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Clinical Protocol

Adoptive Immunotherapy of Cancer with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the GM-CSF Gene

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SCIENTIFIC ABSTRACT

Although it has been well established experimentally that the transfer of sensitized T lymphocytes can mediate potent antitumor effects, extrapolating the principles of adoptive immunotherapy obtained from animal studies to clinical therapy will require the development of innovative techniques to isolate and propagate antitumor effector T cells from cancer patients. Toward this end, we have established culture methods whereby cells from tumor-draining or tumor primed lymph nodes (LN) can be sensitized to acquire therapeutic efficacy. Since these cells do not demonstrate overt antitumor reactivity before culture, they are functionally referred to as "pre-effector" cells. One method to generate antitumor effector T cells involves the sequential activation of pre-effector LN cells with anti-CD3 mAb followed by expansion in low concentrations of IL-2. Animal experiments have demonstrated that the antitumor reactivity of these anti-CD3/IL-2 activated cells are exquisitely tumor-specific and mediate the regression of established tumor in adoptive immunotherapy.

A major obstacle which confronts the clinical application of adoptive immunotherapy is the relatively weak immunogenicity of human cancers which hampers the induction of sensitized pre-effector cells. Recent observations in animal studies indicate the tumors can be genetically altered to enhance the host immune response against native or parental tumor antigens. We found that the transfection of the poorly immunogenic B16BL6 murine melanoma tumor with the GM-CSF gene resulted in the sensitization of immune lymphoid cells when inoculated into the syngeneic host. Draining LN cells removed from these animals and activated by the anti-CD3/IL-2 culture procedure generated potent therapeutic effector cells which mediated the adoptive immunotherapy or established metastatic parental tumors. More importantly, these activated cells were more potent in their therapeutic efficacy compared to similarly derived cells utilizing standard bacterial immune adjuvants. These observations provide the rationale for this clinical protocol to examine autologous tumor cells modified with the GM-CSF gene, which will be utilized as a vaccine to induce pre-effector LN cells in patients with advanced cancers. Human cancers have been postulated to be poorly immunogenic based upon their spontaneous origins. These vaccine-primed LN cells will be activated by the anti-CD3/IL-2 method and subsequently transferred intravenously to patients along with the concomitant administration of IL-2 (360,000 IU/kg q8h × 5 days) to support their survival/function in vivo.

The specific aims of the protocol are: 1) To assess the feasibility and toxicity of adoptive T cell immunotherapy of cancer with anti-CD3/IL-2 activated LN cells that are primed in vivo with GM-CSF modified autologous tumor cells, 2) To evaluate the antitumor efficacy and in vivo immunological reactivity of patients receiving adoptively transferred T cells, and 3) To investigate the in vitro immunological reactivities of the activated T cells that might correlate with their in vivo antitumor function.

NON-TECHNICAL ABSTRACT

This study involves the use of novel methods to treat patients with advanced cancer by manipulation of their immune system. Based on extensive animal studies, we know that the immune system has the potential to recognize tumor cells as being foreign to the body and destroy them. The critical component of the immune system which appears to be involved in the rejection of tumors are lymphocytes. The ability to generate these lymphocytes in the laboratory would be useful for potential therapy of cancer. This approach has been called adoptive immunotherapy and involves the infusion of cancer reactive lymphocytes into patients in order to cause tumor shrinkage.

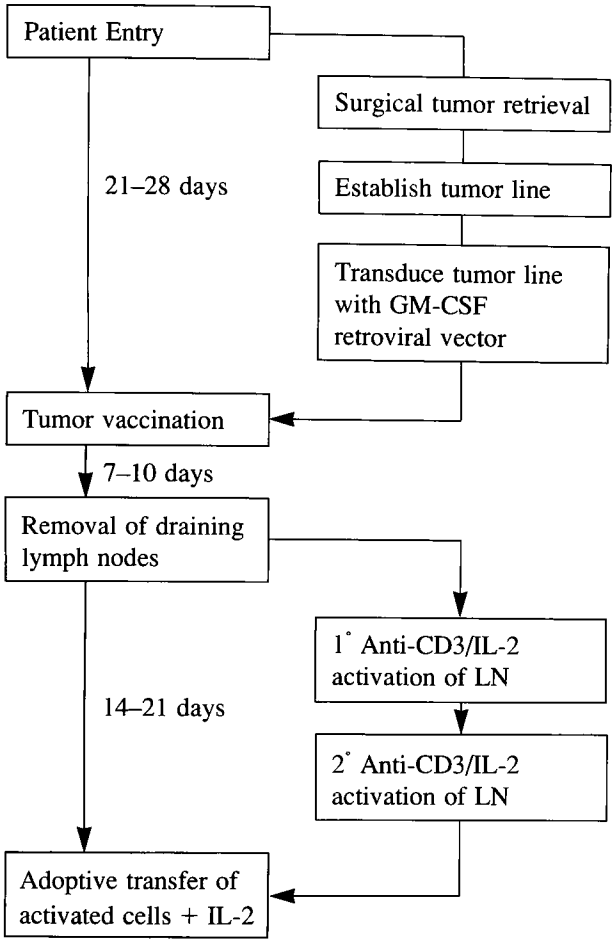
Unfortunately, the generation of immune lymphocytes which can reject human cancers has been extremely difficult. One possible reason why this problem exists is that the foreign proteins present on human tumor cells are very weak in their ability to stimulate the immune system to react against them. Based on our animal studies, we have devised an approach to artificially stimulate immune cells that may be used for the treatment of human tumors that under normal circumstances would not occur. This involves a two-step process. The first step requires vaccination of the patient with their own tumor cells which have previously been removed and genetically engineered to secrete an immune factor called GM-CSF. The tumor cells are irradiated prior to injection into the skin in order to prevent outgrowth of tumor at the site. It has been found in animal studies that the production of GM-CSF by the gene-modified tumor cells promotes an immune response in lymph nodes near the vaccination site. Lymph nodes are small glands of the immune system where lymphocytes congregate. The lymph nodes adjacent to the vaccination sites will be surgically removed approximately 7 to 10 days later and taken to the laboratory for further processing. In the laboratory, cells from the lymph nodes will be stimulated and grown in special flasks by methods we have previously described for a 2 to 3 week period in order to generate a large number of immune lymphocytes. These lymphocytes will be collected and infused back into the patient along with the administration of interleukin-2, another immune protein. The interleukin-2 has been found to promote the antitumor effect of the immune lymphocytes in cancer patients.

This clinical study proposes to address several important questions. These questions include: 1) Can this clinical treatment program be performed as described and what are its side-effects, 2) What antitumor response can be seen with this treatment, and 3) What is the immunological function of the immune lymphocytes, as assessed by laboratory tests.

1.0 Protocol Schema

Schema: Patients with advanced melanoma or renal cell cancer will undergo surgical retrieval of tumor for use as a tumor vaccine. A tumor cell line will be established in culture and subsequently transduced with a retroviral vector containing the GM-CSF gene. The transduced line will be screened for cytokine production and subsequently irradiated with 5,000 cGy. If after 6 patients a specified minimum level of GM-CSF secretion is not achieved by the transduced cells in 3 patients, the investigators will not treat additional patients without returning to the NIH RAC for discussion of the data. Patients will be vaccinated with 10^7–10^8 irradiated transduced tumor cells intradermally in 1 or 2 sites. Surgical removal of draining lymph nodes (LN) 7 to 10 days after vaccination will be performed to

PROTOCOL: Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed in Vivo with Autologous Tumor Cells Transduced with the GM-CSF Gene



obtain lymphocytes for activation with anti-CD3 monoclonal antibody (OKT3) and expansion in IL-2 in vitro. After in vitro activation, these lymphocytes will be adoptively transferred intravenously to the patient followed by the administration of IL-2 (360,000 IU i.v. every 8 h. for 5 days). Fifteen patients with each histology will be treated to gain sufficient information regarding toxicity, antitumor reactivity and immunologic function.

2.0 Objectives

Our laboratory has described a new method of generating sensitized T cells for adoptive immunotherapy utilizing autologous tumor cells genetically modified to secrete GM-CSF. In preclinical studies, the in vivo administration of these tumor cells has been found to elicit sensitized T cells in the draining lymph nodes (LN) against a poorly immunogenic tumor. The subsequent in vitro activation of these LN cells by anti-CD3 mAb and IL-2 has resulted in the generation of T cells capable of mediating regression of established parental tumor metastases. These preclinical studies have formed the basis for a clinical evaluation of similarly derived T cells for patients with advanced melanoma. The objectives of this study are:

2.1 To assess the feasibility and toxicity of adoptive T cell immunotherapy of cancer with anti-CD3/IL-2 activated LN cells that are primed in vivo with GM-CSF modified tumor cells.

2.2 To evaluate the antitumor efficacy and in vivo immunological reactivity of patients receiving adoptively transferred T cells.

2.3 To investigate the in vitro immunological reactivities of the activated T cells that might correlate with their in vivo antitumor function.

3.0 Background and Rationale

Adoptive immunotherapy is defined as the transfer of antitumor reactive cells to the tumor-bearing host which will directly or indirectly mediate the regression of tumor. The therapeutic efficacy of adoptive immunotherapy of cancer with sensitized T lymphocytes is well-documented in animal studies. The application of this approach for the treatment of human cancer poses many theoretical and technical difficulties since it requires the generation of large quantities of antitumor reactive lymphocytes from cancer patients. Despite such limitations, many observations established in animal models of adoptive immunotherapy have been implemented in clinical trials with encouraging results. The demonstration of regression of solid tumors in man following the adoptive transfer of lymphokine-activated killer (LAK) cells or tumor-infiltrating lymphocytes (TIL) in conjunction with IL-2 has established that cellular therapy is a feasible alternative in treating cancers and has encouraged the search for more potent effector cells (1,2).

3.1 Animal studies characterizing the immune T cell response in tumor-draining LN

In spite of the demonstration that many animal tumors possess tumor-specific antigens which are capable of immunizing syngeneic hosts to reject a challenge of an otherwise lethal dose of tumor cells, antigenic tumors often grow in their immunocompetent hosts.

