ANNALS OF SURGERY
Vol. 228, No. 3, 307–319

Durability of Complete Responses in Patients With Metastatic Cancer Treated With High-Dose Interleukin-2

Identification of the Antigens Mediating Response

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Objective

To determine the durability of complete responses in patients with metastatic melanoma or renal cancer treated with high-dose bolus interleukin-2 (IL-2) as well as the factors associated with the development of a complete response and the antigens mediating clinical responses.

Methods

A consecutive series of 409 patients with either metastatic melanoma or renal cancer who were treated with high-dose bolus IL-2 in the Surgery Branch, National Cancer Institute, between September 1985 and November 1996 have been analyzed with a median potential follow-up of 7.1 years. All patients were treated with 720,000 IU/kg administered by 15-minute intravenous infusions every 8 hours for up to 5 days as clinically tolerated per cycle. Two cycles constituted a treatment course. Tumor-infiltrating lymphocytes (TIL) from melanoma patients were used to clone the genes encoding the tumor antigens responsible for clinical responsiveness.

Results

Thirty-three of 409 (8.1%) patients treated with high-dose bolus IL-2 achieved a complete response and 37 (9%) achieved a partial response. Complete regression was seen in 6.6% and 9.3% of patients with metastatic melanoma and renal cancer, respectively. Twenty-seven of these 33 completely responding patients (82%) remain in ongoing continuous complete response from 39 to more than 148 months from the onset of treatment. Tumor regressions were seen at virtually all organ sites. The absence of prior treatment with immunotherapy, the total dose of IL-2 administered, and the maximal rebound lymphocytosis after cessation of IL-2 correlated with achieving a complete response.

Expression cloning techniques have identified a series of tumor antigens that are recognized by TIL grown from resected melanomas. These antigens are mainly melanoma/melanocyte differentiation antigens, although mutated intracellular proteins can also serve as antigens.

Conclusions

Treatment with high-dose bolus IL-2 mediates complete cancer regression in approximately 8% of patients with metastatic renal cancer and melanoma. The great majority of these patients will enter durable complete regressions and appear to be cured of their metastatic cancer. Thus, immunotherapy with high-dose bolus IL-2 should be considered as initial therapy for appropriately selected patients with metastatic melanoma and renal cell cancer. Identification of the tumor antigens mediating clinical response is opening new therapeutic possibilities for cancer treatment.

Patients with metastatic melanoma or metastatic renal cancer experience median survivals of less than 1 year, and virtually all ultimately die of their disease. The recognition in the mid-1980s of interleukin-2 (IL-2) as an immune regulator provided an additional modality for the treatment for these patients, and partial and complete tumor regressions were seen in a subset of patients treated with intravenous IL-2.[1,2]

Long-term follow-up of these responding patients has not been reported. The occasional complete responses reported with the use of combination chemotherapy in patients with

Presented at the 118th Annual Meeting of the American Surgical Association, Palm Beach, FL, April 1998.
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Accepted for publication April 1998.

metastatic melanoma or with the use of alpha-interferon in patients with renal cancer have generally not been durable, and consequently the durability of complete responses in patients responding to IL-2 has been questioned.

In the Surgery Branch, NCI, we began treating patients with high-dose bolus recombinant IL-2 alone in September 1985.[3–5] Since that time, we have treated 409 patients with metastatic melanoma or renal cell cancer using the same regimen. As of March 1998, these patients have a potential median follow-up of 7.1 years, and the longest complete responder has been followed for 12.4 years. This single-institution experience has provided us with the opportunity to assess the durability and possible curative potential of treatment with high-dose IL-2 in patients with these metastatic cancers.

Thirty-three of these patients (8.1%) have undergone a complete regression of all metastatic cancer. Of these 33 patients, 27 (82%) have ongoing complete regressions, from more than 39 to more than 148 months from the onset of treatment. Twenty-five of these patients with ongoing responses have been followed for more than 4 years, and 15 for more than 7 years. Disease has not recurred in any patient with an ongoing complete response of more than 35 months. It thus appears that treatment with high-dose bolus IL-2 can lead to durable, if not curative, complete responses in patients with metastatic melanoma or renal cell cancer. An analysis of the durability of patients undergoing complete regression with this immunotherapy and the possible factors associated with these complete responses and identification of the antigens mediating clinical responsiveness are the subject of this report.

METHODS

Patients

Patients had a diagnosis of metastatic melanoma or metastatic renal cancer and were treated in the Surgery Branch, NCI, between September 1985 and November 1996. All patients had clinically progressive, measurable disease and had received no other therapy for at least 30 days before entering this treatment protocol. This represents a consecutive series of 409 patients treated with high-dose bolus IL-2 as their sole therapy. All protocols were approved by the Institutional Review Board of the National Cancer Institute (NCI), and patients signed an informed consent for participation in these protocols before treatment. Response to treatment and survival were assessed in all patients as of March 1, 1998. Thus, the median potential follow-up from the start of treatment to March 1998 for all patients was 7.1 years.

