Intratumoral injection of IL-12 plasmid DNA – results of a phase I/II clinical trial

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Effective eradication of established tumor and generation of a lasting systemic immune response are the goals of cancer immunotherapy. The objective of this phase I/II study was to assess the safety and toxicity of treatment to metastatic tumor underlying the skin with the DNA encoding interleukin-12 (IL-12). This treatment strategy allowed the patient's own tumor to serve as a source of autologous antigen in the tumor microenvironment. We proposed that IL-12 protein produced by the transfected cells would result in the generation of both a local and systemic antitumor response. The tumor was treated with either three or six intratumoral injections of plasmid containing IL-12 DNA. This treatment strategy resulted in no significant local or systemic toxicity. The treatment did not result in an increase in serum IL-12 protein. The size of the treated lesion decreased significantly (greater than 30\%) in five of the 12 patients. However, nontreated subcutaneous lesions or other disease did not decrease in size.

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Introduction

Effective treatment of malignancies with cytokines has been a goal of many previous clinical trials. An immune response can be generated by the systemic administration of cytokine protein such as interleukin-2 (IL-2), IL-12 or interferon, but the systemic response is both nonspecific and can be associated with significant toxicity. Cytokine gene therapy has been demonstrated to be an effective method for delivery of continuous drug to the tumor microenvironment in murine models. The translation of animal models of cytokine gene therapy into effective human clinical trials, however, has been elusive. The overriding goal of all cytokine trials has been effective delivery of an adequate dose of cytokine to the treated tumor with acceptable toxicity. Many previous cytokine trials have focused on the treatment of patients with significant tumor volume. Cytokine therapy, unlike cytotoxic chemotherapy, may require a small tumor volume to effectively eradicate tumor. We have focused this study on the treatment of patients with relatively small volume disease.

In previous comparative studies, interleukin-12 (IL-12) was the most effective single cytokine in animal tumor models.\textsuperscript{1} IL-12 is a disulfide-linked heterodimer consisting of a p35 and p40 unit. Expression of both proteins is necessary for the action of IL-12. This drug was able to prevent the development of metastases and was able to elicit long-term antitumor immunity.\textsuperscript{2,3} Depending on the tumor model, IL-12 can effect antitumor activity via T cells,\textsuperscript{4,5} natural killer cells \textsuperscript{6-10} or NK T cells.\textsuperscript{11} IL-12 induces other cytokines such as interferon-\gamma (IFN-\gamma)\textsuperscript{12,16} and IFN-inducible protein-10.\textsuperscript{17} IL-12 also exerts an antiangiogenic effect which may also inhibit both metastatic and local tumor growth.\textsuperscript{5,13,14} The systemic administration of IL-12 protein for the treatment of tumor has been studied in both murine models and phase I clinical trials.\textsuperscript{12,19,9,20-22} Systemic IL-12 protein therapy, however, has been associated with dose-limiting toxicity.\textsuperscript{23,24} This toxicity has limited the dose of the drug, and thus the local concentration of IL-12 in the tumor microenvironment.

Many different cytokine cDNA delivery methods have been investigated in preclinical and clinical models. Retroviral vectors have relatively low transfection efficiencies and when given systemically can be toxic.\textsuperscript{25,26} Recombinant adenoviral vectors have been utilized for IL-12 delivery into tumors.\textsuperscript{9,20,26} High doses of systemic adenovirus, however, have been limited by systemic toxicity.\textsuperscript{26,27,29,30} Direct injection of IL-12 cDNA into a tumor may avoid the toxicity of systemic IL-12 protein therapy while still allowing the generation of a sustained tumor-specific systemic immune response.\textsuperscript{2,19,31-37} We have focused our efforts on local expression of IL-12 in the tumor microenvironment after delivery by a nonviral
vector. Nonviral DNA can be inserted into the tumor microenvironment either as plasmid DNA or as DNA complexed to liposome. Limited toxicity has been associated with this type of administration.25.36–40,38.41–43

