Gene Therapy for Malignant Pleural Mesothelioma

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Given the current limitations in therapy for malignant mesothelioma, new approaches are needed. Malignant pleural mesothelioma has several characteristics that make it an attractive target for gene therapy: (1) there is no curative therapy, although some slowing of tumors has been reported with pemetrexid/cisplatin; (2) the pleural space is accessible for biopsy, local (as opposed to systemic) vector delivery, and analysis of treatment effects; and (3) morbidity and mortality are often related to local extension of disease, rather than distant metastases. Therefore, unlike other tumors that metastasize earlier in their course, relatively small increments of improvement in local control in mesothelioma could result in significant survival benefit.

Gene Therapy: Principles and Vectors

Gene therapy is the transfer of genetic material, including complementary DNA (cDNA), full-length genes, RNA, or oligonucleotides into cancer or host cells. The mechanism for transfer of this genetic material is termed the “vector.” Although conceived as a treatment for inherited recessive disorders in which transfer of a normal copy of a defective gene could prevent disease onset or reverse phenotypic expression, it soon became clear that one of the most important targets for gene therapy would be cancer.

A prerequisite for successful gene therapy is efficient gene transfer. A variety of viral and nonviral gene transfer vectors are currently available, which range from replicating and nonreplicating viruses, to bacteria, to liposomes (for reviews see refs. 1 and 2). As summarized in Table 52.1, each of these vectors has certain advantages with regard to DNA carrying capacity, types of cells targeted, in vivo gene transfer efficiency, duration of expression, and induction of inflammation.

Since most of the gene therapy trials for mesothelioma, in both animals and humans, have involved the use of replication incompetent adenovirus, this chapter concentrates on modifications of this vector.
The interested reader is referred elsewhere for details regarding other vector systems (3–6).

Recombinant adenovirus vectors have been derived by genomic deletion of viral gene functions involved in replication (i.e., the E1A/B regions) and provision of these functions in trans via a packaging cell line (7). The deleted gene regions can then be replaced with expression cassettes containing the desired gene allowing high-efficiency transduction in a wide range of target cells (including nondividing cells) and high expression levels of the delivered transgene (8). This vector system offers a number of advantages including good in vivo transduction efficiency, permitting direct gene delivery to many tissue sites, including the pleural space. Although the two primary disadvantages of adenoviruses, transient gene expression and prominent locally and systemic inflammatory responses elicited by virions, are an issue for long-term replacement gene therapy, these inflammatory responses, which include an early “innate” immune response resulting in cytokine secretion, may actually be advantageous for cancer gene therapy.

Several different cancer gene therapy approaches are currently being explored for malignant pleural mesothelioma including the use of “suicide” genes, delivery of tumor suppressor genes, and transfer of immunomodulatory genes (Table 52.2). Several of these have been applied in phase I clinical trials of malignant pleural mesothelioma utilizing a variety of vector systems including recombinant adenovirus, vaccinia virus, and modified ovarian carcinoma cells (9–11). Others remain in the preclinical stage, but with plans for clinical trials in the near future (Table 52.2).

### “Suicide” Gene Therapy

One prominent approach in cancer gene therapeutics is so-called suicide gene therapy. This method involves the transduction of tumor cells with cDNA encoding an enzyme that converts a benign prodrug to a toxic metabolite (12). Administration of the prodrug thus results in selective accumulation of toxin in the tumor cells and cell death. The

