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Cancer targeting using tumor suppressor genes

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TABLE OF CONTENTS

1. ABSTRACT

Conventional cancer treatments include cytotoxic chemotherapies and radiotherapy, which result in significant collateral toxicities. The goal for future cancer treatments is to leverage improved understanding of cancer biology mechanisms and thereby develop targeted drugs that display exquisite tumor selectivity and avoid iatrogenic damage. In this review, we discuss the potential of tumor suppressor genes for development of cancer-selective drugs using the tumor suppressor p53 as an archetype.

2. INTRODUCTION

Gene therapy as a discipline is maturing. This exciting field emerged from the confluence of the understanding of the role of genes in disease and the development of technologies for manipulating these genes. Current practioners of translational gene therapy must be versed in many areas, including virology, immunology, disease specific pathophysiology, pharmacology and classical drug development technologies. In its infancy, gene therapists borrowed from the older disciplines above, but more recently, the field has matured such that contemporary gene therapy research findings are providing new conceptual frameworks for mature fields. Furthermore, as gene therapy progresses we continue to develop new targeted methods for treating a growing number of intractable diseases.

3. SIMILARITIES BETWEEN DEVELOPMENT OF GENE THERAPIES AND MONOCLONAL ANTIBODY THERAPEUTICS

There are clear parallels between the challenges that gene therapy has faced and those encountered by monoclonal antibodies. It is important to recognize that it took more than 20 years from development of the early monoclonal antibody technologies and the recognition that this technology could be harnessed for human therapeutics to the widespread acceptance and actual marketing of antibody based therapies (1,2). In this long intervening period, significant hurdles were overcome, such as the recognition of HAMA (human anti-mouse antibody)

responses in altering antibody pharmacokinetics and toxicity caused by HAMA and development of vascular leak syndrome (VLS) (3). In time, with intensive and focused research efforts, these challenges were mastered and it became clear that HAMA was not the "death knell" for antibody technology. Clinical evaluation of monoclonal antibodies started in the 1980s, however, 89% of murine monoclonals that entered clinical testing between 1980 and 1987 ultimately failed (2). Developments such as antibody grafting, phage display and humanization technologies have allowed an early, potentially "toxic" technology to mature and revitalized the ailing antibody industry (4). Essential elements to this resurgence were the understanding of host responses to these large biologic macromolecules and the development of drugs that provide significant patient benefit; these concepts are exemplified by drugs such as Herceptin, Rituxan, Avastin and Erbitux, which now provide new options to patients who have failed conventional therapies. The approvals and substantial revenues generated by Herceptin, Avastin and Rituxan have invigorated the antibody industry, which has been through a series of peaks and troughs, not dissimilar to those seen in the gene therapy industry. It is interesting to note that in both industries, large pharmaceutical companies were early entrants and committed substantial resources, both on their own programs and also in partnerships with small biotechs, but many changed focus and exited the field when significant challenges were encountered. Consequently in the antibody arena, large pharma has re-entered the fray and either partnered with smaller companies or acquired them to commercialize their antibody products. As the monoclonal product technologies have matured, their sales revenues are striking – from 2003 to 2005, sales of monoclonal therapies were: $5b; $10b and $14 billion, and the current pipeline of monoclonal products in clinical trials exceeds 150 different product candidates (2).One complexity of gene therapy as a discipline is that it encompasses many areas of basic research. A broad array of gene delivery technologies is available and it is clear that practioners must match the disease target with optimal delivery vehicle in addition to selecting the appropriate therapeutic gene. The choices are expansive: vastly different technologies and tolerances for risk:benefit are involved in determining which vector to use for long term stable gene expression for treatment of hemophilia in replacement of Factor VIII or IX; immune reconstitution in SCID or ameliorating symptoms of rheumatoid arthritis. In contrast, transient therapeutic intervention (gene expression) required for killing cancer cells or inhibiting stroke takes advantage of different vector and delivery systems and can generally assume a greater risk profile. Even within the same disease area, different approaches and side effect profiles may be tolerated, such as in the prevention versus treatment of cancer or prevention versus treatment of HIV or HCV infection. Therefore, it is important to recognize that there is no universal delivery system, but rather the therapeutic entity and its delivery must be tailored to the disease target.

