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P192

Comprehensive preclinical characterization of the mechanism of action of EPI-7386, an androgen receptor N-terminal domain inhibitor

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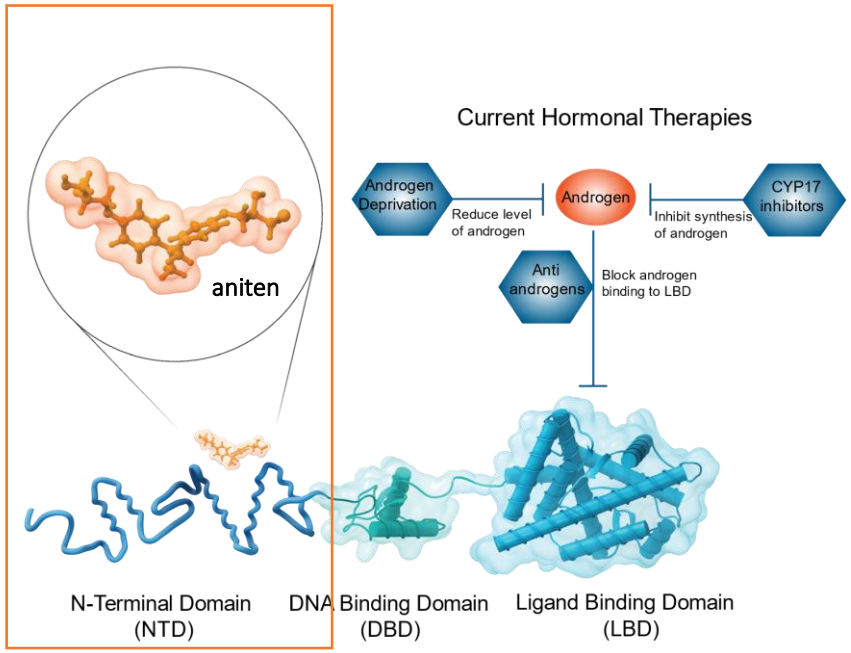
I have the following financial relationships to disclose:

Stockholder in: ESSA Pharma

Employee of: ESSA Pharma

Background

- As a key driver of prostate cancer, androgen receptor (AR) signaling has been a major target of prostate cancer therapy
- All current antiandrogens function through the ligand-binding domain (LBD) of the AR
- Antiandrogen resistance mechanisms generally develop at the LBD, due to mutations in the LBD itself and the expression of constitutively active splice variants of AR that lack the LBD
- Anitens are small molecules capable of targeting the AR N-terminal domain (NTD) that can inhibit transcriptional activity of the AR even in the presence of LBD-driven antiandrogen resistance
- A first-generation aniten and its stereoisomers have been shown specifically to bind to the transactivation unit 5 (Tau5) of AR NTD and block essential protein-protein interactions required for the transcriptional activity of AR[1-3]
- EPI-7386 is a second generation aniten which exhibits high potency, low metabolism, and on-target specificity



Anitens are first-in-class NTD inhibitors of the androgen receptor. Structure of the androgen receptor (AR) and the mechanism of antiandrogen inhibition. The AR comprises three main functional domains: the LBD involved in binding with androgens, the DBD, and the NTD that orchestrates the transactivation of the receptor.

[1]*De Mol et al., ACS Chem Biol, 2016,* [2]*Andersen et al., Cancer Cell, 2010,* [3]*Sadar, Cancer Res, 2011*

Objectives

Interaction of EPI-7386 with N-terminal domain of androgen receptor

- Protein-observed NMR

  monitor chemical shift perturbations observed on purified AR-NTD (13C isotope labeled) NMR spectra upon EPI-7386 binding

- Ligand-observed NMR

  monitor ligand (EPI-7386) changes with NMR upon binding of AR-NTD

  a. Saturation transfer difference (STD)-NMR
  b. T2-CPMG

Target engagement of EPI-7386 with LBD truncated AR-variant in cells

- Cellular thermal shift assay (CETSA)

