Experience with the Use of High-Dose Interleukin-2 in the Treatment of 652 Cancer Patients

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We have administered 1039 courses of high-dose interleukin-2 (IL-2) to 652 cancer patients. Five hundred ninety-six patients had metastatic cancer that either had failed standard effective therapies or had disease for which no standard effective therapy existed, and 56 patients were treated in the absence of evaluable disease in the adjuvant setting. IL-2 was administered either alone (155 patients) or in conjunction with activated immune cells such as lymphokine activated killer (LAK) cells (214 patients) or tumor infiltrating lymphocytes (TIL) (66 patients), with other cytokines such as alpha interferon (a-IFN)(128 patients) or tumor necrosis factor (TNF)(38 patients), with monoclonal antibodies (32 patients), or with the chemotherapeutic agent cyclophosphamide (19 patients). Initial results with the treatment of high-dose IL-2 alone or in conjunction with LAK cells have indicated that objective regressions of cancer can be achieved in 20% to 35% of patients with selected advanced metastatic cancers. Although most responses have been seen in patients with metastatic renal cell cancer, melanoma, colorectal cancer, and non-Hodgkin's lymphoma, many histologic types of cancer have not been treated in significant numbers. These regressions can be durable; of 18 patients achieving a complete response, ten have not experienced recurrence at intervals from 18 to 52 months. Although combinations of IL-2 with TNF do not appear to result in increased responses, there is a suggestion in our initial phase I studies that the combination of a-IFN and IL-2 is more effective than the administration of cytokine alone and this combination deserves further study. Similarly the adoptive transfer of TIL in conjunction with IL-2 also appears to be more effective than the use of IL-2 alone. The toxic side effects in patients treated with high-dose IL-2 are presented and include malaise, nausea and vomiting, hypotension, fluid retention, and organ dysfunction. Treatment-related deaths were seen in 1% of all treatment courses and in 1.5% of patients. These studies demonstrate that a purely immunologic manipulation can mediate the regression of advanced cancers in selected patients and may provide a base for the development of practical, effective biologic treatments for some cancer patients.

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APPROXIMATELY ONE HALF OF all patients who develop cancer will eventually die of metastatic disease despite the best application of surgery, radiation therapy, and chemotherapy. Attempts to develop new approaches to the treatment of metastatic cancer by stimulating immune host defense reactions against the tumor have received substantial attention in recent years. The administration of high-dose interleukin-2 (IL-2), either alone or in conjunction with immune lymphoid cells can result in the regression of metastatic cancer in some patients and these results have spurred additional efforts to improve these treatments.[1–4]

Interleukin-2 is a 15.5 kD glycoprotein that plays a central role in immune regulation.[5] Activation of lymphocytes by specific antigen results in the generation of IL-2 receptors and the subsequent interaction of the lymphocyte with IL-2 leads to cell proliferation resulting in an immune response. IL-2 is only one of a multitude of hormones (cytokines) produced by lymphocytes and monocytes that result in the cascade of immune reactions.

In experimental animals, the administration of IL-2, either alone or in conjunction with other cytokines, monoclonal antibodies, chemotherapeutic agents, or immune lymphocytes, can mediate the rejection of cancers in lung, liver, and subcutaneous tissue.[6–8] The administration of IL-2 in conjunction with alpha-interferon (a-IFN) or tumor necrosis factor-alpha (TNF) resulted in synergistic antitumor activity compared to the use of IL-2 alone.[9,10] The administration of lymphokine activated killer (LAK) cells plus IL-2 similarly appeared more effective than IL-2 alone[11,12] and more recent studies showed that the administration of tumor infiltrating lymphocytes (TIL) plus IL-2 appeared to be 50 to 100 times more effective than the administration of LAK cells plus IL-2.[13]

TREATMENT PROTOCOLS

	Day																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1) Interleukin-2 (I) alone	I	I	I	I	I									I	I	I	I	I
2) Interleukin-2 + Tumor Necrosis Factor (T)	T	T	T	I	I	I	I	I										
3) Interleukin-2 + alpha-interferon (A)	I A	I A	I A	I A	I A									I A	I A	I A	I A	I A
4) Interleukin-2 + Monoclonal Antibodies (M)	I	I M	I M	I M	I									I	I M	I M	I M	I
5) Interleukin-2 + Cyclophosphamide (C)	C	I	I	I	I	I												
6) Interleukin-2 + Lymphokine Activated Killer (LAK) cells	I	I	I	I	I									I LAK	I LAK	I	I LAK	I
7) Interleukin-2 + Tumor Infiltrating Lymphocytes (TIL)	C	I TIL	I TIL	I	I	I								I	I	I	I	I

FIG. 1. General schemata for the use of high-dose Interleukin-2 in immunotherapy protocals.

These studies have lead to the development of a series of clinical trials to test the effectiveness of these immunotherapeutic approaches in patients with cancer. The current paper summarizes our results with the treatment of 652 patients who received 1039 courses of high-dose IL-2 given either alone or in combination with TNF, a-IFN, monoclonal antibodies, cyclophosphamide, LAK cells, or TIL.

