**Digital spatial profiling of pre- and post-treatment GR+ metastatic CRPC from a phase I trial of enzalutamide and a GR antagonist reveals cAMP and MAPK gene expression pathway suppression after treatment**

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**Background:** We and others have reported that glucocorticoid receptor (GR) expression and transcriptional activity are significantly increased following androgen receptor (AR) signaling inhibition (ARSi) in prostate cancer (PC). Interestingly, in this ARSi-resistant context, acquired GR transcriptional activation upregulates oncogenic pathways different from AR. We hypothesized that these acquired GR-specific pathways may contribute to CRPC progression, and that inhibiting them with GR antagonism could reduce progression. To test this, a Phase 1 trial of a GR antagonist (relacorilant) and enzalutamide was performed in men with heavily pre-treated, ARSi-resistant PC (Desai et al., *CCR*, 2024). Here we report spatial gene expression analysis of GR+ tumor cells from pre- and post-treatment biopsies of 3 patients using digital spatial transcriptional profiling (DSP).

**Methods:** To identify significant changes in GR+ tumor regions from men treated with combined GR and AR antagonism, we measured spatial gene expression using the Nanostring DSP platform. Metastatic CRPC exhibited both GR-high and GR-low tumor (Pan-cytokeratin +) by IHC. GR+ tumor regions of interest (ROIs) were identified and confirmed using an RNAscope probe for *NR3C1* (GR). ROIs were segmented into pan-cytokeratin+ (tumor) and pan-cytokeratin- (non-tumor) cells. Whole-transcriptome analysis of tumor (Pan-CK+, NRC31+, CD45-) was analyzed for pre- versus post-treatment differential gene expression.

**Results:** We focused our analysis on transcriptional changes occurring in GR-high tumor to better understand the role of GR antagonism. We found that in in all three patients biopsy pairs, GR-high tumor cell ROIs demonstrated significant gene expression changes following GR antagonism. Biopsies at 28 days post-treatment showed that patients A & B (both progressed within 2 months on treatment) shared commonly regulated genes, while tumor from patient C, with a 5 month response, did not share any differentially expressed genes with the first two tumor DEGs. Patient A's biopsies showed a significant post-treatment downregulation of cAMP and MAPK signaling pathways. In Patient B's tumor, cytokine-cytokine receptor interaction and neuroactive ligand-receptor interaction pathways were also significantly down-regulated. In patient C, significant downregulation of PI3K-AKT, TGF-beta, Rap1 and MAPK signaling were seen post-treatment. Of note, in all three post-treatment samples, we found an increase in the GR-negative ROIs compared to pre-treatment samples, suggesting that GR antagonism may select for resistant tumor cells with low GR expression.

**Conclusions:** In metastatic PC samples from a phase 1 trial of GR and AR antagonism in patients with heavily pretreated, ARSi-resistant PC, we found that several tumor gene expression pathways were reduced following GR antagonism. These transcriptional signatures may reflect GR-mediated oncogenic pathways that evolve post-ARSi and can be targeted by GR antagonism to slow tumor progression. Identifying which GR gene signatures in GR+ PC associate with a clinical response to GR antagonism may help select patients more likely to benefit.

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**Potential conflicts of interest:** Drs. Conzen and Szmulewitz are co-inventors of a patent issued to The University of Chicago for methods and compositions related to glucocorticoid receptor (GR) antagonists and prostate cancer. The authors declare no other conflicts of interest.