<table>
<thead>
<tr>
<th>Vector</th>
<th>DNA-carrying capacity (kb)</th>
<th>Cell range</th>
<th>In vivo gene delivery efficiency</th>
<th>Duration of expression</th>
<th>Co-transfer viral gene elements</th>
<th>Inflammatory response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>&lt;8</td>
<td>Replicating cells only</td>
<td>Low</td>
<td>Stable</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>7–8</td>
<td>Most cells</td>
<td>Moderate</td>
<td>Transient</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>&lt;5</td>
<td>Primarily muscle, liver, and brain</td>
<td>Low</td>
<td>Stable</td>
<td>Minimal</td>
<td>Low</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>&lt;8</td>
<td>Many nondividing cells</td>
<td>Low</td>
<td>Stable</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>Liposome</td>
<td>&gt;10</td>
<td>Most cells</td>
<td>Low</td>
<td>Transient</td>
<td>No</td>
<td>Moderate</td>
</tr>
<tr>
<td>Strategy</td>
<td>Vector</td>
<td>Therapeutic Gene</td>
<td>Molecular Mechanism</td>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide gene</td>
<td>Recombinant, replication deficient, adenovirus</td>
<td>Herpes simplex thymidine kinase</td>
<td>Delivery of enzyme capable of generating toxic metabolite after exposure to ganciclovir</td>
<td>University of Pennsylvania Medical Center, Philadelphia, PA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic immunopotentiation</td>
<td>Replication-restricted vaccinia virus Vaccinia virus</td>
<td>Human IL-2</td>
<td>Augmentation of immune response to tumor</td>
<td>Queen Elizabeth II Medical Center, Perth, Australia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Replication deficient, adenovirus Cationic liposome</td>
<td>Modified SV40 T-antigen Interferon-beta</td>
<td>Stimulation of immune response against SV40+ mesothelioma cells Induction of antitumor immune response</td>
<td>Wayne State University Medical Center, Detroit, MI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prokaryotic DNA</td>
<td>Nonspecific induction of innate and acquired immunity</td>
<td>University of Pennsylvania Medical Center (pending)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination suicide gene/tumor vaccine</td>
<td>Irradiated, allogeneic ovarian carcinoma cells</td>
<td>Herpes simplex thymidine kinase</td>
<td>Generation of toxic metabolite and antitumor immune responses</td>
<td>LSU Medical Center, New Orleans, LA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation compensation</td>
<td>Oligonucleotides Adenovirus Adenovirus</td>
<td>Antisense SV40 Tag Wild-type p14(ARF)/p16 Wild-type p53/Bak</td>
<td>Inhibition of dominant oncogenes Restoration of tumor suppressors Induction of apoptosis</td>
<td>No known clinical protocols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication-competent viral lytic therapy</td>
<td>Replication-restricted adenovirus: ONYX-015</td>
<td>None</td>
<td>Tumor-restricted viral replication and cytotoxicity</td>
<td>No known clinical protocols</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IL-2, interleukin 2; SV40, simian virus 40.
enzymes encoded by the suicide gene are often of nonhuman origin, e.g., the herpes simplex virus-1 thymidine kinase (HSV\textsubscript{tk}) gene, which limits toxicity in normal tissue (13). For instance, HSV\textsubscript{tk} differs enough from mammalian thymidine kinases that transfected malignant cells, but not normal tissue, convert the nucleoside analogue ganciclovir (GCV) to its toxic metabolite. After enzymatic conversion to GCV-monophosphate (GCV-MP) by HSV\textsubscript{tk}, it is rapidly metabolized to GCV-triphosphate (GCV-TP) by endogenous mammalian kinases. Intracellular production of these GCV metabolites causes tumor cell death, or “suicide” (12,14).

**Bystander Effects of HSV\textsubscript{tk} Suicide Gene Therapy**

Although relatively efficient, adenoviral gene transfer is not possible to every tumor cell. Thus the presence of a “bystander effect,” whereby untransfected cells are killed by an indirect mechanism, is extremely important (15). Such a bystander effect has been observed in the HSV\textsubscript{tk}/GCV system (15–19). The nature of this bystander effect is complex and appears to involve passage of toxic GCV metabolites from transduced to nontransduced cells via gap junctions or apoptotic vesicles (20,21), and induction of antitumor immune responses capable of killing tumor cells not expressing the HSV\textsubscript{tk} transgene (15).

**HSV\textsubscript{tk}/GCV Gene Therapy for Malignant Pleural Mesothelioma**

A number of studies showed that an adenoviral vector expressing HSV\textsubscript{tk} (Ad.HSV\textsubscript{tk}), combined with ganciclovir therapy, could be used to kill mesothelioma cells in vitro and in animal models (22–27). Based on these efficacy data and on preclinical toxicity studies showing minimal toxicity (28), a phase I clinical trial for patients with pleural mesothelioma began in November 1995 at the University of Pennsylvania Medical Center. The goals of this trial were to determine the toxicity, gene transfer efficacy, and immune responses generated in response to the intrapleural instillation of Ad.HSV\textsubscript{tk}. Mesothelioma patients who met inclusion criteria (including patent pleural cavities) underwent intrapleural administration of a single dose of Ad.HSV\textsubscript{tk} vector followed by 2 weeks of intravenous GCV (9,29,30). The adenoviral vector used was a so-called first-generation replication-incompetent virus, deleted in the early gene regions E1 and E3 with the HSV\textsubscript{tk} gene inserted in the E1 region.

Twenty-six patients (21 male, five female), ranging in age from 37 to 81, were enrolled in the study between November 1995 and November 1997 (Table 52.3) (29). Intratumoral HSV\textsubscript{tk} gene transfer was documented by immunohistochemistry (IHC) utilizing a murine monoclonal antibody directed against HSV\textsubscript{tk} in all patients treated at dose levels of $3.2 \times 10^{11}$ plaque-forming units (pfu) or greater (29). Ad.HSV\textsubscript{tk}/GCV gene therapy was well tolerated, and a maximum tolerated dose (MTD) was not achieved. Strong antiadenoviral humoral and cellular immune responses were noted, including the generation of high serum and pleural fluid titers of antiadenoviral neutralizing antibodies, the generation of serum antibodies against adenoviral
structural proteins, and increased peripheral blood mononuclear cell proliferative responses to adenoviral proteins (30).

