KARNOFSKY MEMORIAL LECTURE

The Immunotherapy and Gene Therapy of Cancer

By Steven A. Rosenberg

IN THE LAST decade biologic therapy has emerged as a mode of treatment capable of mediating the regression of cancer in some patients. Biologic therapy differs conceptually from surgery, radiation therapy, or chemotherapy because it acts, not by directly attacking the tumor, but by stimulating natural host defense mechanisms to mediate cancer regression.[1,2] The predominant natural host defense mechanism is the immune system.

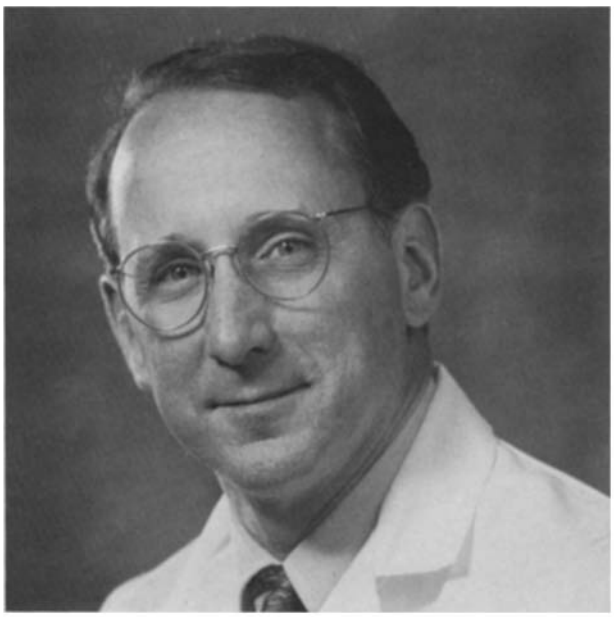
Two factors have converged to enable new approaches to the immune manipulation of the cancer patient in an effort to mediate tumor regression. Substantial progress has been made in understanding the molecular nature of the cellular immune response to cell-surface antigens or to exogenous antigens presented in conjunction with major histocompatibility complex (MHC) molecules. In addition, rapid advances in biotechnology have made it possible to obtain large amounts of biologic molecules that were previously available only in minute quantities. Most prominent among these scarce biologic molecules are the cytokines, which are hormones produced by immune cells that play important roles in regulating the immune system.

We have attempted to develop methods for mediating the rejection of human cancers using immune manipulations with recombinant cytokines and immune cells. One approach we have taken has been referred to as adoptive immunotherapy, which can be defined as the transfer, to the tumor-bearing host, of immunologically reactive cells with antitumor reactivity that can mediate antitumor effects either directly or indirectly. The inability to identify immune cells in the tumor-bearing animal and human that could specifically recognize tumor cells represented the major impediment to the development of adoptive immunotherapy.[3]

The description in 1976 of a hormone called T-cell growth factor (later renamed interleukin-2 [IL-2]) provided new opportunities for identifying cells with antitumor reactivity from a cancer-bearing patient.[4] IL-2 plays a central role in regulating the immune response by mediating the expansion of lymphocyte clones responsive to antigen in vivo.[5]

STUDIES OF IL-2 AND LYMPHOKINE-ACTIVATED KILLER CELLS

Studies with IL-2 attempting to isolate lymphocytes with the specific ability to recognize cancer but not normal cells led us to the description of lymphokine-activated killer (LAK) cells in 1980.[6,7] The incubation of lymphocytes in IL-2 results in the generation of cells capable of lysing fresh tumor cells in short-term, ie, 4-hour, cytotoxicity assays. We performed extensive studies of the biologic characteristics of these LAK cells in both the mouse and human and identified them as non-B, non-T cells that were capable of recognizing cancer cells in a non–MHC-restricted fashion.[8-18] LAK cells, which could be generated from either the normal or tumor-bearing host, appeared to represent a primitive immunosurveillance system capable of recognizing and destroying altered cells. Extensive studies in experimental animal models subsequently demonstrated that the administration of either IL-2 alone or LAK cells plus IL-2 could mediate the regression of established lung and liver metastases from a variety of animal cancers.[19-29] The antitumor effects of both IL-2 and LAK cells were dose-related, and in most models, the combined admin-



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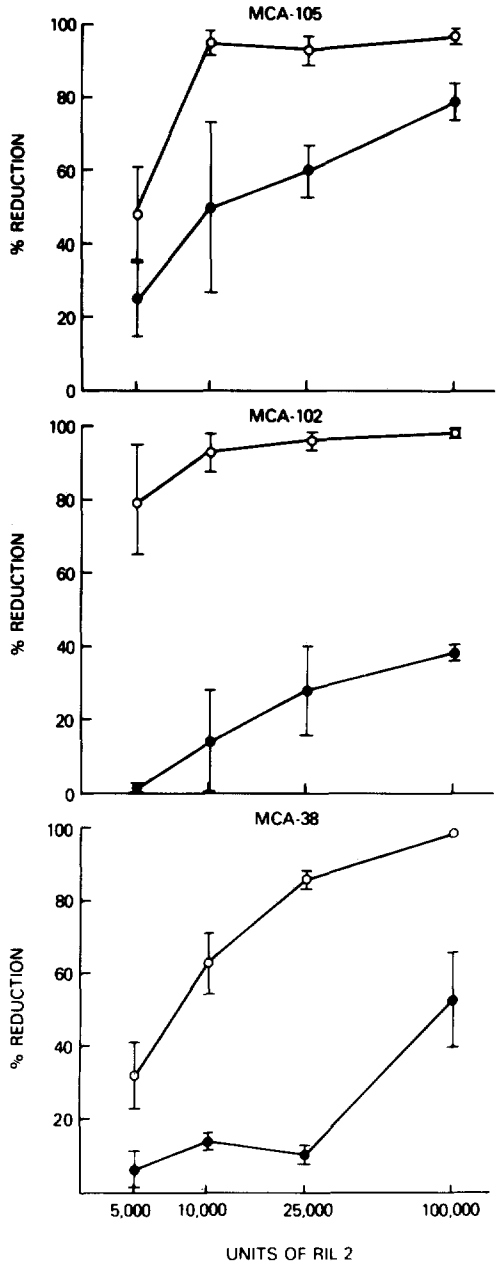

Fig 1. Therapy of established liver metastases in mice using either recombinant IL-2 alone (●) or LAK cells plus recombinant IL-2 (○). Varying doses of recombinant IL-2 were administered intraperitoneally every 8 hours for 5 days. At each dose of recombinant IL-2 more effective reduction in established 3-day liver metastases was seen when LAK cells were also administered. Reprinted with permission from Lafreniere et al.[24]

istration of LAK cells plus IL-2 mediated better antitumor effects than IL-2 alone. An example of one of these early studies in the treatment of established murine liver metastases is shown in Fig 1.[24] A summary of the major findings of the use of either IL-2 alone or LAK cells plus IL-2 in animal therapy models is shown in Tables 1 and 2. These early studies demonstrated that LAK cells could expand in vivo under the influence of their requisite growth factor, IL-2, and maintain their antitumor activity as they multiplied. This basic principle of adoptive immunotherapy influenced many of our subsequent treatments. Activated cells could expand in vivo as long as IL-2 was administered and died in vivo when IL-2 administration was discontinued.[30,31]

The application of these findings to patients with advanced cancer was dependent on a supply of large amounts of IL-2, a problem that was solved when the gene for IL-2 was cloned and expressed in *Escherichia coli.*[32,33] Purification to homogeneity of this recombinant IL-2 and the demonstration that it was similar in all biologic and functional aspects to natural IL-2[34,35] led directly to clinical trials in patients with advanced cancer in whom all available effective treatments had failed.

Although results of studies in animal models predicted that the combined administration of LAK cells and IL-2 would mediate greater antitumor effects than either treatment alone, neither IL-2 nor activated killer cells had been administered to cancer patients. We began phase I studies of the administration of natural Jurkat-cell–derived IL-2 and, later, recombinant IL-2 to explore different doses, routes, and schedules of administration.[36,37] These studies were conducted in 39 patients with advanced cancer, none of whom responded to

Table 1. Immunotherapy of Murine Tumors With IL-2 Alone

1. Liver and lung micrometastases (3-day) from a variety of immunogenic and nonimmunogenic sarcomas, melanomas, and adenocarcinomas can be inhibited by IL-2 administration.
2. Lung macrometastases (10-day) from two immunogenic sarcomas (but not from two nonimmunogenic sarcomas) can be inhibited by IL-2 administration.
3. A direct relationship exists between the dosage of IL-2 and therapeutic effect.
4. High-dose IL-2 administration leads to in vivo lymphoid proliferation in visceral organs. These cells have LAK activity in vitro.
5. The immunotherapeutic effect of IL-2 on 3-day micrometastases is mediated by asialo GM1+ LAK cells. In immunogenic tumors, Lyt 2+ cells also participate.
6. The immunotherapeutic effect of IL-2 on 10-day macrometastases is mediated by Lyt 2+ cells.
7. Immunosuppression with irradiation or cyclophosphamide can inhibit IL-2 therapy against 3-day metastases, but can enhance the effects of IL-2 on 10-day macrometastases.
8. The sensitivity of macrometastases to therapy involving IL-2 appears to be directly related to the expression of MHC antigens (class I) on the tumor.
9. The administration of IL-2 can enhance the therapeutic effect of concomitantly administered LAK cells, TIL, and, specifically, sensitized T lymphocytes.

