Phase I Study of Direct Intrallesional Gene Transfer of HLA-B7 into Metastatic Renal Carcinoma Lesions

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ABSTRACT

Renal cancer cell lines exhibit deficient expression of MHC class I antigens required for appropriate CTL stimulation. Nabel et al. (Proc. Natl. Acad. Sci. USA, 90: 11307–11311, 1993) demonstrated that direct gene transfer of the deficient class I MHC molecule into melanoma cells would stimulate their immune destruction. Stopeck et al. (J. Clin. Oncol., 15: 341–349, 1997) demonstrated the clinical use of this approach in melanoma patients. We investigated the safety and ability to bestow gene expression via intratumoral transfer of escalating amounts of lipid-formulated plasmid DNA encoding for the MHC HLA-B7 gene product Allovecin-7 into metastatic renal cancer lesions.

Fifteen patients with histologically confirmed, HLA-B7-negative metastatic renal cancer received intratumoral injection of Allovecin-7 on an escalating dose schedule. Tumors were evaluated serially by computed tomography scan, ultrasound, and physical examination. Presence of the HLA-B7 gene and protein was determined via PCR, flow cytometry, and immunohistochemical staining in serial biopsy specimens.

HLA-B7 gene, mRNA, or protein expression could be conclusively demonstrated in 8 of 14 patients (57%). Three patients had tumor biopsy to assess the presence of tumor-infiltrating lymphocytes, and all three had higher posttreatment levels of tumor-infiltrating lymphocytes. There were no significant clinical responses or toxicity at the site of injection or at other, noninjected tumor sites.

This study demonstrated that intratumoral injection of Allovecin-7 is safe, feasible, and associated with minimal toxicity. This approach was capable of bestowing gene expression, possible resulting in antitumor CTL response. Despite lack of tumor regression in this series of renal cancer patients, the simplicity and low toxicity of this approach commend it for Phase II studies in renal and other cancers, as well as for transfection with other genes.

INTRODUCTION

The goal of immunotherapy is to stimulate the immune system to recognize and destroy cancerous cells by modifying tumor cells themselves and/or by modifying the host response to tumor cells. Conventionally, immunotherapeutic approaches have included the administration of nonspecific immunomodulating agents such as Bacillus Calmette-Guérin, cytokines, and/or the adoptive transfer of CTLs, which have shown promise in animal models (1–6) and man (7–10). Over the last several decades, there have been many attempts to identify tumor-specific antigens that could serve as targets for cytotoxic antibodies or cell-mediated immunity and to develop vaccines and monoclonal antibodies specifically directed at these preferentially expressed tumor cell surface antigens. Many melanoma-specific antigens have been found, and renal cancer-specific antigens are now beginning to be identified (11, 12).

Early investigations of HLA frequency in renal cancer were mostly family studies that demonstrated significantly increased frequency of certain HLA haplotypes in some affected families (13). More recent work has suggested that HLA antigens on renal cancer cells, as with melanoma cells, may induce autologous CTL (14). These data strengthen the association between HLA and renal cancer by suggesting that tumor-specific mutations of HLA molecules are an impetus for CTL influx. Loss of HLA expression, as seen more often in advanced tumors, is a detriment to immunological surveillance (15–19).

When Nabel et al. (20) introduced into melanoma cells in vivo a gene encoding an allogeneic MHC class I antigen, HLA-B7, there were implications for renal cancer, as well. They showed that expression of allogeneic MHC antigens on tumor cells stimulated immunity against both the transfected cells, as well as previously unrecognized antigens present in unmodified tumor cells. In a preliminary trial of five patients with malignant melanoma treated with intratumoral gene injections via a DNA/lipid complex, evidence of gene transfer (HLA-B7 expression detected by PCR and confirmed by immunohistochemical staining with a monoclonal antibody) was found on biopsy of the injected tumors in four of five patients. Two patients, where cell lines were established from the tumor, showed a cytolytic T cell response by lysing autologous tumor cells. One of the five patients had a partial remission involving cutaneous and visceral disease (20). These data suggested that tumor cell modification with the HLA-B7 gene not only stimulated CTLs and potentially other immune system cells, but that it also could stimulate immunity toward cells that express tumor-associated antigens in association with the patient’s own HLA antigens.

