Protein kinase C alpha expression and resistance to neo-adjuvant gemcitabine-containing chemotherapy in non-small cell lung cancer

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Lay Abstract

Early stage non-small cell lung cancer can be cured with surgery alone in approximately 60% of cases. Trials are currently underway to evaluate whether giving chemotherapy before surgery improves that cure rate. Protein kinase C alpha is a cellular protein involved in regulating cell proliferation and may play a role in resistance of non-small cell lung cancer to chemotherapy. This trial will investigate whether increased levels of protein kinase C alpha are associated with resistance of lung cancer to initial chemotherapy.

We are currently conducting a trial in which patients receive chemotherapy before undergoing surgical removal of early stage non-small cell lung cancer. Patients who are already enrolled in the chemotherapy study will be eligible for this study, in which we will compare levels of protein kinase C alpha in the biopsy specimens from before patients begin chemotherapy and the post-chemotherapy surgical specimens. Up to 70 adults will be enrolled in the chemotherapy study and will therefore be eligible for this study.

Patients will already have provided informed consent for the chemotherapy trial and will provide written informed consent for their biopsies to be used in this study. The decision whether to participate in this study will not affect participation in the chemotherapy study. No additional procedures, treatments, or questionnaires will be used in this study. There are neither additional risks nor additional benefits associated with participation in this study, and the study presents no ethical problems.

A. Study Purpose and Rationale

Lung cancer is a major cause of morbidity and.mortality. Approximately 75% of primary lung malignancies are non-small cell lung cancer (NSCLC). When found at an early stage, without spread to the mediastinal lymph nodes, surgery can provide a cure for NSCLC, but even patients with the best clinical prognosis have at best a 67% 5-year survival rate (for pathological stage T1 NO) with surgical therapy.[1] Adjuvant, or postoperative treatment with chemotherapy, radiation, or both has yielded minimal to no benefit in recurrence or mortality rates. Randomized trials of preoperative, or neoadjuvant, chemotherapy in more advanced disease have shown improvements in cure and survival. [2,3,4] Early experience with neoadjuvant chemotherapy in stage I and II NSCLC has been encouraging, and phase III randomized trials are underway to better define the possible benefit of neoadjuvant chemotherapy compared with surgery alone.

We are currently conducting a multi-center, randomized, phase 11 trial of gemcitabinecontaining neo-adjuvant regimens. The study has received IRB approval and has begun accruing patients with histologically confirmed clinical stage I and 11 non-small cell lung cancers. Patients will receive gemcitabine and either cisplatin or carboplatin in a 6-week regimen of chemotherapy prior to initial surgery. The primary endpoint is pathologic complete response, or the absence of identifiable tumor cells in the postchemotherapy surgical specimen, and based on prior studies of neo-adjuvant regimens, a pathologic complete response rate of at least 20% will indicate a regimen of sufficient efficacy to warrant comparison with surgery alone in a phase III trial. The study will enroll up to 70 patients at three centers depending on preliminary results. Given this expected pathologic complete response rate, we expect 80%

of our patients to have residual tumor at the time of surgery, with demonstrable *in* vivo resistance to gemcitabine and platinum.

Protein kinase C alpha (PKCα) is one of 13 isoforms of a family of serine threonine kinases, appears to play a major antiapoptotic role in lung cancer, and may have some role in resistance to chemotherapy. Protein kinase C alpha and beta over-expression in cell lines from human non-small cell lung cancers is associated with *in* vitro resistance to doxorubicin.[6] An antisense nucleotide that reduces expression of PKCα is currently in phase III trials in late-stage lung cancer.[7,8] It may also be appropriate to use in neo adjuvant regimens for early-stage cancer. The role of PKCα in resistance to gemcitabine containing regimens in early stage lung cancer is unclear. Evidence that PKCα is over-expressed in non-small cell lung cancer that is resistant to initial chemotherapy would strengthen the rationale for using anti-PKCα therapy in early stage lung cancer.

The purpose of this study is to: 1) describe the level of expression of PKCα in prechemotherapy and post-chemotherapy biopsy specimens of non-small cell lung cancer; 2) determine the association between level of PKCα expression and response to chemotherapy with gemcitabine and platinum; and 3) determine whether PKCα expression increases in tumors that are resistant to induction chemotherapy.

B. Study Design and Statistical Analysis

We will attempt to enroll all patients who are in the parent trial. Patients will therefore have histologically confirmed clinical stage I or 11 non-small cell lung cancer; will not have received any prior chemotherapy or radiation therapy for non-small cell lung cancer; will not be receiving other investigational therapy; will be 18 years of age or older, with a Karnofsky Performance Status of ~:70 and adequate pre-treatment lung, hematologic, and renal function; and will have given informed consent to participate in the randomized trial of neo-adjuvant chemotherapy. The parent study will enroll up to seventy patients overall, with up to thirty-five in each arm, and the sample size for this study is therefore limited to seventy patients.

We will describe the mean and standard deviation of relative expression of PKC(X on Western blot of pre-chemotherapy specimens and post-chemotherapy (surgical) specimens. We will assess the relationship between pre-chemotherapy level of PKCOC expression and pathologic complete response with Student's t-test. For patients without pathologic complete response, we will assess the change in PKC(X expression from prechemotherapy to post-chemotherapy specimens with a paired t-test. All p values will be two-tailed, and alpha will be 0.05.

