Multicenter, Randomized, Phase III Trial of CD8+ Tumor-Infiltrating Lymphocytes in Combination With Recombinant Interleukin-2 in Metastatic Renal Cell Carcinoma

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Purpose: **To prospectively evaluate in a multicenter randomized trial the antitumor activity of $CD8^+$ tumor-infiltrating lymphocytes (TILs) in combination with low-dose recombinant interleukin-2 (rIL-2), compared with rIL-2 alone, after radical nephrectomy in metastatic renal cell carcinoma patients.**

Patients and Methods: **Between December 1994 and March 1997, 178 patients with resectable primary tumors were enrolled at 29 centers in the United States and Europe. Patients underwent total nephrectomy, recovered, and were randomized to receive either $CD8^+$ TILs (5×10^7 to 3×10^{10} cells intravenously, day 1) plus rIL-2 (one to four cycles: 5×10^6 IU/m² by continuous infusion daily for 4 days per week for 4 weeks) (TIL/rIL-2 group) or placebo cell infusion plus rIL-2 (identical regimen) (rIL-2 control group). Primary tumor specimens were cultured at a central cell-processing center in serum-free medium containing rIL-2 to generate TILs.**

Results: **Of 178 enrolled patients, 160 were randomized (TIL/rIL-2 group, n = 81; rIL-2 control group, n = 79). Twenty randomized patients received no treatment after nephrectomy because of surgical complications (four patients), operative mortality (two patients), or ineligibility for rIL-2 therapy (14 patients). Among 72 patients eligible for TIL/rIL-2 therapy, 33 (41%) received no TIL therapy because of an insufficient number of viable cells. Intent-to-treat analysis demonstrated objective response rates of 9.9% *v* 11.4% and 1-year survival rates of 55% *v* 47% in the TIL/rIL-2 and rIL-2 control groups, respectively. The study was terminated early for lack of efficacy as determined by the Data Safety Monitoring Board.**

Conclusion: **Treatment with $CD8^+$ TILs did not improve response rate or survival in patients treated with low-dose rIL-2 after nephrectomy.**

J Clin Oncol 17:2521-2529. © 1999 by American Society of Clinical Oncology.

APPROXIMATELY 30% OF renal cell carcinoma (RCC) patients present with metastatic disease, and 20% to 30% of patients who present with clinically localized disease will develop metastatic disease after radical nephrectomy, yielding a 10-year disease-free survival rate of approximately 50%.[1-5] Metastatic renal cell carcinoma (MRCC) is associated with a poor prognosis because it is highly resistant to chemotherapy, hormonal therapy, and radiation therapy. The 5-year survival rate varies from 0% to 20%, depending on cell type and the extent of disease at the time of nephrectomy,[4,6] but it is generally less than 2%.[7] Clinical studies have demonstrated that high-dose intravenous (IV) bolus recombinant interleukin-2 (rIL-2) (ie, 600,000 to 720,000 IU/kg every 8 hours) can induce durable complete remissions (CRs) in patients with bulky disease and multiple visceral metastases, with objective response rates ranging from 13% to 20%. In patients treated with high-dose rIL-2, 1-year survival rates of approximately 55% and 5-year survival rates of 10% to 20% have been reported.[8-11] Comparable response rates and survival have also been observed with regimens of high-dose continuous IV infusion (CIV).[12,13]

However, a limitation to the administration of high-dose IV rIL-2 is the occurrence of acute toxicity, including hypotension, oliguria, pulmonary edema, and dyspnea, related to capillary leak syndrome. The morbidity associated with high-dose IV rIL-2 regimens has led to the investigation of alternative dosage regimens to determine whether durable CRs can be achieved without significant toxicity. Administration of low-dose rIL-2 by IV bolus, CIV, or subcutaneous (SC) injection either alone or in conjunction with recombinant interferon alfa and/or fluorouracil may have activity in the treatment of MRCC, and these regimens are generally better tolerated.[14-18] Moreover, low-dose CIV rIL-2 can produce selective expansion of natural killer cells in vivo with minimal toxicity.[19] This has been described as the most physiologic immunotherapeutic strategy to activate the anticancer immune response.[20] However, further follow-up is required to determine whether CRs associated with low-dose rIL-2 regimens will be as durable as those achieved with high-dose rIL-2 regimens.[17,18,21-23]

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Submitted November 18, 1998; accepted March 19, 1999.

Supported by Rhône-Poulenc Rorer, Collegeville, PA.

