Galeterone Suppresses Castration-Resistant and Enzalutamide-Resistant Prostate Cancer Growth *in Vitro*

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Highlights

- Androgen deprivation therapy remains the standard treatment of metastatic prostate cancer
- Progression to castration-resistant prostate cancer (CRPC) occurs in the majority of patients
- 80% of CRPC Express androgen receptor (AR) and androgen-responsive genes, ➔AR axis remains paradoxically activated despite castration

➢ Enzalutamide (Enz ; MDV3100)

- AR-targeting agent (antagonist) recently approved for metastatic CRPC following docetaxel therapy
- Resistance to enzalutamide has already been observed :
  - Targeting AR using ligand binding inhibitor is short lived
  - Reactivation of AR axis still one of the major drivers of Enz resistance
  - Enz mechanisms of resistance are similar to CRPC
  - Co-targeting strategy will help delay resistance

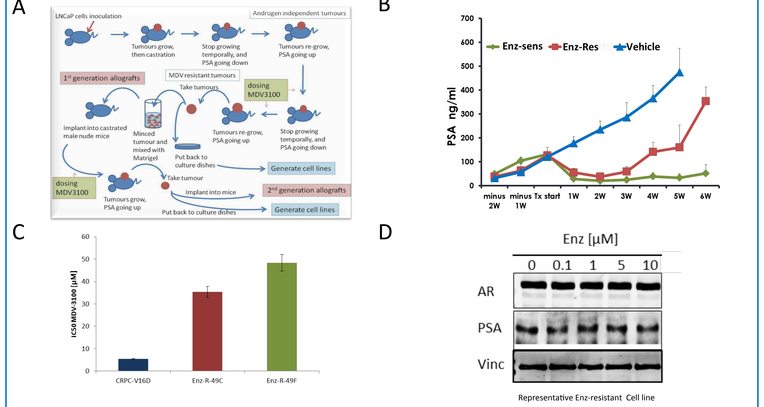
➔ Next major clinical challenge is identifying new therapies that prevent or treat anti-androgen resistance

➢ **Galeterone (TOK-001)**

- is a novel drug that exhibits three mechanisms of action
  - Selective CYP17 lyase inhibitor
  - Androgen Receptor (AR) antagonist
  - Decreases AR levels

- In this study we evaluated the efficacy of **galeterone** to inhibit AR activity in enzalutamide resistant cells

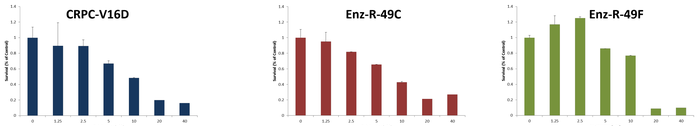
Establishing enzalutamide resistant tumors and cell lines



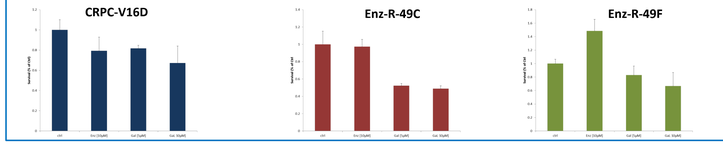
A- Illustration of the approach used to generate Enz-resistant cell lines. In total, 27 cell lines have been established, all have been characterized as AR+/- , PSA+/- and 25 out of 27 are resistant to 10µM Enz when grown in vitro.
B- Androgen sensitive LNCaP PCa cells were inoculated into male athymic nude mice and when the tumor burden reached 150 mm^3 and serum PSA levels was 50ng/ml, mice were randomly divided into treatment groups receiving 10mg/kg daily of enzalutamide or vehicle control. Graph shows serum PSA levels beginning two weeks after castration (minus 2W).
C- Enzalutamide IC_{50} in CRPC and Enz-resistant cell lines
D- Enzalutamide resistant cell lines express AR and PSA ➔ AR axis still activated

