A novel model of prostate cancer suggests enzalutamide functions through the immune system to diminish metastatic growth

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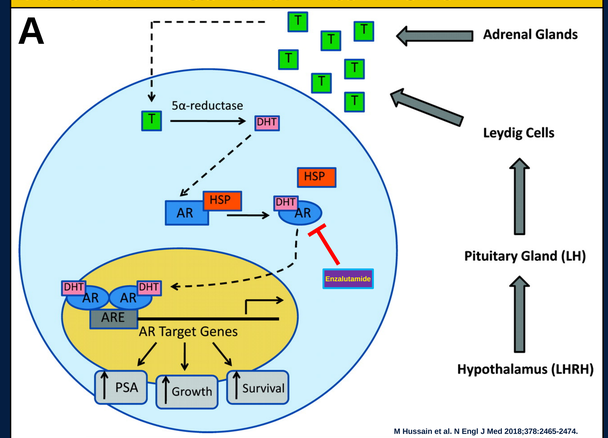
Abstract and Objectives

Purpose: There is tremendous need for improved prostate cancer models. The murine prostate is anatomically and developmentally different from the human prostate, and unlike the human prostate, does not form sporadic tumors, limiting relevance of genetically engineered model systems. Furthermore, engineered models lack the heterogeneity of human disease, rarely (if ever) establish metastatic growth, and tend to be driven in a contrived manner, not at all related to human disease or the human drivers of disease progression. Human xenografts represent alternative models, but they rely on tumor growth in an immunocompromised murine host, preventing the study of tumor-immune interactions and immunotherapy interventions. Consequently, translational progress in prostate cancer research is hampered by the lack of human-derived models that can recapitulate the natural history of the disease—from initiation to metastatic spread—that will respond appropriately to the standard of care hormonal therapies. Accordingly, we generated a prostate cancer xenograft model in a murine system with an intact human immune system to test the hypothesis that humanizing tumor-immune interactions would improve modeling of metastatic prostate cancer, and further-enable improved modeling of hormonal and immune therapies.

Procedure: Male huNOG mice were produced at Taconic Biosciences by engrafting juvenile NOG mice with human CD34+ hematopoietic stem cells. These mice stably develop and maintain multiple human cell lineages, including functional human T-cells. HuNOG and NOG control mice were surgically castrated. One week following castration, castrated and intact control mice were injected subcutaneously with luciferase-transduced 22Rv1 human prostate cancer cells to assay organ-specific metastatic growth. After tumors reached 100mm3, half of the castrated mice were treated with enzalutamide, and then tumor growth was monitored to endpoint. At sacrifice, organs were ex-vivo analyzed for metastatic growth, tumor infiltrating lymphocytes, and splenic immune reconstitution.

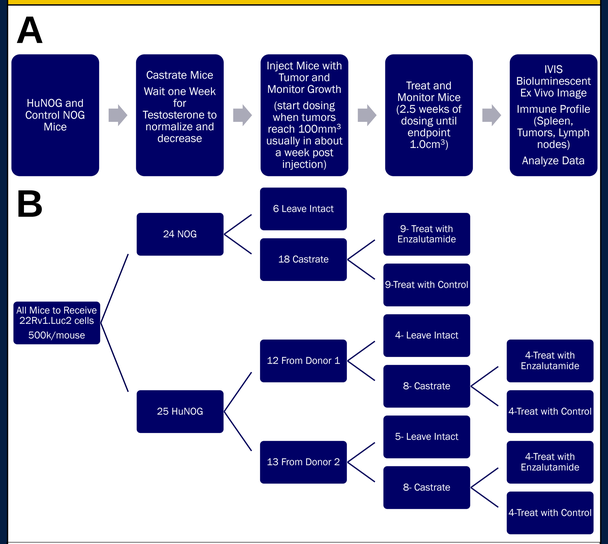
Results: Primary tumor size was not significantly altered across conditions; however, the extent and growth at the secondary sites differed markedly in castrate huNOG vs conventional NOG mice treated with enzalutamide. Furthermore, enzalutamide responses in huNOG and NOG mice were distinct, and associated with increased CD3+ T-cells within tumors of enzalutamide treated huNOG mice, and increased CD3+ T-cell activation, accessed by intracellular interferon-γ. These results illustrate, to the best of our knowledge, the first model of human prostate cancer with metastases to clinically relevant locations, an intact human immune system, that responds appropriately to standard-of-care hormonal therapies.