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0732-183X/99/1708-2521

One strategy to potentially enhance the efficacy of rIL-2 therapy is to combine rIL-2 with adoptive immunotherapy, using lymphokine-activated killer cells or tumor-infiltrating lymphocytes (TILs).[12,24,25] TILs are found in high numbers in RCC tumors and can be expanded ex vivo in the presence of rIL-2, yielding predominantly T lymphocytes.[26,27] Murine models and clinical studies have suggested that TILs plus rIL-2 may act synergistically to activate the cellular immune response and mediate tumor regression.[28-30] In a phase I/II trial at the University of California, Los Angeles, immunotherapy with TILs plus low-dose rIL-2 has produced significant clinical activity in MRCC, with objective response rates of 33% to 35% and 1-year survival rates of 65% to 73%.[31,32] In a pilot study involving 55 patients treated with nephrectomy followed by TILs plus low-dose CIV rIL-2 (2×10^6 IU/m^2/d), 19 patients (34.6%) responded and five (9%) achieved a CR.[32] Moreover, among 23 patients who received CD8$^+$ TILs, the overall response rate was 43.5%. Overall, the median response duration was 14 months, and the actuarial survival rate was 65% at 1 year and 43% at 2 years after radical nephrectomy.

On the basis of this encouraging single-institution study, a randomized, multicenter study was conducted to prospectively compare CD8$^+$ TILs plus low-dose rIL-2 (TIL/rIL-2 group) versus low-dose rIL-2 alone (rIL-2 control group). All patients underwent radical nephrectomy, from which tissue was obtained for generating CD8$^+$ TILs. The rationale for selecting CD8$^+$ TILs was based on the promising results of the pilot study and on previous in vitro characterization of TILs, which suggest that the CD8$^+$ subset has the greatest cytotoxic potential against autologous or allogeneic tumor cells.[26] The goals of the current study were to investigate the safety, efficacy, and feasibility of CD8$^+$ TIL therapy in conjunction with low-dose CIV rIL-2 in a multi-institutional setting.

PATIENTS AND METHODS

Patient Eligibility

Eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, histologic or radiologic documentation of RCC with the primary tumor suitable for resection, bidimensionally measurable metastatic disease, age $\geq$ 18 years, willingness and ability to undergo surgery, willingness and agreement to use contraception, and informed consent. Exclusion criteria were prior rIL-2 therapy, immunotherapy, immunosuppressive therapy, radiotherapy, or chemotherapy within 4 weeks of screening; significant renal dysfunction (ie, serum creatinine level $\geq$ 2.0 mg/dL), significant hepatic dysfunction (ie, serum total bilirubin level $>$ 1.6 mg/dL, ALT $>$ four times normal, and partial thromboplastin time $>$ 1.5 control); inadequate blood counts (ie, hemoglobin count $<$ 8 g/dL, granulocyte count $\leq$ 1,500 cells/mm^3, platelet count $<$ 100,000/mm^3); significant cardiovascular disease (ie, heart failure, ischemia, edema, arrhythmia, myocardial infarction, or hypertension); CNS disease; pleural effusions or ascites; active infection; active peptic ulcer disease; antibodies to human immunodeficiency virus, hepatitis B surface antigen, or hepatitis C; only bone or abdominal metastases; prior history of malignancy within the last 5 years other than basal cell carcinoma or cervical carcinoma-in-situ; serum calcium level greater than 12 mg/dL or symptomatic hypercalcemia; use of corticosteroids or calcium channel and beta adrenergic blockers; women who were pregnant and/or nursing; solitary kidney; significant intercurrent illnesses; and New York Heart Association class III or IV.

Study Design

Between December 1994 and March 1997, MRCC patients were enrolled onto this phase III, double-blind, randomized study at 29 centers (19 university hospitals and three community hospitals in the United States [U.S.] and seven sites in Europe). The study was conducted in compliance with both U.S. Food and Drug Administration laws and European Good Clinical Practice Guidelines and approved by institutional review boards for U.S. sites and by ethics committees for European sites.

After radical nephrectomy and procurement of $\geq$ 10 g of viable tumor tissue, and with pathologic confirmation of MRCC, patients were randomized to treatment with either CD8$^+$ TILs plus rIL-2 (TIL/rIL-2 group) or control infusion plus rIL-2 (rIL-2 control group). Depending on the rate of expansion of the TIL cell cultures, treatment in the TIL/rIL-2 group was generally initiated 4 to 7 weeks after nephrectomy. Treatment in the rIL-2 control group was initiated approximately 5 weeks after surgery. Recombinant human IL-2 (Proleukin; Chiron Therapeutics, Emeryville, CA) was administered to all eligible patients on consecutive days 1 to 4 of each treatment week via CIV using a pump dispensed per institutional policy at a daily dose of 5×10^6 IU/m^2. One treatment cycle consisted of 4 weeks of treatment followed by 2 weeks of rest. After at least 2 hours of rIL-2 therapy on the first day of the first cycle, patients in the TIL/rIL-2 group received a single IV infusion of 5×10^7 to 3×10^{10} CD8$^+$ TILs, and patients in the rIL-2 control group received a placebo (5% human serum albumin) infusion. If fewer than 5×10^7 cells were available, all harvested cells were infused. Patients were hospitalized only during the initial week of the first cycle of therapy to permit close monitoring of adverse events. Thereafter, the pump was initiated by the nursing staff in the outpatient setting and the patient was instructed on how to disconnect it.

