Phase I Trial of Large Multivalent Immunogen Derived from Melanoma Lysates in Patients with Disseminated Melanoma


1Center for Biological Therapy and Melanoma Research, University of California, San Diego, La Jolla, California; 2Lidak (now Avanir) Pharmaceuticals, San Diego, California; and 3Center for Immunology, University of Minnesota, Minneapolis, MN

ABSTRACT

Purpose: The purpose of this research was to determine the toxicity and immunological activity of large multivalent immunogen (LMI), a preparation of tumor cell membranes affixed to amorphous silica microbeads, in patients with melanoma.

Experimental Design: Nineteen patients with metastatic (stage IV) melanoma were entered into the study, of whom 15 received the full 3 months of treatment with LMI. LMI was administered without adjuvant, one-half intradermally (i.d.) and the other half s.c. Because we expected little toxicity, we first treated 2 patients at each dose level, 10-, 30-, or 100-million tumor cell equivalents on weeks 0, 4, and 8, and subsequently randomized the remaining 13 patients to receive treatment with one of those dosage schedules, for a total of 19 patients. Two patients who were registered were found to be ineligible because of brain metastases, and 2 others did not complete the course of treatment for reasons other than toxicity. Thus, 15 patients were fully evaluable. Patients with evidence of a clinical response (at least stable disease at the 12-week checkpoint) had the option of continuing treatment at 4-week intervals. Frequencies of cytolytic T cell precursors against HLA-A2 matched melanoma cells, and delayed-type hypersensitivity to a melanoma membrane preparation before or after treatment. No toxicity of any kind was observed. A degree of clinical effectiveness of LMI was suggested by the elicitation of stable disease in 5 patients at 12 weeks. One patient had >50% regression of a lung nodule but progression of disease to the brain, whereas a second patient had a bona fide partial remission of a 3-cm diameter solitary lung nodule.

Conclusions: LMI was nontoxic, improved immunological reactivity to melanoma cells, and showed evidence of clinical effectiveness (shrinkage of tumor) in 1 patient. Additional studies with LMI with added adjuvant materials, in melanoma and other cancers, appear warranted.

INTRODUCTION

Activation of CD8 T cells by subcellular antigen requires that the antigen be presented on particles of about the same size as cells. Effective in vitro generation of CTL responses or activation of cloned effector CTL was observed when plasma membranes or purified class I MHC proteins were immobilized on 5-μm diameter microspheres; the same antigens were ineffective when presented on smaller particles or in free form (1). Detectable CTL responses were not generated when this form of antigen, termed large multivalent immunogen (LMI), was administered in vivo. However, LMI administration to mice challenged with cells bearing the same antigen resulted in a substantial augmentation of the CTL response to the foreign cells (2). This augmenting effect required that the alloantigen on the LMI was the same as on the cells used for the challenge, and the effector CTL retained specificity for the antigen.

Similar results were obtained when responses to syngeneic tumors were examined, using plasma membranes isolated from the tumor cells to prepare the LMI. Administration of the LMI at the same time as tumor inoculation resulted in generation of detectable tumor-specific CTL activity and a reduction in tumor growth in comparison with untreated controls (2, 3). If administration of LMI was instead delayed until the tumor was established and progressing there was only a marginal effect on tumor growth. However, LMI, given together with low-dose cyclophosphamide, was highly effective in reducing growth of established tumors and extending survival in several tumor models, including fibrosarcomas in a lung metastasis model and P815 growing as a solid s.c. tumor (4). In contrast, free plasma membrane antigen was ineffective in these models when administered either alone or with adjuvant. A significant fraction of mice bearing progressing P815 tumor survived long-term after treatment with cyclophosphamide and LMI, and was immune to
rechallenge with the tumor. These findings suggested that LMI might have therapeutic effects in human tumors in cases where the appropriate plasma membrane antigens could be obtained in sufficient quantities to prepare LMI. This can be accomplished with melanoma cell lines as a source of antigen. Extensive clinical experience in administering antigens from these cell lines (5–8) provided the background for a clinical trial using LMI for antigen delivery.

