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Gene therapy targeting p53

Review Article

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Summary

The product of the p53 gene plays a critical role in the regulation of cell growth. Mutations of this gene are associated with transformation to a malignant phenotype. Correction of the gene defect through transfer of a wildtype p53 gene into malignant cells, or targeting malignant cells with oncolytic viruses (ONYX-015) genetically engineered to proliferate in cells containing mutant genes has been identified as a therapeutic approach by preclinical assessment. Initial clinical trials have confirmed functional activity and expression of the transgene product in Adp53-injected malignant tissue and tumor specific viral proliferation have been observed in patients receiving intratumoral injection of ONYX-015.

I. Introduction

The most common genetic abnormality identified in human malignancy with an occurrence of approximately 60% involves the p53 gene, which is a tumor suppressor gene (Baker, 1990) located on chromosome 18. Disruption of p53 protein production or inhibition of its function is associated with abnormal cellular proliferation and differentiation.

Specific functions of the p53 gene product include upregulation of p21, which is a protein that inhibits cyclin-dependent kinase (CDK), and is necessary for the G1 to S-phase transition. P53 protein also upregulates Bax (a positive regulator of apoptosis), MDM-2 (a negative regulator of p53 function), thrombospondin-1 (inhibitor of angiogenesis), GADD45 (role in DNA repair), and IGF-BP3 (growth regulator) (Harper, 1993; Miyashita, 1995; Dameron, 1994). Extensive analysis of tumors showing evidence of p53 gene dysfunction indicate that abnormal function correlates with poor prognosis in patients with malignancy (Drach, 1998; Horio, 1993; Thorlacius, 1993; Preudhomme, 1997; Lai, 1995).

The purpose of this chapter is to describe data which identifies novel therapeutic approaches targeting correction of the p53 gene via transfection with a wildtype p53 gene using a replication defective adenoviral vector carrier and approaches utilizing oncolytic virus ONYX-015.

II. p53 mutation

Eighty percent of p53 mutations involving solid tumors are point mutations that result in a single amino acid substitution. At first glance, this may not appear to be a significant abnormality, given that the alteration involves less than 1% of the entire molecule. However, many of the aminoacid substitutions result in a charge change (i.e. positive to negative or vice versa), which dramatically alters the three-dimensional structure of the p53 protein. Once altered, receptor-binding affinity is disturbed. As a result, excess p53 protein is produced with accumulation within the nucleus. Normal cells have undetectable levels of p53 protein. Thus, elevated p53 protein expression often indicates the occurrence of a mutated p53 gene although not always (Barnes, 1992; Lehman, 1991).

Other molecules may also be produced by malignant cells which inhibit normal p53 function via binding to the p53 protein, enhancing degradation, or disruption of binding sites. One example of inactivation of p53, which may occur by interaction with another cellular protein, involves the murine double-minute-2 (MDM-2) protein which acts as a false binding site (Teoh, 1997). Another example involving induced degradation is seen in cervical cancer of the p53 protein (Caron de Fromentel, 1992; Vogelstein, 1992; Scheffner, 1992). The majority of

cervical cancers harbor the human papilloma virus (HPV), which enhance degradation of the p53 protein (Howley, 1991). Cervical cancer cells, which are HPV positive and contain the p53 mutation (less than 20%) are particularly aggressive, and such patients have an even more dismal prognosis.

Poor survival prognosis has been observed in patients with cancer of the lung, colon, liver, breast, stomach, cervix, non-Hodgkin's lymphoma, and multiple myeloma who have elevated p53 protein expression or a p53 DNA mutation detected from tumor samples prior to treatment (Drach, 1998; Horio, 1993; Thorlacius, 1993; Preudhomme, 1997). The development of p53 gene mutations may also involve environmental carcinogenic factors (Vogelstein, 1992).

