**Background Text for U01**

**Prostate Cancer Progression Driven Through TP53 and RB loss: An important Model System for our Proposal.**

***TP53/RB1 loss***

Poorly differentiated Neuroendocrine Tumors (NECs), including those that arise *de novo* and those that may evolve after therapy from an epithelial carcinoma as a mechanism of lineage plasticity and adaptive response, are associated with frequent genetic alterations involving the p53 encoding gene *TP53* and the retinoblastoma 1 encoding gene *RB1* (**Figure 2 from PDF**)(REF Rickman et al, 2017 Nature Med PMID28586335).

Both *TP53* and *RB1* are altered in roughly 80% of poorly differentiated NE tumors compared to roughly 40% and 5%, respectively, of adenocarcinomas originating from the same anatomical site. In an exome sequencing analysis of 120 small cell carcinomas of the lung (SCLC), inactivating events involving *TP53* and *RB1* were nearly universal and included mutations, translocations, homozygous deletions, hemizygous losses, copy-neutral losses of heterozygosity (LOH) and LOH at higher ploidy1. In contrast, loss of *TP53* and *RB1* is much less common in lung adenocarcinoma (TP53 alterations present in 46% and RB1 loss in 4%)2-5.

In NEPC, copy number loss of the tumor suppressors *RB1* and mutation or loss of *TP53* are also common (70-90% and 56-67% refs: 6, 7, respectively) and are observed in up to 32% and 31% of castration resistant adenocarcinoma, respectively7. In Merkel cell carcinoma, inactivation of *RB1* and *TP53* is also frequent and occurs in both viral induced (via MCPyV large T antigen inactivation of *RB1* and *TP53*)8 and in UV induced subtypes. Poorly differentiated NE tumors of the pancreas also harbor common *RB1* and *TP53* mutations9

Relevant to this U01 proposal, data from mouse models supports that loss of *TP53* and *RB1* are important steps in the development of poorly differentiated neuroendocrine tumors. The combination of *RB1* and *TP53* alterations can drive small cell carcinoma in both lung and prostate mouse models.

Prostate cancer GEMMs with mutations in *Tp53* and *Rb1* develop tumors that are similar to clinical NEPC10-12. Mice with *Tp53/Rb1* double knock-out develop widespread metastases primarily to the liver but also to the lung, adrenal gland and regional lymph nodes. The *Tp53/Rb1* double knock-out tumors respond initially to androgen ablation (in this case, castration) but became castration-resistant from the early stages of carcinogenesis due to selection of castration–resistant cells associated with an increase in neoplastic cells with a neuroendocrine phenotype (indicated by expression of synaptophysin). Most recently, two concurrent studies provided mechanistic data supporting the role of *Tp53* and *Rb1* combined loss in driving resistance to androgen deprivation therapy (ADT), lineage plasticity and the development of neuroendocrine features10,13 discussed further below.

***Prostate Cancer Progression***

Recent evidence shows that Neuroendocrine prostate cancer (NEPC) can arise in later stages of prostate cancer progression from a preexisting adenocarcinoma during the course of treatment resistance to AR directed therapies. This is as an adaptive resistance mechanism. Although NEPCs retain common prostate cancer genomic alterations, they often lose expression of AR and luminal epithelial prostate cell markers and become less dependent on AR signaling. There are few preclinical systems to model this lineage plasticity. Prostate adenocarcinoma cell lines (such as LNCaP) acquire a neuroendocrine characteristics with some similarities to the few established NEPC cell lines that have been described (e.g. NCI-H66014-16) following exposure to a variety of therapy-like stimuli such as androgen deprivation17 or treatment with cAMP18, IL-619 or fractionated ionizing radiation20 but are post-mitotic limiting extensive study. However LNCaP-AR cells harboring RB1/Tp53 loss results in cellular proliferation that is less dependent on AR signaling and expresses basal and neuroendocrine markers13.

Similar changes have been observed *in vivo*. For example, a well-characterized patient-derived prostate adenocarcinoma xenograft implanted into the subrenal capsule of mice develops small cell NEPC following castration 21, which phenotypically resembles other lines that were generated from bone fide NEPC tumor tissue (e.g. LTL352, LTL37021, LuCAP-4922, UCRU-PR-223 and WISH-PC224). The xenograft retains genomic alterations from its prostate adenocarcinoma precursor21 and an expression profile similar to clinical NEPC samples (e.g. up-regulation of PEG10, NE markers and repression of REST25 and AR signaling)26.