Regulation of AR variant driven gene expression

- Nanostring nCounter System platform using AR custom panel

Effect of EPI-7386 on AR cistrome

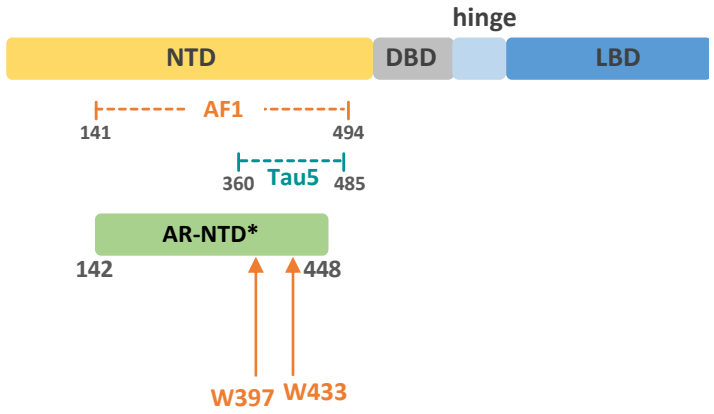
- Chromatin Immunoprecipitation sequencing (ChIP-seq)

EPI-7386 interacts with Tau5 region of AR-NTD:

EPI-7386 induces consistent chemical shift perturbations for two tryptophans in Tau5 region of AR-NTD

This work was conducted in collaboration with ZOBIO

A



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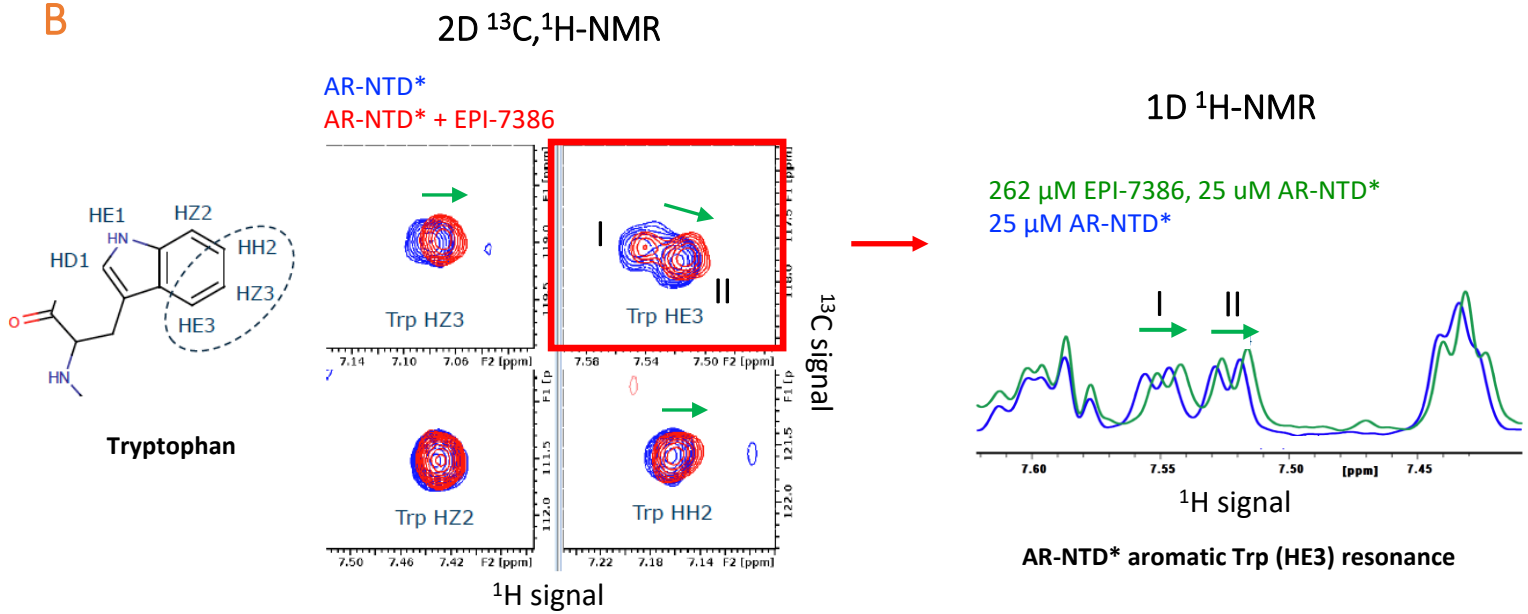

NMR signals shift is observed (green arrows),
indicating interaction between EPI-7386 and W397/W433 in AR-NTD*

(A) Schematic of androgen receptor showing AR-NTD* used in the NMR study. Transcription activation unit 5 (Tau5) contained in AR-NTD* is depicted (shaded in grey), which contains two tryptophans (Trps) interact with EPI-7386 shown in (B).