Malignant cells containing p53 mutations have an increased resistance to death in response to chemotherapeutic agents or ionizing radiation (Lee, 1993), and an increase in metastatic spread (Dutta, 1993). Twenty percent of patients with a p53 mutation have also been found to express antibodies to the mutant p53 protein, although it is unclear whether such patients have an altered prognosis (Crawford, 1982; Caron, 1987; Davidoff, 1992; Winter, 1992; Schlichtolz, 1992).

In conclusion, an understanding of the p53 gene structure and protein function is important in developing therapeutic approaches, and may assist in the understanding of potential activity and toxicity to therapeutic approaches attempting to correct dysfunction of the p53 gene or protein.

III. Adp53 vector

Preclinical studies have reported the introduction of the wildtype p53 gene into human tumor cells with a mutant p53 genotype using a variety of delivery methods including the retroviral vectors, lipid complexes, and adenoviral vectors (Harris, 1996; Wills, 1994; Lesoon-Wood, 1995; Xu, 1997; Blagosklonny, 1996; Zhang, 1995; Nielsen, 1997; Nguyen, 1996). Results demonstrate that the expression of the transgene product provides a normal functioning wildtype p53 protein to the malignant cell, which has been shown to induce tumor regression and improve survival in animal models. Preclinical results also reveal enhanced activity when combined with chemotherapy (Nguyen, 1996; Fujuwara, 1994).

Vectors utilized for adenoviral introduction of the wildtype p53 gene involve wildtype adenovirus containing deletions of the E1 and E3 replication components (Zhang, 1993). Adenoviruses are single-stranded DNA viruses with genomes of approximately 35kB (Takahashi, 1989), which are easily propagated in human cells, and have been associated with minimal pathogenicity. The deletion of the E1 and E3 regions provides empty space (~7KB) where the wildtype p53 gene sequence is inserted (Zhang, 1994). Transfection of several NSCLC cell lines and head and neck cancer cell lines reveal high expression of wildtype p53 protein (the transgene product). Optimal expression is observed at a multiplicity of infection (MOI) of 30-50 plaque-forming units (PFU) per cell (Zhang, 1995; Zhang, 1994). Maximal expression was observed 3 days after transfection and rapidly decreased over the next 5 days. Detection of the transgene product was still observed 15 days following transduction. Similar results were shown *in vitro* and *in vivo*. Transgene expression and normal function has been shown in cell lines of breast cancer, ovarian cancer, colorectal cancer, prostate cancer, the central nervous system, and bladder cancer (Harris, 1996; Wills, 1994; Lesoon-Wood, 1995; Blagosklonny, 1996; Bartek, 1990).

IV. Safety of the Advp53 vector

The Adp53 vector is constructed from a serotype 5 adenovirus. A great deal of data has been accumulated suggesting the safety of this virus (Brandt, 1969). Eighty percent of adults have existing antibodies to adenovirus serotype 5 (Nicholson, 1993), but less 15% of exposed patients become clinically symptomatic. The most common symptoms of an adenoviral serotype 5 infection are flu-like in nature and include cough, gastroenteritis, conjunctivitis, cystitis, and rarely pneumonia. However, these symptoms are rarely seen even in immune compromised patients (Hierholzer, 1992). Oral adenoviral vaccines were given to thousands of military recruits in the 1960s without adverse effects or increase in cancer (Takafuji, 1979). Live adenovirus inocula was also given intratumorally and intra-arterially to patients with cervical carcinoma at the National Cancer Institute in the 1950s (Smith, 1956). No significant toxicities, other than transient fever and malaise, were observed even in subsets of patients treated with steroids and in those in which neutralizing adenovirus antibodies were not present.

