The preclinical characterization and development of EPI-7386, an N-terminal domain androgen receptor inhibitor for the treatment of prostate cancer

Abstract MP79-04
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BACKGROUND

The androgen receptor (AR) pathway continues to drive most castration-resistant prostate cancers (CRPC) even in late stages of the disease through resistance mechanisms, including gain-of-function mutations in the C-terminal ligand-binding domain (LBD), AR amplification and expression of constitutively active truncated AR splice variants lacking the LBD, such as AR-V7.

A new method of inhibiting the androgen pathway is needed to overcome these AR-based mechanisms of resistance. One possibility is through selective inhibition of the N-terminal domain (NTD) of the AR which can inhibit its transcriptional activity even in the presence of LBD-driven anti-androgen resistance.

EPI-7386 represents a new generation of NTD inhibitors (Anitens) and is designed to inhibit transcriptional activity of the AR by interacting with the NTD. In doing so, EPI-7386 is active against both full-length AR and splice-variant AR.

A phase 1 clinical trial of EPI-7386 is beginning and its preclinical efficacy, selectivity, and safety profile are presented.

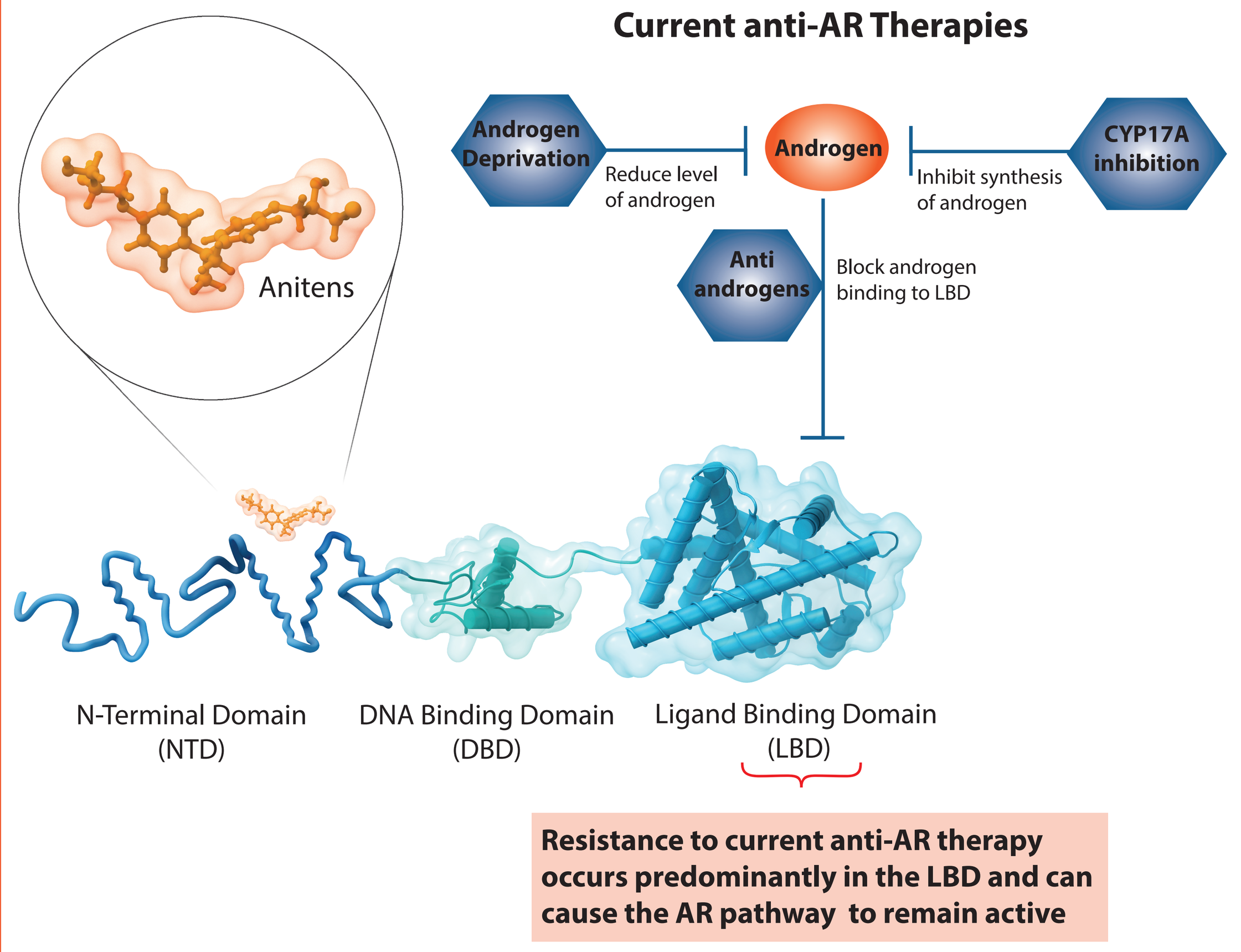

Figure 1: Anitens are first-in-class NTD inhibitors of the androgen receptor
Structure of the androgen receptor (AR) and mechanism of inhibition. The AR is organized in 3 distinct domains: the LBD, involved in binding with androgens, the DBD, and the NTD, which orchestrate the transactivation of the receptor. It was previously demonstrated that the first-generation aniten EPI-002, and its stereoisomeres, specifically binds to the transactivation unit 5 (Tau5) of AR NTD to block essential protein–protein interactions required for transcriptional activity of AR[1-4].

[1] De Mol et al., ACS Chem Biol, 2016. [2] Andersen et al., Cancer cell, 2010. [3] Sadar, Cancer Res, 2011. [4] De Mol et al., Structure, 2018.

EPI-7386 inhibits androgen-induced transcriptional activity

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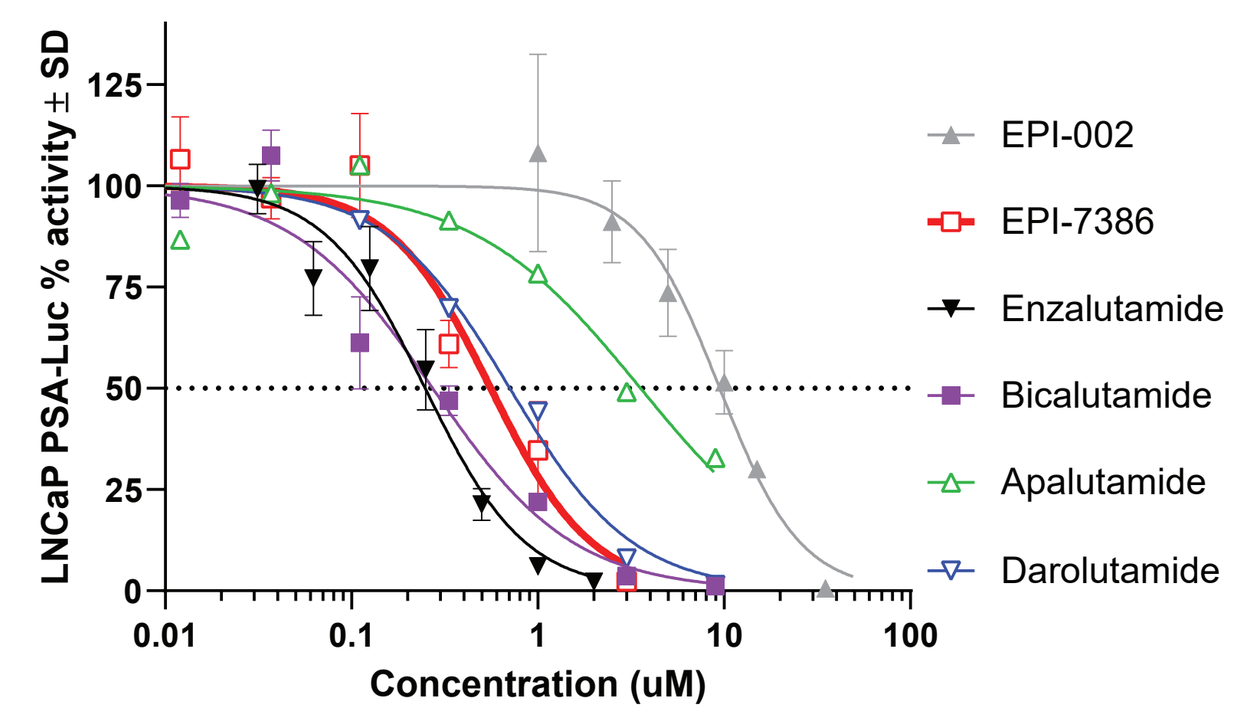