(B) EPI-7386 induces consistent chemical shift perturbations for two tryptophan residues (W397 and W433) in Tau5 indicated in (A). The average environment of the Trp changes when EPI-7386 is present. Labels I and II refer to the corresponding HE3 resonances of the two Trps in the 1D and 2D spectra.

EPI-7386 interacts with AR-NTD:

The interaction of EPI-7386 with AR-NTD is confirmed by STD-NMR

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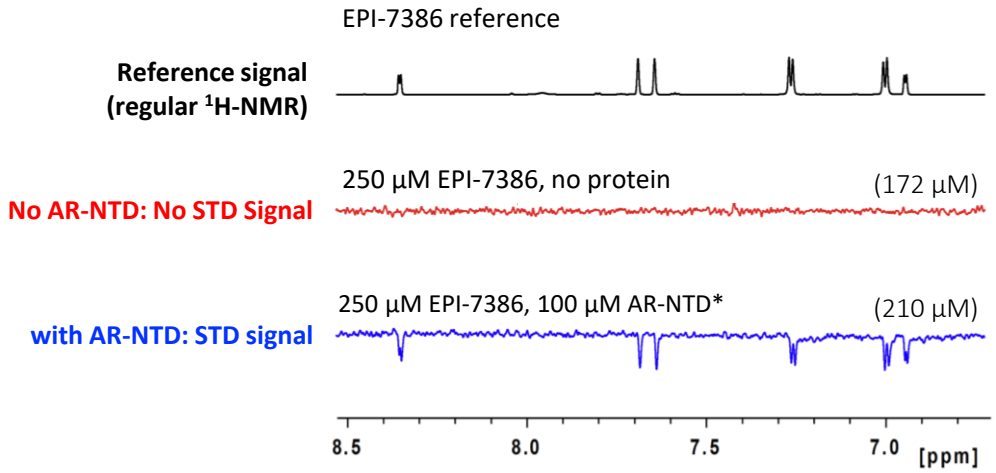
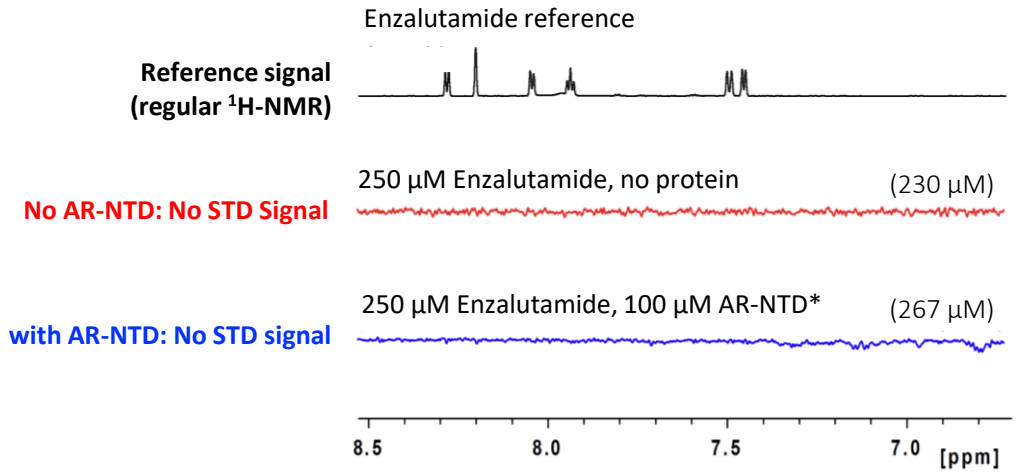
A

EPI-7386



B

Enzalutamide



*(NMR estimated concentration)

(A) EPI-7386 shows a clear STD signal, only in the presence of AR-NTD

(B) Enzalutamide does not show STD signal in the presence of AR-NTD

** Saturation transfer difference (STD)-NMR: In STD-NMR, Ligand protons that are in close contact with the irradiated protein receive a higher degree of saturation, and as a result stronger STD NMR signals can be observed.*

