AACR-NCI-EORTC Virtual International Conference on
MOLECULAR TARGETS AND CANCER THERAPEUTICS
October 7-10, 2021

ONCT-534, an Androgen Receptor (AR) N-Terminal-Domain-Binding Small Molecule Degrader, for the Treatment of AR-Variant 7 (AR-V7)-Positive Castration-Resistant Prostate Cancer.

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Disclosure

Ramesh Narayanan

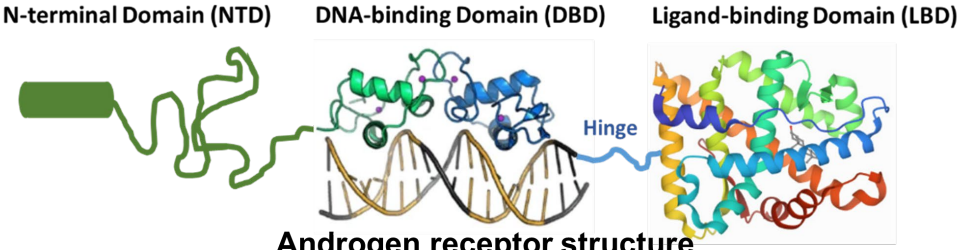
- Consultant for Oncternal Therapeutics, Inc.
- Grant/Research support from National Cancer Institute (R01CA229164) and Oncternal Therapeutics, Inc.
- The ONCT-534 program discussed in this presentation has been licensed to Oncternal Therapeutics, Inc. by The University of Tennessee Research Foundation (UTRF).

James B. Breitmeyer & Gunnar F. Kaufmann

- Employees of Oncternal Therapeutics, Inc.
- Owns equity in Oncternal Therapeutics, Inc.

Background

- Androgen receptor (AR) is an important target for prostate cancer (PCa) therapy as over 85% of PCa are AR-positive[1,2].
- PCa overcomes AR inhibition via several mechanisms, including expression of AR splice variants (AR-SVs), such as AR-V7[3].
- Although AR Ligand-Binding Domain (LBD) is the domain to which ligands bind, the activation function-1 (AF-1) region in the N terminal domain (NTD) exhibits over 70% of AR's transactivation function[4].
- Desired characteristics of next-generation PCa therapies are:
  - Inhibition of mutant AR proteins more potently than existing antagonists.
  - Degradation of AR and mutant AR to prevent activation by intracrine androgens and other signaling molecules;
  - Inhibition and/or degradation of AR-SVs to overcome treatment resistance and aggressive phenotype.
- Current treatments for PCa and castration-resistant prostate cancers (CRPC) are ligand-binding domain (LBD)-binding antagonists or inhibitors of androgen-synthesizing enzymes[5,6].



Androgen receptor structure

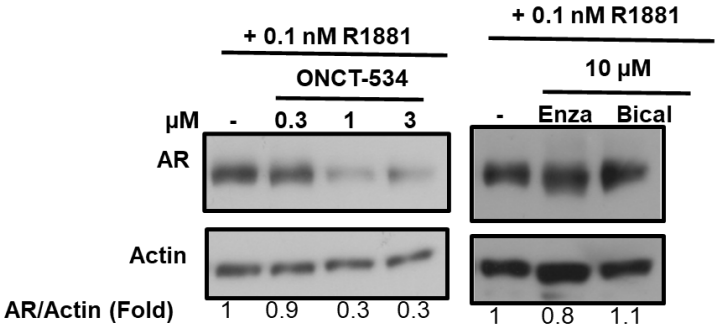
Objective

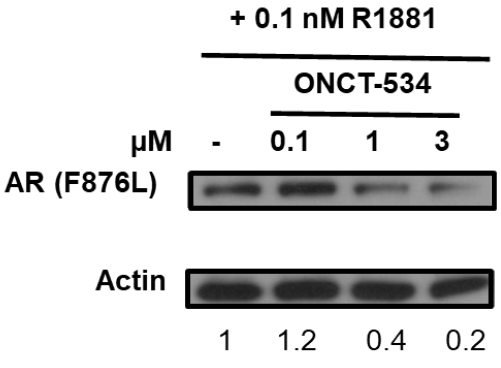
To evaluate the anti-tumor activity of AR N-Terminal-Domain-Binding Small Molecule Degrader, ONCT-534, in AR-splice variants-expressing models of prostate cancer.

