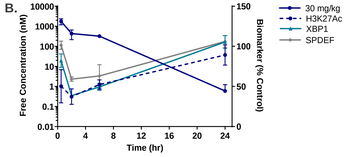

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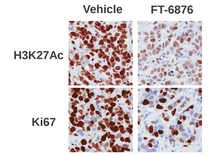

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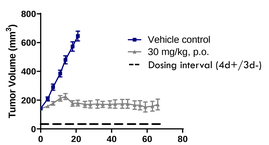

- FT-6876 induced a time and dose dependent PK/PD modulation in MDA-MB-453 xenografts. Free plasma concentration of FT-6876 solid lines, H3K27Ac dotted lines (**Figure 5A**)
- The level of unbound FT-6876 in plasma correlated with modulation of H3K27Ac and ER/AR target genes, (**Figure 5B**)
- Reduction of H3K27 was associated with a reduced proliferation index (Ki-67) by IHC (**Figure 5C**)
- FT-6876 induced tumor stasis in this model of AR+ TNBC (**Figure 5D**)