Apparently, during progressive tumor growth, the innate immune response is insufficient due to the weakness of the antigen and/or the occurrence of tumor-induced immunosuppression. Using several immunogenic tumors, we have demonstrated that LN draining a progressively growing tumor contain T cells that were immunologically sensitized but functionally deficient in expressing antitumor reactivity. This population of cells designated as "pre-effector" cells could nevertheless be further stimulated in vitro to differentiate into potent immune effector cells (3,4). Initially an in vitro sensitization (IVS) procedure was used for the generation of effector cells by the culture of tumor-draining LN cells with excessive numbers of irradiated tumor cells in the presence of IL-2. In IVS, the function of tumor cells was to provide specific antigenic stimulation while IL-2 promoted proliferation of T lymphocytes. In extensive animal studies with the immunogenic MCA 105 and MCA 106 sarcoma tumors, we have documented that IVS cells generated from tumor-bearing mice were capable of curing a significant proportion of animals with grossly visible metastatic disease in the lung and liver (5).

The success of adoptive immunotherapy was critically dependent upon the immunogenicity of the tumor. In animal studies, therapeutic IVS cells could only be generated when the tumors were immunogenic and capable of eliciting transplantation resistance in their syngeneic hosts. It has not been possible to apply this approach to tumors which do not induce systemic immunity. A major concern for cancer immunotherapy in humans is whether or not immunogenic animal tumors represent sufficient models because human tumors originate spontaneously and may be poorly immunogenic. To address this issue, we examined the feasibility of generating immune effector cells with reactivity against poorly immunogenic murine tumors (6,7). The MCA 102 was selected for study because it failed to elicit systemic immunity in syngeneic B6 mice by a variety of immunization procedures including tumor growth and excision, immunization with irradiated tumor cells and with various combination of tumor cells admixed with *C. parvum*. Initial experiments with the MCA 102 tumor indicated that unlike the MCA 105 and MCA 106 tumors, therapeutic effector cells could not be induced from lymph nodes draining a progressive tumor. However, utilizing alternative methods, we found it was possible to generate sensitized lymphocytes with therapeutic efficacy against the MCA 102 tumor. The procedure to achieve this required both *in vivo* tumor priming and IVS. Unlike pre-effector cells sensitized to the weakly immunogenic MCA 105 tumor which were elicited naturally during progressive tumor growth, the process of inducing pre-effector cells to the MCA 102 tumor was an artificial one and required the use of a bacterial adjuvant, *C. parvum*, in addition to tumor cells administered as a vaccine. If properly primed pre-effector cells are induced, the IVS method can provide an environment for them to mature into functional immune cells. Indeed, using this approach, transfer of as few as 1.5×10^7 vaccine-primed IVS cells not only reduced established pulmonary MCA 102 metastases but also prolonged survival and cured tumors in a majority of treated animals (6). Analysis of the distribution of pre-effector cells in various lymphoid organs revealed that their occurrence was regionally restricted and only detectable in lymph nodes draining the tumor vaccine. During the peak response, no evidence of the existence of pre-effector cells in the spleen, mesenteric lymph nodes, PBL or bone marrow was seen. Based on these studies, we have completed a clinical study to evaluate the immunobiology and efficacy of similarly generated primed LN cells in patients with advanced cancer (see Section 3.2).

The requirement for a relatively large number of autologous tumor cells for generating IVS cells in culture was found to be a limiting factor in its clinical application. Hence, we realized that alternate techniques to stimulate and propagate primed pre-effector cells needed to be identified. Since antigen recognition by T cells involves the TCR/CD3 complex for signal transduction and antibodies binding to the complex activate T cells, we examined whether *in vitro* antigenic stimulation could be achieved by using mAb to CD3 (8–10). This possibility was examined with several murine tumor models. A hamster mAb, 145-2C11, directed against the CD3ϵ chain of the murine TCR/CD3 complex has been characterized (11). This antibody has a variety of *in vivo* and *in vitro* immunomodulatory effects on T cells including the induction of nonspecific proliferation. Tumor-draining LN cells activated for 2 days with anti-CD3 (1 μg/ml) alone did not result in significant proliferation. However, sequential activation with anti-CD3 followed by expansion in a low concentration of IL-2 (10 u/ml) for 3 days resulted in approximately 10-fold increase in cell numbers (8). This proliferation was not specific to the tumor-draining LN cells because normal LN cells proliferated to an equivalent degree. In spite of this, our adoptive immunotherapy experiments revealed that the anti-CD3 activated tumor-draining, but not normal, LN cells had antitumor effects.

Numerous reports have demonstrated that the in vitro stimulation of normal T cells with anti-CD3 results in proliferation and generation of cytotoxic effector cells (12,13). However, none of these anti-CD3 stimulated cells demonstrate antigen-specific reactivity probably due to the ubiquitous presence of CD3 on all T lymphocytes. Our experiments with anti-CD3/IL-2 activation of tumor-draining LN cells suggested that specific stimulation of antigen-sensitized pre-effector cells had occurred. We therefore examined the specificity of adoptive immunotherapy mediated by anti-CD3/IL-2 activated cells. Anti-CD3/IL-2 activated MCA 106 tumor-draining LN cells were transferred to mice with 3-day established metastases from three antigenically distinct murine sarcomas (Table 1). Mice lungs were harvested 14 days after tumor inoculation and metastases counted; too numerous to count tumors/lung were scored as >250. There was significant antitumor reactivity against the MCA 106 tumor but not the MCA 102 or MCA 105 tumors. In separate experiments we found that similarly activated LN cells draining the MCA 105, MCA 203 or MCA 102 tumors mediated significant reductions of MCA 105, MCA 203 or MCA 102 metastases, respectively (data not shown) (11).

Next, we examined the feasibility of generating anti-CD3/IL-2 activated effector cells reactive to the poorly immunogenic B16 BL6 melanoma. This subline of B16 melanoma, a spontaneously arisen tumor, is poorly immunogenic by virtue of the inability to elicit systemic immunity in syngeneic B6 mice experimentally. Additionally, therapeutically effective TIL cannot be generated from progressive subdermal tumors (B. Fox, data not shown). Initial experiments with the B16 BL6 tumor indicated that unlike the immunogenic MCA 106, therapeutic anti-CD3/IL-2 activated cells could not be induced from tumor-draining LN. However, it was possible to generate anti-CD3/IL-2 activated lymphocytes with therapeutic efficacy against the B16 BL6 tumor from LN draining an inoculum of tumor cells admixed with *C. parvum* (14,15). As noted in Table 2, the an-

TABLE 1. SPECIFICITY OF ADOPTIVE IMMUNOTHERAPY MEDIATED BY ANTI-CD3/IL-2 ACTIVATED MCA 106 TUMOR-DRAINING LYMPH NODE CELLS

Treatment		*Mean no. pulmonary metastases (SEM)*		
Anti-CD3 activated cells (2×10^7)	*IL-2 (15000 u, ip 2×/day for 4 days)*	*MCA 102*	*MCA 105*	*MCA 106*
–	–	246 (4)	>250	>250
–	+	>250	>250	>250
+	+	217 (28)	>250	0*

*Compared to no treatment or IL-2 alone $p < 0.05$.

TABLE 2. ANTITUMOR EFFICACY OF ANTI-CD3/IL-2 ACTIVATED CELLS DERIVED FROM B16 BL6 DRAINING LN IS DEPENDENT UPON AMOUNT OF *C. parvum*.

Anti-CD3 activated cells	*C. parvum (μgm)*	*Mean no. pulmonary metastases (SEM)*
−	—	>250
+	0	209 (38)
+	12.5	8 (6)*
+	25	<1 (<1)*
+	50	187 (30)
+	100	173 (20)

$p < 0.05$ compared to group not receiving cells.

titumor reactivity of tumor-primed anti-CD3/IL-2 activated LN cells was critically dependent upon the dose of bacterial adjuvant. Activated cells (5×10^7/mouse) were transferred i.v. into mice with 3-day established B16 BL6 pulmonary metastases. IL-2 (15,000 u i.p. bid × 4 days) was started on the day of adoptive transfer.

We also examined the kinetics of pre-effector cell induction after vaccine-priming. In this experiment, mice were inoculated with 10^6 viable or 10^7 irradiated (4,000 cGy) B16 BL6 tumor admixed with 12.5 μgm *C. parvum* s.c. every few days so that draining LN could be obtained on the same day for activation. The development of pre-effector cells in the draining LN was evident as early as day 4 after tumor priming but disappeared after day 14. These findings confirmed that irradiated B16BL6 tumor expressed appropriate tumor antigens and that the host immune response could be modulated to generate anti-CD3/IL-2 activated LN cells against this poorly immunogenic tumor. Based on these data, we are currently conducting a clinical study of similarly generated anti-CD3/IL-2 vaccine primed LN cells in patients with metastatic melanoma and renal cell cancer.

3.2 Clinical experience with human tumor-primed LN cells

Extrapolating methods from our animal models, we initiated a clinical trial to evaluate the antitumor reactivity of tumor-primed IVS-LN cells (16). Patients with advanced melanoma or renal cell cancer were eligible. Patients were tumor-primed by the intradermal inoculation of $1–2 \times 10^7$ irradiated (2,500 cGy) autologous tumor admixed with fresh-frozen Tice BCG (10^7 cfu) in two separate sites. These sites were either on the anterior thigh or axilla in order that draining LN could be surgically removed under local anesthesia 10 days later for subsequent IVS culture. The use of BCG was employed since previous clinical studies demonstrated that it was an effective immune adjuvant in eliciting cellular immune responses when administered in conjunction with irradiated tumor (17,18). The yield of primed LN cells from 10 patients averaged 1.3×10^9 cells with removal of clinically hyperplastic LN. There were no complications associated with the tumor vaccination procedure nor removal of the primed LN.

Upon retrieval of primed LN, the lymphocytes were isolated and placed in IVS culture. IVS cultures were established in gas-permeable culture bags with complete media (CM) containing $1–2 \times 10^5$ LN cells/ml and $1–4 \times 10^5$ irradiated tumor cells/ml. CM consisted of RPMI 1640 with 10% human AB sera, 100 Cetus u/ml IL-2, antibiotics, L-glutamine, non-essential amino acids and sodium pyruvate. Culture bags were incubated at 37°C in 5% CO_2 for 10–15 days. Cells were harvested after tumor cells cleared from the culture and the IVS lymphoid cells reached maximum density. IVS cells proliferated up to 14-fold during an average period of 12 days.

A mean of 6.7×10^9 cells were infused in 10 patients (7 melanoma, 3 renal cell cancer) along with the concomitant administration of IL-2 (180,000 IU/kg q8h for 5 days). Phenotype analysis of the IVS-LN cells revealed 78% T cells which were predominantly $CD4^+$. Seven of 9 patients that received IVS-LN cells developed delayed-type hypersensitivity (DTH) to autologous tumor suggestive that antitumor reactivity was passively transferred. Of the 10 patients treated with IVS-LN cells and IL-2, there was one partial and one minor response, and one patient remains with stable disease at 36+ months.