A key advantage of gene therapy compared to other therapeutic modalities is that the identification of the disease target is intimately linked with identification of the drug. In small molecule drug development, it is not uncommon to identify a disease drug target which requires screening of hundreds of thousands of small molecules to identify a "hit", which then leads to years of medicinal chemistry optimization to select a drug. In monoclonal antibody development, target identification and validation can occur at the genetic level, (such as recognition of Her2/neu over-expression in breast and ovarian cancers) and has no direct association with development of the therapeutic antibody. In contrast, in gene-based therapeutics there is a facile equivalence of "targets as drugs" and thus the discovery process is very rapid. The notion of the gene as a drug also identifies gene therapeutics as "targeted therapeutics". Recent biotech and pharma drug development efforts have focused on identification of targeted therapeutic drugs which exhibit selectivity between tumor and normal tissues and we are beginning to see early promise of this concept in drugs such as Gleevec, Sorafenib and Sutent. By virtue of their screening and identification methodologies, gene therapeutics may serve as the archetype of targeted agents.

A related advantage that has been observed in clinical studies with gene therapeutics is that they generally are very well tolerated and tend to have low toxicities. Use of viral based vectors in hundreds of patients with cancer has resulted in accumulation of a substantial dataset on the safety of these agents. The most common adverse events (AEs) reported with viral gene therapies are low/ moderate grade fevers, arthralgias and nausea, all of which tend to be transient, self-limiting and treatable with analgesics. This AE spectrum is quite consistent with the "flu-like syndrome" commonly reported and importantly, is much more tolerable when compared to conventional agents such as small molecule chemotherapies or radiotherapy which, due to their lack of specificity, cause substantial collateral damage to normal cells and tissues.

4. ROLE OF CLINICAL DEVELOPMENT PLAN IN TRANSLATIONAL RESEARCH

The conventional drug development process benefits from a Clinical Development Plan (CDP), which is used to document and address the specific questions required by regulatory authorities for ultimate approval. Use of this tool allows co-ordination of preclinical and clinical research and ensures that complex and expensive studies such as mechanism of action, toxicology, complete vector sequence analysis, pharmacodynamics and pharmacokinetics analyses are performed in an appropriate temporal order and each clinical study can build upon knowledge gained in prior studies. Gene therapy studies can also benefit from this approach as early adoption of a CDP can serve as a blueprint for studies required by regulatory authorities and will facilitate these interactions (5). Specific guidance documents have been published by the FDA to facilitate gene therapeutic development (6) and similar documents are available from other regulatory agencies. When should these plans be developed and incorporated? This is clearly a complex issue involving funding, regulatory processes and intellectual property considerations; however, we strongly recommend