Restaging of measurable disease by computed tomography (CT) scan was performed every 6 weeks. According to tolerance and response to rIL-2 therapy, patients continued treatment to either CR, disease progression, dose-limiting toxicity, or a maximum of four cycles (24 weeks) of therapy. Treatment was withheld in patients with grade 3 toxicity (excluding granulocytopenia) or grade 2 neurocortical and/or cardiac toxicity according to National Cancer Institute Toxicity Criteria. Upon reversal to $\leq$ grade 1 toxicity, treatment with rIL-2 was resumed at a reduced dose (80% of the dose at occurrence of toxicity). Further reduction to 60% was then allowed as necessary at the investigator's discretion. Patients who experienced grade 4 nonhematologic toxicity or grade 3 neurotoxicity or cardiotoxicity were withdrawn from the study and entered follow-up evaluation.

Patients received 650 mg of oral acetaminophen for body temperature $\geq$ 38.5°C, oral diphenhydramine for rash and pruritus, meperidine (25 to 50 mg oral or IV) or morphine sulfate (4 to 6 mg SC or IV) for chills, 10 mg of oral prochlorperazine for nausea, and diphenoxylate and atropine for diarrhea. Patients were premedicated 30 minutes before the start of rIL-2 infusion and as required thereafter. Prophylactic oral

ofloxacin or its equivalent was administered at the start of rIL-2 administration.

Patients with documented progressive disease who were previously assigned to the rIL-2 control group and who met the rIL-2 eligibility criteria were eligible for cross-over to the TIL/rIL-2 group.

TIL Preparation

Surgery specimens were obtained directly from the operating room on day 0 in a sterile fashion and sent in cold, sterile saline to the central cell-processing center. In the U.S., cell processing occurred at the Ex VT cell-processing center in Torrance, CA. In Europe, cells were processed at the Zentrallaboratorium Center in Bern, Switzerland. The primary tumor was dissected under sterile conditions and digested with overnight stirring in a sterile solution containing collagenase (type IV, 0.1W), hyaluronidase (type V, 0.01), and deoxyribonuclease (type I, 0.002%). The single-cell suspensions were washed three times in cold phosphate-buffered saline and separated ($450 \times g$, 35 minutes) over a Histopaque 1077 (Sigma, St Louis, MO) layer to concentrate the viable cells. The cells were again washed three times in phosphate-buffered saline and finally resuspended in a serum-free medium (5% human serum albumin) containing rIL-2 (1,200 IU/mL). Cells were cultured until $\geq 20\%$ of cells in the unselected cultures were $CD8^+$ by fluorescent cell-sorter analysis. $CD8^+$ lymphocytes were selected using CELLector CD8 T-150 culture flasks (Applied Immune Sciences, Santa Clara, CA) coated with anti-CD8 antibodies. The CELLector flasks positively select adherent $CD8^+$ T cells by immunoaffinity. The nonadherent $CD8^-$ cells were removed by washing. After exposure to medium containing rIL-2 (1,200 IU/mL) and phytohemagglutinin for 3 days, the activated adherent $CD8^+$ cells were removed from the CELLector flasks, transferred to cell culture bags or flasks, and allowed to expand in vitro to reach a total cell number of 4×10^9 to 1×10^{10} cells. The expanded cells were recovered, washed, and resuspended in 5% human serum albumin. Harvested cells were transported to the clinic site only if the cell yield and viability, gram stain, bacterial endotoxin, mycoplasma testing, and bacterial sterility cultures met release criteria. A sample was retained for testing of phenotype, cytokine expression, and cytotoxic activity. Cells collected from patients in the rIL-2 control group were cytopreserved for possible future use.

