Title: **Identifying the Metabolic Signature of Castration-Resistant Prostate Cancer and its Early Metabolic Response to Androgen Pathway Inhibitor ARN-509**

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Intro: Mainstay treatment for patients with metastatic prostate cancer is androgen deprivation therapy (ADT). However, almost all patients eventually develop castration-resistant prostate cancer (CRPC). Recently two potent inhibitors of the androgen receptor (AR), enzalutamide and abiraterone, have been approved for management of patients with CRPC. Currently no reliable clinical or noninvasive imaging method can predict response to therapy, which is critical in guiding treatment decisions. The goal of this research was to assess the metabolic signature of CRPC and its early metabolic response to the androgen pathway inhibitor ARN-509 in order to identify potential imaging techniques that can monitor therapeutic efficacy in patients. Here we assessed glycolysis and oxidative metabolism using a transgenic adenocarcinoma of the mouse prostate (TRAMP) model that is known to mimic therapeutic response in patients.

Methods: Adult male TRAMP with a 0.1–1cc tumor underwent orchiectomy (equivalent to chemically-induced ADT in patients). Mice with <25% increase in tumor volume one-week post-orchiectomy were defined as castration-sensitive prostate cancer (CSPC), and mice with ≥25% increase were defined as CRPC. Mice with CRPC that underwent daily apalutamide (ARN-509) treatment for one week were defined as CRPC+ARN. After treatment, [U-^{13}C]glucose was injected via tail vein over 45min. Tissue was flash-frozen, then extracted to isolate aqueous metabolites. NMR spectra were acquired on an 800Mhz spectrometer. Fractional enrichment (FE)=[^{13}C-metabolite]/[total metabolite].

Results: CRPC tumors had a significantly increased tumor growth (**Fig.1A**) and significantly elevated aspartate FE, glutamate FE, and lactate FE compared to CSPC tumors (**Fig.1B**), indicating upregulated glycolysis and oxidative metabolism in CRPC tumors. While tumor volume increased significantly during the one week of ARN-509 treatment (**Fig.1A**), CRPC+ARN tumors had significantly decreased aspartate FE, glutamate FE, and lactate FE compared to CRPC tumors (**Fig.1B**). In fact the metabolic profile of CRPC+ARN tumors is similar to that of CSPC tumors. While this reduction in metabolism is consistent with what was observed in recently published hyperpolarized [1-^{13}C]pyruvate MR imaging studies after primary ADT in patients, follow up studies will determine whether this the early drop in glycolysis and oxidative metabolism reflects therapeutic response.

Conclusion: This study demonstrated significantly altered glycolysis and oxidative metabolism associated with development of CRPC and its early response to androgen pathway inhibitor ARN-509 using a treatment-driven prostate cancer murine model. These results suggest that a combination of hyperpolarized [1-^{13}C]pyruvate and [2-^{13}C]pyruvate to noninvasively assess therapeutic response in future patient studies using hyperpolarized ^{13}C MRI.

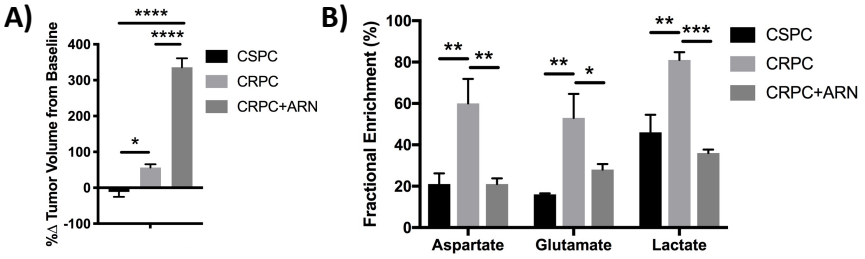

Fig1. (A) Tumor volume and (B) FE from [U-^{13}C]glucose labeling of TRAMP tumors.