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Compound	IC50 (nM)	n
EPI-002	9,580	2
EPI-7386	421	5
Enzalutamide	154	5
Bicalutamide	242	7
Apalutamide	4,540	3
Darolutamide	616	3

Figure 2: Effect against androgen-induced PSA-luciferase activity in LNCaP cells
(A) A dose-dependent decrease in AR-transcriptional activity was demonstrated in LNCaP cells transfected with the PSA reporter gene and incubated with compounds in the presence of androgen (R1881). (B) Summary of IC_{50}s calculated across multiple independent experiments. LNCaP cells bear a T877A mutation on the AR gene that decreases affinity of apalutamide and darolutamide.

AR inhibition is on target, LBD independent and effective in AR-V7 driven models

A

Compound	Cellular proliferation IC50 (uM) LNCaP	PC-3	LNCaP95
EPI-002	9.0	>10	~20
EPI-7386	0.56	>10	3.7
Enzalutamide	0.35	>10	>10

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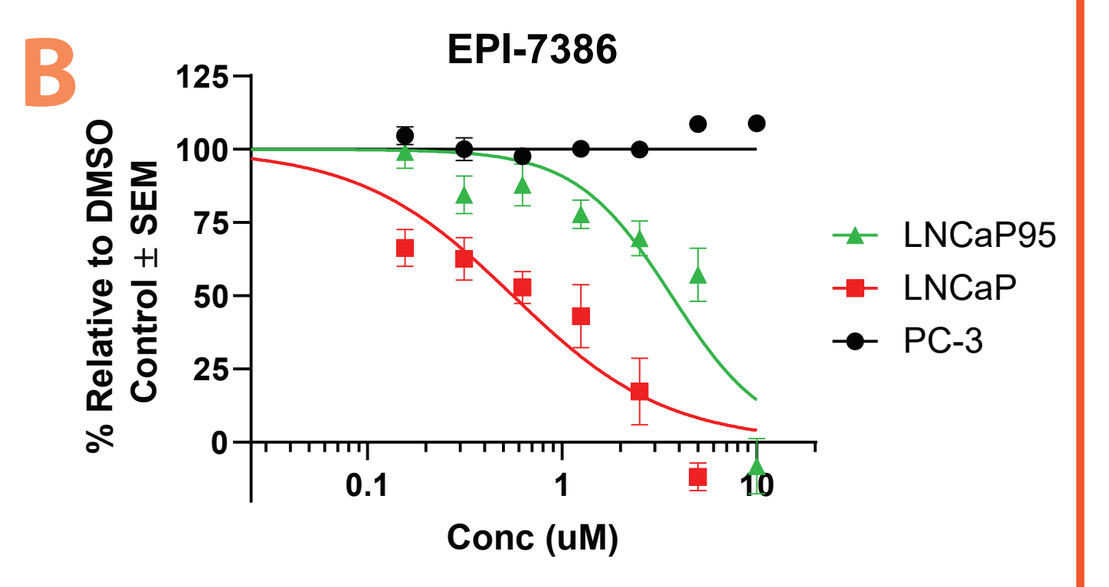

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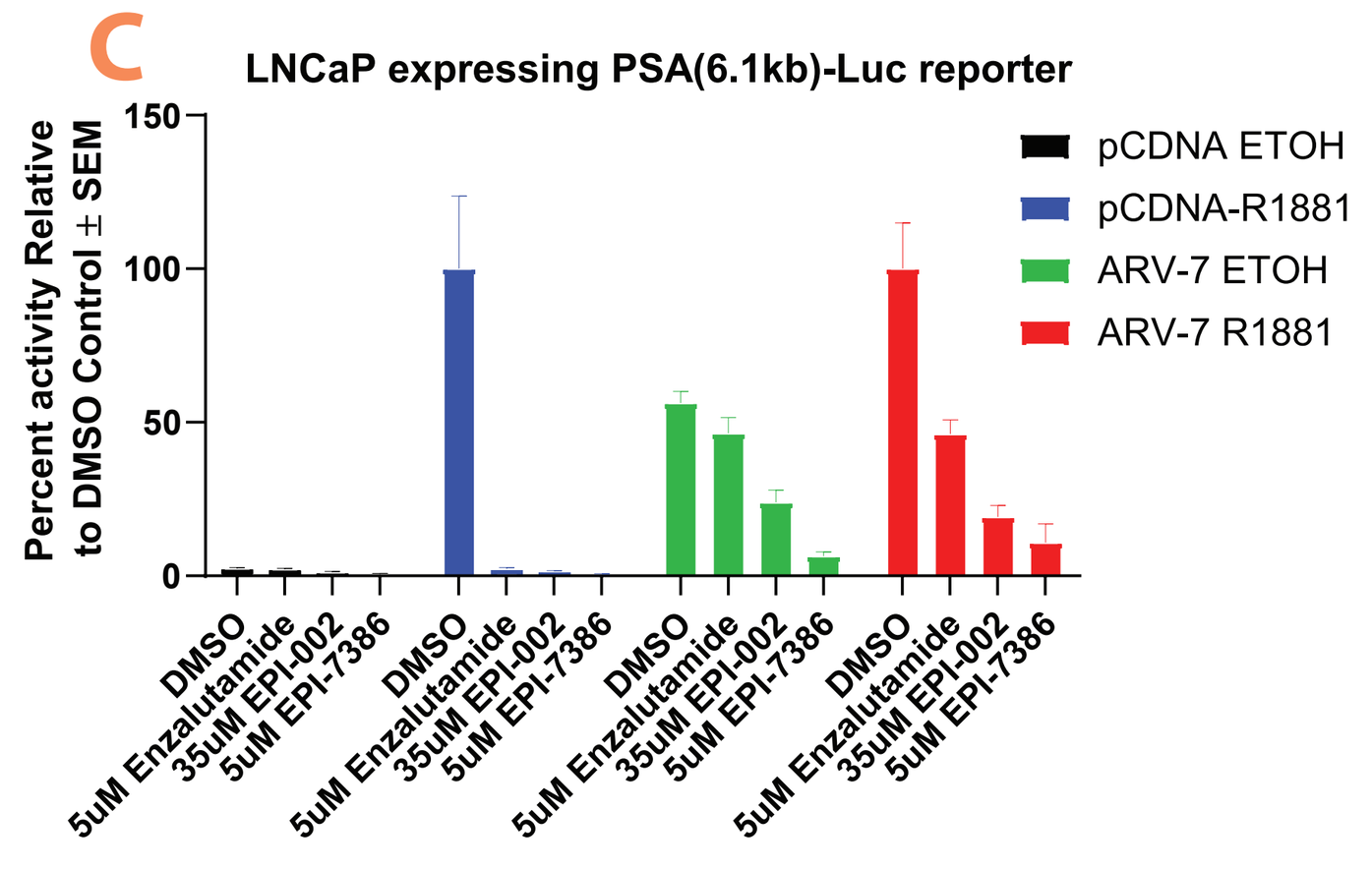