In a small pilot study, five patients (patients 19 to 23) received intravenous corticosteroids around the time of vector instillation. This trial was designed to preliminarily assess the effects of immunosuppression on the degree of intratumoral gene transfer and antiadenoviral immune responses. Decreased fever and hypoxemia were noted in the corticosteroid-treated cohort, but there was also an increased incidence of reversible mental status changes (31). No diminution in antiadenoviral immune responses was demonstrated in the group receiving

Table 52.3. Results of University of Pennsylvania phase I clinical trials of Ad.tk/GCV gene therapy for mesothelioma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage/Vector Post-Rx Gene Tumor</th>
<th>age/sex</th>
<th>cell type</th>
<th>dose (pfu)</th>
<th>survival</th>
<th>transfer</th>
<th>response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62/M IA/E</td>
<td>1 × 10⁷</td>
<td>72 months</td>
<td>SD × 2 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>56/M III/E</td>
<td>1 × 10⁹</td>
<td>8 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>69/M III/B</td>
<td>1 × 10⁹</td>
<td>20 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>66/M II/E</td>
<td>3.2 × 10⁹</td>
<td>11 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>71/M IA/E</td>
<td>3.2 × 10⁹</td>
<td>58 months</td>
<td>SD × 3 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>71/M II/B</td>
<td>1 × 10¹⁰</td>
<td>4 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>70/M II/E</td>
<td>1 × 10¹⁰</td>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>60/M II/E</td>
<td>1 × 10¹⁰</td>
<td>27 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>74/M II/B</td>
<td>3.2 × 10¹⁰</td>
<td>2 months</td>
<td>NP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>60/M III/E</td>
<td>3.2 × 10¹⁰</td>
<td>9 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>37/F IV/E</td>
<td>1 × 10¹ⁱ</td>
<td>16 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>37/M III/B</td>
<td>1 × 10¹¹</td>
<td>2 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>65/F III/E</td>
<td>1 × 10¹¹</td>
<td>10 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>66/F IA/E</td>
<td>3.2 × 10¹¹</td>
<td>50 months</td>
<td>SD × 2 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>60/M IV/B</td>
<td>3.2 × 10¹¹</td>
<td>5 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>69/M IV/E</td>
<td>3.2 × 10¹¹</td>
<td>8 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>70/F IB/E</td>
<td>3.2 × 10¹¹</td>
<td>15 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>69/F IB/E</td>
<td>3.2 × 10¹¹</td>
<td>14 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>75/M II/B</td>
<td>3.2 × 10¹¹</td>
<td>8 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>68/M II/B</td>
<td>3.2 × 10¹¹</td>
<td>1 month</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>71/M II/B</td>
<td>3.2 × 10¹¹</td>
<td>41 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>76/M II/B</td>
<td>3.2 × 10¹¹</td>
<td>33 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>81/M II/B</td>
<td>3.2 × 10¹¹</td>
<td>25 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>71/M II/E</td>
<td>1 × 10¹²</td>
<td>21 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>65/M II/E</td>
<td>1 × 10¹²</td>
<td>5 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>67/M IA/E</td>
<td>1 × 10¹²</td>
<td>22 months</td>
<td>PR (CT)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>67/M II/B</td>
<td>1 × 10¹¹</td>
<td>7 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>53/M III/E</td>
<td>1 × 10¹¹</td>
<td>13 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>30/F IA/E</td>
<td>5 × 10¹¹</td>
<td>37 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>56/F IA/E</td>
<td>5 × 10¹¹</td>
<td>37 months</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>66/M II/B</td>
<td>5 × 10¹¹</td>
<td>9 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>74/M II/B</td>
<td>5 × 10¹¹</td>
<td>19 months</td>
<td>PR (PET)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>64/M II/B</td>
<td>5 × 10¹¹</td>
<td>10 months</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>69/M II/B</td>
<td>5 × 10¹¹</td>
<td>26 months</td>
<td>NP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E, epithelioid; B, biphasic; NP, test not performed; SD, stable disease (CT +/− PET); PR, partial response.

* All patients deceased except patients 1, 29, 30, and 34.
* Received 15 mg/kg/day of GCV × 14 days.
* Received adjuvant corticosteroids.
* Received third-generation E1-/E4-deleted adenoviral vector.
corticosteroids, nor were there any appreciable differences in the degree of intratumoral gene transfer.

Of the 26 patients enrolled in the initial phase I trial, 25 have since died, with a median posttreatment survival of approximately 11 months (Table 52.3). Several patients with stage IA/IB epithelioid mesothelioma had posttreatment survivals of greater than 3 years, with one patient surviving over 4 years. Of the trial participants who are deceased, all had progressive mesothelioma as their primary cause of death, typically with invasion of the mediastinum and the contralateral hemithorax, and transdiaphragmatic extension, as well as with widespread metastatic disease, a fairly common finding in advanced mesothelioma.