Based on the above information, we designed a Phase I trial to investigate the safety, feasibility, and transfection efficacy of intratumoral injection of a lipid-formulated plasmid DNA en-
coding for the MHC HLA-B7 gene product into metastatic renal cancer lesions. We also hypothesized that transfer of HLA-B7 expression would prime T cells to react to this new antigen on tumor cells. As such, presence of TILs and injected tumor/overall clinical response were secondary endpoints.

PATIENTS AND METHODS

Patient Selection. Patients were eligible if they had histologically confirmed metastatic renal carcinoma. Two metastatic lesions, or one metastatic lesion and an intact primary tumor, clearly measurable in two dimensions on CT scan, were required. One metastasis must have measured at least 2.0 cm in greatest diameter and be accessible for intralesional injection. Patients must have been unresponsive to prior standard therapy or have made the decision that other therapy would not be of any benefit. Patients must have been at least 18 years of age, have a Karnofsky Performance Status >70, have an estimated life expectancy >12 weeks, and be HLA-B7 negative. Patients must have agreed to use effective contraception if they were of reproductive potential. In addition, the following laboratory parameters must have been met within 15 days of registration: WBC >3,000/mm³, platelet count >100,000/mm³, hemoglobin >9 g/dl, prothrombin time <1.5× control, serum creatinine <2.0 mg/dl, total bilirubin <1.5 mg/dl (unless due to Gilbert’s disease), and aspartate aminotransferase/alanine aminotransferase <3× the upper limit of normal (unless due to disease). All patients signed a written, informed consent.

Exclusion criteria included positive antibody to HIV, active autoimmune disease, acute or chronic active hepatitis, pregnancy, active infection, or diabetes mellitus not controlled by medical treatment. Patients may not have received radiation, biological therapy, or chemotherapy within 4 weeks (6 weeks medical treatment. Patients may not have received radiation, including interstitial brachytherapy or external beam therapy, or investigational agents within 4 weeks. An adequate recovery interval must have been allowed so as to maximize contact between the study drug and mixed well by repeated inversion. The final concentration of the lipid vial were then transferred into the DNA vial and mixed well by repeated inversion. The final concentration of the DNA vial was 500 μg/ml. All components were stable for at least 8 weeks under recommended storage conditions (DNA, 20°C; DMRIE/DOPE, 4°C). DMRIE/DOPE has been shown in various preclinical models, including the RENCA mouse model, not to have any antitumor effect by itself (46).

Treatment Evaluation. Pretreatment evaluation consisted of a history, physical examination, chest radiograph, electrocardiogram, blood chemistries and urinalysis, tumor measurement (via physical examination, chest X-ray, ultrasound, and/or CT scan) and tumor biopsy guided by ultrasound. Blood was also tested for HLA-B7 phenotype and antinuclear antibodies, as described elsewhere (21). Evaluation of tumor progression was a secondary end point in this clinical trial. During therapy patients were evaluated in the Hematology/Oncology clinic at The University of Chicago Hospital at least every 4 weeks while receiving treatment, every 8 weeks until evidence of tumor progression, and then at 8–12-week intervals thereafter, as long as the patient was able. Evaluation consisted of history, physical examination, blood work, tumor measurement, and tumor biopsy for HLA-B7 gene expression.

Treatment. Because of the concern for inducing HLA-B7 positivity in previously negative patients and resultant side effects, a dose-escalation design was used that set a low threshold for determining a MTD. MTD was defined as the highest dose producing WHO grade two toxicities in less than two of six patients, as per the published protocol (44). Eligible patients were enrolled and treated on one of two schedules. On schedule A, the first group of three patients, group A-1, received a single injection of 10 μg of DNA on day 1. This group was then followed for 30 days to assess toxicity before the next group of three patients, group A-2, was treated at the next higher dose level (50 μg). The same procedure was then followed for the A-2 group before proceeding to the next and highest dose level, 250 μg, with group A-3. Schedule B was to be initiated in new patients after all three patients in the A-2 group had been observed for 30 days and found not to have unacceptable toxicity (WHO grade III or IV). On schedule B, the first group of three patients, group B-1, received 10 μg of DNA on days 1 and 15. If at 30 days no patient had unacceptable toxicity, a second group of three new patients (group B-2) received 10 μg of DNA on days 1, 15, and 30.