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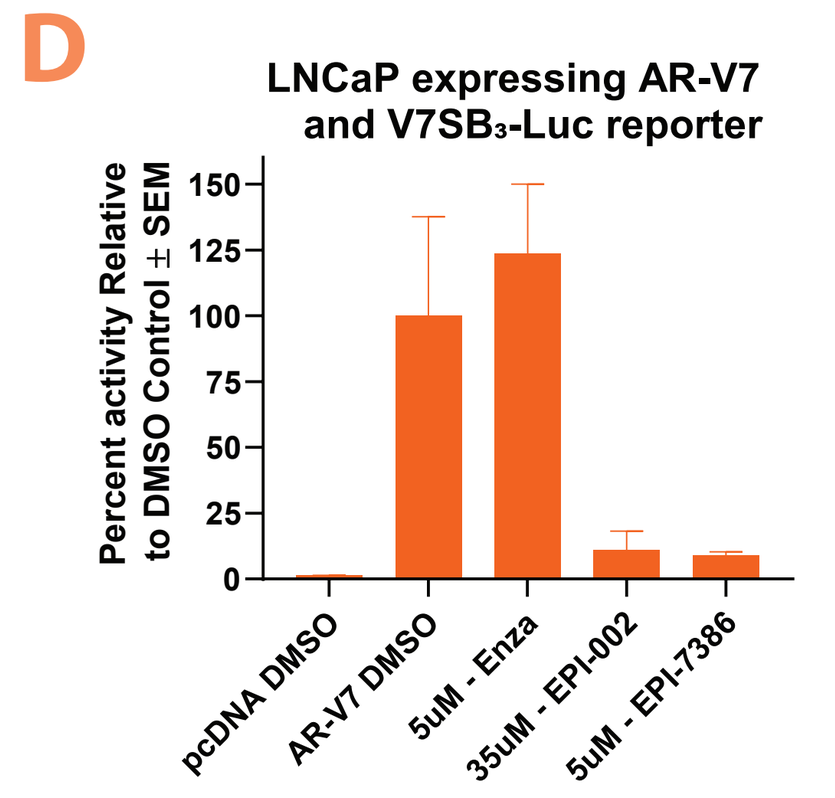

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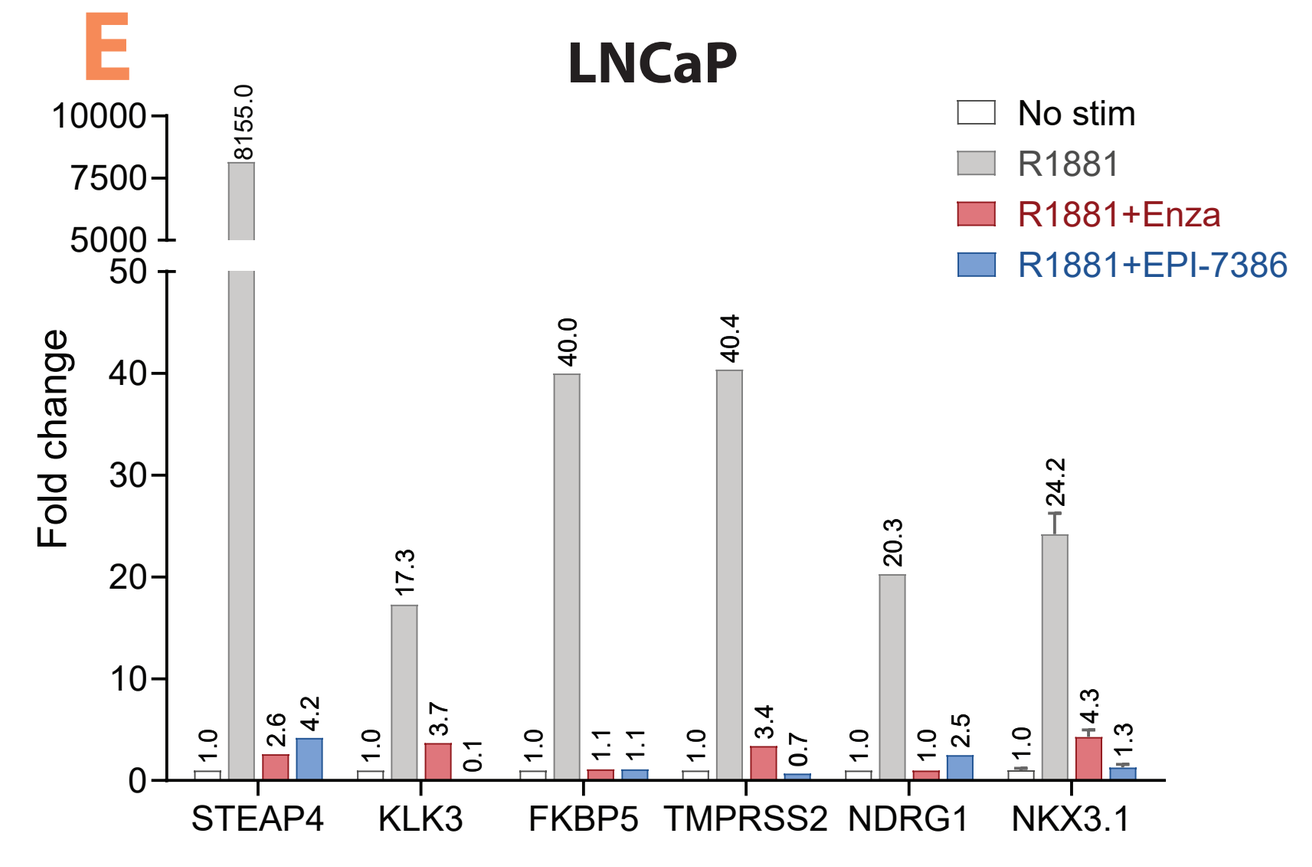

