Novel dual targeting of AKR1C3 and AR/AR variants inhibits androgen signaling and overcomes resistance to antiandrogen treatment

**Background:** AR/AR-V7 and AKR1C3 play key roles in prostate cancer progression and drive resistance to current therapies. Thus far, there are no clinically available therapies to specifically target either AR-V7 or AKR1C3, nor are there dual inhibitors for AR/AR-V7 and AKR1C3. We have developed a number of novel small molecules (LX) that are dual inhibitors of AR/AR-V7 and AKR1C3.

**Methods:** We designed and synthesized a library of novel compounds according to structure based computer modeling. Fourteen were selected for initial study. Cell growth assays were used to assess their efficacy at inhibiting CRPC growth. The effects of the LXs on AR/AR variants and AKR1C3 expression were determined by Western blot. PSA-luciferase assays were used to determine effects on AR signaling activity. RNA-seq was performed on selected LXs (LX-1 and LX-5) based on their improved efficacy over the other LX compounds. Resistant cell sublines generated from C4-2B cells resistant to enzalutamide (MDVR), apalutamide (ApalR), darolutamide (DaroR), or abiraterone (AbiR) were treated with LX-1 or their respective antiandrogen and cell number was determined. Mice bearing VCaP xenograft tumors and LuCaP35CR PDX tumors were treated with LX-1 and the effects on tumor growth were determined.

**Results:** Of the 14 LX compounds, LX-1 had the greatest effect at reducing cell number, AR/AR variant expression, and AKR1C3 activity. LX-5 was the next most effective compound. PSA-luciferase activity was greatly reduced by both LX-1 and LX-5. RNA-seq analysis demonstrated a robust reduction in AR and AR-V7 signaling gene expression by both LX-1 and LX-5. MDVR, ApalR, DaroR, and AbiR cells all showed a reduction in cell number when treated with LX-1. LX-1 inhibited conversion of the testosterone precursor androstenedione into testosterone in AKR1C3 overexpressing C4-2B and LNCaP cells. Additionally, LX-1 treatment reduced testosterone production by LuCaP35CR tumor cells which express high levels of AKR1C3 in the presence of androstenedione in a dose-dependent manner ex vivo. In addition, treatment with LX-1 reduced tumor growth in both VCaP and LuCaP35CR PDX models. Furthermore, LX-1 treatment reduced intratumoral testosterone.

**Conclusions:** We generated novel small molecule inhibitors that dual target AKR1C3 and AR/AR variants. These compounds, specifically LX-1, effectively reduce CRPC growth in vitro and in vivo, suggesting a clinical potential for treating advanced prostate cancer.