Although the elucidation of melanoma-associated peptides (9–11) has led to a greater scientific understanding of the epitopes recognized by CTL, the use of individual peptides by themselves has thus far been insufficient for the most part to elicit either profound immunological or clinical responses. Whole cell-derived melanoma “vaccines” on the other hand elicit either profound immunological or clinical responses. Whole cell-derived melanoma “vaccines” on the other hand elicit either profound immunological or clinical responses.

The microscopic appearance of typical coated silica beads

Over the past 15 years, we have reported our experience with melanoma cell lysates stored frozen at −80°C (“frozen lysates”; Refs. 7, 8), as well as subsequent experience with Melacine, a lyophilized lysate production prepared by Ribi ImmunoChem Research (now Corixa-Montana, Hamilton, MT) from the same cell lines (12). Each study, the lysates were combined with the potent complex microbial adjuvant Detox (Corixa-Montana). Response rates of 20–25% were achieved in our early trials with minimal toxicity with the frozen lysates (7) and 16% with Melacine. In national trials with Melacine the median survivals were on the order of 10–12%. Nonetheless, the median survivals in each of our single institutional studies have been ~13 months, and 8% of all patients have had long survivals measured in years. Nine to 16% of the patients lived at least 1 year in each trial. In 2 cases survival of patients after complete remissions has exceeded 16 years (13).

In all of our studies of active specific immunotherapy, we have focused on the possible involvement of CTL in the immunological response to the tumor. We have attempted to measure the frequency of precursors of CTL in the peripheral blood, i.e., CTL found after one restimulation in vitro, that were reactive against melanoma cells, to determine a correlate of clinical efficacy. There was an association between an increase in the frequency of precursors of CTL (pCTL) against our melanoma cell lines, all of which were fortuitously HLA-A2+, and an objective clinical response, at P < 0.05 (8). Additionally, patients who responded best to the melanoma lysate vaccine were those who had two or three of the following three groups of alleles: (a) HLA-A2 or HLA-A28 (now HLA-A∗6802); (b) HLA-B12, HLA-B44, or HLA-B45; and (c) HLA-C3 (14). Additional studies have shown that those same HLA-class I phenotypes also predict for a more favorable outcome in resected early stage melanoma treated with Melacine, namely, longer disease-free survivals (15). Whereas the involvement of CTL in the rejection of tumors in vivo has not been firmly ascertained for any tumor, we assumed that the generation of melanoma-specific CTL by a multivalent immunogen preparation might be a useful attribute for eliciting a clinical response to metastases.

We decided to test LMI composed of plasma membranes derived from our melanoma cell lysates as a “second-genera-
tion” vaccine, to determine whether LMI also had clinical activity in humans. These studies were also viewed as possible precursors to others with defined complexes of peptides and HLA antigens affixed to the beads, which have been effective in mice (16, 17).

This article reports a Phase I trial of 19 HLA-A2+ patients with disseminated melanoma, exploring the toxicity and immunological activity of LMI comprising cell lysates from our original cell lines attached to amorphous silica beads and stored at ~80°C. This LMI preparation, administered without an immunological adjuvant, proved to have no toxicity, increased the frequency of CTL precursors against melanoma cells, and was associated with objective clinical regression or long-term stability of disease in a proportion of the patients.

MATERIALS AND METHODS

Preparation of LMI. For this study we used the same two melanoma cell lines that were used as frozen lysates in our single-institutional trials and have been the components of Melacine in subsequent multicenter trials. Melanoma cell lines MSM-M1 and MSM-M2 (hereafter abbreviated M-1 and M-2) were isolated in the early 1980s in our laboratory from a s.c. nodule and lymph nodes, respectively, of two patients with disseminated melanoma. They were registered with the American Type Culture Collection as CRL 9822 and 9823, respectively. Stock vials of each melanoma have been stored in liquid nitrogen. New cultures of melanoma cells in RPMI 1640 with 10% human AB serum were made for each new batch of vaccine.

