Galeterone-induced Degradation of the Androgen Receptor Involves Inhibition of a Deubiquitinating Enzyme

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Abstract: #345

Abstract

Galeterone is a highly selective oral small molecule drug candidate that disrupts androgen receptor (AR) signaling. It degrades the AR (IC_{50} ~1μM[1]), is a potent CYP17 lyase inhibitor (<50nM[2,3]), and possesses AR antagonist activity (~600nM[4]). Galeterone-induced AR degradation was observed in models having either full-length AR or known constitutively active truncated forms of the AR receptor that lack the ligand binding domain (LBD), AR-V7 and AR567es. The ligand binding domain (LBD) of the AR is not required for galeterone-dependent AR degradation. Galeterone-induced degradation activity is blocked by co-administration of the proteasome inhibitor MG132. Furthermore, galeterone-induced degradation of AR can be blocked by selective knock-down of the E3 ligases, Mdm2 and CHIP[1]. We utilized a series of biochemical and cell-based *in vitro* studies to further elucidate and characterize additional signaling molecules in the proteasomal dependent mechanism of galeterone-induced AR degradation. We screened a panel of 22 deubiquitinating enzymes (DUBs) *in vitro* and demonstrated that galeterone inhibited enzymatic activity of the DUBs, USP12 and USP46 with IC_{50} in the single digit micromolar range. In addition, we used surface plasmon resonance to demonstrate that dose-dependent inhibition of USP12 and USP46 activity involves direct binding of galeterone to USP12 and USP46 and to each DUB when complexed with UAF1 with a K_D of ≤10μM. Interestingly, USP12 is a co-activator of AR and selective knock-down of this DUB has been shown to increase AR degradation[6]. USP12 and USP46 have been linked to regulation of the PH domain and Leucine rich repeat Protein Phosphatases (PHLPPs) through ubiquitination[7]. PHLPPs dephosphorylate AKT, providing an important regulatory mechanism for controlling the PI3K/AKT pathway. Since it is known that galeterone induces an increase in pAKT and pMdm2, the latter being a substrate of activated AKT, this suggests that inhibition of USP12/USP46 regulates pAKT levels through enhanced degradation of the PHLPPs via increased ubiquitination. These data suggest that a mechanism that differentiates galeterone from other AR targeting agents is through inhibition of USP12/USP46, leading to enhanced AR degradation.

Galeterone: Clinical Development Highlights

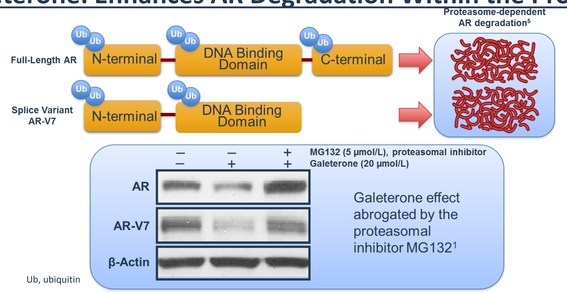
- Clinically meaningful PSA_{50} responses observed in mCRPC patients with C-terminal loss
  - AR-V7 is the most common form of C-terminal loss, and is a biomarker for primary resistance to currently-approved oral therapies for mCRPC (abiraterone/enzalutamide)[8]
- Well-tolerated safety profile
- Pivotal Phase 3 clinical trial of treatment-naïve, AR-V7+ mCRPC patients ongoing
  - Screening eligibility determined using proprietary assay for AR-V7+
  - Primary endpoint: rPFS vs. enzalutamide

Galeterone: Highly Differentiated With First-in-Class MOA

First in Class Potential

	Androgen Receptor (AR) Degrader	CYP17 Inhibitor	AR Antagonist
	Decreases AR Levels Differentiated Mechanism	Inhibits Androgen Synthesis Validated Mechanism	Blocks Androgen Binding Validated Mechanism
GALETERONE	Active in CRPC patients AND supporting data in patients with C-terminal Loss	Improved profile: No Steroids required	Improved Profile: No Seizures to date

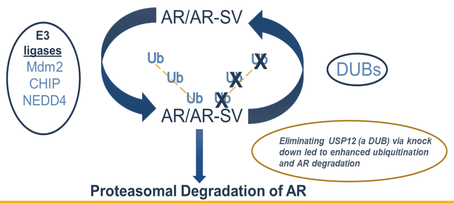
Galeterone: Enhances AR Degradation Within the Proteasome