Abbreviation: TIL, tumor-infiltrating lymphocytes.

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Table 2. Immunotherapy of Murine Tumors With LAK Cells Plus IL-2

1. Liver and lung micrometastases (3-day) from a variety of immunogenic and nonimmunogenic sarcomas, melanomas, and adenocarcinomas can be inhibited by treatment with LAK cells plus IL-2.
2. A direct relationship exists between therapeutic effect and the dosage of IL-2 and the dose of LAK cells.
3. The precursor of the LAK cell effective in vivo is Thy 1-Ig-a-ASGM1.
4. Three-day incubation of splenocytes is optimal for the generation of LAK cells effective in vivo.
5. Immunotherapy of micrometastases with LAK cells and IL-2 is effective in hosts suppressed by total body irradiation or treatment with cyclophosphamide. Therapy is also effective in B mice (thymectomized, lethally irradiated, reconstituted with T-cell–depleted bone marrow).
6. Immunotherapy of micrometastases with allogeneic LAK cells plus IL-2 is effective.
7. LAK cells effective in immunotherapy can be generated from the splenocytes of tumor-bearing mice.
8. Metastases that persist after in vivo therapy with LAK cells plus IL-2 are sensitive to LAK-cell lysis both in vitro and in subsequent in vivo experiments. We have been unable to generate LAK-resistant tumor cells.
9. Administration of IL-2 leads to in vivo proliferation of transferred LAK cells.
10. Diffuse intraperitoneal carcinomatosis can be successfully treated with intraperitoneal LAK cells plus IL-2.
11. LAK cells can mediate antibody-dependent cellular cytotoxicity, and this administration of IL-2 alone or LAK cells plus IL-2 can enhance the in vivo therapeutic efficacy of monoclonal antibodies with antitumor reactivity.

treatment.[38] Similarly, studies were conducted with activated killer cells, first using phytohemagglutinin-activated killer (PAK) cells and then cells activated with recombinant IL-2 (LAK cells) in 27 cancer patients, and none responded to treatment.

After presenting this information to the Food and Drug Administration, we received permission to treat patients with a combination of LAK cells and IL-2. These studies were initiated in November 1984. We had previously demonstrated that the administration of IL-2 led to a rapid decrease in LAK cell precursors in the circulation, which rebounded substantially after discontinuing IL-2 administration.[36,37] We developed a treatment protocol in which IL-2 was administered for one cycle followed by repeated leukophereses and subsequent administration of LAK cells along with IL-2. IL-2 was administered as an intravenous bolus injection every 8 hours. Antitumor effects in cancer patients were seen with this combined treatment.[39] As we gained confidence in the administration of IL-2 and the management of its side effects, we used higher doses of IL-2 alone and began to see antitumor effects with this treatment as well.[40-42]

The results of the treatment of 320 consecutive patients with advanced cancer treated with either IL-2 alone (140 patients) or IL-2 plus LAK cells (180 patients) are listed in Table 3. These data demonstrate that treatment with LAK cells plus IL-2 can mediate objective regressions in patients with kidney cancer, melanoma, colorectal cancer, and non-Hodgkin's lymphoma. Approximately 10% of patients with renal cell cancer and melanoma achieve complete regression of their advanced metastatic disease. Responses to IL-2 alone have also been seen in patients with kidney cancer and melanoma. Few patients with other histologies have been treated, and thus, definitive comments about response rates in other diseases cannot yet be made. The durations of responses in these patients as of March 1991 are shown in Table 4. Of 20 patients who achieved a complete response, nine remain continuously free of disease from 13 to 75 months.

The ability to achieve antitumor responses with both

Table 3. Immunotherapy of Patients With Advanced Cancer Using IL-2 Alone or With LAK Cells

	IL-2				IL-2 + LAK Cells			
Diagnosis	Assessable*	CR	PR	CR + PR (%)	Assessable†	CR	PR	CR + PR (%)
Renal cell cancer	60	5	6	18	72	8	17	35
Melanoma	41	1	9	24	48	4	6	21
Colorectal cancer	12	0	0	0	30	1	3	13
Non-Hodgkin's lymphoma	11	0	0	0	7	1	3	57
Breast cancer	3	0	0	0	1	0	0	0
Sarcoma	1	0	0	0	6	0	0	0
Lung cancer	1	0	0	0	5	0	0	0
Other	7‡	0	0	0	9§	0	0	0
Total	136	6	15	15	178	14	29	24

*Includes all treated patients except four who died of therapy.

†Includes all treated patients except one lost to follow-up and one dead due to therapy.

‡Two patients each with hepatoma and brain cancer; one each with ovarian, pancreatic, and uterine cancer.

§One patient each with cancer of brain, esophagus, ovary, testes, thyroid, gastrinoma, unknown primary; and two patients with Hodgkin's lymphoma.

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Table 4. Duration of Responses (as of 3/91)

Diagnosis	LAK + IL-2		IL-2	
	CR (months)	PR (months)	CR (months)	PR (months)
Renal cell cancer	51+, 47+, 27, 15, 13, 11, 9, 6	45+, 38, 19, 13, 11, 11, 9, 7, 7, 6, 6, 6, 6, 3, 2, 1, 1	51+, 49+, 39+, 38, 36	49+, 43+, 39, 34, 26, 10
Melanoma	75+, 56+, 42+, 13	41, 6, 6, 3, 2, 2	13+	64+, 15, 10, 8, 7, 7, 5, 3, 2
Colorectal cancer	21	11, 6, 6, 2	—	—
Non-Hodgkin's lymphoma	10	55+, 31+, 14	—	—

NOTE. Of 20 patients with CRs, nine remain in CR at 13 to 75 months.
Abbreviations: CR, complete response; PR, partial response.

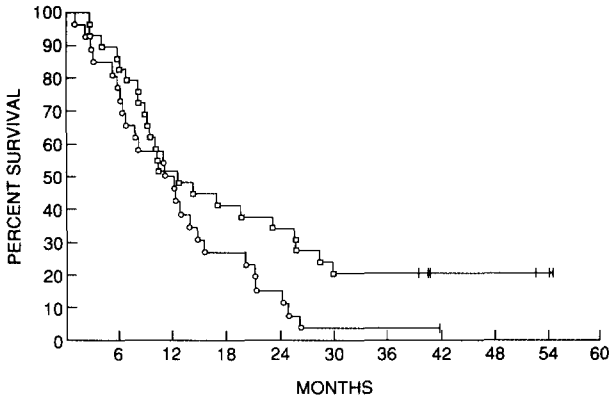

Fig 2. Results of a prospective randomized trial in patients with advanced melanoma who received either IL-2 alone (○) or IL-2 plus LAK cells (□). Patients receiving IL-2 plus LAK cells had a trend toward improved survival (P_2 = .062).

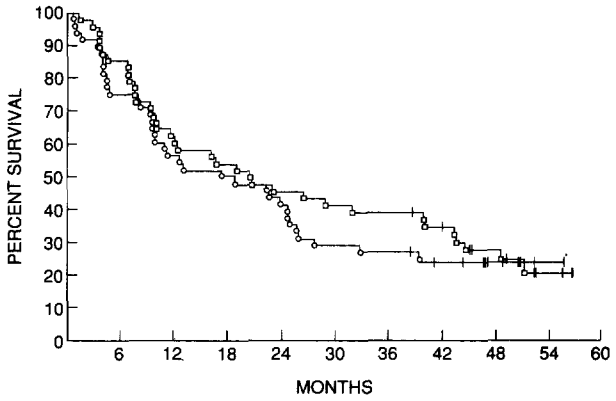

Fig 3. Results of a prospective randomized trial in patients with advanced renal cell cancer who received either IL-2 alone (○) or LAK cells plus IL-2 (□). No difference in long-term survival was seen (P_2 = .55).

IL-2 alone and LAK cells plus IL-2 led us to perform a prospective randomized trial in 181 patients with advanced cancer, the majority of whom had renal cell cancer and melanoma. The survival curves with a median follow-up of 50.2 months are shown in Figs 2 and 3. An example of some of the responses seen in patients treated with LAK cells and IL-2 or IL-2 alone are shown in Figs 4 through 6. Treatment with LAK cells and IL-2 appeared to yield improved survival compared with IL-2 alone in patients with advanced melanoma (P_2 = .06)

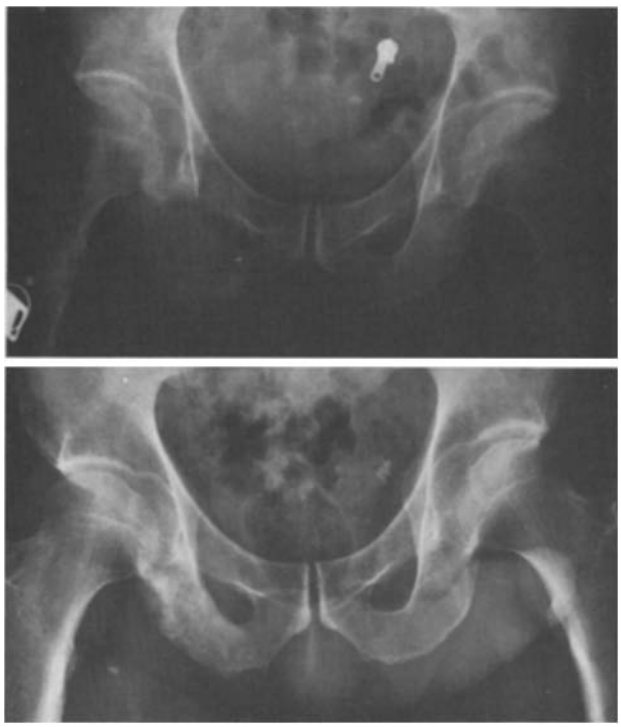

Fig 4. Complete regression of bony metastasis from renal cell cancer in the pubic ramus of a patient treated with LAK cells plus IL-2. This patient underwent complete regression of bony and lung metastases and remains disease-free over 4 years after treatment.