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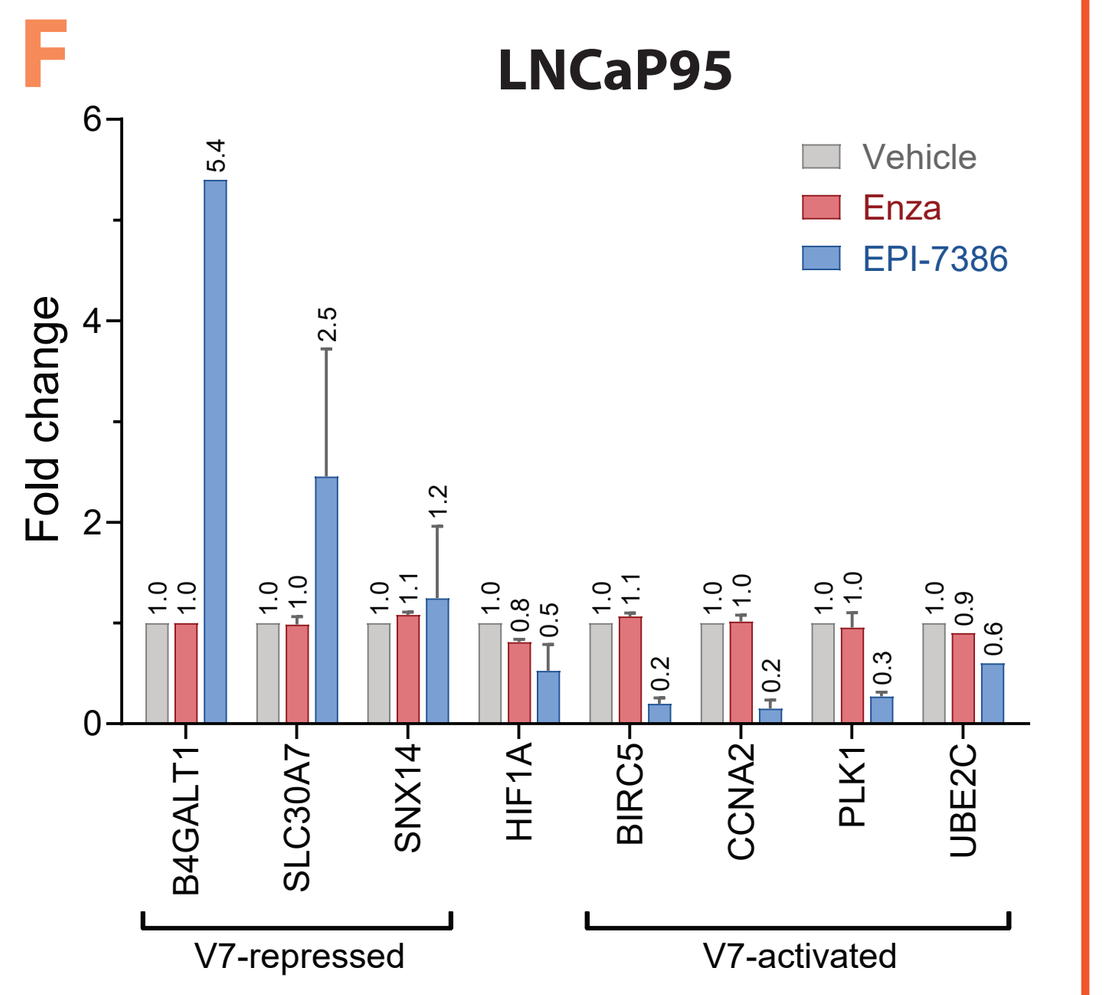

Figure 3: EPI-7386 activity and selectivity in full-length AR and AR-V7 models
(A-B) Androgen-induced proliferation of LNCaP and viability of PC-3 cells was measured with Alamar blue while BrdU was used for LNCaP95 cells. (C) The transcriptional activity of endogenous AR-FL was measured in LNCaP cells using a PSA(6.1kb)-luciferase reporter plasmid which encodes the promoter and enhancer region of the human *PSA/KLK3* gene. (D) The transcriptional activity of ectopic AR-V7 was measured in LNCaP cells transiently transfected with a plasmid encoding AR-V7 (pARV7) and the V7BS3-luciferase reporter (driven by AR-V7). (E) Expression levels of AR-FL regulated genes measured by qPCR in LNCaP cells treated +/- R1881 (1nM) and exposed 24h to 10 uM EPI-7386 or 5 uM enzalutamide. (F) Expression levels of AR-V7 regulated genes measured by qPCR in LNCaP95 cells grown in castrated conditions and exposed 24h to 10uM EPI-7386 or 5 uM enzalutamide.

EPI-7386 inhibition of the AR pathway is similar but slightly different to enzalutamide while the combination with enzalutamide exhibits a broader and deeper response on AR dependent genes

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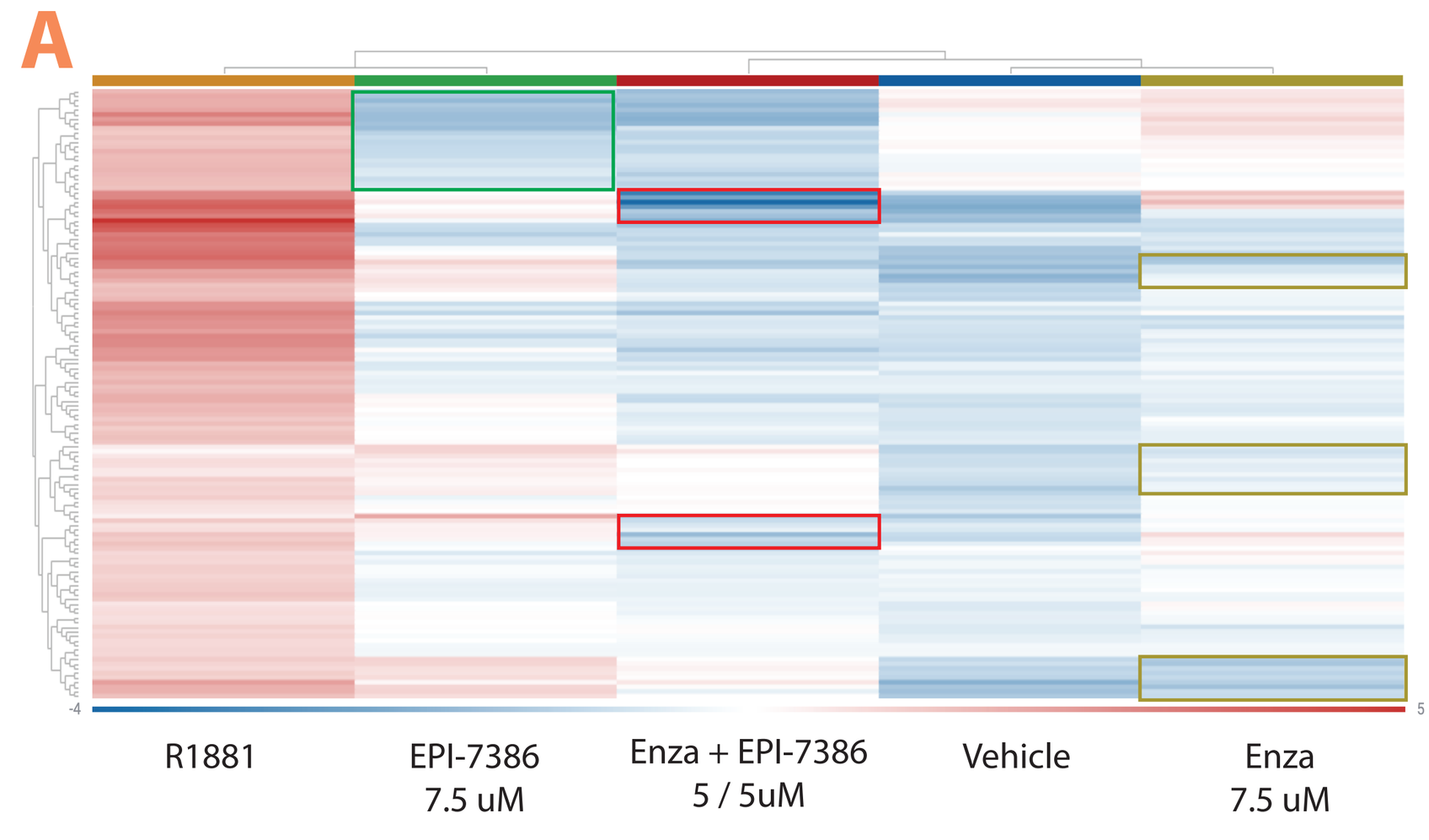