Patients who had received prior IL-2 or who had evidence of concomitant severe respiratory, cardiovascular, or renal disease were not accepted into these trials. Before entry into the protocol, all patients were evaluated with computed tomographic (CT) or magnetic resonance imaging scans of the brain, chest CT scans or full lung tomograms, abdominal CT scans, and radionuclide bone scans. Patients were not eligible for this protocol if they had evidence of central nervous system metastases. Except for the early patients entered into this protocol, all patients older than 50 years of age underwent either a stress electrocardiogram or a stress radionuclide ejection or thallium scan, and patients with evidence of ischemic heart disease or significant arrhythmias on these studies were excluded.

Treatment

Recombinant IL-2 (kindly supplied for this trial by the Cetus Oncology Division, Chiron Corp., Emeryville, CA) was administered intravenously over 15 minutes at a dose of 720,000 IU/kg. Recombinant IL-2 was reconstituted from a lyophilized powder with 1.2 ml of sterile water per vial. Vials also contained 5% mannitol and approximately 130 mcg of sodium dodecyl sulfate per milligram of IL-2. For infusion, IL-2 was diluted in 50 ml of normal saline containing 5% human serum albumin. Patients received IL-2 every 8 hours until grade III/IV toxicity was reached that could not be easily reversed by standard supportive measures. A single treatment course comprised two cycles containing a maximum of 15 doses of IL-2 per cycle, separated by approximately 10 to 15 days. Fifty-nine of these patients received IL-2 conjugated to polyethylene glycol only during the second cycle after an initial cycle of standard high-dose bolus IL-2. In a randomized trial, there was no difference in response rates between patients receiving this latter treatment *versus* patients who received two cycles of high-dose IL-2 alone; thus, these two groups were joined in this analysis.[6] No patient received any other therapy for the 30 days before or during the evaluation of the administration of IL-2.

Patients with any evidence of stable or responding disease received a second course consisting of two cycles of IL-2. IL-2 was routinely administered on a general surgical ward, although some patients were transferred to an intensive care unit for monitoring or if the administration of vasopressors was necessary. All patients received concomitant medications including acetaminophen (650 mg every 4 hours), indomethacin (50 mg every 8 hours), and ranitidine (150 mg every 12 hours) to prevent side effects often associated with IL-2 administration.

Evaluation of Response

All metastatic tumor deposits were measured on either radiologic studies or physical examination, and the product of maximal perpendicular tumor diameters was calculated. Measurements were taken before treatment, 2 months after treatment, and at regular intervals thereafter. A partial response was defined as a 50% or greater reduction in the sum of the products of the perpendicular diameters of all lesions lasting at least 1 month, with no new lesions or growing

lesions. A complete response was defined as the complete disappearance of all disease without the appearance of any new disease, lasting at least 1 month. Any patient not achieving at least a partial response was considered a nonresponder. Response and survival durations were calculated from the time of the first dose of IL-2.

Statistical Analysis

Data were initially evaluated in a univariate fashion. Comparisons between discrete parameters and complete response were assessed using the chi square test or Fisher's exact test if assumptions required for using the chi square test were not met (*e.g.*, expected cell frequency < 5). Comparisons between complete responders and all other patients for continuous parameters were made using the Wilcoxon rank sum test.

Based on previous evaluations (which identified factors having a possible association with complete or partial response), variables were examined according to whether they were believed to be of primary interest in association with complete response, or merely those to be evaluated in an exploratory fashion. Factors considered to be of primary interest were limited to the following: total IL-2 administered, previous immunotherapy, minimum platelet count during therapy, and maximal lymphocyte count during treatment. Because these considerations restricted the main analyses to only four variables, only an adjustment taking four variables into consideration is needed to adjust for multiple comparisons. The method of Hochberg[7] was selected because it correctly holds the overall significance level to 0.05 but is not as restrictive as a Bonferroni procedure, which would have required that every individual probability value (of four) be 0.05/4 or less (or 0.0125 or less) to indicate significance in view of the number of comparisons performed.

In addition to the main variables of interest, additional variables were examined and considered to have only an exploratory, hypothesis-generating association. They are presented without adjustment: initial sites of disease, performance status, sex, race, prior radiation therapy, prior surgery, prior chemotherapy, prior hormonal therapy, nephrotoxicity, cardiotoxicity, neurotoxicity, infection, age, interval from diagnosis to treatment, creatinine (pretreatment, maximum, and difference between these two), lymphocyte count (pretreatment), platelet count (pretreatment), and percentage weight gain. All probability values are two-sided (p_2).

Factors that resulted in univariate associations that were significant ($p_2 \leq 0.10$) were included in logistic regression analysis models. Logistic regression analysis was performed using PROC LOGISTIC of SAS (SAS Institute, Cary, NC) to identify whether a predictive relation exists between complete response and any of the variables identified to be at least moderately associated with complete response.[8] The probability of remaining in complete remission as a function of time, as well as the probability of dying from disease, was determined by the Kaplan–Meier method.