Methods

Patients

The 652 patients in this trial had histologic confirmation of the diagnosis of cancer. Five hundred ninety-six patients had metastatic cancer that either had failed standard effective therapies or had disease for which no standard effective therapy existed. Fifty-six patients were treated in the absence of evaluable disease in the adjuvant setting. Thirty-six patients with malignant melanoma metastatic to draining lymph nodes were treated after resection of the draining lymph node group. Seventeen patients with colon cancer metastatic to the liver were treated after complete resection of hepatic metastases, and three patients with renal cell cancer metastatic to draining lymph nodes were treated after resection of the involved lymph nodes.

All patients with metastatic cancer had evaluable disease and had received no other therapy for their cancer for 1 month before entrance into the protocol or throughout the follow-up period. Patients were excluded from the protocol if they had major illnesses of the cardiovascular, respiratory, or renal systems and if they had any evidence of central nervous system metastases. For the past 3 years all patients older than 50 years entering the protocol underwent either a stress EKG or stress radionuclide ejection scan and patients with any evidence of ischemic heart disease were excluded from these protocols. Evaluation of all patients included CT or MRI scans of the brain, full lung tomograms, or CT scans of the chest, abdomen, and bones.

Treatment

The general outline of the treatment protocols is shown in Figure 1. In general patients received four to seven days of therapy followed by seven to ten days of rest and then an additional four to seven days of treatment. Responding patients, and in later protocols stable patients, generally received additional courses of treatment 2 to 3 months after the initiation of the first course of therapy. A brief summary of each of the individual protocols follows.

IL-2 alone. Recombinant IL-2 was administered intravenously in bolus doses every eight hours.[14] IL-2 (supplied by the Cetus Corporation, Emeryville, CA) was administered at doses of 100,000 U/kg, although fewer patients received doses between 10,000 and 30,000 U/kg. IL-2 (supplied by Hoffman-LaRoche, Nutley, NJ) was administered at doses of 1,000,000 to 6,000,000 U/m^2. Patients received IL-2 for up to five days followed by a seven to ten day rest followed by five more days of treatment.

IL-2 plus Tumor Necrosis Factor-Alpha (TNF). IL-2 and TNF were supplied by the Cetus Corporation (Emeryville, CA). TNF was administered as an intravenous bolus dose once a day on days 1, 2, and 3, followed by IL-2 starting on day 4 every eight hours for up to five days. IL-2 doses varied from 30,000 to 100,000 U/kg and the doses of TNF varied from 50 to 350 ug/m^2. Up to eight patients were entered into each dose schedule to determine the maximum tolerated doses of the combination of IL-2 and TNF.

IL-2 plus Alpha-Interferon (a-IFN). IL-2 and a-IFN were supplied by Hoffman-LaRoche Inc. (Nutley, NJ). IL-2 and a-IFN were administered concurrently, intravenously, every eight hours for up to five days, followed by a seven-to-ten day rest, followed by a second cycle of treatment. As detailed later in some escalating dose regimens, the a-IFN was given once a day. The dose of IL-2 in this procotol varied from 1,000,000 to 6,000,000 U/m^2 and the dose of a-IFN varied from 3,000,000 to 6,000,000 U/m^2.

IL-2 plus monoclonal antibodies (MoAb). IL-2 was administered as in the protocol using IL-2 alone. However monoclonal antibody directed against either melanoma or colorectal cancer antigens were administered intravenously, generally twice daily, during the course of IL-2 treatment.

IL-2 plus cyclophosphamide. A single dose of cyclophosphamide ranging from 10 to 50 mg/kg was administered intravenously after a 12-hour period of intravenous hydration. Twenty-four to 36 hours after the cyclophosphamide, IL-2 administration began as in the protocol using IL-2 alone.

IL-2 plus lymphokine activated killer (LAK) cells. This treatment regimen has been extensively described elsewhere.[1,3,15] Up to five days of IL-2 administration preceded four to five days of leukapheresis to obtain peripheral lymphocytes. LAK cells were generated in culture and reinfused during the second cycle of treatment along with the concomitant administration of IL-2 as in the protocol using IL-2 alone.

IL-2 plus tumor infiltrating lymphocytes (TIL). This protocol has been described in detail elsewhere.[4,16] Patients received a single dose of cyclophosphamide, generally at 25 mg/kg, followed 24 to 36 hours later by the administration of TIL and the concomitant administration of IL-2 as in the protocol using IL-2 alone.

Evaluation of Response to Treatment

A response was considered to be complete if all measurable tumor disappeared. A partial response was defined as a 50% decrease in the sum of the product of the longest perpendicular diameters of all lesions, lasting at least 1 month, without increase in any tumor or the appearance of any new tumor. Any patient who did not achieve at least a partial response is considered a nonresponder in this analysis. The response durations were computed from the time of the first dose of IL-2 until disease progression.

Results

Between November 1984 and March 1989, 652 patients with cancer underwent treatment with 1039 courses of immunotherapy using high-dose IL-2. Eleven of these patients were treated in two separate protocols and each protocol was evaluated separately. Five hundred ninety-six patients had metastatic cancer and 56 patients were treated after resection of all disease in the adjuvant setting.

