Enzalutamide-resistant LNCAP AR^{F877L} mutation KI model

WuXi AppTec, WuXi Biology, Oncology & Immunology Unit

2023.05

Outline

- **Background**
  - AR antagonists binding location and current AR mutations leading to the resistance
  - Strategies to overcome resistance to AR antagonists
  - Development of AR^{F877L} mutation edited LNCAP FGC cell line via KI method
- ***In vitro & in vivo* validation of Enzalutamide-resistant LNCAP AR^{F877L} KI model**

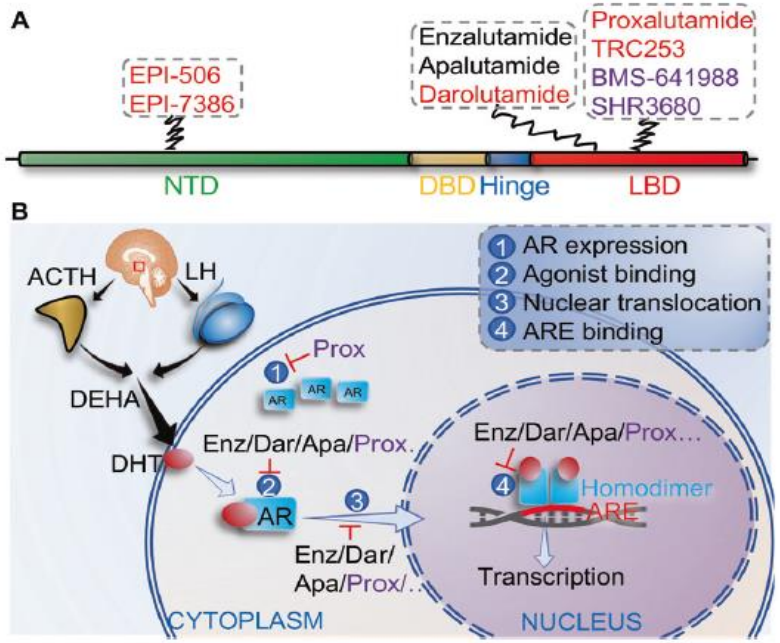
AR antagonists binding location and current AR mutations leading to the resistance

- Persistent androgen receptor (AR) activation drives therapeutic resistance to second-generation AR pathway inhibitors and contributes to the progression of advanced prostate cancer.
- One resistance mechanism is point mutations in the ligand binding domain (LBD) of AR that can transform antagonists into agonists.
- The AR^{F877L} mutation, identified in patients treated with enzalutamide, confers resistance mechanism to enzalutamide.

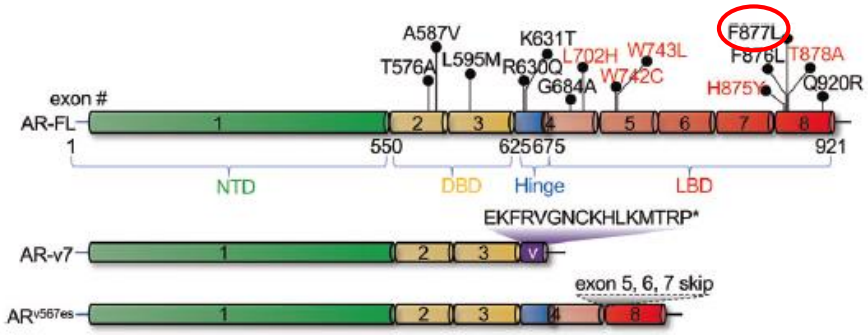
Table 1. Timeline for the development of AR antagonists for prostate cancer.

Generic name	Other name	Approval date (or clinical stage)	Treatments
Fist-generation			
Flutamide	Eulexin	27 Jan 1989	mCRPC
Bicalutamide	Casodex	04 Oct 1995	mCRPC
Nilutamide	Nilandron	09 Sep 1996	mCRPC (combined with surgical castration)
Second-generation			
Enzalutamide	MDV3100	31 Aug 2012	mCRPC
		13 Jul 2018	nmCRPC
		16 Dec 2019	mCSPC
Apalutamide	ARN-509	14 Feb 2018	nmCRPC
		17 Sep 2019	mCSPC/mCRPC
Darolutamide	ODM-201	30 Jul 2019	nmCRPC
Candidates in clinical trials			
Proxalutamide	GT-0918	Phase II (recruiting)	mCRPC
BMS-641988		Phase I (closure)	CRPC
TQB3720		Phase I (recruiting)	mCRPC
SHR3680	Rezvilutamide	Phase I/IIA (complete)	mCRPC
TRC-253		Phase I/IIA (complete)	mCRPC

Information is taken from the websites ClinicalTrials.gov *(https://clinicaltrials.gov/ct2/home)* and Drugs@FDA: FDA-Approved Drugs *(https://www.accessdata.fda.gov/scripts/cder/daf/)*.