Response Assessment

Patients were evaluated for response after each treatment cycle. A CR was defined as the complete disappearance of all clinically detectable disease for a minimum of 4 weeks. Positive bone scans had to revert to normal or show sclerotic healing of lytic metastases, if present. Partial response (PR) was defined as a $\geq 50\%$ decrease in the sum of the product of the two greatest perpendicular diameters of all measurable marker lesions for at least 4 weeks. Any increase of less than 25% or decrease of less than 50% throughout the period of treatment was considered stable disease. For both PR and stable disease no simultaneous progression of assessable disease or appearance of any new lesion could occur, nor could there be worsening of existing lesions or appearance of new ones on bone scan. Progressive disease (PD) was defined as a $\geq 25\%$ increase in the size of one or more marker lesions over baseline or over the smallest size observed, or the appearance of new lesions. Worsening of existing lesions or the appearance of new lesions on bone scan was considered PD. Objective responses were confirmed by an independent group of radiologists who reviewed the CT scans of responding patients in a blinded fashion. Overall survival was measured from the time of nephrectomy to the time of death or to the last follow-up assessment. Patients who were alive at the time of the last follow-up or who were lost to follow-up were censored.

Statistical Methods

The primary efficacy variable was the proportion of patients responding to treatment (CR + PR). Secondary efficacy variables were time to disease progression, durability of response, and survival. An evaluation of the relationship between the patient's clinical response and the phenotype, cytokine profile, and cytotoxic activity of the cultured TILs was planned.

The primary efficacy analysis was based on the intent-to-treat (ITT) population. The sample size of 166 patients was estimated based on the results of the pilot phase I/II trial. Using the 90% confidence interval for response from that trial, it was assumed that RCC patients with an ECOG PS of 0 or 1 who received treatment with rIL-2 would have a complete plus partial response rate of approximately 15%, and that patients treated with rIL-2 plus $CD8^+$ TILs would have a complete plus partial response rate of approximately 36%. This trial was powered to detect a difference of this magnitude.

Logistic regression analysis was used to evaluate the proportion of patients responding to treatment. Chi-square statistics (alpha = 0.048) were used to evaluate the hypothesis of conditional independence of the treatment group and response, controlling for ECOG PS. Fisher's exact test was used to compare the proportion of responders between the two treatment groups. To evaluate the effect of rIL-2 plus $CD8^+$ TIL therapy on survival, a Cox proportional hazards model was used with treatment group and ECOG PS as variables. If survival and ECOG PS were not statistically significant variables, survival curves were estimated by the Kaplan-Meier method.[33]

RESULTS

Patient Characteristics and Disposition

A total of 178 patients presenting with MRCC were enrolled. After radical nephrectomy, 160 patients were randomized (81 to the TIL/rIL-2 group, 79 to the rIL-2 control group). Eighteen patients (10%) were not randomized because of pathology other than MRCC (transitional cell carcinoma, n = 5; leiomyosarcoma, n = 3; adrenal cortical carcinoma, n = 1; collecting duct carcinoma, n = 2; neuroectodermal tumor, n = 1), insufficient tissue available for resection (n = 5), or unresectable tumor (n = 1) (Fig 1). Twenty patients (12.5%) were randomized but did not receive rIL-2 treatment because of either surgical complications (n = 4), operative mortality (n = 2), or failure to meet eligibility criteria for rIL-2 therapy after nephrectomy (n = 14). Therefore, 72 patients (88.9%) in the TIL/rIL-2 group and 68 patients (86.1%) in the rIL-2 control group received the first cycle of rIL-2 therapy. Only 30 patients (37%) in the TIL/rIL-2 group and 28 patients (35%) in the rIL-2 control group were eligible for a second cycle of therapy. In the majority of cases, treatment was discontinued after the first cycle due to PD; seven patients were removed from study at the end of cycle 1 for reasons other than PD, including intercurrent illness (n = 1), voluntary withdrawal without adverse events (n = 3), unacceptable toxicities

