Steroid sulfatase stimulates intracrine androgen synthesis and is a therapeutic target for advanced prostate cancer

Background: DHEAS is the most abundant steroid in blood circulation and significant concentrations of DHEAS are present in prostate cancer patients even after ketoconazole or abiraterone therapy, suggesting that this may act as a depot for downstream androgen production. Steroid sulfatase (STS) catalyzes the hydrolysis of DHEAS to biologically active DHEA, which is further metabolized to active androgens that bind the androgen receptor (AR) leading to cell proliferation. Currently the role of STS in AR signaling and CRPC is largely unknown. This study determines the role of STS in AR signaling and explores the potential of targeting STS to overcome castration resistance in prostate cancer.

Methods: Quantitative rt-PCR and Western blots were used to detect expression of STS and AR. STS was downregulated using siRNA specific to STS. Stable cell lines overexpressing STS were generated and characterized. RNA-seq was performed on the stable clones. The steroid profiles of the cells were analyzed by LC-MS using the Thermo Scientific Vanquish UPLC/AB Sciex Qtrap system. Testosterone was assessed by ELISA in tumor extracts and cell culture media. Eleven potent STS inhibitors (SI) were synthesized and characterized. Prostate cancer cell sensitivity to SI was tested using cell growth assays and clonogenic assays. Efficacy of two SI was tested *in vivo* in castration relapsed VCaP xenograft tumor models.

Results: STS is overexpressed in CRPC patients and resistant prostate cancer cells including VCaP and C4-2B MDVR. Stable STS overexpression in C4-2B and LNCaP cells increases the levels of androgen. This resulted in increased cell growth and PSA expression *in vitro*. Inhibiting STS with siRNA or SI suppresses cell growth and AR signaling. STS overexpression in C4-2B and LNCaP cells promoted resistance to enzalutamide and this could be reversed by STS siRNA and SI. SI significantly suppressed AR transcriptional activity, suggesting that inhibition of STS activity by SI downregulates AR signaling. RNAseq analysis determined that enrichment of AR and AR variant signaling gene sets was reduced by SI-1 and SI-2. SI-1 and SI-2 significantly suppressed the growth of relapsed VCaP tumors, reduced intratumoral testosterone, and improved enzalutamide treatment *in vitro* and *in vivo*.

Conclusions: These studies suggest that STS drives intracrine androgen synthesis and prostate cancer proliferation. Targeting STS represents a therapeutic strategy to treat CRPC and improve second generation anti-androgen therapy.