The binding location of AR antagonists and roles in inhibition of AR-mediated transactivation.



Recurrent AR mutations and alternative splicing variants lead to AR antagonist resistance.

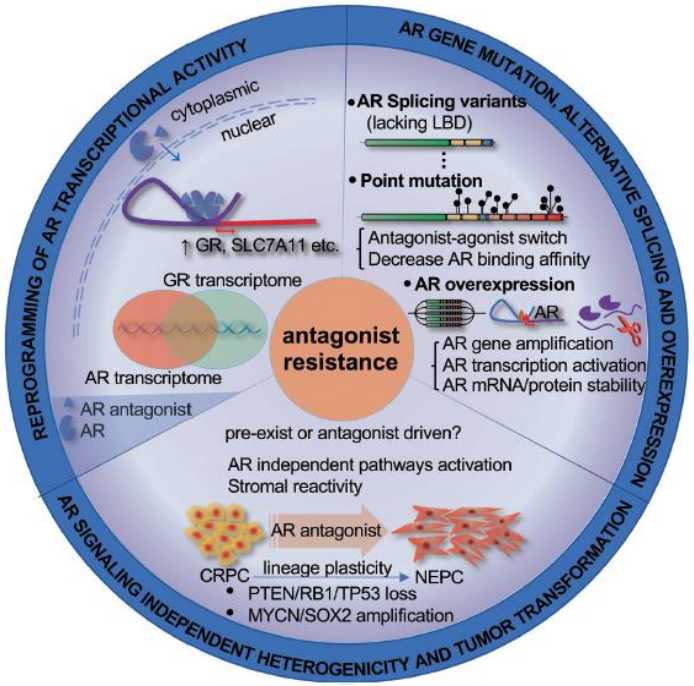
Cell Death Dis. 2022 Jul; 13(7): 632.

Strategies to overcome resistance to AR antagonists

Development of novel AR-targeted therapies

Overcoming resistance to AR antagonists:

- LBD inhibitors bind with AR^{F877L} mutation: Darolutamide, Proxalutamide, TRC-253
- N-terminal inhibitors (NTD)and DNA-binding Domain (DBD) inhibitors: EPI-7386, EPI-506, SBF-1
- AR-targeted PROTACs: ARV-110
- AR-targeted CRISPR-Cas13 system



Mechanisms of resistance to androgen receptor inhibitors in prostate cancer.

Novel AR targeted therapies.

Agents/technologies	Mechanisms and preclinical/clinical evidence
AR DBD inhibitors	AR binding to the DNA via its DBD is an essential step in the regulation of gene transcription by both full-length and variant forms of AR [163]. AR DBD inhibitors can effectively inhibit the activity of truncated ARVs and repress PCa growth in vitro and in vivo [129, 134, 164].
AR NTD inhibitors	The AR NTD is essential for AR transactivation, and NTD deletion renders AR transcriptionally inactive [165]. A phase I trial has established the safety of EPI-506 and provides proof of concept for targeting the AR NTD [133].
AR-targeted PROTACs	PROTACs technology has emerged as a promising approach for targeted therapy in various diseases, particularly in cancer [136]. ARV-110 targets AR and is safe and has efficacy in mCRPC patients [137, 138]. A phase I/II dose escalation study is currently recruiting mCRPC patients to assess the tolerability and safety of ARV-110 (NCT03888612).
AR-targeted CRISPR-Cas13	CRISPR/Cas13 targeting of oncogenes has been proven to repress the growth of multiple types of cancer in vitro and in vivo [147–149].