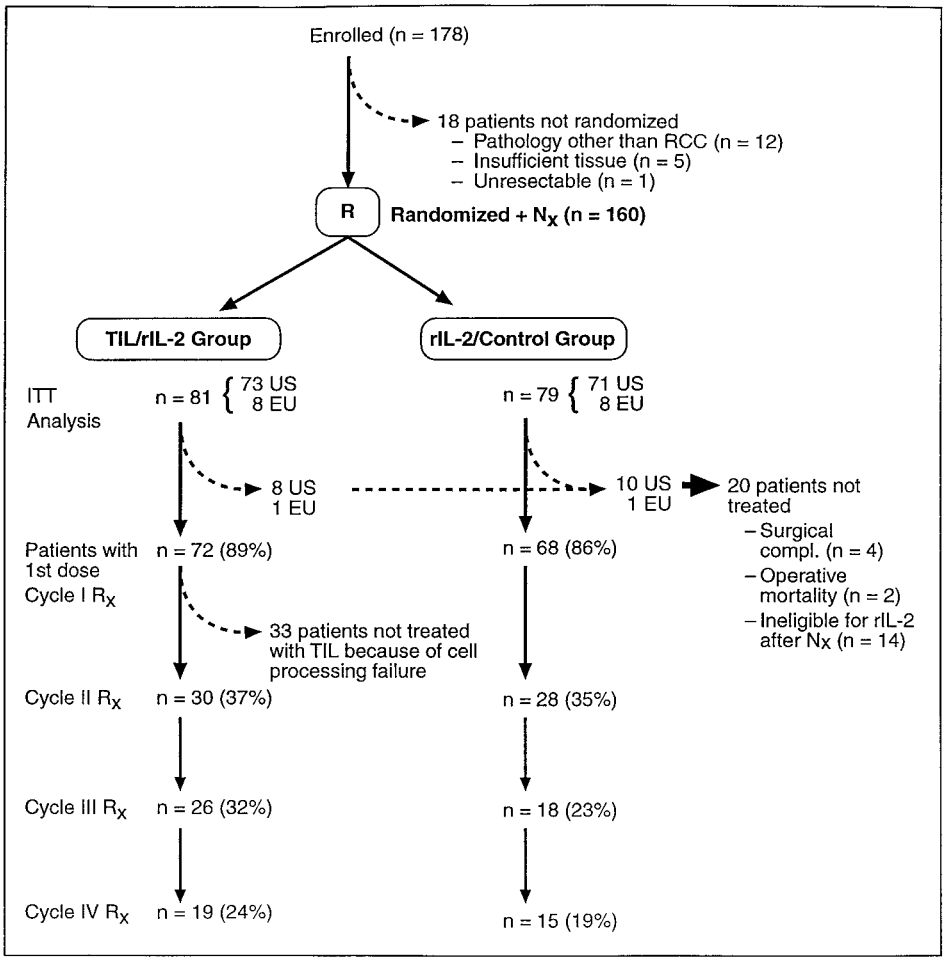

Fig 1. Number of patients enrolled, receiving nephrectomy, randomized, and receiving treatment. Dotted arrows (---►) indicate patients excluded from treatment. Abbreviations: N_x, nephrectomy; EU, Europe; R_x, therapy.

(n = 2), and unblinding as a result of mistaken diagnosis of PD (n = 1). Of 81 patients randomized to the TIL/rIL-2 group, 33 patients (41%) did not receive $CD8^+$ TILs due to factors related to cell processing, including inadequate numbers of $CD8^+$ TILs or poor cell viability.

The characteristics of randomized patients in both treatment groups were comparable for age (mean, 55 to 56 years), ECOG PS, time from diagnosis to surgery, postoperative tumor staging, renal vein involvement, and sites of metastases (Table 1). There was, however, a greater proportion of females in the rIL-2 control group (32.9%) compared with the TIL/rIL-2 group (13.6%). In the TIL/rIL-2 group and the rIL-2 control group, renal vein involvement was observed in 25.9% and 29.1% of patients, inferior vena caval extension in 11.1% and 21.5%, lymph node involvement in 39.5% and 36.7%, and multiple organ metastases in 44.4% and 57% of patients, respectively. All patients underwent radical nephrectomy. Bone metastases were not identified as a separate risk category.

Response to Treatment: ITT Analysis

The overall response rate by treatment group and ECOG PS is summarized in Table 2. Of eight responders in the TIL/rIL-2 group, three patients (7.9%) had an ECOG PS of 0 and five patients (11.6%) had an ECOG PS of 1, for an overall response rate of 9.9%. Of nine responders in the rIL-2 control group, five patients (14.3%) had an ECOG PS of 0 and four patients (9.1%) had an ECOG PS of 1, for an overall response rate of 11.4%. Using a logistic regression model, the difference in overall response rate was not statistically significant between the treatment groups ($P = .753$), and ECOG PS was also not predictive of response ($P = .894$). The odds ratios were 0.851 for treatment group and 1.07 for ECOG PS, indicating a similar likelihood of response regardless of TIL treatment or ECOG PS. However, only 39 (48%) of 81 patients in the ITT population who were randomized to the TIL/rIL-2 group actually received TIL therapy. Because of the lack of efficacy, as determined by the Data Safety Monitoring Board

