Galeterone-induced Degradation of the Androgen Receptor Involves Inhibition of Deubiquitinating Enzymes

Daniel T. Dransfield, Nivedita Namdev, Douglas B. Jacoby and Karen J. Ferrante

Tokai Pharmaceuticals, 255 State Street, Boston, MA 02109 www.tokaipharma.com

Abstract: #1234

Abstract

Galeterone is a highly selective oral small molecule drug candidate that disrupts androgen receptor (AR) signaling through degradation of the AR[1], is a potent CYP17 lyase inhibitor[2,3], and possesses AR antagonist activity[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, suggesting the LBD of the AR is not required for galeterone-dependent AR degradation. Further, galeterone-induced AR degradation activity is blocked by co-administration of the proteasome inhibitor MG132[1]. Along these lines, galeterone-induced AR degradation 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 methodologies to further elucidate and characterize additional signaling molecules participating 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 selectively inhibited enzymatic activity of two of the DUBs, USP12 and USP46. Separately, we used surface plasmon resonance to demonstrate a dose-dependent direct binding of galeterone to each of USP12 and USP46, alone or when pre-complexed with UAF1. USP12 is a co-activator of the AR, and selective knock-down of this DUB has been shown to increase AR degradation[6]. USP12/USP46 have also been linked to regulation of phosphatases (PHLPPs) through ubiquitination[7]. PHLPPs dephosphorylate AKT, providing an important regulatory mechanism for controlling the PI3K/AKT pathway. 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 may regulate pAKT levels through enhanced degradation of PHLPPs via increased ubiquitination. These data support a differentiating mechanism of galeterone from other AR targeting agents whereby inhibition of USP12/USP46 leads 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 in treatment-naïve, AR-V7+ mCRPC patients ongoing
  - Screening eligibility determined using proprietary assay for AR-V7+
  - Primary endpoint: superior rPFS vs. enzalutamide

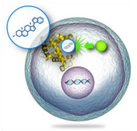
Galeterone: First-in-Class MOA

First in Class Potential

Androgen Receptor (AR) Degrader

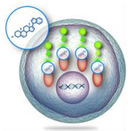
Decreases AR Levels
Differentiated Mechanism

CYP17 Inhibitor



Inhibits Androgen Synthesis
Validated Mechanism

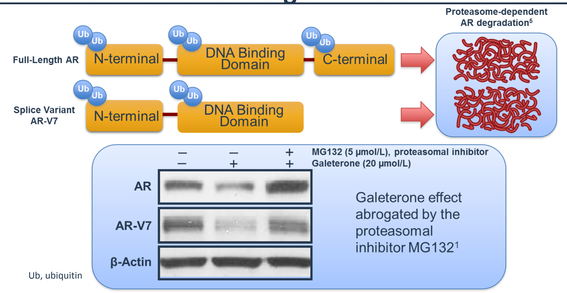
AR Antagonist



Blocks Androgen Binding
Validated Mechanism

- Active in CRPC patients AND supporting data in patients with C-terminal loss

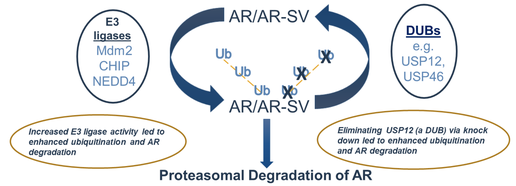
Galeterone: Enhances AR Degradation Within the Proteasome



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

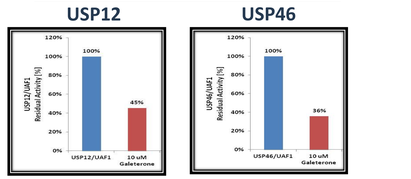
- Proteins are "tagged" for degradation by **ubiquitin (Ub)**
- Tagging is mediated by **E3 ligases** and increases proteasomal degradation

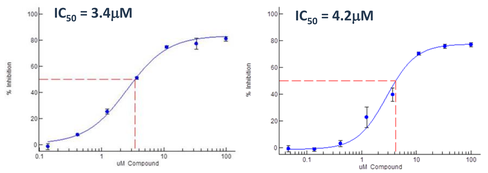
- **Deubiquitinating enzymes (DUBs)** remove ubiquitin tags to decrease proteasomal degradation



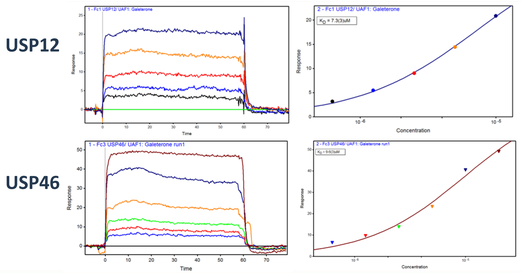
- Galeterone regulates AR levels through modulation of the E3 ligases, Mdm2 and CHIP[1]
- DUB USP12 is a novel coactivator of the AR[6]
- DUBs USP12/USP46 regulate the interaction between the AR and the Akt pathway[7]
- We tested the effect of galeterone on a panel of DUBs (USP2 catalytic domain, USP5, 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*

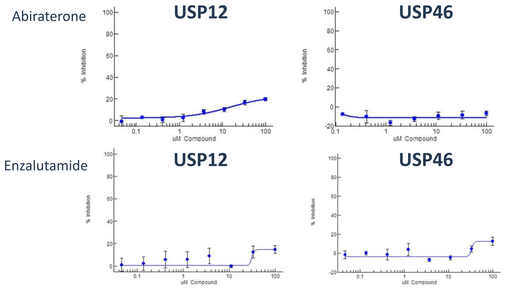




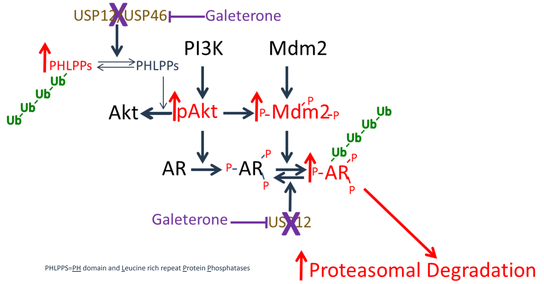
SPR Studies Demonstrate Galeterone Binds Dose-dependently to USP12 and USP46



Abiraterone and Enzalutamide Do Not Inhibit USP12 and USP46 Activity



Proposed Mechanism of Galeterone-Induced AR Degradation



Summary

- Galeterone induces proteasomal degradation of AR/AR-V7 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 activity

1. Kwegyir-Afful AK et al. *Oncotarget* 2015;6(29):27440
2. Tokai Research Reports (TK-001, TK-002)
3. Bruno RD et al. *Steroids*. 2011;76(12):1268
4. Purushottamachar P et al. *J Med Chem*. 2013;56(12):4880
5. Lin H-K et al. *EMBO J*. 2002;21:4037
6. Burska UL et al. *JBC*. 2013;288(45):32631
7. McClurg UL et al. *Oncotarget* 2014;5(16):7081
8. Antonarakis E, et al. *NEJM* 2014;371(11):1028

ddransfield@tokaipharma.com

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