Characteristics of these patients are shown in Table 1. Most patients were between the ages of 30 and 60 and all but 16 patients had received previous treatment for their cancer. Six hundred seven patients had undergone surgical therapy, 183 had received chemotherapy, and 120 had received radiation therapy. Two hundred seventy-four patients had received at least two forms of treatment and 96 patients had received three treatment modalities. The majority of the patients treated in these protocols had malignant melanoma or metastatic renal cell cancer; these two diagnoses comprised 477 of the 652 patients. Ninety-nine patients had colorectal cancer, 21 had metastatic breast cancer, and 18 had non-Hodgkin's lymphomas. Less than ten patients with any other histology were treated. Most patients had good performance status, which was required for entrance into these clinical protocols.

All patients in these studies were accrued as of March 1989 and follow-up is included to that date as well. Response data is presented only for those patients having at least 2 months of follow-up after the last dose of immunotherapy.

The characteristics of the immunotherapy treatments are shown in Table 2. Six hundred seventy-eight of the 1039 treatment courses were given at a dose of 100,000 U/kg every eight hours, which is the maximum tolerated dose of IL-2. Few patients can receive more than 15 doses at this dose level. Doses of IL-2 and the cumulative IL-2 dose for each of the protocols are shown in Table 2. The number of patients receiving LAK cells or TIL in each protocol and the number of cells received is also shown.

Our initial studies dealt with the administration of high-dose IL-2 given either alone or in conjunction with the adoptive transfer of LAK cells.[1-3] The results of these protocols, presented in Tables 3 and 4, of the treatment of 307 patients represents an update of previously published results on 212 patients[8] with an additional 20 months of follow-up. The results of patients treated with IL-2 alone include all patients with the exception of four who died of therapy-related complications and 21 who were treated in the absence of evaluable disease in the adjuvant setting (Table 3). Of these 130 patients, the only two diseases with appreciable numbers of patients are renal cell cancer and melanoma and the objective response rates in these diseases were 22% and 24%, respectively. No objective responses were seen in 12 colorectal cancer patients or 11 patients with non-Hodgkin's lymphoma. No more than three other patients with any single histology were treated, and thus no comments could be made about the effectiveness of this therapy in patients with other histologic types of cancer.

A similar update of our treatment results in 177 patients with advanced cancer treated with LAK cells and IL-2 is

TABLE 1. *Characteristics of Patients Receiving Immunotherapy*

Interluekin-2 Plus		Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
		Number of Patients							
Total		155	38	128	32	19	214	66	652*
Sex	Male	91	21	83	19	11	137	40	402
	Female	64	17	45	13	8	77	26	250
Age	11–20	3	2	1	—	—	3	—	9
	21–30	10	4	6	—	4	22	11	57
	31–40	34	9	19	8	7	42	17	136
	41–50	54	11	42	7	2	63	16	195
	51–60	39	8	42	13	4	61	20	187
	61–70	15	4	18	4	2	23	2	68
Previous Treatment	None	4	—	7	—	—	4	1	16
	Surgery	139	38	119	31	19	199	62	607
	Chemotherapy	48	9	27	17	7	64	11	183
	Radiotherapy	27	10	21	4	2	45	11	120
	Hormonal	12	1	3	1	—	7	2	26
	Immunotherapy	20	5	13	4	4	27	17	90
	Any 2 or more	64	18	49	19	10	90	24	274
	Any 3 or more	25	5	12	5	3	35	11	96
Diagnosis	Melanoma	60	15	53	12	13	66	51	270
	Renal cell	58	10	51	—	3	74	11	207
	Colorectal	14	7	10	20	—	46	2	99
	Breast	4	5	5	—	3	2	2	21
	NH lymphoma	11	—	—	—	—	7	—	18
	NSC lung	1	—	1	—	—	5	—	7
	ST sarcoma	1	—	2	—	—	4	—	7
	Brain	2	—	—	—	—	1	—	3
	Esophageal	—	—	1	—	—	1	—	2
	Gastrinoma	—	1	—	—	—	1	—	2
	Liver	2	—	—	—	—	—	—	2
	Ovarian	1	—	—	—	—	1	—	2
	Prostate	—	—	2	—	—	—	—	2
	Small bowel	—	—	2	—	—	—	—	2
	Testicular	—	—	1	—	—	1	—	2
	Ewing's	—	—	—	—	—	1	—	1
	Hodgkin's	—	—	—	—	—	1	—	1
	Osteosarcoma	—	—	—	—	—	1	—	1
	Pancreatic	1	—	—	—	—	—	—	1
	Thyroid	—	—	—	—	—	1	—	1
	Unknown primary	—	—	—	—	—	1	—	1
Performance†	0	109	34	112	26	11	132	53	477
	1	36	3	13	3	6	60	11	132
	2	8	1	3	3	1	21	1	38
	3	2	—	—	—	1	1	1	5

* Eleven patients are in two protocols.