Cloning of the Genes Encoding Melanoma Antigens Responsible for Cancer Regression

To identify the melanoma antigens that appeared to be responsible for cancer regression, tumor-infiltrating lymphocytes (TIL) were grown from resected melanomas, and TIL capable of selectively recognizing the autologous cancer *in vitro* were identified using techniques previously described.[9,10]. Selected TIL were administered to cancer patients along with IL-2 to identify the TIL populations that were associated with tumor regression *in vivo*.[11] These TIL were then used to identify the genes encoding the tumor antigens recognized by the TIL.[12] cDNA libraries were prepared from melanoma cell lines and used to transfect target lines bearing the appropriate class I restriction element. The transfectants were assayed for recognition by TIL previously shown to be associated with tumor regression *in vivo*. The genes capable of mediating regression by TIL after transfection were then sequenced, and the amino acid sequence of the encoded proteins was identified.[13,14]

RESULTS

Patient Characteristics

Between September 1985 and November 1996, 409 patients (182 with metastatic melanoma and 227 with metastatic renal cancer) received a total of 673 courses of therapy with high-dose bolus IL-2 in the Surgery Branch, NCI (Table 1). Fifty-nine of these patients also received second cycles of IL-2 conjugated to polyethylene glycol and are included in this analysis. These 409 patients represent a consecutive series of patients who had high-dose IL-2 alone during this interval. Patients who received high-dose IL-2 in conjunction with cell therapy such as lymphokine activated killer cells or TIL or with other cytokines were not included in this study.

Most patients ranged between the ages of 30 and 60. All but one patient had an Eastern Cooperative Oncology Group performance status of 2 or less. All but 12 of the 409 patients had undergone previous therapy. Sixty-three had received chemotherapy and 101 had received prior immunotherapy that did not include IL-2. Thirty-eight percent of the patients had two or more treatments for their cancer, and 13% had three or more different treatments.

Response to Therapy

The response rates and response durations are presented in Tables 2 and 3, respectively. Of 182 melanoma patients, 12 experienced a complete regression and 15 a partial regression for an overall response rate of 14.8%. Of 227

Table 1. CHARACTERISTICS OF PATIENTS TREATED WITH HIGH-DOSE BOLUS IL-2

	Melanoma (%)	Renal Cancer (%)	
	(Number of Patients)		Total (%)
Total	182 (100)	227 (100)	409 (100)
Sex			
Male	123 (68)	156 (69)	279 (68)
Female	59 (32)	71 (31)	130 (32)
Age (years)			
11–20	3 (2)	2 (1)	5 (1)
21–30	28 (15)	10 (4)	38 (9)
31–40	60 (33)	28 (12)	88 (22)
41–50	46 (25)	77 (34)	123 (30)
51–60	31 (17)	83 (37)	114 (28)
61–70	14 (8)	27 (12)	41 (10)
Performance			
0	147 (81)	169 (74)	316 (77)
1	29 (16)	47 (21)	76 (19)
2	6 (3)	10 (4)	16 (4)
3	– –	1 (0)	1 (0)
Prior treatment			
None	2 (1)	10 (4)	12 (3)
Surgery	177 (97)	215 (95)	392 (96)
Chemotherapy	48 (26)	15 (7)	63 (15)
Radiotherapy	29 (16)	20 (9)	49 (12)
Hormonal	1 (1)	7 (3)	8 (2)
Immunotherapy	66 (36)	35 (16)	101 (25)
Any 2 or more	97 (53)	60 (26)	157 (38)
Any 3 or more	37 (20)	16 (7)	53 (13)

patients with renal cell cancer, 21 experienced a complete regression and 22 a partial regression for an overall response rate of 19%. Thus, of the 409 total patients, 33 experienced a complete and 37 a partial response for an overall response rate of 17.1%.

The response durations are shown in Table 3, with a median potential follow-up of 7.1 years. All patients who experienced a partial regression ultimately progressed; the median duration of partial response for patients with melanoma or renal cancer was 7 and 11 months, respectively. In contrast, of 33 patients who experienced a complete response, 27 have continuous ongoing complete responses at 39 to 148 months. Of the 12 completely responding patients with melanoma, 2 had recurrent disease at 16 and 12 months, with the remaining patients in ongoing complete response at 70 to 148 months. Of the 21 completely responding patients with metastatic renal cancer, 4 relapsed at 19, 19, 23, and 35 months, with the remaining 17 patients in ongoing complete response at 39 to 134 months. The actuarial curves reflecting the duration of response and survival of patients who experienced a complete response to treatment are shown in Figure 1. No patient who attained a complete response suffered recurrent disease beyond 3 years. Two patients with renal cell cancer died with no evidence of renal cancer in the midst of an ongoing complete regression and were censored at the time of death in Figure 1. One patient died at 87 months of a myocardial infarction, and a second patient died at 46 months of an acute myelocytic leukemia. There were 5 complete responders in the 59 patients who received second cycles of polyethylene glycol-conjugated IL-2, for a complete response rate similar to that seen in patients who received IL-2 in all treatment cycles.

The exact site and sizes of lesions for all completely responding patients with metastatic renal cancer or metastatic melanoma are shown in Tables 4 and 5, respectively. Complete regression of tumor in the lung, liver, bone, adrenal gland, lymph nodes, and subcutaneous sites was seen. Only one patient with melanoma and one patient with renal cell cancer had a single metastatic lesion; all other patients had multiple metastases.