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R1881-activated genes	Pathway Implication	Gene
Co-downregulated by Enza and EPI (74)	HALLMARK_Androgen response (p adj=3.25x10^{-70}) GO_Reproduction (p adj=3.1x10^{-10}) GO_Regulation of Cell population Proliferation (p adj=3.1x10^{-10})	
Strongly downregulated by EPI only(22)	GO_Cell Cycle Phase Transition (p adj=2.22x10^{-16}) GO_DNA Replication Initiation (p adj=3.95x10^{-16})	ORC6, CDC45, MCM4, CCNA2, E2F1, NDRG1, CDC6, AKAP12, CCNE2, POLA2, HIST2H2AB, FANCD2, TYMS, CREB3L4, CDK2, BRCA1, ERCC6L, CAMKK2, PLK1, ORC1, UBE2C, HIST1H2BN
Strongly downregulated by Enza only (25)	HALLMARK_TNFa Signaling via NFkB (p adj=3.82x10^{-7}) HALLMARK_EMT (p adj=38.21x10^{-4})	STK17B, PTPN21, SEC24D, ID2, SAT1, SLC16A6, KLF4, TNFAIP8, WIPI1, DNAJB9, HERPUD1, B4GALT1, CALU, ZBTB10, ITGAV, LMAN1, B2M, PGM3, GHR, ABHD3, ERRFI1, SNAI2, CENPN, STK39, HERC3, IQGAP2, ALDH1A3, UAP1
Strongly downregulated by Enza + EPI combo (11)	HALLMARK_Androgen Response (p adj=3.82x10^{-7})	KLK3, NKX3-1, KLK2, TMPRSS2, GUCY1A3, ZBTB16, RRP9, HES6, KLK4, ENDOD1, ABHD2, SMAGP

C

	Gene	Log2 Fold Change 7.5 Enza	7.5 EPI	5/5 Combo
1	KLK2	-1.71	-2.71	-6.63
2	FKBP5	-5.01	-4.47	-6.09
3	TMPRSS2	-2.66	-3.32	-5.68
4	KLK3	-1.24	-2.42	-5.40
5	NCAPD3	-4.32	-4.40	-5.03
6	NKX3-1	-1.72	-2.28	-4.54
7	NDRG1	-2.04	-4.17	-4.37
8	STEAP4	-4.22	-4.22	-4.22
9	FAM105A	-3.37	-2.78	-4.18
10	AKAP12	-1.91	-3.84	-4.10
11	PMEPA1	-3.05	-2.16	-4.05
12	PLPP1	-2.48	-3.53	-3.97
13	SNAI2	-3.97	-1.79	-3.97
14	ACSL3	-3.43	-3.62	-3.90
15	ERRFI1	-4.51	-2.76	-3.90
16	CDC6	-1.20	-3.60	-3.86
17	ELL2	-3.39	-3.05	-3.81
18	CENPN	-3.31	-2.13	-3.79
19	RHOU	-3.94	-3.03	-3.78
20	EAF2	-3.32	-3.81	-3.52

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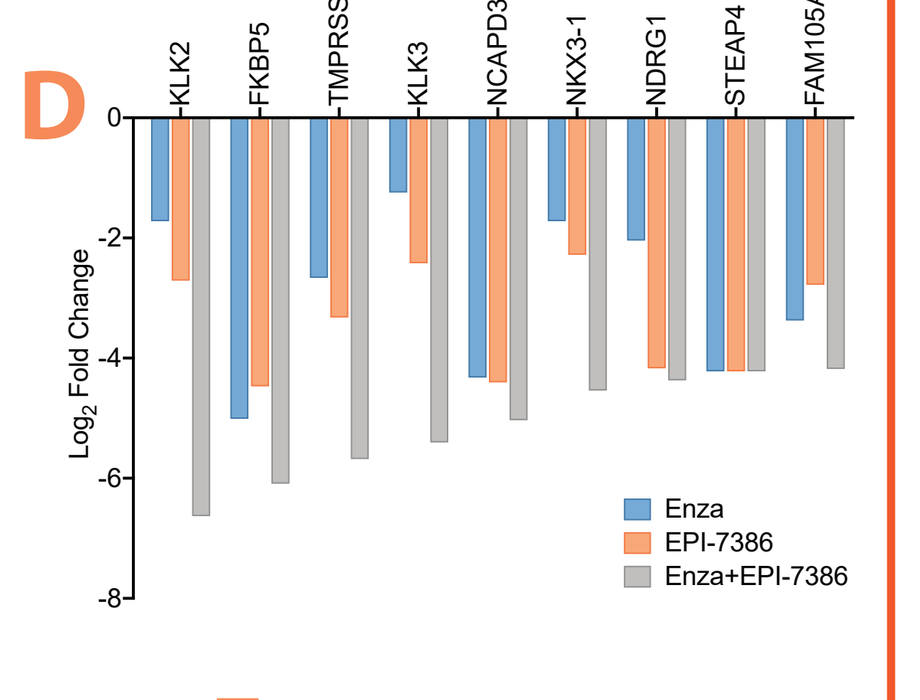

