The establishment of AR cell-based and biochemical assays facilitates novel drug discovery in prostate cancer and breast cancer

Xiaoyan Wang, Ziwei Zhang, Dandan Hu, Haiting Dai, Jie Yang,Tiejun Bing
Innovation R & D, ICE Bioscience InC.

Innovative CRO+Explorer
ICE Bioscience

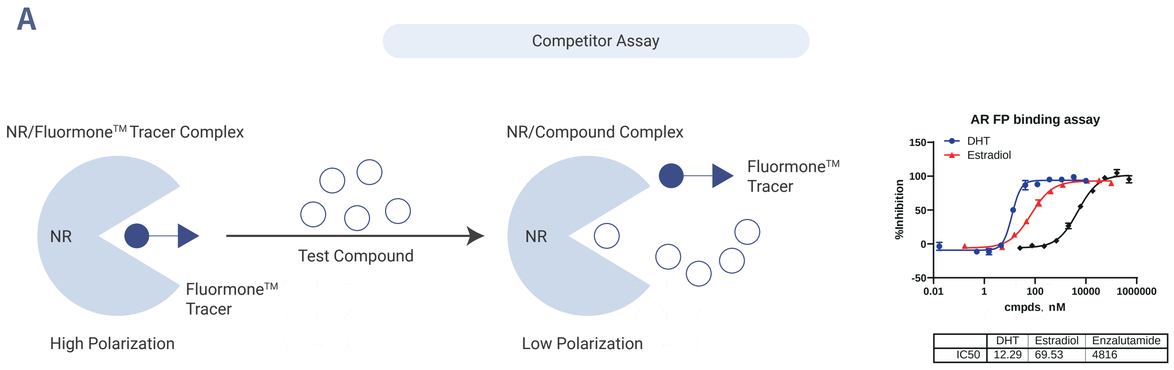
Introduction

Androgen Receptor (AR) belongs to the steroid receptor subfamily of the nuclear receptor superfamily. As a transcription factor, AR is responsible for regulating the physiological effects of androgens, such as testosterone and dihydrotestosterone (DHT), plays a pivotal role in the initiation and advancement of prostate cancer (PCa) and breast cancer (BC), with therapeutic approaches primarily focusing on the modulation of the AR signaling pathway.

We constructed an integrated experimental cascade, including biochemical and cell-based assays to conduct the high throughput hit-to-lead compound screening. Meanwhile, CDX modes are constructed for promisingly conducting in-vivo experiments and biomarker detection. Thus, ICE supports multiple approaches for helping the drug discovery and development of AR to facilitate the treatment of PCa and BC.

AR Binding Assay

A



	DHT	Estradiol	Enzalutamide
IC50	12.29	69.53	4816

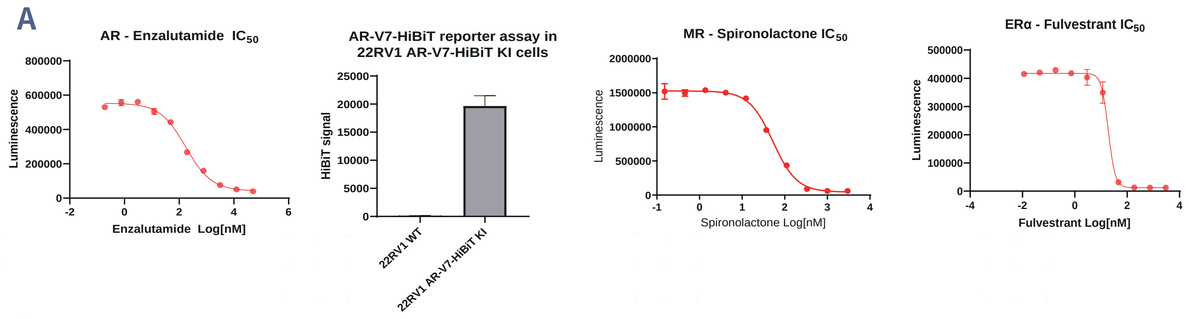
B

Num.	Assay
1	AR-LBD FP Antagonist assay
2	GR-LBD HTRF Agonist assay
3	GR-LBD HTRF Antagonist assay
4	PPARα-LBD HTRF Agonist assay
5	PPARα LBD HTRF Antagonist assay
6	PPARγ-LBD HTRF Agonist assay
7	PPARγ-LBD HTRF Antagonist assay