The toxicities of treatment seen in these patients were similar to those seen with the use of high-dose IL-2 in many previous publications from our group. There were four treatment-related deaths; however, these deaths all occurred in our early experience with IL-2 (among the first 69 patients). There were no treatment-related deaths in the last 340 patients treated with high-dose IL-2 in this study.

Prognostic Factors Associated With Complete Response

Analyses of four principal parameters, as well as a large number of exploratory patient demographic, pretreatment, and treatment-related factors, were performed to attempt to identify factors that might predict or be associated with complete response among patients treated with high-dose IL-2 alone. Of the four principal factors analyzed, three showed a statistically significant association with complete

Table 2. RESPONSE OF PATIENTS TREATED WITH HIGH-DOSE BOLUS IL-2

Diagnosis	Total	Complete Response	Partial Response	Total
		(Number of Patients [%])		
Melanoma	182	12 (6.6)	15 (8.2)	27 (14.8)
Renal cancer	227	21 (9.3)	22 (9.7)	43 (19.0)
Total	409	33 (8.1)	37 (9.0)	70 (17.1)

Table 3. DURATION OF RESPONSE IN PATIENTS TREATED USING HIGH-DOSE BOLUS IL-2

Diagnosis	Complete Response (Months)*	Partial Response
Melanoma	148+, 96+, 95+, 93+, 91+, 84+, 80+, 71+, 71+, 70+, 16, 12	35, 31, 19, 10, 10, 8, 8, 7, 7, 5, 5, 5, 4, 4, 2
Renal cell cancer	134+, 126+, 123+, 94+, 94+, 90+, 87+, 86+, 86+, 79+, 70+, 64+, 63+, 60+, 49+, 46+, 39+, 35, 23, 19, 19	52, 30, 30, 22, 20, 17, 16, 15, 14, 14, 13, 11, 9, 8, 8, 7, 7, 6, 4, 4, 4, 4

* "+" indicates ongoing response, as of March 1, 1998.
Of 33 patients with complete response, 27 remain in complete response at 39 to 148 months.

response when appropriately corrected for the number of factors analyzed (Table 6). Although 25% (101 of 409) of all patients had received prior immunotherapy, only 2% of the patients who received prior immunotherapy had complete responses ($p_2 < 0.05$), strongly suggesting that patients who received prior immunotherapy were less likely to respond than patients who had not been so treated. This was the only pretreatment factor among the many analyzed that had any clear association with complete response in these patients (see Table 6).

Two principal treatment factors, however, also were associated with the tendency to achieve a complete response. The total amount of IL-2 administered in the first treatment course was significantly higher in patients achieving a complete response ($p_2 < 0.05$). After the cessation of high-dose IL-2, most patients developed a rebound lymphocytosis approximately 2 to 5 days later. This maximal lymphocyte count was also significantly higher in patients experiencing a complete response than in all other patients ($p_2 < 0.05$). Minimal platelet count was not associated with complete response ($p_2 = 0.67$). None of the other treatment-related factors listed in Table 6 had a significant association with complete response in these 409 patients.

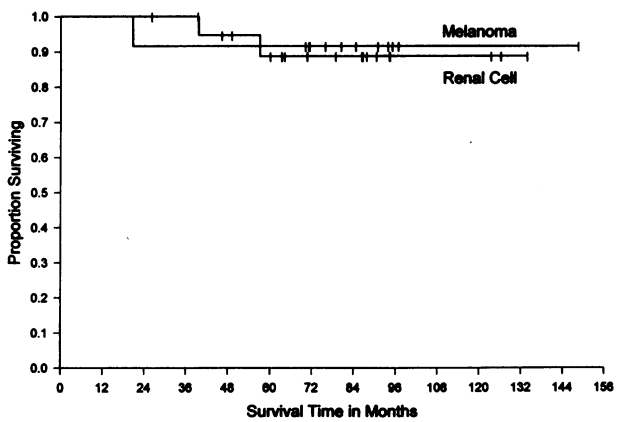

Figure 1. Complete response to treatment with high dose IL-2. Actuarial curve of the probability of dying from disease in patients with metastatic melanoma (n = 12) or renal cancer (n = 21) who underwent a complete response.

Logistic regression analyses failed to identify any important set of variables that could reliably predict complete response. This is because of both the relatively small fraction of total patients who were complete responders (8.1%) and because of the limited number of parameters identified in the overall evaluation that were potentially associated with complete response.

Identification of the Genes Encoding Cancer Regression Antigens

Using expression cloning techniques, a series of genes have been identified that encoded antigens recognized by TIL that had previously been shown to be associated with tumor regression *in vivo*. Many of these genes have been previously described.[13–23] A summary of the melanoma antigens recognized by TIL is shown in Table 7. Genes have been identified that are restricted by HLA-A2, A24, and A31. Most of these genes represent normal nonmutated melanoma/melanocyte differentiation antigens, indicating that these melanoma patients have broken tolerance to these cell proteins. Two of the genes are encoded by alternative open reading frames of normal genes, and one of the antigens (beta-catenin) represents a single base mutation of a gene encoding a protein involved in cell adhesion.