Assuming a pathologic complete response rate of 15%, we will have approximately ten patients with pathologic complete response and sixty patients with residual tumor after chemotherapy. Our study will therefore have the ability (with a power of 80% and alpha = 0.05) to detect a difference of 0.97 standard deviations in initial PKCa levels between patients who achieve pathologic complete response and those who do not. Similarly, we will have the ability to detect a difference of 0.37 standard deviations between levels of PKCa in pre-chemotherapy and post-chemotherapy specimens in the sixty patients without a pathologic complete response. The mean and standard deviation of Western blot levels for PKCa in biopsy specimens of NSCLC are not known prior to this study.

C. Study Procedure

Biopsy specimens will be available from most patients prior to starting chemotherapy, and from all patients after chemotherapy. We will have access to all specimens from surgery done at Columbia-Presbyterian, the primary site for the original study, and we may have access to specimens from surgery done at other sites. Pathologic complete response is defined as the absence of viable tumor cells on light microscopy of routine sections in the pathology laboratory. We will perform a Western blot on all specimens to quantify the relative expression of PKCa.

We will not perform any medical procedures in addition to those necessary for the parent trial. No additional follow-up will be necessary.

D. Study Drugs

This study will not involve any drugs in addition to those used in the parent trial.

E. Medical Devices

This study will not involve any investigational medical devices.

F. Study Questionnaires

We will not gather any additional information other than PKCα levels.

G. Study Subjects

Inclusion criteria:

1) Patients enrolled in the parent study with an evaluable pre-chemotherapy biopsy specimen will be eligible for inclusion in this study.
2) Patients must be able to provide informed consent for participation in this study.

Inclusion criteria for the parent study:

1) Histologically confirmed non-small cell lung cancer
2) Stage I or 11 disease with a negative mediastinal evaluation (all mediastinal lymph nodes < 1 cm by CT scan and negative PET scan if available, with negative mediastinoscopy if either CT or PET is positive) and no signs or symptoms of systemic spread
3) No prior chemotherapy or radiation therapy for non-small cell lung cancer. No prior resection of lung disease within the five years before registration
4) Not receiving other investigational therapy
5) Karnofsky Performance Status ≥70
6) Age 18 years or older
7) Calculated post-resection FEV_1 > 40% predicted and calculated post-resection D_LCO/V_A > 40% predicted.
8) Absolute granulocyte count ≥1.5 x 10^9/L and platelet count ≥ 120 x 10^9/L
9) Serum creatinine within normal limits, or estimated or calculated creatinine clearance ≥65 ml/min
10) No prior malignancy, except treated basal cell or squamous cell skin cancer, *in situ* cervical cancer, other cancer from which the patient has been disease-free for 5 years or in which the stage and nature of malignancy is unlikely to affect 3-year survival
11) Able to give written informed consent
12) Suitable candidate for surgery

Exclusion criteria for the parent study:

1) Stage III tumor or stage II tumor involving the superior sulcus
2) Post-obstructive pneumonia or other serious infection, serious medical condition, or prior allergic reactions to drugs containing cremophor
3) Pregnancy or nursing

H. Recruitment of Subjects

The primary investigator for the parent trial will approach patients regarding inclusion in this trial.

I. Confidentiality of Study Data

All biopsy specimens will be labeled with the patient's unique code number from the parent study and not with the patient's name or other identifying information. Biopsy specimens will be destroyed after the completion of the study.

J. Potential Conflict of Interest

There are no potential conflicts of interest.

K. Location of the Study

The study will take place in the Department of Oncology at CPIVIC.

L. Potential Risks

There are no additional potential risks to subjects as a result of participating in this trial.

M. Potential Benefits

There are no benefits to the subjects from participation in this trial.

N. Alternative Therapies

No therapy is provided as part of this trial, and the alternative is not participating in the trial.

O. Compensation to Subjects

No compensation will be provided.

P. Costs to Subjects

Subjects will not incur any additional costs as a result of participating in this study.

Q. Minors as Research Subjects

This study does not involve minors.

R. Radiation or Radioactive Substances

This study does not involve exposure of subjects to radiation.

S. References:

1. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710-17.
2. Martini N, Kris MG, Flehinger BJ, et al. Preoperative Chemotherapy for Stage IIIa (N2) Lung Cancer: The Sloan-Kettering Experience with 136 patients. Ann Thorac Surg 1993;55:1365-74.

3. Rosell et al. A randomized trial comparing preoperative chemotherapy plus surgery with surgery alone in patients with non-small cell lung cancer. N Engl J Med 1994;330:153-58.
4. Roth et al. A randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small-cell lung cancer. J Natl Cancer Inst 1995; 86:673-680.
5. Pisters KMW, Ginsberg RJ, Firoux DJ, et al. Induction chemotherapy before surgery for early-stage lung cancer: a novel approach. J Thorac Cariovasc Surg 2000; 119:429-39.
6. Volm M, Pornmerenke EW. Associated expression of protein kinase C with resistance to doxorubicin in human lung cancer. Anticancer Research 1995;15:463-66.
7. Marcusson EG, Yacyshyn BR, Shanahan WR, Dean NM. Preclinical and clinical pharmacology of antisense oligonucleotides. Molecular Biotechnology 1999; 12: 1 -11.
8. McKay RA, Miraglia LJ, Cummins LL, et al. Characterizqation of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C-α expression. J Biol Chem 1999;274:1715-22.