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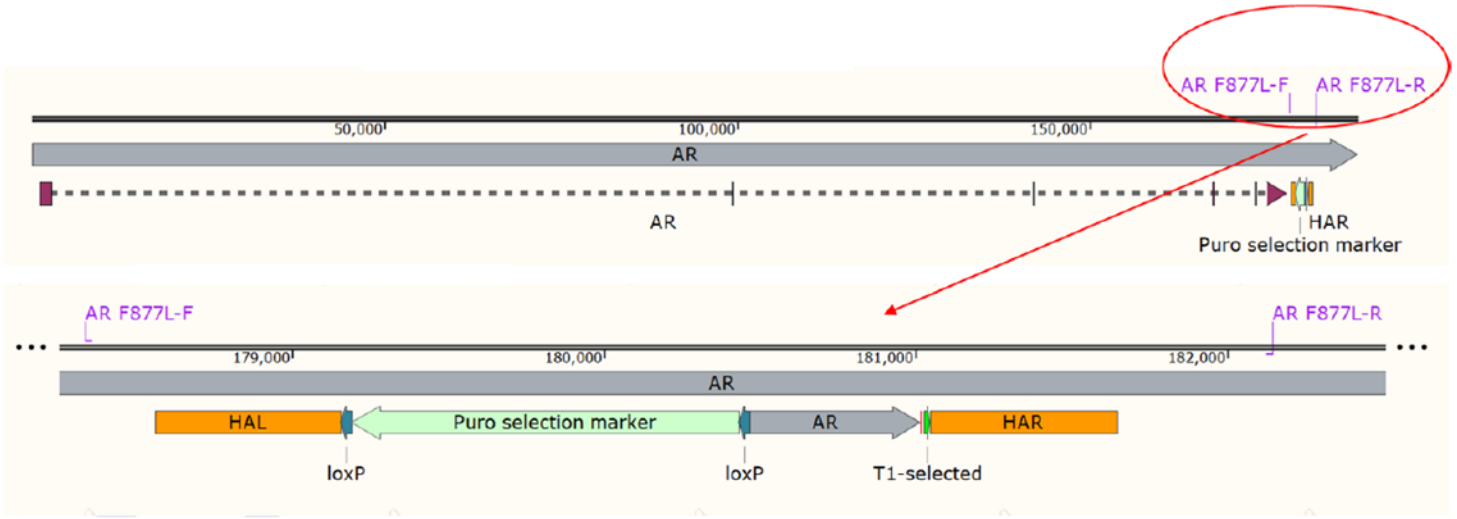
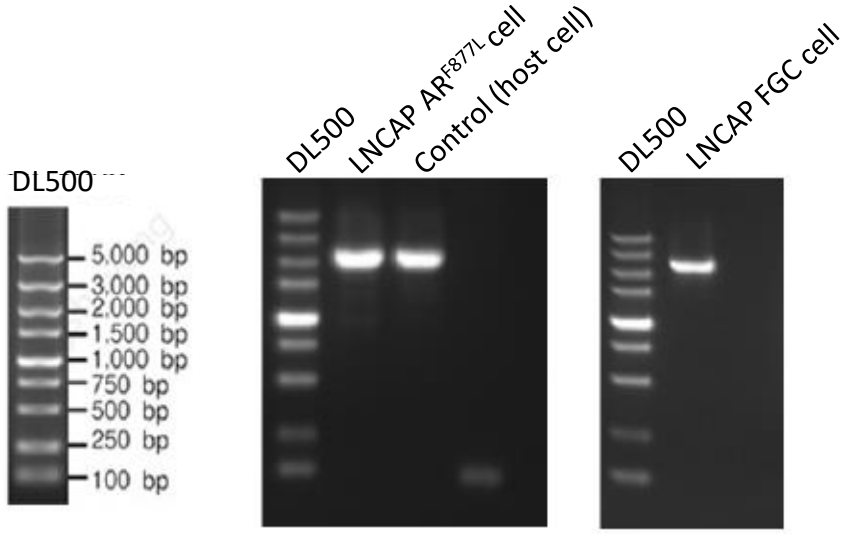
Development of AR^{F877L} mutation edited LNCAP FGC cell line via KI method