DISCUSSION

Patients with metastatic melanoma or renal cell cancer have a poor prognosis, and no curative systemic treatment for these patients has been available. Chemotherapy, often a combination of agents, or alpha-interferon has been reported to induce objective cancer responses in a small percentage of patients.[24,25] These responses have often not been durable, and most patients suffer recurrent disease at short intervals. The administration of recombinant IL-2 showed significant antitumor activity in animal models, and these studies led to an evaluation of IL-2 administration as a therapy for human cancer.[1,2] Although initially evaluated for the treatment of patients with a wide variety of malignancies,[3] significant early responses were seen in patients

Table 4. PATIENTS WITH RENAL CELL CANCER: CHARACTERISTICS OF COMPLETE RESPONDERS

Patient Age/Sex	Site of Tumor	Size of Tumor, Cm × cm	Duration of Response, mo*	Current Status†
53/M	Lung	1.9 × 1.6 2.9 × 2.9 1.0 × 1.0 1.9 × 1.0 and other sizes	134+	Alive, NED
39/M	Lung (hilum) Lung	2.6 × 2.6 1.7 × 1.7 1.5 × 1.5 1.3 × 1.3 and other sizes	87+	Dead, NED (leukemia)
57/M	Lung (hilum) Lung Lymph node	6.1 × 5.6 4.7 × 5.4 2.7 × 1.9 1.0 × 1.0 and other sizes 1.0 × 1.0 1.0 × 1.0	123+	Alive, NED
44/M	Lung Intraperitoneal Lymph node 	1.3 × 0.3 5.2 × 5.2 2.6 × 3.0 2.6 × 1.3 4.0 × 5.0	46+	Dead, NED (myocardial infarction)
48/M	Liver	6.1 × 4.0	94+	Alive, NED
62/M	Lung	1.4 × 1.4 1.1 × 1.1 1.0 × 1.2 1.0 × 1.0 and other sizes	35	Dead with disease
61/M	Lung	1.7 × 2.0 2.9 × 5.0 and other sizes	75	Alive, NED
57/M	Lung Lymph node	0.9 × 0.9 1.9 × 2.0 0.0 × 1.0 1.2 × 1.3 6.0 × 5.2 7.1 × 5.6	86+	Alive, NED
48/M	Lung	5.1 × 6.0 2.5 × 3.4 2.2 × 2.8 1.8 × 1.6 and other sizes	19	Dead with disease
46/M	Lung	2.4 × 2.4 0.7 × 0.7 3.2 × 4.0 2.0 × 2.4	19	Alive with disease
51/F	Lung	1.0 × 1.1 0.7 × 1.2 1.6 × 1.8	64+	Alive, NED
++58/F	Lung Lymph node	1.7 × 1.6 6.4 × 6.1 1.4 × 1.5 2.0 × 2.0 1.0 × 1.5	126+	Alive, NED
41/M	Lung	1.3 × 2.0 1.3 × 1.7 1.0 × 1.3 1.0 × 1.2 and other sizes	94+	Alive, NED
++45/M	Lung Lung (hilum) Lymph node	0.8 × 1.0 2.3 × 2.3 1.6 × 1.8	90+	Alive, NED

(*continues*)

Table 4. (*continued*)

Patient Age/Sex	Site of Tumor	Size of Tumor, Cm × cm	Duration of Response, mo*	Current Status†
67/M	Lung	0.6 × 0.7	79+	Alive, NED
	Liver	7.4 × 7.6		
		5.1 × 5.3		
		3.0 × 3.2		
		3.0 × 3.1		
		2.3 × 2.7		
45/M	Lung	2.1 × 2.4	70+	Alive, NED
		1.2 × 1.7		
		1.3 × 1.3		
		0.7 × 0.8 and other sizes		
41/M	Lung	6.3 × 5.8	63+	Alive, NED
		2.6 × 4.2		
		2.6 × 3.5		
		1.9 × 2.5 and other sizes		
42/M	Lung	0.9 × 1.1	60+	Alive, NED
		2.7 × 2.9		
		0.6 × 0.8		
54/M	Liver	13.5 × 15.4	49+	Alive, NED
	Lymph node	3.1 × 5.8		
52/M	Lung	3.3 × 5.2	39+	Alive, NED
		2.2 × 2.6		
		1.5 × 1.7		
		1.6 × 1.9		
50/M	Lung	1.0 × 1.1		
		1.0 × 1.1		
		1.0 × 1.0		
	Lung (hilum)	2.2 × 3.0 and other sizes	23	Alive with disease

*+ Indicates a patient in continuing complete remission.
† NED indicates no evidence of disease.
++ Received IL-2 plus PEG/IL-2.
NED = no evidence of disease.

with metastatic melanoma and metastatic renal cancer, and the great majority of patients treated in subsequent experimental protocols have one of these diagnoses. In most reported series, objective response rates of 15% to 25% were seen, with 5% to 10% of patients exhibiting complete responses. Based on this early information, the FDA licensed IL-2 in May 1992 for use in the treatment of patients with metastatic renal cancer.

