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Dual-Action Androgen Receptor Inhibitors are N-Terminal Domain Binding Androgen Receptor (AR) Antagonists and Degraders for the Treatment of AR and AR-Splice Variant-Positive Castration-Resistant Prostate Cancer

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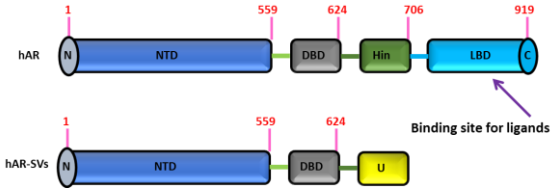
Introduction

- Androgen receptor (AR) is the primary therapeutic target that is expressed in over 85% of prostate cancer (PC).
- PC that relapses from hormonal treatment (castration-resistant prostate cancer (CRPC)) is aggressive and contributes to majority of PC-related deaths.
- Second-generation AR antagonists and androgen-synthesizing enzyme inhibitors have significantly extended the survival of CRPC patients.
- After a brief period of response, resistance to AR-pathway inhibitors develops by various mechanisms.
- Expression of ligand-binding domain (LBD)-null AR splice variants (AR-SVs) has been identified as one of the mechanisms by which resistance to AR-pathway inhibitors develops in CRPC. No approved drugs inhibit AR-SVs.
- **The objective of this work is to evaluate N-terminal domain-binding Dual Acting AR Inhibitors (DAARIs) in AR-SV-positive preclinical models.**



Summary

- DAARIs ONCT-534 and ONCT-505 bind to the NTD, degrade AR & AR-SV proteins, and inhibit AR activity.
- DAARIs exhibit sustained AR inhibition in PC cells with maximum effect observed long-term treatment.
- DAARIs inhibit AR and AR-SV–positive cell line and patient-derived PC xenografts.

Conclusions: DAARIs could be promising next-generation AR-targeting drugs for the treatment of AR-SV-positive CRPCs.

Disclosure: RN, SP, DH, YH, DDM are inventors in DAARI patents. JBB and GFK are employees of Oncternal Therapeutics, Inc. (NASDAQ: ONCT), San Diego, CA and receives compensation and holds equity. RN is a consultant to ONCT. DAARI program is licensed to ONCT.

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DAARIs Degrade and Antagonize AR and AR-SV

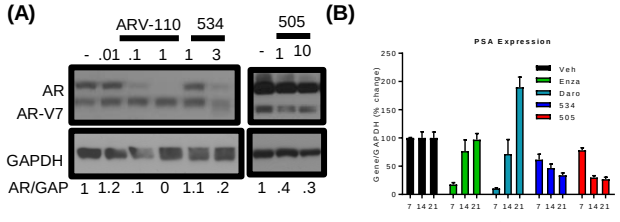


Figure-1: **A.** DAARIs (ONCT-534 and ONCT-505) destabilize AR and AR-V7 in 22RV1 cells, while PROTAC ARV-110 destabilized only AR, but not AR-V7. **B**. DAARIs have a distinct temporal inhibition of AR function (AR-target gene FKBP5) with sustained inhibition observed in 3-Dimensional LNCaP culture treated for 21 days.

DAARIs Bind to the N-Terminal Domain of the AR

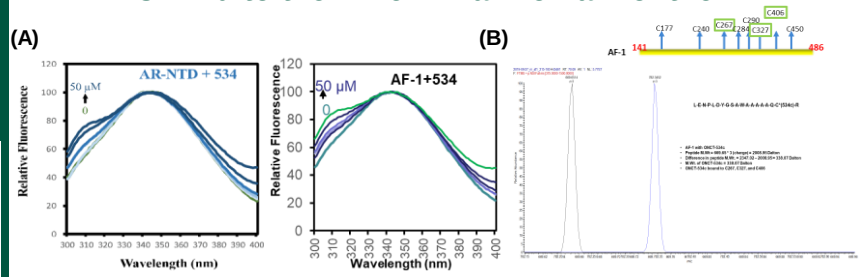


Figure-2: A. DAARI ONCT-534 binds to the N-terminal domain of the AR as shown by intrinsic fluorescence. **B**. Mass Spectrum of a covalent molecule designed from ONCT-534 confirms the binding region in the AF-1 domain. Binding amino acids are shown above the spectrum.

