

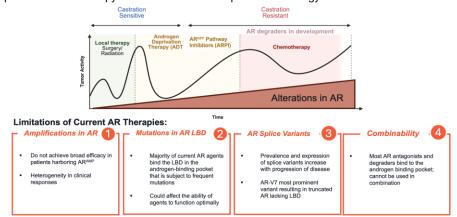
Discovery of Helicon Peptides for the Selective Degradation of the Agonist-Bound Conformation of Androgen Receptor (ARON) in Prostate Cancer

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Background

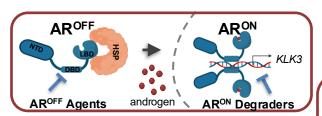
The androgen receptor (AR) is a critical driver of prostate cancer, regulating genes that control tumor growth, survival, and progression. Androgen deprivation therapy (ADT) is a cornerstone of treatment, but many patients eventually develop castration-resistant prostate cancer (CRPC), in which AR signaling 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.

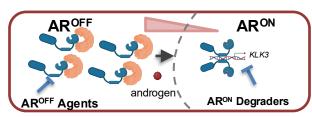


Currently approved agents and the majority of those in development bind to the inactive conformation of AR that is not androgen bound (AROFF), preventing activation of the protein, rather than targeting it in the active, agonist-bound conformation that is responsible for driving transcription and tumorigenesis. 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 a site distinct from the ligand binding pocket targeted by AR^{OFF} agents. Binding to a unique LBD site outside of ligand binding pocket enables us to selectively degrade the AR^{ON} agonist-bound pool.



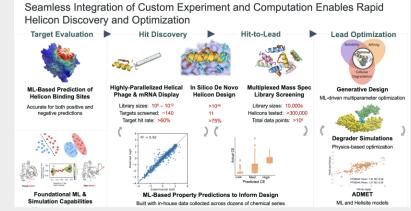


Key advantages of AR^{on} Helicondegraders:

- Known LBD point mutations do not alter Helicon binding
- Amplified AR does not act as a decoy
- Ability to combine with current SoC AR^{OFF} agents

Computational *De Novo* Discovery and Optimization

Using a combination of *de novo* computational and structure-based molecular design, we discovered compounds that bind to the AR LBD in its agonist-bound conformation, at a site distinct from the ligand binding pocket targeted by AR^{OFF} agents.



- Customized high-throughput chemistry on 1000's of non-natural amino acids and 100's of staples
- Proprietary high-quality data from in vitro and in vivo biological data
- Custom Al/ML models and physics-based modeling connects biology to Helicon binding sites on targets of interest and to navigate vast chemical space

ARON Helicons Bind to Agonist-bound LBD

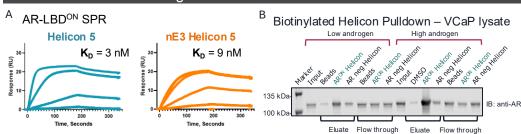


Figure 1. AR^{on} helicons bind to agonist bound AR LBD. (A) AR^{on} helicons bind recombinant, agonist-loaded LBD with high affinity as measured by SPR (B) AR pulldown by biotinylated helicons from VCaP lysate is strongly increased by treating cells in culture with high androgen concentration prior to generating lysate.

Degradation of ARON Decreases AR Signaling and Cell Proliferation

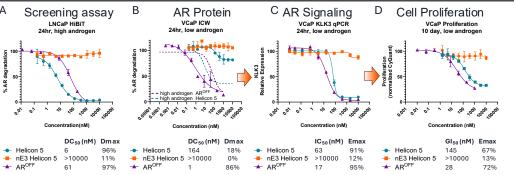


Figure 2. AR^{ON} helicons are nanomolar potency AR degraders that reduce AR dependent transcription and cell proliferation in VCaP cells. (A) AR degradation in LNCaP cell lines expressing AR-HiBiT (10nM R1881) and (B) VCaP In Cell Western (no added androgen) (C). KLK3 transcript levels measured by qPCR (D) Cell proliferation after 10 days as measured by DNA-contentusing CyQUANT (no added androgen).

ARON Helicons Preferentially Reduce Nuclear AR

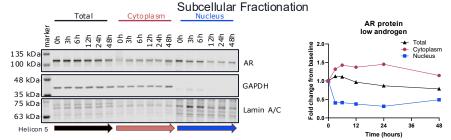


Figure 3. Subcellular fractionation of VCaP cells treated with ARON degrader demonstrates preferential degradation of nuclear pool of AR

ARON Degraders results in selective, E3/UPS-dependent AR degradation

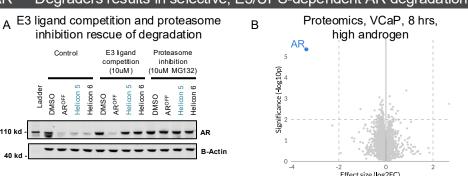


Figure 4. AR^{on} helicons selectively degrade AR in E3 ligase and UPS-dependent manner in LNCaP cells (A) Competition with 10x excess E3 ligand blocks helicon or proteasome inhibition with MG132 block helicon degrader activity. (B) Proteomics profiling comparing helicon 5 with vehicle control

AR^{ON} degraders demonstrate combination potential with ARPI SoC

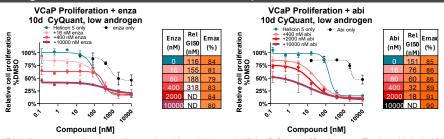


Figure 5. AR^{on} helicons were combined with either enzalutamide or abiraterone in 10-day CyQuant proliferation assay. Enzalutamide had additive effects to helicon across most of the concentration range. Abiraterone combination had synergistic effects, and shifted the helicon dose response left

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 combined with SoC ARPIs such as enzalutamide and abiraterone without observing antagonistic effects

Acknowledgments

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