Additional Phase I Trials of Ad.HSVtk Gene Therapy for Mesothelioma

Based on these results, two additional phase I trials were initiated. In the first trial, a second-generation Ad.HSVtk vector containing deletions in the E1 and E4 regions with preservation of the E3 region was used. Based on animal studies, this vector was chosen for diminished cytopathic effects and reduced cellular immune responses (32) and, since two replication-necessary genes are deleted, simple recombination could not produce replication competent virus in the vector production process.

Five patients were treated under this protocol, starting at a dose one log lower than the highest dose used with the E1/E3-deleted Ad vector ($1.5 \times 10^{13}$ viral particles). Dose-related gene transfer was detected in all patients at both dose levels via immunohistochemistry using an anti-HSVtk monoclonal antibody. Of the five patients treated in this second phase I trial, there are two surviving (patients 29 and 30), both of them treated at the higher dose level of $5.0 \times 10^{13}$ particles of Ad.HSVtk (Table 52.3). Each of the patients had stage I epithelioid mesothelioma at diagnosis. Both have had clinically and radiographically stable disease without other antitumor therapy for 54 months after treatment. Patient 29, a 34-year-old woman, has demonstrated diminished tumor metabolic activity on serial follow-up 18-fluorodeoxyglucose positron emission tomography (18-FDG-PET) scans (Fig. 52.1). This delayed decrease in tumor metabolic activity several months after completion of the gene therapy protocol suggests the induction of a secondary immune bystander effect induced by Ad.HSVtk/GCV (33).

The third trial involved gradual dose escalation of ganciclovir in combination with intrapleural delivery of the E1/E4-deleted Ad vector. One cohort of three patients was treated with $3.0 \times 10^{13}$ particles of Ad.HSVtk (E1/E4-deleted) and 7.5 mg/kg ganciclovir IV b.i.d. (15 mg/kg/day). All three patients tolerated the treatment well. One of three patients is still alive, albeit with evidence of significant tumor progression, now 26 months after treatment (Table 52.3). No durable clinical responses were noted in any of the three patients treated in this protocol.
Lessons Learned

Based on our clinical trial experience, the Ad.HSVtk/GCV suicide gene therapy approach showed some potential for the treatment of malignant mesothelioma, as well as other localized malignancies. Unfortunately, these phase I trials were halted because of the death of a participant in a gene therapy trial for ornithine transcarbamylase (OTC) deficiency at the University of Pennsylvania Medical Center utilizing a similar adenoviral vector backbone (34). Nonetheless, one of the most valuable aspects of our trial has been the identification of specific challenges that need to be addressed, such as gene transfer efficiency.

Using the current strategy, therapeutic efficacy could only be expected in patients with relatively small tumor burdens (small
nodules or diffuse, “thin” tumors). An alternative treatment schema maximizing the vector-to-tumor cell ratio would involve surgical “debulking” to minimize tumor mass, followed by adjuvant administration of Ad.HSV\textit{tk}/GCV. Another method of improving intratumoral gene transfer would be repeated administration of vector and GCV (i.e., three doses over a 3-week period). Completed studies in immunocompetent mice with established peritoneal tumors by our group (35) and others (36) showed marked increases in efficacy after multiple intraperitoneal injections of Ad.HSV\textit{tk}, each followed by a course of GCV. Importantly, data from our initial clinical trials suggest that gene transfer is possible even in patients with titers of anti-Ad neutralizing antibodies of up to 1:500, as would be expected with repeated administration of Ad vector.

Another approach to the gene transfer problem is to maximize the efficacy of the expressed HSV\textit{tk} enzyme. “Molecular remodeling” of the HSV\textit{tk} enzyme has allowed increasing specificity for GCV and a cyclovir (ACV) and concomitantly decreased thymidine utilization (37). These HSV\textit{tk} mutants show increased ACV- and GCV-mediated cytotoxicity, and enhanced bystander effects in mixing experiments (38,39). We have produced adenoviral vectors containing the mutated HSV\textit{tk}s and demonstrated enhanced cell killing and augmented bystander effect in in vitro and in vivo tumor models (40).

**Suicide Gene Vaccines**

A growing body of evidence supports the hypothesis that in most models tested, treatment with HSV\textit{tk}/GCV results in an immunologic bystander effect that enhances antitumor cytotoxicity both at the site of vector delivery as well as at distant, nontransduced tumor sites (15,19,41–43). We believe that we have seen evidence of this immune bystander effect in our mesothelioma phase I clinical trials with the progressive decline in tumor metabolic activity seen on PET scan in patient 29 over 36 months posttreatment (Fig. 52.1). This putative antitumor immune reaction may result from nonapoptotic HSV\textit{tk}/GCV-mediated tumor necrosis, a type of cell death that releases so-called danger signals that then activate significant cellular immune responses (43,44). Generation of these danger signals may be enhanced by transduction of tumor cells with the HSV\textit{tk} gene plus a cytokine gene, such as the gene for interleukin-2 (IL-2). Augmented tumor cytotoxicity has been reported with HSV\textit{tk} plus IL-2 in a number of tumor models (45).