Background: AR Signaling in Prostate Cancer



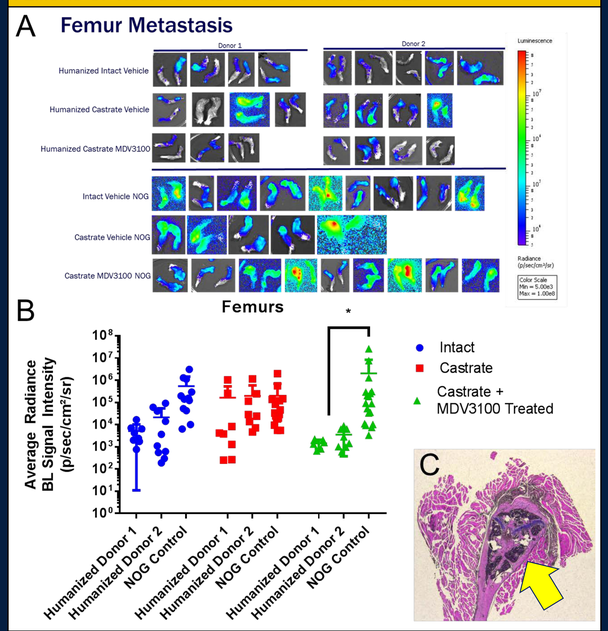
A. Testosterone (T), the main androgen produced by the body, is predominantly released by the Leydig cells of the testes, but also in small amounts by the adrenal glands. Free circulating testosterone can enter prostate cells, where it can be converted to its more potent metabolite, dihydrotestosterone (DHT), which in turn binds to the AR protein. Testosterone itself can bind to the AR as well, but DHT has about 10 times the affinity. The AR is a nuclear transcription factor that can activate and regulate the expression of many genes involved in growth and proliferation. AR binds to androgens, resulting in a conformational change, where it dissociates from heat shock proteins in the cytoplasm, and localizes to the nucleus. In the nucleus, the AR binds to specific DNA sequences, called androgen responsive elements, via the DNA-binding domain, promoting further association of factors into a complex, which leads to gene transcription. Various genes are regulated by the AR, including kallikrein-related peptidase 3 (KLK3/PSA), which is one of the best cancer biomarkers available. Enzalutamide is an FDA approved competitive inhibitor of DHT/T and antagonizes AR. Adapted from “Molecular Alterations during Progression of Prostate Cancer to Androgen Independence,” Clinical Chemistry. Diamandis et al 2011.
B. Kaplan-Meier curve from clinical data published by Hussain et al. illustrating that among men with non-metastatic, castration-resistant prostate cancer with a rapidly rising PSA level, enzalutamide treatment led to a clinically meaningful and significant 71% lower risk of metastasis or death than placebo. Adverse events were consistent with the established safety profile of enzalutamide.

Experimental Design



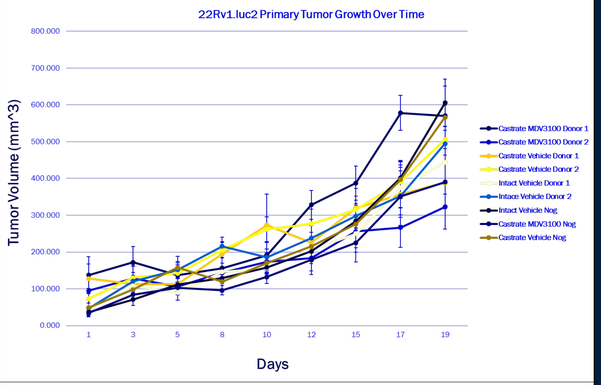
Experimental timeline (A) and conditions with animal numbers (B) for assessment of for the xenograft of 22Rv1.luc2 cells (transduced with Promega luciferase2 ® for bioluminescent imaging). 22Rv1 are the most aggressive prostate cancer cell line in vivo that still has AR expression. They express mutant (H875Y), and wild type full length AR and many splice variants including V7, some of which are stably expressed through genetic alterations. 22Rv1 respond weakly to both anti-androgens and androgens, but are generally considered enzalutamide resistant. Isolated from a patient derived xenograft made from a primary tumor from a patient with extensive bone metastases upon disease presentation. In mice, these cells metastasize and colonize clinically relevant sites such as the bone, liver, and lymph nodes.