Figure 1. A. AR protein was purified to apply in detection the binding of DHT, Estradiol, Enzalutamide and AR by competitor binding assay. B. Besides competitor assay, we also developed Co-activator binding Assay to detect the binding between agonist/antagonist and NHRs, eg. GR , PPARα and PPARγ...

Cellular Assay

1.NHRs reporter assay



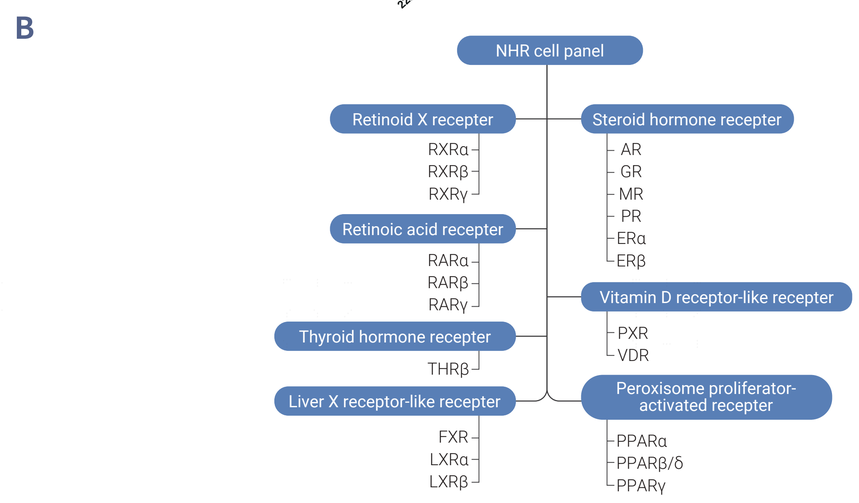
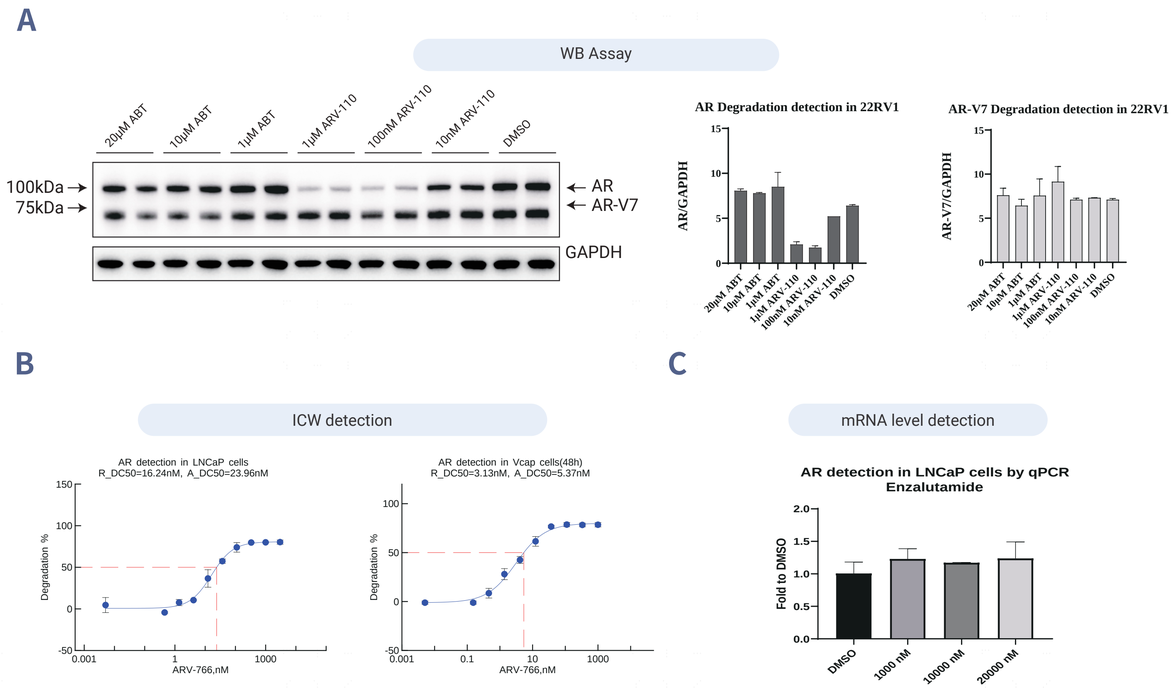


