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TITLE: Investigation of the AKT/PKB Kinase in the Development of  
Hormone-Independent Prostate Cancer

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<b>13. ABSTRACT (Maximum 200 Words)</b> Our laboratory has been interested in the role of Akt in the development of hormoneindependent cancers. Using a breast cancer cell model, we previously demonstrated that tumors with a constitutively active Akt are resistant to anti-hormone therapy. In this study we have expanded upon our preliminary observations in the breast model into <i>in vitro</i> prostate cancer models to determine the molecular and biological mechanisms underlying these findings. In our first year of this study, we found that treatment with an Akt inhibitor prevented the progression of LNCaP cells to a state of androgen-independence. The results of these studies could have a significant impact on clinical approaches for the treatment of recurrent prostate cancer. Currently, progression of prostate cancer to androgen independence remains the primary obstacle to improved survival with this disease. In order to improve overall survival, novel treatment strategies that are based upon specific molecular mechanisms that prolong the androgen-dependent state and that are useful for androgen-independent disease need to be identified. The results of our studies suggest that targeting the Akt pathway may provide such a strategy, resulting in increased survival among patients with recurrent disease.				
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## INTRODUCTION

Our laboratory has been interested in the role of Akt in the development of hormone-independent cancers. Using a breast cancer cell model, we have demonstrated that tumors with a constitutively active Akt are resistant to anti-hormone therapy. In this study we will expand our preliminary observations in the breast model into *in vitro* and *in vivo* prostate cancer models and determine the molecular and biological mechanisms underlying these findings.

## BODY:

*Task 1: To determine whether the level of phospho-Akt within the tumor is a predictor of eventual development of hormone-refractory disease.*

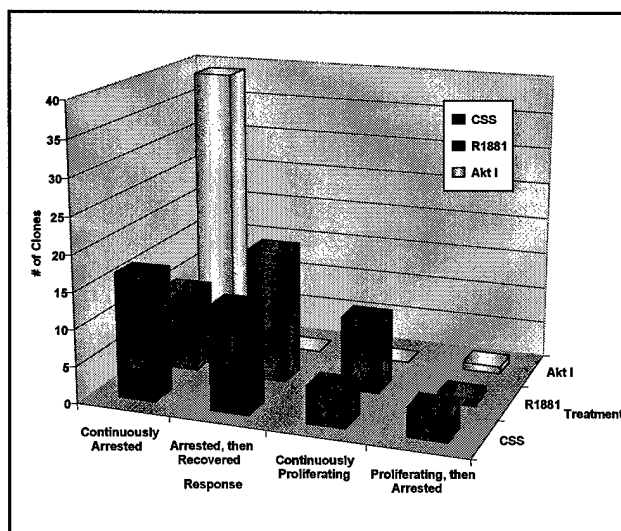
Perform immunohistochemical staining and analyses of paraffin-embedded core prostate biopsies from two cohorts of patients: 1) those that **did** develop hormone refractory metastatic disease and 2) those that **did not** develop hormone refractory metastatic disease

We are currently compiling the biopsy samples that will be evaluated for this Specific Aim. Once we have finished collecting the samples we will initiate the immunohistochemical studies.

*Task 2: To investigate in vitro whether Akt signaling is a critical component of one of the mechanisms by which prostate cancer progresses to a condition of hormone independence.*

Culture LNCaP and CRW-R1 cells under conditions of hormone ablation with and without co-treatment with an Akt inhibitor

Because of results in our breast cancer cells, we pursued studies investigating the effects of exposure to an Akt inhibitor on prostate cancer progression in a model of hormone ablation. The underlying hypothesis of these studies is that inhibition of Akt signaling in LNCaP cells during the period of androgen ablation will inhibit progression to a hormone-refractory state. We used an *in vitro* model that mimics many of the features of prostate cancer progression, and gives rise to androgen-independent cell sublines. Forty (40) single-cell subclones were isolated from a population of heterogeneous LNCaP cells that were cultured in complete FBS-containing medium. Each of the 40 subclones was then separated into five treatment groups:



**Fig. 1. LNCaP growth response to androgen-depletion.** 40 LNCaP subclones were each grown long-term under conditions of androgen-depletion (CSS, blue), CSS media supplemented with the 1 nM of the synthetic, nonmetabolizable androgen R1881 (R1881, red), and CSS media supplemented with 10 $\mu$ M of the Akt inhibitor I (Akt I, yellow). Clones were assessed at weeks 5 and 10, and determined to be either arrested or proliferating at each point.