Work was conducted in animal models exploring the most significant serious clinical toxicity to live adenovirus (pneumonia). A unique strain of cotton rats (*gigmodon hispidus*) has been shown to consistently develop pulmonary infection in response to inoculation with adenovirus serotype 5 (Pacini, 1984). Pathogenicity was related to the dose of the viral inoculum. Additional safety testing has been conducted in mice and cotton rats in which high doses of adenovirus were injected locally and systemically. Animals developed minor histopathologic changes in several organs, but no pulmonary toxicity was observed (Pacini, 1984; Ginsberg, 1991). However, inflammatory infiltrates related to p53 have been observed in the lungs of animals given high doses of Adp53 directly

to the bronchial airway (Zhang, 1995; Ghosh-Choudjury, 1985; Englehardt, 1993; Rich, 1993; Ginsberg, 1990). The resulting inflammatory responses were characterized by interstitial infiltration of neutrophils, and monocytes within 1-2 days after exposure (Ginsberg, 1990; Prince, 1993). This early inflammatory process was felt to be mediated by local elaboration of various cytokines such as tumor necrosis factor, IL-1 and IL-6 (Prince, 1993). An additional inflammatory response also occurs within 3-7 days. At this time, peribronchial infiltration of lymphocytes is observed. Direct exposure of the lung with low concentrations of the adenovirus vector does not appear to be associated with pulmonary toxicity (Simon, 1993; Yei, 1994).

The possibility of adenoviral replication competency developing after vector injection also appears to be negligible, given the construction design of the vector (Zhang, 1995). However, complete inhibition of DNA replication solely from E1 deletion has not been 100% successful (Englehardt, 1993; Rich, 1993). This necessitates intense monitoring of the Adp53 clinical material for replication competency. Repeat sequencing of the product reveals that the wildtype p53 genotype does not undergo mutation changes during manufacturing. Expression of the transgene product also does not appear to be toxic. Studies performed *in vitro* looking at Adp53 transfection of non-malignant fibroblasts and human bronchial epithelial cells in comparison to malignant head and neck tumor cells indicate no change in p53 expression in non-malignant cells. These data suggest that normal cellular p53 expression is not altered by transfection with Adp53. The growth rate and morphology of the non-malignant fibroblasts and bronchial epithelial cells was not altered following transfection with Adp53 (Zhang, 1995). Theoretical concerns regarding oncogenicity of adenoviruses are also unlikely. The life cycle of an adenovirus does not require integration into the host genome, thus, foreign genes delivered by adenoviral vectors are expressed episomally and have low genotoxicity (Zhang, 1995). DNA from thousands of human tumors have been analyzed for the presence of adenovirus DNA and no integrated viral DNA has been isolated from any human tumor (Green, 1979). Long- and short-term safety of adenoviral injection has been shown in several animal models (Lesoon-Wood, 1995; Zhang, 1995; Nielsen, 1997; Englehardt, 1993; Simon, 1993; Yei, 1994; Xu, 1998; Gomez-Foix, 1992; Le Gal La Salle, 1993).

In humans, (-GAL vector injection was administered to patients with endobronchial lung cancer. Evidence of replication competent adenovirus was studied in caretaker staff samples. Specifically, 73 staff provided 78 blood samples, 272 urine samples, and 193 samples to study antibody formation or the presence of replication competent adenovirus. No replication competent adenovirus was detected, and elevated antibody formation did not inhibit gene expression with repeat injections (Tursz, 1996).

Adenoviral vectors with E1 and E3 deletion containing the *E-coli* cytosine deaminase gene have also been administered to normal individuals to study immune response (Harvey, 1998). Six volunteers received intradermal injections of 10^6, 10^7, or 10^8 PFU (2 patients per group). Five of the 6 volunteers showed a rapid increase in anti-Ad5 neutralizing antibody titers above baseline. The peak antibody response occurred 2 weeks after vector injection. Erythema occurred at the site of injection with maximum induration of approximately 7mm by Day 3, and complete disappearance of induration by Day 10. Skin biopsies of the erythema revealed T-cell, B-cell and a macrophage infiltrate. Vector DNA was detected in biopsies of patients who received the 10^8 dose on Day 3, but no evidence of vector DNA was detected on Day 28. No systemic toxicity was observed in any of the normal volunteers (Harvey, 1998).