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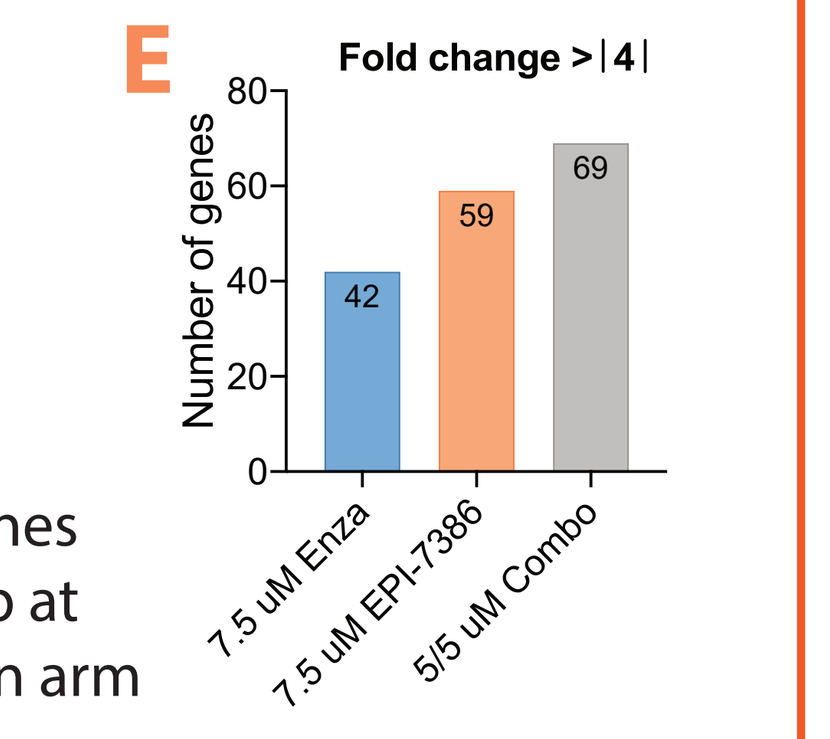


Figure 4: Transcriptomic analysis from Nanostring androgen receptor panel (585 custom AR dependent genes) in LNCaP cells
(A) Heat map showing expression of R1881-activated genes (> 2 fold) in LNCaP cells treated for 24 hrs, in conditions described in Fig. 3E. (B) Table summarizing pathways and genes specfic to each treatment arms. (C) 20 most downregulated genes in the EPI-7386 5 uM / enzalutamide 5 uM combination treated group compared to single agent treated group at 7.5 uM. All conditions were compared to R1881-stimulated conditions only. (D) Graphical representation of Log2 fold change in the 9 most down regulated genes in combination arm (5/5 uM) vs each single agent (7.5 uM). (E) Number of genes showing > 4 fold change in the EPI-7386 / enzalutamide combination treated group compared to single agents.

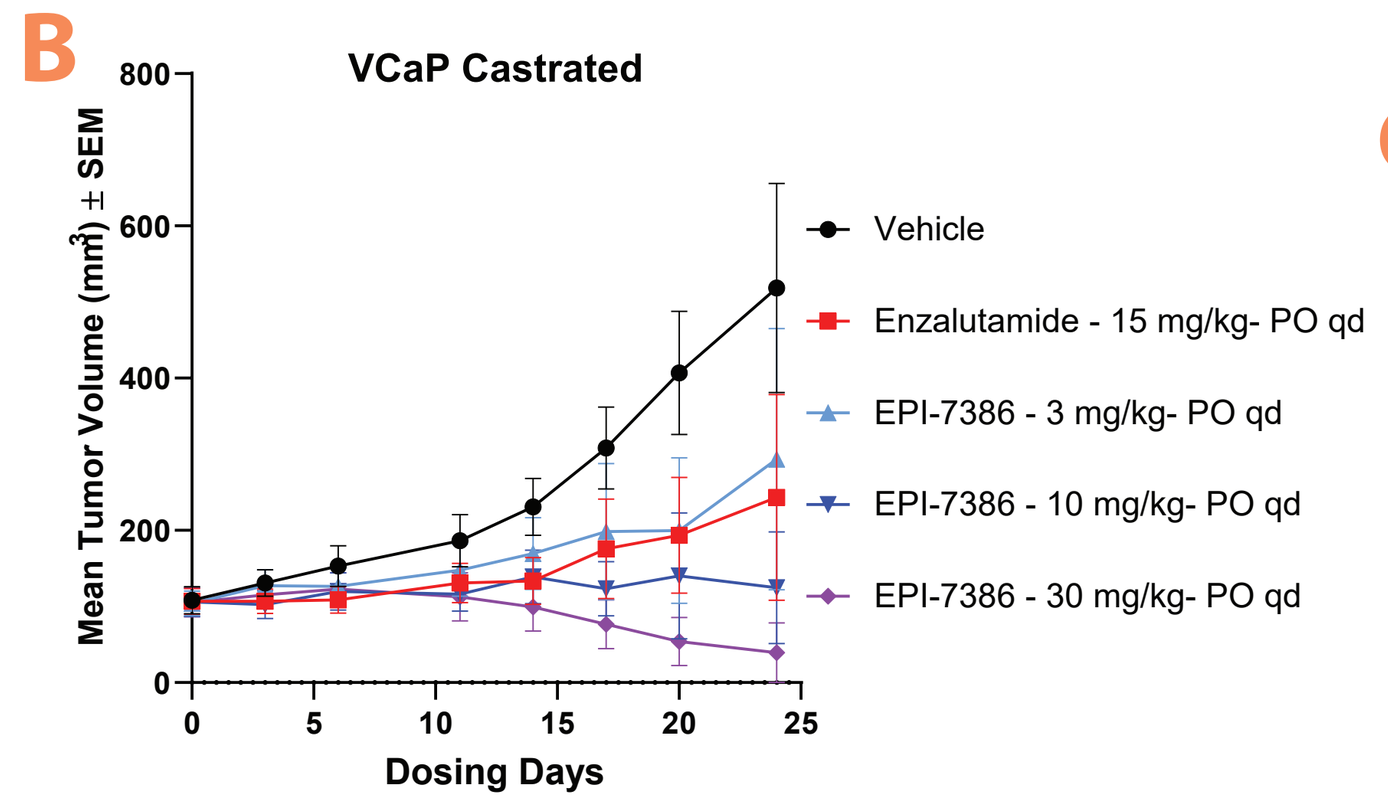
EPI-7386 is active in a variety of castrate-sensitive and resistant prostate cancer xenograft models

A

in vivo model	AR status	Male Mice Condition	Compound	Dose (mg/kg)	Formulation	Regimen	TGI (%)	p value
LNCaP	AR FL	Castrated	Enzalutamide	30	Solution	qd24	>100%	p<0.0001
			EPI-7386	60			93%	p<0.0001
VCaP	Amplified AR Some AR-V7	Castrated	Enzalutamide	15	Solution	qd24	66%	p<0.05
			EPI-7386	3			54%	p<0.01
			EPI-7386	10			95%	p<0.0001
			EPI-7386	30			>100%	p<0.0001
LNCaP95	High level AR-V7 Other oncogenic pathways	Castrated	Enzalutamide	15	Solution	qd24	<0%	NS
			EPI-7386	30			54%	p<0.0001
22Rv1	High level AR-V7 Other oncogenic pathways	Castrated	EPI-7386	30	Solution	qd28	48%	p<0.0001
HID28	AR +, PSA +	Castrated	Enzalutamide	15	Solution	qd28	<0%	NS
	AR-V7 unknown		EPI-7386	60	Suspension		58%	p<0.05
PC-3	Non functional AR Other oncogenic pathways	Intact	Enzalutamide	15	Solution	qd28	0%	NS
			EPI-7386	30			0%	NS

Statistics: 2-way ANOVA with Dunnett correction for multiple comparisons. NS: Non significant. TGI: Tumor Growth Inhibition

B



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Dose (mg/kg)	Mouse strain	Day of dosing	Cmax (ng/mL)	AUC0-last (ng·h/mL)
3	CD-1	1	6,610	87,200
10	CD-1	1	16,300	220,000
30	CD-1	1	45,300	534,000
30	Nude / SCID / SCID Beige / NOG	1	38,750	475,000
30	Nude / SCID / SCID Beige / NOG	7	31,000	282,000