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	rIL-2 + TIL (n = 81)	rIL-2 Control (n = 79)
Age, years		
Mean	56	55
Range	20-77	16-85
Sex, %		
Male	86.4	67.1
Female	13.6	32.9
ECOG performance status, %		
0	46.9	44.3
1	53.1	55.7
Time from Dx to surgery, %		
< 30 days	32.1	38.0
30-60 days	37.0	31.7
60-90 days	19.8	22.8
> 90 days	9.9	7.6
Postoperative tumor staging		
Extent of primary tumor, %		
T2	19.8	24.1
T3a	46.9	53.2
T4	30.9	21.5
Renal vein involvement, %	25.9	29.1
IVC extension, %	11.1	21.5
Lymph node involvement, %	39.5	36.7
Tumor completely resected, %	92.6	98.7
Sites of metastases, %		
Lung only	42.0	34.2
Single organ (not lung)	13.6	8.9
Multiple organs	44.4	57.0

Abbreviations: Dx, diagnosis; IVC, inferior vena cava.

using the data from 80 patients, the study was terminated early. As such, no data are available concerning response durations or the number of complete responses.

Survival: ITT Analysis

The 1-year overall survival rates were similar in the TIL/rIL-2 group (55%) and the rIL-2 control group (47%) ($P = .551$). Median survival was 12.8 months in the TIL/rIL-2 group, with 38 patients (46%) censored, versus 11.5 months in the rIL-2 control group, with 35 patients (44%) censored (Fig 2). The ECOG PS was not predictive of improved overall survival ($P = .121$). Twenty-nine patients in the rIL-2 control group with PD were eligible for crossover to the TIL/rIL-2 group; however, because of the early study termination, these data were not collected.

Table 2. Response (CR + PR) by Treatment Group and ECOG PS (ITT analysis)*

	rIL-2 + TIL (n = 81)		rIL-2 Control (n = 79)	
ECOG PS	No. of Patients	%	No. of Patients	%
0	3/38	7.9	5/35	14.3
1	5/43	11.6	4/44	9.1
Total	8/81	9.9	9/79	11.4

*$P = .753$, ITT analysis.

TIL Characteristics

The characteristics of the infused TILs are summarized in Table 3. The quality of the cell cultures with respect to the proportion of $CD8^+$ cells varied from 5% to 99% (mean, 84.8% ± 23.3%); however, the majority of cultures were highly enriched for $CD3^+CD8^+$ cells. In general, the TIL cell preparations for infusion showed great variability in terms of cell numbers and phenotypic characteristics. Unfortunately, no functional characterization of the TIL cultures was performed. The relationship between the characteristics of the TIL cultures and patient clinical outcomes was also not analyzed because of the large number of cell culture failures, which led to reduction in sample size and number of responders.

Safety

Postoperative complications, including small bowel obstruction (n = 1), cardiac arrest (n = 1), liver failure (n = 1), and cerebrovascular accident (n = 1), were responsible for the exclusion of four patients from treatment with rIL-2. Operative mortality, defined as death within 30 days of surgery, from any cause, occurred in two patients (1.25%). PD associated with clinical deterioration accounted for 14 patients (7.9%) being excluded from therapy. Adverse events occurring in ≥ 50% of patients in the ITT analysis were PD, asthenia, fever, pain, and nausea, with most events designated as not serious. The most common serious adverse events by body system were related to the body as a whole and included PD, asthenia, carcinoma, fever, pain, and sepsis (Table 4). Serious hypotension and cardiac side effects occurred in 0% to 6% of treated patients. The number of serious adverse events was comparable between treatment groups. There were no side effects specifically associated with TIL therapy.

The total number of deaths in each of the groups was identical (n = 43). PD accounted for the greatest number of deaths in both groups, resulting in 35 of 43 deaths in the TIL/rIL-2 group and 43 of 43 deaths in the rIL-2 control group. The additional eight deaths in the TIL/rIL-2 group were due to cardiac and cardiopulmonary arrests, respiratory failure and arrest, pulmonary embolism, and unknown cause. One death in the rIL-2 control group was due to multiple health problems caused by PD. These deaths were designated by the investigator as not directly related to treatment.

