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Prostate Cancer RIPTAC™ Therapeutics Demonstrate Activity in Preclinical Models of Enzalutamide-Resistant Prostate Cancer

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Background

Resistance to Androgen Receptor Signaling Inhibitors (ARSIs) in prostate cancer occurs in almost all patients and is driven by many heterogenous bypass resistance mechanisms including genomic alterations in AR and increases in AR expression. In the metastatic castration-resistant (mCRPC) setting, more than 80% of patients harbor amplifications of the AR gene or the upstream enhancer region of DNA[1]. New therapies are urgently needed to tackle the disease, especially in its advanced, drug resistant, and most lethal form. **R**egulated **I**nduced **P**roximity **Ta**rgeting **C**himeras or RIPTAC™ therapeutics are a new class of heterobifunctional small molecules invented by Halda Therapeutics[2]. RIPTAC Therapeutics recruit a tumor-specific targeting protein (TP) into a stable intracellular ternary complex with a protein essential for cell survival (Fig. 1). This results in tumor-specific abrogation of the essential protein (EP) function, and selective killing of cancer cells while sparing non-TP expressing healthy cells. Applied to prostate cancer, our RIPTAC technology leverages selective AR expression to abrogate the function of an undisclosed EP effector.

Regulated Induced Proximity Targeting Chimera (RIPTAC Therapeutic)

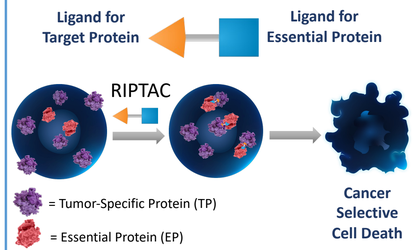


Fig. 1 RIPTACs are hetero-bifunctional small molecules designed to selectively kill cancer cells that express a particular TP. The RIPTAC mechanism involves formation of stable intracellular ternary complexes between the TP and EP. Complex formation involves the formation of neo-protein-protein interactions and abrogation of the EP function which results in selective cancer cell killing.

Table 1. RIPTACs Offer Advantages Over Existing Modalities

RIPTACs expand precision medicine opportunity space	RIPTACs	Small Molecule Inhibitors	Protein Degraders	Bispecifics / ADCs / CAR-T, mAb
Drug activity independent of target function	✓			✓
Treat nontarget-based resistance mechanisms	✓			✓
Potential to treat "undruggable" and "druggable" oncoproteins	✓		✓	✓
Novel pharmacology with ternary complex and neo protein-protein interaction	✓		✓	
Oral dosage form, low COGS	✓	✓	✓	
Intracellular targets	✓	✓	✓	

Summary

We describe H001 and H003, two novel orally bioavailable hetero-bifunctional small molecule RIPTAC therapeutics for prostate cancer. They recruit Androgen Receptor (AR) as the TP and an undisclosed EP into a stable ternary complex, thereby abrogating the EP function and leading to cell death selectively in FL-AR positive cells. RIPTACs were designed as part of a Structure-Activity Relationship (SAR) campaign, in order to optimize cellular ternary complex formation between AR and EP, AR-selective cell killing, efficacy, and oral bioavailability.

RIPTACs are Structurally Enabled and Form Ternary Complexes in Prostate Cancer Cells

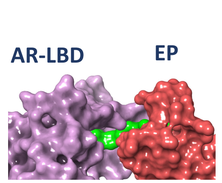


Fig. 2 X-Ray crystal structures of AR:RIPTAC:EP ternary complexes have been determined to enable structure-aided drug design (SADD).

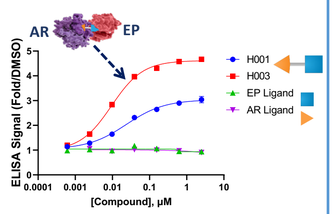


Fig. 3 RIPTACs form intracellular ternary complexes in VCaP cells (H001 EC_{50} = 24 nM, H003 EC_{50} = 9 nM). Neither the AR ligand nor the EP ligand by itself induces ternary complex formation.

