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 heterogeneous 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. New therapies are urgently needed to tackle the disease, especially in its advanced, drug resistant, and most lethal form. Regulated Induced Proximity Targeting Chimeras (RIPTACs) are a new class of heterobifunctional small molecules invented by Hilda Therapeutics®. 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.

Table 1. RIPTACs Offer Advantages Over Existing Modalities

| RIPTAC | mAb | Drug action | Drug selectivity | Target engagement | Drug development | Safety
|---|---|---|---|---|---|---|
| mAb | TP | Selective | TP | TP | mAb | mAb
| TP | EP | Tumor-specific | predominantly | predominantly | mAb | mAb

Fig. 3 RIPTACs form intracellular ternary complexes in VCaP cells (mAb, EC50 = 1.7 nM; TP, IC50 = 9 nM). Neither the AR ligand nor the EP ligand by itself induces ternary complex formation.

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

We describe H001 and H003, two novel orally bioavailable heterobifunctional small molecule RIPTACs 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 FLAR positive cells. RIPTACs were designed as part of a Strategy-Relationship (SAR) campaign, in order to optimize cellular ternary complex formation between AR and EP, and selective cell killing, efficacy, and oral bioavailability.

Fig. 4 The designed and synthesized RIPTACs were evaluated for efficacy in a panel of patient-derived xenografts. The panel of patients included a diverse set of TP and EP expression. The RIPTACs were shown to be selective for their respecive TP and EP, indicating the potential for preclinical and clinical development.

RIPTACs Are Apoptotic in FL-AR™ Expressing Prostate Cancer Cell Lines

Fig. 5 FL-AR™ expressing 22Rv1 cells were derived from the patient AR-V7+ Enzalutamide resistant line. H001 and H003 are highly apoptotic in 22Rv1 cells expressing FL-AR (H001 EC50 = 1.3 nM; H003 EC50 = 4.6 nM) as determined by XTMTripliCation™. Live cells were visualized with Hoechst 33258 stain and 22Rv1 cells were expressed low FL-AR levels, as determined by the Enzymex 3340 assay.

H001 Induces AR Downregulation in Vivo" "BRCaness" in VCaP Model

Fig. 7 The end products and plasma PSA reduction were observed with H001 and H003 at 30MPM closed oral in a VCaP tumor model in castrated mice. Efficacy at this dose was supported by both tumor volume complex formation and tumor EP inhibition in a 24 h PFC study.

RIPTACs Demonstrate Superior Oral In Vivo Efficacy to Enzalutamide in an AR™, V7" Castrate VCP Model

Fig. 6 RIPTAC H001 forms ternary complexes in cell lysates requiring doxorubicin or clinically relevant AR antagonists (ARPs). RIPTACs in cell lysates with ARPs. RIPTACs in cell lysates with ARPs. RIPTACs in cell lysates with ARPs. RIPTACs in cell lysates with ARPs. RIPTACs in cell lysates with ARPs.

Conclusions and Future Directions

• RIPTACs are a novel heterobifunctional small molecule therapeutic modality with applications in prostate cancer.
• RIPTACs 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 induce an undisclosed essential protein selectively in prostate cancer cells.
• RIPTACs can induce apoptosis selectively in AR™ 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-doing an AR LBD inhibitor, demonstrating AR dependence of EP inhibition.
• RIPTACs downregulate genes involved in homologous recombination repair, inducing BRCAAness. Combination therapy with PARP inhibitors will be explored.
• Hilda’s Prostate Cancer RIPTAC program has demonstrated oral exposure in rats and will begin IND-enabling studies in 2023.

Methods

TREx293 cells were purchased from Thermofisher. All other cell lines were purchased from ATCC. Protein purification and structural studies were performed in collaboration with Seluxia (Poland). PSA ELISA kit was purchased from Bachem. CellTiterGlo and Caspase 3/7 GLO kits were purchased from Promega.

References


**Treatments for Castration-Resistant Prostate Cancer. Cell 2018, 175, 719-738 (doi:10.1016/j.cell.2018.06.038)

Regulated Induced Proximity Targeting Chimeras (RIPTACs) is a Novel Heterobifunctional Small Molecule Therapeutic Strategy for Killing Cancer Cells Selectively. Saslun, 2020 (11): 1332/2013 (EOL: 03/02/2020)