This method of causing mesothelioma tumor destruction via the immunologic bystander effects of HSV\textit{tk}/GCV gene therapy, a presumptive suicide gene vaccine, was studied in a phase I clinical trial conducted by Schwarzenberger and colleagues (10,46) at the Louisiana State University (LSU) Medical Center in New Orleans (Table 52.2). The protocol designed by the LSU investigators consisted of the intrapleural instillation via an indwelling pleural catheter of an irradiated ovarian carcinoma cell line retrovirally transfected with HSV\textit{tk} (PA1-STK cells), followed by systemic administration of GCV (10).
Schwarzenberger and colleagues hypothesized that the PA1-STK cells would migrate to areas of intrapleural tumor after instillation, undergo necrotic cell death after exposure to GCV, and generate immune responses that would facilitate killing of adjacent mesothelioma cells. Antimesothelioma immune responses in this system are related to the local generation of proinflammatory cytokines, which, in turn, summon an influx of cytotoxic lymphocytes to the area producing hemorrhagic tumor necrosis (10,46). In the patients treated to date, minimal side effects have been seen, while preliminary findings showed significant posttreatment increases in the percentage of CD8 T lymphocytes in pleural fluid (46).

Cytokine Gene Therapy for Mesothelioma

There has been significant interest in the delivery of genes encoding for proinflammatory cytokines to the pleural space of patients with malignant mesothelioma. One of the rationales for cytokine gene therapy is that exogenous cytokines are known to have direct antiproliferative effects on mesothelioma cells, as well as the ability to activate intrapleural and intratumoral immune effector cells in vivo. Expression of cytokine genes by tumor cells generates a high level of intratumoral cytokines in paracrine fashion, inducing powerful local cytokine effects without significant systemic toxicity. Prolonged local cytokine expression can induce activation of tumor-associated dendritic cells (DCs) to express major histocompatibility complex (MHC) tumor antigen complexes in conjunction with co-stimulatory molecules. These activated DCs can then migrate to regional lymph nodes where they stimulate proliferation of tumor-specific CD8 and CD4 lymphocytes, inducing antitumor cytotoxicity at distant sites of tumor. In addition, some proinflammatory cytokines, such as IL-2, have the capability of direct intratumoral activation of CD8+ tumor infiltrating lymphocytes, overcoming tolerance signals to produce tumor-specific cytotoxic T lymphocytes (CTLs). Increased intratumoral IL-2 may also activate natural killer (NK) cells and lymphokine-activated killer cells (LAKs). Animal experiments have shown that injection of IL-2-transduced tumor cells increases specific antitumor activity, generates systemic responses to the parental tumor, augments the immune response against autologous tumor, and causes rejection of rechallenged tumor cells (47,48).

Several published phase I and phase II clinical trials have documented mesothelioma tumor responses to intrapleural infusion of IL-2, type I interferons [interferon-α and interferon-β (IFN-β)], and type II interferons [interferon-γ (IFN-γ)] (49–55). In particular, Boutin and colleagues (52,53) at the Hôpital de la Conception in Marseilles, France, demonstrated significant response rates in pleural mesothelioma after intrapleural instillation of IFN-γ, including several complete pathologic responses in patients with stage IA disease (tumor limited to the parietal and diaphragmatic pleural surfaces) (52,53).
The first human clinical trial of direct intratumoral delivery of cytokine genes in malignant pleural mesothelioma using this method of in vivo genetic immunotherapy was conducted by investigators at Queen Elizabeth II Hospital in Perth, Australia, using a recombinant vaccinia virus (VV) expressing the human IL-2 gene (Table 52.2). A vaccinia vector was chosen because of its large genome, proven safety in human vaccines, and availability of anti-VV antibodies for evaluation of vector-induced immune responses. In addition, insertion of the IL-2 gene into the thymidine kinase region of the VV rendered it partially replication-restricted, allowing for relatively more expression in tumor cells. The VV–IL-2 vector at a dose of $1 \times 10^7$ pfu was serially injected into palpable chest wall lesions of six patients with advanced malignant mesothelioma. Toxicities were minimal, and there was no clinical or serologic evidence of spread of vaccinia virus to patient contacts. No significant tumor regression was seen in any of the patients, and only modest intratumoral T-cell infiltration was detected. VV–IL-2 messenger RNA (mRNA) was detected by reverse-transcriptase polymerase chain reaction (PCR) in serial tumor biopsies for up to 6 days after injection, but declined to low levels by day 8. The prolonged nature of IL-2 gene expression in this trial was remarkable, considering the fact that significant serum titers of anti-VV neutralizing antibodies were generated in all patients (56).