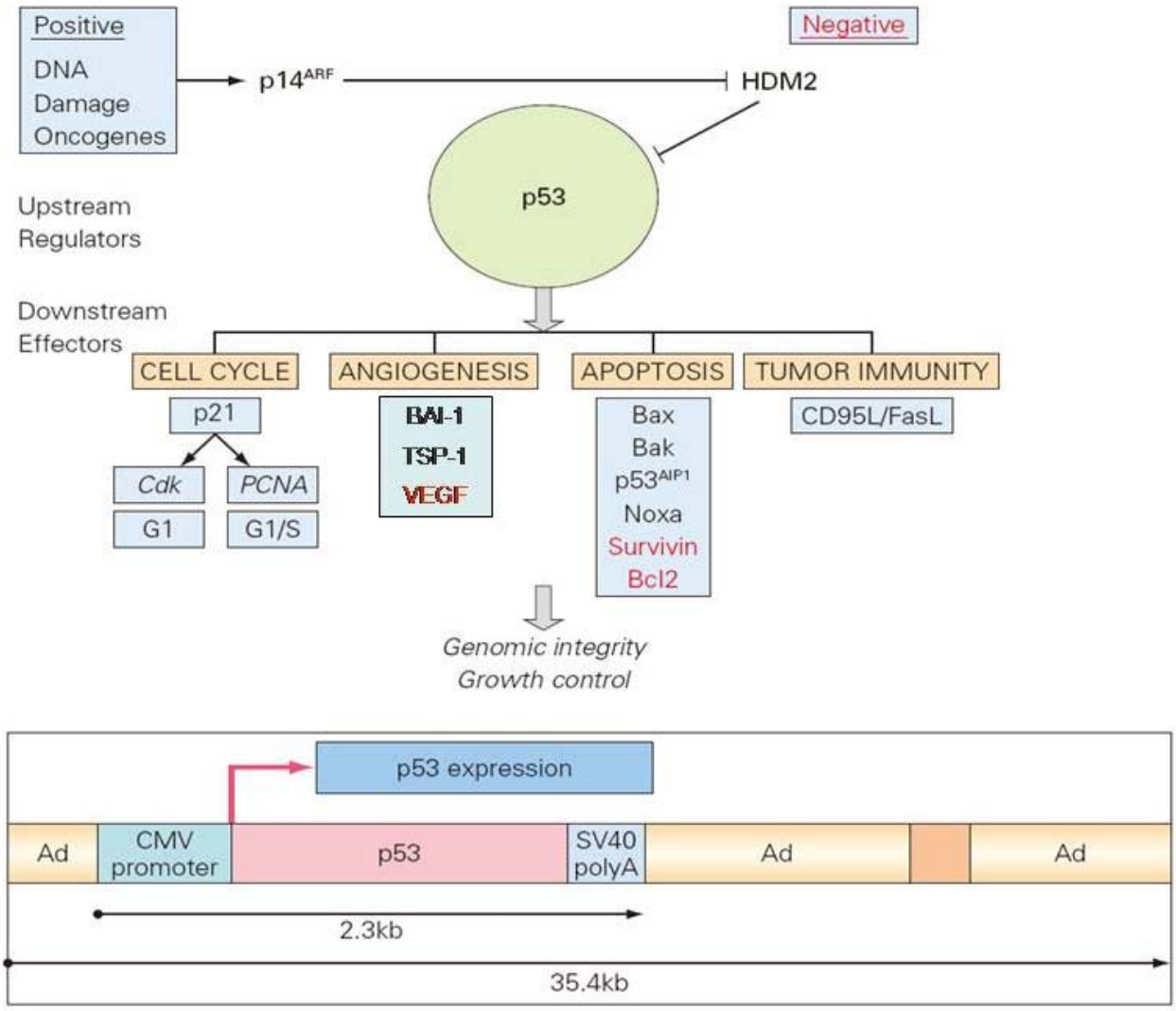

Figure 1. p53 regulatory pathways and map of Advexin Genome.

developing draft CDP documents once a decision is made to move from "proof of concept" to translational research. Clearly, these plans are "living" documents and prone to multiple changes, however, early adoption will not guarantee success, but they can improve chances and reduce the potential of unwanted surprises.

5. CASE STUDY: Advexin® (Ad-p53)

Below, we review a gene therapy clinical development case study using Advexin (Ad-p53) as a representative example. There are currently many gene therapy drug candidates at different stages of development and each has its own unique challenges and opportunities. The mechanisms of action employed by gene drugs are distinct and each may be appropriate for combination with conventional drugs. However, the results obtained from the Advexin preclinical and clinical studies may help to illuminate the path and guide development of additional genetic therapies.

5.1. Rationale and preclinical studies of tumor suppressor gene replacement

The p53 gene is a critical tumor suppressor that plays a key role maintaining the integrity of cellular DNA. p53 regulates progression through the cell cycle and in the presence of DNA damage, functions as a regulatory node to either facilitate DNA repair, or initiate apoptotic cell death when the damage is too extensive (7, 8). The primary mode of action of p53 is transcription modulation; p53 activates or represses expression of hundreds of target genes involved in regulation of cell cycle arrest ($p21^{WAF1/CIP1}$), apoptosis (*bax, bcl-2*), and/or DNA-repair processes (9,10). Additionally, p53 inhibits neovascularization by regulating expression of several key proteins in the process, including VEGF, BAI1, TSP1 (11, 12), and bFGF-binding protein (13, 14).