Cell culture, isolation of membranes, and preparation of LMI were performed at Charles River Laboratories (Wilmington, MA). Frozen melanoma cells were thawed, washed three times in (pH 7.4) PBS, lysed by nebulization, and centrifuged twice at low speeds to pellet nuclei and mitochondria, to obtain a crude cell membrane preparation. Plasma membranes were then enriched by sucrose density gradient ultracentrifugation and subsequently concentrated by a second ultracentrifugation. The final membrane preparations were stored in a REVCO freezer at −80°C until use. ODS1 beads were sterilized by autoclaving, suspended in DM5O at 1 × 109 beads/ml, vortexed, and then sonicated to remove aggregates. Membranes were thawed and diluted in Dulbecco’s PBS to a concentration of 0.6 mg/ml. Beads in DM5O were then slowly added to the membrane suspension while sonicating both tubes, using 108 beads/0.3 mg membrane. The bead/membrane mixture was sonicated for an additional 30 s and incubated on a rotator at 4°C for 90 min. The coated beads were pelleted by centrifugation, washed, counted, and resuspended in DPBS at 109 beads/ml. Equal numbers of M-1/ODS1 and M-2/ODS1 LMI were mixed for injections. Final product testing for coated beads in vials before release for use included the following nine parameters: sterility, general safety (guinea pig test), absence of presence of endotoxin by the Limulus assay, identity, and potency (flow-cytometric positivity of >50% compared with negative control antibody, using three HLA-ABC-reactive monoclonal antibodies), bead concentration, pH, bead size, volume in container, and appearance.

The microscopic appearance of typical coated silica beads
is illustrated in the scanning electron micrograph presented in Fig. 1. An adherent central CD8+ CTL is also shown for a comparison of size and to exemplify its interaction with the beads.

HLA typing of both cell lines had been performed previously in the laboratories of Dr. Paul Terasaki (University of California, Los Angeles, Los Angeles, CA) and Dr. Fritz Bach (University of Minnesota, Minneapolis, MN). We incubated each cell line with IFN-γ for 18 h before sending the cells to Dr. Terasaki’s laboratory to up-regulate the expression of HLA-class I and II molecules. HLA-class I molecules were determined by serotyping, whereas HLA-class II were determined by the PCR. Melanoma M-1 is amelanotic, nearly tetraploid, grows slowly, and expresses HLA-A2, HLA-B12, -B62, and HLA-C3, and the HLA class II antigens DR4, DR10, DRw53, and DQ8. It also expresses the ganglioside GD3 but not GD2. Melanoma M-2 is smaller and nearly diploid, but has a small percentage of hypodiploid cells and trisomy at chromosome 7. It is highly pigmented, grows rapidly, expresses HLA-A28, -A31, -B51, -B60, -C2, and -C6, and is consistently devoid of HLA class II antigens by serological assays. However, genes coding for HLA class II antigens are present.

We must mention for completeness that after the study was in progress, a repeat serological typing and analysis by the PCR for HLA antigens on the tumor cell lines revealed somewhat different results. Specifically, M-1 was typed as HLA-A11, -A11; HLA-B60, -B60--; and HLA-C3, -C3 by PCR. Results with M-2 were much closer to the original data. Thus, PCR found HLA alleles HLA-A28 and -A31. The B locus by PCR was HLA-B51, -B60, as before, but here repeat serotyping found HLA-B78, -B60, and -Bw6. The C locus remained HLA-C3 by both methods. Thus, it is possible that the LMI preparation was of non-HLA-A*0201+ melanomas. However, the discrepancies between PCR and repeated serotyping, as well as from a multiplicity of previous serotypings for M-1, underscore the notorious difficulty of HLA typing tumor cells by any means. Such tumor cells are often characterized by variable or absent expression of HLA molecules, even after 18-h incubation in the presence of IFN-γ. At the time of the study, we were forced to assume that our original HLA-class I and II typing in two highly regarded laboratories was as accurate as these attempts to repeat them.

**The Phase I Trial.** The purposes of the Phase I trial were to determine whether: (a) there was significant toxicity of the LMI preparation itself, in the absence of an immunological adjuvant; (b) LMI could immunize patients against the immunizing melanomas; and (c) as a secondary goal, LMI could lead to measurable clinical responses in patients with advanced (disseminated, stage IV) melanoma. We selected patients who were HLA-A2+, matching the haplotype of one of the components of
Clinical Cancer Research