Intratumoral injection may generate not only a local immune response but also a systemic immune response.29,38,43 We have demonstrated previously in a murine model that delivery of IL-12 cDNA to the tumor microenvironment of established adenocarcinoma resulted in tumor regression.34 This antitumor response also resulted in protection of the animal from subsequent tumor challenge with the same tumor, but not a different type of tumor. We have compared both the injection of naked DNA and the injection of IL-12 cDNA complexed to cationic lipids and found no difference in antitumor efficacy in a murine model. In this study, we treated subcutaneous tumor with repeated injections of IL-12 cDNA. We previously demonstrated in a murine toxicity study that there was no toxicity associated with this regimen. We further demonstrated that a dose of 50 μg per treatment was necessary and sufficient for tumor eradication that multiple treatments were more effective than a single treatment, and that complex with lipid had no impact of antitumor efficacy. IL-12 protein was not detectable systemically in any of our murine models. The dosage of IL-12 cDNA and the schema for treatment in the current report was chosen to mirror our murine studies. Although the human is obviously larger than the mouse, the tumors we targeted were similar in size to our murine models.

Materials and methods

Study design

Written informed consent was obtained according to institutional and federal guidelines. Twelve adult patients were enrolled sequentially and treated with intratumoral injection of human IL-12 DNA. Pretreatment biopsy confirmed the diagnosis of malignancy. Eligible patients had cutaneous or subcutaneous metastases (between 0.5 and 2.5 cm in size) with no available effective standard therapy. There was no restriction to entry based on tumor type. Patients could not have received chemotherapy or immunotherapy within the last 60 days. Other relevant inclusion and exclusion criteria included: Eastern Cooperative Oncology Group performance status of 0 or 1, life expectancy greater than 3 months, WBC > 3000/μl, platelets > 80 000/μl, creatinine < 2.0 mg/dl and bilirubin < 2.0 mg/dl. Patients with active infections requiring treatment including HIV infection were ineligible. No patients with organ allografts were allowed. Those patients with a history of treatment with steroids (other than intermittent use as antiinflammatories or as topical agents) in the last month or an ongoing requirement for steroid therapy were excluded. Patients with evidence of an active second malignancy were not eligible. Pregnant or lactating women and men refusing to use measures to prevent pregnancy while receiving treatment were excluded. Concurrent treatment with antineoplastic therapy, colony-stimulating factors or other investigational drugs was not allowed during the vaccine treatment course.

If two subcutaneous tumors were present, one was treated (designated lesion 1) and one was observed (designated lesion 2). These lesions were left in situ to observe the local and systemic effect of the treatment. If three or more subcutaneous masses were present we planned to disperse a nontreated tumor for delayed-type hypersensitivity (DTH) testing. However, none of the 12 patients in this study had more that two accessible tumors, thus DTH testing with autologous tumor was not performed.

IL-12 tumor treatment was performed by a dedicated study nurse. Two treatment strategies were employed in two groups of six patients each. Treatment strategies between groups differed only by the number of drug administrations per patient. Patients were treated sequentially first in group 1 (patients 1–6) then group 2 (patients 7–12). Group 1 patients received treatments three times a week on days 1, 3 and 5. If the patient had a local response or stable disease during the first course of therapy, then a second course of three injections was performed beginning on day 28. Group 2 patients received 2 weeks of treatment 3 times a week on days 1, 3, 5, 8, 10 and 12. All injections were to the same lesion. Patients with stable disease or responding disease were eligible for retreatment with the same regimen (six treatments over 12 days) beginning on day 28.

Evaluation of toxicity and response

Pre-study physical exam, lab work and disease assessment were performed including IL-12 and IFN-γ serum levels. Patients were observed for local and systemic toxicity on each day of treatment, 1 week following treatment, and day 29 after the vaccination. Restaging of the patient’s disease and long-term toxicity evaluation were performed on day 29, day 55, and at 3, 6 and 12 months. Serum IL-12 levels were evaluated on injection days 1, 3, 5, and day 12 from the first injection. Serum anti-DNA antibody levels (anti-double strand, anti-single strand and anti-nuclear antibody) were evaluated before entry, day 29, and at 3, 6 and 12 months.