† Eastern Cooperative Oncology Group criteria.

presented in Table 4. This represents all of our treated patients except one lost to follow-up, one who died of therapy related complications, and 35 who were treated without evidence of disease in the adjuvant setting. Of 72 patients with renal cell cancer and 48 patients with metastatic melanoma, response rates were 35% and 21%, respectively. One complete response and four partial responses were seen in 30 patients (17%) with metastatic colorectal cancer and four objective responses were seen in seven patients with non-Hodgkin's lymphoma (57%). Too few patients with other histologic types of cancer were treated to draw any conclusions about efficacy in other histologic types of cancer.

Tumor responses were seen in these patients at a variety of sites including lung, liver, bone, subcutaneous tissue, skin, and circulating tumor cells. When tumor at a single site regressed, regression generally occurred at all sites. Mixed responses were rare and were categorized as nonresponses. Maximum follow-up in these patients now extends to 52 months. The duration of responses as of March 1989 in patients treated with IL-2 and LAK/IL-2 is shown in Table 5. The limited duration since the onset of these

TABLE 2. *Characteristics of the Immunotherapy Treatments*

Interleukin-2 Plus		Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients		155	38	128	32	19	214	66	652*
Number of Courses		236	85	210	35	30	348	95	1039
Dose IL-2	100	212	22	—	31	30	297	86	678
(CC: U/kg × 10^{-3})	60	—	21	—	—	—	—	—	21
	30	7	42	—	4	—	21	8	82
	20	4	—	—	—	—	9	—	13
	10	1	—	—	—	—	21	1	23
(HL: U/m^2 × 10^{-6})	6.0	7	—	12	—	—	—	—	19
	4.5	5	—	104	—	—	—	—	109
	3.0	—	—	85	—	—	—	—	85
	1.0	—	—	9	—	—	—	—	9
Number IL-2 doses	1–10	52	50	25	10	11	30	57	235
	11–20	129	35	137	22	19	235	33	610
	21–30	51	—	48	3	—	54	5	161
	31–40	4	—	—	—	—	12	—	16
	41+	—	—	—	—	—	17	—	17
Cumulative IL-2 dose	1–500	17	52	—	2	—	18	25	114
(CC: U/kg × 10^{-3})	501–1000	39	21	—	11	11	54	34	170
	1001–2000	122	12	—	21	19	238	32	444
	2001–3000	42	—	—	1	—	31	4	78
	3001–4000	4	—	—	—	—	6	—	10
	4001–5000	—	—	—	—	—	1	—	1
(HL: U/m^2 × 10^{-6})	1–50	4	—	65	—	—	—	—	69
	51–100	5	—	134	—	—	—	—	139
	101–150	3	—	11	—	—	—	—	14
Number of cell doses	1–5	7	—	—	2	—	306	85	400
	6–10	—	—	—	—	—	34	—	34
	11–14	—	—	—	—	—	3	—	3
Cumulative cells	0.1–5.0	—	—	—	—	—	41	11	52
(×10^{-10}	5.1–10.0	1	—	—	—	—	139	16	156
	10.1–15.0	3	—	—	—	—	101	11	115
	15.1+	3	—	—	2	—	62	47	114

* Eleven patients are in two protocols.

clinical efforts precludes definitive comments about the duration of responses, although it is interesting that 14 of the responding patients have remained in sustained response for more than two years. Of 18 patients in complete remission, ten remain in complete remission at 18 to 52 months. It thus appears that at least some patients with

TABLE 3. *Results of Immunotherapy in Patients with Advanced Cancer (March 1989)*

Cancer Diagnosis	Treatment with IL-2 (number of patients) Evaluable*	CR	PR	CR + PR (%)
Renal	54	4	8	22
Melanoma	42	0	10	24
Colorectal	12	0	0	—
Non Hodgkin's Lymphoma	11	0	0	—
Breast	3	0	0	—
Other†	8	0	0	—
Total	130	4	18	17

* Includes all treated patients except four that died of therapy and 21 treated in adjuvant setting.

† Two patients each with hepatoma and brain cancer; one each with sarcoma, lung, ovary and pancreas.

TABLE 4. *Results of Immunotherapy in Patients with Advanced Cancer (March 1989)*

Cancer Diagnosis	Treatment with LAK/IL-2 (number of patients) Evaluable*	CR	PR	CR + PR (%)
Renal	72	8	17	35
Melanoma	48	4	6	21
Colorectal	30	1	4	17
Non-Hodgkin's lymphoma	7	1	3	57
Sarcoma	6	0	0	—
Lung	5	0	0	—
Other†	9	0	0	—
Total	177	14	30	25

* Includes all treated patients except one lost to follow-up, one died of therapy and 35 treated in adjuvant setting.

† One patient each with cancer of breast, brain, esophagus, ovary, testes, thyroid, gastrinoma, Hodgkin's, unknown primary.