Figure 2. A. AR overexpression stable cell and 22RV1 AR-V7-HiBiT KI cell were generated for AR inhibitors and PROTAC validation by reporter assay. B. Multiple NHR receptors(MR,ER,GR...) cell lines were established to form NHR panel for compound screening by reporter assay.

2. AR protein level detection .



D

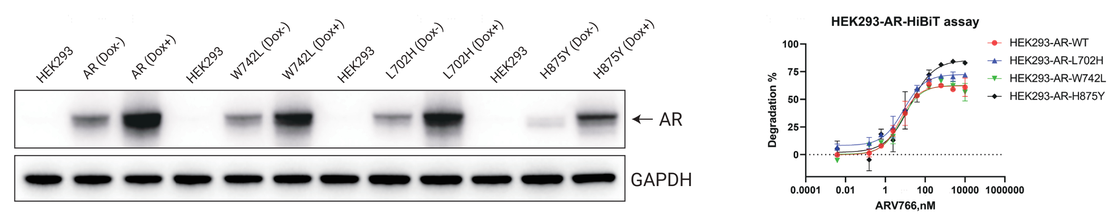


Figure 3. A. Different concentration of ABT and ARV-110 were used to incubate with 22RV1 cell, and the AR and AR-V7 protein were detected by western blot. B. An anti-AR antibody was utilized to assess the protein level of the AR in both LNCaP and VCaP cells after a 7-day exposure to the enzalutamide, as determined by the ICW assay. C. Enzalutamide did not influence the AR transcript in LNCaP cell. D. The HEK293-AR point mutant HiBiT cell lines was confirmed by WB, the protein level can be induced by Dox, ARV766 shown activity in AR mutation degradation detect in different cell lines.

3. Cell proliferation assay and resistent cell line establishment

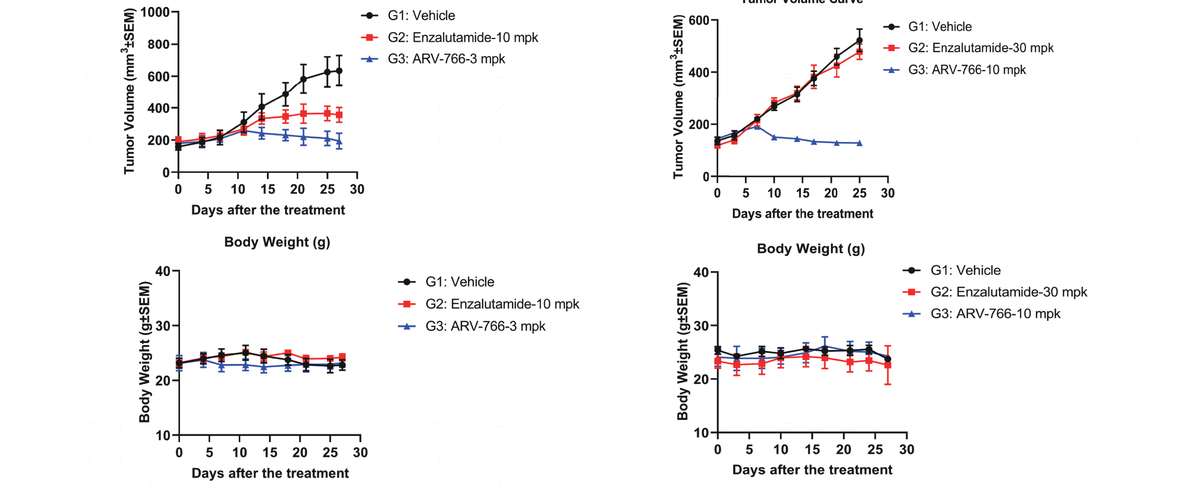


Figure 4. LNCaP and VCaP cells were applied in the test of enzalutamide and ARV-766 by proliferation assay at different treatment time.