IL-2 is a cytokine produced by human T lymphocytes and plays a central role in the regulation of immune reactions. IL-2 has no direct impact on cancer cells; thus, all the antitumor effects of IL-2 administration result from its ability to modulate immune reactions. The exact mechanism of antitumor action of IL-2 is not known, although it is thought that IL-2 stimulates the activation and expansion of immune T cells with T-cell receptors capable of recognizing putative tumor antigens on the surface of melanomas or renal cancers.[1] Recent studies have begun to identify the molecular nature of these tumor antigens. Thus, the mechanism of action of IL-2 administration appears to be different from conventional treatments such as surgery, radiation therapy, and chemotherapy, which directly affect cancer cells. The durability of responses using these latter conventional treatments in patients with metastatic melanoma and renal cancer is quite limited; thus, the durability of complete responses in patients with these diseases treated with IL-2 was of concern.

Many approaches to the administration of IL-2 to patients with metastatic cancer have been explored, including the use of IL-2 alone at a wide variety of doses, given intravenously or subcutaneously, given in conjunction with antitumor cells such as lymphokine activated killer cells or TIL or in conjunction with other cytokines such as alpha-interferon, IL-4, tumor necrosis factor, or others.[1,2] None of these combinations of IL-2 with other agents has been conclusively shown to be more effective than treatment with IL-2 alone. Although antitumor responses have been seen with IL-2 administered at a variety of doses, the only schedule of IL-2 approved by the FDA includes dosages of 600,000 to 720,000 IU/kg administered by intravenous bolus infusion every 8 hours to patient tolerance.

The major question addressed in this paper deals with the durability of complete responses seen in patients with metastatic renal cancer and melanoma treated with high-dose bolus IL-2. The first patient in this series was treated on September 19, 1985, and the median potential duration of

Table 5. PATIENTS WITH MELANOMA: CHARACTERISTICS OF COMPLETE RESPONDERS

Patient Age, y/Sex	Site of Tumor	Size of Tumor, Cm × cm	Duration of Response, mo*	Current Status†
37/F	Lung	2.1 × 1.7	148+	Alive, NED
		0.8 × 1.0		
		0.8 × 0.8		
		0.8 × 0.8 and other sizes		
	Lymph node	5.5 × 7.1		
	Subcutaneous	3.8 × 4.5		
44/F	Subcutaneous	Multiple (<1 cm)	96+	Alive, NED
32/M	Lymph node	1.5 × 2.1	91+	Alive, NED
	Subcutaneous	2.5 × 3.0		
		3.0 × 3.5		
		6.0 × 7.0		
29/M	Lung (hilum)	1.8 × 2.5	84+	Alive, NED
49/M	Lung	0.4 × 0.4	80+	Alive, NED
	Lymph node	1.8 × 2.1		
62/M	Lung	2.8 × 2.9	16	Alive with disease
		2.5 × 2.0		
		1.7 × 1.8		
		1.0 × 1.2 and other sizes		
	Adrenal	1.2 × 1.5		
	Subcutaneous	1.8 × 1.8		
		1.0 × 1.2		
		1.0 × 1.0		
		0.8 × 0.8 and other sizes		
36/M	Lung	1.9 × 1.7	71+	Alive, NED
		3.0 × 2.8		
		2.0 × 1.8		
		1.7 × 1.8 and other sizes		
	Subcutaneous	1.0 × 1.0		
		1.0 × 0.5		
		2.5 × 1.5		
		0.3 × 0.3		
61/F	Lung	2.1 × 1.9	71+	Alive, NED
		1.5 × 1.5		
		1.3 × 1.3 and other sizes		
	Subcutaneous	1.0 × 1.0		
		1.0 × 1.5		
35/F	Lung	2.4 × 2.4	12	Alive with disease
		1.0 × 1.0 and other sizes		
62/F	Subcutaneous	1.5 × 2.5	70+	Alive, NED
		1.5 × 1.4		
		1.5 × 1.8		
		2.5 × 2.5 and other sizes		
	Bone			
++62/F	Lung	0.6 × 0.7	93+	Alive, NED
		0.7 × 0.7		
	Lung (hilum)	2.0 × 3.0		
	Liver	3.3 × 3.0		
		1.5 × 2.2		
		1.5 × 2.1		
++64/M	Cutaneous	2.0 × 1.6	95+	Alive, NED
		1.0 × 0.8		
		0.5 × 0.5		
		2.2 × 1.3		

*+ Indicates a patient in continuing complete remission.
† Ned indicates no evidence of disease.
++ Received IL-2 plus PEG/IL-2.
NED = no evidence of disease.

