

## TRAMP MODEL DESCRIPTION

The TRAMP model was generated in the C57BL/6 inbred strain by microinjection of a construct harboring a minimal rat probasin (PB) -426/+28 regulatory element to direct expression of the SV40 early genes (T and t antigens; Tag) to prostatic epithelium in a developmentally and hormonally regulated fashion. The rationale for building this construct was that the Tag encoded T oncoprotein would bind and abrogate the function of the tumor suppressor genes Rb and p53 that are often mutated or lost in clinical prostate cancer. As well, Tag can bind Bub 1, a spindle assembly checkpoint protein. In addition, the little t antigen would modulate activity of protein phosphatase 2A (PP2A) that has been implicated in the activities of a number of cellular pathways involving TOR, e1F4E, MAPK, AKT and RSK. Hence, the specific aim was to directly test the hypothesis that inactivation of p53, Rb and Bub1 would lead to genomic instability, inhibition of DNA repair, aberrant cellular signaling and abrogated cell cycle check point control leading to prostate cancer.

Expression of the PB-Tag transgene in prostate tissue can be detected as early as 4 weeks of age (Gingrich, Barrios et al. 1999). The earliest pathology is consistent with prostatic intraepithelial hyperplasia (PIN) and the mice can display well-differentiated adenocarcinoma as early as 12 weeks of age when expression of the transgene is maximal (Gingrich, Barrios et al. 1999; Kaplan-Lefko, Chen et al. 2002). Over the next 6-week period the TRAMP mice commonly display moderately differentiated carcinoma and ultimately develop poorly differentiated carcinoma by the time they reach 24 to 30 weeks of age. Distant site metastasis, both hematogenous and lymphatic, have been detected as early as 12 weeks of age and by the time the mice are 24-30 weeks of age the incidence of metastasis approaches 100% (Gingrich, Barrios et al. 1996; Kaplan-Lefko, Chen et al. 2002).

Clinical prostate cancer is initially androgen dependent and tumor development in the TRAMP model is also initially dependent on androgen. When TRAMP males are castrated at 6 weeks of age approximately 50% of mice will progress to androgen independent disease (Eng, Charles et al. 1999). However, when TRAMP males are castrated at 12 weeks of age 70-80% will ultimately develop androgen independent disease (Gingrich, Barrios et al. 1997; Kaplan-Lefko, Chen et al. 2002). The castrated mice typically develop poorly differentiated adenocarcinoma and can exhibit twice the incidence of metastasis as intact littermates. Most recently, we have observed, isolated and characterized specific somatic mutations in the androgen receptor (AR) in tumors arising in the TRAMP mice (Han, Foster et al. 2001). The nature and incidence of these mutations correlates with the hormonal status of the mice and most of these mutations coincide with mutations observed in clinical disease (Buchanan, Greenberg et al. 2001; Buchanan, Yang et al. 2001).

Phenotypic variability in pathologic progression has been observed clinically and in the TRAMP model. This variability appears to be a function of genetic background and is consistent with the hypothesis that specific modifier genes or gene sets exist in the mouse. Tumors obtained from C57BL/6 TRAMP were mostly derived from the lateral lobes that often invaded into the urethra and seminal vesicles resulting in seminal vesicle obstruction. However, tumors obtained from [TRAMP x FVB]F1 mice arise from the dorsolateral lobes as more spherical, highly vascularized masses. It is interesting to note that C57BL/6 TRAMP mice frequently survive beyond 40 weeks of age while [TRAMP x FVB]F1 mice rarely survive beyond 33 weeks of age (Gingrich, Barrios et al. 1999).

Although the significance of the neuroendocrine component of clinical disease is controversial, it is apparent that tumors displaying characteristics of a neuroendocrine phenotype are usually associated with advanced and lethal cancers. Most recently, we have reported on the emergence of a neuroendocrine phenotype in the TRAMP mice (Kaplan-Lefko, Chen et al. 2002). By following expression of synaptophysin, it has been determined that the development of the neuroendocrine phenotype is a stochastic late event in the TRAMP system and consistent with an epithelial to neuroendocrine transition. Hence, in TRAMP, advanced stage poorly differentiated cancers with neuroendocrine features do not arise from neuroendocrine cells, rather, they are they are the consequence of transformation of epithelial cells.

By virtue of the fact that TRAMP was generated in an inbred genetic background makes this model system ideal for immunobiological studies to understand and characterize the role of the immune system in the natural history of prostate cancer and an ideal platform to investigate immunotherapeutic strategies. To this end, a number of studies have already been published which serve to underscore the utility of the TRAMP system for immunobiology. For example, the TRAMP derived cell lines were used to demonstrate how enforced expression of B7 costimulatory molecules could make prostate cancer cells mimic antigen presentation cells (Kwon, Hurwitz et al. 1997) and this could translate into an in vivo immunotherapy using a strategy based on an anti-CTLA4 antibody (Hurwitz, Foster et al. 2000). It should be noted that these studies have since been translated into Phase I clinical trials for melanoma and prostate cancer. Furthermore, the TRAMP system has been used to demonstrate how similar strategy could be used to eliminate residual metastatic disease following surgery (Kwon, Foster et al. 1999).

Clearly, the TRAMP system serves as a paradigm for discovery-based investigations designed to identify and characterize novel targets and as for pre-clinical trials designed to test novel strategies for the prevention, intervention and regression of progressive prostate cancer.

#### **Literature Cited:**

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