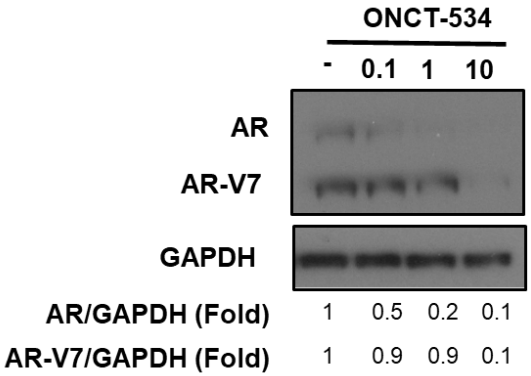
In Vitro and *In Vivo* Properties[7] of ONCT-534

Assay	ONCT-534
In vitro AR LBD binding (nM)	1172
In vitro AR transactivation IC_{50} (nM)	180-270
In vitro AR Degradation (% at 1 µM)	60-90
In vivo P.D. (Hershberger)-Effective dose (mg/kg) rats – 50% effect	20
In vivo xenograft- Effective dose (mg/kg) rats	20
PK ($t_{1/2}$)	Not reached
PK (AUC_{0-24}) h*ng/ml	5 mg/kg = 6860 20 mg/kg = 37000 30 mg/kg = 62000 60 mg/kg = 77500
G-Protein Coupled Receptor panel	Minimal activity
Kinome panel	Minimal activity
Nuclear Hormone Receptor panel	Minimal cross-reactive

ONCT-534 Degrades AR Full Length and Splice Variants

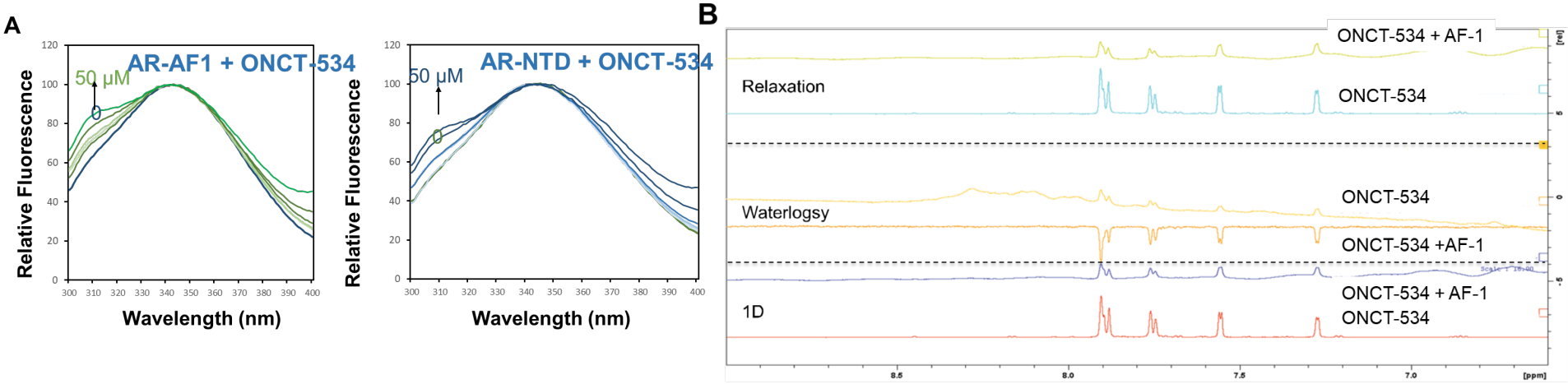






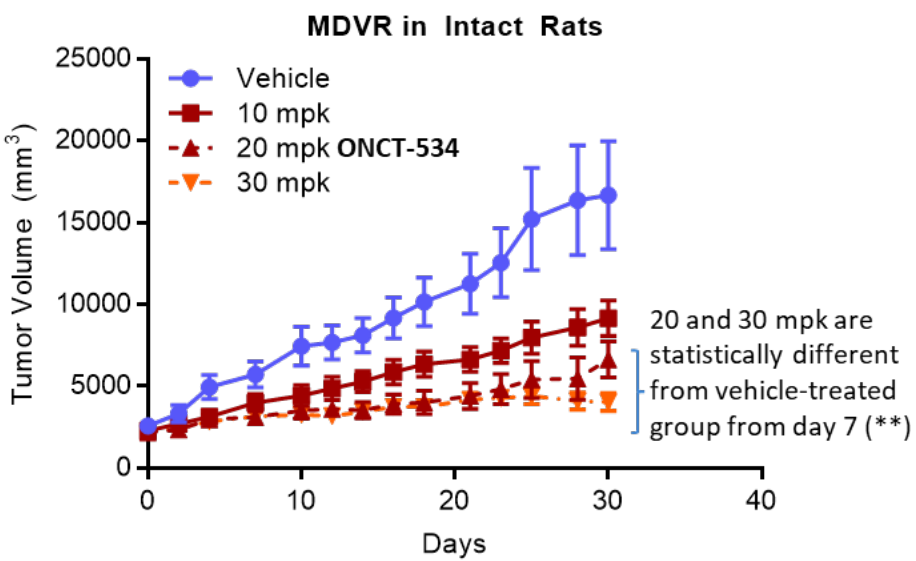
Cells (LNCaP, MR49F, and LNCaP-V7) plated in charcoal-stripped serum-containing medium were treated as indicated in the respective figures for 20-24 hrs. Cells were harvested, protein was extracted, run on an SDS-PAGE gel, and Western blotted with AR and actin/GAPDH antibodies[7].

