

1.0 INTRODUCTION

1.1 Background

Despite aggressive campaigns to curb tobacco smoking and progress in the use of novel agents and combined modality treatment programs, relatively little objective progress has been made in reducing the incidence or the mortality of lung cancer over the last ten years. It is clear that new modalities are needed.

Recent advances in the cellular and molecular biology of lung cancer have identified many genetic alterations that represent potential new molecular targets for cancer therapy (1). Cells are chronically faced with decisions to divide, differentiate, or undergo programmed cell death (apoptosis). Two major classes of genes are known to affect this process: dominant oncogenes and recessive oncogenes (tumor suppressor genes). Mutations have been identified in many of these genes in lung cancer. Examples of dominant oncogenes are Ras which is activated by a point mutation or the MYC family, which is frequently found to be overexpressed due to gene amplification or regulatory alterations. Tumor suppressor genes frequently found to be mutated in lung cancer include p53, usually altered by a point mutation combined with loss of the associated wild type allele, or RB which is usually inactivated by deletions.

The p53 gene encodes a nuclear phosphoprotein which controls cell proliferation and suppresses neoplastic transformation. The wild type p53 protein delays S-phase entry in the case of DNA damage, allowing for DNA repair and preventing the propagation of mutations and chromosomal rearrangements to the next cell generations (2, 3). Normal cells tolerate this cycle arrest, but cancer cells that are driven by activating mutations in dominant oncogenes undergo apoptosis and die. Thus in order for cancer cells to survive, they often mutate p53 and are thus resistant to this sort of apoptotic cell death (4). One copy of the chromosomal region 17p13 which contains p53 is frequently deleted in both SCLC and NSCLC, and mutational inactivation of the remaining allele occurs in more than 90 percent of SCLC and 50 percent of NSCLC (5-8). Furthermore reintroducing a wild type p53 gene into lung cancer cells, including BAC, dramatically inhibits tumor cell growth and promotes tumor cell death despite the presence of mutations in multiple other genes (6). The object of this proposal is to take advantage of this property of p53 and the biology of bronchioloalveolar carcinoma (BAC) to test the feasibility of the therapeutic re-introduction of p53 via a recombinant adenovirus.

1.2 Ad- p53

Ad-p53 is a non-replicating adenoviral vector which encodes a wildtype p53 gene driven by the CMV promoter. The Ad-p53 backbone is an E1-partial E3-deleted human adenovirus type 5 serotype. E1 and E3 gene products modulate viral replication and host immune responses. Adenoviruses are double stranded DNA viruses with a known tropism for aerodigestive epithelium and are linked to only transient, minor respiratory disease in humans (9). These viruses enter cells via receptor mediated endocytosis. Although they migrate to the cell nuclei, where they express their genes, they remain extrachromosomal and do not integrate into the host genome. The transient nature of gene expression and the lack of a significant potential for insertional mutagenesis after adenovirus gene delivery allow selective molecular intervention with a low risk of stable integration of the recombinant vector into non malignant cells.

Transduction of many different therapeutic genes using recombinant adenoviruses has led to significant anti-tumor effect in several animal models. Preclinical *in vitro* and *in vivo* murine studies in head and neck cancer and non small lung cancer demonstrated a significant anti-tumor effect of Ad-p53. Initial experiments in which the H358 (a p53-null BAC cell line), H322 (p53-mutant), and H460 (p53-wild type) human non small cell lung cancer cell lines were treated with Ad-p53 resulted in significant inhibition of cell growth in the H358 and H322 lines (10). Over 100 lung and head and neck cancer patients have now been treated with Ad-p53 by direct injection, without significant toxicity and with objective evidence of responses (11).

1.3 Bronchiolo-alveolar Carcinoma

This is a distinct subtype of adenocarcinoma of the lung that is increasing in incidence (12, 13). It particularly affects younger people and non-smokers, causing the loss of more years of productive life than the other types of lung cancer more associated with heavy tobacco abuse, such as SCLC and squamous cell NSCLC. It is defined as a malignant neoplasm of the lung that 1) occurs in the absence of another primary adenocarcinoma; 2) has no central bronchogenic source; 3) is peripheral in location; 4) has an intact pulmonary interstitium; and 5) exhibits malignant cells growing along alveolar septae. It appears to arise from type 2 pneumocytes, grows along alveolar septa by lepidic ("scale-like") growth, and shows little if any desmoplastic or glandular change. BAC usually presents in three forms, a solitary peripheral nodule, multifocal disease, and a rapidly progressive pneumonic form, which appears to spread from lobe to lobe ultimately encompassing both lungs with little early evidence of distant metastases. The radiographic appearance of BAC is varied, with a single peripheral nodule or a diffuse infiltrate being the most common radiographic finding at presentation (14).

Patients with stage 1 and 2 BAC are treated like other NSCLCs, but local (intrapulmonary) failures are common due to the superficial spread typical of this disease. Several reports suggest that Stage 3 and 4 BAC should be treated in a more supportive fashion, as these tumors are felt to be chemoresistant and radioresistant (13, 15, 16).

1.4 Rationale

P53 overexpression is correlated with the presence of a mutant protein in non-small cell lung cancer (17). Thirty six percent of Bronchio-alveolar lung cancer show abnormal p53 accumulation (18). There is evidence (both clinical and laboratory) that abnormal accumulation is not necessary for a response to the introduction of wild-type p53 (wtp53). Direct transfer of the tumor suppressor gene via a recombinant adenovirus (Ad-p53) to induce apoptosis and cell death is thus a potentially attractive anti cancer therapeutic approach for this tumor type. We have shown that reintroduction of wild-type p53 into a BAC cell line (H358 which is p53 null and does not overexpress the protein) by stable transfection results in massive apoptosis and growth inhibition (6). We and others have obtained similar results in this cell line by p53 delivery via recombinant adenovirus (10, 19).

The diffuse multilobar involvement by thin sheets of tumor cells inside the airways makes BAC frequently impossible to resect completely, and local progression is frequently the life-limiting process in this disease. It is also very resistant to chemotherapy and incurable if not completely resectable. This biology, however, makes BAC particularly amenable to gene therapeutic approaches whose vectors typically do not penetrate or diffuse into large solid tumor masses well. In particular, endobronchial delivery of recombinant wtp53 adenovirus via bronchoalveolar lavage may be a highly efficient means of gene transfer and a highly effective local therapeutic in this disease. Effective local palliation could well translate into significant clinical benefit. While BAC is only a subset of all NSCLC, the large numbers of NSCLC deaths each year and the complete lack of good therapeutic options for unresectable or recurrent BAC make the potential clinical impact of this therapy significant.

This trial is a "pilot" phase 1 trial of an Adenovirus p-53 gene delivered via bronchoalveolar lavage in patients with locally advanced Bronchiolo-alveolar lung carcinoma. It is considered a pilot study as only a single involved lobe would be treated to assess the feasibility of this approach without exposing a larger portion of the lung to the potentially toxic effects of the recombinant adenovirus. It is anticipated that another phase I study evaluating the safety of treating all involved lobes would be called for if the current study proves the approach to be feasible. In this study, escalating doses are to be given in 3 patient cohorts until the practical dose limit or maximally tolerated dose is achieved. Patients would be monitored for toxicities, response to treatment and biopsies will be performed to evaluate the level of induced apoptosis at the end of the treatments.