TABLE 5. *Duration of Responses (as of March 1989) Expressed in Months*

	LAK/IL-2		IL-2	
Diagnosis	CR	PR	CR	PR
Renal	30+, 27+, 23+, 15, 13, 11, 9, 6	36+, 21+, 19, 13, 11, 11, 9, 7, 7, 6, 6, 6, 3, 2, 1, 1	34+, 28+, 27+, 25+	27+, 27+, 25+, 21+, 21+, 19+, 15+, 3+
Melanoma	52+, 32+, 18+, 13	30+, 6, 6, 3, 2, 2	—	41+, 15, 12, 11, 10, 8, 7, 5, 3, 2
Colorectal	21	11, 6, 6, 2	—	—
Non-Hodgkin's lymphoma	10	31+, 20, 8+	—	—

Of 18 patients with complete remission, 10 remain in CR at 18 to 52 months.

metastatic cancer experience durable complete and partial remissions when treated with this form of immunotherapy.

As patients accrued in protocols containing IL-2 and IL-2 plus LAK cells, we explored the administration of combinations of cytokines in murine models. Substantial synergistic effects were seen in models of established murine tumors using the combinations of TNF with IL-2 and a-IFN with IL-2.[9,10] In these murine models a direct relationship existed between antitumor response and the amount of both IL-2 and either TNF or a-IFN administered.

We thus began phase I clinical trials in patients with advanced cancer to seek the maximum tolerated dose of the combined administration of either IL-2 and TNF or IL-2 and a-IFN. An example of the design of the IL-2/TNF trial is shown in Table 6. Three to eight patients were entered into an escalating dose trial in which the IL-2 was varied between 30,000 and 100,000 U/kg q8h after three daily doses of TNF given at doses of 50 to 300 ug/m^2. A single patient was treated at 350 ug/m^2, although this dose proved to be intolerable due to hypotensive side effects. It thus appears that the maximum tolerated dose of both IL-2 and TNF was 100,000 U/kg of IL-2 q8h for up to five days after three daily doses of TNF at 300 ug/m^2. The response of patients in this phase I trial is shown in Tables 6 and 7. One complete response in a patient with metastatic melanoma and three partial responses were seen in patients with metastatic renal cell cancer. The complete responder is still in remission at 14 months and one of the partial responses is continuing at 13 months; two other partial responses progressed at 5 and 7 months, respectively (Table 6). This response rate is not different from that expected from the use of IL-2 alone.

A similar escalating dose trial was conducted using the combination of IL-2 and a-IFN in patients with advanced cancer. This trial, which used Hoffman-LaRoche IL-2 (compared to IL-2 from Cetus Corporation used in previous trials) involved escalating doses of IL-2 and a-IFN. A-IFN was administered at a dose of 3,000,000 U/m^2 q8h concurrent with the administration of 1,000,000 U/m^2 of IL-2 q8h (in six patients), 3,000,000 U/m^2 q8h (in 32 patients) and 4,500,000 U/m^2 q8h (in 26 patients). In addition 30 patients received a-IFN at a dose of 6,000,000 U/m^2 q8h with the concurrent administration of IL-2 at 4,500,000 U/m^2 q8h, and 21 additional patients received 6,000,000 U/m^2 of a-IFN once a day along with the administration of 6,000,000 U/m^2 of IL-2 q8h. Patients received two cycles of treatment, as shown in Figure 1, to a maximum of 15 doses per cycle. Preliminary results of treatment in this protocol are shown in Table 7. Although follow-up is short, it appears that increased response rates are seen in patients accrued at higher doses of a-IFN and IL-2 with response rates at 41% in patients with renal cancer and melanoma at the highest dose escalation. We have not yet reached the maximum tolerated doses in this protocol and accrual is continuing.

In mice bearing advanced tumors we showed that the administration of high-dose IL-2, in conjunction with cyclophosphamide, mediated more potent antitumor responses than treatments with IL-2 alone or cyclophosphamide alone.[17] We thus conducted a phase I study using escalating doses of cyclophosphamide from 10 mg/kg to 50 mg/kg in conjunction with high-dose IL-2. The results in 19 patients treated in this phase I protocol are shown in Table 7. Only two partial responses were seen in patients with melanoma and although the number of patients treated was small, it does not appear that higher response rates are seen compared to the use of IL-2 alone. It should be noted, however, that the three tumor types treated in this study, renal cell cancer, melanoma, and patients with heavily pretreated breast cancer, are not responsive to cyclophosphamide.

TABLE 6. *IL-2/TNF Protocol*

Dose			
IL-2 ($\times 10^{-5}$ μ/Kg)	TNF (μg/m^2)	Number of Patients	Responses
30	50	3	1 PR (5 months)
30	100	3	
30	150	3	1 PR (7 months)
30	200	3	1 CR (14+ months)
30	300	5	
60	200	3	1 PR (13+ months)
60	250	3	
60	300	3	
100	300	8	
100	350	1	
Total		38	4

TABLE 7. *Results of Immunotherapy in Patients with Advanced Cancer (accrued by March 1989)*

	(Number of Patients)								
	Cy/IL-2			TNF/IL-2			aIFN/IL-2		
Diagnosis	Total Evaluable	CR	PR	Total Evaluable*	CR	PR	Total Evaluable†	CR	PR
Renal	3	0	0	10	0	3	46	4	11
Melanoma	13	0	2	15	1	0	44	3	13
Colorectal				6	0	0	10	0	1
Non-Hodgkin's lymphoma									
Sarcoma							1	0	0
Lung adenocarcinoma							1	0	0
Breast	3	0	0	5	0	0	5	0	0
Esophageal							1	0	0
Testicular							1	0	0
Hepatoma							1	0	0
Small intestine							1	0	0
Gastrinoma				1	0	0			
Prostate									
Total	19	0	2	37	1	3	113	7	25

* Includes all treated patients except for one with colorectal cancer found to have a simultaneous non-Hodgkin's lymphoma and received chemotherapy.