This initial study indicated that vaccine-primed LN cells harbored tumor reactive lymphocytes and that additional studies to generate larger numbers of cells might be required to evaluate their efficacy. A limiting factor for generating larger numbers of IVS cells was the amount of available tumor for the culture procedure. Therefore, we developed an alternate in vitro method to generate immune cells from tumor-primed pre-effector cells without the requirement of tumor stimulator cells (see Section 2.1). This alternative method utilized the stimulation of pre-effector cells by an anti-CD3 mAb for 48 hours followed by their expansion in IL-2 (10 u/ml). From our animal studies, this activation procedure resulted in the expansion and maturation of highly specific effector T cells.

Utilizing this approach we initiated a second clinical study (19). Patients were primed to autologous tumor utilizing the same vaccination procedure as was used for the IVS protocol. Tumor-primed LN cells were harvested 10 days after vaccination and activated by the anti-CD3/IL-2 method. Since it was anticipated that the primed LN would expand approximately 5 to 6-fold after the first activation procedure, further activation utilizing the identical procedure would be performed a second time. Animal studies in our laboratory have demonstrated that the antitumor efficacy of these activated cells were maintained during a second activation procedure. We anticipated that this would enable at least a 30-fold expansion of 10^9 primed LN cells which could be obtained from each patient. Not only would this represent a significantly larger number of immune cells generated for each patient, it would also increase the number of patients who could potentially be treated since large numbers of tumor cells were not required.

For anti-CD3 activation, lymphocytes were suspended in CM at a concentration of 2×10^6 cells/ml in flasks coated with immobilized anti-CD3 mAb (OKT3). After 2 days of activation the cells were harvested, washed and expanded in IL-2. Expansion in IL-2 was accomplished by resuspending 3×10^5 anti-CD3 activated cells/ml in fresh CM containing 10 u/ml of IL-2. Cells were grown to maximum density and then re-cultured in a secondary anti-CD3/IL-2 activation procedure. This activation scheme is illustrated in Appendix A with a patient who had 5×10^8 LN cells activated in vitro over a 13-day period to a final cell number of 1.4×10^{11} effector cells.

To date 21 patients (11 melanoma and 10 renal cell) have been treated on this protocol. A median of 10^{11} activated cells

were administered in conjunction with IL-2 (360,000 IU/kg q8 × 6d). Phenotype analysis of the anti-CD3/IL-2 activated LN cells revealed that $CD8^+$ T cells were preferentially activated. Among the 11 patients with melanoma, 1 had a partial response to treatment. Of note, among the 10 patients with renal cell cancer, 2 had a complete, 2 partial and 1 minor responses to therapy. Among the 6 patients with responses, 4 developed DTH reactivity to autologous tumor. By contrast, among the remaining 15 patients, only 4 developed DTH reactivity to autologous tumor.

3.3 Rationale for proposed clinical trial to utilize GM-CSF gene modified tumor cells to prime LN in vivo

Conceptually, the modulation of tumor immunogenicity by genetic manipulation can be categorized into two categories: 1) the expression of novel immunogenic antigens on the cell surface which will stimulate the host immune response (i.e., allogeneic MHC antigens, B7, etc.), or 2) the elaboration of a gene product by the tumor cell which will facilitate the host immune response in a paracrine fashion. Several recent studies have demonstrated that the transduction of murine tumor cells with genes encoding cytokines (IL-2, IL-4, IL-6, IL-7, IFNγ, TFNα and G-CSF) alters the tumorigenicity of the cells with regression of inoculated transduced cells (20–24). Moreover, many of these studies have demonstrated that animals vaccinated with transduced tumors are resistant to a subsequent challenge of parental non-transduced tumor; which suggests that immune cellular responses against parental tumor antigens are upregulated. Dranoff et al. reported that the transduction of the murine GM-CSF gene into the B16-F10 melanoma tumor significantly enhanced the immunogenicity of the parental tumor (24). The parental tumor was characterized to be poorly immunogenic by virtue of the inability to induce systemic immunity with the inoculation of irradiated tumor cells. By contrast, vaccination with irradiated GM-CSF secreting tumor cells induced specific immunity requiring both $CD4^+$ and $CD8^+$ cells. The mechanism for this immunomodulatory effect is not known, and it has been postulated that GM-CSF may enhance antigen presentation by stimulating or inducing the recruitment of antigen presenting cells (APCs) to the site of tumor.

Because of our interest in developing approaches to generating immune T effector cells against poorly immunogenic tumors, we have proceeded to investigate the immunobiologic properties of B16-BL6 melanoma transduced to secrete GM-CSF. Utilizing limiting dilution techniques, clones of the B16-BL6 tumor were developed, one of which termed, D5, was used for transduction with a GM-CSF retroviral vector. Transduced tumor cell clones were subsequently generated; and clone D5G6, which secreted approximately 450 ng/ml/10^6 cells/24 h, was selected for further study. In animal studies, the tumorigenicity of the D5G6 was minimally depressed compared to wild-type tumor. All animals inoculated with D5G6 developed tumors which lead to the eventual death of the animals. This was distinctly different from the studies reported by Dranoff et al. where viable transduced tumor (expressing 300 ng/ml/10^6/48 h) inoculated s.c. into normal hosts spontaneously regressed (24).

It appeared that the B16-BL6 tumor we utilized in these studies was less immunogenic than the B16-F10 reported by Dranoff et al. (24) since protective immunity could not be established by vaccination of animals with GM-CSF transduced tumor. For example, 10^6 irradiated (5,000 cGy) D6 or D5G6 tumor cells were inoculated s.c. into normal hosts in a vaccination protocol. At this irradiation dose tumor cells were not tumorigenic, but were still capable of producing GM-CSF (data not shown). Vaccinated mice were challenged with 10^5 viable wild-type tumor 21 days later, along with a group of naive animals. There was no difference in tumor challenge outgrowth between animals vaccinated with wild-type tumor compared to D5G6. These observations confirmed the poorly immunogenic nature of the D5G6 clone; but also demonstrated that secretion of GM-CSF by tumor cells did not induce systemic immunity.

Despite the inability to induce systemic immunity, D5G6 tumors elicited a marked intratumoral host response. Histologic analysis of the cellular infiltrate present in established 9-day D5G6 tumors was significantly different compared to wild-type tumor. Hematoxylin and eosin staining of cytospin preparations of tumor digests demonstrated significant neutrophilic infiltration of the D5G6 tumors compared to parental tumor. Enumeration of cells in these preparations revealed 4% neutrophils in D5 tumors compared to 23% neutrophils in D5G6. Immunohistochemical staining with Mac-1a mAb documented a marked increase in monocytes/macrophages infiltrating the D5G6 tumor compared to D5. This indicated that the secretion of GM-CSF in the local microenvironment elicited an influx of inflammatory cells and professional APCs. We proceeded to examine the immune function of LN cells derived from animals inoculated with D5 or D5G6 tumor.

If antigen processing and/or presentation was enhanced in D5G6 tumors, we hypothesized that the antitumor reactivity of draining LN cells would also be upregulated. There were significant differences in the cellular composition of LN draining transduced tumors versus wild-type tumor. By day 9 of s.c. tumor growth, there was a marked increase in the percent of Mac-1^+ (M1/70^+:monocytes/mØ, neutrophils) or Mac-2^+ (M3/38^+:monocytes/MØ) cells in the D5G6-draining LN compared to a D5-draining LN (Figure 1). **[Ed.: Figures not reproduced.]**

Based upon the *in vitro* functional assays developed by our laboratory for evaluating pre-effector cell activity, we examined the reactivity of anti-CD3/IL-2 activated TDLN cells derived from animals bearing D5G6 or D5. Animals with established 9-day s.c. tumors had TDLN harvested and activated by the anti-CD3/IL-2 method. Anti-CD3/IL-2 activated cells were assessed for cytotoxicity and cytokine release. Using the 48h long-term cytotoxicity assay, D5G6-draining LN mediated significantly greater cytotoxicity of parental tumor targets compared to D5-draining LN (Figure 2). Moreover, activated D5G6-draining LN cells secreted larger quantities of GM-CSF and IFNγ after *in vitro* restimulation with autologous tumor compared with D5-draining LN cells. This was assessed by stimulating 3×10^5 activated LN cells with 2×10^5 irradiated wild-type tumor cells per well in a 24-well plate. Each well contained CM with 4 u/ml of IL-2 added. There was no secretion of cytokines by effector cells or tumor cells alone. Supernatant was collected daily for 6 days for measurement of GM-CSF and IFNγ by ELISA. At all time points, activated D5G6-draining LN cells released larger quantities of GM-CSF (Figure 3) and

TABLE 3. COMPARISON OF D5G6 VERSUS WILD TYPE D5 IN SENSITIZING TDLN

Treatment[a]		*Mean no. lung metastases (SEM)*	
Cells	*IL-2*	*Exp 1*	*Exp 2*
A. None	−	>250	>250
B. None	+	>250	>250
C. D5	+	>250	>250
D. D5G6	+	87 (32)[b]	31 (27)[b]

[a]6–7 × 10^7 cells/animal i.v.; 10,000 u IL-2 i.p. bid × 4 days after cell transfer.
[b]$p < 0.01$ compared to groups A, B, C.

IFNγ (data not shown) compared to D5-draining LN cells. These data suggested that tumor reactivity to parental tumor was significantly enhanced *in vivo* by the paracrine secretion of GM-CSF during tumor growth.

The *in vitro* antitumor reactivity of activated TDLN from animals bearing D5G6 was significantly enhanced compared to the reactivity mediated by D5-draining LN cells; and suggested that they may be therapeutically functional *in vivo*. We utilized our adoptive immunotherapy model to evaluate this possibility. Anti-CD3/IL-2 activated TDLN from mice bearing 10-day D5G6 or wild-type D5 s.c. tumors were adoptively transferred to mice with established 3-day pulmonary D5 metastases. Approximately 21 days later, lung metastases were enumerated. Progressive D5 tumor did not elicit pre-effector cells in draining LN as previously observed for this poorly immunogenic tumor. By contrast, D5G6 tumors induced a significant pre-effector cell response in the TDLN which was demonstrated by the regression of pulmonary metastases mediated by activated TDLN cells (Table 3).