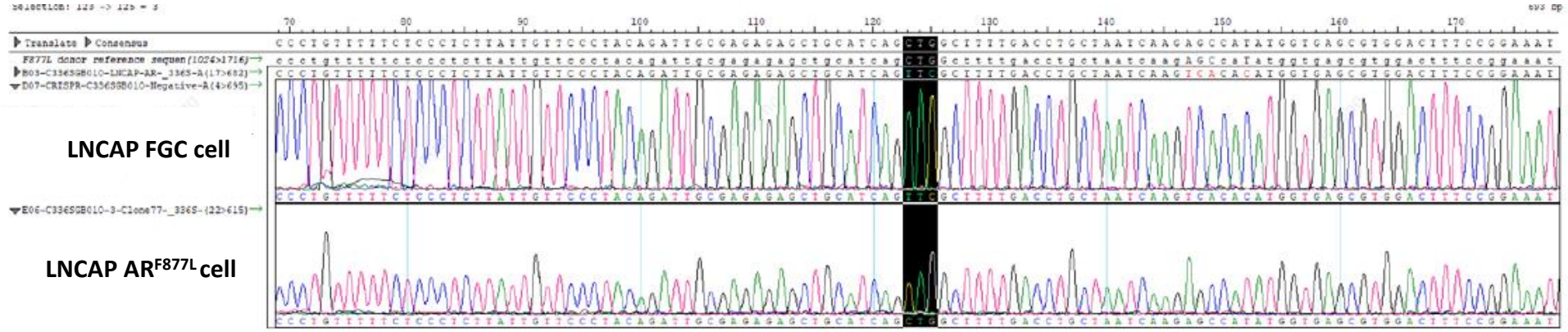
Isogenic clone generation and sanger sequencing screening



Map of primers for modified single clone validation



Map of electrophoresis of PCR products

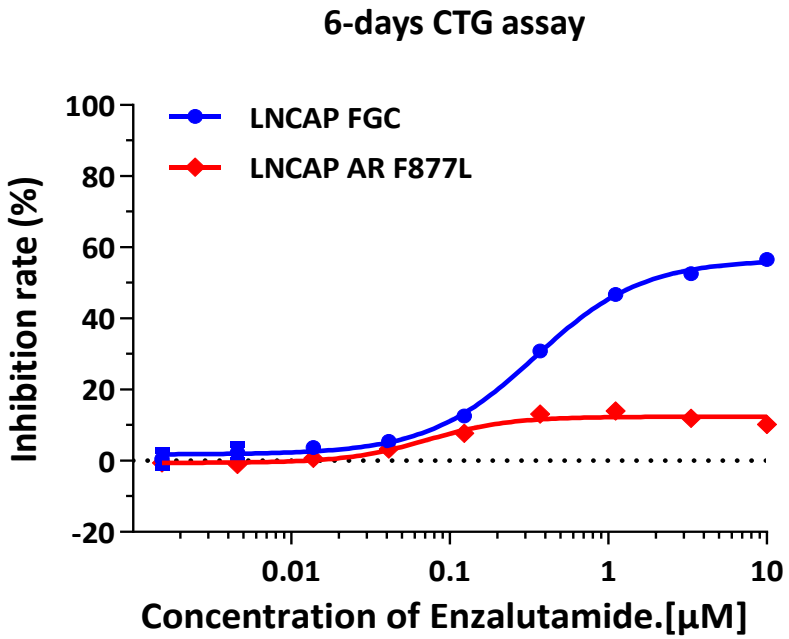


Identification of modified single clones by Sanger sequencing.

- LNCAP AR^{F877L} was validated as full-allelic modified single clone, without selection marker integration. Highlighted area indicates the intended mutation “TTC” to “CTG”.