† Excludes patients with less than two months follow-up.

Similarly animal models have shown that the combination of specific monoclonal antibodies plus high-dose IL-2 can mediate synergistic antitumor effects.[18] These studies led us to design escalating dose phase I studies of the use of monoclonal antibodies and IL-2 in cancer patients. These studies are currently in their escalating dose phase and no antitumor responses have been seen.

Murine tumor models have indicated that the administration of TIL in conjunction with IL-2 is 50 to 100 times more potent than is the administration of LAK cells and IL-2 in mediating the regression of a variety of established murine cancers.[13] We have recently reported the results of treatment with TIL and IL-2 in patients with malignant melanoma.[4] In preliminary studies it appears that approximately 50% of patients with metastatic melanoma show objective responses to this latter treatment. This preliminary work has recently been published and will not be considered further here, although these patients are considered in the overall evaluation of the toxicity of treatment.

Toxicity of Immunotherapies Containing High-Dose IL-2

The toxicities associated with the administration of high-dose IL-2 are directly related to the dose of IL-2 administered. We have previously reported the toxicity of high-dose IL-2 used either alone or in conjunction with LAK cells in 212 patients with advanced cancer.[3,8] In this paper we update this data to report the toxicity of the administration of high-dose IL-2 either alone or in combination with other cytokines, monoclonal antibodies, cyclophosphamide, or immune lymphocytes in 652 patients receiving 1039 courses of treatment (Table 8). This tabulation includes all patients we have treated, including those with metastatic cancer, and the 56 patients treated in the absence of evaluable disease in the adjuvant setting. All patients received high-dose IL-2 and the contribution to toxicity of the additional agents varied somewhat depending on the dose of the additional agent administered. Systemic symptoms were common and many patients experienced nausea and vomiting, diarrhea, and malaise during therapy. All patients received acetaminophen, indomethacin, and ranitidine to allievate these symptoms. Transient organ dysfunctions were common and elevations of bilirubin and creatinine were often found. These elevations returned to normal with a median of four days after discontinuing treatment.

Many of the side effects associated with high-dose IL-2 treatment are similar to those seen in patients with sepsis, including a decrease in peripheral vascular resistance, an increase in cardiac index, tachycardia, oliguria, and hypotention. A capillary permeability increase led to fluid extravasation into soft tissues and a weight gain of more than 5% of the total body weight in most patients. Vasopressors were used early in the treatment course to reduce the fluid requirement necessary to maintain urine output and blood pressure.

Ten patients died of therapy-related complications, which represented 1% of all treatment courses and 1.5% of all patients.

Discussion

In this paper we present our efforts exploring the use of high-dose IL-2 in conjunction with other immunotherapeutic manipulations to develop effective immunother-

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19		2	3	1	12	5	42
Weight gain (% body weight)								
0.0–5.0	106	23	65	8	9	117	49	377
5.1–10.0	78	41	111	22	10	148	26	436
10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.

apies for the treatment of patients with cancer. Results of the use of high-dose IL-2 alone or in conjunction with LAK cells demonstrate that a purely immunologic manipulation is capable of mediating the regression of established cancer in humans. Although the earlier use of interferon to mediate tumor regression may have an immunologic basis, the direct antiproliferative effects of the interferons suggest that these agents may be acting directly on the tumor rather than stimulating the host reaction to the tumor.[19]

The treatment of patients with advanced cancer using IL-2 and LAK cells can result in durable clinical remissions even in patients with advanced tumor burdens (Table 5). In experimental animal models the use of IL-2 alone can mediate tumor regression, although the administration of LAK cells results in a more substantial antitumor effect at all doses of IL-2 administered.[11,12] We have recently completed a prospective randomized trial of 181 patients randomized to receive either IL-2 alone or IL-2 plus LAK cells. Although the overall response rates are similar in these two regimens, the incidence of complete responses is greater in patients who received LAK cells plus IL-2 ($p = 0.04$). Follow-up is short and further evaluation of this question is in progress.

In the development of these immunotherapies, experiments in animal models have played an important role in predicting the appropriate design of clinical trials in humans with advanced cancer. These animal models revealed a substantial therapeutic synergy between IL-2 and TNF and between IL-2 and a-IFN.[9,10] In the phase I study of TNF plus IL-2, we have reached the limiting toxicity of the combined administration of these agents and we have not seen a suggestion of increased activity due to the combination. Clinical trials of TNF alone in cancer patients have yielded disappointing results and the reason for the activity of TNF in animal models but not in humans is not well understood.[20] In our initial phase I efforts it does appear that a-IFN and IL-2 may have additive or synergistic activity in patients with advanced cancer, although additional patients must be entered at the maximum tolerated doses to draw definitive conclusions.