The Future of Genetic Immunotherapy for Mesothelioma

Several other candidate cytokine genes are being evaluated for therapeutic effectiveness in animal models of mesothelioma. Caminschi and colleagues at (57) Queen Elizabeth II Medical Center in Perth, have investigated genetic alteration of murine mesothelioma cell lines with the gene for interleukin-12 (IL-12), one of the most active immunomodulatory cytokines. This same group previously demonstrated that systemic administration of exogenous IL-12 induced strong antitumor immune responses in mice bearing syngeneic mesothelioma tumors. The Perth group showed that injection of murine mesothelioma cells transfected with the IL-12 gene (AB1–IL-12) did not produce tumors in immune-competent mice, but did so in athymic nude mice, implicating a T-cell–dependent mechanism of IL-12 activity. Immune-competent mice challenged with AB1–IL-12 were protected from subsequent challenge with parental tumor not expressing IL-12, demonstrating induction of long-term immunity. In addition, AB1–IL-12 injection reduced the incidence of tumor development from parental cell challenge at a distant site (58).

Innate and adaptive antitumor immune responses can also be elicited by delivery of nonspecific immunostimulatory genes. As an example of this paradigm, Lukacs and colleagues (59) transferred mycobacterial heat shock protein gene (HSP-65) via a cationic liposome into the abdominal cavities of mice bearing intraperitoneal sarcomas.
resulting in a significant antitumor response. Interestingly, Lanuti and colleagues (60) in our laboratory, found that the antitumor effects of heat shock protein gene transfer via cationic liposomes could be reproduced in a syngeneic murine model of mesothelioma, but appeared to be related to nonspecific effects of lipid-plasmid DNA (pDNA) complexes. Rudginsky and colleagues (61) at Genzyme Corp. (Framingham, MA) further explored the potential of prokaryotic DNA induction in mesothelioma cells. They conducted a series of experiments confirming antitumor responses and increased survival with liposomal delivery of fragments of bacterial plasmid DNA, genomic *Escherichia coli* DNA, and synthetic CpG oligonucleotides. No increased survival or tumor reductions were seen with liposomal delivery of eukaryotic DNA or with methylated bacterial DNA. Therefore, the unmethylated CpG motifs of prokaryotic DNA play a crucial role in the development of innate and adaptive antitumor immune responses. Based on these studies, therefore, a case could then be made for a straightforward clinical trial of intrapleural delivery of nonspecific lipid-pDNA in patients with mesothelioma (Table 52.2).

As previously mentioned, the type I (α, β) and type II (γ) interferons have been shown to have clinical antitumor activity when administered exogenously to patients with pleural mesothelioma. Interferon-β, for example, has potent antiproliferative in vitro effects on mesothelioma cells, and strong immunostimulatory actions in animal models, but is limited in clinical use by toxicity of systemic administration (62). Odaka and colleagues (63,64) at the University of Pennsylvania Medical Center, therefore investigated the effects of IFN-β gene therapy in murine models of mesothelioma. The Penn investigators showed that a single intraperitoneal (i.p.) injection of a recombinant adenovirus, engineered to express the murine IFN-β gene (Ad.muIFN-β), can eradicate small syngeneic murine mesothelioma tumors in >90% of animals tested. Intraperitoneal Ad.muIFN-β gene therapy resulted in significant reduction of subcutaneous tumors at a distant site, as well. These effects of Ad.muIFN-β were clearly shown in several experiments to be mediated by CD8+ T lymphocytes. Additional studies have shown that the combination of intratumoral treatment with Ad.IFN-β, followed (in 3 days) by surgical debulking, led to a high cure rate in very large tumors. Based on these promising preclinical studies and a toxicology trial performed in mice showing a good safety profile, we have initiated a phase I clinical trial of intrapleural delivery of Ad.muIFN-β for the treatment of mesothelioma (Table 52.1). If the phase I trial shows safety, a phase II “neoadjuvant” immunotherapy/surgical approach will be proposed.

**Induction of Apoptosis**

One of the primary approaches to cancer gene therapy research over the past decade has been mutation compensation—the replacement of absent or mutated tumor suppressor genes responsible, at least in part, for the malignant phenotype of the cancer cell. Intratumoral delivery
of the wild-type p53 gene, for example, has been the most frequent method of experimental gene therapy of solid tumors, as mutations in the p53 tumor suppressor gene account for the majority of genetic abnormalities in solid tumors. Most mesotheliomas, however, contain wild-type p53 and a normal copy of the cell cycle regulator pRb. The most common molecular abnormality found in pleural mesotheliomas is absent expression of the cyclin-dependent kinase (CDK) inhibitor, p16INK4a. This mutation can lead to unmitigated progression through the cell cycle despite the presence of normal pRb expression and wild-type p53, and therefore, the development of a neoplastic phenotype (65).