Table 1 Characteristics of patients on study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, sex</th>
<th>Sites of metastasis</th>
<th>Previous treatment other than surgery</th>
<th>Dose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44 M</td>
<td>Lymph nodes</td>
<td>RT*</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>57 M</td>
<td>Spleen, liver, lymph nodes</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>52 M</td>
<td>Subcutaneous</td>
<td>Chemo</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>58 M</td>
<td>Subcutaneous</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>65 F</td>
<td>Lung, lymph nodes</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>28 M</td>
<td>Subcutaneous</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>66 F</td>
<td>Adrenal</td>
<td>Chemo, RT</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>44 M</td>
<td>Subcutaneous, lymph nodes</td>
<td>Chemo, RT</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>53 M</td>
<td>Lung</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>36 M</td>
<td>Subcutaneous, lymph nodes, lung, kidney</td>
<td>Chemo</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>51 M</td>
<td>Lung</td>
<td>Chemo</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>57 M</td>
<td>Lung</td>
<td>Chemo</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>47 F</td>
<td>Lung, lymph nodes, subcutaneous</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>69 F</td>
<td>Lung</td>
<td>Chemo</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>58 M</td>
<td>Lung, spleen, lymph nodes</td>
<td>Chemo</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>70 M</td>
<td>Subcutaneous</td>
<td>Chemo, RT, marimastat</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>47 M</td>
<td>Lymph nodes</td>
<td>None</td>
<td>2</td>
</tr>
</tbody>
</table>

* Chemo, chemotherapy; RT, radiation therapy.

the LMI. This was done because of our finding that HLA-A2+ patients were among those who responded best to our frozen lysates (14) and because it permitted testing for CTL precursors directed against the component HLA-A2+ melanoma M-1.

Other eligibility criteria included: age >18 years old, no concurrent therapy, no previous treatment with a melanoma vaccine, no chemotherapy or radiation within 4 weeks (6 weeks for a nitrosourea), Karnofsky performance status of at least 70%, no brain metastases by magnetic resonance imaging, non-pregnant, and signed informed consent. The trial was reviewed and approved by the Institutional Review Board of the university.

Prior treatment received by the patients is shown in Table 1. Nine had received chemotherapy, and 4, radiation therapy, but none had had immunological therapy. One patient (#16) had received antiangiogenesis treatment with Marimastat (British Biotech, Inc., London, United Kingdom) in Canada.

As an initial screening for the possibility of severe toxicity, we treated 6 patients, 2 at each of the 3 doses of 10-, 30-, and 100-million tumor cell equivalents at monthly intervals for three doses. Because there was little or no toxicity at any level, we then entered 13 patients randomized to receive one of the three dose levels, for a total of 19 patients. This also circumvented the possibility of treating the first group of patients with a dose that was too low to be effective. Two patients who were registered were found to be ineligible on further workup (brain metastases) and did not receive treatment. Two others chose not complete the course of treatment, neither of them because of toxicity. Thus, 15 patients were fully evaluable for immunological reactivity and toxicity.

One-half of the preparation was given in 0.1–0.2 ml by intradermal (i.d.) injection and the other half in 0.9–1.8 ml into two matching sites by s.c. injection. The extremities were used exclusively for these injections. These routes were used to promote interaction of the LMI with the T cells, and perhaps dendritic cells, that migrated to the sites of injection in those locations.

Patients whose disease was at least stable at the 12-week evaluation point were given the option of continuing on treatment with monthly doses of LMI.

Immunological Measurements. We measured the frequency of reactive pCTL against the HLA-2+ M-1 cell line by limiting dilution analysis (7, 18). In brief, these assays entailed obtaining peripheral blood mononuclear cells (PBMCs) from 50 ml of heparinized blood by Ficoll-Hypaque gradient centrifugation and resuspension in RPMI 1640 with 3% human AB serum. The PBMCs were washed and resuspended in RPMI 1640 with 10% human AB serum. Cells (1.5 × 10^6) were irradiated with 2000 cGy for use as autologous feeder cells. Thirty-two replicate wells containing a range of 3 × 10^4 to 5 × 10^4 responder cells, 3 × 10^4 feeder cells, and 6 × 10^3 irradiated M-1 melanoma target cells, with 25 IU of interleukin 2 in RPMI 1640 +10% human AB serum were then set up. The cultures were fed on day 6 with fresh medium containing 25 IU/ml of IL-2, and allowed to incubate for a total of 9–10 days, at which time cytotoxicity was measured. A standard 6 h/37°C 51Cr release assay was used to judge cytotoxicity, with 8000 labeled M-1 cells/well. After incubation, 100 μl of supernatant fluid was harvested from each well, and released radioactivity was measured in a gamma scintillation counter. A well was considered positive if the value of cpm released exceeded the mean control values by >2 SDs. The number of cells plated that corresponded to 37% negative wells on a plate provided an estimate of frequency, indicating one CTL in that population according to the Poisson distribution. A computer program was used to calculate the 95% confidence intervals for pCTL frequency.