In addition to the use of combination cytokines, animal models have indicated that the combined use of IL-2 with chemotherapeutic agents such as cyclophosphamide,[17] or with monoclonal antibodies directed against antigens on the tumor cell surface may synergize with IL-2 administration.[18] These animal models included tumors that were sensitive to cyclophosphamide in contrast to the human tumors treated in our patients who were either nonresponsive to chemotherapy or failed available chemotherapies. We have not seen a suggestion of combined therapeutic activity in combining cyclophosphamide and IL-2 in these patients, although it is possible that a synergy would be seen if an effective chemotherapy were used. Ongoing trials in the Surgery Branch, NCI, are exploring the use of 5-fluorouracil plus leucovorin in conjunction with IL-2 or monoclonal antibodies plus and IL-2 in the treatment of patients with metastatic colorectal cancer.

We have sought means to identify cells with greater therapeutic potency for the adoptive immunotherapy of patients with advanced cancer, and a recent clinical trial has suggested that TIL may be more effective than LAK cells in the treatment of patients with metastatic melanoma.[4] These TIL, which are T cells, in contrast to LAK, which are null cells, often exhibit unique lytic activity, *in vitro*, to autologous melanoma cells and not to other normal cells from that patient or to allogeneic melanoma cells.[16,21] The *in vitro* activity of these TIL provides the best evidence now available that at least some patients with growing cancers can mount immune reactions to their malignancy. Continued work with these TIL is in progress.

The use of high-dose IL-2 alone or in combination regimens provided us with the opportunity to analyze the toxic side effects of this treatment in 652 patients who received 1039 courses of immunotherapy. The major side effects of IL-2 result from its tendency to induce a capillary leak syndrome, from the growth of lymphocytes in visceral organs throughout the body, and from stimulation of the secretion of other cytokines by cells of the immune system.[22–25] Many of the side effects of IL-2 resemble those seen in patients with septic shock and it is possible that some of these side effects are due to the stimulation by IL-2 of the secretion of TNF by mononuclear cells. These side effects are quite transient and reverse readily after IL-2 therapy is discontinued.

Since the beginning of our experience with high-dose IL-2, 1.5% of patients died due to treatment-related causes. This rate is substantially lower than that associated with the use of virtually any combination chemotherapy in standard use in patients with advanced cancer. Thus although the toxicity of the administration of IL-2 can be severe, it is readily managed in the great majority of patients and high-dose IL-2 either alone or in combination with other agents can be safely administered. Supporting this contention is the fact that one treatment related fatality was seen in 94 patients treated in a multi-institution trial using high-dose IL-2 and LAK cells and by the safe use of LAK/IL-2 in other reported trials.[26–29]

Current clinical efforts are being devoted to a continued exploration of combination cytokine therapy and the use of immunotherapy in patients with decreased tumor burdens. In these latter efforts immunotherapy is being used in combination with surgery, radiation therapy, and chemotherapy. One approach with substantial promise for improving the effectiveness of adoptive cellular therapy

is our ability to modify TIL by gene insertion. Traffic studies with Indium^{111}-labeled TIL have indicated that these TIL can "home" to tumor deposits and thus TIL might represent ideal packets to deliver toxic substances directly to the tumor site.[30] We have recently received authorization from the Recombinant DNA Advisory Committee, the National Institutes of Health, and the Food and Drug Administration to perform gene transfer studies in humans using TIL. In initial clinical trials, genes coding for neomycin resistance will be transduced into TIL using retroviral vectors to enable studies of the long-term distribution and survival of TIL. Future experiments are planned to explore the therapeutic potency of TIL modified by genes coding for other cytokines such and TNF, a-IFN, and perhaps IL-2 itself.

The use of high-dose IL-2 in the treatment of patients with cancer administered either alone or in combination with other agents represents an approach to the biologic therapy of patients with advanced cancer that is in the early stages of its development and, we hope, may provide a base for the development of practical effective therapies for the treatment of patients with cancer.

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Discussion

DR. CHARLES M. BALCH (Houston, Texas): Dr. Rosenberg has made major contributions in this important and evolving area of research involving biologic therapy. His research represents a blend of both excellent preclinical models and well-controlled clinical trials. I have a few comments relevant to the lymphokines he discussed today and would like to ask two questions.

(Slide) The battlefield for host–tumor relationships is within the tumor, and here again Dr. Rosenberg has done pioneering work in examining TIL and exploring their use as a treatment strategy. There is a profound defect of the lymphocytes that are recovered from such cancer as melanoma and renal cell carcinoma in that they cannot bind or kill autologous tumor cells. However this deficit can be corrected *in vitro* by adding Interleukin-2, and Dr. Rosenberg proposes that at least in some patients you can augment cellular immune responses *in vivo* as well as by administering Interleukin-2 in combination with other lymphokines.