A lack of functional p53 protein can, therefore, allow the accumulation of genomic instability, resulting in unregulated proliferation of damaged cells and tumor

Table 1. Cell lines in which Advexin inhibits proliferation and/or increases apoptosis

Indication	Cytotoxicity [1]
Squamous Cell Carcinoma of the Head and Neck	12/12
Non-Small Cell Lung Cancer	12/12
Breast	7/7
Colorectal	8/8
Prostate	4/4
Cervical	8/8
Osteosarcoma	3/3
Esophageal	4/4
Hepatocellular Carcinoma	2/2
Pancreatic	7/7
Ovarian	7/7
Glioma	6/8
Endometrial	1/1
Bladder	3/3
Multiple Myeloma	7/7
Normal cells	0/8

[1]Number of cell lines showing cytotoxicity in response to Advexin / Number of lines tested

formation (15). Aberrant p53 pathways are present in virtually all cancer cells, either by mutation/deletion of the p53 gene, or by abnormal regulation of p53 gene expression, stability, or function in the absence of p53 gene mutations (7, 10). Mutations have been detected in over 50% of human cancers tested, and up to 70% of non-small cell lung cancer (NSCLC) and squamous cell carcinoma of the head and neck (SCCHN) (16-18). Other alterations of this pathway include inactivation or sequestration of the wild-type p53 gene product (e.g., inactivation via over-expression of MDM2 – see Figure 1), inability to activate p53 protein (e.g., via post-translational modifications), and mutations of downstream p53 targets (e.g., inactive enzymes in the apoptotic cascade) (17). Importantly, the presence of altered protein function or mutation of the p53 gene has been associated with poor clinical outcomes in patients with several types of cancer (19,20) and the presence of p53 mutations or disrupted p53 pathways correlates with resistance to chemotherapy and radiation.

Advexin® is an adenoviral vector, derived from adenovirus serotype 5 (Ad5), which mediates overexpression of the human wild type tumor suppressor protein p53 under the control of the CMV promoter (Figure 1) (21). The E1 region of the parental Ad5 DNA is deleted, thus preventing replication and expression of adenoviral genes. Numerous preclinical studies have demonstrated that transduction of cancer cells with a replication-incompetent adenoviral vector carrying the wild-type p53 gene (Ad-p53; Advexin), increases apoptosis and decreases proliferation of cancer cells with no apparent effect on normal cells (22). These studies have also shown that p53 sensitizes cancer cells to the effects of chemotherapy or radiation therapy and indicate that p53 may have utility both as monotherapy as well as a component of combination regimens. Significantly, increases in apoptosis and decreases in cancer cell proliferation have been demonstrated following administration of Advexin without observable effects on normal cells (23-25) - see Table 1. Clinical studies have demonstrated that Advexin is safe and more easily tolerated than chemotherapy or radiation treatment. Initial clinical trials designed to assess the safety and tolerability of Advexin in patients with a variety of cancers had favorable outcomes, with safety profiles that are superior to those of chemotherapy and radiation (26-29).

Numerous studies have shown Advexin to be effective in animal tumor models, including SCCHN, NSCLC, breast, colorectal, prostate, cervical, ovarian, esophageal, bladder, glioma, hepatocellular carcinoma and osteosarcoma. Early work using *ex vivo* models demonstrated that Advexin reduced the tumorigenicity of cells from several cancer types (e.g., 22, 28). In later studies, intratumoral (IT) injection of Advexin into established SQ human tumor xenografts in nude mice resulted in a reduced growth rate or regression of tumors derived from a wide range of tumor types, including SCCHN, NSCLC, colorectal cancer, and breast cancer. Advexin is effective against both p53 mutant and p53 wild-type xenograft tumors. As one would expect based on the broad spectrum of Advexin effects *in vitro*, Advexin appears to be effective in nearly all *in vivo* cancer models tested, with the possible exception of p53 wild-type gliomas (22). Some effects seen *with in vivo* models were dramatic, such as the complete inhibition of tumor growth after IT Advexin administration into human cervical cancer xenografts in nude mice reported by Hamada *et al.* (30). Most *in vivo* efficacy studies have been performed in SQ xenograft models, but Advexin also inhibits growth in disseminated xenograft cancer models and in orthotopic and syngeneic models.