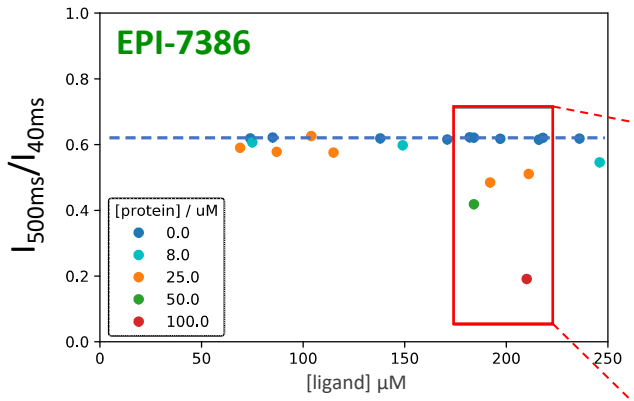
EPI-7386 interacts with AR-NTD:

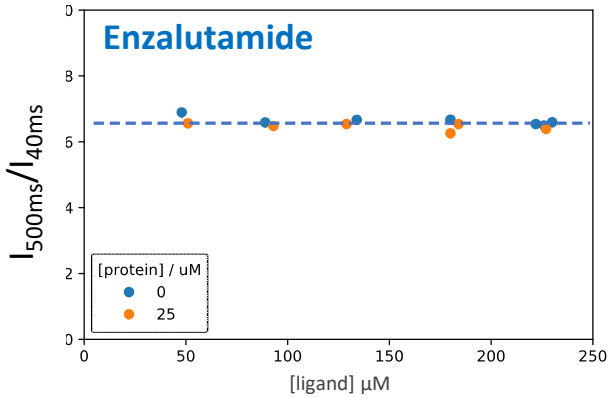
The interaction of EPI-7386 with AR-NTD is confirmed by T2-CPMG experiment

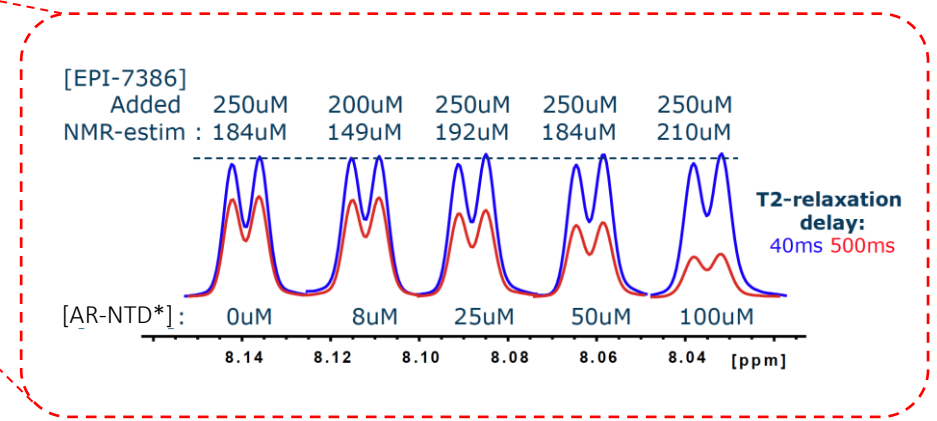
This work was conducted in collaboration with ZOBIO

- AR-NTD induces T2 relaxation of EPI-7386 in dose dependent manner
- AR-NTD does not induce T2 relaxation of enzalutamide

Mean T2-CPMG I_{500ms}/I_{40ms} aromatic peaks







The T2 relaxation of EPI-7386 increased in an AR-NTD concentration dependent manner, while we do not see this effect for Enzalutamide, a LBD binder.