We have previously reported that the generation of pre-effector cells reactive to B16-BL6 can be induced in mice by the inoculation of tumor cells admixed with *C. parvum* (14,15). These observations have served as a rationale for our current clinical studies evaluating T cell therapy with activated LN cells primed *in vivo* by autologous tumor cells admixed with the bacterial adjuvant BCG (16). We proceeded to compare the efficacy of D5G6 tumor versus D5 admixed with *C. parvum* (12.5 μgm) in the induction of pre-effector cells in TDLN. Animals were inoculated with D5, D5 + *C. parvum*, or D5G6 tumor cells (10^6/animal); and TDLN harvested 9 days later. After anti-CD3/IL-2 activation, the TDLN cells were transferred into mice with established 3-day parental D5 pulmonary metastases. The results of 3 independent experiments are summarized in Table 4. As before, progressive D5 tumor did not sensitize pre-effector TDLN cells. Animals inoculated with D5 plus *C. parvum* resulted in a pre-effector cell response in TDLN in 2 of 3 experiments. More importantly, D5G6 elicited pre-effector cells in the TDLN were significantly more effective in mediating tumor regression compared to LN cells draining D5 alone or D5 + *C. parvum* inoculation sites in 3 of 3 experiments. The results of these latter studies lend credence to the use of genetically modified tumor cells, which secrete GM-CSF, as an approach to generate human T cells for clinical therapy.

TABLE 4. COMPARISON OF DIFFERENT METHODS TO SENSITIZE TDLN REACTIVE TO D5 TUMOR

Treatment[a]		*Mean no. lung metastases (SEM)*		
LN cells	*IL-2*	*Exp 1*	*Exp 2*	*Exp 3*
A. None	−	>250	>250	189 (32)
B. None	+	>250	>250	155 (32)
C. D5	+	>250	173 (22)[b]	161 (40)
D. D5 + *C. parvum*	+	98 (38)[c]	40 (14)[c]	93 (53)
E. D5G6	+	1 (1)[d]	5 (5)[d]	1 (1)[d]

[a]6–7 × 10^7 cells/animal i.v.; 10,000 u IL-2 bid × 4 days after cell transfer.
[b]$p < 0.01$ compared to A, B.
[c]$p < 0.01$ compared to A–C.
[d]$p < 0.01$ compared to A–D.

4.0 Drug Information

4.1 Interleukin-2

The recombinant IL-2 (Proleukin^R) used in this protocol is manufactured by Chiron Therapeutics, Emeryville, CA. Proleukin^R is a protein manufactured by recombinant technology and is similar to the native IL-2 protein found in humans. Proleukin^R is supplied as a lyophilized cake in 5 ml vials containing 1 mg of protein. The lyophilized powder will be stored in a refrigerator. Each vial should be reconstituted with 1.2 ml of sterile water and gently swirled without excess foaming. When reconstituted each ml contains 1 mg (18 million IU) of Proleukin^R and is stable for 48 hours when stored in the refrigerator or at room temperature. For intravenous injection, Proleukin^R can be reconstituted in volumes of 50 to 500 ml of 5% dextrose solution with 0.1% human albumin. It is recommended that in-line filters not be used for delivery of Proleukin^R since bioassays have shown significant loss of IL-2 activity after filtration. Reconstitution with sterile water or normal saline solutions for intravenous injection should be avoided because of precipitation. In addition, Proleukin^R should not be mixed with other drugs. The lyophilized material is stable at 2–28°C for 18 months from the date of manufacture. This material should be protected from excessive exposure to light during extended storage.

4.2 OKT 3 (NSC# 618843)

OKT 3 is a murine monoclonal antibody (mAb) to the CD3 antigen on human T cells and is manufactured by Ortho Pharmaceutical Corp. It has been used extensively as an intravenously administered immunosuppressive agent in organ transplant patients. The mAb is a biochemically purified IgG_{2a} immunoglobulin. It is supplied in a 5 ml ampule (Ortho clone OKT 3^R) containing 5 mg (1 mg/ml) of mAb in a sterile, colorless solution which may contain a few fine translucent protein particles. Each ampule contains a buffered solution (pH 7.0 ± 0.5) of monobasic sodium phosphate (2.25 mg), dibasic

sodium phosphate (9.0 mg), sodium chloride (43 mg) and polysorbate 80 (1.0 mg) in water. Intact ampules will be stored in the refrigerator, since ampules do not contain preservative, they will be discarded 8 hours after opening.

4.3 Retroviral vector: MFG-S-GM-CSF

MFG-S-GM-CSF is a Moloney Murine Leukemia virus which encodes for human GM-CSF and was developed by Dr. Richard Mulligan (Somatix Therapy, Corporation, Alameda, CA) (25). Somatix Therapy has agreed to supply FDA-approved clinical grade viral particles to be used in this study. Data on MFG-S has been presented and approved previously by the NIH RAC in Protocol 9303-040 for renal cancer. The vector has been approved by the FDA under IND BB5229. In this vector, Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR) sequences are used to generate both a full length viral RNA necessary for the generation of viral particles and a subgenomic mRNA analogous to the Mo-MuLV env RNA, which is responsible for the expression of huGM-CSF. The vector retains both sequences in the viral gag region shown to improve the encapsidation of viral RNA and the normal viral 5′ and 3′ splice sites necessary for generation of the subgenomic RNA. DNA sequences encoding human GM-CSF have been inserted between Nco 1 and Bam H1 sites in such a way that the initiation codon of the inserted sequences is placed precisely at the position normally occupied by the initiation codon for env translation, and minimal 3′ non-translated sequences are included in the insert. Unlike many vectors commonly in use, MFG-S contains no sequences encoding a selectable marker. The complete nucleotide sequence of MFG-S-huGM-CSF is attached. The psiCRIP cell line will be utilized for the packaging of recombinant genomes into virus particles.

High titer stocks of recombinant viruses suitable for clinical use are generated from cultures of cells propagated in a closed loop perfusion system designed for the mass cultures of anchorage dependent cells. To ensure and document the quality and safety of the recombinant virus, an FDA approved, extensive testing program has been established for all production lots.

5.0 Eligibility Criteria

5.1 Eligible patients will include those with the following at the time of treatment with systemic IL-2 (see Appendix B):

a. Histologically proven metastatic melanoma or renal cell cancer. For vaccine development, at least 3–4 gm of tumor need to be available for transduction. This tumor tissue should be from a readily accessible site.
b. Performance status ECOG 0–1 (see Appendix C).
c. No symptomatic or acutely life-threatening tumor which is judged likely to require intervention with alternative modalities for 3 months.
d. Non-tumor involved superficial inguinal or axillary lymph nodes which are surgically accessible.
e. Absolute WBC $>3000/mm^3$.
f. BUN <25 mg, creatinine ≤ 1.8 mg%.
g. Liver function tests: total bilirubin ≤ 2.0 mg.dl, SGOT ≤ 70 IU/L, SGPT ≤ 90 IU/L.
h. 18 years or older.

5.2 Patients will be excluded for:

a. Prior second malignancy (except squamous or basal cell skin cancer)
b. Previous severe reactions to any blood product.
c. Chronic renal disease with creatinine $\geq 2\times$ normal.
d. Previous chemotherapy, radiation therapy, or steroids within 4 weeks.
e. Brain metastasis.
f. Active infection requiring treatment or unexplained febrile illness, any collagen disease or autoimmune process.
g. History of ischemic or congestive cardiac disease requiring chronic medication (NYHA class III, IV) or evidence of ischemic change or venticular ectopy (>4/min) on electrocardiogram, or evidence of type II AV block. Any evidence of prior or current cardiac disease as determined by stress test and EKG.
h. Functional respiratory impairment (FEV_1 <2.0 liters or <75% predicted).
i. Pregnancy, or men and women of procreation potential who refuse to take precautions to avoid pregnancy.
j. Patients who are HIV positive.

6.0 Treatment Plan

Patients with advanced melanoma or renal cell cancer will undergo surgical retrieval of tumor from a readily accessible site for use as a tumor vaccine. A tumor cell line will be established in culture and subsequently transduced with a retroviral vector containing the GM-CSF gene. The transduced line will be screened for GM-CSF production and subsequently irradiated with 5,000 cGy. Patients will be vaccinated with 10^7–10^8 irradiated transduced tumor cells intradermally in 1 or 2 sites. Surgical removal of draining LN 7 to 10 days after vaccination will be performed to obtain lymphocytes for in vitro activation with anti-CD3 monoclonal antibody (OKT3) and expansion in IL-2. After in vitro activation, these lymphocytes will be adoptively transferred intravenously to the patient followed by the administration of IL-2 (360,000 IU i.v. every 8 h. for 5 days). Fifteen patients with each histology will be treated to gain sufficient information regarding toxicity, antitumor reactivity and immunologic function. Retreatment will be considered if the patient has a response and cryopreserved LN cells are available. This retreatment will be performed 2 months after completion of the last therapy and will involve repeat infusion of activated lymphocytes and IL-2. The procedures for tumor preparation, LN cell preparation and anti-CD3/IL-2 activation have been previously reviewed and approved by the FDA (IND 3860) for prior clinical studies.

6.1 Preparation of single cell suspensions and cultures from solid tumors

Resected tumors will be collected at surgery and transported in cold (4°C) RPMI 1640 media (GIBCO) until processing. Necrotic tumor and connective tissue will be trimmed and the remaining specimen will be minced to approximately 3–4 mm^3 pieces with scissors in HBSS containing 2.5 units/ml

hyaluronidase, 0.5 mg/ml collagenase and 0.05 mg/ml deoxyribonuclease. All enzymes are obtained from Sigma Chemical Co., St. Louis, MO in lyophilized form. The enzymatic digestion of solid tumors will be carried out at room temperature for 3–5 hours with constant stirring in sterile trypsinizing flasks containing magnetic stirring bars. The stirring plate will be set between 3–4 to allow turbulence without foaming. Routinely one gram of tumor specimen will be digested in a minimum volume of 10 ml enzyme mixture. The resulting cell suspension will be centrifuged (500 g, 15 min.), filtered through a double layer of no. 100 nylon mesh and resuspended in 100 ml RPMI 1640 medium containing 10% heat-inactivated human AB serum. If there are significant residual tumor pieces remaining in the trypsinizing flask they will be digested by repeating the above protocol. An aliquot of harvested tumor cells will be sent for cytologic analysis and for bacteriological testing. The remaining tumor cells will be cryopreserved in 90% human AB serum plus 10% dimethylsulfoxide at −178°C in liquid nitrogen for patient skin testing and in vitro assays. Each freezing vial will contain 2 to 4 × 10^7 tumor cells. The procedures for tumor procurement and preparation will be carried out by sterile techniques.