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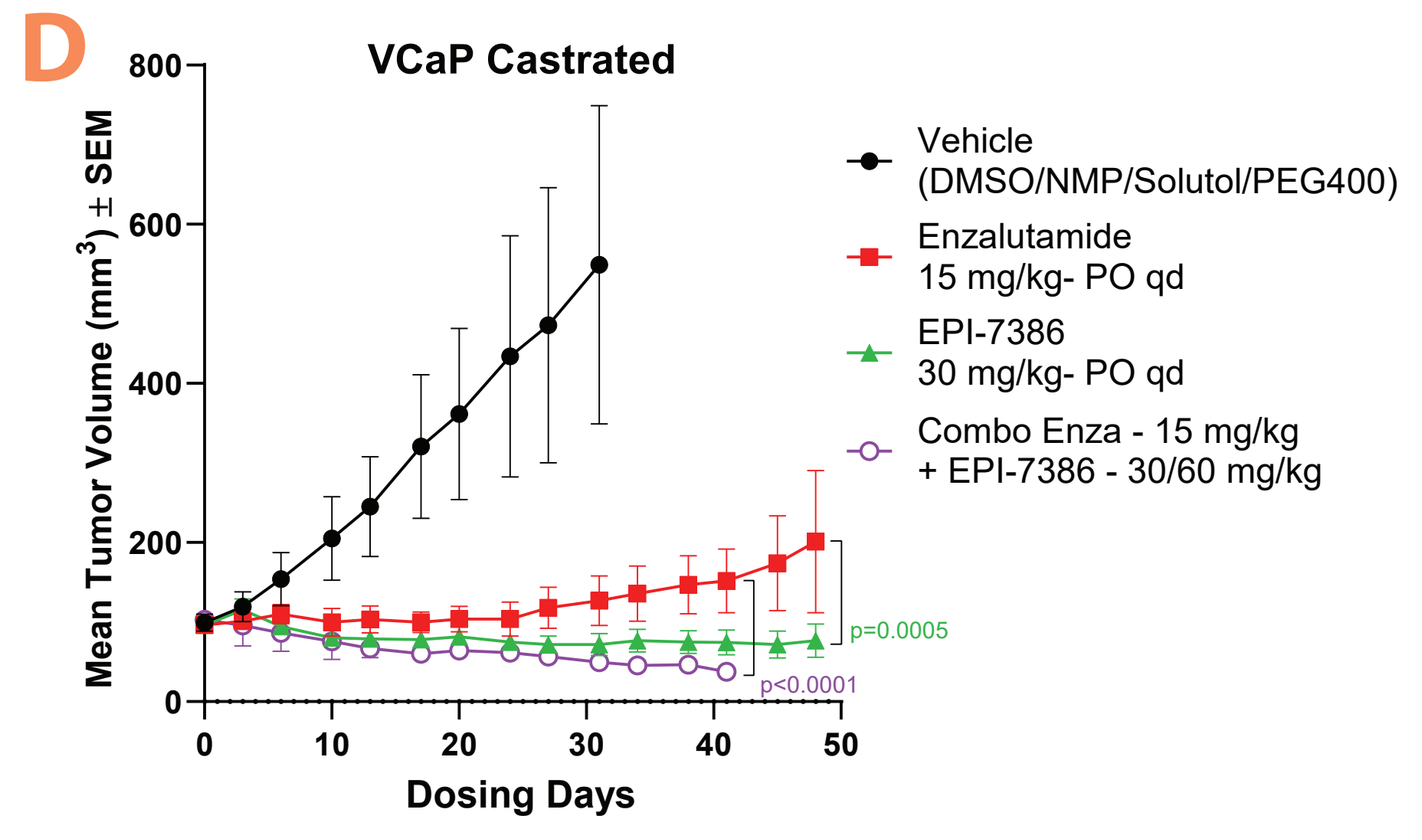


E

Arm	Progressive Disease (PD)	Stable Disease (SD)	Partial Response (PR)	Complete Response (CR)
Enza (15 mg/kg)	4 (57%)	3 (43%)	0 (0%)	0 (0%)
EPI-7386 (30 mg/kg)	1 (14%)	5 (71%)	0 (0%)	1 (14%)
COMBO (Enza+EPI-7386)	0 (0%)	1 (20%)	4 (80%)	0 (0%)

Figure 5: *In vivo* activity in CRPC xenograft models
(A) Summary of *in vivo* activity of EPI-7386 in a subset of prostate cancer xenograft models bearing various AR-dependent and independent tumors. (B) Example of dose response activity obtained in castrated male SCID Beige mice bearing VCaP tumors. (C) EPI-7386 PK parameters in different strains of mice. (D) Tumor growth of EPI-7386 in combination with enzalutamide in castrated male SCID Beige mice bearing VCaP tumors. (E) Summary of preclinical responses in the VCaP study.

EPI-7386 was well tolerated in rat and dog tox studies and is predicted to achieve high exposures in humans

A

Male SD rat 28-days GLP tox

Dose (mg/kg/day)	AUC0–24h D23 (ng·h/mL)	Major Findings	Conclusion
60	1,119,000	Well tolerated and non-adverse	Below NOAEL
120	1,640,000	Well tolerated and non-adverse	NOAEL
240	2,350,000	Adverse body weight and food consumption loss. No other significant clinical or anatomical pathology findings	HNSTD

NOAEL: No-observed -adverse-event level; HNSTD: Highest non-severe toxic dose. Repeat dose showed no accumulation between D1 and D23.

