

## **A novel model of prostate cancer suggests enzalutamide functions through the immune system to diminish castration resistant and metastatic growth**

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**Background:** There is tremendous need for improved prostate cancer (PCa) models. The murine prostate is anatomically and developmentally different from the human prostate, and does not form sporadic tumors. Furthermore, engineered mouse models lack the heterogeneity of human disease, are often driven in a contrived manner, and rarely (if ever) establish metastatic growth. Human xenografts represent an alternative, but they rely on tumor growth in an immunocompromised host, preventing the study of tumor-immune interactions and immunotherapies. Accordingly, we generated PCa xenograft models in a murine system with an intact human immune system to test the hypothesis that humanizing tumor-immune interactions would improve modeling of metastatic PCa, and further-enable improved modeling of hormonal and immunotherapies.

**Methods:** Male huNOG mice were produced at Taconic Biosciences by engrafted juvenile NOG mice with human CD34+ hematopoietic stem cells. These mice stably maintain multiple human cell lineages, including functional human T-cells. We utilized two human PCa xenograft cell line models transduced with luciferase to assay organ-specific metastatic growth. First, castrated and intact control mice were injected subcutaneously with 22Rv1 cells. When tumors reached >100mm<sup>3</sup>, half of the castrated mice were treated with enzalutamide (enza), then tumor growth was monitored to endpoint. Additionally, VCaP tumor-bearing mice were castrated when the tumors were ~200 mm<sup>3</sup> in size, and once the tumor grew back, they were randomized and treated with enza and/or the anti-PD-1 antibody pembrolizumab (pembro), with vehicle controls. At sacrifice, organs were *ex-vivo* analyzed for metastatic growth, tumor infiltrating lymphocytes, and splenic immune reconstitution.

**Results:** With 22Rv1, subcutaneous tumor size was not significantly altered across conditions; however, the extent and growth at the secondary sites decreased markedly in castrate huNOG vs conventional NOG mice treated with enza. VCaP xenograft tumors showed marked decreases in growth with enza and pembro treatments in huNOG mice, and no effect was seen for either of these treatments in NOG mice. Furthermore, enza responses in huNOG and NOG mice were distinct, and associated with increased CD3+ T-cells within tumors of enza treated huNOG mice, and increased CD3+ T-cell activation, accessed by intracellular interferon- $\gamma$ .

**Conclusions:** These results illustrate, to the best of our knowledge, the first model of human PCa that metastasizes to clinically relevant locations, has intact human immune system, and responds appropriately to standard-of-care hormonal therapies.

**Conflict of Interests:** Taconic Biosciences supplied the animals for this study.

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