Response to drug administration at the site of cutaneous or subcutaneous lesions was defined by measurement of percentage change in the longest diameter of the lesion. To be assigned a status of stable disease, the target lesion must have remained stable at day 29. Stable disease was defined (based on RECIST criteria) as neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.44 Partial response was defined as greater than 30% decrease of the longest diameter of the target lesion, taking as reference the baseline sum longest diameter with no increase in other disease that met criteria for progressive disease. Progressive disease was defined as a 20% increase in the sum of the longest diameter of the target or nontarget lesion, taking as reference the smallest sum longest diameter recorded since the baseline measurement, or the appearance of one or more new lesions.
The disappearance of all target lesions would yield a status of complete response.

The primary objective of the study was to determine toxicity. Local and systemic toxicity were evaluated according to the National Cancer Institute’s Common Toxicity Criteria Version 2.0, Final 1/30/1998. Local toxicity at the injection site was defined as erythema, local pain, warmth and/or skin ulceration or pruritus. Local toxicity was graded as follows: grade 1, erythema or induration <20 mm; grade 2, erythema or induration 20 mm, but no ulcer; grade 3, <1 cm ulcer or painful regional adenopathy requiring narcotic pain relief; grade 4, ulcer >1 cm or permanent dysfunction related to local toxicity. Systemic toxicities were monitored by patient report of constitutional symptoms, and laboratory evaluations of blood dyscrasias, electrolyte imbalances and liver function studies.

IL-12 cDNA

The human IL-12 DNA expression plasmid vector utilized in this protocol pNGVL3-mIL12 was developed by Dr Gary Nabel of the National Gene Vector Lab (University of Michigan). The hIL-12 p35 and p40 coding regions were amplified via polymerase chain reaction (PCR) using wild-type human DNA as a template. The amplified DNA was subcloned into a pUC19 vector containing the cytomegalovirus (CMV) intermediate early promoter, intron A, and a bovine growth hormone (BGH) polyadenylation region. The IL-12 p35 and p40 subunits were separated by an internal ribosomal entry site and driven by a single CMV promoter. Plasmid DNA was purified in the absence of ethidium bromide or penicillin derivatives by using a commercially available column chromatography method according to manufacturer’s protocol (Qiagen, Chatsworth, CA). The activity of this plasmid was confirmed by detection of IL-12 protein after in vitro transfection of human tumor cells. The IL-12 vector was manufactured by the national gene vector lab and distributed by the pharmaceutical management branch, Cancer Center Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute.

Intratumoral administration of IL-12 cDNA. The dose of IL-12 plasmid cDNA per injection was identical to the effective dose utilized for treatment of murine tumors of up to 1 cm. Each treatment involved a single injection of 50 mcg of plasmid in 0.76 ml of saline. Injections were performed directly into the tumor and the tumor/skin interface with a 27-gauge needle. No escalation of tumor dose per injection was allowed as this was the first utilization of this cDNA in a clinical trial. The second cohort of patients however received twice as many injections of the plasmid over a 2-week course.

Cytokine production

Serum was collected for evaluation of IL-12 and IFN-γ on days 3, 5, 8 and 15 in group 1 and days 3, 5, 8, 10 and 17 in group 2. A commercially available ELISA kit was used for these evaluations.