The frequencies were expressed as pCTL per 10,000 PBMC. These measurements were made three times at baseline, before each monthly dose of LMI and at 2-week intervals at least to day 56. A frequency of >3 SDs from the mean pretreatment values was considered to be significantly elevated over baseline, i.e., unlikely to be due to chance alone (2-sided P < 0.01).

Delayed-type hypersensitivity (DTH) was assessed with a panel of common microbial antigens (Merieux, Paris, France) before treatment in all of the patients to rule out those with...
Phase I Trial of Large Multivalent Immunogen

Table 2 pCTL* frequencies and clinical outcome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose level</th>
<th>Clinical outcome</th>
<th>Individual values Mean, SD</th>
<th>Peak pCTL frequency # per 10^6 PBMC Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 PD</td>
<td></td>
<td>0.88, 2.2</td>
<td>17 (d28) Increased</td>
</tr>
<tr>
<td>2</td>
<td>1 NE</td>
<td></td>
<td>1.2, 0.46, 0.54</td>
<td>None Unchanged</td>
</tr>
<tr>
<td>3</td>
<td>2 PD</td>
<td></td>
<td>0.26, 0.6</td>
<td>6.2 (d56) Increased</td>
</tr>
<tr>
<td>4</td>
<td>2 Stable, 15 mo</td>
<td></td>
<td>0.34, 2.58, 0.4</td>
<td>5.8 (d56); 10.58 (d84) Increased</td>
</tr>
<tr>
<td>5</td>
<td>3 PD</td>
<td></td>
<td>N.D., N.D., 1.02</td>
<td>8.54 (d23) Increased</td>
</tr>
<tr>
<td>6</td>
<td>3 Shrinkage of nodule (PR), but progression to brain metastases</td>
<td></td>
<td>0.66, 8.48</td>
<td>24.06 (d56) Increased, prolonged elevation</td>
</tr>
<tr>
<td>7</td>
<td>3 PD</td>
<td></td>
<td>3.76, N.D.</td>
<td>6.88 (d70) Unchanged</td>
</tr>
<tr>
<td>8</td>
<td>1 NE</td>
<td></td>
<td>2.58, 0.9, 0.86</td>
<td>Insufficient data</td>
</tr>
<tr>
<td>9</td>
<td>2 PD</td>
<td></td>
<td>4.74, 3.4, 8.04</td>
<td>11 (d56) Unchanged</td>
</tr>
<tr>
<td>10</td>
<td>3 PD</td>
<td></td>
<td>8.39, N.D., 12.9</td>
<td>20.64 (d42) Increased</td>
</tr>
<tr>
<td>11</td>
<td>2 PR</td>
<td></td>
<td>11.52, 9.3, 12.12</td>
<td>27.8 (d42) Increased</td>
</tr>
<tr>
<td>12</td>
<td>1 PD</td>
<td></td>
<td>16.8, 9.6, 16.8</td>
<td>None Unchanged</td>
</tr>
<tr>
<td>13</td>
<td>2 Stable, 12 mo</td>
<td></td>
<td>14.4, 2.4</td>
<td>None Unchanged</td>
</tr>
<tr>
<td>14</td>
<td>3 PD</td>
<td></td>
<td>20.6, 4.7</td>
<td>None Unchanged</td>
</tr>
<tr>
<td>15</td>
<td>1 Stable, 13 mo</td>
<td></td>
<td>12.65, 3.78</td>
<td>77 (d69) Increased</td>
</tr>
<tr>
<td>16</td>
<td>1 Stable, 2 mo, voluntarily left study at that point</td>
<td></td>
<td>5.34, 1.96</td>
<td>54 (d14), 26 (d70) Increased</td>
</tr>
<tr>
<td>17</td>
<td>2 Stable, 2 mo, voluntarily left study at that point</td>
<td></td>
<td>17.35, 3.25</td>
<td>&gt;74 (d14, 42, 56) Increased</td>
</tr>
</tbody>
</table>

*pCTL, precursor to CTL; PD, progressive disease; PR, partial remission; Stable, <50% decrease and <25% increase in sum of products of diameters of all lesions; NE, not evaluable: did not complete study; PBMC, peripheral blood mononuclear cells.