Finally, if serious viral infection does develop, therapeutic approaches are available. Wildtype adenovirus dissemination has been seen in organ transplant recipients, however, in most cases, the viremia has been eliminated with the use of intravenous Ribavirine (Liles, 1979), although occasionally Ribavirine has not been successful (Mirza, 1994).

V. Preclinical studies with Adp53

Early preclinical studies with Adp53 vector in lung cancer initially utilized the H358 cell line. In one study, 50 mice received injections of 2 x 10^6 H358 cells, which had been previously transfected with Adp53 *in vitro*. Eighty percent of control animals developed tumors within 2-3 weeks; however, none of the p53 transfected cells evolved into malignant lesions 6 weeks after injection. Other work with the Adp53 vector involved the use of H326 cells which were derived from a highly aggressive squamous cell NSCLC lesion. This cell line contains a p53 point mutation (Zhang, 1994; Georges, 1993). Inoculation of 2 x 10^6 H326 cells into the trachea of mice followed by inoculation with Adp53 vector, control vector, or control vehicle, reveals that only 2 of the 8 Adp53-treated mice developed tumors 6 weeks after treatment with a mean tumor volume of $8mm^3$, whereas 7 of 10 of the treated mice, and 8 of 10 of the control vector treated mice developed tumors where the mean volume exceeded $30mm^3$ within 6 weeks after inoculation. Subsequent approaches exploring the use of Adp53 in combination with Cisplatin revealed enhanced activity.

Animal models have been designed to test whether transfection of head and neck cancer cells with Adp53 may alter response to radiation, chemotherapy or have direct effects. In one model, Adp53 was transfected into a radioresistent human cell line GSQ-3 (squamous cell carcinoma of head and neck). Wildtype p53 protein was shown to be expressed in high levels and have functional activity in the transfected cells (Xu, 1998). A dose of 10^8 PFU was shown to be sufficient to induce tumor regression without evidence of systemic toxicity (Liu, 1994; Yamamoto, 1998). Animal studies in other tumor xenograph models (ovarian, breast, prostate) have also shown activity following Adp53 injection (Sheikh, 1995; Eastham, 1995; Mujoo, 1996).

VI. ONYX-015 preclinical studies

P53 protein mediates cell cycle arrest via apoptosis if foreign DNA synthesis is occurring within a cell from viral replication (Debbas, 1993; Grand, 1994; Lowe, 1997). DNA tumor viruses such as certain adenoviruses, SV40 and human papilloma virus incode proteins which inactivate p53, thereby allowing their own replication (Debbas, 1993; Lechner, 1992; Gannon, 1987). Specifically, a 55dDa protein from the E1B region of adenovirus serotype 5 binds and inactivates p53 (Barker, 1987). Inability to block p53 function with deletion of the E1B region would enable the p53 protein to maintain its function thereby inhibiting viral replication. The ONYX-015 virus is a DNA adenovirus which was constructed with an E1B deleted region so that it no longer produces the 55kDa protein. In this manner, the virus would not be expected to proliferate in normal cells, but it would be expected to have extensive proliferative capacity in tumor cells which are either p53 mutant or have disrupted p53 function (Bischoff, 1996).

