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14. ABSTRACT
In this proposal, we focus on, and have preliminary data to support, a novel approach targeting metabolism (by inhibition of glycolysis) to compliment ongoing clinical efforts to abrogate growth factor and apoptotic pathways in prostate cancer. The proposed clinical and laboratory studies are relevant to prostate cancer, given preliminary data demonstrating the importance of modulation of the glycolytic pathway in prostate cancer cell lines independent of activation of Akt and overexpression of Bcl-2, both common mechanisms of resistance in prostate cancer. We have put together an effective translational team capable of both laboratory efforts and development of clinical trials to develop this program and test this novel paradigm. In this regard, the proposal will involve both preclinical and clinical development. The potential of this proposal includes the reassessment of a metabolic pathway to begin a totally new area of cancer therapeutics in which we abrogate the critical nutrient pathway required for the development of oncogenic mechanisms of resistance.

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INTRODUCTION:

In this proposal, we focus on, and have preliminary data to support, a novel approach targeting metabolism (by inhibition of glycolysis) to compliment ongoing clinical efforts to abrogate growth factor and apoptotic pathways in prostate cancer. The proposed clinical and laboratory studies are relevant to prostate cancer, given preliminary data demonstrating the importance of modulation of the glycolytic pathway in prostate cancer cell lines independent of activation of Akt and overexpression of Bcl-2, both common mechanisms of resistance in prostate cancer. We have put together an effective translational team capable of both laboratory efforts and development of clinical trials to develop this program and test this novel paradigm. In this regard, the proposal will involve both preclinical and clinical development. The potential of this proposal includes the reassessment of a metabolic pathway to begin a totally new area of cancer therapeutics in which we abrogate the critical nutrient pathway required for the development of oncogenic mechanisms of resistance.

The work is innovative, since this approach has not been adequately tested in the clinic, and, therefore, represents the start of a potentially new class of agents. Prior studies demonstrated the dependence of early tumor growth and progression on anaerobic metabolism through glycolysis. In fact, the preference for tumor cells to depend on glycolysis over normal cells is the basis for the successful development of FDG-PET imaging. Despite these prior data, clinical development of agents that target glycolysis has been limited with initial concern over the lack of a therapeutic window. However, more recent studies have demonstrated that abnormal growth factor and apoptotic pathways, required by tumor cells to resist multiple insults, can drive tumor cells to even further dependence on glycolysis, supporting a rationale for selectivity of abrogating glycolysis in tumor cells compared to normal cells. Using a co-culture model that could detect the growth effect of autocrine stimulation by prostate tumor cells, two dimensional (2D) in-gel electrophoresis (DIGE), and Mass spectrometry, we found that initial changes consisted exclusively of induction of multiple glycolytic enzymes. We also created isogenic cell lines derived from rat prostate epithelial cells in which we introduced a Bcl-2 expression vector along with a constitutively active form of Akt, myr-Akt, H-Ras, and K-Ras. We found that 2-deoxyglucose, an inhibitor of glycolysis, decreased the expression of glycolytic enzymes in the co-culture model, inhibited cell growth at concentrations below what can be obtained safely in humans, and had cytotoxicity independent of Bcl-2 overexpression and Akt activation. We therefore hypothesize that 2-deoxyglucose will be safe and active in patients with prostate cancer, and abnormal cell pathways found commonly in prostate cancer, such as constitutive activation of Akt and overexpression of Bcl-2, will increase sensitivity to inhibition of glycolysis. To test this hypothesis we have the following specific aims:

1. To inhibit glycolysis in patients with prostate cancer in a phase I/II study of 2-deoxyglucose.

2. To determine the mechanism of inhibition of tumor cell growth through modulation of glycolysis.

BODY:

Based on our prior data, as noted above, we therefore hypothesize that 2-deoxyglucose will be safe and active in patients with prostate cancer, and abnormal cell pathways found commonly in prostate cancer, such as constitutive activation of Akt and overexpression of Bcl-2, will increase sensitivity to inhibition of glycolysis. Although this report covers the period of 10/04 to 10/05, the project was delayed in starting secondary to required approvals and revisions of the clinical trial, resulting in a revised statement of work, which was approved. We have had additional delays secondary to a delay in contract negotiations with the company for drug supply. We have approached the company and the negotiations are again in process; additionally, we plan to submit for a RAID application for the agent, if drug supply becomes a problem through the company. We have, therefore, revised the statement of work to allow for this new timeline, funding the laboratory studies in year one and two, and the clinical trial starting in year 3 of the grant. To determine the effect of bcl-2 and Akt on 2DG induced cytotoxicity, we already set up an isogenic model using rat prostate epithelial cells transformed with E1A (RP) and transfected with either a human bcl-2 expression vector (RP-B), Myr-Akt, a constitutively active form of Akt, (RP-Akt), the combination (RP-B/Akt), and vector control (RP-V). Using immunoblot, we validated these cell lines demonstrating increased Bcl-2 overexpression in RP-B vs. RP-V, and phosphorylated Akt in RP-Akt vs. RP-V. Using a lactic acid assay, we demonstrated decreased lactate production with treatment by 2DG in all cell lines ($p<0.01$). Using MTT assay we demonstrated a 51% decrease in cell viability in RP-B, 36% in RP-Akt, 62% in RP-B/Akt, compared to only 12% in the control RP-V ($p< 0.001$) with 2.5mM 2DG, a concentration achievable in man in prior clinical studies. These data support that 2DG cytotoxicity was increased by overexpression of Bcl-2 and activation of Akt, known mechanisms of resistance in prostate cancer. These data were presented, and published as an abstract in proceedings, at The American Society of Clinical Oncology (DiPaola et al. Abstract #9535). Laboratory studies are ongoing, as is the approval process for the clinical trial planned in year 3 of the grant.

KEY RESEARCH ACCOMPLISHMENTS

Publication and presentation of abstract to the American Society of Clinical Oncology, 2005.

REPORTABLE OUTCOMES:

PASCO 2005 abstract, as noted above.

CONCLUSIONS:

As planned, we initiated laboratory studies and published and presented initial results. A manuscript has been submitted for publication. The statement of work was revised and approved to fund the clinical trial in year three, given delays in contracts and trial approval process.