As observed with *in vitro* studies, the anti-tumor effects of Advexin in animal models correlate with exogenous p53 expression, induction of p21 and mdm2 protein expression, and induction of apoptosis and/or decreased proliferation of cells within the tumor. Ohtani *et al.*, using a SQ NSCLC tumor model, demonstrated increased expression of p53, p21, MDM2, Noxa, and p53AIP1, and increased apoptosis, following a single IT injection of Advexin (31).

5.2. Clinical Experience with Advexin

A total of 28 Phase 1, 2, and 3 clinical studies have been conducted using Advexin; 23 have been finalized, completed or closed and 5 are ongoing. Of these, 16 are monotherapy studies and 6 have combined Advexin with chemotherapy or radiation. Patients in these studies had advanced cancers, most commonly lung cancer or SCCHN, although studies were also performed in patients with prostate cancer, breast cancer, colorectal cancer and other solid tumors. Advexin typically has been administered via intratumoral injection, although 17 patients in clinical studies have been treated intravenously. The majority of patients in these clinical trials have received multiple cycles of Advexin therapy (28,29), and the results of these have demonstrated the safety, tolerability and utility of Advexin as monotherapy and in combination with chemotherapy, radiation and surgery.

These studies also identified a number of prognostic indicators that may be used to identify patients most likely to benefit from Advexin therapy. Below, we summarize the clinical experience of Advexin as a monotherapy (SCCHN), and in combination with chemotherapy (LABC) and radiotherapy (NSCLC).

5.2.1. Advexin as Monotherapy

Advexin has been evaluated as monotherapy for several types of cancer, including recurrent, unresectable, locally advanced SCCHN and radiation-resistant, locally advanced esophageal cancer (32, 33). In this cohort of heavily pretreated patients, Advexin monotherapy was well tolerated with evidence of clinical activity.

Patients with recurrent, unresectable, locally advanced SCCHN have a poor prognosis and recurrent disease is usually considered incurable. Median overall survival after first relapse in patients with recurrent SCCHN is dismally short regardless of the treatment: 6 months if treated with chemotherapy as monotherapy, and 6-9 months for patients treated with combination therapy with platinum- or taxane-based regimens (34). The rationale for use of a p53-targeted therapy in treatment of SCCHN stems from loss of p53 function in approximately 70% of patients with SCCHN, which has been associated with tumorigenesis and resistance to radiation and chemotherapy.

We conducted three Phase II trials in this patient population, two studies used a higher dose of Advexin (5 x 10^{11} to 2.5 x 10^{12} viral particles (vp)/injection) and one used a lower dose (1-4 x 10^{9} vp/injection). Patients had histologically-confirmed SCCHN, with cytologically confirmed recurrence after first-line therapy administered with a curative intent (≥ 50 Gy radiotherapy and/or surgery with or without chemotherapy). The total area of all measurable lesions had to be ≤30 cm^2, and the sum of the longest diameter of each measurable lesion had to be ≤10 cm.

Results from three Phase II trials with 217 patients were combined to determine the overall rate of best lesional response to Advexin treatment in patients evaluable for objective response. Overall, 10% of treated lesions showed a CR or PR, a result comparable to conventional chemotherapies, however Advexin treated patients showed significantly reduced toxicity. Furthermore, 20% of patients showed durable tumor growth control lasting longer than 3 months. Dose response and survival analyses for patients with recurrent/refractory SCCHN treated in the high dose and low dose Phase II trials revealed clinical benefit, as defined by durable tumor growth control. In patients who received at least one cycle of treatment, high dose for Advexin provided survival advantage, as compared to treatment with low dose Advexin. This suggests a dose-response effect induced by Advexin in this patient population. Multivariate analyses conducted on studies in SCCHN patients identified a long progression free interval after initial therapy (≥ 12 months) as the major prognostic factor for all efficacy outcomes (32). The size of treated lesions (≤25 mm) was a favorable prognostic factor for both tumor response and tumor growth control, while prior irradiation of target lesions was a prognostic factor for the latter. Absence of ulcerated and/or necrotic lesions, and baseline tumor-pain identified tumors more suitable for intra-lesional Advexin treatment and were independent factors for response. Applying these selection criteria, subgroups of patients in these studies were defined; these groups exhibited overall response rates of 20%-30% and tumor growth control rates of 50%-60%, depending on the degree of selection.

