ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
Laboratory of Comparative Genomics

ISAG 2006 Poster Winner

# A Porcine Model of T-cell Lymphoma


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## ABSTRACT

Non-Hodgkin's lymphoma (NHL) is a disease of increasingly serious proportions. The highest incidence rate in the world is seen in the United States and Canada, with forty to fifty thousand new cases per year in the U.S. NHL is the sixth most common cancer and the sixth most common cause of cancer death, accounting for 4% of all cancers and 4% of cancer-related deaths. There is a clear need for relevant animal models of lymphoma that provide preclinical tools. Our objective is to create a porcine model of lymphoma analogous to the human disease. Genetically engineered porcine tumors may prove to be invaluable for: (1) determining the efficacy of anti-cancer drugs; (2) studying the process of tumorigenesis; and (3) producing cancer in a genetically compliant animal model that is physiologically more similar to humans than rodents. We have previously shown that solid tumors could be readily induced in immunosuppressed pigs via the expression of proteins disrupting the p53 tumor suppressor pathway, and activating c-Myc and Ras pathways, all of which are commonly corrupted in human cancers. In an attempt to induce a less immunogenic tumor with a defined phenotype retroviral vectors encoding four genes (Cyclin d1, CDK 4, c-Myc, and H-Ras) were constructed and injected SC into the mammary area and/or behind an ear, or IV via the ear vein. Both challenge routes induced T-cell lymphoma in the absence of immunosuppression. The effects of vector dose responses are currently being evaluated and future studies will attempt to induce tumors in cloned animals, thus producing tumors that can be transferred to any number of identical animals to study the process of tumorigenesis and cancer


## INTRODUCTION

- **Current limitations in cancer biology have delayed the transition from basic science to patient benefit.**
- **Mouse models are the predominant choice currently for cancer studies. However, the differences between murine and human tumorigenesis and pharmacodynamics prevent adequate comparisons to human cancers.**
- **Achieving an understanding of the pathogenesis of lymphoma in a model similar to humans is key to developing successful treatments.**
- **Previous research demonstrates that solid tumors can be readily induced in immunosuppressed pigs via the expression of proteins disrupting the p53 tumor suppressor pathway, and activating the c-Myc and Ras pathways, all of which are commonly corrupted in human cancers.**

## RESULTS


**Table 1. Mortality rate for pigs injected with retroviruses encoding cyclin D1, CDK4$^{R24C}$, c-Myc$^{T58A}$, and H-Ras$^{G12V}$.** Two of 16 pigs demonstrated visible signs of disease development and were found dead or euthanized prior the to endpoint of the study. Necropsies showed pigs had developed aggressive T-cell lymphoma. Remaining pigs euthanized two months after injection were also diagnosed with T-cell lymphoma, albeit a less aggressive form.

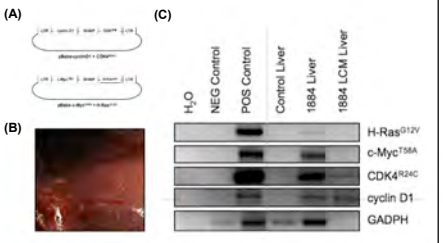



**Figure 1.** (A) Retroviral constructs used to induce cancer in the pig. (B) Lymphomas (light-colored masses) in the liver. (C) Expression of the indicated transgenes by RT-PCR from RNA isolated from the liver.

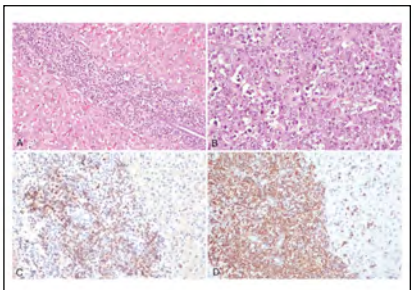


**Figure 2. Immunohistochemical analysis of porcine liver samples**. Liver samples collected from pig injected with retroviruses encoding cyclin D1, CDK4$^{R24C}$, c-Myc$^{T58A}$, and H-Ras$^{G12V}$ contained visible tumor cells that (A) when stained with hemotoxylin and eosin, were found to be invading the septa of the liver and (B) have multiple nucleoli of varying size and shape, nuclear hyperchromicity, and abnormal mitotic activity. Tumor cells (C) labeled with an anti-CD3 Ab confirmed the T-cell origin of the lymphoma, and (D) labeled positive for vimentin intermediate filaments, consistent with the mesenchymal origin of the T-cells.