Detection, quantification and histological validation of metastases from 22Rv1 luciferase tagged cells in the femurs of huNOG and NOG control mice.



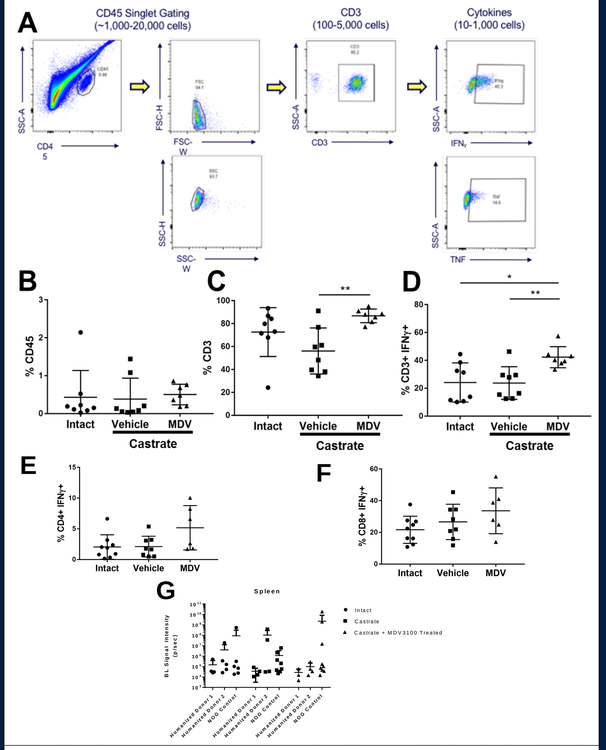
HuNOG and NOG control mice were surgically castrated. One week following castration, castrated and intact control mice were injected subcutaneously with luciferase-transduced 22Rv1 human prostate cancer cells to assay organ-specific metastatic growth. At sacrifice, organs were ex-vivo analyzed for metastatic growth using the IVIS bioluminescence system [PerkinElmer, images taken of signal intensity overlaid on top of an image of the limbs seen in (A)]. Quantification of average signal intensity per unit area (B). No significant changes in the metastatic outgrowth are seen in any condition except there is a significant increase in the metastatic burden of NOG control mice castrated and treated with enzalutamide, and a significant decrease in the outgrowth seen in the huNOG mice treated with enzalutamide (B). Histological validation (H&E stain) of the femoral metastases confirmed by a pathologist (Dr. Rahul Manan, MD), with cancer cells seen in both the bone marrow and matrix of the epiphyseal head of a mouse femur (C – yellow arrow indicates 22Rv1 tumor mass).

Subcutaneous “primary” tumor growth of 22Rv1 cells is unaffected neither by the presence of an intact immune system, castration, nor enzalutamide treatment in NOG or huNOG mice



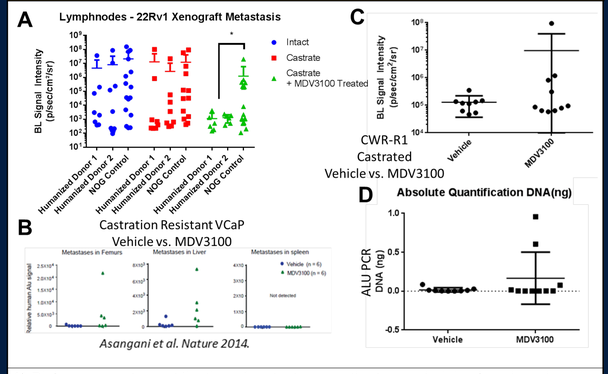
Tumor volume measurements plotted as a function of time (Days) after tumor palpability post-injection of 5x105 22Rv1 cells subcutaneously into the flanks of intact or castrated NOG or huNOG mice with two unique donor stem cells (donor 1 or donor 2). Starting on day 1, castrated mice were randomized within a cohort, and then treated with enzalutamide (10mg/kg- MDV3100) or vehicle control (vehicle), which was also given to the intact mice. No significant changes were seen at endpoint between any of the treatment groups, with either donor, immunocompromised control, nor AR-antagonist treatment; however, there is variability in the tumor sizes within each of the cohorts.