5.2.2. Combination Approaches

5.2.2.1 Advexin and chemotherapy for treatment of Non-Small Cell Lung Cancer (NSCLC)

Non Small Cell Lung Cancer (NSCLC) accounts for nearly 80% of all lung cancers, and one third of patients diagnosed with NSCLC present with locally advanced, unresectable tumors. Despite advances in chemotherapy and the recent approval of biologic therapies gefitinib and erlotinib HCl, the 5-year survival rate for all lung cancers is only 15% (American Cancer Society). Two-year survival for patients with advanced disease ranges from 20% (stage III) to 5% (stage IV), and treatments for patients with advanced disease frequently result in severe side effects that may significantly decrease quality of life. Cisplatin is the most active single agent in NSCLC, and the drug is a mainstay of combination chemotherapy for this disease. Although several other chemotherapy agents have shown evidence of activity in NSCLC, their use has not increased median survival and is associated with significant toxicity (35). Mutations in the p53 gene have been detected in approximately 70% of NSCLC samples tested, and preclinical studies demonstrated activity of Advexin in combination with chemotherapy (36, 37). This provided the rationale for evaluation of the toxicity and antitumor activity of Advexin, delivered via computed tomography-guided percutaneous or bronchoscopic injection into NSCLC tumors obstructing the airway. The first Advexin study in lung cancer was conducted by Swisher *et al*, who treated 28 NSCLC patients with intratumoral injections of 10^{6} to 10^{11} pfu, and demonstrated wt-p53 transgene expression that was consistent with antitumor activity in a subset of patients (38). Below, we review two clinical studies evaluating Advexin in this population of patients.

A two-arm Phase I study was conducted to evaluate the feasibility, safety, humoral immune response and biologic activity of multiple IT injections of Advexin, and to characterize the pharmacokinetics in patients with advanced NSCLC. Fifteen patients with life expectancy >12 weeks, histologically confirmed NSCLC resistant or refractory to standard therapies, with lesions accessible to repeated injection and measurable disease with p53 mutations were enrolled (DNA mutation or protein over-expression). Patients in one arm (n=9) received escalating doses of Advexin monotherapy (1 x 10^{6} to 1 x 10^{11} plaque-forming units), administered by fine-needle injection using a bronchoscope; the other arm (n=6) evaluated Advexin (escalating doses ranging from 1 x 10^{9} to 1 x 10^{11} pfu), administered on day 4 of a 28-day schedule, in combination

which intravenous (IV) cisplatin (80 mg/m2 over 2 hours) administered on day 1 (39). Patients received a total of up to 14 courses of study treatment, and were monitored for adverse events and clinical effects.

Results of this study support the feasibility and safety of IT Advexin, alone or in combination with Cisplatin, in patients with advanced NSCLC. Of the 15 patients enrolled, 13 were assessable for efficacy: 1 patient had a partial response, while 10 patients had stable disease (3 of these lasting > 9 months), and 2 patients had progressive disease. Symptomatic improvement included reduction in dyspnea, cough, and hemoptysis, observed in 4 (26.7%) patients (39). There was no dose-limiting toxicity associated with the study treatment, and no patient was withdrawn from the study due to adverse effects. Of the adverse events (AE) reported, the most common was a transient, self-limited, fever. Hematologic toxicity was limited (1 incidence of leukopenia, and 3 incidences of grade 2-3 anemia). Transient, mild increases in liver ALT and AST were observed in one patient treated with monotherapy.