Identification and localization of a specific metastasis for biopsy/injection using ultrasound (or CT scan if not visible by ultrasound) was carried out by the Department of Diagnostic Radiology at The University of Chicago. Tumor lesions were selected for treatment if they were accessible to intralesional administration by direct needle injection. Sonographic visualization aided drug injection when needed, and multiple injections (of a constant amount) into a single tumor site were allowed so as to maximize contact between the study drug and the tumor cells. Immediately after injection, a blood sample was obtained to check serum enzymes, blood chemistries and cell counts.

HLA-B7 Gene Expression Determination. Tumor biopsy was done before treatment (on day 1), at days 15 and 30, and approximately every 8 weeks thereafter. Biopsy tissue was assayed for the presence of the HLA-B7 gene by PCR amplification (to determine the presence of HLA-B7 DNA) and RT-PCR (to determine the presence of HLA-B7 mRNA). Expression of the gene protein product was determined through flow cytometry and immunohistochemically via staining with anti-

3 The abbreviations used are: DMRIE, 1,2-dimyrystilloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide; DOPE, dioleoyl phospahtidylethanolamine; MTD, maximum tolerated dose; TIL, tumor-infiltrating lymphocyte; RT-PCR, reverse transcription-PCR.

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HLA-B7 antibodies ME-1, BB7.1, and GSP5.3, as described in detail elsewhere (21).

Assay for Tumor-infiltrating Lymphocytes. When sufficient materials were present, attempts to assess the development of cytolytic T-cell response to tumor cells were made. Tissue was frozen in liquid nitrogen and sent to a central laboratory facility at The University of Arizona Cancer Center (Tucson, AZ). Microscopic inspection of biopsies was performed and judged on a relative scale (from 0 to 4+) concerning the presence of tumor-infiltrating T cells, with appropriate positive and negative controls as described elsewhere (21).

RESULTS

Patient Characteristics. Fifteen patients with histologically confirmed metastatic renal carcinoma were enrolled on study and treated with intratumoral gene injection. Table 1 portrays a summary of patient characteristics.

Toxicities. Serious systemic toxicities (grade III or IV) related to the study drug were not observed in any patient. There was one grade II increased antinuclear antibody felt to be probably drug related. The patient had neither symptoms referable to joints nor clinical signs of autoimmune disease, and follow-up measurement of the antinuclear antibody was not obtained due to the patient’s deteriorating malignancy. There were several grade I or II toxicities, including pain and/or bleeding at the biopsy/injection site, fatigue, anemia, and nausea, for which symptomatic therapy only was administered. One patient experienced grade II pain and bleeding complications from tumor injection and chose to discontinue therapy. The MTD was not achieved according to the defined criteria. Nonetheless, administration of the lowest dose of the study drug, 10 μg, was safe and capable of inducing expression of the gene product (vide infra). This lack of ability of higher Allovectin-7 doses to induce tumor regression or to increase HLA-B7 expression was discussed by the collective investigators who agreed that further attempts to deliver doses of HLA-B7 beyond 250 μg into renal cancer lesions was not likely to be beneficial. Therefore, the trial was stopped.

Efficacy of Gene Transfer. Demonstration of gene transfer was conclusively demonstrated in 8 of 14 patients (57%). There was a patient in the 250 μg group who had a positive PCR result at week 8 with no other positive results to confirm gene expression. Another patient in the 10 μg × 2 group had only one positive RT-PCR result at week 6 with no other confirmatory positive results. Lastly, a third patient in the 10 μg × 3 group had a single positive PCR result at day 29 with other measurements of gene expression negative or not able to be performed. These patients were considered not to have conclusive evidence of HLA-B7 expression. One patient withdrew after 4 weeks on study due to bleeding complications and was not, therefore, able to be included in the analysis. Table 2, thus, summarizes the efficacy of Allovectin-7 in bestowing gene expression as measured by various means. Plasmid DNA was detected in 6 of 14 tumors (43%), and mRNA was detected in 2 of 14 tumors (14%) after gene injection. HLA-B7 protein expression was detected in 4 of 12 tumors (33%) by flow cytometry and 4 of 6 tumors (66%) by immunohistochemical staining.