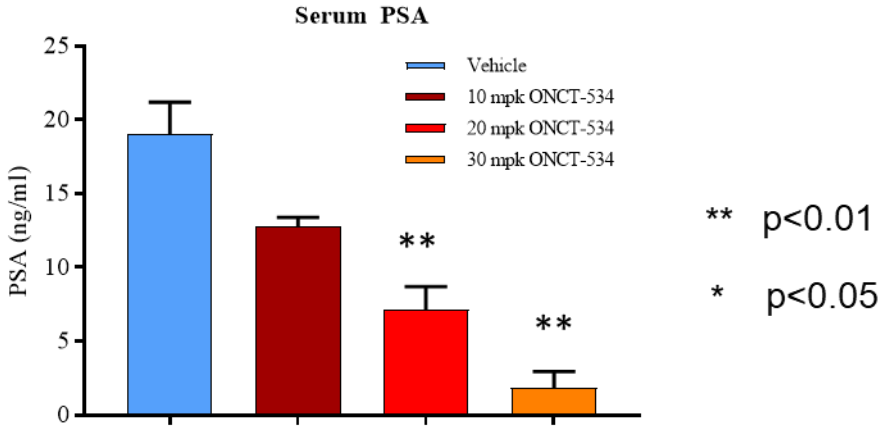
ONCT-534 Interacts with AR-AF-1 Domain



Biophysical studies suggest that ONCT-534 interacts with AR AF-1[7]. **A.** Fluorescence polarization studies with purified AR AF-1. **B**. NMR with purified AR AF-1 protein in the presence or absence of ONCT-534.

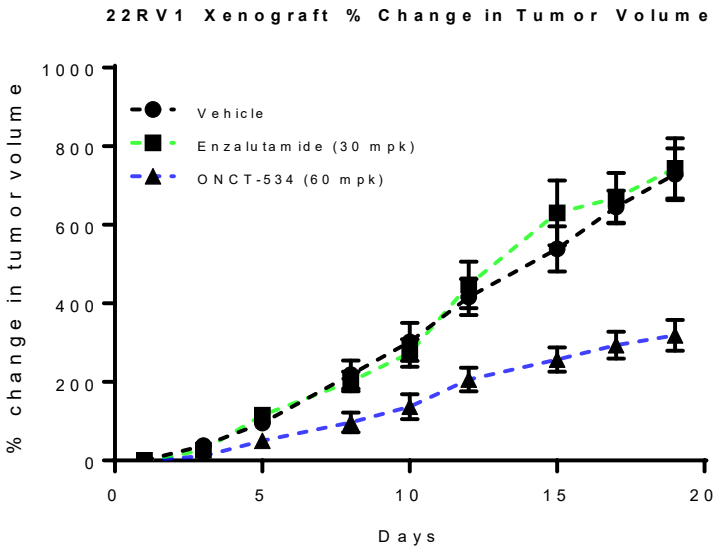
ONCT-534 Inhibits Enza-Resistant PCa Xenograft in Intact Models





Enzalutamide-resistant VCaP cells were implanted in ***intact*** immunocompromised rats[7]. Once the tumors grew to 1500-2000 mm^3, the animals were randomized and treated orally. Tumor volume was measured thrice weekly. Serum PSA was quantified at the end of the study.

