

## **Preclinical Trials for Prostate Cancer**

### **The Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) Model System**

#### **Overview:**

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 (Greenberg, DeMayo et al. 1995). The rationale for building this construct was that Tag can bind and abrogate the function of Rb and p53 (Ludlow 1993) (Ali and DeCaprio 2001) that are often mutated or lost in clinical prostate cancer. As well, Tag can bind Bub 1, a spindle assembly checkpoint protein (Cotsiki, Lock et al. 2004) while the little t antigen can 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 (Chen, Possemato et al. 2004) (Van Hoof and Goris 2004). Hence, the specific aim was to directly test the hypothesis that abrogation of p53, Rb and Bub1 functions could result in genomic instability, inhibition of DNA repair, aberrant cellular signaling and cell cycle check point control causing the initiation, and progression of autochthonous prostate cancer.

Expression of the PB-Tag transgene in prostate tissue can be detected as early as 4 weeks of age. The earliest pathology is 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. Over the next 6-week period the TRAMP mice commonly display progressive forms of adenocarcinoma and will 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 (Gingrich, Barrios et al. 1996) and by the time the mice are 24-30 weeks of age the incidence of metastasis may approach 100% (Gingrich, Barrios et al. 1999) (Kaplan-Lefko, Chen et al. 2003). Based, in part, on these properties the TRAMP model received US Patent 5,907,078 on May 25, 1999.

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. 2003). The castrated mice typically develop poorly differentiated adenocarcinoma and can exhibit twice the incidence of metastasis as intact littermates.

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 are mostly derived from the lateral lobes that often invaded into the urethra and seminal vesicles resulting in seminal vesicle obstruction. However, tumors obtained from [C57BL/6 TRAMP x FVB] F1 mice arise from the dorsolateral and ventral lobes as more spherical, highly vascularized masses (Gingrich, Barrios et al. 1999). It is interesting to note that C57BL/6 TRAMP mice frequently survive beyond 40 weeks of age while [C57BL/6 TRAMP x FVB] F1 mice rarely survive beyond 33 weeks of age (Kaplan-Lefko, Chen et al. 2003).

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. 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 (Kaplan-Lefko, Chen et al. 2003). 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 that may actually facilitate growth in the androgen depleted environment (Evangelou, Winter et al. 2004).

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 can be exploited as a paradigm for discovery-based investigations designed to identify and characterize novel targets (Huss, Hanrahan et al. 2001) (Huss, Barrios et al. 2003) (Uzgare, Kaplan et al. 2003) (Maddison, Huss et al. 2004) and as for pre-clinical trials designed to test novel strategies for the prevention, intervention and regression of progressive prostate cancer (Wechter, Leipold et al. 2000) (Gupta, Ahmad et al. 2000) (Huss, Barrios et al. 2003).

### **Study Considerations**

An essential component of a pre-clinical trial is identification of the appropriate “window of opportunity” for each compound or therapy. The intrinsic progressive spontaneous autochthonous nature of the TRAMP system facilitates prevention, intervention and regression trials. Since the mice are born disease free but equally predisposed to develop prostate cancer, TRAMP mice can begin receiving therapy in a prevention setting as early as 4-6 weeks of age when they are first weaned and screened. In most cases, prevention studies initiated by week 8 can be completed by weeks 12-16 by scoring for significant reduction in PIN and early invasive lesions. Early intervention therapy would likely initiate by 12 weeks of age, a time at which TRAMP mice display either PIN or well-differentiated prostate cancer, and conclude by 16-20 weeks looking for significant evidence of inhibition or progression, reduction in disease burden or elimination of disease. Intervention therapies can be conducted in both the intact and castrated states. Late stage intervention or regression trails can also be conducted in TRAMP, usually initiated at 18-20 weeks and concluding by 32 weeks.

### **Statistical Considerations**

The appropriate number of mice should be accrued to each cohort to provide 80% power to detect a difference between cohorts, based on a t-test with a two-sided 0.05 level of significance. In general, we would recruit:

- 17 mice per cohort if the expected change in the primary outcome measure between test and control is 1 standard deviation unit (50% expected change);
- 29 mice per cohort if the expected change in the primary outcome measure between test and control is 0.75 standard deviation unit (38% change);
- 37 mice per cohort if the expected change in the primary outcome measure between test and control is 0.67 standard deviation units (33% change) or,
- 64 mice per cohort if the expected change in the primary outcome measure between test and control is 0.5 standard deviation unit (25% change).

Most of our studies are empowered to predict a 50% change between test and control (17 mice) with a few additional mice to account for occasional sample loss during analysis. Since castration at 12 weeks leads to a 100% response and approximately 70% failure, cohorts testing response in castrates may need to be adjusted accordingly.