Establishment of Enzalutamide-resistant LNCAP AR^{F877L} KI cell line

In vitro validation of LNCAP AR^{F877L} KI cell line

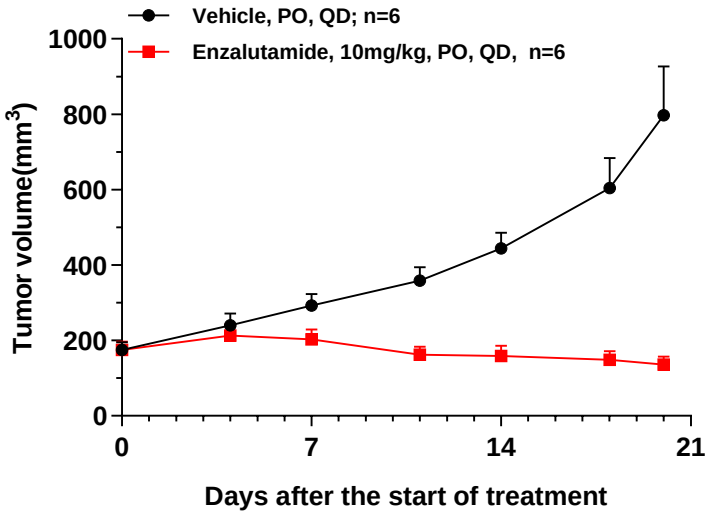


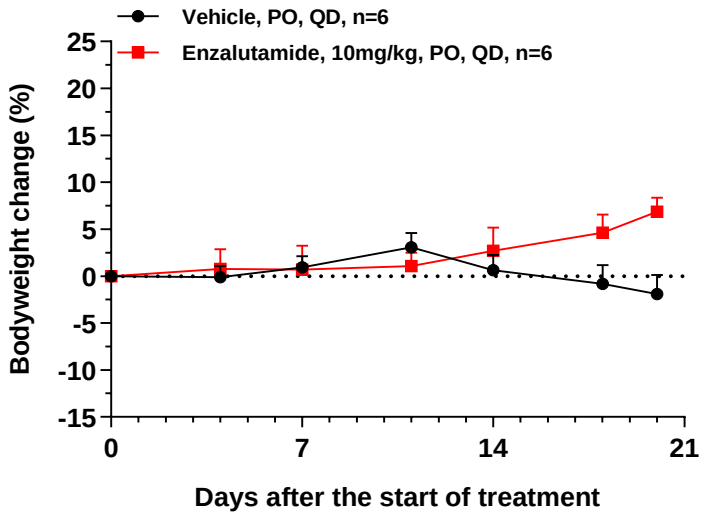
- LNCAP AR^{F877L} KI cell line is validated to be resistant to Enzalutamide, compared with LNCAP FGC cell line.

Compound	Cell line	AbsIC50 (μM)	ReIC50 (μM)	Bottom (%)	Top (%)
Enzalutamide	LNCAP FGC	1.643	0.344	0.503	56.580
	LNCAP AR^{F877L} KI	NA	NA	NA	13.953

Establishment of Enzalutamide-resistant LNCAP AR^{F877L} KI model

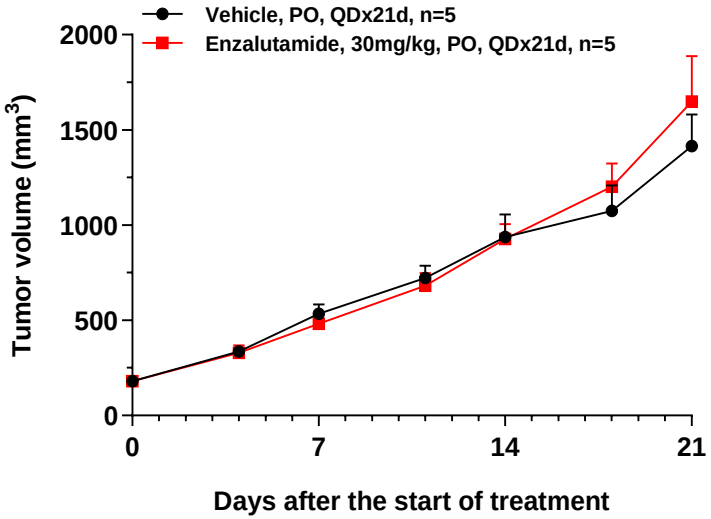
In vivo validation of Enzalutamide on LNCAP FGC and LNCAP AR^{F877L} KI model in castrated animals

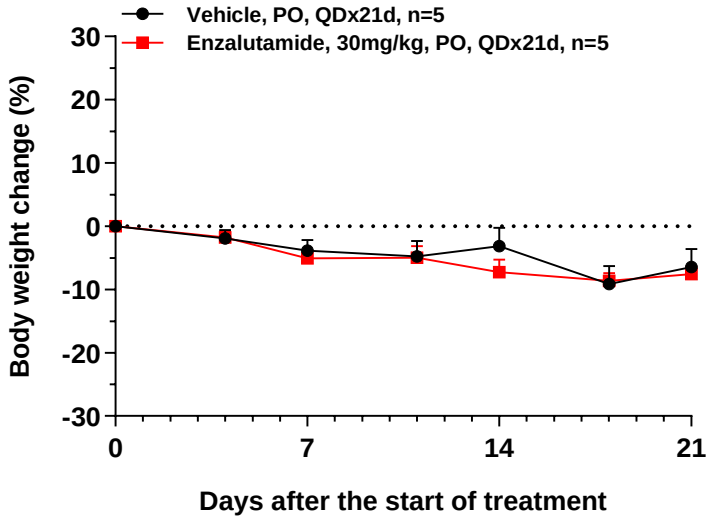




Castrated LNCAP FGC model

Enzalutamide showed **106% TGI** on PG-D20.





Castrated LNCAP AR^{F877L} KI model

Enzalutamide showed **-19% TGI** on PG-D21.

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