We have studied these TIL extensively and found an extraordinary diversity of the immune responses. However some patterns emerge as shown in this study of more than 120 human tumors in which we classified the subtypes of lymphocytes that emigrated into the tumor and their growth rate with Interleukin-2. In melanoma most of these cells are T-cells with a cytotoxic phenotype ($CD8^+$). There are almost no NK cells. Renal cell carcinomas, on the other hand, have both T-cell subsets and NK-cells, as do sarcomas. Breast cancers and colon cancers are different yet again. The cytotoxic capacity of these cells is also quite different among various human cancers. Thus TIL from distant metastatic melanoma has an efficient level of cytotoxicity that is restricted to the patient's own tumor (*i.e.*, they cannot kill allogeneic cells). TIL from lymph node metastases from melanomas are very inefficient cytotoxic effector cells. TIL from renal cell carcinomas are different from those in melanomas because these lymphocytes have the capacity to kill both autologous and allogenic tumor target cells.

Because biologic therapy is an indirect approach to eliminate cancers by augmenting an immune rejection response, one would expect that there would be some variations from tumor to tumor and site to site, and would also emphasize an important part of Dr. Rosenberg's treatment strategy in that he used multiagent immunotherapy using agents with different mechanisms of action.

I would like to ask two questions. First do you have any idea about the nature of the functional defect of these tumor-infiltrating lymphocytes that appear to be overcome *in vitro* by adding back pharmacologic doses of Interleukin-2? Second what is the relative contribution of the expanded lymphocytes that are included in the Interleukin-2 regimens? That is how do you know that the TIL or LAK cells are contributing significantly to these tumor responses *in vivo*? Do you have data now from your studies, either by trafficking or clinical trials, showing the relative contribution of adoptive immunotherapy compared to the therapeutic effect of using the lymphokines alone?

DR. DONALD L. MORTON (Los Angeles, California): To Dr. Rosenberg must go the credit for ushering in the modern era of immunotherapy with cytokines, in combination with adoptive immunotherapy with lympoid cells. I admire Dr. Rosenberg, not only for his scientific advances, but especially for his tenacity and hard work in dealing with 652 critically ill patients with hopeless malignancy who have undergone the toxicity he has described with IL-2. The response rates of 21% to 35% in disseminated melanoma and renal cell cancer are impressive. The even higher response rates of 35% to 50% with alpha interferon and IL-2 and TIL-IL-2 are even more significant when one considers that these tumors are refractory to chemotherapy.

It is perhaps significant that these responses are all or none, which is different from chemotherapy in which there are often only partial responses in some metastatic sites. I want to ask Dr. Rosenberg two questions: Does he have an explanation for this all-or-none phenomenon, and does he know the target structure to which the IL-2 LAK cells are directed?

Our own work has concentrated on active specific immunotherapy with tumor cell vaccines, and I thought this might be a chance to give the Association a brief update. We have used a whole cell vaccine composed of three allogeneic melanoma cell lines irradiated to 10,000 rads and administered intradermally. Low-dose cyclophosphamide has been used as an immunomodulator and compared this with tumor cell vaccine alone. The early results in the 300 mg/M^2 dosage shows no difference between tumor cell vaccine alone and tumor cell vaccine with cyclophosphamide.

However if one compares the results we have seen with vaccine immunotherapy with those previously seen with chemotherapy in disseminated melanoma, we see that the patients receiving vaccine with or without cyclophosphamide do significantly better. The median survival for chemotherapy is 6 to 9 months *versus* 16 months for immunotherapy; the 40-month survival for immunotherapy is 30% *versus* 5% for chemotherapy.

We began to see a rise in antibody titer to one or more of the seven melanoma-associated cell surface antigens 4 to 8 weeks after the start of immunotherapy. Regression of evaluable disease begins at about 3 months.

(Slide) This is a patient with extensive recurrent melanoma over the ear, face, and neck, refractory to chemotherapy and radiation therapy treated with this vaccine. After 4 months, partial regression is observed. After 10 months it is almost complete, except for some small, residual disease on the cheek, which is completely gone at 19 months. This patient is free of disease at 36 months.

Of 25 patients treated with evaluable disease who were treated, we have had two complete regressions and an overall response rate of 25%. It is interesting that the responses we see with active specific immunotherapy are slower in their evolution, but are of more durable duration than the responses we see with chemotherapy.

DR. JEROME J. DECOSSE (New York, New York): May I ask Dr. Rosenberg a point of clarification. Were any of the patients described in your talk also treated with either radiation therapy or chemotherapy and can you exclude an effect of these other modalities?

DR. JONATHAN L. MEAKINS (Montreal, Canada): Last year Dr. Wilmore presented a paper that showed that ibuprofin could control some of the symptoms associated with IL-2, and the question was raised at that time whether that would affect any of the antitumor effects of LAK cells and IL-2 or other cytokines.

I wonder if Dr. Rosenberg could tell us whether he has been looking at this. It may have very real implications for the acceptability of this form of therapy as well as its more general applicability in other than very highly specialized centers.

DR. THOMAS C. MOORE (Los Angeles, California): Dr. Rosenberg's report of his innovative work with lymphokines alone and in combination and his use of tumor-infiltrating lymphocytes is most impressive. It is not unreasonable to assume that these lymphocytes are in tumors for a reason and Dr. Rosenberg and his associate have made important advances in exploring this intriguing potential.

I wish to ask Dr. Rosenberg if he and his associates have considered