In addition to cryopreservation, a tumor cell line will be initiated by suspending approximately 60 ml of complete media (CM) in 150 cm^2 flasks with 1–5 × 10^6 freshly dissociated tumor cells/flask. CM is composed of RPMI 1640 with 10% human AB serum, 2 mM glutamine and 50 μg/ml gentamicin. Flasks are incubated at 37°C in 5°CO_2. It is anticipated that only 3–4 gm of tumor will be required from each patient for therapy.

6.2 Transduction of tumor cells with the MFG-S-GM-CSF viral particles

All procedures for tumor transduction will be performed in the Human Applications Laboratory of the CRC or MSRB I, Room 1522. These are BL-2 facilities certified by the Institutional Biosafety Committee. FDA certified, frozen virus supernatant will be thawed in preparation for tumor cell transduction. Tumor cells will be plated at 0.5–1 × 10^6 cells per T-75 flask and incubated at 37°C in a 5% CO_2 incubator for 18h in CM. Transduction will be performed by aspirating the CM and replacing with 10 ml of MFG-S-GM-CSF viral particles containing 35 μgm/ml of DEAE-dextran (Sigma) and incubated for 4 h. The viral particles are then aspirated and replaced with 14 ml of CM. GM-CSF production will be assayed 24h later by ELISA. If protein production is of high enough quantity to prepare a tumor cell vaccine (ie, greater than 40 ng/mL/10^6 cells/24 hours), the cells will be washed, harvested, and used for vaccine preparation as described below at 72h. A minimum of 10^7 cells/vaccine will be prepared which should represent at least 400 ng of GM-CSF produced per 24 h. The minimum level of GM-CSF production represents an equivalent amount of GM-CSF production identified in the animal model to enhance T cell sensitization in the draining LN (ie. 400 ng/24 h).

6.3 Vaccination with GM-CSF modified tumor cells

Gene-modified tumor cells will be harvested and irradiated with 5,000 cGy. The cell suspension will be adjusted so that a volume of 0.5 ml HBSS contains 10^7–10^8 irradiated tumor cells. The tumor cells will be placed on ice until injection. If 10^8 cells are not available, fewer may be given but not less than 10^7 will be injected per site. Patients will be immunized i.d. with 0.5 ml of the cell suspension in one or two separate sites drained by an accessible nodal basin (i.e. inguinal or axillary region). An india ink tatoo will be placed to mark each site of the injection. Lymph nodes draining the sites of vaccine will be selected so that the chosen LN group will not have clinically apparent metastases. Before immunization, patients will have been skin tested with a standard panel of microbial recall antigens (Merieux multi-test) as well as irradiated (10,000 cGy) irradiated autologous tumor cells in graded doses. These skin tests are performed to determine the immunocompetence of the patient as well as obtain pre-treatment evaluation of cutaneous reactivity to autologous tumor antigens.

6.4 Preparation of single cells from lymph nodes

Patients will undergo excision of hypertrophied LN draining the site 7 to 10 days after the vaccination. This can be performed under local anesthesia as an outpatient procedure. Lymph nodes will be transported to the laboratory in sterile containers containing HBSS. Single cell suspensions will be prepared mechanically by mincing with scissors to approximately 2 mm^3 pieces in cold HBSS. Cells will be dissociated by pressing pieces of LN through a sterile steel mesh with the blunt end of a 10 ml syringe plunger. The resultant cell suspension will be filtered through a double layer of no. 100 nylon mesh. After washing the cells in HBSS × 3, they will be placed in anti-CD3 activation cultures immediately and the remaining LN cells will be cryopreserved in liquid nitrogen for subsequent analysis and possible use in retreatments. All procedures will be carried out under sterile conditions.

6.5 Anti-CD3 activation and expansion

Animal studies of adoptive immunotherapy have clearly demonstrated that the numbers of transferred effector cells are directly related to the antitumor effects which were observed. Clinical studies which have reported tumor responses from the adoptive transfer of LAK or TIL cells have generally administered 10^{10} to 10^{11} effector cells (1,2). From our current clinical studies, we have been able to generate these numbers of human activated LN cells utilizing the proposed anti-CD3/IL-2 activation procedure (see Section 2.2). This required culturing cells an average of 15 days and subjecting the draining LN cells to a primary and secondary culture procedures. The methods for the primary and secondary culture procedures utilized in this clinical study are described below:

6.5.a Primary Anti-CD3 Activation: For primary anti-CD3 activation, 5 × 10^8 lymphocytes will be suspended in 250 ml of complete media (CM) and cultured in 24-well tissue culture plates pre-treated with OKT3. Plates are coated with mAb by adding 400 μl of diluted OKT3 (1 μg/ml) in sterile 0.05 M borate buffer, pH 8.6 per well and stored overnight at 4°C. Prior to use, the plates are washed of excess mAb with PBS. CM is X-Vivo-15 (Whittaker) supplemented with 10% human AB serum (Sigma). After 48 hours of activation the cells are harvested and expanded in IL-2.

Expansion in IL-2 is accomplished by suspending 3 × 10^5 anti-CD3 activated cells/ml by diluting the primary activation media with X-Vivo-15 containing 5% human AB serum sup-

plemented with 60 IU/ml of IL-2 (Chiron Therapeutics, Emeryville, CA). These cells are expanded in 3000 ml culture flasks (Lifecell 3000, Fenwal, Deerfield, IL) each containing 500–850 ml of media. When cell density reaches approximately 10^6 cells/ml, an additional 2 liters of serum-free X-Vivo-15 is added to each flask. Cells are grown to a maximum density (approximately 2×10^6 cells/ml) which is anticipated to take 5 days based on our current experience.

6.5.b Secondary Anti-CD3 Activation: The procedure to perform the secondary anti-CD3 activation represents a slight modification of the primary culture procedure. Briefly, 2×10^8 anti-CD3 activated cells derived from the primary culture procedure will be suspended in 100 ml of CM in 150 cm^2 flasks with immobilized OKT3 mAb. After 1 day, the cells are harvested, washed and expanded in serum-free media (X-Vivo-15) containing IL-2 (60 IU/ml) using 3000 ml culture bags as previously described. Cells are harvested after a 3–4 day period when cells reach their maximum density. It is anticipated that each patient will require 50–100 L of CM to generate anti-CD3 activated cells from primary and secondary culture procedures. In order to handle large-scale tissue cultures, we plan to use automated systems for solution transfer and cell harvesting. A solution transfer pump, life cell tissue culture bags and the Celltrifuge II (Fenwall, Deerfield, IL) will be utilized.

6.5.c Harvesting Conditions: One out of ten bags will be tested for sterility 48 hrs. before harvesting by obtaining an aliquot of cells and sending it for culture by the Microbiology Laboratory of the University Hospital. Bacterial monitoring will include thioglycolate broth, chocolate agar and sheeps blood agar. Cell suspensions from the culture bags will be harvested and washed in phenol-free HBSS using the Celltrifuge II (Fenwal). A gram stain of the final cell pellet will be evaluated to confirm that there are no organisms prior to infusion.

6.6 Adoptive cellular therapy and IL-2 administration

The lymphocytes will be suspended in infusion media consisting of 190 ml normal saline, 10 ml human albumin (concentration 25%, final concentration of 1.25%) and 450,000 IU IL-2. IL-2 will be administered i.v. to enhance the activity of the activated cells and will be given 3 times daily for five days starting on the day of cell transfer. Infusions of IL-2 will be 360,000 IU/kg and will commence immediately following cell transfer. This schedule and dose of IL-2 has been associated with manageable and reversible side effects according to our previous experience with this regimen. The patients will be treated as inpatients in the Clinical Research Center.

7.0 Toxicities to be Monitored/ Dosage Modifications

7.1 Vaccination with retroviral transduced tumor cells

Inoculation with a retrovirus-transduced tumor cell vaccine could theoretically pose risks to the patient which include: a. Possible exposure to replication competent retrovirus, b. Possible adverse effects from provirus integration, c. Growth of the inoculated tumor at tumor vaccine site, d. Systemic toxicity from GM-CSF production by the tumor cell vaccine, and e. Generation of autoimmunity by the tumor cell vaccine.

7.1.a Possible Exposure to Replication Competent Retrovirus: Retrovirus transduction of tumors for vaccine preparation will only be performed with FDA approved retrovirus stocks in a BL-2 facility located in the Clinical Research Center at The University of Michigan or Dr. Chang's laboratory Room 1522, MSRB I. All retrovirus stocks will be safety tested and documented to be free of replication competent virus, bacterial, viral, and fungal adventitial agents before use in tumor transduction for clinical trials.

7.1.b Possible Adverse Effects from Provirus Insertion: The tumor cells will be irradiated prior to administration to patients. This should provide for the complete loss of ability of the tumor cells to divide and or persist in the patients. Thus no growth of the tumor cells is expected, and therefore any possible mutagenesis from provirus integration should not result in harmful effects. If any tumors do grow at the inoculation site, they will be biopsied, and assayed for GM-CSF production, and for abundance of provirus sequences.

7.1.c Growth of the Inoculated Tumor at Tumor Vaccine Site: These patients will receive up to 10^8/irradiated transduced tumor cells intradermally in one or two sites. Tumor cells will be irradiated with 5,000 cGy. In our previous experience, inoculations of 2×10^7 irradiated (2,500 cGy) tumor cells admixed with BCG has not resulted in tumor outgrowth in more than 20 patients. Berd et al. has also reported an extensive experience in 64 melanoma patients treated with multiple injections of $1–2.5 \times 10^7$ irradiated (2,500 cGy) autologous tumor cells plus BCG without evidence of tumor outgrowth at the injection sites (17). The vaccine sites will be carefully monitored, and if tumor growth does occur the sites have been selected so that they can easily be excised with minimal morbidity. A small chance does exist, however, that if this site does grow that it might lead to the spread of injected tumor cells to other sites of the body. This unlikely event should not negatively influence the prognosis of this patient population which already has documented metastatic disease with limited life expectancy.