Table 6A. PARAMETERS SIGNIFICANTLY ASSOCIATED WITH COMPLETE RESPONSE TO TREATMENT WITH HIGH-DOSE IL-2

	Complete Responders (n = 33)	Noncomplete Responders (n = 376)	P_2*
Prior immunotherapy (number):			
Yes	2	99	0.009
No	31	276	
Total IL-2 in first course (IU/kg):			
Mean (± SEM)	11,171 ± 624	9710 ± 183	0.024
Median	11,520	9360	
Maximum lymphocytes (per mm^3):			
Mean (± SEM)	8048 ± 900	6514 ± 668	0.017
Median	5970	5343	

* Applying the Hochberg correction, these p values are significant at the 0.05 level.

follow-up for all patients in this series was 7.1 years. This series, the largest single-institution experience with this agent, thus provides the opportunity to evaluate, with prolonged follow-up, a consistently treated series of patients with these two diagnoses.

Of 182 melanoma patients, 12 (6.6%) underwent a complete response, as did 21 of 227 (9.3%) patients with renal cell cancer. Of these 33 patients undergoing a complete response, only 6 have had recurrent disease. The remaining 27 patients (82%) have continuous ongoing complete regressions at more than 39 to more than 148 months from the onset of treatment; 25 of these patients have ongoing complete responses for more than 4 years and 15 for more than 7 years. As shown in Tables 4 and 5, most patients had multiple metastatic lesions, and tumor regressions were seen at sites throughout the body, including the lung, liver, lymph nodes, bone, and subcutaneous and cutaneous tissues. It thus appears that the great majority of patients with metastatic renal cell cancer or melanoma who undergo a complete response will experience a durable and perhaps curative tumor regression. Thirty-seven of the 409 patients in this series experienced an objective partial response; in 14 of these patients, the response lasted more than 1 year and in 5 patients more than 2 years, but all partial responders ultimately suffered recurrent disease.

Attempts were made to identify the characteristics of the subset of patients undergoing a complete response. An extensive analysis was performed of prognostic factors in patients treated with high-dose IL-2, comparing complete responders to all other patients. This revealed three parameters that correlated with complete response. Patients who had received prior immunotherapy with any modality other than IL-2 had a lower chance of experiencing a complete response (2 of 101; 2%) than did patients who had not previously received immunotherapy (31 of 307; 10.1%). It thus appeared that patients who underwent failed immune manipulations before IL-2 administration had an altered ability to respond effectively to treatment with high-dose bolus IL-2. The total amount of IL-2 administered also was significantly higher in complete responders. This corroborated animal data indicating a direct relation between the amount of IL-2 administered and antitumor responses. IL-2 causes a transient decrease of lymphocytes in the peripheral circulation, which then rebounds after IL-2 administration is stopped. The magnitude of this lymphocyte rebound was the third criterion studied that directly correlated with a tendency toward complete response. Thus, except for prior treatment with immunotherapy, there were no pretreatment factors among the many listed in Table 6 that could predict which patients were likely to experience a complete response when treated with high-dose bolus IL-2.

The incidence and durability of complete responses in patients treated with high-dose bolus IL-2 was corroborated by an analysis of data accumulated by the Chiron Corp. of 310 patients with metastatic renal cancer or melanoma treated at 21 different institutions with a regimen almost identical to that used in the Surgery Branch, NCI.[26–28] The only significant difference between the management of patients in these institutions and at the NCI was the use of 600,000 IU/kg of IL-2 instead of the 720,000 IU/kg used in Surgery Branch studies. In these non-Surgery Branch patients, 17 of 310 (5.5%) achieved a complete response, and 10 of these 17 patients have continuous ongoing complete responses at 67 to 114 months. This provides a response rate and durability comparable to those seen in Surgery Branch studies. Thus, combining Surgery Branch and non-Surgery Branch studies, 50 of 719 patients (7%) have experienced a complete response, and 37 of 50 (74%) of these complete responders have continuous ongoing responses at more than

Table 6B. PARAMETERS THAT SHOWED NO SIGNIFICANT ASSOCIATION WITH COMPLETE RESPONSE TO TREATMENT WITH HIGH-DOSE IL-2

Pretreatment Factors	Treatment Factors
Sex	Maximum creatinine
Race	Maximum bilirubin
Age	Maximum white blood count
Performance status	Minimum platelets
Prior surgery	Wight gain
Prior radiotherapy	Renal toxicity
Prior chemotherapy	Cardiovascular toxicity
Prior hormonal therapy	Neurologic toxicity
Sites of disease	Infectious toxicity
Time from diagnosis to treatment	
Pretreatment bilirubin	
Pretreatment creatinine	
Pretreatment white blood count	
Pretreatment lymphocyte count	
Pretreatment platelet count	

Table 7. MELANOMA ANTIGENS RECOGNIZED BY TIL

Name of Antigen	Alternate Name(s)	TILs Used for Identification	HLA Restriction	Immunodominant Epitopes	Characteristics
MART-1	Melan A	1235 & others	A2	AAGIGILTV	Normal differentiation antigen
gp100	HMB-45	1200 & others	A2	KTWGQYWQV	Normal differentiation antigen
	HMB-50			ITDQVPFSV	
	NK1/betab			YLEPGPVTA	
		888	A24	VYFFLPDHL	Intronic sequence
Tyrosinase	–	1138 & 1374	A2	YMDGTMSQV	Normal differentiation antigen
		888 & 1413	A24	AFLPWHRLF	Normal differentiation antigen
p15	–	1290	A24	AYGLDFYIL	Post-transcriptional control
TRP-1	gp75	586	A31	MSLQRQFLR	Translated from alternate open reading frame
TRP-2		586	A31	LLGPGRPYR	Normal differentiation antigen
ESO-1		586	A31	ASGPGGGAPR	Translated from alternate open reading frame
β-catenin		1290	A24	SYLDSGIHF	Single base mutation

2 years. No patient with a complete response in either series has recurred after 3 years.