DAARIs Regress Enza-Resistant Tumors in CDX Models

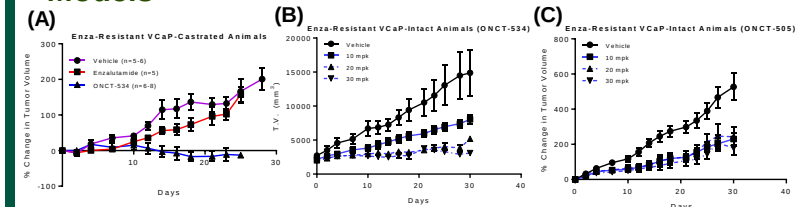
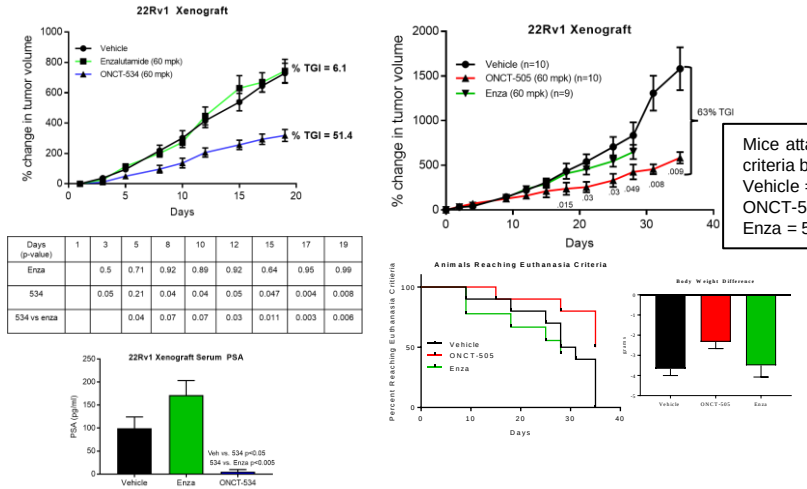


Figure-3: Enzalutamide-resistant VCaP cells were implanted in SRG rats (n=5-8). Animals were either castrated (A) or left intact (B & C) and were treated orally with 60 mpk ONCT-534 (A), dose response of ONCT-534 (B) or ONCT-505 (C). Tumor volume and body weight were measured thrice weekly.

DAARIs Inhibit AR-SV-Positive PC Cell Line-Derived Xenograft (CDX)



Days (p-value)	1	3	5	8	10	12	15	17	19
Enza		0.5	0.71	0.92	0.89	0.92	0.84	0.95	0.99
534		0.05	0.21	0.04	0.04	0.05	0.047	0.004	0.008
534 vs enza			0.04	0.07	0.07	0.03	0.011	0.003	0.006

Mice attaining euthanasia criteria before 28 days:
Vehicle = 5/10
ONCT-505 = 2/10
Enza = 5/9

Figure-4: A. 22Rv1 cells were implanted in SRG immunocompromised rats. Once the tumors are palpable, the animals were castrated, and the tumors were allowed to regrow as CRPC. Animals (n=7-10) were randomized and treated orally. Tumor volume was measured thrice weekly. Serum PSA was measured in serum collected at the end of the study. **Right**. 22Rv1 cells were implanted in NSG mice and a xenograft study was performed as indicated for the left panel.

DAARIs Inhibit AR-SV-Positive Patient-Derived Xenograft (PDX)

Details of LuCaP 86.2 PDX cells
- obtained from bladder metastasis
- expresses AR and AR v567es splice variant.
- No response to AR-targeting agents, such as Enza or Abi
- Response to docetaxel.

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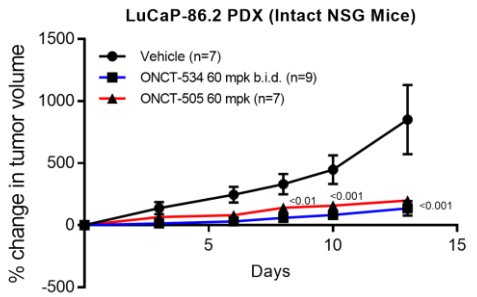

Figure-5: LuCaP 86.2 PDX tumor pieces were implanted into intact NSG mice. Once the tumors reached 100-300 mm³, the animals were randomized and treated orally.