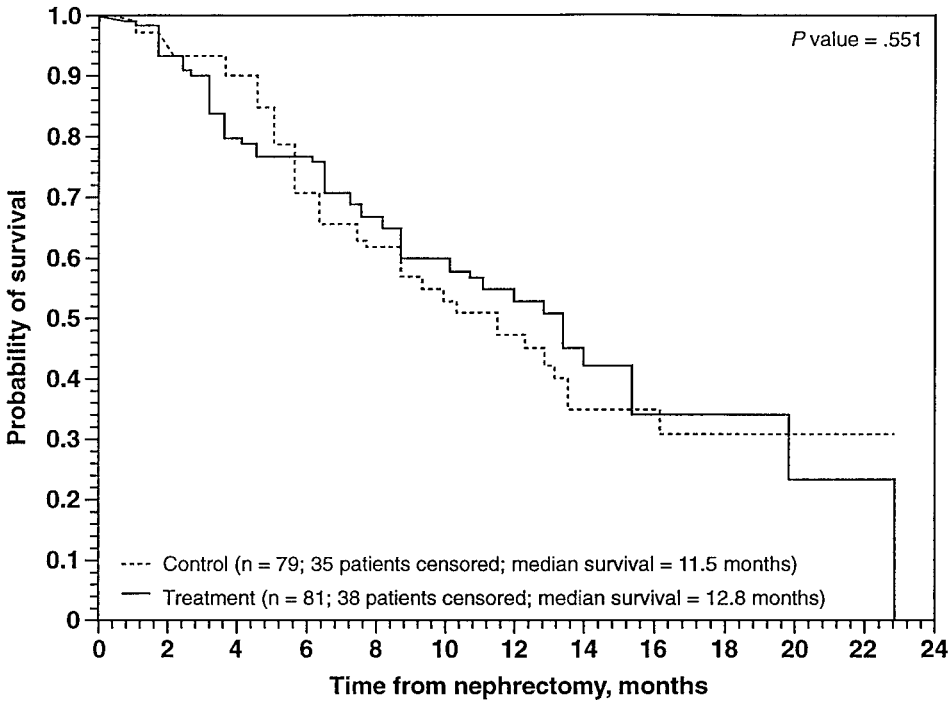

Fig 2. Overall survival for all patients by treatment group, based on the ITT analysis.

DISCUSSION

Low-dose rIL-2 regimens continue to be of interest in the treatment of MRCC for their potential to yield meaningful responses with lower toxicity; however, low-dose regimens have not yet been demonstrated to induce durable CRs as effectively as high-dose rIL-2 therapy. In the current study, we treated patients with nephrectomy followed by low-dose CIV rIL-2 with and without $CD8^+$ TILs. Selection of $CD8^+$ TILs was intended to enhance the proportion of cytotoxic cells capable of recognizing major histocompatibility complex class I-restricted target antigens. In vitro studies have demonstrated that TILs cultured for 3 weeks in the presence of rIL-2 are primarily $CD3^+CD8^+$ cells and exhibit maximum cytotoxic activity; the proportion of $CD8^+$ cells thereafter declines with a concomitant decrease in their cytolytic potential.[26]

Table 3. Characteristics of $CD8^+$ TIL Infusion Product

Variable	No. of Observations	Mean	SD	Minimum	Maximum
Total no. of cells, $\times 10^6$	38	10,118	9,220	60	49,300
Cell viability, %	38	91.0	7.1	69.0	98.0
$CD3^+$, %	39	91.8	17.4	20.8	99.1
$CD8^+$, %	39	84.8	23.3	5.5	99.2
$CD4^+$, %	39	9.0	17.8	0.0	94.1
$CD56^+$, %	39	30.1	22.0	6.7	88.9
$CD3^+/CD8^+$, %	39	76.6	32.8	0.0	99.4
$CD8^+/CD56^+$, %	39	22.3	19.6	0.0	81.5

In the primary ITT analysis, the overall response rate for both groups combined was 10.6%, and the 1-year survival rate was 55% in the TIL/rIL-2 group and 47% in the rIL-2 control group. This multicenter study did not confirm the treatment benefit associated with $CD8^+$ TILs plus rIL-2 compared with rIL-2 therapy alone that was observed in the pilot study.[32] Because the response rates in the two groups were comparable, this could be attributed in part to the large proportion of patients (41%) in the TIL/rIL-2 group who did not receive $CD8^+$ TIL therapy because their cell cultures failed to yield sufficient numbers of viable cells. In contrast, 23 (96%) of 24 patients in the pilot study were treated with $CD8^+$ TILs. The technical limitations related to $CD8^+$ TIL processing also resulted in great variability in phenotypic characteristics and cell numbers between TIL preparations (Table 3).