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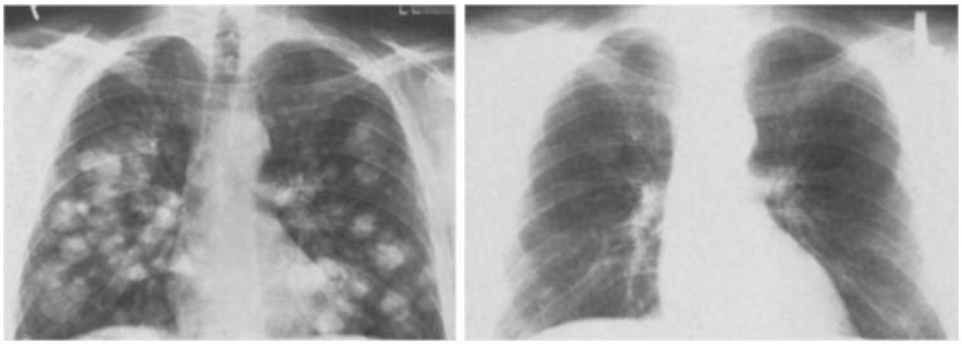

Fig 5. Regression of lung metastases in a patient with advanced malignant melanoma treated with recombinant IL-2 alone.

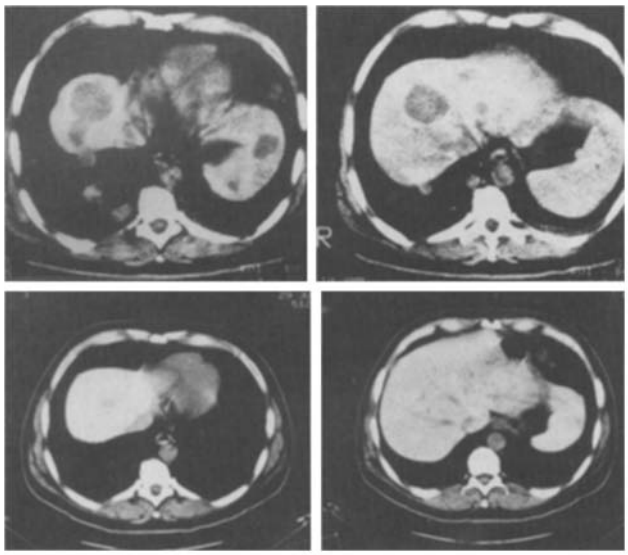

Fig 6. Objective regression of liver and splenic metastases in the same patient shown in Fig 5.

but no difference in patients with kidney cancer (P_2 = .55).

The mechanism of action of these antitumor responses in humans appears to be similar to that seen in the mouse. After administration of this immunotherapy, extensive lymphoid infiltrates could be seen in tumors in conjunction with tumor destruction. An example of a subcutaneous melanoma lesion biopsied before and after treatment with LAK cells and IL-2 is shown in Fig 7.

The administration of large doses of recombinant IL-2 can be associated with substantial side effects. The major toxicity of IL-2 appears to result from an increased membrane permeability induced by IL-2 that leads to fluid and colloid loss into visceral organs and soft tissues.[43] These side effects and their mechanisms have been extensively studied and reported.[42,44-62] A summary of the side effects of treatment with 1,039 treatment courses in our first 652 patients treated with a variety of regimens involving high-dose IL-2, either alone, with activated killer cells, or with other cytokines is shown in Table 5. Treatment-related mortality for the

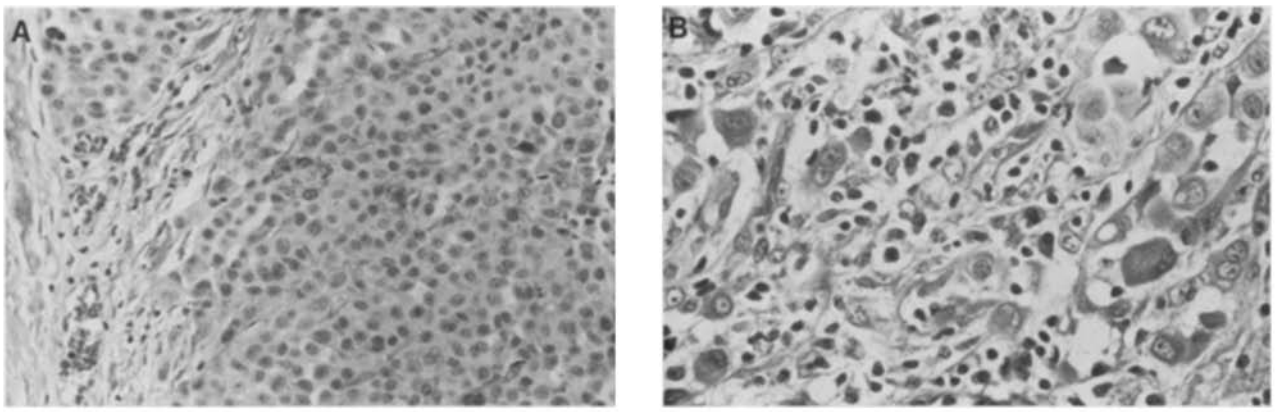

Fig 7. The histologic appearance of subcutaneous melanoma deposits in a patient being treated with LAK cells and IL-2: (A) pretreatment, (B) posttreatment. There is an extensive infiltrate of lymphocytes and evidence of tumor destruction after treatment.

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Table 5. Toxicity of Treatment With IL-2

No. of patients	652
No. of courses	1,039
Chills	399
Pruritus	180
Necrosis	5
Anaphylaxis	1
Mucositis (requiring liquid diet)	30
Alimentation not possible	4
Nausea and vomiting	666
Diarrhea	596
Hyperbilirubinemia, maximum (mg/dL)	
2.1-6.0	547
6.1-10.0	179
10.1+	83
Oliguria	
< 80 mL/8 hours	347
< 240 mL/24 hours	42
Weight gain (% body weight)	
0.0-5.0	377
5.1-10.0	436
10.1-15.0	175
15.1-20.0	38
20.1+	13
Elevated creatinine, maximum (mg/dL)	
2.1-6.0	637
6.1-10.0	85
10.1+	10
Hematuria (gross)	2
Edema (symptomatic nerve or vessel compression)	17
Tissue ischemia	2
Respiratory distress	
Not intubated	67
Intubated	41
Bronchospasm	9
Pleural effusion (requiring thoracentesis)	17
Somnolence	114
Coma	33
Disorientation	215
Hypotension (requiring pressors)	508
Angina	22
Myocardial infarction	6
Arrhythmias	78
Anemia requiring transfusion (no. of units transfused)	
1-5	377
6-10	95
11-15	24
16+	14
Thrombocytopenia, minimum (/μL)	
< 20,000	131
20,001-60,000	361
60,001-100,000	285
Central line sepsis	63
Death	10

entire series was 1.5% of patients. These data do not reflect the first 66 patients treated in the phase I studies, in which no fatalities occurred. As we have gained experience with the administration of high-dose IL-2, treatment-related morbidity and mortality has decreased, and we have not had a treatment-related fatality in the last 383 patients treated with high-dose IL-2. Shortly after our results were published, the National Cancer Institute (NCI) initiated studies by the IL-2 Working Group using LAK cells and IL-2. Similar treatment results were obtained, and one treatment-related mortality was experienced in the first 94 patients treated.[63] A more recent report of 515 patients treated by this working group with a variety of high-dose IL-2–related therapies showed a 3.5% treatment-related mortality. High-dose IL-2 can be administered safely either alone or with activated killer cells, although experience is required with the unique nature of the toxicities resulting from the administration of this biologic agent. A variety of other groups have confirmed the antitumor activity of IL-2 alone and LAK cells plus IL-2.[63-76]

STUDIES OF TUMOR-INFILTRATING LYMPHOCYTES

Our studies with LAK cells and IL-2 led us to search for immune cells with improved therapeutic effectiveness. In 1986, we described a class of T lymphocytes with unique antitumor activity that we called tumor-infiltrating lymphocytes (TIL).[77,78] TIL are cells that infiltrate into growing tumors and can be grown by culturing single-cell suspensions obtained from tumors in IL-2. Although lymphocytes comprise only a small subpopulation of the cells in a cancer nodule, some of these lymphocytes contain IL-2 receptors—presumably because of their interactions with tumor antigens—and grow under the influence of IL-2. Although tumor cells also grow in the culture, the lymphocytes capable of eliminating the tumor cells have a selective growth advantage. After 2 to 3 weeks of culture, pure populations of lymphocytes without contaminating tumor cells are obtained.

