# Discovery of Helicon™ Peptides that Selectively Degrade the Agonist-Bound Androgen Receptor ($AR^{ON}$) in Prostate Cancer

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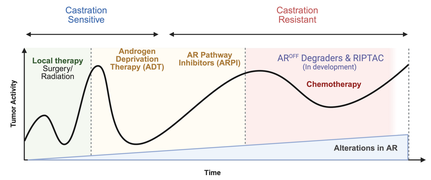
## Background

The androgen receptor (AR) is a critical driver of prostate cancer, regulating genes that control tumor growth, survival, and progression. Androgen deprivation therapy (ADT) is a cornerstone of treatment, but many patients eventually develop castration-resistant prostate cancer (CRPC), in which AR signaling remains active through mutations, amplification, or splice variants. Thus, AR remains a prime therapeutic target, and drug discovery has focused on next-generation AR antagonists, degraders, and other approaches to overcome resistance. Advances in targeting AR biology continue to shape the landscape of prostate cancer therapy and drive innovation in precision oncology.



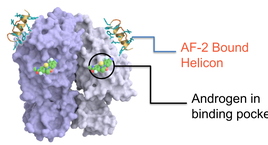

**Limitations of $AR^{OFF}$ agents:**

| 1 *Amplifications in AR* | 2 *Mutations in AR LBD* | 3 *AR Splice Variants* | 4 *Combinability* |
|---|---|---|---|
| ▪ Do not achieve broad efficacy in patients harboring $AR^{AMP}$<br>▪ Heterogeneity in clinical responses | ▪ Majority of current AR agents bind the LBD in the androgen-binding pocket that is subject to frequent mutations<br>▪ Affects the ability of agents to function optimally | ▪ Prevalence and expression of splice variants increase with progression of disease<br>▪ AR-V7 most prominent variant resulting in truncated AR lacking LBD | ▪ Most AR antagonists and degraders bind to the androgen binding pocket<br>▪ Potential antagonism prevents their use in combination |

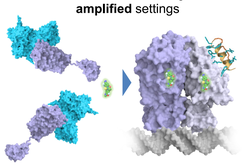
Currently approved agents and the majority of those in development bind to the inactive conformation of AR that is not androgen bound ($AR^{OFF}$), preventing activation of the protein, rather than targeting it in the active, agonist-bound conformation that is responsible for driving transcription and tumorigenesis. Tumors that retain AR pathway dependence while treated with these inhibitors respond with adaptive activation of AR signaling, which in turn is associated with relapse and disease progression.

## Our Unique Approach to Targeting AR

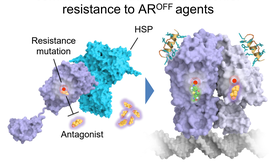
Our Helicon-based bifunctional degraders bind to the AR LBD in its agonist-bound conformation, at the AF2 pocket, distinct from the ligand binding pocket targeted by $AR^{OFF}$ agents. Binding to the AF2 pocket enables us to selectively degrade the $AR^{ON}$ agonist-bound pool.



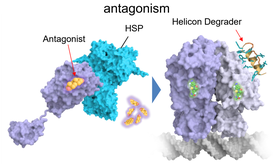

$AR^{ON}$ degraders may have stoichiometric advantage in **amplified** settings




$AR^{ON}$ degraders are not susceptible to common LBD **mutations** that confer resistance to $AR^{OFF}$ agents




$AR^{ON}$ and $AR^{OFF}$ agents can be **combined** for superior AR antagonism




## $AR^{ON}$ Helicons Bind to Agonist-bound LBD

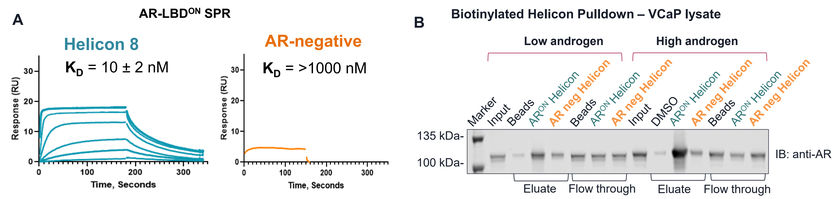



**Figure 1**. $AR^{ON}$ Helicon and associated negative control bind to agonist loaded AR. (A) $AR^{ON}$ Helicons bind recombinant, DHT-loaded LBD with high affinity as measured by SPR, with the matched AR negative control that is unable to bind. (B) AR pulldown by biotinylated Helicons from VCaP lysate is strongly increased by treating cells in culture with high androgen concentration (30nM DHT) prior to generating lysate.

## Degradation of $AR^{ON}$ Reduces AR Signaling and Inhibits Cell Proliferation

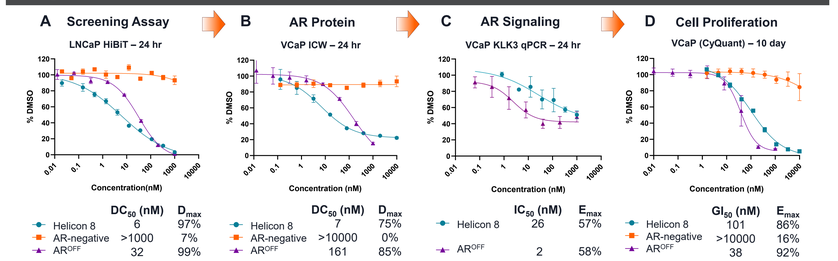



**Figure 2**. $AR^{ON}$ Helicons are nanomolar potency AR degraders that reduce AR dependent transcription and cell proliferation. (A) AR degradation in LNCaP cell lines expressing AR-HiBiT (10nM R1881), (B) VCaP In Cell Western (ICW), (C) *KLK3* transcript levels measured by qPCR, (D) cell proliferation after 10 days measured by DNA-content using CyQUANT. VCaP cells were conditioned with 1 nM R1881 for at least 10 days before the start of all assays.