RIPTACs Demonstrate Potent Anti-Proliferative Activity and Effector Inhibition in AR-Expressing Cells

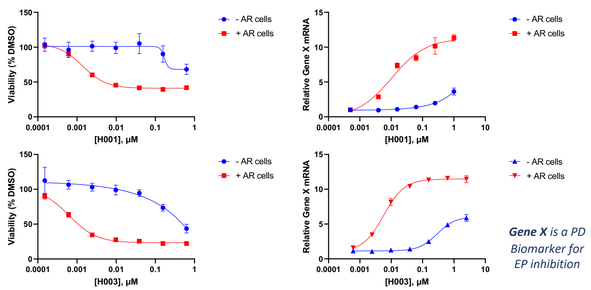
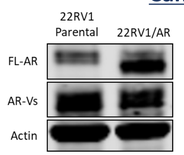


Fig. 4 TRex293 cells expressing doxycycline-inducible AR were treated with increasing concentrations of H001 and H003 in the presence or absence of doxycycline to determine the dependency of cellular proliferation and PD modulation on AR-expression. Viability was determined using a CellTiterGlo assay and Gene X mRNA levels were determined using qRT-PCR.

RIPTACs are Apoptotic in FL-AR^{hi} Expressing Prostate Cancer Cell Lines



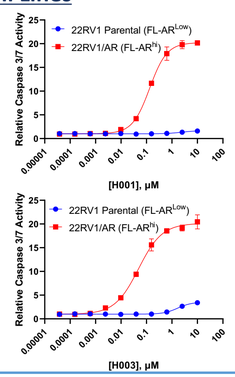


Fig. 5 FL-AR overexpressing 22RV1/AR cells were derived from the parental AR-V7+, Enzalutamide insensitive line. H001 and H003 are highly apoptotic in 22RV1 cells overexpressing FL-AR (H001 EC_{50} = 113 nM, H003 EC_{50} = 48.5nM) as compared to the parental 22RV1 cell line that expressed low FL-AR levels, as determined by the Caspase 3/7 Glo assay.

H001 forms AR:RIPTAC:EP Ternary Complex in Mutant AR Expressing Cells

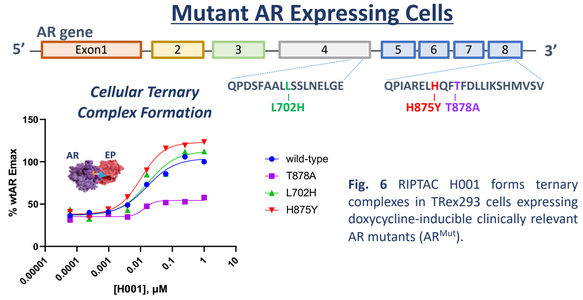
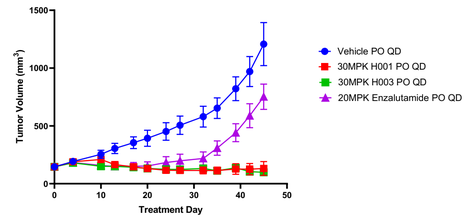


Fig. 6 RIPTAC H001 forms ternary complexes in TRex293 cells expressing doxycycline-inducible clinically relevant AR mutants (AR^{Mut}).

RIPTACs Demonstrate Superior Oral *In Vivo* Efficacy to Enzalutamide in an AR^{amp}, V7^{+} Castrate VCaP Model



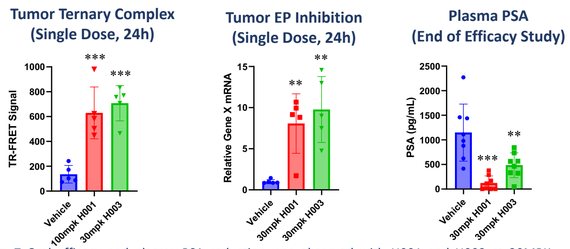


Fig. 7 Oral efficacy and plasma PSA reduction was observed with H001 and H003 at 30MPK dosed daily in a VCaP tumor model in castrated mice. Efficacy at this dose was supported by both tumor ternary complex formation and tumor EP inhibition in a 24-hr PK/PD study.