In vitro, TIL obtained from methylcholanthrene-induced murine sarcomas, as well as from the MC-38 colon adenocarcinoma, specifically lyse the tumor from which they are derived and not other tumors.[79] More recently, we have demonstrated specific cytokine release when these TIL are in contact with their autologous tumors.[80,81] TIL are thus unique in their ability to identify cancer-associated antigens in vitro.

Extensive in vivo studies demonstrated that TIL were from 50 to 100 times more effective in treating established 3-day lung and liver metastases than were LAK cells.[77] An example of the titration of the antitumor effectiveness of TIL and LAK cells is shown in Fig 8. The results of extensive studies of the mechanism of this

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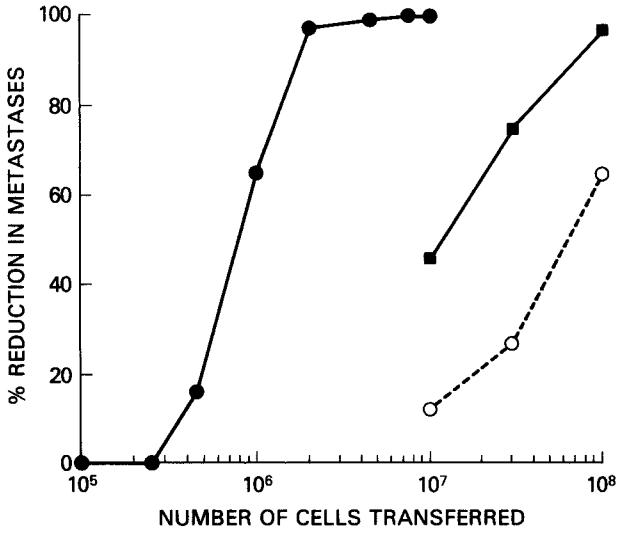

Fig 8. Titration of LAK cells and TIL administered with IL-2 in mice bearing 3-day lung metastases from a transplantable sarcoma[77,78]: ●, TIL, one treatment; ○, LAK cells, one treatment; ■, LAK cells, two treatments. TIL appear to be 50 to 100 times more potent than LAK cells in reducing established lung metastases. Reprinted with permission from Rosenberg et al[77] (*Science* 233:1318-1321, 1986). Copyright 1986 by the AAAS.

immunotherapeutic effect of TIL and IL-2 in mice with established tumors are listed in Table 6.[80-89] TIL are classic T cells. In the mouse they are almost exclusively CD8+, but human TIL can be both CD4+ and CD8+. We have recently shown that the specific secretion of interferon gamma by TIL cocultured with tumor appears to be the best in vitro correlate of the in vivo antitumor effectiveness of TIL.[80] Non-lytic TIL that can specifically secrete interferon gamma when in contact with the autologous tumor are effective in treating established lung micrometastases. In recent studies, we have shown that the administration of antibodies to interferon gamma or tumor necrosis factor (TNF) can abrogate some of the therapeutic effectiveness of TIL, thus suggesting a very potent role for cytokine secretion in this phenomenon.[80]

We and others have successfully grown TIL from approximately 80% of over 300 human cancers, including melanoma, renal cell cancer, colon cancer, breast cancer, bladder cancer, neuroblastoma, lymphomas, and other tumor types.[90-107] From approximately one third of patients with melanoma, TIL can be derived that have the ability to lyse specifically the autologous melanoma and not other melanomas or normal cells from the same patient.[90-92] An example of this phenomena is shown in Fig 9.[90] More recently, we have demonstrated that TIL can also recognize unique tumor antigens on the tumors of some patients with melanoma, breast cancer, colon cancer, and lymphomas as evidenced by the secretion of specific cytokines when cocultured with the autologous tumor.[108] An example of the specific recognition of breast cancer antigens by this technique is shown in Fig 10. Extensive studies of the in vitro characteristics of human TIL have been performed (Table 7).

The ability of TIL to recognize unique cancer-associated antigens has made the study of the biologic characteristics of these determinants possible. A study of the lytic recognition of a variety of HLA-type melanomas by TIL demonstrated that melanomas from patients that share MHC antigens are often cross-recognized by TIL, as would be expected for an MHC-restricted T-cell reaction.[109,110] The example of this phenomenon shown in Table 8 demonstrates the recognition of a melanoma antigen restricted by the HLA-A24 class I determinant. TIL from melanoma patient no. 888 recognized autologous tumor and all three allogeneic melanomas from patients bearing the HLA-24 determinant, but did not recognize a normal Epstein-Barr virus (EBV)-transformed line from an HLA-24–positive patient or 16 other tumors that were unmatched at any HLA locus.

In these studies we found that the HLA-A2 class I determinant was frequently used as an MHC restriction element in the recognition of melanoma antigens. To study this phenomenon further, the HLA-A2 gene was transfected into the allogeneic melanomas of six dif-

Table 6. Immunotherapy of Murine Tumors With TIL and IL-2

1. The administration of TIL plus IL-2 is 50 to 100 times more effective than LAK cells plus IL-2 in reducing established (day 3) lung micrometastases.
2. The administration of TIL plus IL-2 plus cyclophosphamide is effective in treating mice with advanced lung (day 14) or liver (day 8) metastases. All three agents are required for effective treatment.
3. The phenotype of TIL effective in vivo is CD3+, CD4−, CD8+.
4. TIL raised from tumors that express class I antigens only after transfection with genes coding for class I can mediate the regression of micrometastases from the parental tumor after treatment of the mouse with interferon gamma.
5. Local tumor irradiation synergizes with TIL and IL-2 administration in mediating the regression of established metastases. Local irradiation can substitute for cyclophosphamide.
6. TIL with improved antitumor activity in vivo can be generated from tumor suspensions by using immunomagnetic beads to isolate lymphocytes followed by incubation of lymphocytes in low-dose IL-2.
7. In mice cured of lung micrometastases by administration of TIL, live TIL can be identified in vivo 3 months after injection.
8. The specific secretion of interferon gamma by TIL when cultured with tumor is the best in vitro correlate of the in vivo antitumor effectiveness of TIL. Nonlytic TIL that specifically secrete interferon gamma can effectively treat established lung micrometastases.

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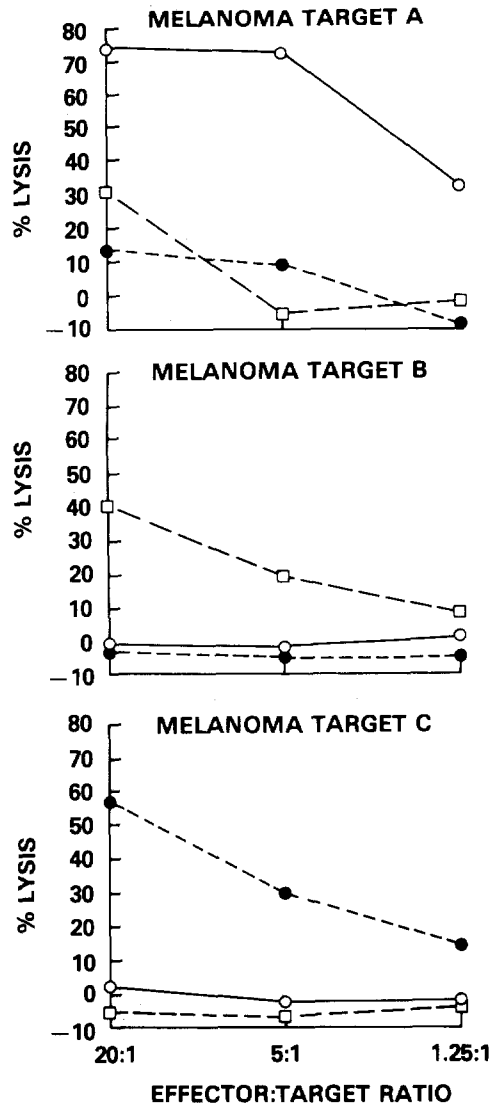

Fig 9. Specific TIL lysis of autologous melanoma and not other allogeneic melanomas. TIL from three patients (○, patient A; □, patient B; ●, patient C) were tested simultaneously against melanoma from these patients. Only the autologous tumors exhibited significant lysis. Reprinted with permission from Muul et al.[90]

ferent HLA-A2–negative patients, and HLA-A2–expressing and –nonexpressing clones of these melanomas were isolated and tested for lysis by an HLA-A2–restricted melanoma TIL. The allogeneic melanomas of all six patients shared tumor antigens uncovered by insertion of the HLA-A2 normal class I gene. Thus, it appears that human melanomas bear at least some antigens that are commonly expressed between individuals and are recognized in an MHC-restricted fashion.

The presence of shared tumor antigens provides the possibility that these antigens might be used for active immunization of patients against their own tumors. Libraries of cDNA have been prepared from human melanomas and are being transfected into nonantigen-expressing autologous lines or transformed fibroblast lines in an attempt to identify the antigen recognized by TIL. The identification of such cancer antigen genes might enable the insertion of the gene into viruses for use in the immunization against cancer in patients with advanced disease or for the primary or secondary prevention of cancer. These efforts are being actively pursued in the laboratory.