An open-label, dose escalating, Phase I trial was conducted on patients with advanced NSCLC harboring p53 mutations, as determined by DNA sequencing. 24 patients were enrolled, and treated with IT injection of Advexin (1×10^6 to 1×10^{11} pfu) on day 4 and IV Cisplatin (80 mg/m^2) on day 1. Apoptosis was measured using TUNEL assay. Vector dissemination and biodistribution was monitored. The study reports a best overall response of stable disease in 17 patients (74%), partial responses in 2 patients (9%), and progressive disease in 4 (17%). Consistent with p53 apoptotic functions, the mean apoptotic index in the tumor increased four-fold (P=0.011). As in the study described above, the most common AE attributable to the study treatment was transient, self-limiting fever, reported in 8 patients (33%). No changes in mean vital sign parameters, hematologic function, electrolytes, renal or liver function were observed. There was no detectable dose-related effect on toxicity. The study concluded that IT injection of Advexin in combination with Cisplatin was well tolerated, and reported evidence of its clinical activity (40).

Taken together, these clinical results support the use of direct bronchoscopic injection of Adp53 into endobronchial NSCLC in combination with platinum chemotherapy. Further, Advexin demonstrated relief of airway obstruction in heavily pretreated patients, thus supporting the use of Advexin in combination with chemotherapy or as monotherapy for localized lesions interfering with patient's quality of life.

5.2.2.2. Neoadjuvant Advexin and chemotherapy for treatment of Locally Advanced Breast Cancer (LABC)

Breast cancer is the second-leading cause of cancer death in women, and it is estimated that 211,240 new cases of breast cancer will be diagnosed in 2005 in the United States and that 40,870 people will die of the disease (American Cancer Society). Locally advanced breast cancer (LABC) represents approximately 10-30% of all primary breast cancers diagnosed. Approximately 50% of patients with LABC express alterations of p53 in their tumors. Management of LABC has evolved, such that standard of care now incorporates neoadjuvant (pre-operative or induction) chemotherapy as part of the multimodality approach (41). The taxanes and anthracyclines are considered to have the highest level of activity against breast cancer (BC), although as single agents they fail to produce a response in about half of BCs. Standard of care for this population of patients involves Induction Chemotherapy (IC), which is increasingly favored for management of LABC (disease stages IIA-IIIB) because: 1) it allows chemosensitivity testing, 2) it can downstage size of primary tumor and render it operable; 3) depending on the responses to primary systemic therapy, it may allow for breast-conservation surgery to be performed 4) allows for elimination of occult systemic metastases (41). Aberrations in p53 gene sequence or protein expression levels are frequently observed in primary breast tumors, particularly in LABC, and p53 dysfunction has been associated with poor prognoses, more aggressive tumors, early metastasis, chemoresistance and decreased survival (42). Additionally, preclinical studies have shown that Advexin may increase chemosensitivity, especially to drugs that induce DNA damage (36, 37). These observations provided the rationale for evaluating the efficacy of Advexin in combination with chemotherapeutic agents, in this case an anthracycline (doxorubicin) and a taxane (docetaxel).

Our group has conducted a prospective, open-label Phase II study to assess the safety, efficacy and biological activity of the combination of doxorubicin and docetaxel with the intratumoral injection of Advexin in patients with newly diagnosed inoperable LABC (stage IIIB-IIIC) (44). Patients received four to six 3-week cycles of study treatment (IT injection of Advexin, at (2.5×10^{12} vp on day 1-2). Chemotherapy (doxorubicin 50 mg/m^2 IV was followed by docetaxel 75 mg/m^2 IV) was administered on day 1, after administration of Advexin. Patients that achieved clinical remission following study treatment were treated with surgery, followed by radiation therapy. Adjuvant hormonal therapy was given to those patients with hormone receptor-positive disease.