WS-medium (normal control), CSS-only medium, CSS plus 1 nM of the synthetic, nonmetabolizable androgen R1881 containing medium (control), CSS plus 10 $\mu$ M of the Calbiochem Akt inhibitor I, or CSS plus 10  $\mu$ M of the PI3 kinase inhibitor LY294002. Androgen-deprived medium was prepared by adding 10% charcoal-stripped serum (CSS) instead of untreated FBS [whole FBS-containing medium (WS)]. Cells were cultured for 10 weeks, but assessed every 2 weeks for proliferation and growth arrest. Data is presented for the results obtained at weeks 5 and 10 (Fig 1). The salient result from these studies was that treatment with the Akt

inhibitor prevented the progression of LNCaP cells to a state of androgen-independence. As seen in Fig. 1, all but one of the clones exposed to the Akt inhibitor arrested by week 5, and never recovered. Conversely, only seventeen (43%) of the clones in the charcoal-stripped alone group (CSS) continuously arrested. Fourteen (40%) of the clones in the CSS group arrested, but then recovered, suggesting that this subset is now hormone-independent. Approximately 13% (5 of 40) of the clones in the CSS group never arrested, suggesting *de novo* resistance. Supplementation with the synthetic androgen R1881 decreased the percentage of clones that were continuously arrested compared to the CSS group, (only 28% compared to 43%), while increasing the number of clones that either recovered or continuously proliferated (18 (45%) and 10 (25%), respectively). In **Specific Aim 3**, we will expand upon these *in vitro* studies to determine in an animal model of prostate cancer progression whether treatment with an Akt inhibitor will have the same preventive effect.

Evaluate cells for cell cycle, morphological, and molecular status  
Cells are currently being evaluated for morphological and molecular status

*Task 3: To investigate in vivo whether Akt signaling is a critical component of the mechanism by which prostate cancer progresses to a condition of hormone independence.*

Initiate implantation of CWR22 tumor xenografts  
Tumors will be implanted within the next month

Carry out treatment and tissue harvesting regimens  
Molecular, immunohistochemical and cell cycle analyses of harvested tissues  
Perform statistical analyses

#### **KEY RESEARCH ACCOMPLISHMENTS:**

- Development of several AR-positive hormone-independent prostate cancer LNCaP sublines
- Demonstration that inhibition of the Akt pathway prevents the progression to hormone independence

#### **REPORTABLE OUTCOMES:**

Currently the data is being developed for presentation at the AACR-sponsored "Frontiers in Cancer Prevention Research", October 30 - November 2, 2005 in Baltimore, MD, as well as publication, probably in Cancer Research.

#### **CONCLUSIONS:**

As part of our on-going studies to better understand the role of the Akt kinase pathway in the progression of prostate cancer, we have found that treatment with an Akt inhibitor inhibited almost all progression to hormone independence in an *in vitro* model of androgen ablation. These results suggest a critical role for Akt signaling in prostate cancer progression. Cells are currently being evaluated for morphological and molecular status, especially those that directly correlate with hormone dependence. Results of the *in vitro* studies will be confirmed using an animal model of prostate cancer progression in studies scheduled for the upcoming year.

The results of these studies could have a significant impact on clinical approaches for the treatment of recurrent prostate cancer. Currently, progression of prostate cancer to androgen independence remains the primary obstacle to improved survival with this disease. In order to improve overall survival, novel treatment strategies that are based upon specific molecular mechanisms that prolong the androgen-dependent state and that are useful for androgen-independent disease need to be identified. The results of our studies suggest that targeting the Akt pathway may provide such a strategy, resulting in increased survival among patients with recurrent disease.