ONCT-534 Inhibits AR-V7-Positive 22RV1 CRPC Xenograft



Days (p-value)	1	3	5	8	10	12	15	17	19
Enza		0.5	0.71	0.92	0.89	0.92	0.64	0.95	0.99
ONCT-534		0.05	0.21	0.04	0.04	0.05	0.047	0.004	0.008
ONCT-534 vs enza			0.04	0.07	0.07	0.03	0.011	0.003	0.006

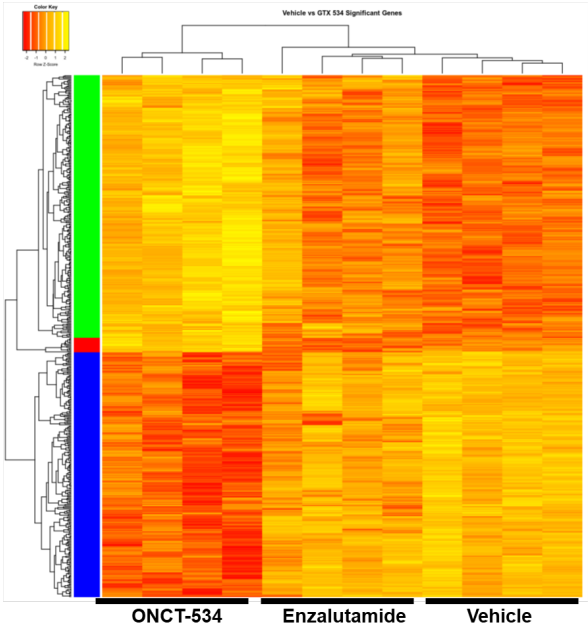
Drug Concentration

Model	Dose (mpk)	ONCT-534 (nM)		Enzalutamide (nM)	
		Serum	Tumor	Serum	Tumor
22RV1	60	2928	6727	12430	12843

22RV1 cells expressing AR-V7 splice variant were implanted in castrated SRG immunocompromised rats. Once the tumors grew to 1500-2000 mm^3, the animals were randomized and treated as indicated. Body weight and tumor volume were measured thrice weekly. At the end of 21 days of treatment and 24 hours after last treatment, the animals were sacrificed, and tumors were preserved for further analysis (n=6-8/group). Drug concentration in serum and tumor were measured.

ONCT-534 Alters Transcriptome in 22RV1 Tumors and Serum PSA of Tumor-Bearing Rats

A



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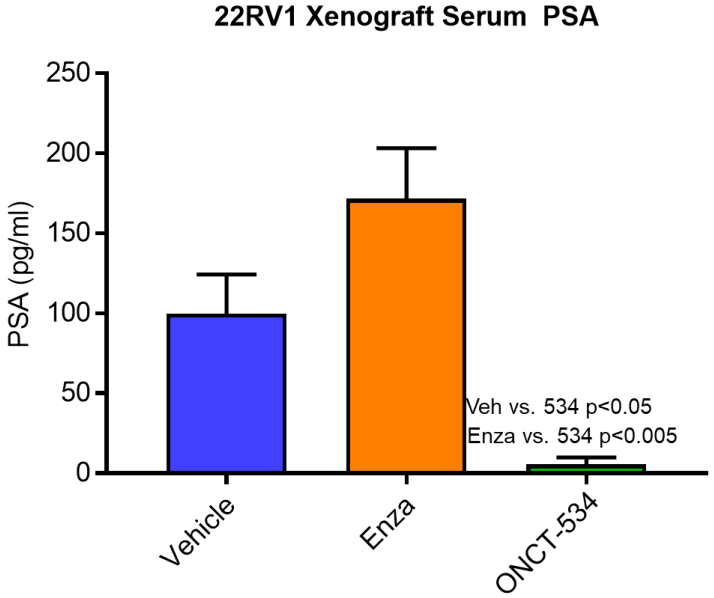

A. RNA was extracted from 22RV1 tumors and microarray was performed. ONCT-534 altered the expression of 936 genes, while enzalutamide altered the expression of only 4 genes. **B**. PSA was measured in the serum of tumor-bearing rats.

Summary

- ONCT-534 may provide a promising next-generation treatment option for CRPC and for the clinically important emerging class of pan-resistant splice variant-expressing prostate cancers[8,9].
- The notable features of ONCT-534 are
  - Binding to the AR-AF-1 domain and inhibition of transcriptional activity.
  - Induction of degradation of both AR full length proteins and AR-SV proteins, including AR-V7.
  - Anti-tumor activity in CRPC and intact xenograft.
  - Potent anti-tumor activity in AR-V7-positive tumor xenografts in castrated animals
- ONCT-534 minimally cross-reacts with GPCRs and kinome and possesses necessary drug-like properties.

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