The increased efficacy of murine TIL in treating established cancers in mice and the ability to generate TIL from a variety of human tumors led to a pilot trial of

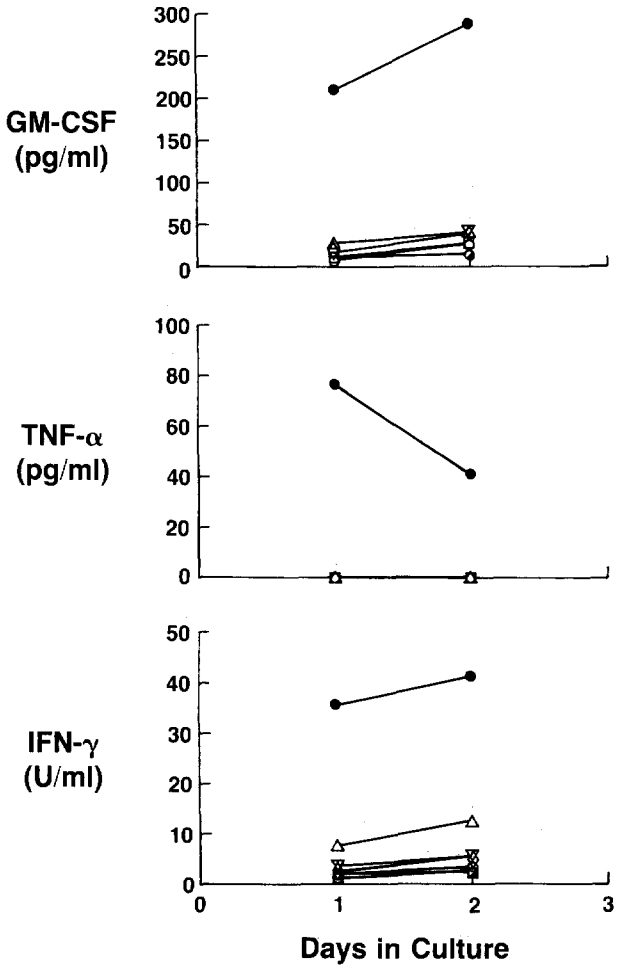

Fig 10. Demonstration of unique tumor antigens on breast cancer as evidenced by specific cytokine secretion by TIL. TIL from a patient with breast cancer secreted granulocyte-macrophage colony-stimulating factor, TNF alfa, and interferon gamma only when cocultured with the autologous breast cancer (●) and not with an autologous B-cell line (△) or with four other allogeneic breast cancers: □, ▽, ◇, and ◐. Reprinted with permission from Schwartzentruber et al.[108]

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Table 7. In Vitro Studies of Human TIL

1. TIL can be grown in IL-2 from approximately 80% of human cancers of a variety of histologic types including melanoma, renal cell cancer, colon cancer, breast cancer, ovarian cancer, and others.
2. TIL with specific cytolytic activity for fresh cancer cells can be grown from approximately one third of patients with melanoma.
3. TIL are mainly CD3+ and can be either CD8+, CD4+, or mixtures of both. Smaller numbers of CD56+ TIL can also be present.
4. Specific lysis of TIL can be inhibited by antibodies to CD3 or to MHC class I molecules.
5. Growth of TIL from melanoma patients in IL-2 plus IL-4 results in increased in vitro lytic specificity for autologous melanoma.
6. Incubation of cultured tumors in interferon gamma increases their susceptibility to lysis by TIL.
7. TIL with lytic specificity for autologous tumor have not been obtained from patients with colorectal cancer, breast cancer, or sarcomas and only rarely from patients with renal cell cancer.
8. Direct positive panning techniques using antibody-coated flasks can be used to separate and grow highly purified subpopulations of CD4+ and CD8+ TIL.
9. Repeated immunoselection using TIL with specific lysis can be used to identify tumor lines resistant to lysis by autologous TIL. These immunoselected tumor can be used to identify multiple tumor antigens on a single tumor.
10. Shared tumor antigens on allogeneic melanomas that are recognized in an MHC-restricted fashion can be identified by testing lysis of specific TIL on panels of HLA-type melanoma cultures. HLA-A2 is a common restriction element in the recognition of melanoma antigens.
11. MHC-restricted recognition of shared melanoma antigens on allogeneic melanomas was demonstrated by using HLA-A2–restricted specific TIL to lyse allogeneic melanomas transfected with the gene for HLA-A2.
12. Nonlytic TIL with specific immune recognition of human tumor antigens can be recognized by the specific release of cytokines (such as granulocyte-macrophage colony-stimulating factor, TNF, and interferon gamma) after incubation with autologous tumor. Specific reactivity has been identified in patients with melanoma and breast cancer.
13. In vitro lysis of autologous tumor by TIL exhibits mild but significant correlation with clinical response.

TIL therapy in 12 patients with advanced cancer.[111] In prior murine adoptive immunotherapy models in which bulk tumor was treated, immunosuppression with either cyclophosphamide or total body irradiation was necessary to achieve optimal therapeutic effects.[112,113] Thus, in the pilot trial, patients received TIL with varying doses of IL-2 with or without cyclophosphamide. After this pilot trial, a phase II trial was initiated in patients with metastatic melanoma using TIL with high-dose IL-2.[114] The results of this trial are shown in Table 9. Response rates of 36% to 39% were seen in patients in whom IL-2–containing regimens had previously failed. In preliminary results, it does not appear that the administration of cyclophosphamide is required to achieve the therapeutic effects of TIL and IL-2, although a randomized trial will be needed to confirm this.

In conjunction with these trials, studies were conducted to determine whether TIL accumulate in tumor deposits. TIL were labeled with indium-111 and infused along with the administration of IL-2.[115,116] Clear tumor localization was seen in 13 of 18 radionuclide scan series. In addition, biopsies of tumors and normal skin showed a slow accumulation of indium-111 TIL in cancer deposits compared with normal tissue (Fig 11). These studies showed that TIL recirculate and specifically localize in cancer deposits and provided a basis for future experiments using gene-modified TIL in the treatment of human cancer.

After these early studies with IL-2, LAK cells, and TIL, attempts were made to find predictors of antitumor response. An analysis of responding and nonresponding lesions to IL-2–based immunotherapies in humans suggested a direct relationship between the expression of class II antigens on the tumor-cell surface and the response of individual lesions.[117] These observations led to experimental studies in mice using IL-2 combined with interferon alfa to upregulate MHC antigens on tumor cells.[118] Thus, tumors that were relatively resistant to treatment with IL-2 or interferon alone were treated with the combination of these two agents at a variety of doses. Only the combination of IL-2 and interferon alfa was capable of reducing the number of established metastases in these mice. Further studies showed that the addition of TIL to IL-2 and interferon alfa increased antitumor effectiveness compared with the use of IL-2 and interferon alone or the use of TIL and IL-2 alone.

A phase I study was undertaken in 201 humans with advanced cancer who received escalating doses of IL-2 and interferon alfa that suggested that the combination might be more effective in the treatment of patients with advanced kidney cancer and melanoma than either of these agents alone[119] (Table 10). A conclusive test of this hypothesis, however, would require a prospective randomized trial.

A variety of experimental leads for improving immunotherapy with IL-2 or TIL are listed in Table 11. The combination of TIL plus local radiation therapy has shown synergistic activity against localized tumor deposits in mice.[83] Possible methods for generating TIL with improved effectiveness involve the use of lymphocyte subpopulations or clones or restimulation with tumor in vitro. TIL with enhanced in vitro lytic specificity have been raised in humans using IL-2 in combination with interleukin-4 (IL-4).[120] Recently, Yang et al have shown

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Table 8. Cytolytic Activity of Patient No. 888 TIL Against HLA-Matched and Unmatched Targets

% Lysis*	Autologous	Unmatched	HLA-A1†	HLA-A24	HLA-B22	HLA-B52	HLA-Cw1	HLA-Cw7
> 10	888; 29 ± 2; (6) 888-mel; 46 ± 3; (14)			878; 12 ± 1; (7) 809; 14 ± 1; (6) 501-mel; 16 ± 1; (9)				(501)
≤ 10	888-EBV; −8 ± 5 888, fibroblast 1	894; 2 ± 0 890; 0 889; 0 865; 2 851‡; −1 ± 1 822; 2 ± 1; (6) 816; 1 796; −1 745; 6 734; 1 697; 0 ± 1 583-EBV; −4 ± 2 553; 2 526-mel, −1 ± 0; (7) Z2B; −1 Daudi; −1 ± 1; (17)	790; 1 537-mel; −2 ± 1; (6) 537-EBV; −4 ± 2; (3) 397-mel; −1 ± 1; (6)	501-EBV; −2 ± 0	544-EBV; −3		(537-mel) (537-EBV)	890; 1 ± 1; (3) 882; 0 ± 1 864; 3 ± 2; (3) 836; 2 ± 1; (4) 619; 0 624-mel; −1 ± 2; (4) 586-mel; −1 ± 1; (11) 586-EBV; −12 ± 7 551-EBV; −2 ± 5 (790) (501-EBV)

NOTE. Data indicate target cells; mean % lysis ± SEM; (n), which is 1 where no SEM is indicated or 2 unless otherwise specified. Target cells are fresh cryopreserved melanomas unless otherwise indicated. Targets matching more than one TIL HLA locus are indicated in parentheses.