Kratzke and colleagues at the University of Minnesota School of Medicine have demonstrated that reexpression of p16INK4a in mesothelioma cells in vitro and in vivo results in cell cycle arrest, cell growth inhibition, apoptosis, and tumor reduction (65). In addition, the Minnesota investigators have recently shown that repeated administration of an adenoviral vector expressing wild-type p16INK4a into established human mesothelioma xenografts in athymic nude mice resulted in prolongation of survival compared with controls receiving saline or an Ad vector expressing the marker gene lacZ (66). Successful application of this technology to human clinical trials is dependent on the development of more efficient means of tumor cell transduction.

Investigators at the Thoracic Oncology Laboratory, University of California at San Francisco (UCSF) Cancer Center, are targeting another common mutation in mesotheliomas for mutation compensation gene therapeutic approaches. Jablons and colleagues at UCSF have demonstrated that homozygous deletion of the INK4a/ARF locus is common in human mesotheliomas (67). The p14(ARF) protein encoded by the INK4a/ARF locus promotes degradation of the p53 binding protein called MDM2, which functions to bind p53 and inactivate it. Thus, production of the ARF protein prevents MDM2-mediated neutralization of p53 and favors p53-mediated cell cycle arrest. Deletion of the INK4a/ARF locus abrogates p14(ARF) protein expression, which leads to higher levels of MDM2. MDM2 binds p53 and inactivates it, leading to unchecked progression through the cell cycle. The UCSF group transfected human mesothelioma cell lines with an adenoviral vector encoding for human p14(ARF) cDNA (Ad.p14). Overexpression of p14(ARF) within the mesothelioma cells led to increased amounts of p53 and p21, and dephosphorylation of pRb. In addition, Ad.p14 inhibited mesothelioma cell growth via induction G1-phase cell cycle arrest and apoptotic cell death (67).

Despite the fact that most mesotheliomas have wild-type p53 (wt-p53), the function of p53 in mesothelioma cells may be abnormal secondary to binding of p53 by inhibitor proteins such as MDM2 and simian virus 40 (SV40) large-T antigen. Therefore, there may be a rationale for gene therapy of mesothelioma via overexpression of wt-p53 within the cell. Giuliano and colleagues (68) in Chieti, Italy, performed a series of experiments in which they transfected human mesothelioma cells with a replication-deficient adenoviral vector carrying the wt-p53 gene. They demonstrated greater than 80% inhibition of tumor cell growth in vitro at a multiplicity of infection (MOI) of 25 with docu-
mentation of induction of apoptosis in the dying tumor cells. In addition, Giuliano and colleagues showed that ex vivo transfer of the \( wt-p53 \) gene to mesothelioma cells inhibited growth of tumor implants in nude mice. In immunodeficient mice with established human mesothelioma xenografts, intratumoral injection of the \( wt-p53 \) gene inhibited tumor growth and prolonged survival. It is not inconceivable, therefore, to consider human clinical trials of Ad \( wt-p53 \) gene therapy in mesothelioma akin to those conducted in lung cancer, head and neck cancer, and metastatic colon cancer (Table 52.2).

Another method of inhibiting mesothelioma cells is the introduction of “downstream” promoters of apoptosis such as the proapoptotic Bcl-2 family member Bak. Pataer and colleagues (69) at M.D. Anderson Cancer Center in Houston co-delivered binary adenoviral-Bak/GV-16 vectors into \( wt-p53 \) positive and mutated \( p53 \) mesothelioma cell lines in vitro, along with binary Ad.\( lacZ \)/GV-16 control vectors. The M.D. Anderson group demonstrated marked induction of apoptosis and decreased cellular viability in both \( p53 \) “sensitive” and “resistant” cell lines with \( Bak \) gene transfer, but not with \( lacZ \) delivery. Thus, gene transfer in vivo with proapoptotic Bcl-2 family members would be a reasonable strategy for future mesothelioma gene therapy clinical trials. Alternatively, inhibition of endogenous inhibitors of apoptosis is a possible approach. Xia et al (70) have recently shown widespread expression of the inhibitor of apoptosis, survivin, in mesothelioma. Interestingly, antisense oligonucleotides to survivin, induced apoptosis in mesothelioma cell lines overexpressing survivin.

**SV40: Is There a Role in Therapy for Mesothelioma?**

One of the most remarkable developments in mesothelioma research over the past several years has been the discovery of simian virus 40 (SV40) sequences in mesothelioma tumor specimens from the United States and several European countries. SV40, a nonhuman polyomavirus that was a contaminant of some polio vaccines in the 1950s and 1960s, carries the ability to transform normal cells via the oncogenic properties of its large-T antigen (Tag), and can induce the formation of mesotheliomas in hamsters after injection into the pleural space or peritoneal cavity (71). Laboratory analysis of a subset of human mesotheliomas has demonstrated coimmunoprecipitation of SV40 Tag with tumor suppressor gene products, such as the p53 and pRB proteins (72). The presence of SV40 Tag within tumor cells binding and inactivating wild-type p53 and pRB may explain the unusually high rate of wild-type p53 and pRb within mesotheliomas, unlike most other solid tumors.