7.1.d Systemic Toxicity from GM-CSF Production by the Tumor Cell Vaccine: If gene modified tumor cells grow and produce GM-CSF, then patients may be exposed to systemic GM-CSF. In therapy, if a palpable tumor nodule were evident at 1 cm in size, approximately 10^9 cells would be present and secreting at least 40 μgm of GM-CSF/24 hrs (minimum required level of GM-CSF secretion is 40 ng/10^6 cells/24 hr). There is extensive clinical experience with giving GM-CSF in patients. Continuous infusion of GM-CSF to doses of 6,500 μgm/m^2/24h over a 2 week period have been reported to be associated with dose-dependent increases in circulating monocytes (25). Other reports of continuous intravenous GM-CSF up to 32 μg/kg/24h for 2 weeks (i.e. in a 60 kg patient = 1920 μg/24h) in bone marrow transplant patients was associated with accelerated recovery of total leukocyte and granulocyte counts (26). Edema, weight gain and myalgias occurred in all patients at this latter dose. We will monitor for GM-CSF effects by measurement of serum levels and circulating monocytes and granulocytes.

7.1.e Generation of Autoimmunity by the Tumor Cell Vaccine: If GM-CSF production within an autologous tumor unlocks immunity to several self antigens, it may predispose the patient to develop autoimmunity and/or an autoimmune like syndrome. Tumor vaccines have been used throughout this century

without such an effect, and in our BCG/autologous cell trial we have not seen evidence of clinical autoimmunity. We will measure anti-DNA antibodies to monitor for this possibility.

7.2 Cellular Therapy/IL-2 Administration

In all clinical studies of adoptive immunotherapy the major toxicities have been generally attributable to the infusion of high-dose IL-2 (i.e. 720,000 IU/kg i.v. q8h). At this dose, the most prominent side effects are hypotension, leaky capillary syndrome, fluid retention, oliguria, respiratory distress and mental confusion (1,2). Since the side effects are dose related, it is anticipated that the toxicity associated with this proposal should be less since the dose of IL-2 being administered is 360,000 IU/kg i.v. q8h for a total of 5 days. With our current clinical studies, we have treated 14 patients with activated vaccine-primed LN cells and IL-2 at the proposed dosage without life-threatening toxicities.

Medications are used to overcome some of the side effects of treatment. Fever, chills and malaise are significantly reduced by the use of acetaminophen (650 mg every six hours) and indomethacin (25 mg every six hours). Many patients receive hydroxyzine hydrochloride, an antihistamine, for treatment of a generalized erythematous rash. Patients are also maintained on ranitidine (150 mg orally bid) for prophylaxis against gastrointestinal bleeding. Patients receiving anti-hypertensive medications will have them discontinued during treatment with IL-2.

Toxicities of treatment will be recorded daily during treatment and graded on a 0–4 scale as defined by the NCI Common Toxicity Criteria (0 = no toxicity, 4 = life threatening, see Appendix D). For grade 4 toxicity, treatment will be discontinued. Based on our clinical experience and the experience reported by other groups. The dose modifications related to individual organ toxicity will be followed as indicated in Appendix E. Doses of IL-2 which are held will be restarted at 50% reduction from the previous dose if the specific toxicity resolves to the next acceptable lower grade.

8.0 Study Parameters

8.1 Pretreatment patient evaluation

a. Complete history and physical examination
b. Chem-21 blood chemistry panel
c. CBC, differential, PT, PTT, platelet count
d. Urinalysis
e. HB_sAg, HTLV III Antigen
f. Anti-DNA antibodies
g. Chest CT scan
h. Abdominal CT scan
i. Head CT scan
j. Bone scan
k. Pregnancy test (if woman of child-bearing age)
l. Stress myocardial function tests for patients >65 years of age
m. PFT's for patients with a smoking history >15 pack years

8.2 Adoptive cellular therapy/IL-2 administration

Daily CBC, differential, platelet count, Chem-21

8.2 Post-treatment patient evaluation

a. History and physical examination with chest x-ray, Chem 21, and CBC every month post-treatment until responsive status is determined.
b. Chest and abdominal CT scan as appropriate to follow evaluable disease monthly for 2 months.

8.4 Immunologic studies: will be performed pre and post treatment (1 and 2 months after therapy)

a. Recall skin tests: Merieux multi-test
b. Cutaneous hypersensitivity to tumor: i.d. 10^4, 10^5 and 10^6 irradiated tumor cells (10,000 cGy) in the contralateral extremity
c. Anti-DNA antibodies

8.5 Assessment for adverse effects due to the retroviral transduced tumor cells

Serum GM-CSF assays and monocyte/granulocyte counts will be performed prior to tumor vaccination, at the time of LN harvest, at the time of adoptive cellular therapy; and at 1 and 2 months after completion of therapy.

9.0 Criteria for Evaluation/Removal from Study/Retreatment

Definition of responses: to be determined after a minimum of 4 weeks; and should last a minimum of 4 weeks.

9.1 Complete tumor response (CR) is defined as disappearance of all signs, symptoms, biochemical, and radiographic evidence of tumor. In the case of accessible cutaneous or subcutaneous metastases, tissue biopsy of at least one known site of tumor is requested.

9.2 Partial response (PR) is defined as a reduction of all measurable tumor lesions by 50% of the product of the two greatest perpendicular diameters (sum of all evaluable tumors), without the appearance of new tumor lesions or the concurrent progression of any previously defined lesions.

9.3 Minimal response (MR) is defined as a reduction of all measurable tumor lesions by ≥25% but <50% of the product of the two greatest perpendicular diameters (sum of all evaluable tumors), without the appearance of new lesions or the concurrent progression at any sites of known disease.

9.4 Stable disease (SD) includes all with response less than described for progressive disease.

9.5 Progressive disease (PD) is defined as an increase of all measurable tumor lesions by ≥25% of the product of the two greatest perpendicular diameters (sum of all evaluable tumors), or the appearance of new lesions.

9.6 Patients that recur at any site will be considered as failures of that treatment and other standard or experimental treatments will be considered as appropriate for their disease. If a tumor response (PR or CR) is evident by 2 months post treatment, then retreatment with activated cells from cryopreserved material during the following month will be offered to the patient. If cryopreserved LN are not available, then retreatment with IL-2 alone will be offered.

10.0 Statistical Considerations

Fifteen patients will be enrolled into this study for each tumor histology. This has been deemed adequate to determine several in vitro and in vivo immunologic parameters which are being followed. Cytotoxicity, proliferation and cytokine release (ie. GM-CSF and IFNγ) to autologous tumor will be measured of the vaccine-primed LN in vitro. Evidence of tumor-specific responses in these assays would be a basis for a phase II study since an antitumor effect may be related to the quantitative requirement of larger numbers of adoptively transferred cells. Similarly, if there is evidence for the development of cutaneous hypersensitivity to autologous tumor after adoptive immunotherapy in the majority of patients, then a phase II study is justified.

11.0 Ethical and Regulatory Considerations

The following will be observed for compliance with Food and Drug Administration regulations involving the conduct and monitoring of clinical investigations; they also represent sound research practice:

11.1 Institutional review

This study will be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56). The protocol and informed consent form for this study will be approved in writing by the appropriate Institutional Review Board (IRB). The IRB will be from an institution which has a valid Multiple Project Assurance, Single Project Assurance or Cooperative Oncology Group Assurance on file with the Office for Protection from Research Risks, National Institutes of Health. The institution must be in compliance with regulations of the Food and Drug Administration and the Department of Health and Human Services.

Significant changes to the protocol, as well as a change of principal investigator, will also be approved by the Board and documentation of this approval provided to the study monitor. Records of the Institutional Review Board review and approval of all documents pertaining to this study will be kept on file by the investigator and are subject to FDA inspection at any time during the study. Periodic status reports will be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 3 months of study completion or termination. The investigator will maintain an accurate and complete record of all submissions made to the Institutional Review Board, including a list of all reports and documents submitted.

11.2 Drug accountability

For each drug supplied for this study an accountability ledger containing current and accurate inventory records covering receipt, dispensing, and the return of study drug supplies will be maintained. The ledger will be maintained routinely for all studies regardless of study design, and will identify for each shipment the subject number (as applicable) and the quantity of drugs contained in the shipment. The ledger will consist of drug Accountability Record Forms supplied by the NCI. One form for each investigational drug used on each research protocol will be kept. A separate drug Accountability Form will also be maintained for each different strength or dosage form of the particular drug being used.

Drug supplies will be kept in a secure, limited access storage area under the recommended storage conditions. During the course of the study, the following information will be noted on the accountability ledger; the identification code of the subject to whom drug is dispensed, the date(s) and quantity of drug dispensed to the subject, and the date(s) and quantity of drug returned by the subject; subjects should return empty containers to the investigator, and the return noted on the ledger. These Accountability Forms will be readily available for inspection and are open to FDA inspection at any time.

11.3 Reporting "ADR" (adverse drug reactions) (NCI 12/89)

The Federal (NCI and FDA) guidelines and conditions under which an "ADR" should be reported summarized in this section. The appropriate forms will be completed and submitted to both the NCI and to the University IRB Committee. This reporting will be performed according to the guidelines listed below.

11.4 University of Michigan Cancer Center reporting requirements of "ADR"

In addition to notifying the appropriate Federal and Group or Drug Company monitors, the University of Michigan Medical Center and the VA Medical Center both have internal notification procedures to follow. A copy of the completed form will be sent to the following groups: Nursing, Pharmacy, IRB Committee and appropriate medical staff.

If the "ADR" is an unknown reaction which has not been previously reported for the drug in question, the consent form will also be modified to include the previously-unknown toxicity. If this is necessary, the modified consent will be submitted immediately to the IRB Committee.

If the protocol in question is sponsored by the Cancer Center or one of the programs within the Cancer Center, the Protocol/Data Management section of the Clinical Trials Office will carry out the appropriate procedures for the "ADR" notification to both federal and hospital departments. This includes reporting adverse events to CTEP according to NCI guidelines for phase I trials.