Abbreviations: mel, cultured melanoma line; EBV, EBV-transformed B-cell line; ^{51}Cr, radioactive isotope of chromium; Ag, antigen.

*Lysis in 4-hour ^{51}Cr-release assays at effector:target ratio of 40:1. Values ≤ 10% are not considered significant.

†Target donor HLA Ag matching TIL effector HLA Ag.

‡Fresh sarcoma target.

that murine TIL with far greater in vivo efficacy can be generated using low-dose IL-2 (ie, 10 to 20 U/mL) compared with the higher-dose IL-2 currently in use (1,000 U/mL).[84] Each of these modifications in TIL preparation requires evaluation as it may lead to the generation of TIL with improved therapeutic effectiveness in humans.

Cytokine hormones are being described with increasing frequency, and many have potent immune-stimulating activity. We have recently demonstrated that the administration of interleukin-6 (IL-6) can successfully treat lung micrometastases in animal models.[121] Interleukin-7 (IL-7) can be used to generate cells with antitumor activity from draining lymph nodes in mice.[122] These agents warrant evaluation in humans with advanced cancer. The combined use of monoclonal antibodies and LAK cells, which mediate potent antibody-dependent cellular cytotoxicity, leads to increased therapeutic effect in murine models.[123-125] Finally, studies combining these immunotherapy approaches with chemotherapy regimens[126] or after surgery to reduce tumor burden represent alternate approaches worthy of investigation in humans.

Table 9. TIL Treatment of Patients With Melanoma

	NR	Objective Response (PR + CR)	PR + CR (all)	%
No previous IL-2				
IL-2 + CY	17	11	11/28	39
IL-2 (no CY)	7	4	4/11	36
Previous IL-2				
IL-2 + CY*	3	3	3/6	50
IL-2 (no CY)	4	1	1/5	20

NOTE. Excludes patients with brain metastases at start of treatment (all NR).

Abbreviations: NR, no response; CY, cyclophosphamide.

*Excludes two patients who received IL-2 at 30,000/kg; both were NR.

GENE THERAPY FOR CANCER

Attempts to alter immune reactivity in the cancer-bearing host have recently turned to genetic manipulation of immune cells and tumor cells.[127] Gene therapy can be defined as a therapeutic technique in which a functioning gene is inserted into the cells of the patient to correct an inborn genetic error or to provide a new function to the cell. The development of techniques for inserting and expressing foreign genes in eukaryotic cells and an increased understanding of the regulation of gene expression have opened new possibilities for cancer therapy based on these gene transfer techniques.

Our previous studies demonstrating that TIL accumu-

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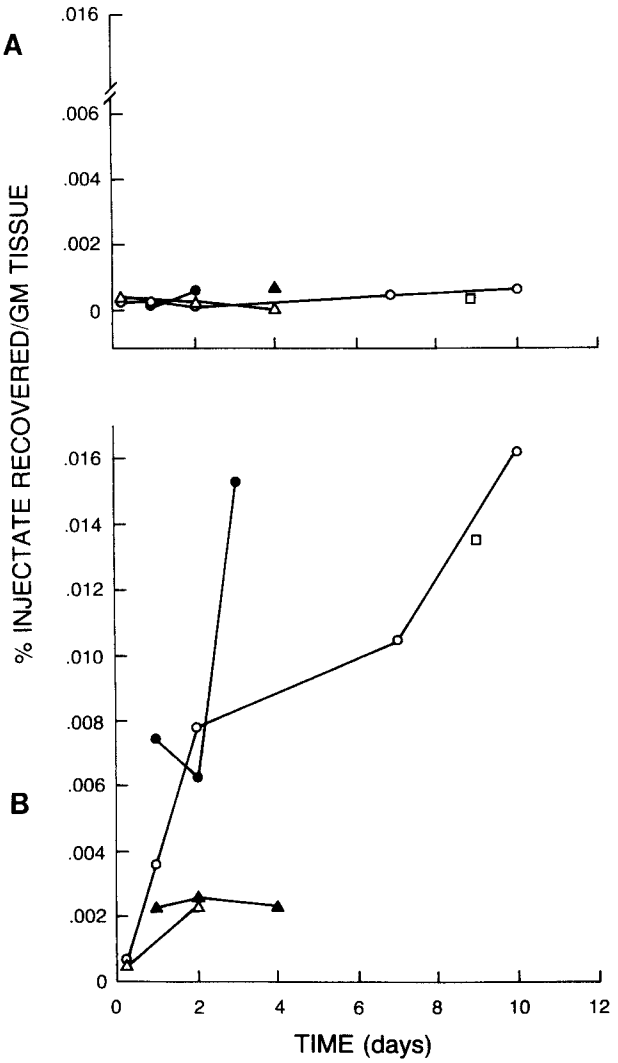

Fig 11. After intravenous injection, TIL accumulate in tumor deposits. Indium-111–labeled TIL were administered to patients (○, ●, △, ▲), and normal tissue (A) and tumors (B) were biopsied. There was no accumulation in normal tissue, but a slow accumulation of labeled TIL was seen in the tumor of these four patients. Reprinted with permission from Fisher et al.[115]

lated in tumor deposits suggested that TIL could be used as vehicles to deliver to the tumor-site molecules that would increase their antitumor effectiveness.[115,116] Using gene transfer techniques, new properties could be introduced into TIL to enhance existing functions or to generate a kind of lymphocyte that normally did not exist in nature. Along with my colleagues (W. French Anderson, National Heart, Lung and Blood Institute; R. Michael Blaese, NCI), we have begun to study genetic modification of TIL in an effort to increase their therapeutic efficacy.

These studies were designed in two phases. The first was an attempt to insert a gene coding for neomycin phosphotransferase that would mark these cells with a bacterial enzyme that would render them resistant to the antibiotic neomycin and, thus, enable us to perform studies of the long-term distribution and survival of TIL in humans. These studies were conceived as a preliminary effort to demonstrate the safety and efficacy of using retroviral-mediated gene transduction to introduce genes into TIL and as a preparation for introducing genes that could improve TIL therapeutic efficacy. Although most physical techniques for introducing genes into the DNA of eukaryotic cells are very inefficient and at best can introduce genes into only one in 10,000 cells, the use of retroviral vectors can introduce genes with an efficiency up to 30% to 50%.[127-130] After infection of the cell, viral RNA is transcribed to DNA, which randomly integrates into the genome. The integrated provirus is indistinguishable from other cellular genes and replicates as does normal DNA during mitosis. In the early 1980s techniques were developed for generating replication-incompetent retroviruses using packaging cell lines. These cell lines can produce genetically modified virus lacking the genes for virally encoded proteins so that the retrovirus can infect subsequent cells but cannot itself form new virus. The retroviral vector that we used was derived from a murine Moloney leukemia virus genetically engineered to contain the gene for neomycin phosphotransferase.[131,132]

A potential danger of using a retrovirus to insert genes into human cells was the possibility that replication-competent virus might be produced by recombination of vector sequences with viral coding sequences present in the packaging cell line. Careful modification of the packaging cell lines and the retroviral vectors were performed to minimize the possibility of this helper virus generation.[131,132] The random incorporation of the retroviral-coded DNA into the genome could also activate sequences in the transduced cells that could result in autonomous growth, and this required careful evaluation before reinfusion of cells into patients.

The use of this neomycin phosphotransferase marker gene in TIL provided many advantages that did not exist using the indium-111 label. Indium-111 has a half-life of 2.8 days and the combination of the natural decay of the isotope and the spontaneous release of the label from the cell severely limited the time that these cells could be used to study cell survival in vivo. Autoirradiation of the cell resulting from indium-111 incorporation led to potential damage of TIL that could alter their function. Neomycin resistance genes appeared to represent an ideal label that would be incorporated into the genome of the cell without altering its function and would

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Table 10. Treatment of Patients With Advanced Cancer Using Interferon alfa Plus IL-2

		Melanoma				Renal Cell Cancer				Colorectal Cancer				Other	Total			
IFN (U/m^2)	IL-2 ($\times 10^6$)	NR	PR	CR	% Response	NR	PR	CR	% Response	NR	PR	CR	% Response	NR	NR	PR	CR	% Response
3	1	2	0	0	0	3	0	1	25	—	—	—	—	—	5	0	1	17
3	3	11	2	1	21	9†	2	1	25	2	0	0	0	3§	25	4	2	19
3	4.5	5	4	0	44	4‡	2	0	33	5	0	0	0	5§	19	6	0	24
6	4.5	8*	4	2	43	8	3	2	38	1	1	0	50	—	17	8	4	41
6 ($\times 1$)	3	2	2	1	60	6**	4	0	40	1	0	0	0	4¶	13	6	1	35
6 ($\times 1$)	6	31	3	3	16	11††	5	1	35	5	0	0	0	5#	51	8	4	19
Total		59	15	7	27	41	16	5	34	14	1	0	7	17	131	32	12	25

*Plus one unassessable patient.
†Plus one patient dead of treatment.
‡Plus one unassessable patient.
§One patient each with lymphoma, prostate, and testicular cancer.
‖Three patients with breast cancer; one esophageal cancer; one sarcoma.
¶One patient each with breast, sarcoma, small bowel, and prostate cancer.
#Two patients with breast cancer; one small bowel; one sarcoma; one Von Hippel Lindau syndrome.
**Plus one patient treated with kidney in place; no response.
††Plus 11 patients treated with kidney in place; one CR, one PR, nine NR.

replicate along with the cell so that daughter cells would have an equal amount of label. Further, this marker could be used to reisolate the infused cells because it would confer resistance to an antibiotic in cells not bearing the gene. In addition, the polymerase chain reaction technique could be used to detect as few as one modified cell in 10,000 normal cells.