Other patient-derived prostate cancer xenograft models that show a mixed adenocarcinoma and NEPC phenotype have been described (e.g. MDA PCA 14427) and androgen deprivation therapy (ADT) increases the number of NE cells in these models (e.g. PC-310 and PC-29528 and CWR2229). These models all share the phenotypic alterations associated with NEPC (e.g. loss of AR and increase in the NE markers synaptophysin, chromogranin A and/or neuronal specific enolase). The loss of *RB1* (e.g. NEPC MDA PCA 14427) or expression of mutant TP53 (e.g., WISH-PC224) are also features of the NEPC molecular program that are expressed in these xenografts6,7,12,30. Although these NEPC xenograft models bare the phenotypic hallmarks of clinical NEPC tumors they are limited in terms of their ability to spontaneously metastasize and therefore are not ideal for studying NEPC metastatic niche characteristics.

In mice with a prostate lacking *Rb1 and Pten*, prostate tumors develop that contain heterogeneous populations of tumor cells, some cells that express high levels of the luminal epithelial marker Krt8, high levels of AR and low levels of the NE cell marker synaptophysin that is a marker of a more luminal like phenotype, and others that express high synaptophysin and low luminal markers10. The existence of both luminal-like cells and NE-like cells within primary and metastatic tumors suggests that these cancers went through a stage of lineage plasticity. Detailed lineage tracing and longitudinal analyses of these lesions (from prostatic intraepithelial neoplasia (PIN) to invasive carcinoma) suggests that the primary and metastatic tumor cells were most likely derived from a single neoplastic cell clone and that the NE tumor cells arose later. Blocking AR signaling through castration was associated with recurrent disease and the acquisition of spontaneous loss-of-function Tp53 mutations suggesting that Tp53 cooperates with Rb1 to maintain the NE phenotype.

Another *Tp53*/*Rb1* mouse knock-out in a model of human AR-signaling dependent prostate cancer cells (LNCaP-AR) resulted in a similar castrate resistant, reversible lineage plasticity of cells that displayed NEPC features13. Gene expression analyses of the *Rb1/Pten* double knock out GEMM tumors revealed altered expression of gene sets related to stem cells and epigenetic reprogramming, including increased expression of SRY (sex determining region Y)-box 2 (Sox2) and Ezh210. SOX2 induces expression of neuroendocrine markers13. Furthermore, loss of *RB1* in mouse embryonic fibroblasts results in the induction of the pluripotency transcriptional program including SOX231. However *RB1* loss alone is insufficient to induce SOX2 or neuroendocrine gene expression and lineage plasticity10,13. Using another model of AR pathway inhibitor–resistant prostate cancer, the neural transcription factor BRN2 (encoded by POU3F2) was shown to regulate the expression of SOX2 and to drive the NEPC phenotype32.

Although the data from the different GEMM tumors and cell lines with mixed populations of NE, mesenchymal and luminal tumors cells do not definitely exclude other mechanisms for emergence of NE-tumor cells, collectively **they show *TP53/RB1* loss enhances lineage plasticity of prostate tumor cells that arose from independent tumorigenic alterations.** For adenocarcinomas such as prostate cancer, loss of luminal identity and the enrichment of more basal, mesenchymal, or NE-like phenotypes when, as Sawyers and colleagues suggested, faced with a selective pressure (e.g., potent anti-androgen therapy) is one model of drug resistance. Within this context, the epithelial tumor cell does not need to “trans-differentiate” (i.e., undergo a full transition from one lineage to a completely different lineage) but to revert to a more plastic state. Given the broad spectrum of tumor types that display lineage plasticity, recent data7,10,13,33 suggests that reversing or delaying lineage transformation with targeted therapy may provide a clinical benefit to a larger number of patients than previously appreciated.

The first evidence in prostate cancer that neuroendocrine regions arise by transdifferentiation of luminal adenocarcinoma cells was through a recent lineage-tracing experiment of genetically engineered mice34. This GEM with loss of Trp53 and Pten failed to respond to abiraterone, and display accelerated progression to tumors resembling treatment-related CRPC with neuroendocrine differentiation (CRPC-NE) in humans. Lineage-tracing of these mice was performed using a R26R-YFP reporter allele. In this model YFP is only expressed by luminal cells and their descendants in NPp53 tumors. This study found that nearly all synaptophysin positive cells co-expressed YFP, demonstrating that these cell were derived from luminal cells, and not from neuroendocrine or basal cells. Therefore, Zou et al show by lineage-tracing that these neuroendocrine-like cells arise by transdifferentiation of luminal prostate adenocarcinoma cells, underscoring the significance of lineage plasticity as a mechanism of drug resistance.

In summary, we posit that understanding regulation of TP53 and RB will provide important insights into prostate cancer progression and will be useful potentially for other cancer types such as lung and bladder.

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