## $AR^{ON}$ Helicons Selectively Deplete Nuclear AR

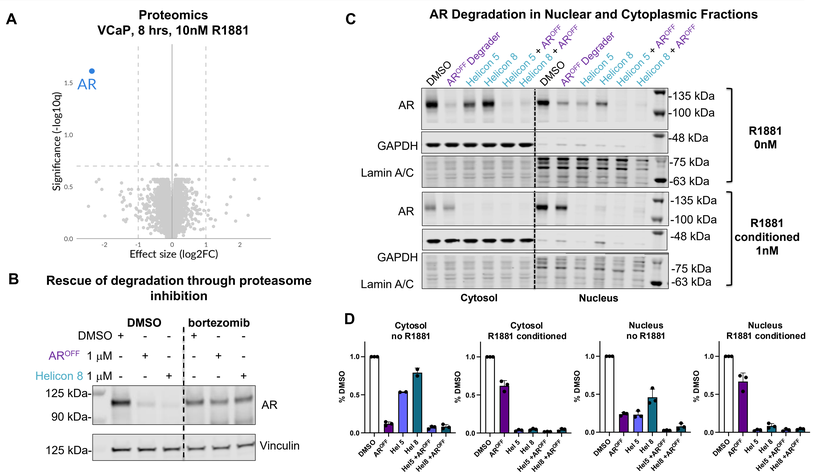



**Figure 3**. $AR^{ON}$ Helicons selectively degrade AR and preferentially reduce nuclear AR. (A) Proteomics profiling comparing Helicon 8 with vehicle control, (B) Proteosome inhibition with bortezomib blocks AR degrader activity. (C) Subcellular fractionation of cells treated with $AR^{OFF}$ degrader, Helicon 5, Helicon 8, or a combination $AR^{OFF}$ degrader and Helicon. (D) Quantitation of immunoblots in (C). All cell data presented was obtained from VCaP cells.

## $AR^{ON}$ Degraders Demonstrate Combination Potential with ARPI SoC Agents

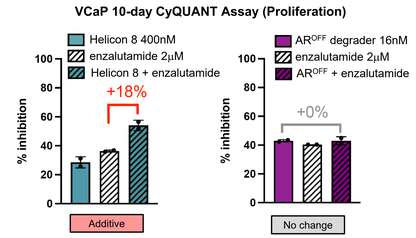



**Figure 4**. $AR^{ON}$ Helicons exhibit additive anti-proliferative effects with $AR^{OFF}$ antagonists. Enzalutamide had additive effects when combined with Helicon 8 across most of the concentration range in a 10-day CyQUANT proliferation assay. No additivity was observed for an $AR^{OFF}$ degrader.

## $AR^{ON}$ Degrader Exhibits *In Vivo* anti-tumor Activity with Associated AR Protein Reduction

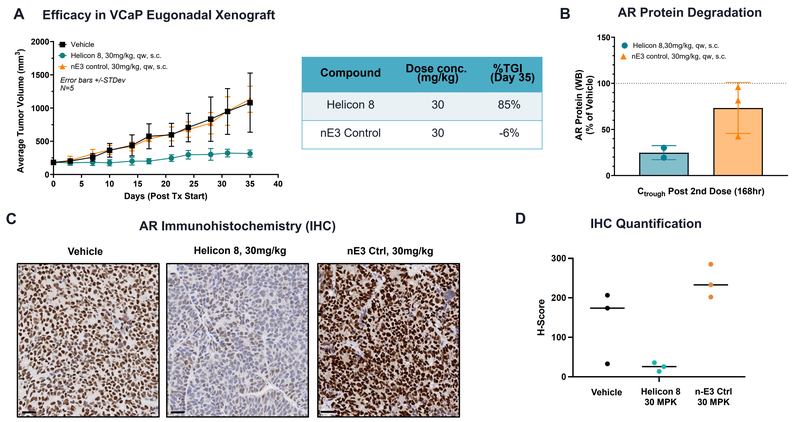



**Figure 5**. *In vivo* efficacy and AR degradation of $AR^{ON}$ degraders in a VCaP eugonadal xenograft model (A) Tumor growth inhibition following treatment with Helicon degraders compared to E3-incompetent negative control. (B) Reduction of AR protein levels in tumors following dosing. (C) Representative AR IHC images across treatment groups. (D) AR staining intensity H-score quantification across treatment groups. Size bar 20 μm

## Conclusions

- We have developed Helicon-based bifunctional degraders of the $AR^{ON}$ pool of the androgen receptor with potent binding affinity and degradation
- Our Helicon-based degraders are E3-dependent and selective for AR
- $AR^{ON}$ degradation is sufficient to inhibit AR signaling and cell proliferation, despite constituting only a portion of total AR under low androgen conditions
- *In vitro* evidence suggests $AR^{ON}$ degraders can be additively combined with SoC ARPIs such as enzalutamide without observing antagonistic effects, which is not possible with $AR^{OFF}$ degraders that bind to the same pocket
- $AR^{ON}$ degraders demonstrate *in vivo* tumor growth inhibition with associated AR protein reduction, supporting effective targeting of agonist-bound $AR^{ON}$ pool and validating its importance in maintaining PCa tumor maintenance

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