RESULTS

Toxicity. There was only minor toxicity of the LMI preparation in the absence of an admixed adjuvant. Four patients experienced local soreness at the site of injection, but without granulomas or sterile abscesses. No allergic or anaphylactic reaction was noted, nor were there significant fevers or chills. No patient was removed from study because of inability to tolerate the treatment, and no changes in complete blood counts or serum chemistries were noted on serial measurements throughout the course of the study.

Generation of pCTL. Stimulation of immunological reactivity to M-1 by LMI was noted in 9 of 15 evaluable patients (Table 2). A rise of >2-fold to as much as 10-fold was common, usually with peak frequencies of 6–20 pCTL/10,000 PBMCs. Peak elevations usually occurred on days 42 and beyond, although some patients had a rise by 28 days. Patients 15–17 reached a very high frequency of 54–77 cells/10,000 PBMCs, or >1 reactive CTL in 200. Elevations were equally distributed among patients receiving all three of the doses.

Comparisons among the three groups by one-way ANOVA and Mann-Whitney nonparametric rank sum statistics (for level 1 versus 2, level 2 versus 3, and level 1 versus 3) showed no significant differences. Neither the baseline values nor, more importantly, the peak values after immunization at each dosage level of LMI differed significantly from one another (P > 0.05). For example, for peak pCTL: level 1 versus 2, P = 0.8413; level 1 versus 3, P = 0.8413; and level 2 versus 3, P = 0.4206.

As we have stated, the differences between our original HLA class I typing and the repeat PCR determinations make it possible that the pCTL results represented alloreactivity at least in part, rather than purely antimelanoma reactivity.

DTH. None of the patients exhibited DTH to melanoma antigens either before or after the immunization regimen, regardless of the dose level of the treatment or the amount of LMI injected i.d. This failure to detect DTH was similar to our previous experience with melanoma lysates and adjuvant (7) but very different from the experience of others, such as those who administered shed materials from melanoma cells (19).

Clinical Outcomes. No immunological adjuvant was used with LMI to measure the toxicity of the preparation alone. In addition, clinical efficacy was not a primary end point of the trial. Nonetheless, 5 patients entered the maintenance phase of the treatment, with at least stable disease at the critical 3- and 4-month (12- and 16-week) evaluation points. Patient #6 had >50% shrinkage of a lung nodule, but progression of disease to the brain. Patient #11 had a bona fide partial remission of his single 3-cm diameter lung nodule on computed tomography scans, his only site of disease (Fig. 2, A–C). He remained alive,
in remission, and on-study for 13.2 months before being treated on another protocol. The other 4 patients had stable disease, of whom 2 survived for 9 and 10 months, respectively.

**DISCUSSION**

LMI represents a potentially important variation in the production of cancer vaccines. Tumor cell membranes adherent to microscopic sized beads, presenting a panoply of tumor epitopes associated with MHC molecules, is a modification of methods in which irradiated whole cells or cell lysates are used. The “pseudocells” created by this method may stimulate CTL directly, but are of a size (5 μm) where uptake by macrophages or dendritic cells is possible. That, in turn, may induce CTL responses through cross-priming. We did not biopsy the sites of injection to investigate this possibility here, but histological studies at Lidak showed a significant mononuclear cellular infiltrate at such sites in mice. The method is adaptable to the presentation not only of cell membranes but of MHC-peptide complexes (16, 17), which provides an alternative method of immunization of individuals with the same haplotype(s).

