Clinical Protocol "Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype *p53* and Cisplatin"

1.0 OBJECTIVES

1.1 To determine the maximum tolerated dose of the wild-type *p53* adenovirus vector given with and without cisplatin in patients with refractory non-small cell lung cancer.

1.2 To determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment approach.

1.3 To document observed antitumor activity of this treatment approach.

2.0 BACKGROUND AND RATIONALE

2.1 Molecular events in NSCLC

Lung cancer remains the leading cause of cancer deaths in the United States where it kills more than 140,000 people annually. Recently, age-adjusted mortality from lung cancer has surpassed that from breast cancer in women. Although implementation of smoking-reduction programs has decreased the prevalence of smoking, lung cancer mortality rates will remain high well into the 21st century[1]. Unfortunately, all current treatment modalities, including radiation therapy, surgery, and chemotherapy, have limited effectiveness. The rational development of new therapies for lung cancer will depend on an understanding of the biology of lung cancer at the molecular level. Research in our laboratory has identified critical molecular events leading to NSCLC development and progression. The goal of this research is to directly modify the cancer cell to eliminate the expression of gene products which are responsible for the maintenance or progression of the malignant phenotype or to restore in normal form deleted or mutated gene products that suppress the characteristics of the malignant phenotype.

The purpose of this protocol is to investigate molecular mechanisms that may influence the growth and progression of human lung cancer; our goal is development of therapeutic agents specifically targeted at the molecular level. The most common lung cancer histologies (80%) are grouped under the term non-small-cell lung cancer (NSCLC) and include squamous, adenocarcinoma, and large-cell undifferentiated. Many of the current data on the molecular biology of lung cancer come from the study of the more uncommon small-cell lung cancer (SCLC). SCLC can be distinguished from NSCLC by the neuroendocrine features of the cells; SCLC is very responsive to chemotherapy but recurs rapidly after treatment. NSCLC also may serve as a model for other carcinogen-induced malignancies. The approaches and observations developed in this study may be applicable to other types of epithelial cancers.

Abundant evidence has accumulated that the process of malignant transformation is mediated by a genetic paradigm[2]. The major lesions detected in cancer cells occur in dominant oncogenes and tumor suppressor genes. Dominant oncogenes have alterations in a class of genes called proto-oncogenes, which participate in critical normal cell functions, including signal transduction and transcription. Primary modifications in the dominant oncogenes that confer the ability to transform include point mutations, translocations, rearrangements, and amplification. Tumor suppressor genes appear to require homozygous loss of function, by mutation, deletion, or a combination of these for transformation to occur. Some tumor suppressor genes appear to play a role in the governance o f proliferation by regulation of transcription it is possible that modification of the expression of dominant and tumor suppressor oncogenes may influence certain characteristics of cells that contribute to the malignant phenotype.

Despite increasing knowledge of the mechanisms involved in oncogene-mediated transformation,

little progress has occurred in developing therapeutic strategies that specifically target oncogenes and their products. Initially research in this area was focused on dominant oncogenes, as these were the first to be characterized. DNA-mediated gene transfer studies showed acquisition of the malignant phenotype by normal cells following the transfer of DNA from malignant human tumors. Activated oncogenes of the *ras* family were identified by this technique with transfection of human DNA into mouse NIH 3T3 cells. More recently a class of tumor suppressor genes have been identified. Mutation or deletion of both copies of a tumor suppressor gene is required to eliminate its function and cause the cell acquire characteristics of the malignant phenotype.

Tumor Suppressor Gene Mutations in Lung Cancer

The *p53* gene is the most frequently mutated gene yet identified in human cancers. It is mutated in over 50% of human NSCLC[3]. The *p53* gene encodes a 375-amino-acid phosphoprotein that can form complexes with viral proteins such as large-T antigen and E1B[4]. Missense mutations are common for the *p53* gene and are essential for the transforming ability of the oncogene. The wildtype *p53* gene may directly suppress uncontrolled cell growth or indirectly activate genes that suppress this growth. Thus, absence or inactivation or wildtype *p53* may contribute to transformation. However, some studies indicate that the presence of mutant *p53* may be necessary for full expression of the transforming potential of the gene. Mutations of *p53* are common in a wide spectrum of tumors[5-8]; they occur in both NSCLC and SCLC cell lines and fresh tumors[8,9].