The toxicity of the administration of high-dose bolus IL-2 has been well documented.[29] Largely because IL-2 causes a vascular leak syndrome, transient dysfunctions in multiple organ systems can be seen and have been well documented. As experience with the administration of high-dose bolus IL-2 has accumulated, the safety of administration has significantly increased. Although four treatment-related deaths were seen in this series, they occurred in the first 69 patients treated; we have experienced no treatment-related deaths in the last 340 patients treated. Indeed, in considering the entire Surgery Branch experience using high-dose IL-2, either alone or in conjunction with other cytokines or immune cells, a recent analysis of 1241 patients revealed an overall treatment mortality of 0.7%, with no treatment-related deaths in the last 680 consecutive patients treated with high-dose bolus IL-2. Thus, high-dose bolus IL-2 can be safely administered, and based on the results of this long-term study, this therapy offers patients with metastatic melanoma and renal cell cancer a chance at a durable, if not curative, complete regression of their malignancy.

In an attempt to explain the cellular and molecular basis of tumor regressions in these patients, studies were undertaken to identify the genes encoding melanoma antigens responsible for tumor regression. A series of candidate antigens have been identified. Many of these antigens are melanocyte/melanoma differentiation antigens, although a mutated antigen has also been described. Identification of the genes encoding these melanoma regression antigens has opened new possibilities for the development of immunotherapies for patients with melanoma. A series of clinical protocols have been initiated in which patients are being immunized with recombinant adenovirus, vaccinia virus, or fowlpox virus encoding the MART-1 or gp100 melanoma antigens, administered along with IL-2. In addition, clinical trials using immunization with the immunodominant peptides from these antigens administered in incomplete Freund's adjuvant have also been initiated. Immunologic and clinical studies resulting from these clinical trials may provide insight into the immunologic mechanisms involved in tumor destruction and allow for the rational design of improvements in these immunotherapies.

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Discussion

DR. FRANCIS D. MOORE, SR. (Boston, Massachusetts): I would like to ask two questions and make one suggestion. The first question is one of histology. Dr. Rosenberg, do you have any needle biopsies or information on your patients while they are under treatment to demonstrate increased infiltration with lymphocytes or any change in the staining of those lymphocytes or in the activity of those lymphocytes to indicate that it is truly lymphocytic activation which is producing these responses? That seems like a simple, naive question, but I would like to know if you can confirm histologically that the response to IL-2 stimultes lymphocyte attack on the neoplasms.

The other question has to do with the illness produced by giving IL-2. You naturally didn't have time to go into that in detail. Several of your patients were in the intensive care ward while they were under treatment. I would like to know the nature of the response to IL-2. How sick does it make patients? And in what way does it make them sick?

Now, my suggestion is based on the fact that in the second half of this century, two new approaches to malignancy have been very encouraging. And they have both been pursued from their initial discovery to their present-day use by Fellows of this Association. One is the immunologic approach pursued by Dr. Rosenberg and several other of our colleagues. The other is based on neovascularization in tumors, an approach first described by Dr. Folkman, who is here this morning. I would just express the hope and the suggestion that Rosenberg and Folkman would study the two modalities together. A tumor which has been compromised by immunotherapy might be especially vulnerable to treatment directed toward its neovascularization. Such might be done with endostatin or somatostatin. Vice versa, a tumor that has had its vascular supply compromised might be very sensitive to immunological therapy. I hope they will get together and work out a combined approach. Thank you for a wonderful paper.

DR. STEVEN A. ROSENBERG (Bethesda, Maryland): Thank you. When I came to the Peter Bent Brigham Hospital as an intern in 1963, Dr. Moore was the chief there. He was then and remains now the smartest person I know. As surgeons, we all stand taller because of his accomplishments. Thank you, Dr. Moore, for all you have done for me.

In answer to the questions: We have had the opportunity to biopsy lesions sequentially during the course of treatment in patients that have multiple subcutaneous or cutaneous nodules and in those patients we can obtain snapshots of what is occurring as the tumor disappears. We see increasing infiltration of lymphocytes into the lesions as they regress. Recently we have developed techniques for growing lymphocytes and tumor from fine needle aspirates so we can study the same lesions sequentially during the course of treatment.

Interleukin-2 administration is associated with toxicity. The earliest physiologic change we see is a decrease in systemic vascular resistance which can be associated with hypotension and tachycardia. We have learned to effectively control these side effects. In the first 157 patients tested we had four treatment-related deaths but, recently we have treated over 800 consecutive patients with no treatment related mortality. I believe treatment with high-dose IL-2 can be administered safely by those with experience, certainly as safely as most combination chemotherapies in common use.