LMI administration alone, without any additional adjuvant, was sufficient to stimulate increased CTL precursors in the blood of most patients (Table 2). However, only modest clinical effects were observed. In studies of murine tumor models it was found that LMI were only effective for treatment of established tumors when preceded by chemotherapy with low-dose cyclophosphamide (4). It was decided to omit cyclophosphamide because this was intended to be purely a study of the new LMI material, as sponsored by Lidak, for additional development. Now that the safety of LMI therapy has been established in this study it is feasible to combine LMI and immunomodulatory (low-dose) chemotherapy Such trials for melanoma and renal carcinoma (using autologous tumor antigen) have been initiated at the University of Minnesota (Minneapolis, MN). In addition, ongoing studies of LMI effects in murine models have sug-

---

1 P. Morrow, unpublished observations.


---

Fig. 2 Computed tomography scans of the chest showing partial remission of a 3 cm in diameter solitary pulmonary metastasis under therapy with large multivalent immunogen (LMI) in patient 11. A, pretreatment films on 8/19/96 with lesion of 3.0 × 2.8 cm. B, films taken 6 months later on 2/11/97 during LMI treatment. Lesion is now 2.5 × 1.8 cm. C, films on 5/5/97 showing continued shrinkage (to 2.0 × 1.5 cm) and decreased density of the lesion. The patient received no therapy other than LMI.
gested additional improvements of LMI for tumor immunotherapy. We demonstrated recently that defined peptide/class I MHC protein complexes could be immobilized on microspheres to make LMI that were effective in reducing the growth of a tumor bearing the same peptide antigen (17). In addition, co-stimulatory ligands such as CD80 (B7–1) and CD86 (B7–2) can be incorporated onto the microspheres (16), and preliminary experiments suggest that this may increase the efficacy of the LMI.\(^3\) Finally, studies of the mechanism(s) by which LMI augments CTL responses are under way to determine, for example, whether this involves direct recognition of the antigen on the microspheres or, alternatively, whether cross-presentation by host antigen-presenting cells might be involved.

In one important respect LMI resembles the original lysate vaccine preparations reported from our institution in 1988 and 1990, which were all stored frozen at \(-80^\circ\text{C}\) before use in patients. Melanoma, a lyophilized material stored at \(4^\circ\text{C}\) refrigeration temperature, was produced to permit interstate shipment and subsequent storage in hospital pharmacies, which generally do not have low temperature freezers. A formal extensive comparison between the frozen and lyophilized versions has never been performed, leaving some uncertainty whether the former was in fact more effective than the latter. Whether LMI can achieve the same high response rates, in the range of 20–25\%, that were found in the earliest trials of frozen lysates remains to be determined in Phase II and most critically in Phase III trials.

Additional clinical trials are warranted with LMI, in which a complex microbial adjuvant such as Detox is used. Alternatively, one might explore whether the addition of individual adjuvant cytokines such as granulocyte macrophage colony-stimulating factor or interleukin 12 is useful in studies with randomized groups receiving LMI. The sequential use of cytokines such as interleukin 2 after vaccination is also theoretically interesting. Whereas LMI may well stimulate CD8\(^+\) CTL by direct interaction, the addition of an adjuvant might enable it to stimulate CD4\(^+\) helper T cells as well. The failure of LMI to elicit DTH in this study may perhaps have been due to poor representation of HLA-class II-presented epitopes or poor elicitation of dendritic cells to serve as intermediaries for stimulating CD4\(^+\) T cells. The importance of CD4\(^+\) T cells in rejection of tumors is being increasingly appreciated. Our immunohistological observations of tumor nodules in the process of being rejected has shown that CD4\(^+\) T cells predominate over CD8\(^+\) T cells (3/2 or 3/1; Ref. 20). Moreover, CD4\(^+\) T cells can by themselves overcome a tumor in mice (21) and have demonstrable cytotoxicity in humans (22). It is conceivable that in vivo the major effector cells may be CD4\(^+\) T cells, acting in concert with macrophages attracted to the site. In any event, the stimulation of CD4\(^+\) cells may be a worthwhile extension of LMI technology.

In addition, because a number of epitopes stimulating CTL and helper T cells have been identified in cancers other than melanoma, such as MUC1 (23–25), HER-2/neu (c-erbB2/neu: 26), and MGD50 (27) in breast and ovarian cancers, LMI is potentially applicable as a vehicle for treating a variety of tumor types.

\(^3\) J. Curtsinger and M. Mescher, unpublished observations.

REFERENCES


Phase I Trial of Large Multivalent Immunogen Derived from Melanoma Lysates in Patients with Disseminated Melanoma