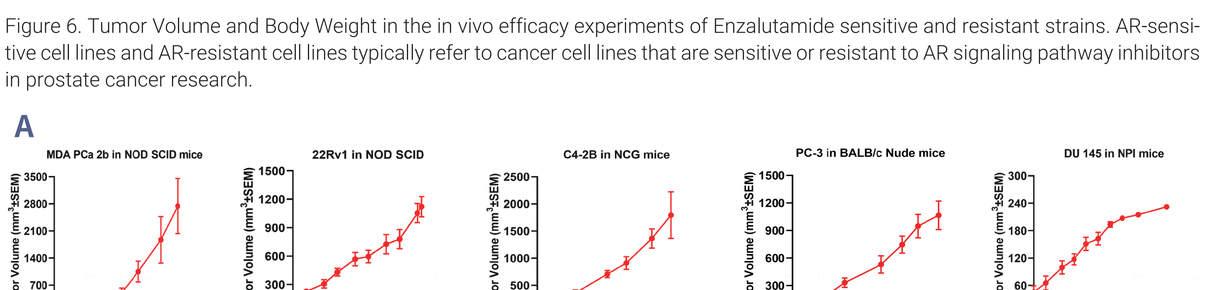


Figure5. A. Enzalutamide was tested in LNCaP C4-2B and in LNCaP C4-2B resistant cell separately. B. Docetaxel was tested in 22RV1 and in 22RV1 docetaxel resistant cell.

In Vivo CDX Model

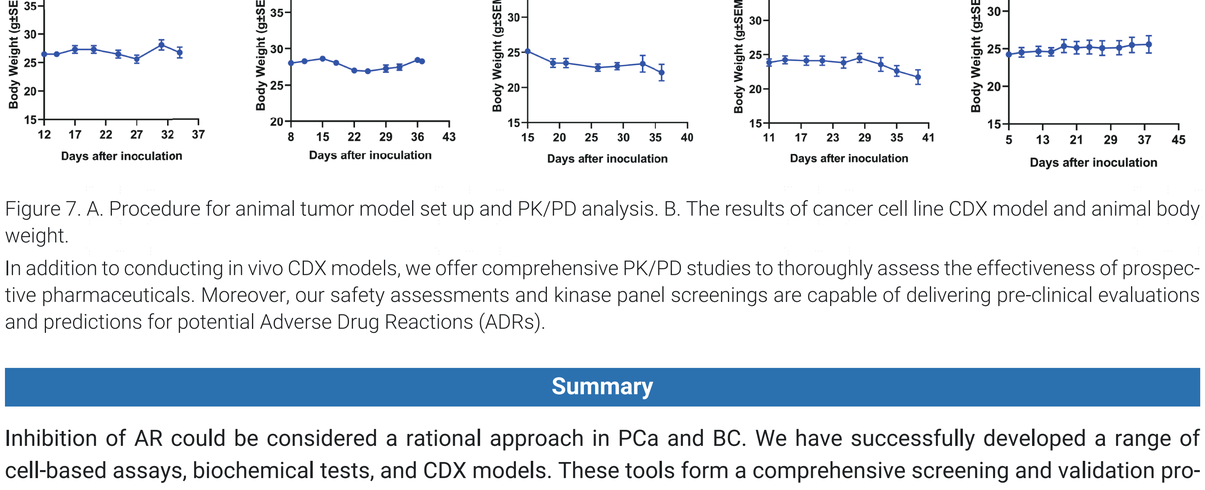


Figure 6. Tumor Volume and Body Weight in the in vivo efficacy experiments of Enzalutamide sensitive and resistant strains. AR-sensitive cell lines and AR-resistant cell lines typically refer to cancer cell lines that are sensitive or resistant to AR signaling pathway inhibitors in prostate cancer research.