The potential role for SV40 as a causative factor in mesothelioma oncogenesis and proliferation has inspired several new experimental gene therapy approaches. Schrump and Waheed (73), at the thoracic oncology branch of the National Cancer Institute, have shown that antisense oligonucleotides designed to abrogate SV40 Tag expression induce apoptosis and enhance sensitivity to chemotherapeutic agents
in SV40 (+) mesothelioma cells in vitro. Another strategy has been advocated by Imperiale and colleagues (74) at the University of Michigan and Wayne State University Medical Centers, who are developing a genetically engineered vaccine to SV40 Tag. SV40 is an excellent candidate for antigen-specific immunotherapy, because Tag is a viral antigen that should not induce immune tolerance, unlike most other tumor antigens. The Michigan group has created a recombinant, truncated version of Tag (mTag), modified to exclude the domains involved in oncogenic function: the J domain and the p53 and pRB binding domains. They have cloned the mTag gene into a vaccinia vector (vac-mTag), and have demonstrated significant antitumor immune responses in Balb/c mice carrying Tag(+) tumors. A phase I dose-escalation safety and toxicity trial in patients with Tag-expressing mesotheliomas is planned (Table 52.2) (74).

**Replicating Viruses**

An additional mechanism of maximizing intratumoral gene transfer would be to produce adenoviral vectors capable of selective replication in mesothelioma cells. In this approach, tumor killing could occur via two mechanisms: direct tumor lysis due to viral replication or augmentation of transgene delivery, such as HSVtk. Widespread dissemination would likely be precluded by the intact host immune response (75).

One approach to make such tumor selective virus is to substitute the adenoviral E1 promoter with promoters for mesothelioma-related proteins, such as manganese-superoxide dismutase (MnSOD), calretinin, and mesothelin (76–79). Work by Kinnula’s group (76) in Finland, has shown that MnSOD is very highly expressed in human malignant mesothelioma tissues and cell lines. Calretinin is a 29-kd calcium-binding protein that is expressed primarily in the nervous system, but high levels of expression have also been noted in cells of mesothelial origin (77,78). Mesothelin is a 40-kd surface protein of unknown function that is expressed only on the tissues forming the pleural, pericardial, and peritoneal membranes (79). Another approach is to use more general tumor-selective promoters, such as promoters responsive to the transcription factor E2F (80) or the survivin gene (81). Our group has recently generated such an E2F-driven virus and showed that it selectively replicated in and lysed tumor cells, compared with non-transformed cells (80).

ONYX-015 is a conditionally replication competent adenovirus lacking the E1b 55-kd gene, and therefore can only replicate in tumor cells lacking functional p53. One of the functions of E1b 55-kd is to bind and inactivate wild-type p53. Clinical trials of ONYX-015 in patients with cancers of the head and neck and lung have shown evidence of tumor reduction with minimal toxicity. As described above, in mesothelioma, unlike many other solid tumors, genetic alterations in p53 are uncommon, but functional inhibition of p53 can be achieved via deletions in the INK4a/ARF locus. The UCSF group demonstrated
in vitro cytotoxicity of ONYX-015 on mesothelioma cell lines lacking p14(ARF), and increased resistance of these same cell lines to ONYX-015 after transfection of the tumor cells with Ad.p14 (82).

To date, replicating adenoviruses have proven safe in phase I clinical trials, but have had limited efficacy. With further refinements or with combinations with other treatments (such as chemother- or radiation therapy), this approach could prove useful in the future.

Summary

Gene therapy for mesothelioma is in its infancy, yet the results of recent phase I clinical trials and ongoing preclinical studies offer significant promise for the future. Intrapleural and intratumoral injections of viral and nonviral vectors encoding therapeutic genes have proved safe in humans with evidence of intratumoral gene transfer and expression of therapeutic proteins. Anecdotal tumor responses have been seen in suicide gene therapy trials, either as a result of direct cytotoxicity or via induction of bystander immunologic phenomena. Our group is vigorously pursuing an immuno-gene therapy approach with an adenovirus expressing interferon-β. Expanding knowledge of the cellular and molecular abnormalities responsible for the carcinogenesis of mesothelioma has led to the development of new gene therapy approaches targeting oncoproteins and mutant tumor suppressor genes. Implementation of these experimental modalities on a routine basis for mesothelioma patients remains several years in the future. Nevertheless, the lack of significant benefit from standard anticancer treatments in the disease argues strongly for patient enrollment in clinical studies of various gene therapy approaches to determine safety, toxicity, and efficacy, as well as to guide future laboratory investigation.

References