Because foreign genes had never been inserted into humans, an extensive review process was undertaken before beginning these gene transfers, including review by the Clinical Research Committees of the NCI, the National Heart, Lung and Blood Institute, the National Institutes of Health Biosafety Committee, the Recombinant DNA Advisory Committee and its Gene Therapy Subcommittee, and the Food and Drug Administration (FDA). A variety of studies were performed and presented to these review groups.[133,134] We demonstrated that the neomycin phosphotransferase gene could be inserted and expressed in human TIL, that the phenotype and function of marked TIL were not significantly altered, that we could detect the marked gene in animal models, and that there was low risk to the patient and no risk to health care personnel or the public in the use of these techniques. Insertion of the gene into human TIL was assessed by Southern blot analysis. We demonstrated that the gene was expressed and that these transduced cells were resistant to exposure to G418, a neomycin analog normally lethal to all eukayrotic cells (Fig 12). Permission was given to treat 10 patients with life expectancies of up to 90 days.

Table 11. Experimental Leads for Improving Immunotherapy

1. Enhancing in vivo effectiveness of TIL
   a. Combination with other cytokines
   b. Combination with local radiation therapy
2. Generating more effective TIL
   a. Lymphocyte subpopulations or clones
   b. Repeated in vitro stimulation
   c. Low-dose IL-2
   d. Culture in IL-2 plus IL-4
   e. Modify TIL by gene transfer
3. Immunize with gene-modified tumor
   a. Cytokine genes
   b. MHC genes
4. Application of new cytokines
   a. IL-6
   b. Cytokine combinations
5. Monoclonal antibodies
   a. +IL-2 + LAK cells (LAK cells mediate ADCC)
   b. +M-CSF
6. Synergy of chemotherapy + IL-2

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; M-CSF, macrophage colony-stimulating factor.

The first patient received an infusion of gene-modified cells on May 22, 1989. We have now treated 10 patients with advanced melanoma using these gene-modified cells. The results of the first five patients have been published previously.[135] These patients had extensive melanoma with multiple lesions including metastases in brain, lung, liver, subcutaneous tissue, adrenal gland, and other sites (Table 12). The characteristics of the infused gene-transduced and nontransduced TIL administered are listed in Table 13. The primary goal of these studies was the detection of these gene-marked cells in blood and tumor samples. A summary of the polymerase chain reaction analysis of the distribution of gene-modified TIL in peripheral blood and in tumor in the first five patients is shown in Fig 13. We have been

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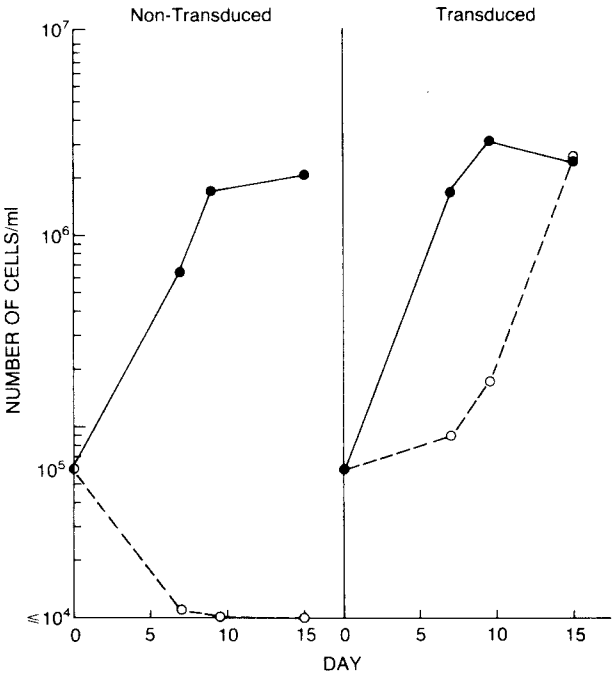

Fig 12. Transduced TIL become resistant to exposure to G418. Nontransduced TIL grow in the absence (●) but not in the presence (○, 0.4 mg/mL) of G418, a neomycin analog. Transduced TIL grow well in the presence and absence of G418.

able to detect gene-modified cells in peripheral blood at times up to 189 days and in tumor up to 64 days after cell infusion.[135] In general, however, gene-modified cells are only found in the circulation for the first 3 weeks after infusion, which corresponds to the time that IL-2 is administered. Two of the first five patients had objective responses to gene-modified TIL. The patient we previously reported had undergone complete regression of multiple subcutaneous, lung, and mucosal melanoma deposits remains alive and free of disease beyond 2 years.

All of the safety studies performed on the retroviral supernatant used for transduction and the transduced cells infused into the patient and multiple tests on patients at varying intervals after the infusion of transduced cells revealed no safety problems of any kind. No patient was ever exposed to replication-competent retrovirus, as evidenced by sensitive S+ L− 3T3 amplification assays or tests for antibody to envelope proteins in patient serum.[135]

These studies demonstrated that retroviral-mediated gene transduction could be safely used to modify genetically cells used for infusion into patients and led to two applications to attempt to improve the therapy of established disease. Because lymphocytes appeared to be suitable vehicles for introducing genes, clinical efforts led by Drs R. Michael Blaese and W. French Anderson were initiated to insert the gene for adenosine deaminase into children with severe combined immunodeficiency, resulting from a deficiency of this enzyme. A second series of efforts were devoted to the gene therapy of cancer. An implication of our studies using gene transduction into TIL was the concept that gene modification might be used to design TIL with new properties that could increase their therapeutic effectiveness. Several possibilities existed for the genetic modification of TIL and some of these are listed in Table 14. The first

Table 12. Patient Characteristics

Patient No.	Age (years)/ Sex	Primary Site	Prior Treatment	Tumor Harvest: Site	Size (cm)	Total No. of Cells Obtained ($\times 10^{-7}$)	No. of Cells to Start Culture* ($\times 10^{-7}$)	% Lymphocytes	% Tumor Cells	Sites of Assessable Disease
1	52/M	Neck	Wide local excision	Lymph node	4 × 4 × 2	33	12	15	85	Lung, liver, spleen
2	46/F	Finger	Amputation finger lymph node dissection	Lymph node	5 × 5 × 3	157	50	70	30	Lymph nodes, intramuscular
3	42/M	Back	Wide local excision	Lymph node	6 × 5 × 4	205	120	31	69	Lung, subcutaneous
			Lymph node dissection	Subcutaneous	2 × 2 × 2					
			Melanoma vaccine	Subcutaneous	2 × 2 × 2					
			IL-2/interferon alfa							
4	41/M	Chest	Wide local excision	Subcutaneous	2 × 1 × 1	41	4	39	61	Lung, liver, lymph nodes, subcutaneous, brain
			Lymph node dissection	Subcutaneous	5 × 4 × 4					
			IL-2/interferon alfa							
5	26/F	Arm	Wide local excision	Subcutaneous	2 × 2 × 2 to 5 × 4 × 2	71	15	16	84	Lung, lymph nodes, subcutaneous

NOTE. Reprinted with permission from Rosenberg et al.[1]

*Denotes the number of cells used to start the cultures that were ultimately administered to the patient.

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Table 13. Characteristics of Infused Cells

Patient No.	Cells Transduced/ Day of Transduction	No. of Cells Transduced ($\times 10^{-8}$)	Multiplicity of Infection*	Total Days of Growth	Fold Expansion†	Doubling Time‡ (days)	No. of Cells Infused ($\times 10^{-10}$)	Estimate of Cells Transduced (%)	Cycle Administered	No. of IL-2 Doses to Patient
1	No	—	—	36 and 37	63,400	2.5	22.8	—	1	7
	Yes/13	1.8	2.3	60	16,100	7.5	7.1	1	2	
2	No	—	—	65	209	3.5	0.2	—	1	15
	Yes/19	2.5	1.3	65	5,030	3.5	13.2	11	1	
3§										
A	No	—	—	35	874	2.8	10.8	—	1	13
	Yes/12	1.8	1.6	48	35,100	2.5	14.5	1	2	
B	No	—	—	35	324	2.3	3.5	—	1	
	Yes/12	1.4	1.6	48	21,400	3.5	5.5	1	2	
4	No	—	—	36	8,470	2.0	26.0	—	1	10
	Yes/16	1.8	1.7	36	9,480	2.0	3.3	4	1	
5	No	—	—	30	18,900	2.0	15.0	—	1	7
	Yes/8	0.6	1.6	30	5,250	2.3	6.2	10	1	

NOTE. Reprinted with permission from Rosenberg et al.[1]

*Ratio of number of virions to number of TIL during the transduction procedure.

†Calculated fold expansion of cells administered. Not all cultured cells were given; some cells were diverted for experimental studies or lost to contamination. Nontransduced cells shown here are those in conjunction with the transduced cells administered to the patient.

‡Varied during culture growth; doubling time at time of final cell infusion is presented here.