Table 1 Patient demographics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Tumor type</th>
<th>Location</th>
<th>Other immunotherapy</th>
<th>Other tumor</th>
<th>Time from dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>76</td>
<td>MM</td>
<td>E</td>
<td>N</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>F</td>
<td>72</td>
<td>MM</td>
<td>E</td>
<td>N</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>72</td>
<td>RCC</td>
<td>H&amp;N</td>
<td>Y</td>
<td>Intraabdominal, brain</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>32</td>
<td>MM</td>
<td>T</td>
<td>Y</td>
<td>N</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>71</td>
<td>MM</td>
<td>H&amp;N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>53</td>
<td>MM</td>
<td>T</td>
<td>N</td>
<td>Lung</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>59</td>
<td>MM</td>
<td>T</td>
<td>N</td>
<td>Lung, brain</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>47</td>
<td>MM</td>
<td>T</td>
<td>Y</td>
<td>Lung, axilla</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>72</td>
<td>MM</td>
<td>T</td>
<td>Y</td>
<td>Lung</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>73</td>
<td>MM</td>
<td>E</td>
<td>N</td>
<td>N</td>
<td>18</td>
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<tr>
<td>11</td>
<td>M</td>
<td>75</td>
<td>MM (ocular)</td>
<td>H&amp;N</td>
<td>N</td>
<td>Lung, bone</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>24</td>
<td>MM</td>
<td>T</td>
<td>Y</td>
<td>Small bowel, oral</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male.
Tumor type was either malignant melanoma (MM) or renal cell carcinoma (RCC). Location of the treated lesion was extremity (E), head and neck (H&N) or trunk (T). Five patients had treatment with other immunotherapy all before study entry. All patients had subcutaneous disease; seven had other disease at the time of study entry. The time (in months) from initial demonstration of metastatic disease to study entry varied from 1 month to more than 3 years.

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Table 2 Toxicity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment</th>
<th>Local toxicity</th>
<th>Systemic toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Two courses of three injections</td>
<td>Grade 1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>One course of three injections</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>One course of three injections</td>
<td>Grade 1</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Two courses of three injections</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>One injection</td>
<td>Grade 4 pulmonary embolism</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>One course of three injections</td>
<td>Grade 1 night sweats</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>One course of six injections</td>
<td>None</td>
<td>Grade 1 nasal congestion</td>
</tr>
<tr>
<td>8</td>
<td>One course of six injections</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>One course of six injections</td>
<td>Grade 1 diarrhea</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>Two courses of six injections</td>
<td>None</td>
<td>Grade 1 dyspepsia/ fatigue</td>
</tr>
<tr>
<td>11</td>
<td>Two courses of six injections</td>
<td>None</td>
<td>Grade 1 fatigue</td>
</tr>
<tr>
<td>12</td>
<td>Two courses of six injections</td>
<td>None</td>
<td>Grade 1 pain tachycardia</td>
</tr>
</tbody>
</table>

Local toxicity was evaluated at the site of IL-12 cDNA treatment. No patient had any laboratory evidence of systemic toxicity (elevated anti-DNA antibodies, electrolyte, coagulation or hematologic changes).

day of his first treatment. He was removed from the study, although the pulmonary embolus was not felt to be related to the treatment. This subject experienced a second adverse event (tracheal bronchitis and hemoptysis secondary to tracheal bronchitis). This adverse event, like the first, was thought to be unrelated to the study drug. We were unable to detect IL-12 or IFN-γ in the serum of any of these patients at any time point tested. We also did not detect anti-DNA antibodies in any patient at any time point. No patient had more than two treatable lesions, thus evaluation by DTH testing with autologous tumor was not feasible.

**Antitumor response**

There were no complete tumor responses (Table 3). Three of six group 1 subjects had stable disease after the first cycle of therapy (day 29). Two of these subjects with stable disease then received a second series of three injections of IL-12 cDNA spaced identically. One subject (patient 2) with stable disease declined further therapies due to logistical problems with travel to our facility. Two subjects were found to have progressive disease following day 29 and protocol therapy was discontinued. One of these subjects had radiological evidence of new pulmonary disease. This finding was later identified pathologically as a second primary tumor (lung cancer) rather than progression of melanoma. One subject in group 1 received only one injection. This patient suffered an adverse event (pulmonary emboli) several hours after his first IL-12 cDNA injection. Although the event was not thought to be related to the study drug, he was removed from the study and received no subsequent investigational agent. Of the two patients in group 1 who went on to have a second series of injections, one maintained stable disease (patient 3) and one had progressive disease at day 55.