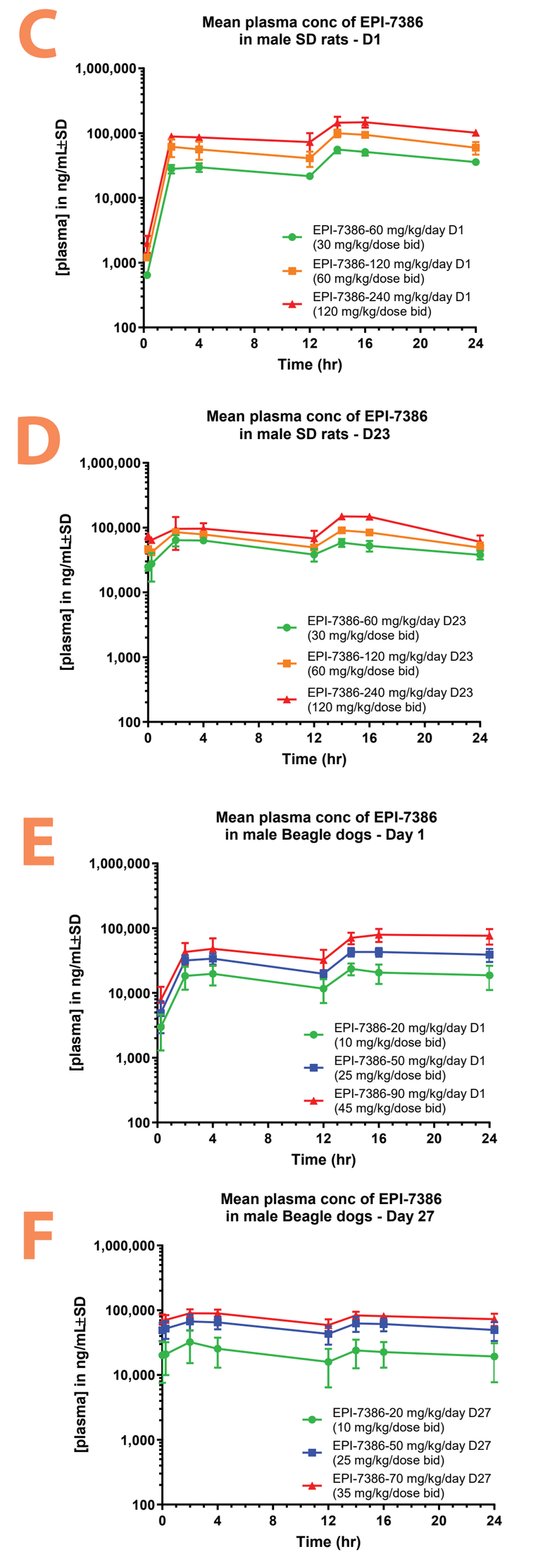
B

Male Beagle dog 28-days GLP Tox

Dose (mg/kg/day)	AUC0–24h D27 (ng·h/mL)	Major Findings	Conclusion
20	529,000	Well tolerated and non-adverse	NOAEL
50	1,350,000	Well tolerated and non-adverse	Below HNSTD
90/70	1,850,000	Adverse body weight and food consumption loss. No other significant clinical or anatomical pathology findings	HNSTD

NOAEL: No-observed -adverse-event level; HNSTD: Highest non-severe toxic dose. Repeat dose showed minimal accumulation between D1 and D27 . Anti-androgen effects observed on target organs.

C–F



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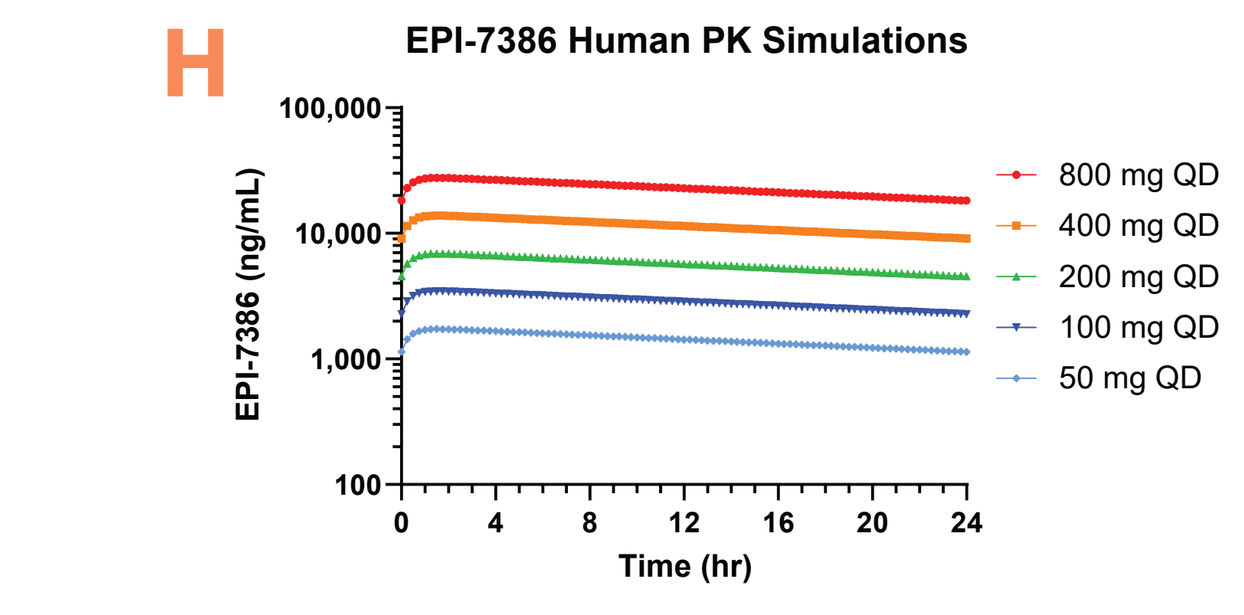
Species	GLP Toxicology Study Reference Dose (mg/kg/day)	Converted to BSA (mg/m²)	Applied Safety Factor	Estimated Human MRSD mg/m²	mg/day
Rat	240	1,440	10	144	233
Dog	70	1,400	6	233	378

Abbreviations: BSA = body surface area; MRSD = maximum recommended starting dose. Conversion for mg/kg to mg/m²: rat (mg/kg) × 6; dog (mg/kg) × 20 . MRSD = 1/10 of STD 10 (mg/m²) in rodents or 1/6 high dose (mg/m²) in non-rodents. Conversion factor from mg/m² to mg/day in humans is 1.62 m² for a standard adult body surface area

H



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Dose (mg)	Cmax (ng/mL)	AUC0-24 (ng*hr/mL)
50	1,729	34,320
100	3,458	68,639
200	6,915	137,278
400	13,830	274,556
800	27,659	549,113

AUC and Cmax calculated at steady state

Figure 6: Toxicology overview, human projected exposure and solid form of EPI-7386
(A) 28-day GLP TK and tox conclusion in male Sprague-Dawley rats. (B) 28-day GLP TK and tox conclusion in male Beagle dogs. (C-F) TK graph of EPI-7386 concentration in plasma over time, in D1 in rats (C), D23 in rats (D), D1 in dogs (E) and D27 in dogs (F). (G) Estimated human maximum recommended starting dose for phase 1 calculated from tox studies. (H) Human estimated PK curves and (I) PK parameters at steady state, from 50-800 mg per day, based on in vitro in vivo correlation .

First in human clinical study

Patient population
mCRPC patients progressing on standard of care (including the latest antiandrogens)

Study design
- 3+3 design
- n = ~ 18 patients for dose escalation
- n = ~ 10 patients for dose expansion

Study endpoints
- Recommended Phase 2 dose (RP2D)
- Safety and PK
- PSA response

Correlative studies
- CTC conversion
- CTC AR-V7
- ctDNA

Timeline
- FPI anticipated of 2Q 2020

CONCLUSION

- Clinical candidate EPI-7386 displays preclinically:

a. Similar potency *in vitro* to the 'lutamides in full length AR models

b. LBD independent inhibition of AR demonstrated by activity on AR-V7 driven gene expression

c. Specific activity on the AR transcriptome, similar but different to enzalutamide, while displaying broader and deeper AR inhibition when combined together with enzalutamide

d. Activity in several *in vitro* and *in vivo* CRPC cell lines, including enzalutamide resistant models

e. Dose response activity with a minimal active exposure ~ 80,000 ng*h/mL in mouse VCaP xenograft models

f. Tolerability in 28-days tox studies in rats and dogs at AUC ≤ 2,000,000 ng*h/mL, with activity seen on androgen-sensitive target organs

g. Favorable human PK parameters supporting QD dosing

h. Initial clinical starting dose of 200mg

- **EPI-7386 IND allowed by the FDA with the first-patient-in planned for June 2020**