An Oral RIPTAC™ Therapeutic for 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]. The death toll from prostate cancer in the USA is nearly 35,000/yr[2], and median overall survival for metastatic castration-resistant prostate cancer is less than two years. New therapies are urgently needed to tackle the disease, especially in its advanced, drug resistant, and most lethal form.

Regulated Induced Proximity Targeting Chimeras or RIPTAC Therapeutics™ are a new class of heterobifunctional small molecules developed by Halda Therapeutics[3]. 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 normal non-TP expressing 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)

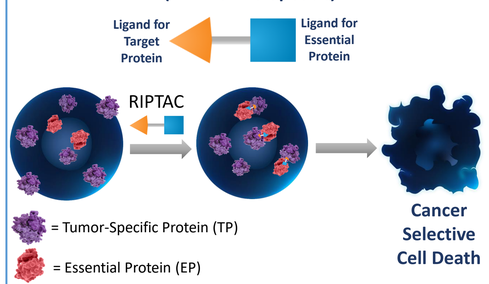


Fig. 1 RIPTACs are heterobifunctional small molecules designed to selectively kill cancer cells that express a particular TP. The RIPTAC mechanism involves formation of stable intracellular ternary complexes between the TP and a protein essential for cell survival (EP) as the effector protein. Complex formation involves the formation of neo-protein-protein interactions and abrogation of the EP function resulting in cancer cell killing.

Summary

We describe here H001, a novel orally bioavailable heterobifunctional small molecule RIPTAC Therapeutic for prostate cancer. It recruits Androgen Receptor (AR) as the TP and an undisclosed Essential cellular Protein (EP) into a stable ternary complex with FL-AR (full length), thereby abrogating the EP function and leading to cell death selectively in FL-AR positive cells. H001 was 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, and oral bioavailability.

H001 Treatment Results in AR:RIPTAC:EP Ternary Complex Formation in VCaP Cells

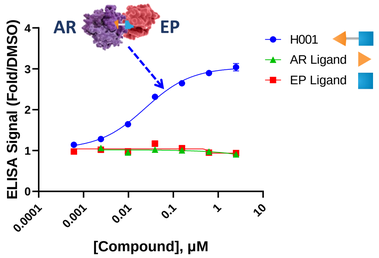
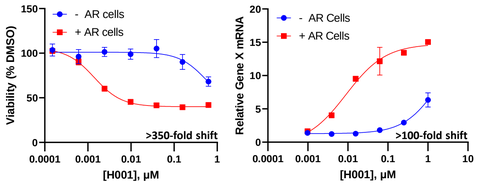


Fig. 2 Intracellular ternary complexes can be detected following lysis using a sandwich ELISA (H001 EC_{50} = 24 nM). Endogenous AR is immunoprecipitated and the presence of EP in the complex is detected using an anti-EP antibody. Neither the AR ligand nor the EP ligand by itself induces ternary complex formation.

H001 Demonstrates Potent Anti-Proliferative Activity and Effector Inhibition in AR-Expressing Cells



Gene X is a PD Biomarker for EP inhibition

Fig. 3 TRex293 cells expressing doxycycline-inducible AR were treated with increasing concentrations of H001 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.

RIPTAC Therapeutic Design is Aided by Ternary Complex X-Ray Crystallography

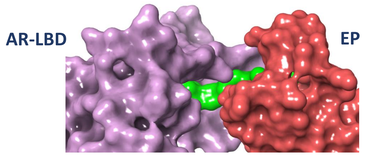
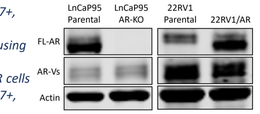


Fig. 4 X-Ray crystal structures of AR:RIPTAC:EP ternary complexes have been determined to enable SADD. Significant conformational rearrangement in AR-LBD is observed as well as significant protein-protein interactions.

H001 is Active in FL-AR+ Prostate Cancer Cell Lines, Independent of Their Dependence on FL-AR Signaling

- *LnCaP95 cells are FL-AR+, AR-V7+, Enzalutamide insensitive*
- *LnCaP95 AR-KO cells prepared using CRISPR/Cas9*
- *FL-AR overexpressing 22RV1/AR cells derived from the parental AR-V7+, Enzalutamide insensitive Line*



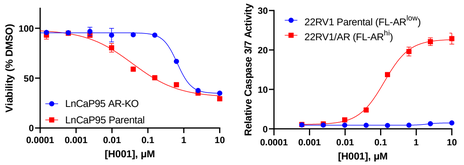
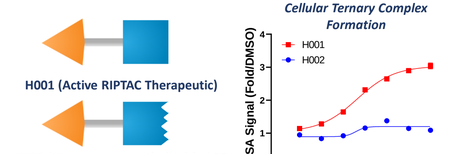


Fig. 5 H001 shows enhanced activity in parental LnCaP95 cells (GI_{50} = 37 nM) compared to AR knockout cells in a CellTiterGlo assay. Similarly, H001 is highly apoptotic in 22RV1 cells overexpressing FL-AR (EC_{50} = 126 nM) as compared to the parental 22RV1 cell line that expressed low FL-AR levels, as determined by the Caspase 3/7 Glo assay.

H001 Activity in Prostate Cancer Cells is Dependent on Ternary Complex Formation, and not FL-AR Inhibition



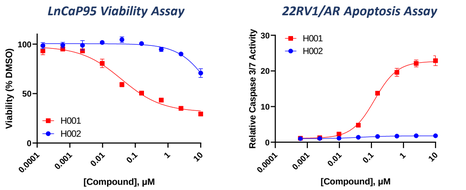
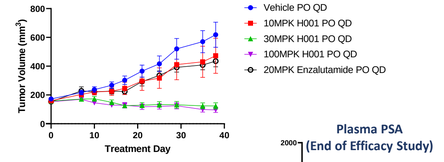


Fig. 6 H002 is a control molecule with poor EP binding but no change to the AR targeting moiety. H002 induces significantly weakened ternary complex formation, and is inactive in both LnCaP95 and 22RV1/AR cell lines

H001 Demonstrates Superior Oral *In Vivo* Efficacy to Enzalutamide in an AR^{amp}, $V7^{+}$ Castrate VCaP Model



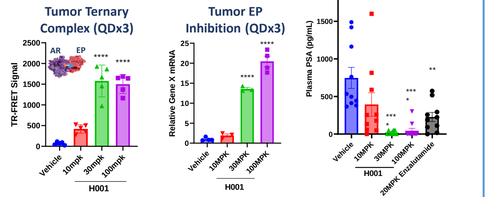
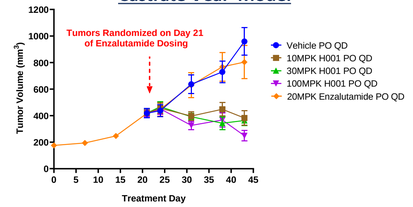
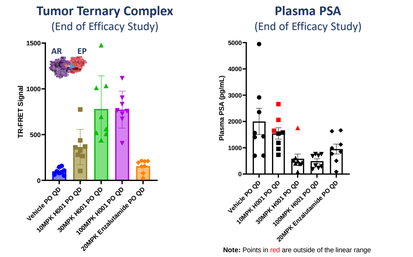


Fig. 7 Oral efficacy and plasma PSA reduction is observed with H001 at 30MPK dosed daily in a VCaP model in castrated mice. Efficacy at this dose is supported by both tumor ternary complex formation and tumor EP inhibition in a 3-day PK/PD study.

H001 is Efficacious in an Enzalutamide-Adapted Castrate VCaP Model





Note: Points in red are outside of the linear range

Fig. 8 Oral efficacy and plasma PSA reduction are observed with H001 in VCaP tumors randomized after daily dosing with Enzalutamide for 21 days. Efficacy is observed concomitantly with trimer complex formation in the tumor

H001 and not H002 is Active Against Castrate VCaP Tumors, Confirming RIPTAC MoA *In Vivo*

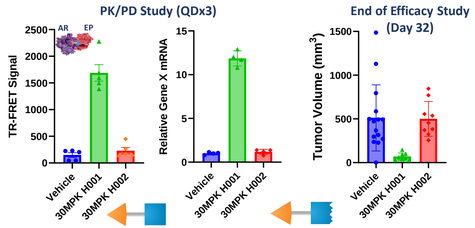


Fig. 9 H001 but not the control molecule H002, with disabled EP binding, leads to ternary complex formation and EP PD modulation in tumors following oral dosing in mice. Similarly, H002 is not efficacious in the castrate VCaP model as demonstrated by end of study tumor volumes, suggesting that AR binding alone is insufficient for efficacy.

Conclusions and Future Directions

- RIPTAC Therapeutics 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, in this case AR.
- H001 is an AR binding RIPTAC that blocks the function of an undisclosed essential protein selectively in prostate cancer cells.
- H001 is potently anti-proliferative against AR-V7+ prostate cancer cell lines that are insensitive to AR signaling inhibitors like Enzalutamide.
- H001 is orally bioavailable and efficacious in AR^{amp}, AR-V7+ Enzalutamide-adapted prostate cancer mouse models.
- Halda's Prostate Cancer RIPTAC program has demonstrated oral exposure in rat/dog and will begin IND-enabling studies in 2023.

Methods

LnCaP95 cells were licensed from Johns Hopkins University. 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]SEER Database

[3]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)