Three of six group 2 subjects were found to have progressive disease on day 29 of therapy and were not retreated. Three subjects had either stable disease or a decrease in tumor size on day 29 and received a second series of six injections of cDNA. On day 56 of therapy, one patient had progressive disease, and two had stable disease. One of those subjects (patient 11) had progressive disease at a distant site, although there was a partial response at the injected site.

Five patients were alive at last follow-up. Only one of these five patients did not have progressive disease (patient 4). Although serial biopsy was not required for this study, two patients (patients 3 and 4) underwent resection of the treated tumor on day 43 (patient 3) and day 180 (patient 4). The tumor volume by palpation was similar to the pretreatment volume in patient 3 and was 43% larger in patient 4. The nodule in patient 4 was unchanged from the 3-month evaluation to the 6-month evaluation. Patient 4 was rendered disease free and has had no further recurrent disease 60 months after.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Lesion number</th>
<th>Change in diameter (%)</th>
<th>Total number of injections</th>
<th>Response</th>
<th>Local progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>-75</td>
<td>6</td>
<td>PD</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-75</td>
<td>80</td>
<td>PD</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-36</td>
<td>3</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>-43</td>
<td>6</td>
<td>PD</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>ND</td>
<td>1</td>
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<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-28</td>
<td>3</td>
<td>SD</td>
<td>N</td>
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<td>6</td>
<td>PD</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>-30</td>
<td>6</td>
<td>PD</td>
<td>Y</td>
</tr>
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<td>1</td>
<td>-11</td>
<td>6</td>
<td>PD</td>
<td>Y</td>
</tr>
<tr>
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<td>1</td>
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<td>12</td>
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<td>11</td>
<td>1</td>
<td>-80</td>
<td>12</td>
<td>SD</td>
<td>N</td>
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<tr>
<td>12</td>
<td>1</td>
<td>33</td>
<td>12</td>
<td>PD</td>
<td>Y</td>
</tr>
</tbody>
</table>

Abbreviations: IL-12, interleukin-12; ND, not determined; PD, progressive disease; SD, stable disease.

The response of both treated and nontreated subcutaneous lesions to local IL-12 cDNA therapy was determined by comparing the longest diameter of tumor before and after therapy. Lesion 1 is the treated lesion in all cases. Lesion 2 was not treated by local injection in all cases. Initial treatment for patients 1–6 consisted of three injections of IL-12 cDNA. Two of the six patients (1 and 4) were re-treated with three more injections. Initial treatment for patients 7–12 consisted of six injections of IL-12 cDNA. Patients 10–12 had six more injections. Response was determined on day 28 (patient 1–6) or day 54 (patient 7–12). Response was defined by RECIST criteria. Local progression at the 3-month evaluation Y – yes; N – no.
Figure 1  Local antitumor efficacy. Three patients (patients 1, 7, and 8) had more than one subcutaneous tumor. In each patient lesion 1 is the treated lesion and lesion 2 is the nontreated lesion. The relative effect of IL-12 cDNA injection on tumor area is shown. The impact of therapy is depicted as the percentage difference in area from the pretreatment volume. The pretreatment volume is normalized as 100% in all cases. Day 58 data are only available for patient 1.

treatment. No necrosis or lymphocytic infiltrate was noted in either specimen. Three of our patients (patient 2, 10, and 11) did not have local disease progression at the 3-month evaluation.

Despite the lack of a clinically relevant systemic partial response, the IL-12 DNA treatment did demonstrate local antitumor activity. Five of the 12 treated patients had a partial response (>30% reduction in area) in the treated tumor (Table 3). Three of these patients (patient 1, 11 and 12) were retreated (two had progression at other sites). The treated tumor continued to decrease after the second course of therapy in two of the three retreated patients. There was no decrease in the size of tumor at distant sites in any patient. In fact, for the three patients with a second subcutaneous lesion (patients 1, 7 and 8), a response was noted at the treated site in all three of these patients, but the tumor nodule at the second site either increased in size or was stable (Figure 1).