A total of 8 of 14 tumors (57%) were positive for expression of HLA-B7 by at least one method.

TIL Activity. Only three patients were able to have tumor biopsies analyzed for the presence of TILs. All three patients had no evidence of gene expression and no evidence of TILs on pretreatment biopsy. Two patients (one in the 10 μg group and one in the 250 μg group) developed 1+ TIL activity at day 30 and day 15, respectively. The first patient died of rapid progressive disease, and the second patient had a stable injected tumor, but progressive systemic disease. Neither patient had subsequent TIL measurement. The third patient (in the 10 mg group) developed 1+ TIL activity at day 30 that increased to 3+ at week 8. This patient had stability in both the injected tumor and overall disease.

Clinical Results. Of the 14 patients assessed for a clinical response, none achieved any significant reduction of tumor burden. As seen in Table 3, median change in tumor size was +12% (range, −21% to 67%). One patient did not have a posttreatment measurement because of death due to progressive disease. Seven patients experienced stable disease while on study, whereas the remaining eight patients had progression of disease resulting in four deaths. The posttreatment tumor measurements were taken at a median of 9 weeks after pretreatment measurements (range, 4–12 weeks). The median survival of all 15 patients was 31 weeks from the date of first treatment.

DISCUSSION

The vast majority of malignancies arise in immunocompetent hosts, suggesting that tumor cells escape normal host de-
fenses by a wide variety of mechanisms. One such mechanism may include deficient expression of MHC class I antigens, resulting in lack of CTL stimulation. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (22–25). The resulting MHC antigen deficiency, seen in a wide variety of solid tumors, is associated with tumor invasiveness (26, 27). However, only 18% of renal cancers in a study by Gastl et al. (28) demonstrated partial or complete loss of MHC class I antigen expression, and these were found only in metastatic specimens (28). Several tumor cell lines that exhibit low levels of class I MHC proteins become less oncogenic when the relevant class I MHC antigen gene is introduced into them (29–31). Furthermore, tumor cells that express a class I MHC gene confer immunity in naive recipients against the parental tumor (32, 33). However, the absolute level of class I MHC expression is not the only factor that influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression, did not display altered tumorigenicity compared with the parent line (34). These studies collectively demonstrate that deficient MHC class I expression may be one of many mechanisms by which tumor cells escape normal host immune surveillance. Correction of this deficiency may only partially increase host immune response against tumors.

Direct gene transfer of the deficient MHC class I gene would be an efficient method of correcting such a deficiency and potentially controlling local tumor growth and spread. The introduction of recombinant genes directly into tumors in vivo eliminates the need to establish cell lines from each patient and, thus, avoids costly delays. Early studies focused on the demonstration that specific reporter genes could be expressed in vivo (35, 36). Subsequent studies were designed to determine whether specific biological responses could be induced at sites of recombinant gene transfer. The human MHC HLA-B7 gene was used to elicit an immune response in the iliofemoral artery using a porcine model (37). Expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall and induced a granulomatous mononuclear cell infiltrate response at the sites of genetic modification. This response began 10 days after introduction of the recombinant gene and resolved by 75 days after gene transfer. A specific cytolytic T cell response against the HLA-B7 molecule, however, was persistent. This study provided one of the first indi-