Initial studies testing the ONYX-015 virus involved incubation of virus with RKO human colon cancer cell lines which have normal p53 function and a subcloned line of RKO, which has a mutant p53 gene. The ONYX-015 virus replicated as efficiently as the wildtype adenovirus in the subclone lacking functional p53 protein, however, the cytopathic effects of ONYX-015 are reduced by 2 logs in the parent tumor line harboring normal p53 function (Bischoff, 1996). Cell lines resistant to ONYX-015 have also been made sensitive through transfection and expression of the E1B 55dDa gene (Bischoff, 1996). Cytopathic effects of ONYX-015 have also been shown in other malignant cells, which have abnormal p53 function, involving the breast, cervix, colon, central nervous system, liver, ovary, pancreas and head and neck region (Heise, 1997). Potential infectivity of ONYX-015 was tested against wildtype adenovirus by infecting non-malignant (normal p53 functioning) human microvascular endothelial cells, airway epithelial cells, and mammary epithelial cells. Wildtype adenovirus showed cytopathic effects at a MOI as low as 0.01 virus particles within 8-10 days, whereas cytopathic effects of ONYX-015 virus were not observed until MOIs of >100 virus particles were achieved. Thus, safety and antitumor activity appear to be related to the dose of virus infused. Several studies involving oncolytic viruses other than ONYX-015 have been performed *in vitro* and *in vivo* in human patients without significant toxicity (Kenney, 1994; Russell, 1994; Asada, 1974; Smith, 1956). Unfortunately, the difficulties in characterizing viral load led to inconsistent results and there was no suggestion of efficacy. Preclinical studies with the ONYX-015 virus *in vivo* were performed to confirm direct tumor cell lysis through local injection and systemic infusion, and to determine whether or not tumor lysis is observed in response to viral replication (Yang, 1994).

In animal human xenographt studies, intratumor injection of ONYX-015 virus has been tested in cervical cancer (C33 cervical carcinoma cells) and head and neck cancer (HLaC laryngeal carcinoma cells), both of which have a p53 functional deficiency (Heise, 1997). Significant tumor growth inhibition was observed compared to controls. Mice achieving a complete response remained disease-free for 4-6 months before sacrifice. U87 glioblastoma tumors, which do not have a p53 mutation, were not affected by injection with the ONYX-015 virus. Evidence of viral proliferation based on histochemical staining for adenovirus exon protein was confirmed in the sensitive tumors, but not in the U-87 tumors. Additional studies comparing vehicle versus chemotherapy (5-FU or Cisplatin), ONYX-015 alone, or ONYX-015 plus chemotherapy, were carried out (Heise, 1997). Median survival in mice receiving ONYX-015 plus 5-FU was further improved compared to control or ONYX-015 alone. Similar results were seen in combination with Cisplatin.

Systemic injections of ONYX-015 at a dose of 10^8 PFU were also injected for 10 days into the tail vein of nude mice implanted with C33-a or HCT116 human xenographt tumors. Tumor growth was significantly inhibited in the C33-a tumors with ONYX-015 treatment by 55% compared with growth in mice injected with vehicle solution ($p=0.004$). Comparison of intravenous ONYX-015 virus (IV for 5 days) plus 5-FU (IP for 5 days) in mice showed that 6 of 7 mice had complete tumor regression following the combination, whereas only 2 of 7 mice achieved complete tumor regression following 5-FU treatment alone. The median tumor volume on day 40 was 93(L in the mice receiving ONYX-015 plus 5-FU. However, mice receiving 5-FU alone had a median tumor volume of 461(L, compared to ONYX-015 alone with a tumor volume of 671(L, and saline alone with a tumor volume of 748(L. No significant toxicity was observed.

Results suggest that both intratumor and intra-venous infusion of ONYX-015 when combined with chemotherapy was safe and effective in inducing tumor regression and prolonging survival.

VII. Human studies with Adp53

The first trial published to explore gene transduction of the p53 gene via intratumor injection in humans utilized a retroviral vector. In this trial, 9 patients (median age 68) with NSCLC were treated (Roth, 1996). Four received retrovector p53 gene via bronchoscopic injection, and 5 were treated via a percutaneous injection with CT guidance. Eight of the 9 patients treated had a point mutation, and 1 had a frame shift mutation of the p53 gene. Vector transduction was confirmed in 8 patients by PCR analysis, and 6 patients showed induction of apoptosis (TUNEL assay). Three patients showed evidence of tumor regression (all 3 of these patients received endobronchial injections). No toxic effects were attributed to the vector. Retroviral sequences were not detected in non-injected pulmonary tissue analyzed by PCR, and no evidence of replication competent retrovirus was detected. Unfortunately, low transduction efficiency associated with the retroviral vector was a major limiting factor.

