Discovery and characterization of first-in-field BRN2 inhibitors as a treatment strategy for Neuroendocrine Prostate Cancer

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Introduction: Resistance to newly developed androgen receptor pathway inhibitors (ARPIs), such as Enzalutamide (ENZ), rapidly emerges. In particular, a subset of patients who relapse following ARPI therapy their dependence on AR signaling and emerge with neuroendocrine features. These tumors, termed treatment induced neuroendocrine prostate cancer (t-NEPC), carry an extremely poor prognosis and, to date, treatment remains decades old cytotoxic chemotherapies. Recently our group identified the neural transcription factor *BRN2* as a major clinically relevant driver of NEPC and targeting *BRN2* is a promising strategy to prevent neuroendocrine differentiation or treat NEPC.

Methods/Results: *In silico* screening of small molecules was conducted on a model of BRN2 which was that was validated with the first-in-field crystal structure of BRN2 DNA binding domain. On the basis of the model, several small molecules were identified that showed direct binding to BRN2 and inhibited its reporter activity. Pharmacokinetic studies measured stability and bioavailability of med-chem optimized lead compound (BRN2i) that significantly reduced tumor growth in multiple xenograft models with no measurable side-effects.

In silico modeling showed a 7Å "closing" in the DBD once it was bound to BRN2i, this shift translated to reduced interaction with DNA by chromatin fractionation and ChIP-seq, thus confirming the mode of action for BRN2i is through loss of DNA binding. Loss of BRN2 binding reduced expression of direct downstream genes and several known targets in NEPC like ASCL1, SOX2 and PEG10 as well as reducing cell proliferation in $42D_{ENZR}$ (t-NEPC), NCI-H660 (NEPC) and NEPC organoids through cell cycle dependent mechanisms. These results were validated with CRISPR/Cas9 mediated knockout of BRN2, demonstrating on-target specificity for the BRN2i.

Conclusion: The described work aims to lay the pre-clinical foundation for the integration of BRN2 targeted therapies into the treatment landscape to improve survival for patients suffering from small-cell neuroendocrine prostate cancer.

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