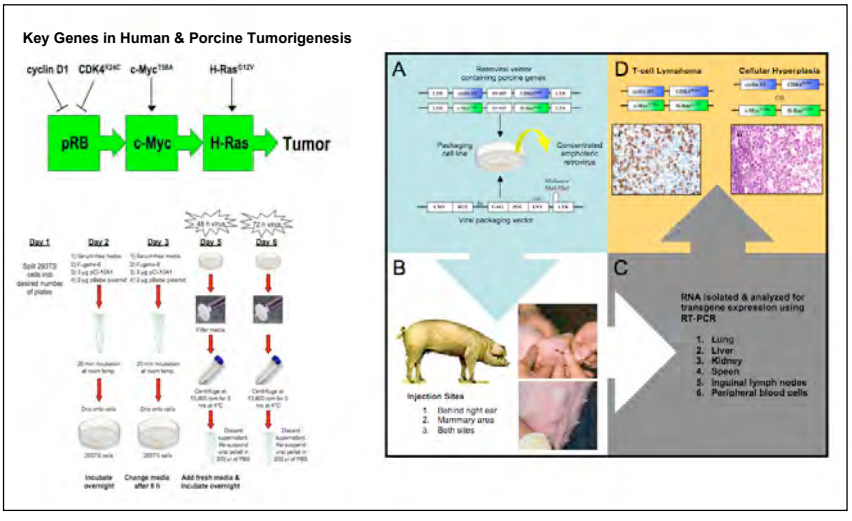
## OBJECTIVES

- **Develop methods to induce genetically defined tumor formation by altering pathways commonly disrupted in human cancers.**
- **Develop a porcine model of lymphoma analogous to the human disease.**

| Injection site[a] | Viral concentration[b] | Diagnosis[c] | Endpoint[d] |
|---|---|---|---|
| Ear & mammary | 1x | Widespread neoplasia | d15 |
| Ear & mammary | 1x | Cellular hyperplasia | d113 |
| Ear & mammary | 1x | Cellular hyperplasia | d109 |
| Ear | 1x | Normal tissues | d105 |
| Mammary | 2x | Widespread neoplasia | d16 |
| Mammary | 2x | Cellular hyperplasia | d61 |
| Mammary | 3x | Cellular hyperplasia, Neoplastic cells in blood | d70 |
| Mammary | 3x | Cellular hyperplasia, Atypical cells in blood | d77 |
| IV | 1x | Cellular hyperplasia | d42 |
| IV | 1x | Cellular hyperplasia | d45 |
| IV | 2x | Normal tissues | d69 |
| IV | 2x | Normal tissues | d75 |
| IV | 3x | Cellular hyperplasia, Atypical cells in blood | d63 |
| IV | 3x | Cellular hyperplasia, Atypical cells in blood | d80 |

**Table 2. Tumor progression is independent of viral concentration and injection site.**
[a] Subcutaneous injection site in area dorsal to right ear and/or in mammary area near the third teat on the right side of the body; intravenous injection into right ear vein
[b] 1x = 40, 10 cm plates; 2x = 80, 10 cm plates; 3x = 120, 10 cm plates
[c] Necropsy and microscopic analysis
[d] Animals were euthanized a minimum of two months following initial injection unless disease progression resulted in an earlier death.

## MATERIALS AND METHODS




## CONCLUSIONS

- **A porcine model of T-cell lymphoma was successfully created by genetically modifying pathways commonly disrupted in human cancers.**
- **This genetically compliant animal model is physiologically more similar to humans than rodents and supports adjunct therapies.**
- **The ability to manipulate gene expression *in vivo* and rapidly generate pathologically accurate tumors makes this model an ideal preclinical system for the generation of novel therapeutics and testing new drugs for the treatment of lymphoma and other types of diseases.**
- **The rapid development of novel technologies for manipulating the pig genome has allowed and will allow, in the next few years, to recreate in the pig virtually any pathological condition whose molecular basis has been elucidated in humans.**

## FUTURE DIRECTIONS

- **Expand methods to support a preclinical model for testing of novel therapeutics, experimental therapies and imaging.**
- **Develop cell-based solid tumor models from known tissue of origin and with designated clinical outcomes in the absence of immunosuppression.**
- **Establish a porcine model for breast cancer using retroviral injection methods.**
- **Generate a transgenic pig model that will be used to induce tissue-specific, conditionally expressed solid tumors.**
- **Induce tumors in cloned animals, thus producing tumors that can be transferred to any number of identical animals to study the process of tumorigenesis and cancer phenotypes.**

## REFERENCES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**Acknowledgements: This work was supported in part by grants from the USDA (USDA/NRI-CSREES AG2001-35205-11698, USDA-ARS AG58-5438-2-313), Elsa U Pardee Foundation, Duke Comprehensive Cancer Center, and NIH (CA94184).**