First-in-Class AR-V7/AR-fl Small Molecule Degrader for Prostate Cancer Therapeutics

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Metastatic castration resistance prostate cancer (mCRPC) is a lethal disease due to the development of resistance to standard-of-care (SoC) treatment, namely androgen receptor (AR) signaling inhibitors and taxanes. Treatment resistance occurs partly due to the expression of constitutively active AR splice variants, that hijack AR signaling even after castration. AR-V7, the most prevalent variant, is expressed in approximately 75% of patients with mCRPC, and confers resistance to SoC. Currently, there is no AR-V7 pharmacologic inhibitor, limiting patients' therapeutic options. Thus, AR-V7 inhibition remains an urgent clinically unmet need.

To address this gap, we conducted a high-throughput small molecule AR-V7 inhibitor phenotypic screen of 170K compounds and identified hit **compound 7907**, **as a dual AR-V7 and AR full-length (AR-fl) protein degrader**, with unique chemotype compared to all known AR modulators. Hit to lead optimization by medicinal chemistry and SAR studies identified **lead ARV/AR degraders** (**ARVD** hereafter) with increased potency (nM range) compared to 7907.

Mechanistically, ARVD compounds did not affect AR-V7/AR-fl transcription, but rather shortened their half-life leading to degradation within 4hr of treatment through Cullin-RING E3 ligase activity. ARVD activity was blocked by the proteasome inhibitor, bortezomib, suggesting E3-mediated proteasomal degradation as the mechanism of action (MoA). Cryo-EM analysis identified conformational changes of AR-fl in the presence of ARVD indicating direct binding, which was confirmed by cellular thermal shift assays. Serial mutagenesis of AR-V7/AR-fl identified that ARVD binds at the N-terminal domain (NTD), shared by both AR isoforms, specifically interacting with TAU1 and AF1 NTD functional subdomains but not with TAU5. Preliminary molecular docking experiments further supported this binding. Importantly, ARVD compounds reversed enzalutamide resistance *in vitro*, addressing a crucial therapeutic need. ARVD target specificity was demonstrated by dose-dependent cytotoxicity enhancement in AR-V7-positive cells across various PC cell lines with different AR-V7/AR-fl expression profiles and lack of activity in AR-null or normal prostate epithelial cells. Additionally, four top ARVD compounds were selected for *in vivo* evaluation based on on-target activity, drug-likeness, and formulation. Preliminary pharmacokinetics studies showed good stability and oral bioavailability for ARVD-15 and ARVD-64, which are currently undergoing efficacy testing in enzalutamide-resistant mCRPC xenografts expressing endogenously both AR-V7/AR-fl. Ongoing experiments include an E3-ligase CRISPR screen and proximity ligation with mass spectrometry to identify the E3-ligase(s) involved, while SILAC proteomics are used to assess AR isoform degradation, re-synthesis rates, and potential off-target effects. Collectively, these data support a molecular glue degrader MoA, offering a unique therapeutic approach for targeting "undruggable" proteins like AR-V7, which lacks a ligand-binding domain or contains intrinsically disordered regions.

In summary, our drug candidates offer **dual AR-V7/AR-fl inhibition in a single treatment**, has the potential to not only benefit patients with mCRPC but also patients with hormone-sensitive disease, and delay the expression of AR-V7.

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