An option for specific targeting of tumor suppressor genes is replacement of a deleted or mutated tumor suppressor gene. Progress in the understanding of the critical genes involved in tumor development and in technology for altering gene expression logically led to our studies of techniques for achieving these options.

The working hypothesis that we developed is that reversal of a single altered genetic event in the cancer cell can potentially reverse critical features of the malignant phenotype of that cell. This finding has important therapeutic implications. Cancer cells have multiple genetic alterations. Therapy directed toward oncogenes will be practical only if therapeutic effects occur with targeting of one or two genes. It is unlikely that any therapy targeting oncogenes or their products will be absolutely specific for cancer cells. If other genes can compensate for loss of normal function by a specific oncogene mediated by an antisense construct, the harmful effects of the therapy will be reduced. Studies from our laboratory indicate that reversal of a single genetic alteration has profound effects on the growth and tumorigenicity of lung cancer cells[10,11]. Additional support for this concept comes from a recent study by Soriano and coworkers[12] in which transgenic mice were created that lacked a functional *c-src* proto-oncogene. The resulting developmental defect in the mice was osteopetrosis. The ubiquity of *c-src* its high degree of conservation among species, and its role in mitosis suggest that inactivation would be lethal, but this was not the case; viable mice were recovered. A possible explanation is that other closely related nonreceptor tyrosine kinases such as yes and *fyn* can compensate for loss of *c-src*. Introduction of a single copy of a wildtype tumor suppressor gene into normal cells would be unlikely to have adverse effects if it occurred during therapy directed at replacing inactivated tumor suppressor genes in cancer cells.

Data on transfection of an antisense K-*ras* expression vector indicated that inhibition of expression of a single oncogene reduced the growth rate of cancer cells and tumorigenicity in nu/nu mice. However, transfected cells retained viability, as did cells with no endogenous K-ras mutation that were also transfected with the construct. The wt*p53* appears dominant over the mutant gene and will select against proliferation when transfected into cells with the mutant gene[11,13]. Normal expression of the transfected wt*p53* does not affect the growth of cells with endogenous wt*p53.* Thus, such constructs might be taken up by normal cells without adverse effects. This protocol will study regional delivery of wt*p53* to lung cancer cells in patients with unresectable obstructing endobronchial cancers, unresectable local cancers, and malignant pleural effusions not treatable by conventional therapies. The efficiency of delivery and gene expression will be evaluated both in lung cancer cells and in normal cells in vivo. This is of importance for the design of constructs that may be useful therapeutically. The effects of these constructs on clinical progression of the cancer will be studied.

These approaches may lead to cancer therapy based on direct alteration of gene expression in cancer cells. Current therapy relies on attempts to kill or remove the last cancer cell. However, tumor cell dormancy is an established phenomenon making effective killing highly unlikely. Although inhibition of expression of some oncogenes may be lethal to the cancer cell, in some cases cell replication will slow or cease, thus rendering these cancers clinically dormant. Even if absolute specificity is not achieved, single oncogenes or tumor suppressor genes may still be important targets, because it is likely that adverse effects to normal cells will be minimal.

Programmed cell death, also known as apoptosis, shows a characteristic pattern of DNA fragmentation resulting from cleavage of nuclear DNA and is considered to be a selective process of physiologic cell deletion. It has recently been reported that the wt-*p53* gene is involved in mediating programmed cell death of some types of tumor cells including human lung cancer cells[14-16]. The loss of *p53* function may contribute to resistance to a variety of chemotherapeutic agents[17]. Our studies showed that H358a cells, which have a homozygous *p53* deletion, were resistant to cisplatin *in vitro*. Recombinant adenovirus-mediated wt-*p53* gene transfer into monolayer cultures or multicellular tumor spheroids of H358a resulted in a marked increase in cellular sensitivity to cisplatin. The dying *p53*-transduced cells showed apoptosis with specific DNA fragmentation. Direct injection of *p53* adenovirus into H358a tumors subcutaneously implanted in *nu/nu* mice, followed by intraperitoneal administration of cisplatin, induces massive apoptotic destruction of established tumors. These results support the use of this strategy in a clinical trial.