** T2-CPMG: T2 relaxation of NMR signals increases at increasing rotational correlation time, and thus size of molecules. Binding to a larger protein causes a small molecule to have a higher apparent molecular size and increase T2 relaxation.*

EPI-7386 interacts with LBD truncated AR variant, AR-V567es:

EPI-7386 induces thermal shift of AR-V567es and inhibits AR-V567es driven gene expression

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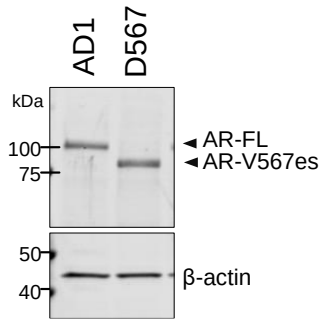
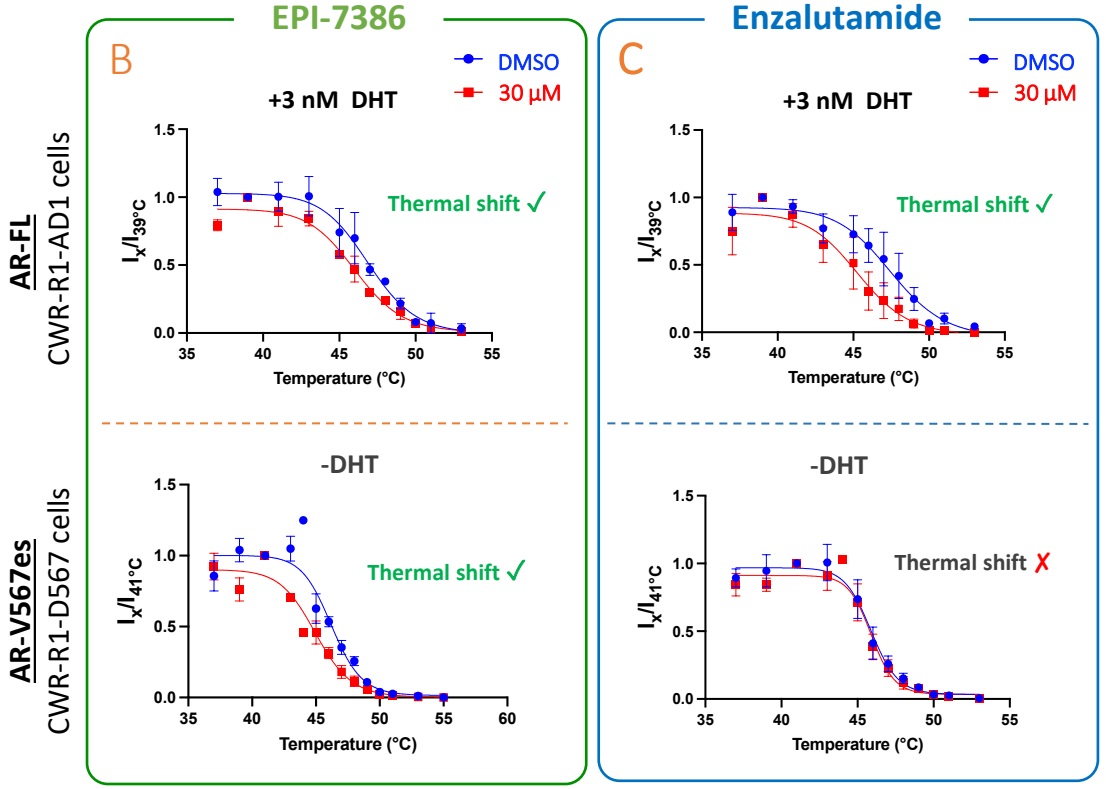


- CWR-R1-AD1 expresses **only** AR-FL
- CWR-R1-D567 expresses **only** AR-V567es



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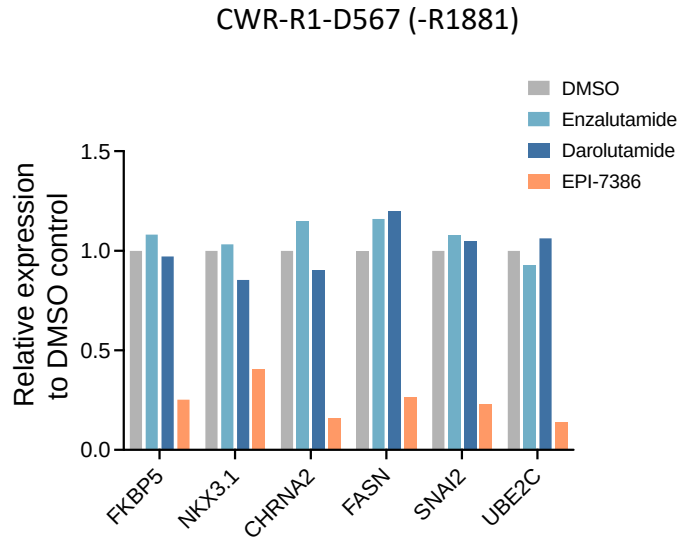


This work was conducted in collaboration with PELAGO BIOSCIENCE

(A) Western blot showing expression of AR-FL and AR-V567es in CWR-R1-AD1 and CWR-R1-D567 cells, respectively.