Proteasomal Degradation of AR: Balance of E3 Ligase and DUB Activities

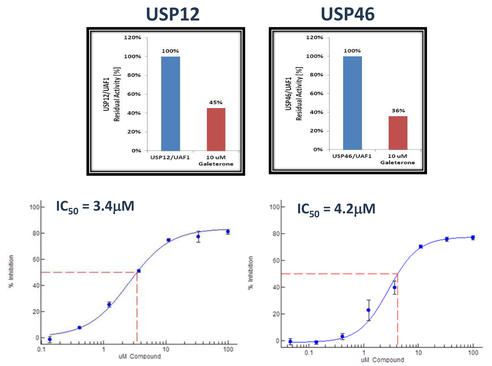
- Proteins are "tagged" for degradation by **ubiquitin (Ub)**
- Tagging is mediated by **E3 ligases** and increases proteasomal degradation

- **Deubiquitinating enzymes (DUBs)** remove ubiquitin tags to decrease proteasomal degradation

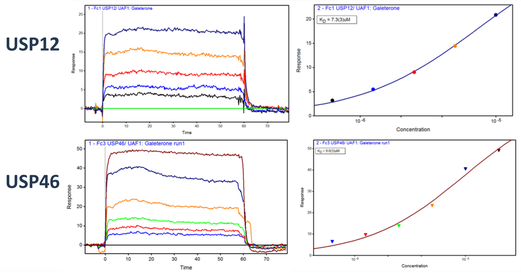


- Galeterone regulates AR levels through modulation in Mdm2 and CHIP[1]
- DUB USP12 is a novel coactivator of the AR[6]
- DUBs USP12/USP46 regulate the interaction between the AR and the Akt pathway[7]
- We tested the effect of galeterone on a panel of DUBs (USP2 catalytic domain, USP5, USP25, USP7, USP8, UCHL1, UCHL3, USP9x, USP20, USP19, USP28, USP1/UAF1, USP12, USP12/UAF1, USP46, USP46/UAF1, CYLD, UCHL5, Otubain 2, OTUD3 catalytic domain, Cezanne, Yod1, SARS-PLPro, Trabid, AMSH) in a single point (10μM) *in vitro* biochemical assay

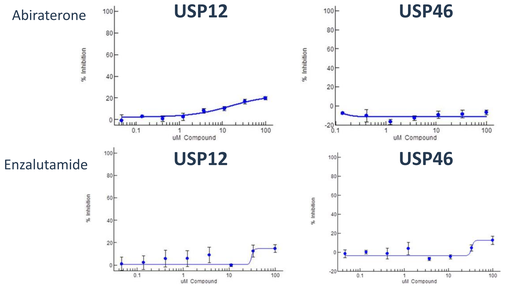
Galeterone Inhibits USP12 and USP46 Enzymatic Activity *In Vitro*



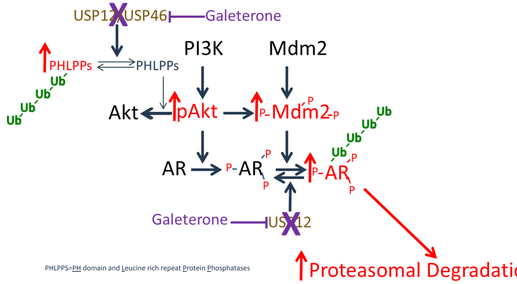
SPR Studies Demonstrate Galeterone Binds Dose-dependently to USP12 and USP46



Abiraterone and Enzalutamide Do Not Inhibit USP12 and USP46 Activity



Proposed Mechanism of Galeterone-Induced AR Degradation



Summary

- Galeterone induces proteasomal degradation of AR by changing the balance between ubiquitination and deubiquitination
- Galeterone binds to and inhibits USP12/USP46, thereby enhancing proteasomal degradation of AR/AR-V7
  - Decreases deubiquitination
- Galeterone induces proteasomal degradation of AR/AR-V7 through modulation of the E3 ligases, Mdm2 and CHIP
  - Increases ubiquitination
- Neither abiraterone or enzalutamide induced AR degradation or inhibited USP12/USP46

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