Several studies with Adp53 were subsequently initiated. One Phase I trial investigating tolerability of Adp53 in NSCLC was recently completed. Fifty-two patients with advanced NSCLC who had previously failed conventional treatment were entered into trial (Swisher, 1998). Adp53 doses were escalated from 10^6 to 10^{11} PFU and injected monthly into a single primary or metastatic tumor by bronchoscopy (12 patients) or computed tomographic (CT) guidance (40 patients). Patients were treated by direct assignment with or without Cisplatin ($80mg/m^2$) given IV over 2 hours prior to Adp53 injection. Each patient received up to 6 courses of treatment and median follow-up was 9.9 months. Vector-specific deoxyribonucleic acid (DNA) was detected by PCR, and p53 transgene expression was determined by reverse transcriptase PCR and immunohistochemistry. Vector was present in plasma within 30 minutes of injection, and decreased in the next 60 minutes (Timmons, 1998). No replication competent adenovirus was detected in any body fluids tested. Antibody titers increased in patients receiving at least 2 doses and remained elevated for several months after completion of injections. In patients who received Cisplatin, the apoptotic index increase from 0.124 to 0.034 ($p=0.011$) when compared to baseline in samples harvested after the first course of Adp53 injection. The TUNEL assay showed an increase in the number of apoptotic cells in 11 of the 15 evaluable patients, a decrease in 2 patients, and no change in 2 patients (Nemunaitis, 1998). Anti-adenoviral type 5 IgG antibody response ((2-fold increase) was shown in 19 of 20 evaluable patients following course 1. Cytopathic effect assays (CPE) also revealed the presence of Adp53 vector in plasma within 30 minutes of intratumor injection in all 16 patients tested. Tumor biopsies collected 3 days post-treatment demonstrated p53 transgene expression by RT-PCR in 10 of 17 (58%) patients receiving vector dose levels (3×10^{10} PFU, and only 8 of 27 (30%) patients who received the lower dose level. Toxicity attributed specifically to the vector was limited to transient fever and nausea. Cisplatin-related toxicity was not observed in any greater frequency than it would be expected when Adp53 gene vector was not combined with Cisplatin. Four patients fulfilled a definition of partial response (PR) (8%), 33 patients (64%) experienced stable disease for a transient period of time (minimum 1 month), 11 patients (20%) had progressive disease, and 4 patients (8%) were not evaluable for response (Nemunaitis, 1998; Swisher, in preparation; Nemunaitis, 1998). Overall, median survival was 149 days. The difference in survival between the patients who received Cisplatin or Adp53 + Cisplatin did not achieve statistical significance. Six of 12 patients with endobronchial-injected lesions had sufficient tumor regression to open obstructed airways.

The conclusion of this trial is that Adp53 endobronchial or CT-guided injections at a dose of 10^{11} PFU in patients with NSCLC are safe and well tolerated. The maximum tolerable dose of the vector has not been reached. This therapy can be administered monthly, alone or with Cisplatin with no increase in Cisplatin-related toxicity. Immune response to the Adp53 vector does not limit continued injections, and there is evidence of objective activity and clinical benefit.

Additional work exploring the same Adp53 vector was done in head and neck cancer (Clayman, in press). In this trial, patients with recurrent or refractory squamous cell carcinoma of the head and neck region with a performance status of 0-2 were eligible for trial. Results of this trial concluded that repeated intratumoral injections of up 10^{11} PFU was safe and well tolerated. Transgene expression occurred despite evidence of adenovirus antibody response. Peri- and post-operative Adp53 injection had no adverse effect on surgical morbidity and/or wound healing. Evidence of activity based on tumor regression following injection of Adp53 was observed (1 CR, 2 PRs) (Clayman, in press; Wilson, 1998).