The outcome of this trial underscores the challenges associated with the application of TILs in the broader clinical setting. Although some single-institution studies have demonstrated objective tumor regression associated with TIL plus rIL-2 therapy, results have been variable, possibly because of disparity with respect to rIL-2 administration, TIL preparation, or patient selection.[24,32,34-37] Further randomized studies are necessary to evaluate the clinical

Table 4. Incidence of Serious Adverse Events (grade 3/4) by Body System Occurring in More Than 5% of the ITT Population (n = 160)

Adverse Event	rIL-2 + TIL (%)	rIL-2 Control (%)
Whole body		
Asthenia	2	14
Fever	3	8
Pain	14	8
Unassessable reaction	8	5
Sepsis	5	10
Cardiovascular		
Embolus	6	3
Digestive		
Anorexia	2	6
Nausea	3	10
Vomiting	6	10
Ileus	3	10
Metabolic/nutritional		
Dehydration	3	6
Hypercalcemia	8	6
Respiratory		
Apnea	6	2
Dyspnea	13	6
Pleural effusion	5	6
Pneumonia	8	8

benefit of TIL therapy in combination with standard immunotherapeutic protocols. Such studies must clearly establish the technology and delineate its technical challenges, so that the technology does not become a dependent variable. In practical terms, special care must be applied to standardizing the techniques used for tumor handling and transport, tumor processing to yield single-cell suspensions, and CD8+ TIL expansion, selection, and harvesting.

The substantial number of cell culture failures and lack of efficacy based on the data from 80 patients resulted in early termination of the trial. Because of the small number of responders, the relationship between the phenotypic and cytotoxic profile of TIL cultures and the clinical response could not be analyzed. Belldegrun et al[28] designed a study to identify predictors of response to TIL/rIL-2 therapy. Although no significant differences between responders and nonresponders were found with respect to the in vitro characteristics of tumor TILs (including phenotype), their study did suggest that clinical response to TIL/rIL-2 therapy may be associated with patients' natural immune status at baseline.[28] Many investigators are currently studying different aspects of CD8+ TIL function, which should eventually advance the clinical application of TIL therapy.[28,38-40]

The results of the current study further underscore the safety of nephrectomy before systemic rIL-2 therapy. Moreover, the high incidence of renal vein involvement, inferior vena caval extension, and multiple organ metastasis in a large proportion of patients enrolled on the current study did not preclude them from undergoing successful nephrectomy. The surgical complication rate was only 5% and, overall, only 12.5% of patients were ineligible for rIL-2 therapy after nephrectomy. Pathologic examination of tumor tissue revealed that 10% of patients entered onto the study had a histology other than RCC. This was indeed surprising, given that histologic or radiologic documentation of RCC was required to meet the eligibility criteria. Therefore, physicians should consider pathologic confirmation of RCC in all patients before preimmunotherapy debulking nephrectomy or immunotherapy followed by debulking nephrectomy.

Most investigators agree on the value of palliative nephrectomy in alleviating symptoms such as relentless pain and intractable hemorrhage.[41] However, the role of nephrectomy in the management of advanced MRCC remains controversial because of the occurrence of surgical complications and postoperative disease progression, which may preclude subsequent rIL-2 therapy. The proportion of nephrectomized patients who fail to receive planned rIL-2–based treatment because of disease progression, deterioration of PS, or perioperative morbidity or mortality generally ranges from 10% to 40%.[24,42,43] While this may seem a substantial risk and has led many to advocate initial systemic therapy with the primary tumor in situ, careful selection of patients can reduce this risk to a reasonable level. When patients treated at the University of California, Los Angeles, were carefully selected based on ECOG PS, perioperative complications led to ineligibility for systemic rIL-2 therapy in only 8% to 13% of patients.[32,44] Walther et al and Rackley et al have reported similar complication rates (13% and 16%, respectively).[42,43] These studies suggest that, based on strict eligibility criteria, nephrectomy does not result in an unacceptable level of rIL-2 treatment ineligibility. A randomized phase III study by the Southwest Oncology Group is currently evaluating the clinical benefit of nephrectomy before systemic immunotherapy and will no doubt help to clarify these issues.

In conclusion, therapy with low-dose CIV rIL-2 plus CD8+ TILs has not demonstrated any improvement in response rate or survival compared with low-dose CIV rIL-2 therapy alone, when administered to MRCC patients after radical nephrectomy in this multicenter randomized trial. However, based on the ITT analysis, 1-year survival rates in both treatment groups compared favorably with other published trials, including trials of high-dose IV bolus rIL-2 therapy, although a direct comparison cannot be made based on these data. This trial has further shown that, at the present time, it may not be technically feasible to deliver TIL

therapy in the broader clinical setting; however, further refinement of cell-culture techniques may ultimately result in the broader application of this treatment modality.

ACKNOWLEDGMENT

We are grateful to Chiron Therapeutics for providing rIL-2 for these studies.

APPENDIX

We acknowledge the significant contributions of the following investigators: Robert P. Whitehead, MD, Texas Tech University Health Sciences Center; Seth Lerner, MD, Baylor College of Medicine; Kim A. Margolin, MD, City of Hope National Medical Center; H. Kim Lyerly, MD, Duke University Medical Center; Robert Flanigan, MD, