Targeting RNA Splicing in Race-Related Aggressive and Lethal Prostate Cancer

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The focus of most studies of the molecular mechanisms of prostate cancer (PCa) has been on DNA mutation and aggregate gene expression in tumors from patients of European ancestry. This approach has uncovered a limited number of drivers that are therapeutically actionable. Our work addresses the urgent need to determine new molecular mechanisms underlying ancestry-related aggressive and lethal PCa and develop new treatments for such cancers to improve outcomes for PCa patients, including African Americans (AAs), Veterans, and other high-risk populations.

Alternative/Aberrant RNA Splicing (AARS) is emerging as a major driver of abnormal phenotypic heterogeneity. Our team has identified novel ancestry-related differentially expressed RNA splice variants contributing to PCa aggressiveness and drug response and associating with PCa survival, including PI3Kδ. In addition, we have identified a novel metabolic RNA splice variant switch, involving glutaminase, contributing to PCa progression and therapeutic resistance. Despite its role in cancer, AARS has been largely ignored in drug development. Towards being able to specifically modulate the aforementioned targets, for therapeutic application, our team is developing novel splice-switching oligonucleotides (SSOs) and RNA-targeted small molecules. Specifically, we are developing a novel therapeutic SSO, targeting AR-V7 that inhibits PCa cell growth and restores sensitivity to secondary hormonal therapy. In addition, we have purchased and synthesized RNA-biased small molecules, developed selective small molecules for therapeutically relevant RNA targets and identified RNA secondary structures best differentiated by small molecules.

We aim to extend these findings by 1) Evaluating the therapeutic efficacy of PI3Kδ inhibition in PCa by conducting an *in vivo* xenograft/PDX trial and correlative science to identify candidate RNA splice variant predictors of response. 2) Initiating a human lead-in trial of glutaminase inhibition in AA and white metastatic castration-resistant PCa patients, including Veterans, and prospectively estimating PSA and associating candidate RNA splice variant predictors of response, and conducting an *in vivo* PCa xenograft/PDX trial to test a combination regimen of glutaminase inhibition and enzalutamide. 3) Identifying available targeted therapeutic agents that inhibit ancestry-related aggressive PCa based on dysregulated RNA splicing pathways. 4) Determining responses of PCa xenografts/PDX to a novel SSO targeting *AR* RNA splicing. 5) Developing novel SSOs to modulate additional candidate AARS events critical to ancestry-related aggressive and lethal PCa. 6) Developing ligands that bind candidate RNA splice sites and alter splicing in PCa.

The rationale for and impact of this study is that it will establish new AARS-related targets, therapeutic agents against such targets and predictors of response in ancestry-related aggressive and lethal PCa. Our findings will challenge the paradigm of what constitutes driver biology and actionable targets in such cancers. Our ultimate goal is to improve outcomes for men of all ancestries with lethal PCa, including Veterans, and mitigate PCa disparities for AAs.

The authors declare no potential conflicts of interest.

This work was supported by a DoD Prostate Cancer Research Program Health Disparity Research Award PC131972, NIH/NCI Feasibility Studies to Build Collaborative Partnerships in Cancer Research P20 Grant CA202925, and NIH/NCI Basic Cancer Research in Cancer Health Disparities R01 Grant CA220314.