12.0 Bibliography

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GUIDELINES FOR REPORTING OF ADVERSE DRUG REACTIONS

Phase II and III Studies			
Unknown reaction[2,4,5]		*Known reaction*[2,4,5]	
Grades 2–3	*Grades 4–5*	*Grades 1–3*	*Grades 4–5*
Written report to IDB within 10 working days[3]	Report by phone IDB within 24 hours.[1] Written report to follow within 10 working days	Not to be reported to IDB as ADRs. These toxicities should be submitted as part of study summary.	a) Written report to IDB within 10 working days. b) Grade 4 myelo-suppression not to be reported, but should be submitted as part of study results. c) Grade 5 Aplasia leukemia patients—Written report within 10 working days to IDB[6]

Phase I Studies		
Unknown reaction[2,4,5]	*Known reaction*[2,4,5]	
Any Grade	*Grades 1–3*	*Grades 4–5*
The 1st occurrence of any *previously unknown* chemical event should be reported to the IDB by phone within 24 hours[1] and a written report submitted within 10 working days[3]	Not to be reported to IDB as ADRs. These toxicities should be submitted as part of study summary.	All life-threatening —OR— fatal events are reported by phone to the IDB within 24 hours

[1]Telephone number available 24 hours daily: 301/496-7957 (recorder after hours).
[2]See attached Common Toxicity Criteria (Appendix I).
[3]Report to: Investigational Drug Branch
P.O. Box 30012
Bethesda, MD 20824
[4]A list of all known toxicities can be found in the protocol document (Background Section or Informed Consent), and the manufacturer's package inserts.
[5]Reactions judged *definitely* not treatment related should not be reported. However, a report shall be submitted if there is only a reasonable suspicion of drug effect.
[6]Grade 4 unknown and known reactions not to be reported. Report Grade 5 unknown and known reaction of aplasia in leukemia patients within 10 working days IF clearly related to experimental drug (i.e., single agent therapy). Otherwise, do not report.

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APPENDIX B: ELIGIBILITY CHECKLIST

Protocol: Adoptive Immunotherapy of Cancer with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the GM-CSF Gene

NAME _____
last first middle initial

REG # _____ DATE ON STUDY _____

All answers must be "YES"

Y____N____ Does the patient have histologically proven metastatic melanoma, incurable by standard modalities? (check applicable site)
Y____N____ Does the patient have a performance status ECOG 0–1? (Performance status _____)
Y____N____ Does the patient have non-tumor involved superficial inguinal or axillary lymph nodes which are surgically accessible?
Y____N____ Is the patient a woman of childbearing age who is willing to take precautions to avoid pregnancy? (Put N/A if question is not applicable)
Y____N____ Does the patient have an absolute WBC <3000/microliter? (WBC = _____)
Y____N____ Does the patient have a BUN < 25 mg and creatinine ≤1.8 mg%? (BUN = _____ and Creat = _____)
Y____N____ Does the patient have liver function tests with total bilirubin ≤2.0 mg.dl, SGOT ≤70 IU/L and SGPT ≤90 IU/L? (T.bili = _____, SGOT = _____, SGPT = _____)
Y____N____ Is the patient 18 years or older? (Age = _____ years)
Y____N____ Has the patient given informed consent?

All answers must be "NO"

Y____N____ Does the patient have a symptomatic or acutely life-threatening tumor which is judged likely to require intervention with alternative modalities for 3 months?
Y____N____ Has the patient had a prior second malignancy (except squamous or basal cell skin cancer)?
Y____N____ Has the patient had any previous severe reaction(s) to any blood product?
Y____N____ Has the patient had chronic renal disease with creatinine ≥2× normal?
Y____N____ Has the patient had previous chemotherapy, radiation therapy, specific and non-specific immunotherapy or steroids within 4 weeks?
Y____N____ Does the patient have brain metastasis?
Y____N____ Does the patient have an active infection requiring treatment or unexplained febrile illness, any collagen disease or autoimmune process?
Y____N____ Does the patient have a history of ischemic or congestive cardiac disease requiring chronic medication (NYHA class III, IV)? (NYHA Class _____)
Y____N____ Does the patient have evidence of ischemic change or ventricular ectopy (>4/min) on electrocardiogram, or evidence of type II AV block?
Y____N____ Does the patient have any evidence of prior or current cardiac disease as determined by stress test and EKG?

Y____N____ Does the patient have functional respiratory impairment (FEV_1 <2.0 liters or <75% predicted)? (FEV1 = _____ liters -or- _____% predicted)
Y____N____ Is the patient pregnant or of child-bearing age?
Y____N____ Is the patient HIV positive?
Y____N____ Does the patient have a diagnosis of leukemia or lymphoma?

APPENDIX C. KARNOFSKY AND ECOG PERFORMANCE CRITERIA

Karnofsky		*ECOG*	
Activity	*Score %*	*Grade*	*Activity*
Normal, no complaints	100		
Normal, only minor signs/symptoms	90	0	Fully active, able to carry on all predisease activities without restrictions
Normal activity, but effort	80		
		1	No physically strenuous requires activity, but ambulatory and able to carry out light or sedentary work (eg., office work, light house work)
Unable to do active work, but able to care for self	70		
Able to care for most needs, requires occasional help	60		
		2	Ambulatory/capable of all self-care, unable to perform any work activities Up and about more than 50% of waking hours
Requires frequent medical help and considerable assistance	50		
Disabled, needs special assistance	40		
		3	Capable of only limited care and self-care, confined to bed or chair more than 50% of waking hours
Severely disabled, needs hospitalization, death not imminent	30		
Very sick, hospitalized, support needed	20		
		4	Completely disabled, active totally confined to bed or chair. Cannot carry on any self-care.
Moribund	10		
Dead	0	0	Dead

APPENDIX E. IL-2 DOSE MODIFICATION

NCI common toxicity (see Appendix)	*Dose modification*		
	Grade:	*2*	*3*
Blood/bone marrow:			
WBC/Hgb/granulocytes/lymphocytes		—	—
PLT		—	Hold
Hemorrhage:		Hold	Hold
Infection:		Hold	Hold
Gastrointestinal:			
Nausea/Vomiting/Diarrhea/ Stomatitis		—	—
Liver:			
Bilirubin/SGOT/SGPT/ Alk phos		—	—
Liver, clinical		—	Hold
Kidney/bladder:			
Creatinine/proteinuria		—	—
Hematuria		—	Hold
Alopecia:		—	—
Pulmonary:		—	Hold
Heart:			
Cardiac dysrrhymias		Hold	Hold

NCI common toxicity (see Appendix)	*Dose modification*		
	Grade:	*2*	*3*
Cardiac		—	Hold
Cardiac ischemia		Hold	Hold
Pericardial		Hold	Hold
Blood pressure:			
Hypertension		—	Hold
Hypotension		—	Hold
Neurologic:			
Neurosensory		Hold	Hold
Neuromotor		—	Hold
Neurocortical		Hold	Hold
Neurocerebellar		Hold	Hold
Mood		—	—
Headache		—	Hold
Constipation		—	—
Hearing		—	—
Vision		—	Hold
Skin:		—	—
Allergy:		—	Hold
Fever in absence of infection:		—	—
Local:		—	—
Weight gain:		—	—
Metabolic:			
Hyperglycemia		—	Hold
Hypoglycemia		Hold	Hold
Amylase		—	Hold
Hypercalcemia		—	Hold
Hypocalcemia		—	Hold
Hypomagnesemia		—	Hold
Coagulation:			
Fibrinogen		—	Hold
PT		—	Hold
PTT		—	Hold

The University of Michigan Medical School and the Clinical Research Center

CONSENT FORM

(To be read by the Patient and explained to the Patient by his or her Physician).

PROTOCOL: *Adoptive Immunotherapy of Cancer with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the GM-CSF Gene*

PRINCIPAL INVESTIGATOR: Alfred E. Chang, M.D.
CO-INVESTIGATORS: D. Keith Bishop, Ph.D.
Brian J. Nickoloff, M.D., Ph.D.
James J. Mulé, Ph.D.
Vernon K. Sondak, M.D.

PROTOCOL NUMBER: _____ DATE: _____

PATIENT NAME: _____ HOSPITAL NO.: _____

INTRODUCTION

I am being invited to participate in a research study at the University of Michigan Medical Center. I understand that several general principles apply to all who take part in any experimental studies:

1. My participation in this study is voluntary.

2. I may not personally benefit from this study, but knowledge may be gained from it that will benefit others.
3. I may withdraw from the study at any time for any reason without jeopardizing my further care.
4. I meet all the eligibility requirements of this study.
5. Consent for this research study will require my signature and the signature of the treating physician and a witness.
6. I may take any period of time to consider entrance into this study.

I am aware of the nature of the study, the risks, inconveniences, discomforts, and other information which are discussed in the following sections. This study is seeking to enter 15 patients with melanoma and 15 with kidney cancer.

DESCRIPTION OF RESEARCH

I have a melanoma or a kidney cancer which cannot be cured by medicine, surgery or radiation. This is a study, to test a new approach that may help to fight this disease in future patients. Because this approach is experimental, I may not derive any direct benefit from it. I understand that the purpose of this study is to determine the safety of this new approach which will attempt to cause tumor shrinkage. My case had been reviewed by the University of Michigan Melanoma or Urology Multidisciplinary Tumor Boards and has been deemed appropriate for consideration of this research study.

I will have removal of a sample of my own tumor which will be subsequently processed in a laboratory. The tumor will be modified in the laboratory by inserting a gene into the cells which will cause them to make a protein called granulocyte/macrophage colony stimulating factor (GM-CSF). GM-CSF is an immune hormone normally produced by the body. After this laboratory manipulation which will take 2 or 3 weeks, I will be injected with GM-CSF modified tumor cells in one or two skin sites of my thigh or underarm areas. These modified tumor cells will be irradiated prior to injection into the skin in order to prevent their growth. About 7 to 10 days later I will undergo removal of lymph nodes that are adjacent to these injected skin sites in order to grow cells called lymphocytes that will later be given back to me as part of my treatment. The lymphocytes will be returned to me through an intravenous line placed in a blood vessel. In addition to these lymphocytes, I will receive interleukin-2 (IL-2), a protein normally made by the body's immune system that can help the immune cells function in my body.

PROCEDURES TO BE UNDERTAKEN

Before starting this research, I will have a number of tests to determine if I quality for the study. These studies may include the following: 1) x-rays of the brain, chest and abdomen; 2) blood tests; 3) testing my blood for the antibodies to human immunodeficiency virus (HIV) that causes AIDS, a separate informed consent will be given to me for this test. If I am found to have these antibodies, I may not participate in this study. If I do qualify, I will undergo a series of procedures.

I understand that the first step will require the surgical removal of one of my metastatic tumors from a readily accessible site in order to grow the tumor cells in a test tube. This surgical procedure will probably be done under local anesthesia but may require general anesthesia. My tumor cells will then be modified by insertion of genetic material using a mouse virus which will result in their production of GM-CSF, a protein normally made by the body's immune system which can help stimulate immune cells.