Galeterone decreases survival in CRPC and Enz-resistant Cell lines

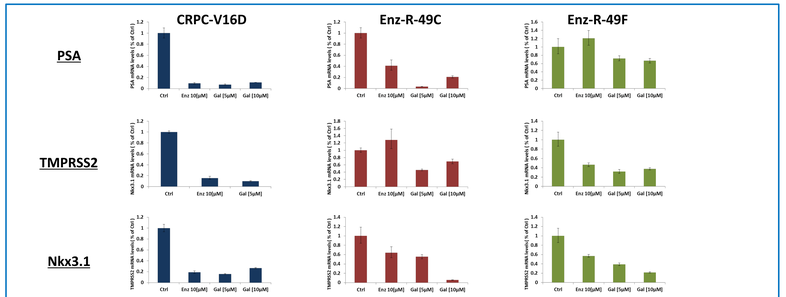
➢ CRPC and Enz-Resistant cells respond to galeterone between 5 and 10µm



➢ At 5µM and 10µM galeterone has a greater effect then enzalutamide in CRPC and Enz-R cells

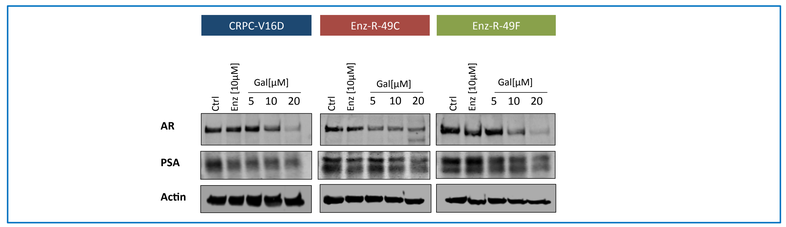


Galeterone reduces AR-dependent genes mRNA expression in Enz-resistant cells

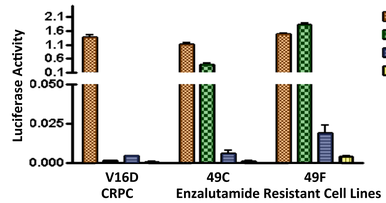


Total RNA was extracted from cultured cells after 48 hours of treatment using TRIzol reagent (Invitrogen Life Technologies, Inc.). mRNA expression was normalized to GAPDH levels as an internal standard, and the comparative cycle threshold (Ct) method was used to calculate relative quantification of target mRNAs. Each assay was conducted in triplicate.

Galeterone induces AR degradation in CRPC and Enz-resistant cells

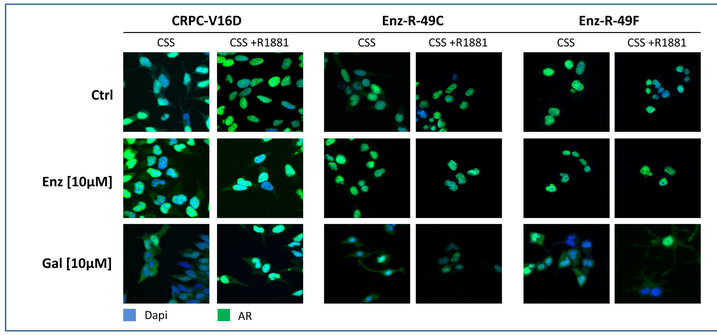


Galeterone induces a drastic decrease in Probasin luciferase reporter (AR) activity in CRPC and enzalutamide resistant cells



CRPC-V16D, Enz-R-49F and Enz-R-49C cells were transfected with Probasin luciferase reporter along with *Renilla* plasmid and then treated with enzalutamide or galeterone for 24h. All experiments were carried out in triplicate wells.

Galeterone Inhibits AR nuclear translocation in CRPC and enzalutamide resistant cells



Conclusion

- Galeterone has anti-proliferative effects in CRPC cells but most importantly, in enzalutamide resistant cell lines
- The resistant cell lines are still responding to Galeterone; no significant difference in IC_{50} between Enz-resistant and CRPC cells
- Galeterone decrease AR dependent genes expression in CRPC and Enz-resistant cell lines
- Compared to enzalutamide treatment, galeterone induces a drastic decrease in Probasin luciferase reporter (AR) activity in CRPC and Enz-resistant cells
- Compared to enzalutamide treatment, galeterone causes a greater reduction of AR nuclear translocation in CRPC and Enz-resistant cells

➔ **Galeterone is a potent inhibitor of the AR pathway and may represent the next generation of hormone therapy for patients with not only CRPC but also enzalutamide resistant disease**

tōkai

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