### Table 2 Detection of HLA-B7 expression in tumor biopsies

<table>
<thead>
<tr>
<th>Dose (µg) of HLA-B7 DNA</th>
<th>DNA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>mRNA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Protein expression (FACS)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Protein expression (IHC)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Total (positive for one or more)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1/3</td>
<td>1/3</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>50</td>
<td>2/3</td>
<td>1/3</td>
<td>1/2 (1 N/A&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>1/3</td>
<td>2/3</td>
</tr>
<tr>
<td>250</td>
<td>2/3</td>
<td>0/3</td>
<td>1/3</td>
<td>All 3 N/A</td>
<td>2/3</td>
</tr>
<tr>
<td>10 × 2</td>
<td>1/3</td>
<td>0/3</td>
<td>0/3</td>
<td>All 3 N/A</td>
<td>1/3</td>
</tr>
<tr>
<td>10 × 3</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1 (1 N/A)</td>
<td>All 2 N/A</td>
<td>0/2</td>
</tr>
<tr>
<td>Total</td>
<td>6/14 (43%)</td>
<td>2/14 (14%)</td>
<td>4/12 (33%)</td>
<td>4/6 (66%)</td>
<td>8/14 (57%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> DNA expression determined via PCR.

<sup>b</sup> mRNA expression determined via RT-PCR; rapid degradation of message may lead to negative results.

<sup>c</sup> Immunohistochemical staining of tumor biopsies for HLA-B7 expression.

<sup>d</sup> Assay of tumor biopsy not performed because of lack of sufficient tissue.

### Table 3 Clinical results

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Injected lesion site</th>
<th>Dose (µg)</th>
<th>Tumor size pre-tx. (mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Tumor size post-tx. (mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Delta size</th>
<th>Injected tumor status</th>
<th>Disease status (while on study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.c.</td>
<td>10</td>
<td>1980</td>
<td>2400</td>
<td>21%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>Lung</td>
<td>10</td>
<td>10400</td>
<td>&gt;10400&lt;sup&gt;g&lt;/sup&gt;</td>
<td>-50%</td>
<td>P&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>Pancreas</td>
<td>10</td>
<td>2750</td>
<td>2400</td>
<td>-13%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>S+</td>
</tr>
<tr>
<td>4</td>
<td>Rib</td>
<td>50</td>
<td>2100</td>
<td>2100</td>
<td>0%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Renal fossa</td>
<td>50</td>
<td>13200</td>
<td>9520</td>
<td>-21%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>s.c.</td>
<td>50</td>
<td>1200</td>
<td>1200</td>
<td>0%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>Pleura</td>
<td>50</td>
<td>5900</td>
<td>4750</td>
<td>-19%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>S</td>
</tr>
<tr>
<td>8</td>
<td>Pancreas</td>
<td>250</td>
<td>1500</td>
<td>2100</td>
<td>-40%</td>
<td>P&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>9</td>
<td>Node</td>
<td>250</td>
<td>624</td>
<td>550</td>
<td>-12%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>10</td>
<td>Renal fossa</td>
<td>250</td>
<td>16150</td>
<td>17100</td>
<td>6%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>S</td>
</tr>
<tr>
<td>11</td>
<td>Liver</td>
<td>10 × 2</td>
<td>24600</td>
<td>28000</td>
<td>14%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>12</td>
<td>Kidney</td>
<td>10 × 2</td>
<td>15600</td>
<td>19500</td>
<td>25%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>13</td>
<td>Renal fossa</td>
<td>10 × 2</td>
<td>1680</td>
<td>2800</td>
<td>67%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>14</td>
<td>Kidney</td>
<td>10 × 3</td>
<td>4550</td>
<td>5025</td>
<td>10%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>15</td>
<td>Liver</td>
<td>10 × 3</td>
<td>10260</td>
<td>12850</td>
<td>25%</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>P</td>
</tr>
</tbody>
</table>

<sup>f</sup> S, stable disease; P, progressed on therapy; +, alive with disease currently.

<sup>g</sup> Tumor unable to be assessed accurately after injection due to death from progressive disease.

<sup>c</sup> Patient withdrew after 4 weeks on study due to bleeding complications.
cations that direct gene transfer of a specific recombinant gene could induce an immunological response to the products of that gene at a specific site in vivo.

To determine whether direct gene transfer might be appropriate for the treatment of cancer, a murine model was used. Direct gene transfer of an allogeneic MHC gene into a murine tumor elicited an immune response not only to the foreign MHC gene products, but also to previously unrecognized tumor-associated antigens. These immune responses were T cell dependent, and these tumor-associated proteins were recognized within the context of the self-MHC. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (38). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors has potentially therapeutic effects on injected and distant tumors.