§Two separate cultures transduced and expanded separately. Culture A was started in AIM-V medium; culture B was started in RPMI 1640 plus 10% human serum.

gene we selected was TNF because animal and laboratory experiments suggested that TIL secreting large amounts of TNF might provide therapeutic benefit to patients with established cancer.

TNF is an extremely effective therapeutic agent for the treatment of established mouse tumors in the skin and liver.[136-139] These tumors can undergo regression within hours after injection of a single dose of TNF. However, extensive studies in humans have failed to reveal any benefit of TNF administration in humans. In our own phase I studies, 39 patients were treated with escalating doses of TNF to maximum tolerance, and no antitumor effects were seen.[42] There is a substantial difference in the dose of TNF tolerated by mice and humans. Approximately 400 mg/kg are required to mediate tumor regression in mice. However, humans will tolerate only 8 to 10 mg/kg before life-threatening hypotension supervenes. Because TIL accumulate in tumor deposits, we hypothesized that TIL could be used to produce large amounts of TNF at the site of tumor without exposing the entire patient to TNF. Our calculations predicted that in humans we could achieve at the

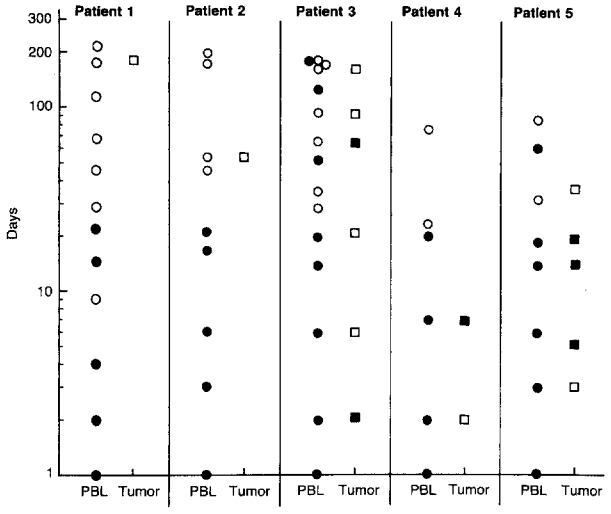

Fig 13. Results of polymerase chain reaction assays of peripheral blood lymphocytes (PBL) and tumor from cancer patients after infusion of transduced TIL. Consistent demonstration of transduced TIL in the circulation was seen for the first 3 weeks after TIL administration, which corresponded to the time of IL-2 administration. (○, □) Negative; (●, ■) positive. Reprinted, by permission of *The New England Journal of Medicine* (323:570-578, 1990).[135]

Table 14. Possible Genetic Modifications of TIL to Improve Antitumor Activity

Tumor necrosis factor (interferes with blood supply)
Interferon alfa or gamma (upregulated MHC antigens)
Other cytokines such as IL-6, IL-1 alfa, IL-7, RANTES (modulates immune response to tumors)
Fc receptor (mediates antibody-dependent cellular cytotoxicity)
Chimeric T-cell receptors (constant region of T-cell receptor plus variable region of monoclonal antibody alters specificity of T cell)
IL-2 receptor (increase sensitivity to administered IL-2)

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tumor site, two to three times the 400 μg/kg required to see tumor regression in mice.

With this aim in mind, we selected a retroviral construct in which the TNF gene was promoted by the viral long terminal repeat promoter, and the neomycin resistance gene was promoted by the SV40 early promoter. These retroviral vectors were supplied to us by Genetic Therapy Inc (Rockville, MD) and Dr Michael Kreigler (Cetus Corp, Emeryville, CA). Extensive testing in vitro revealed that TIL transduced with this TNF vector could secrete high levels of TNF. After considerable review, a protocol for administering these TNF gene-modified TIL was agreed upon by the review groups and the FDA. This protocol schema is shown in Table 15.

Increasing doses of TNF-TIL are given without IL-2 starting at very low doses (10^8 cells) and escalated to 10^{11} cells. After these infusions are completed, the dose of TNF/TIL is dropped by a factor of 10 and then administered along with IL-2.

On January 29, 1991, the first two patients with advanced melanoma were treated with TNF gene-modified TIL. The first four patients to receive TNF/TIL are shown in Table 16. Only one of these patients has received gene-modified TIL along with IL-2. No side effects have been seen due to the TNF/TIL. Results are very preliminary, and it will take approximately 2 years to accrue 50 patients to evaluate this therapeutic approach. As of October 1, 1991, one of the patients treated with TNF-TIL alone without IL-2 died of progressive disease 7 months after the last TIL infusion. The only patient to receive TNF-TIL along with IL-2 has shown an objective regression of tumor, which is continuing at the present time.

We are currently exploring a variety of other means for genetically modifying TIL to increase their therapeutic effectiveness (Table 14). The introduction of other cytokines such as interferon alfa or gamma can upregulate MHC antigens and may synergize with TIL activity. The introduction of Fc receptors into TIL may allow their use in conjunction with monoclonal antibodies to mediate antibody-dependent cellular cytotoxicity. A particularly intriguing application of TIL is the introduction of chimeric T-cell receptors to modify the antigenic range of these cells. These chimeric receptors consist of the constant region of the T-cell receptor plus the variable region of the monoclonal antibody. Gross et al[140] have shown that the introduction of these chimeric T-cell receptors into mouse T-cell hybridomas can alter the antigenic specificity of these cells.[140] The use of this technology in conjunction with the use of TIL could potentially increase the range of TIL activity to tumors for which monoclonal antibodies exist, and we are actively exploring this potential application. The introduction of IL-2 receptors into TIL might increase their sensitivity to IL-2 and, thus, enable us to use lower levels of administered IL-2.

More recently we have begun studies of the genetic modification of cancer cells to increase their immunogenicity. A variety of investigators have shown that introduction of cytokine genes into tumor cells can increase

Table 15. Protocol Schema for Administration of TNF-Gene Modified TIL

1. Escalate TNF TIL twice weekly; no IL-2:
   10^8 cells
   3×10^8
   10^9
   3×10^9
   10^{10}
   3×10^{10}
   10^{11}
   3×10^{11}
2. Reduce cell dose to 1/10 maximum-tolerated dose. Escalate as in (1) every 3 weeks with 180,000 IU/kg IL-2.
3. After three patients discuss with the FDA (possibly start at a higher cell dose with IL-2).

Table 16. Gene Therapy With TIL Transduced With the Gene for TNF

Patient No.	Age (years)/ Sex	Sites of Metastatic Disease	Secretion of TNF by TIL (pg/10^6 cells/24h)	Cell Doses Administered
1	30/F	Brain, subcutaneous, adrenal	141	1.1×10^8
				3.0×10^8
				1.0×10^9
				3.0×10^9
				5.4×10^9
2	44/M	Lymph nodes, subcutaneous, intramuscular	517	1.0×10^8
				3.1×10^8
				1.1×10^9
				3.0×10^9
3	53/F	Liver, subcutaneous, retroperitoneum	125	1.0×10^8
				3.0×10^8
				9.6×10^8
				3.0×10^9
				1.0×10^{10}
				2.4×10^{10}
				1.0×10^{11}
				1.0×10^{10} + IL-2
				2.8×10^{10} + IL-2
				9.0×10^{10} + IL-2
4	25/M	Brain, lung, subcutaneous, mediastinum	115	1.0×10^9
				1.0×10^{10}

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the recognition of tumor by the host immune system.[141-148] The cytokine genes used in these studies have included IL-4, IL-2, interferon gamma, TNF, and granulocyte-macrophage colony-stimulating factor. The introduction of genes coding for MHC antigens can also increase tumor immunogenicity. Our work is centered on introducing the genes for TNF and IL-2 into tumor cells. Mouse tumor cells transduced with the TNF gene grow for a short time and then spontaneously regress. This regression is dependent on the presence of CD4+ and CD8+ cells, as has been demonstrated by antibody-depletion studies. We have thus designed a clinical protocol for the treatment of patients with advanced cancer. The protocol schema is outlined in Table 17. Tumor is excised from patients, and tissue culture lines established. Genes for either TNF or IL-2 are inserted into these tumor cells, and these tumor-cell vaccines are then used to immunize the patient against their autologous cancer. In addition to this active immunization, draining lymph nodes will be excised 3 weeks after tumor-cell injection grown in culture and used for adoptive immunotherapy.

The studies described in this report demonstrate that it is possible to manipulate the host immune system to mediate the regression of cancer in some patients with advanced disease. The immune manipulations we are currently using are in the infancy of their development and as increased knowledge accrues concerning the nature of the immune response to human tumors, more sophisticated approaches should be possible. The fact that at least some bulky human tumors can be impacted by immunologic maneuvers is encouraging for future progress in this field. With future developments, it is hoped that these early approaches can be developed into effective, safe, and practical treatments for patients with cancer.

Table 17. Protocol Schema for the Use of Tumor Cells Transduced With the Genes for TNF or IL-2 for the Treatment of Patients With Advanced Cancer

1. Tumor resected as part of standard treatment.
2. Tissue culture line established.
3. Cytokine gene transduced into culture, and cultures selected in G418 (cytokine > 100 pg/10^6 cells/24h).
4. Inject 2×10^8 gene-modified tumor cells subcutaneously into right thigh and 2×10^7 cells intradermally at two nearby sites.
5. Three weeks later, remove draining lymph node and grow in vitro in IL-2.
6. Adoptive immunotherapy using these cells plus IL-2.

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