FACS-based Whole-genome CRISPRi Screen to Uncover STEAP1 Regulators in Prostate Cancer

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Background
Six-Transmembrane Epithelial Antigen of the Prostate 1 (STEAP1) is a transmembrane protein frequently overexpressed in prostate cancer (PC). Antibody-based therapies targeting STEAP1 have shown promising preclinical and clinical outcomes. Despite its widespread and PC-specific overexpression, the molecular regulation and biological function of STEAP1 remain poorly understood. Elucidating the mechanisms that govern STEAP1 expression and its role in PC progression could provide valuable insights into disease mechanisms, improve patient selection for STEAP1-targeted therapies, and inform combination or sequential treatment strategies.

Methods
To investigate STEAP1 regulation and function, we first compared its expression to established PC driver genes and observed a correlation between STEAP1 and the androgen receptor (AR) as well as several AR-regulated genes. Using pathway analyses in the Decipher (localized prostate cancer) and West Coast Dream Team (WCDT, metastatic prostate cancer) clinical cohorts, we examined dysregulated pathways in patients with high STEAP1 expression. Additionally, we performed a FACS-based whole-genome CRISPR interference (CRISPRi) screen in C4-2B cells using an anti-STEAP1 antibody to identify regulators by comparing STEAP1-high and STEAP1-low expressing subsets. Functional validation of selected regulators was performed using small-molecule inhibitors with therapeutic potential. We also performed in vivo co-culture experiments of C4-2B cells with anti-STEAP1 bi-specific T cell engager/STEAP1-CAR-T cells and selected inhibitors to observe the synergistic effects.

Results
In the WCDT dataset, STEAP1 was highly expressed in AR-positive metastatic prostate cancer subtypes, irrespective of neuroendocrine differentiation, but was significantly lower in AR-negative subtypes. STEAP1 expression strongly correlated with several AR-regulated genes, including STEAP2, KLK2, NKX3-1, KLK3, ARHGAP6, SLC45A3, and PMEPA1. Pathway analyses in both localized and metastatic PC cohorts revealed dysregulated metabolic pathways, particularly lipid metabolism, in STEAP1-high patients. The CRISPRi screen identified several potential targetable regulators of STEAP1, including MYC, SWI/SNF chromatin remodeling complexes. Knockdown of genes such as GNB2L1, SWI/SNF complex members, and MYC increased STEAP1 expression, whereas depletion of ZIC4, ALAS1, FBXO48, and HOXB13 reduced expression. Notably, ZIC4 inhibition nearly abolished STEAP1 surface expression. Analysis of WCDT ATAC-seq data revealed 3,340 predicted ZIC4 binding sites genome-wide, including a footprint at the STEAP1 promoter, supporting its role as a direct transcriptional regulator.

Conclusions
Our findings identify novel regulators of STEAP1 expression in prostate cancer and reinforce its association with androgen receptor–driven metastatic castration-resistant prostate cancer subtypes. This work provides mechanistic insight into STEAP1 regulation and highlights opportunities to develop rational

combination strategies—for example, pairing epigenetic modulators with STEAP1-targeted agents—to enhance efficacy, overcome resistance, and refine patient selection for STEAP1-based therapies.

Funding Acknowledgements:

UCSF Paul Calabresi K12 Career Development Program in Clinical Oncology (K12CA260225) (CEC)

Prostate Cancer Foundation Young Investigator Award (CEC)

Benioff Initiative for Prostate Cancer Research (CEC)

UCSF SPORE Career Enhancement Program (CEC)

UCSF Prostate Cancer Program Pilot Award (SZ)

Stephen & Faith Brown Endowed Professorship (CEC)

Conflicts of Interest:

CEC receives research funding from Boston Scientific