(B) EPI-7386 induces thermal shift of both AR-FL and AR-V567es melting curves, with a detectable and reproducible destabilization.

(C) In contrast, enzalutamide only induced destabilization of AR-FL and had no effect on AR-V567es thermal stability (B, bottom).

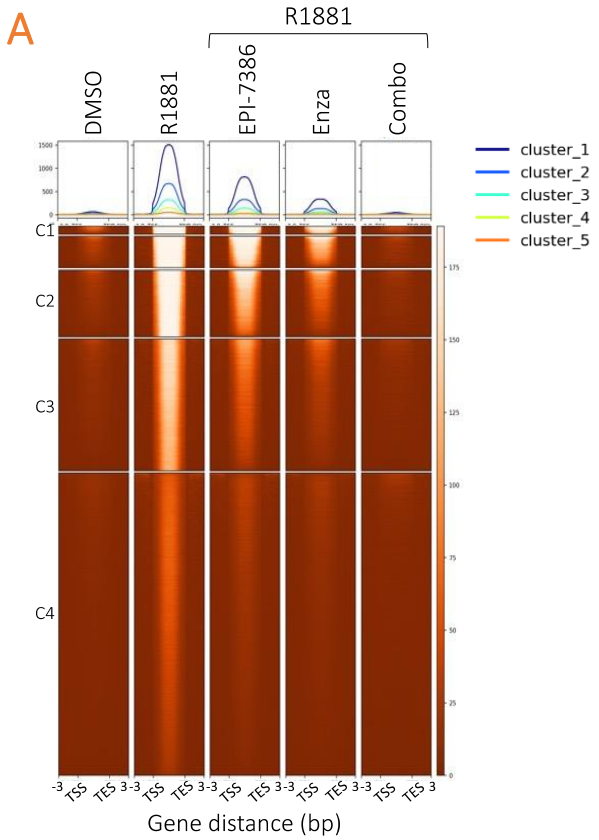
(D) EPI-7386 inhibits gene expression driven by LBD truncated AR variant, AR-V567es, while both enzalutamide and darolutamide which bind to LBD do not.

EPI-7386 inhibits genome-wide androgen induced AR binding:

EPI-7386 fully abrogates genome-wide androgen induced AR occupancy in combination with enzalutamide

**EPI-7386, enzalutamide, or combination treatment was done in presence of 1nM R1881*

A



B

AR peaks

treatment	Rep #1	Rep #2
DMSO	26,666	24,464
R1881, 1nM	121,340	131,546
EPI-7386, 10 µM	79,746	86,306
Enza, 10 µM	54,307	51,795
Combo, 10 + 10 µM	38,728	25,772

C

Enrichment of ARE

treatment	P-value	% sequence with ARE Motif
DMSO	1e-53	6.98
R1881, 1nM	1e-476	27.86
EPI-7386, 10 µM	1e-292	19.96
Enza, 10 µM	1e-223	16.11
Combo, 10 + 10 µM	1e-10	3.60

D

Treatment	Term	Adjusted p-value
EPI-7386	UV Response Down	7.15e-5
	Androgen Response	8.51e-4
Enzalutamide	UV Response Down	2.81e-4
	Androgen Response	0.0598
Combo	UV Response Down	7.27e-4
	Androgen Response	0.0297

(A,B) Heatmap view of AR ChIP-seq signals around AR peaks detected in LNCaP cells (A) and AR peak numbers (B) with treatments as indicated.