Discussion

We demonstrate that local injection of IL-12 cDNA can be safely used to treat subcutaneous tumor. We postulated that IL-12 gene therapy could potentially avoid the systemic toxicity associated with IL-12 protein immunotherapy. No evidence of any significant local or systemic toxicity was demonstrated in this study at the dose of IL-12 cDNA we evaluated. This confirms predictions of prior murine studies from our group and others of local tumor treatment with IL-12 DNA or cells engineered to express IL-12.2,3,19,27,33,34,36,45-49 We also demonstrate that this regime did not result in detectable systemic concentrations of IL-12 or IFN-γ. In previous murine studies, we were also unable to demonstrate systemic levels of IL-12 at the same dose evaluated in this study.50 Thus, the lack of detection of IL-12 in blood samples was not unexpected and is consistent with the lack of systemic toxicity we demonstrated in this study.

In contrast to our murine studies, however, no significant systemic antitumor effect was noted with this dose regimen. The reason for this lack of systemic antitumor effect is not clear. This may be a consequence of inadequate local levels of IL-12 protein. The dose of IL-12 was chosen to mirror our murine studies. We chose a dose for this clinical trial that was effective with tumors of somewhat similar size in mice, and that was recommended for safety considerations, based on murine safety data and on discussions with the FDA.50 It is possible that with a higher local dose of IL-12 we would have generated a stronger local effect and may have induced a systemic antitumor effect. We did not measure local IL-12 protein expression to confirm the presence and the amount of gene expression, although this was confirmed in vitro before this study. We were concerned that removal of the tumor/cytokine would remove the stimulus for a systemic response. We had previously demonstrated expression of the murine IL-12 protein in vivo in an experiment designed to mimic this clinical trial and thus felt there was little to be gained by repeatedly biopsying our treatment site. Many previous studies have also had difficulty demonstrating systemic antitumor efficacy in patients using regimens that have demonstrated systemic antitumor efficacy in murine transplanted syngeneic tumor models. The lack of a systemic effect may indicate that the cytokine-induced immune response shows differential efficacy in our murine models and in the clinical treatment of the tumors we elected to approach due to differences in the tumor or tumor/host relationships in the murine model and in our patients. Generally, the patients that we treated had cutaneous recurrences of melanoma that perhaps may not be the appropriate tumor target for this drug. The local tumor shrinkage, albeit transient, suggests however that IL-12 did have at least local antitumor efficacy in these patients with cutaneous recurrences of melanoma. We attempted to select patients with relatively small amounts of disease, but the systemic immune response may have still been overwhelmed by the tumor burden of these patients.

The treated lesion, however, did respond to intratumoral IL-12 cDNA therapy (>30% tumor shrinkage in five of the 12 patients treated). The local treatment generated a local antitumor effect without discernable toxicity. The three responding lesions that were retreated continued to decrease in size in all patients after the second course of treatment. Thus, despite a lack of systemic effect the local expression of IL-12 seems to result in an antitumor response in the tumor microenvironment. This could be due to either the ability of IL-12 to generate a tumor-specific (T-cell mediated) immune response, to less specific immune effects mediated by IL-12 responsive innate effectors or to the antiangiogenic effects of IL-12. We detected no correlation between tumor response to IL-12 cDNA and any of the
demographic or treatment data we evaluated (tumor location, tumor burden, prior immunotherapy, local toxicity, systemic toxicity or number of treatments).

One mechanism that injection of IL-12 cDNA may be inducing is due to the prolonged sustained slow release of IL-12 protein by the cells that have taken up the IL-12 cDNA. This same result has been achieved in murine models by the slow release into tissue of IL-12 encapsulated microspheres.\textsuperscript{21} We anticipate that similar clinical effects would be induced by intratumoral injection of IL-12 cDNA or IL-12 protein encapsulated microspheres.

In summary, we demonstrate that a dose of IL-12 cDNA with demonstrated efficacy in murine models can be administered without toxicity to subcutaneous tumor in humans. We demonstrated a local antitumor response in the treated lesion, but no systemic antitumor efficacy.

Abbreviations

IL, interleukin; IFN, interferon.

References


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