One hundred percent of patients achieved clinical PR and underwent subsequent surgery; radiological assessment of response showed 79% median reduced primary tumor volume, and median reduced size of 67% for nodal disease. After 35 months of follow-up, 92% of the treated patients are alive and 83% have survived without evidence of disease recurrence. Overall clinical responses with a greater than 50 percent reduction in tumor size were seen following the combined therapy in all of the patients. The highly significant antitumor activity induced in the primary lesion, promising OS (82% at 3 years), and the 100% resectability rate, suggests that treatment with Advexin in conjunction with anthracycline-based IC dramatically reduces chemoresistance (37, 43). The results of the therapy with the addition of Advexin are better than what would be expected from neoadjuvant chemotherapy treatment alone. In a novel finding, activation of a local

immune response at the site of the tumor was observed. Treated tumors were infiltrated with cells of the immune system that are known to participate in immune responses against tumors, which may be useful in controlling local disease as well as disease outside the breast.

This study is the first to indicate safety and efficacy of a gene-based neoadjuvant therapy in breast cancer. The 100% PR rate observed in this study has not been observed in any other neo-adjuvant therapy trial for primary LABC. These data suggest that Advexin may be combined with neoadjuvant chemotherapy to further reduce tumor size and improve patient outcomes by facilitating complete surgical tumor removal. In addition, the results add to the very favorable safety profile observed in other Advexin clinical trials in patients with later stages of cancer and support clinical applications of Advexin in earlier phases of disease management. These data also suggest that Advexin can enhance the clinical benefit of chemotherapy without increasing this treatment's toxicity.

5.2.2.3. Advexin in combination with Radiation Therapy for treatment of NSCLC

A prospective, single arm, Phase II study was conducted to evaluate the feasibility and mechanisms of apoptosis induced by IT bronchoscopic injection of Advexin (3 x 10^{11} to 1 x 10^{12} vp, administered on days 1, 18 and 32) in combination with radiation therapy (60 Gy, starting on day 4, administered over 6 weeks) (45). The dose of Advexin was escalated in cohorts of 3 for the first 9 patients, while subsequent patients were treated with 3 x 1012 vp. A total of 19 patients with histologically proven, non-metastatic, measurable, stage I-III NSCLC were enrolled; 9 of them had locoregional advanced NSCLC (stages IIIA-B). Patients were ineligible for chemoradiation or surgery because of significant comorbidities, age, or obstructed bronchi. The primary endpoint of the study was local control at 3 months after completion of radiation therapy, as assessed by CT scan (16 patients) and biopsy (3 patients). The study reports that 89% of the patients completed the study treatment. CT and bronchoscopic findings at the tumor site revealed complete response in 1 (5%), partial responses in 11 (58%), stable disease in 3 (16%), and progressive disease in 2 patients (11%); 2 patients were not evaluable due to progression or death. At the time the study was reported, 5 patients were alive 34-48 months after study initiation, and 11 had developed distant metastases. Median time to progression had not been reached for loco-regional disease and was 9.2 months for metastatic disease.

The high number of pathologic negative biopsies (63%) and ORR are highly uncommon in this patient population (44, 45). Importantly, combination of Advexin and radiation did not increase toxicity as compared with previously reported results for radiation alone, and no dose-limiting toxicities were observed in the study (44). These data suggest that Advexin can provide loco-regional control of NSCLC for patients who are not candidates for surgery or chemoradiation, and have few treatment options.

6. CONCLUSIONS

The dataset of patients treated with gene therapies for cancer now numbers greater than 1,000 and the overwhelming conclusion supports that this modality is safe, although improved methods for determining biologic activity of these agents are warranted. It is intriguing that multiple clinical studies have indicated improvements in survival, yet these results are not always correlated with increased objective response rates. Similar results have recently been noted for other biological agents, and suggest that alternate (more biologically-based) imaging techniques may reflect the true utility of these agents. Recent focus has turned to use of metabolic imaging such as PET-CT to indicate tumor cell viability and proliferation rather than conventional tumor size measurements. Adoption of these imaging technologies will likely benefit both the understanding of gene drugs as well as help to optimize their application in cancer.

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Abbreviations: VLS: vascular leak syndrome; HAMA: human anti-mouse antibody; AEs: adverse events; CDP: Clinical Development Plan; SCCHN: squamous cell carcinoma of the head and neck; NSCLC: non-small cell lung cancer; Ad5: adenovirus serotype 5; IT: intratumoral; vp: viral particles; LABC: locally advanced breast cancer

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