

## Patient-derived organoid models of neuroendocrine prostate cancer

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**Background:** One emerging mechanism of treatment resistance in advanced prostate cancer involves epithelial plasticity, associated with the development of small cell or neuroendocrine carcinoma (NEPC) features and loss of AR dependence. The development of effective treatment strategies for patients with NEPC represents a clinical unmet need. There are few preclinical models of NEPC to study NEPC biology and to test new therapies. To address this gap, we developed, maintained, and characterized patient-derived organoids and organoid-PDX models derived from metastatic tumor biopsies from patients with NEPC.

### Methods:

Two patients with widely metastatic small cell NEPC were enrolled and consented for metastatic biopsy under an IRB approved protocol. Liver and bone fresh-tumor biopsies were washed, enzymatically digested and then seeded in Matrigel (BD) droplets. Organoids were maintained using protocols described in Gao et al, Cell 2014. Organoids were characterized by histology and at the genomic (WES), transcript (RNA-seq), and protein level (IHC). Organoids were subcutaneously injected in NSG mice to generate PDX models. Lentiviral infection was performed in tumor organoids using shRNAs to knock down EZH2. In vitro and in vivo drug testing was performed using GSK126/GSK503 compounds. In vitro drug library screening was performed using a cell viability assay (CellTiter-Glo®).

**Results:** The two NEPC tumor organoids (PM154, PM155) developed from bone and liver, respectively, were histologically identical to their matched tumor biopsy, strongly expressed classical neuroendocrine markers (eg. chromogranin, synaptophysin) and lacked AR expression by IHC. Direct comparison of patient-organoid and matched tumor data showed high concordance at the genomic level, and transcriptome analysis demonstrated significant clustering of NEPC organoids with NEPC tumors in the Beltran et al, Nature Med 2016 cohort. Histology and molecular features of organoids were stable and maintained at ~24 months of passaging. Similar to patients, the histone methyltransferase EZH2 was highly expressed in both NEPC organoids compared to adenocarcinoma cell lines and organoids, and EZH2-repressed target genes down-regulated. Knockdown of EZH2 or treatment with GSK126/GSK503 in 2-dimension (2D) and 3-dimension (3D) tumor organoids decreased viability in vitro, decreased tumor growth in vivo (PDX), and modulated neuroendocrine/neuronal and stem cell pathways. High throughput organoid drug screening results will be presented.

**Conclusions:** Patient-derived tumor organoids of NEPC represent clinically relevant model systems to understand biology and to test emerging drug targets.

**Conflict of Interest:** The authors declare no conflicts of interest.

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