After making these modified tumor cells, they will be irradiated in the test tube and then injected into the skin of my thigh or underarm area in order to stimulate an immune reaction in nearby lymph nodes against my tumor. The injections will be done with a standard needle and syringe. Approximately 7 to 10 days later, the lymph nodes adjacent to the injected skin sites will be surgically removed. This procedure will probably be done under local anesthesia. The lymph nodes will be taken to the laboratory where the cells from them will be further stimulated in the test tube and grown into larger amounts. After 2 to 3 weeks, the lymph node cells should have grown sufficiently enough to transfuse them back into me along with the administration of IL-2. This part of the research will require hospitalization for several days. I am aware that it will be necessary to place an intravenous catheter through a vein either on the upper chest or in the neck that will be threaded into a central vein in my body. This intravenous catheter is placed under local anesthesia at the bedside. The lymph node cells will be infused once through this intravenous catheter over a 30 minute period and will be followed by 15 minute IL-2 infusions given every 8 hours for up to 15 doses. The IL-2 is a protein normally produced by the body which is given to allow the injected lymph node cells to remain alive.

I understand that at different times in the study, biopsies of superficial tumors or the skin injection sites may be performed. These biopsies would be performed under local anesthesia and would not occur more than two times.

RISKS AND SIDE EFFECTS

I know that there are potential side effects and risks to these procedures which are summarized below. Besides the side effects listed below, unforeseeable risks may also occur as part of this research study. If there are life-threatening side-effects which occur on this study, my participation may be terminated without my consent.

Surgical removal of tumor and lymph nodes:

These procedures may be associated with discomfort which should be minimized by an appropriate anesthetic agent. It is possible I may develop bleeding or an infection at the site of the procedure, I will receive a separate surgical consent document for these procedures.

Skin injection with gene-modified tumor cells:

I will be injected with gene-modified tumor cells into the skin in an attempt to stimulate an immune response against my tumor. This may cause mild discomfort at the site of injection. One possible side effect may be growth of tumor at the skin injection site. The tumor cells will have been irradiated prior to the injection procedure in an effort to avoid this problem. If tumor does grow at the injection sites, they will be removed surgically in order to minimize problems in these areas.

I understand the potential risks associated with receiving cells modified by this gene. The genetic material was inserted into my tumor cells with the use of a mouse virus. I have been informed that even though the mouse virus used to insert the gene into my tumor cells cannot grow and is considered harmless to me, it is possible that events could occur within the cell that allow the virus to grow. To minimize this possibility, the virus used to modify the cells has been tested extensively prior to use. I am aware that there is a remote chance that the use of this virus may cause a new cancer. Because this procedure is relatively new, it is possible that despite extensive precautions, other unforeseen problems may occur including the very remote possibility of death.

The gene-modified tumor cells I will receive are designed to produce an immune hormone called GM-CSF. This hormone is naturally produced in the body in small amounts. However, the gene-modified tumor cells which are injected into my skin may produce too much GM-CSF. This might lead to side effects which include muscle pains and fluid retention. I will be monitored for this possible side effect by obtaining blood samples on a periodic basis to measure GM-CSF levels and blood counts. If it becomes apparent that I am experiencing problems related to GM-CSF production by the tumor cells, they will be surgically removed.

Lymph node cells/IL-2 infusions:

For the administration of my lymphocytes it will be necessary to place an intravenous catheter through a vein either on the upper chest wall or in the neck that will be threaded into a central vein in the body. The risks involved in having such a central venous catheter include collapse of the lung, infection, or development of a blood clot in the vein. Infusion of the cells into my vein is performed over a 30 minute period. The administration of these cells may cause side effects including fever, chills and some shortness of breath but I have been informed that these effects last for only a few hours.

I am aware that the administration of IL-2 can cause significant side effects. These include weight gain from fluid retention, shortness of breath, a drop in blood pressure, infections, nausea, diarrhea, fever, chills, fatigue, anemia, thrombocytopenia, abnormal heart beats, abnormalities of liver and kidney function and confusion. I understand that this latter potential side effect of confusion can range from forgetfulness and disorientation to coma, an unresponsive state. I have been informed that these side effects require close monitoring in the hospital. If any serious side effect occurs I understand the treatment will stop and appropriate medical actions will be taken. I am aware that treatment with IL-2 may cause my death. Less than 1% of patients who have received IL-2 have died as a result of the treatment.

GENE TRANSFER ASPECTS OF THIS STUDY

This research study utilizes a new technology which involves the insertion of a gene into my tumor cells to alter their immunological reactivity. There have been no previous experience with the use of this genetic material in patients. I understand that even though the mouse virus used to insert the gene into my tumor cells cannot grow and is considered harmless to me, it is possible that events could occur within the cell that allow the virus to grow. To minimize this possibility, the virus used to modify the cells will be tested extensively prior to use. I am aware that there is a remote chance that the use of this virus may cause a new cancer. Because this procedure is new, it is possible that despite extensive precautions, other unforeseen problems may occur including the remote possibility of death.

This research may have unforeseeable risks to an embryo or fetus. If I could potentially father a child or bear a child, I will not participate in this research study unless I am employing a medically acceptable form of birth control for a 3 month period after receiving the gene-modified tumor cells.

To permit evaluation of long-term safety and effectiveness of this gene therapy study, I agree to cooperate in long-term follow-up that extends beyond the active phase of the study. I will provide Dr. Chang with any future changes in my address or telephone number in order that long-term follow-up will be possible.

Due to the nature of the study which involves genetic alteration of some of my tumor cells, I have been informed that vital information could be obtained from an autopsy examination in the event of death in the near future. I am aware that a request for autopsy should I die will be requested from my family, and I will advise my family about this possibility.

FOLLOW-UP

After I have completed the research protocol, I will be required to return to the University of Michigan Medical Center for follow-up studies approximately four and eight weeks after hospital discharge. Tests used to decide if my tumor responded to the research will be similar to those I had before beginning the research. I will also undergo skin testing at these time intervals as well as prior to start of the research. Some of these skin tests will be done to determine if I react to my own tumor cells. This will involve the injection of irradiated tumor cells in my skin. If my disease worsens after this protocol, I will be eligible for other protocols and will receive treatment as indicated by my disease or referred elsewhere for such treatment. I understand that this research may attract attention from the media. I have been informed that every effort to protect my confidentiality will be made. Because of media interest, however, I realize there is a significant chance that information concerning me and this research may appear publicly without my consent.

By two months after the first cycle, if I have evidence of tumor shrinkage or my tumors remain unchanged it is possible that additional cycles will be offered. I understand repeat cycles would consist of receiving additional lymph node cells which had been frozen and stored from my initial procedures; along with the administration of IL-2. Repeat cycles would not entail any further surgical procedures to remove tumor or lymph nodes. My physicians feel that the risks of my disease are much greater than the risks of the research as outlined above. Furthermore, my physicians have considered my individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy or other chemotherapeutic treatments are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with me because this study does not preclude their use.

POTENTIAL BENEFITS FOR PARTICIPANTS

I understand that there may be no direct benefit to me from study participation, and there is a possibility my condition may become worse. In the past, lymphocyte and IL-2 infusions have resulted in some tumor shrinkage in some individuals but not in others. Even those tumors that exhibit shrinkage may show regrowth after a short period of time. A better understanding of immunity to cancer may be gained by my participation that would benefit other patients.

ALTERNATIVE THERAPIES

There are no known cures for patients with my disease. I am aware that alternative treatments available to me include chemotherapy or other experimental treatments. The option also exists to receive no treatment at this time.

OTHER PERTINENT INFORMATION

1. Confidentiality. I understand that when results of a study such as this are reported to medical journals or at meetings, the identification of those taking part is withheld. Medical records are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users (ie., qualified representative of pharmaceutical companies), only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records.
2. Policy regarding research-related injuries. I understand that in the unlikely event a physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment of injuries or side effects directly related to this experimental treatment will be provided at no cost to me. Additional medical treatment will be provided in accordance with the determination by the University of responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research. Other additional costs incurred by my participation in this study may occur (ie., travel expenses) which I will be responsible for.
3. I will not be paid to take part in this study.
4. Laboratory tests and scans will be ordered to determine my eligibility for study and whether I have had a response to the treatment. Items which are not covered by insurance which relate to this research protocol will be free. The cost of tests and treatments unrelated to this study will be charged for. I understand that insurance coverage cannot be guaranteed for all tests and treatments.
5. This consent form does not include consent relating to the risks of any surgical procedures. Any surgical procedures performed will require a separate consent form.
6. If there are any new significant findings which develop over the course of this research which may affect your willingness to participate, you will be provided these findings. If you do not participate, or do not complete the study it will not affect your care at the University Hospital, and you may continue to be treated by your doctor.

7. I am free to withdraw my consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in my care.

If you have questions pertinent to this research, you should contact Alfred E. Chang, M.D., at 313/936-4392.

If you feel that you have a research-related injury, contact Alfred E. Chang, M.D., at 313/936-4392.

The following numbers are for your use if medical problems develop during or after treatment:

Office: 313/936-4392
Clinical Research Center: 313-936-8090
Voice Mailbox: 313/936-4392 (after office hours)
Doctor or Nurse: 313/936-6267 (This is hospital paging operator. Ask for the physician on call for Surgical Oncology).

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456.

I have fully explained to the patient, _____ the nature of the research program described above and such risks involved in its performance.

_____
Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that since my participation is voluntary, I can refuse this study without in any way prejudicing my future medical care. In signing this consent form, I agree to this research protocol, and I understand that I will receive the best supportive care even if not participating in this research. I also understand that my doctors can stop this protocol if they feel the risks in my case have increased, or to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have retained a copy of this consent form.

I understand that the University will provide first-aid medical treatment in the unlikely event of physical injury resulting from procedures. Treatment of injuries or side effects directly related to experimental treatment will be provided in accordance with the University's determination of its responsibility to do so. The University does not however, provide compensation to a person who is injured while participating as a subject in research.

_____
Signature of Patient

_____
Date

_____
Witness

This article has been cited by:

1. Alfred E. Chang , Qiao Li , D. Keith Bishop , Daniel P. Normolle , Bruce D. Redman , Brian J. Nickoloff . 2000. Immunogenetic Therapy of Human Melanoma Utilizing Autologous Tumor Cells Transduced to Secrete Granulocyte-Macrophage Colony-Stimulating FactorImmunogenetic Therapy of Human Melanoma Utilizing Autologous Tumor Cells Transduced to Secrete Granulocyte-Macrophage Colony-Stimulating Factor. *Human Gene Therapy* **11**:6, 839-850. [Abstract] [PDF] [PDF Plus]
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