This technology evolved with an improved cationic lipid formulation, DMRIE/DOPE, which allows for 2- to 7-fold greater transfection efficiency in vitro versus the formulation used by Nabel et al. (45). Also, the new formulation did not aggregate at high concentrations, allowing 10–100 times more DNA to be introduced. Inclusion of the β-2 microglobulin gene allowed increased expression of the complete HLA-B7 histocompatibility molecule. Lack of β-2 microglobulin gene expression, as occurs on some human malignant melanoma cells, limits the ability of cells to stably express the complete I molecule on the cell surface and, thus, may contribute to resistance to the desired immune response (41–43).

This Phase I study demonstrated that intratumoral injection of plasmid DNA is safe, feasible, and associated with only local toxicity. Transfection of tumor cells was demonstrated by detection of HLA-B7 expression in tumor biopsies in 8 of 14 patients. Evidence for biological response was difficult to evaluate secondary to insufficient quantity of tissue to assess presence of TILs. However, the three patients with TIL measurement after treatment did have an increase in activity, presumably due to gene transfer because the tumors did not have evidence of TILs before treatment. As well, the patient with the most TIL activity had stability in injected tumor and overall disease. Importantly, however, correlation of gene expression with a specific T cell response against the protein product of that gene can not be conclusively made given the paucity of TIL data. Future studies must determine whether the gene expression transferred by this amount of HLA-B7 DNA is capable of inducing a specific T cell response that translates into clinical benefit.

There were no significant clinical responses in our study group. The University of Arizona Cancer Center has conducted an identical trial involving intratumoral injection of the study drug into metastatic melanoma lesions. Of 14 assessable patients, 7 had measurable tumor shrinkage, with 5 of 7 patients showing >50% decrease of the injected tumor nodule, with no responses occurring in noninjected lesions. Responses occurred in patients in all dosage and injection schedule groups, and there were no laboratory or clinical parameters that correlated with response (21). Another Phase I trial of Allovecin-7 was conducted at the University of Cincinnati Medical Center (Cincinnati, OH) in patients with advanced squamous cell carcinoma of the head and neck. Four of the nine evaluable patients had significant tumor shrinkage resulting in durable partial responses, one of which was a near complete remission that continues more than 17 months after gene therapy (39). The clinical responses observed may be due to the inherent susceptibility of melanoma or squamous cell carcinoma tumors of the head and neck to host antitumor response or other as yet undefined factors. The lack of clinical response in our study group may reflect differing T cell responsiveness to ‘natural’ expression of HLA-B7 and tumor cells modified to express this molecule. This difference may be due to the need for other costimulatory molecules besides β-2 microglobulin or other requirements in the T cell/tumor cell interaction, such as the need for other treatments (i.e., concurrent cytokines or T cell infusion) to boost the immune response. Also, identification of specific antigens associated with renal carcinoma, as in melanoma, could further our understanding of how to better stimulate an immune response. Finally, the small number of patients in this preliminary study may not be sufficient to show benefit, especially in certain subgroups as yet undefined.

The knowledge gained via these and other trials is a requisite step toward the ultimate goal of stimulating a more specific host antitumor response via in situ manipulation of tumor cells. This approach would hopefully lead to an improved clinical response to immune modulating agents and reduce the use of nonspecific systemic therapy and its inherent toxicity. Vical Inc. has recently developed a plasmid DNA encoding the interleukin-2 gene complexed with the DMRIE/DOPE lipid mixture. This product, Leuvectin, has undergone Phase I testing successfully, and Phase II testing is underway. In the Phase II trial, 14 renal cancer patients were treated and 2 patients experienced clinical partial responses of the injected lesions persisting beyond 13 months (40). These early results, if confirmed, may suggest the usefulness of gene-based immunotherapy. The use of Allovecin-7 in combination with Leuvectin or other immunomodulating agents may be warranted and is under consideration for patients with renal cancer.

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