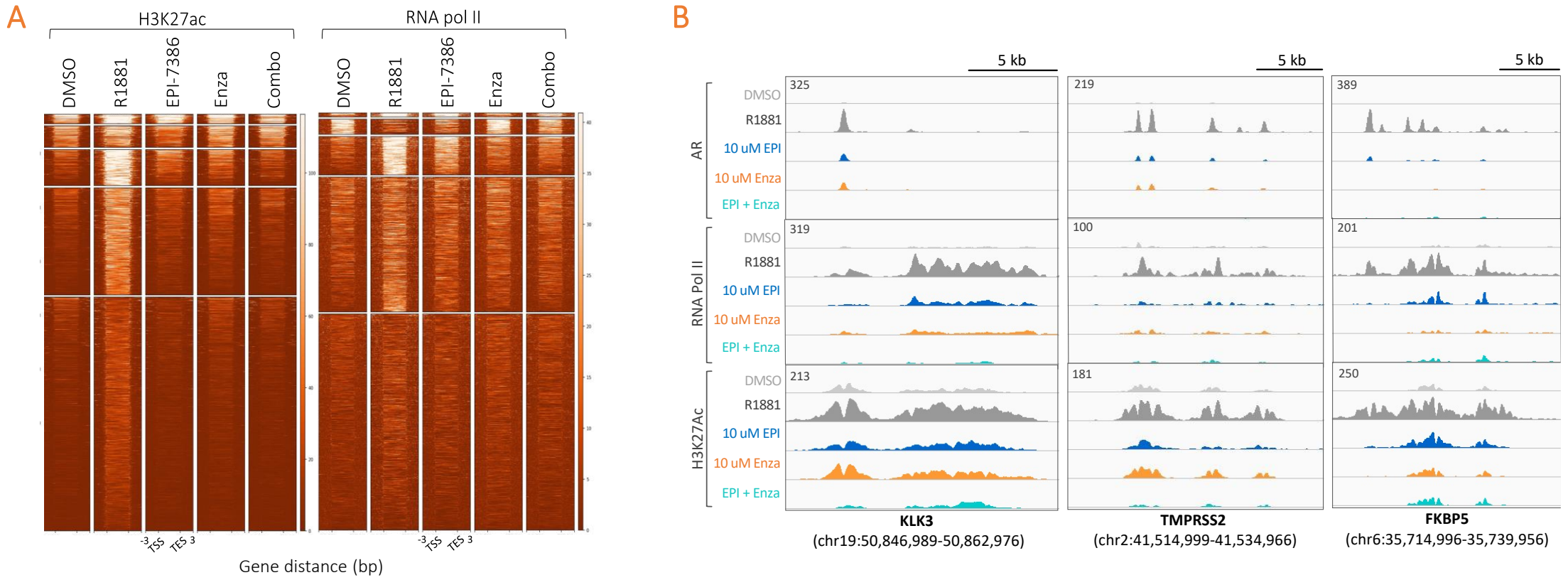
(C) Enrichment of androgen response element (ARE) in each treatment condition.

(D) Top enriched gene sets (Hallmark) associated with AR peaks lost by EPI-7386, enzalutamide, or combination treatment as compared to R1881.

EPI-7386 inhibits androgen-induced changes at the AR cistrome:

EPI-7386 reduces R1881-induced H3K27 acetylation and RNA polymerase II recruitment to AR binding sites

**EPI-7386, enzalutamide, or combination treatment was done in presence of 1nM R1881*



(A) Heatmap visualization for H3K27ac and RNA polymerase II signal at AR-binding sites. While indicated treatments induced changes in H3K27 acetylation and RNA polymerase II recruitment to AR binding site, no global changes were detected upon treatments.

(B) Gene track view of AR, RNA polymerase II, and H3K27ac ChIP-seq signal at KLK3, TMPRSS2. and FKBP5 loci.

Summary

Objectives	Outcome	Evidence
AR-NTD Binding	✓	Orthogonal NMR approaches consistently confirm EPI-7386 binding to Tau5 region of the AR NTD
Target engagement	✓	Cellular thermal shift assay confirms the engagement of EPI-7386 with both AR-FL and splice variants lacking LBD, including AR-V567es in cells
Gene expression	✓	EPI-7386 Inhibits AR-V567es driven gene expression
Genomic AR occupancy	✓	EPI-7386 inhibits androgen-induced changes at AR cistrome and completely abrogates AR binding in combination with enzalutamide

- A Phase 1 dose escalation clinical trial (NCT04421222) of EPI-7386 in men with mCRPC progressing on standard of care therapies including second generation anti-androgens is underway.
- A Phase 1/2 combination study of EPI-7386 and enzalutamide in men with mCRPC will soon be underway.