Others have explored the use of Adp53 vectors in head and neck cancer and other tumor types such as colon cancer and ovarian cancer. In another Phase I trial using a different Adp53 vector (SCH-58500), 16 patients with head and neck cancer received escalated doses ranging from 7.5×10^9 PFU to 7.5×10^{12} PFU (charts of patients received single or multiple intratumor injections). The median age of patients entered into this trial was 60.5 years. Ten of 16

patients had elevated serum IgG to p53 protein following injection, and p53 transgene expression was confirmed in a subset of patients. Toxicity attributed to the vector was limited to Grade 1/2 fever (11 patients) and injection pain (6 patients). One patient achieved a PR which correlated with the induction of apoptosis and transgene expression (Agarvala, 1998).

Another trial utilizing SCH-58500 was performed in patients with colorectal cancer with liver metastasis. In this trial, 16 patients received hepatic arterial infusion of Adp53 vector. A single dose was administered prior to laparotomy. Patients received escalating dose levels ranging from 7.5×10^9 PFU to 2.5×10^{12} PFU. Adverse events included fever in 15 of 16 patients, and headache in 3 of 16 patients. Transgene expression was confirmed in normal liver and tumor. No responses specifically attributed to the Adp53 therapy alone were observed, however, 12 patients subsequently received FUDR and 11 achieved a 50% reduction in disease, suggesting the potential for sequential therapeutic approaches to be considered in trial designs utilizing Adp53 (Agarvala, 1998).

SCH58500 was also given to 18 patients with advanced NSCLC. Patients received escalating doses ranging from 10^7 to 10^{10} PFU. No serious adverse events were observed. Only one patient required hospitalization for prolonged persistent flu-like symptoms. Transgene expression was confirmed in patients who received higher dose levels. In 4 of the 6 patients who showed evidence of wildtype p53 expression, progression of transient local disease was stabilized following injection with Adp53 (Schuler, 1998).

VIII. Human trials with ONYX-015 virus

Several trials with ONYX-015 virus in treatment of head and neck cancer were recently reported. These trials suggested that ONYX-015 is well tolerated except for transient low-grade fever and that antitumor activity is observed.

Preliminary Phase I studies indicated that intratumor ONYX-015 injections are well tolerated and viral proliferation has been confirmed in malignant cells by electron microscopy. The duration of tumor response appeared to be greater in patients receiving multiple injections compared to a single injection per cycle (every 21 days). The optimal dose suggested for Phase II investigation was 1×10^{10} PFU given for 5 days every 21 days (unpublished results).

Phase II studies performed in refractory head and neck patients utilized a dose of 1×10^{10} PFU of ONYX-015 daily x 5 days every 3 weeks via intratumor injection (Kirn, 1998). Injections were given throughout the perimeter of the tumor, and the volume of the injected medium was normalized to 30% of the target tumor volume. Neutralizing antibodies were found in 10 of 20 Phase II treated patients prior to injection, and the p53 gene sequence was mutated in 7 of 13 patients. There was also a suggestion of increased response in patients with tumor sized of (5cm in diameter. Thirty-seven percent of patients with tumor (5cm achieved a complete response or partial response compared to 0% of patients with tumor >5cm (n=30). The most frequent side effect observed in the Phase II trial was pain at the injection site and it occurred in 32% of patients. Transient fever and chills occurred in 28%, nausea in 8%, and confusion in 4% of patients. Despite these preliminary results and with the trial not yet completed, results are sufficient to determine that the ONYX-015 virus is well tolerated at a dose of 10^{10} PFU given to 5 consecutive day every 3 weeks. Subsequent studies exploring ONYX-015 virus (1×10^{10} PFU daily x 5 days every 3 weeks) combined with chemotherapy (Cisplatin 100mg/m^2, IV on day 1; and 5-FU 800-1,000mg/m^2 by continuous infusion per day on days 1-5 every 3 weeks) were thus initiated. Patients with recurrent head and neck cancer who had not previously been exposed to chemotherapy or radiotherapy in the recurrent tumor setting were entered into trial. At the time of the preliminary analysis (Kirn, 1998), 10 patients had been treated and 9 of 10 patients achieved a partial response or complete response. Despite being preliminary, the data is very encouraging particularly when compared to expected response rates, in which similar patients receiving chemotherapy without ONYX-015 virus would be expected to achieve a 30-40% partial or complete response rate, and would not be expected to have a median survival >9 months.