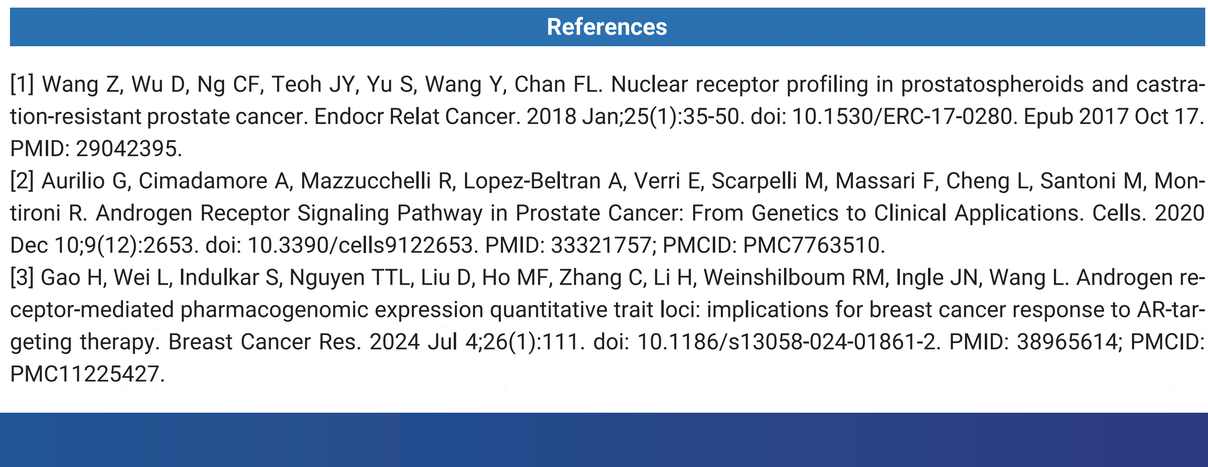


Figure 7. A. Procedure for animal tumor model set up and PK/PD analysis. B. The results of cancer cell line CDX model and animal body weight.

In addition to conducting in vivo CDX models, we offer comprehensive PK/PD studies to thoroughly assess the effectiveness of prospective pharmaceuticals. Moreover, our safety assessments and kinase panel screenings are capable of delivering pre-clinical evaluations and predictions for potential Adverse Drug Reactions (ADRs).

Summary

Inhibition of AR could be considered a rational approach in PCa and BC. We have successfully developed a range of cell-based assays, biochemical tests, and CDX models. These tools form a comprehensive screening and validation process for AR-targeted compounds. This integrated approach facilitates the study of AR mechanisms and enables efficient, thorough screening of potential AR inhibitors, thereby expediting the discovery of innovative therapeutics.

References

[1] Wang Z, Wu D, Ng CF, Teoh JY, Yu S, Wang Y, Chan FL. Nuclear receptor profiling in prostatospheroids and castration-resistant prostate cancer. Endocr Relat Cancer. 2018 Jan;25(1):35-50. doi: 10.1530/ERC-17-0280. Epub 2017 Oct 17. PMID: 29042395.

[2] Aurilio G, Cimadamore A, Mazzucchelli R, Lopez-Beltran A, Verri E, Scarpelli M, Massari F, Cheng L, Santoni M, Montironi R. Androgen Receptor Signaling Pathway in Prostate Cancer: From Genetics to Clinical Applications. Cells. 2020 Dec 10;9(12):2653. doi: 10.3390/cells9122653. PMID: 33321757; PMCID: PMC7763510.

[3] Gao H, Wei L, Indulkar S, Nguyen TTL, Liu D, Ho MF, Zhang C, Li H, Weinshilboum RM, Ingle JN, Wang L. Androgen receptor-mediated pharmacogenomic expression quantitative trait loci: implications for breast cancer response to AR-targeting therapy. Breast Cancer Res. 2024 Jul 4;26(1):111. doi: 10.1186/s13058-024-01861-2. PMID: 38965614; PMCID: PMC11225427.