Immunological Profiling of 22Rv1 subcutaneous tumors in huNOG mice castrated and/or treated with enzalutamide (MDV).



A) The Flow Cytometry gating strategy to analyze T-cells and their activation within the tumor. Disassociated tumors cells were stained with human α-CD45 (for Leukocytes) and α-CD3+ (T-cells) were co-stained with intracellular IFN-γ and TNF-α (markers of activation), and positively gated for FACS analysis. Quantification of the percentage of cells stained in each of the hormone and AR-antagonist treatment conditions and gated for CD45+ leukocytes (B), CD3+ T-Cells (C), and IFN-γ (D). Statistically significant increases in the intratumoral CD3+ T-Cells and their activation are seen with enzalutamide treatment, and not in other groups. There are no significant systemic changes as assayed by the number splenic of CD4+ helper T-Cells (E) and CD8+ Cytotoxic T-Cells (F) in these mice the different hormonal and enzalutamide (MDV) treated conditions; however, a caveat to these data is the fact that there are increased splenic metastases in the hormonally intact and a castrate only mice (G).

The paradoxical increase in detectable metastases seen in immunocompromised mice treated with enzalutamide is reversed in the presence of an intact immune system.



A) Bioluminescent quantification of metastases in the tumor draining lymph nodes in 22Rv1 prostate tumor-bearing mice, similar to femoral quantification on Figure 1. A statistically significant decrease in lymph node metastases is seen in mice with human immune systems treated with enzalutamide. Contrastingly, we see increases in detectable metastases in two independent cell line xenograft models. B) Subcutaneous xenografts of castration resistant VCaP cells treated with enzalutamide (MDV3100 10mg/kg) in castrated CB17 SCID mice show increases in the detection of human specific ALU repeats at a variety of organ sites (adapted from Asangani et al. Nature 2014). Subcutaneous luciferase-tagged CWR-R1 tumors show increased metastasis in castrated CB17 SCID mice treated with enzalutamide (10mg/kg), assessed by bioluminescent (BL) signal (average radiance (C), and confirmed by human specific ALU-repeat PCR (D).

Conclusions and Acknowledgements

We are, to our knowledge, the first to have a **human** model of prostate that is capable of responding to standard of care (AR-targeted) therapies, metastasizes to clinically relevant locations, has an intact human immune system, and can model many distinct human prostate cancers (not just one genetically engineered mouse model).

- **Primary tumor size is not altered between any condition in a significant manner; however the extent and growth at the secondary site is different in the mice with humanized immune systems.**
  - **This suggests that the effects seen are mediated by the immune system.**
  - **Also, this is in concordance with previously published data that suggests that AR-signaling is immunosuppressive.**
- **This has no measurable effect on the primary tumor size, but metastatic burden in the enzalutamide treated mice is decreased in a model of cancer that is enzalutamide resistant.**
  - **This seems to be what occurs in patients given recently published clinical data that enzalutamide may prevent metastatic growth in patients.**
- **There is coupled by a paradoxical increase in the metastatic burden in the immunocompromised controls**
  - **Previous work done in CWR-R1 and VCaP cells by the group holds up the trend that in an immunocompromised host, MDV3100 increases metastases.**
    - **This is most likely due to selection for resistant and aggressive metastatic clones in these cells.**
- **The AR-antagonist enzalutamide leads to increase T-infiltration and increased T-cell activation within the tumors**
- **More work is needed to characterize the model, and investigate potential synergy between the androgen antagonism and immunotherapy using huNOG mice.**
  - **Androgen signaling has long been reported to be immunosuppressive, future mechanistic studies will illustrate how enzalutamide may be acting on the immune system to directly activate and promote an anti-tumor response.**

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