H001 *In Vivo* PD Modulation is Dependent on AR Binding

Pre-dosing with Enzalutamide competes off RIPTAC AR binding, thus blocking ternary complex formation and EP Inhibition

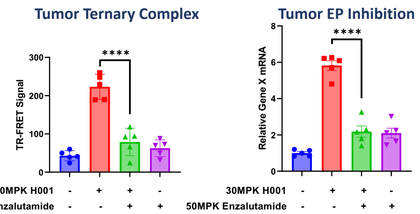
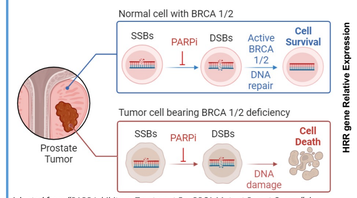


Fig. 8 Pre-dosing for 3 days with Enzalutamide occupies the AR LBD and prevents RIPTAC binding. Trimer complex formation and EP inhibition 24h post single H001 dose are significantly attenuated.

H001 Induces "BRCAness" in VCaP Model



Adapted from "PARP Inhibitors: Treatment For BRCA Mutant Breast Cancer", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

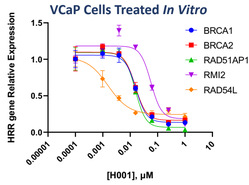
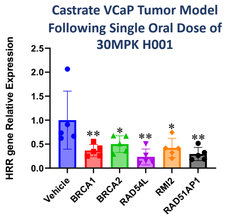


Fig.9 PARP inhibitors can induce synthetic lethality in cancer cells with homologous recombination repair (HRR) pathway defects such as alternation of breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2). H001 decreased BRCA1, BRCA2 and related HRR gene expression in the VCaP model, both *in vitro* and *in vivo*. Combination therapy using PARP inhibitors will be explored.



Conclusions and Future Directions

- RIPTACs are a novel heterobifunctional small molecule therapeutic modality with applications in prostate cancer.
- RIPTACs act by abrogating the function of a pan-essential protein selectively in tumor cells by sequestering it in a stable ternary complex with a tumor-specific targeting protein.
- H001 and H003 are AR binding RIPTACs that inactivate an undisclosed essential protein selectively in prostate cancer cells.
- RIPTACs can induce apoptosis selectively in AR^{hi} prostate cancer cells.
- H001 demonstrates ternary complex formation with clinically relevant AR mutants.
- Multiple RIPTACs have been demonstrated to be orally efficacious *in vivo*, with preclinical activity in models of enzalutamide resistance.
- RIPTAC activity in vivo can be competed off by pre-dosing with an AR LBD inhibitor, demonstrating AR dependence of EP inhibition.
- RIPTACs downregulate genes involved in homologous recombination repair, inducing BRCAness. Combination therapy with PARP inhibitors will be explored.
- Halda's Prostate Cancer RIPTAC program has demonstrated oral exposure in rat/dog and will begin IND-enabling studies in 2023.

Methods

TREx293 cells were purchased from Thermo Fisher. All other cell lines were purchased from ATCC. Protein purification and structural studies were performed in collaboration with Selvita (Poland). PSA ELISA kit was purchased from Abcam. CellTiterGlo and Caspase 3/7 Glo kits were purchased from Promega.

References

[1]Genomic Hallmarks and Structural Variation in Metastatic Prostate Cancer. Cell **2018**, *174*, 758-769 (doi 10.1016/j.cell.2018.06.039)

[2]Regulated Induced Proximity Targeting Chimeras (RIPTACs): a Novel Heterobifunctional Small Molecule Therapeutic Strategy for Killing Cancer Cells Selectively. Biorxiv **2023** (doi 10.1101/2023.01.01.522436)