These preliminary results suggest that ONYX-015 replicates in recurrent refractory head and neck cancer, and that ONYX-015 is well tolerated following intratumor injection alone, or when combined with chemotherapy.

ONYX-015 is also being explored at escalating dose levels in patients with gastrointestinal tumors metastatic to liver (Bergsland, 1998). Patients with metastatic disease to the liver were administered intratumoral injections through CT guidance. The starting dose level was 1×10^8 PFU. Injections were given one time every 21 days. Patients not showing progressive disease were eligible for continued injections. A total of 16 patients and 29 injections had been administered at the time of this preliminary analysis, and the dose level of 1×10^8 PFU was reached without evidence of dose-limiting toxicity. Minor toxicities such as flu-like symptoms were observed in 11 patients, transient elevation and coagulation times were observed in 7 patients, lymphopenia in 5, and transient liver function enzyme elevations was observed in

4 patients. Response assessment after cycle 1 revealed 2 patients with minor responses, 9 patients with stable disease, and 4 patients with progressive disease. This is an ongoing trial in which patients are continuing to receive injections, and thus far it can be concluded that the treatment is well tolerated, although evidence of activity remains to be determined.

Others have also performed Phase I exploration of ONYX-015 in patients with unresectable carcinoma of the pancreas (Mulvihill, 1998). In another trial, escalating doses of ONYX-015 were administered to patients with to patients with unresectable pancreatic cancer. Sixteen patients received a total of 36 injections. At baseline, 5 of 13 tumors assessed contained mutant p53 gene sequences, and 9 of 10 patients had neutralizing antiadenoviral antibodies. All patients showed escalation of antiadenoviral antibodies following injection. One patient developed Grade 3 hyperbility rubimenia following the injection, otherwise no other Grade 3-4 toxicities were observed at dose levels up to 10^{10} PFU. Grade 1-2 flu-like symptoms were reported in all patients. Four patients had minor regressions following the initial cycle of treatment with a 35-45% decrease in disease, 7 patients had stable disease, and 3 patients had progressive disease. Two patients reported a decrease in pain following injection. Preliminary conclusions are that the intratumor injection of ONYX-015 was well tolerated. Continued injections are ongoing.

IX. Conclusions

Results of clinical trials performed are encouraging and shown good tolerability to a variety of Adp53 vectors and confirm that the transgene product expressed from the transfected vector is functional and associated antitumor activity in small numbers of patients. Unfortunately, therapy at this time is limited to direct intratumor injection. If immunologic difficulties leading to vector neutralization can be overcome, safety data suggest that systemic infusion of Adp53 vector may be well tolerated. Studies to limit immunoreactivity to the Adp53 vector through inhibition of the immune response or alteration of the vector or other gene transfer vehicles are ongoing. For instance, using a ligand lyposome complex, wildtype p53 gene was efficiently delivered both *in vitro* and *in vivo* in murine squamous cell head and neck cancer models. Injection of the ligand/lyposome complex with the wildtype p53 gene was shown to be taken up in both head and neck and prostate tumors. Transfection was higher in malignant tissue than surrounding normal tissue. Furthermore, enhanced activity was shown following treatment with radiotherapy after ligand/lyposome encapsulated wildtype p53 injection or IV infusion (Pirollo, 1998), without significant toxicity (Joshi, 1998).

Overall, preliminary results of Phase I studies indicate that the p53 gene transfer through intratumoral injection using replication vectors is well tolerated, associated with antitumor activity at dose levels equal to and above 1×10^9 PFU. Data also suggest that administration of multiple injections and combination with chemotherapy or radiotherapy may enhance the overall antitumor effect. Phase II trials to determine efficacy are ongoing.

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