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Targeted Degradation of the Androgen Receptor in Prostate Cancer

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Abstract

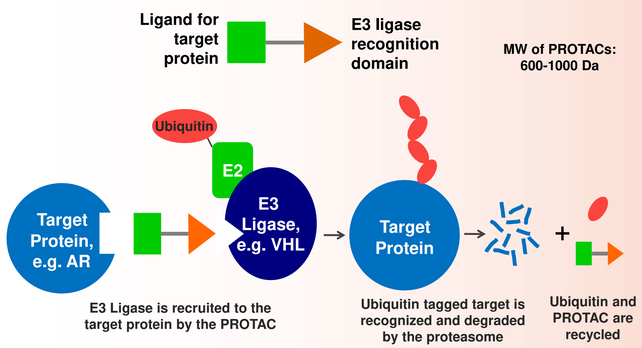
Progression of prostate cancer in patients treated with anti-androgen therapy usually involves several mechanisms of enhanced Androgen Receptor (AR) signaling, including increased intratumoral androgen synthesis, increased AR expression and AR mutations. We have developed a protein degradation technology called PROTAC (PROteolysis TArgeting Chimera), which uses bi-functional molecules that simultaneously bind a target of choice and an E3 ligase. PROTACs, via induced proximity, cause ubiquitination and degradation of the targeted, pathological protein. As opposed to traditional target inhibition, which is a competitive process, degradation is a progressive process. As such, it is less susceptible to increases in endogenous ligand, target expression, or mutations in the target. Thus this technology seems ideal for addressing many mechanisms of AR resistance in patients with prostate cancer.

AR PROTACs were shown to degrade AR in LNCaP and VCaP cells, with low nM to pM potency, and had a >90% reduction in AR concentration (Dmax). Degradation was rapid, with 50% of AR lost within 15 minutes and maximal degradation observed by 4 hours. The degradation process in cells was specific, as the PROTAC activity can be competed with excess E3 ligand and PROTACs with an inactive epimer for E3 ligase binding did not degrade AR. AR PROTACs induced rapid apoptosis and cell death in VCaP cells. In LNCap and VCaP cell systems, AR PROTACs were anti-proliferative under conditions in which enzalutamide was inactive, such as increasing concentrations of the AR agonist R1881 and cells containing the AR-F876L mutation. AR PROTACs typically exhibited good pharmacokinetic properties, with $t_{1/2}$ values of several hours and bioavailability of >50% after ip or sc injection. In mice, AR PROTACs demonstrate in vivo activity, including reduction of AR protein levels, prostate involution and tumor growth inhibition.

In summary, PROTACs designed to degrade AR are potent, specific, active in vitro and in vivo, and have cellular efficacy superior to enzalutamide. Targeted degradation of AR may provide a novel mechanism for providing efficacious therapy for patients with prostate cancer for whom current therapies have failed.

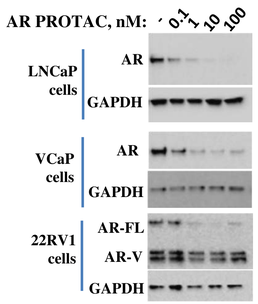
PROTAC: PROteolysis TArgeting Chimera

- **Technology developed by Prof. Craig Crews, Yale University**
- **Platform licensed to Arvinas in 2013**

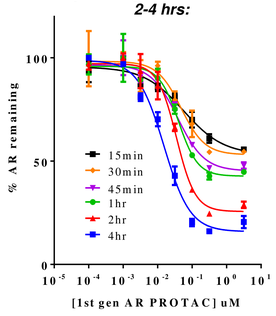


VHL recruiting Androgen Receptor (AR) PROTACs: potent, rapid, VHL specific and selective

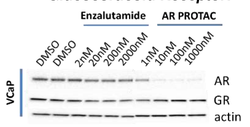
Sub-nM AR degrader:



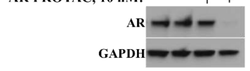
Complete degradation reached in 2-4 hrs:



AR PROTAC does not degrade Glucocorticoid Receptor:

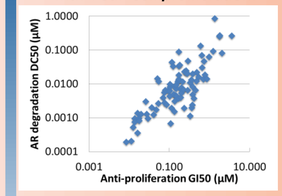


Blocking VHL binding abolishes AR PROTAC activity:

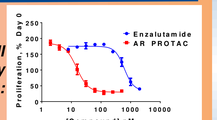


Functional characterization of AR PROTACs *in vitro*

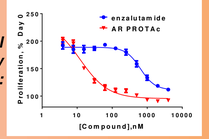
AR degradation potency correlates with VCaP cell proliferation:



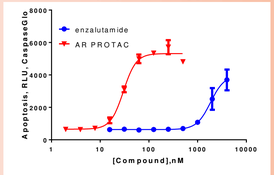
VCaP cell proliferation assay with 0.1 nM R1881:



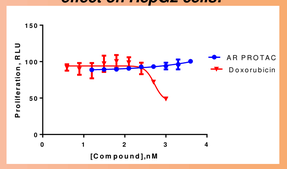
LNCaP/AR cell proliferation assay with 0.1 nM R1881:



Apoptosis in VCaP cells with 0.1 nM R1881:

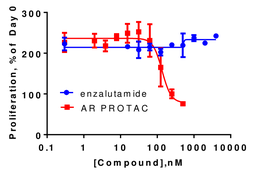


AR PROTAC has no antipoliferative effect on HepG2 cells:

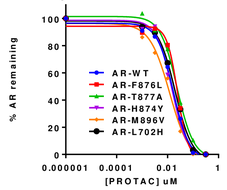


AR PROTAC retains potency in high androgen milieu and across AR mutations

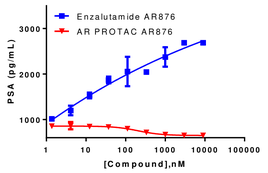
VCaP cell proliferation in 1 nM R1881:



AR PROTAC degrades all clinically relevant AR mutations:



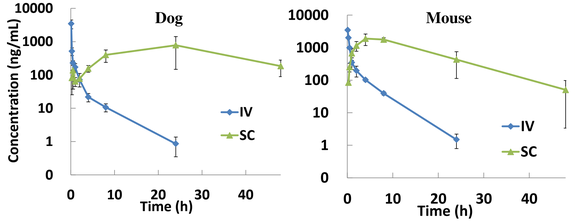
AR PROTAC demonstrates no agonist properties in LNCaP/AR-F876L



AR mutation	agonist
F876L	Enzalutamide, ARN-509
T877A	Glucocorticoids, progesterone
H874Y	Glucocorticoids, progesterone
M896V	N/A
L702H	Glucocorticoids

AR PROTACs exhibit favorable pharmacokinetic (PK) profile – representative curves shown

Single dose PK by 1 mpk intravenous (IV) or 10 mpk subcutaenous (SC) route

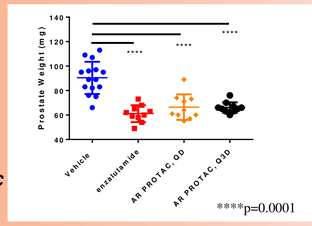


AR PROTACs lead to mouse prostate involution

Ten day mouse prostate involution assay treatment arms:

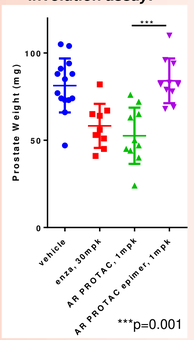
- *Enzalutamide: 30 mpk, PO, QD*
- *AR PROTAC: 3 mpk, SC, QD*
- *AR PROTAC: 3 mpk, SC, Q3D*

Robust efficacy observed with daily and intermittent dosing of AR PROTAC



Stereoselective activity of AR PROTACs in vivo

Ten day mouse prostate involution assay:



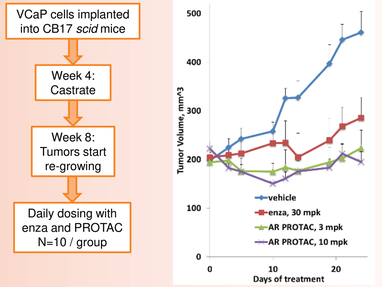
Plasma PK shows equal exposures for AR PROTAC and its epimer:

Drug	Dose	Plasma Conc (nM) 4 hr Post Dose
Enza	30 mg/kg PO	59900
AR PROTAC	1 mg/kg IP	280
AR PROTAC epimer	1 mg/kg IP	290

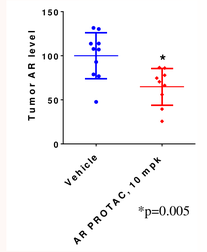
- ***The AR PROTAC and its epimer have different configurations at one stereogenic center in the VHL binding moiety. The epimer does not bind VHL, does not degrade AR and does not show in vivo efficacy.***

AR PROTAC demonstrates antitumor activity in prostate cancer xenograft model

VCaP xenograft in castrated mice. AR PROTAC was delivered SC QD:



AR degradation in VCaP xenograft 8 hrs post last dose:



Summary

The AR PROTACs demonstrate pM AR degradation potency and consistent functional activity in various *in vitro* and *in vivo* systems thought to represent the shortcomings of current prostate cancer treatment regimens.

Complete degradation of AR provides a novel mechanism to address mCRPC:

- **Degradation is ideally suited for AR-amplified mCRPC**
- **AR PROTACs target AR irrespective of its mutational status and binding partners**
- **Since PROTACs only need to make a transient interaction with their targets, AR PROTACs retain efficacy in high androgen environment**
- **AR PROTACs are effective against growth factor activated AR**