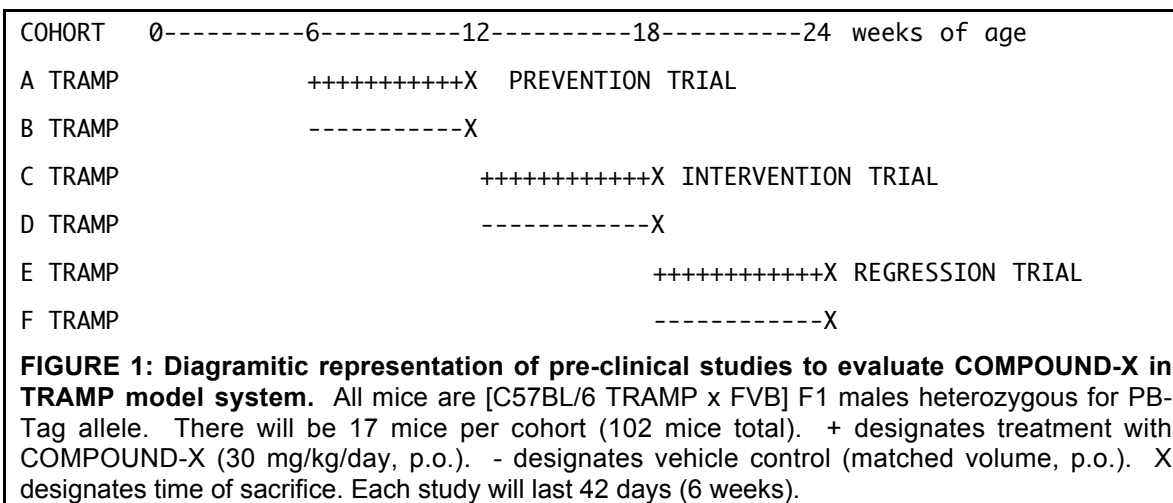
**The following Specific Aims are given solely as a general guide to help design your specific trial.**

To test the hypothesis that COMPOUND-X can be used to treat prostate cancer in vivo, the following three pre-clinical trials are proposed:

- Study 1. The ability of COMPOUND-X to prevent prostate cancer will be determined in TRAMP mice.
- Study 2. The ability of COMPOUND-X to inhibit prostate cancer progression will be determined in TRAMP mice.
- Study 3. The ability of COMPOUND-X to cause the regression of established prostate cancer will be determined in TRAMP mice.

**Methods:**

The goal of these pre-clinical trials will be to test the ability of COMPOUND-X to prevent the initiation, inhibit the progression, or cause the regression of prostate cancer in an autochthonous model system. To this end, cohorts of mice (17 mice per cohort), will be treated with COMPOUND-X for various times as shown in Figure 1.



**Drug Required**

Anticipate the amount of COMPOUND-X required assuming a daily dose of 30mg/kg p.o. :

$$30 \text{ mg/kg/mouse/day} \times 0.04 \text{ kg/mouse} \times 51 \text{ mice} \times 126 \text{ days} = 7712 \text{ mg}$$

In this model, 8 to 10 grams of COMPOUND-X would be the minimal amount required for these trials. However, if we were to try to dose a cohort for a longer term (i.e. from 6 to 18 weeks) or to try more than one dose, or in combination with castration then more compound could be required.

**Analysis and Perspective**

At sacrifice, all mice will be analyzed for the incidence and nature of tumor burden, as well as metastases. Sections prepared from treated and control mice would be analyzed for the levels of COMPOUND-X. If COMPOUND-X prevents the initiation and progression or induces regression of formation of adenocarcinoma and metastatic disease in TRAMP mice, then these results would support testing COMPOUND-X in clinical trials.

### Parameters To Be Evaluated In All Animal Studies:

1. **Body weight:** All mice should be weighed weekly from 6 weeks of age until sacrifice. Since transgenic tumor bearing mice are often observed to be cachectic, a comparison of body weights between transgenic and non-transgenic mice should be used to assess the impact of COMPOUND-X on the gross weight of the animals;
2. **Genitourinary (GU) tract weight:** Upon sacrifice, the GU tract would routinely be harvested from each mouse and weighed. The GU weight is used as a measure of tumor volume. We have previously demonstrated that GU weight can be increased by 20% at 12 weeks of age in TRAMP mice compared to non-transgenic littermate. A comparison of GU weights would be used to assess the impact of COMPOUND-X on tumor volume;
3. **Time to palpable tumor:** Animals would be palpated abdominally for tumor formation on a weekly basis from 6 weeks of age until sacrifice. The time to palpable tumor should be recorded;
4. **Incidence of metastasis:** All mice would be examined upon autopsy for the appearance of metastatic lesions. The location and gross appearance of these lesions would be noted. The tissues would be harvested, fixed in 4% paraformaldehyde, embedded in paraffin and sectioned for histological analysis;
5. **Pathological tumor grade:** Sections would be cut from formalin fixed paraffin embedded samples on a microtome at 5mm and stained with hematoxylin and eosin. The dorsolateral and ventral prostate would also be harvested from non-transgenic mice as control tissue. We apply our established a grading system and pathologists would confirm grading.

**Note Regarding Imaging:** We are currently establishing a non-invasive imaging facility at the Fred Hutchinson Cancer Center. This will allow serial and longitudinal image analysis of tumor progression by CT, MRI, fluorescence and luminescence (in selected model systems). PET imaging could be arranged through the University of Washington. Proposals employing non-invasive imaging should be thoroughly discussed prior to inception.

### Difficulties and Time Line

It is anticipated that most studies could be completed within 52 weeks. Given a certain level of proficiency and experience with transgenic husbandry only few difficulties would be anticipated.

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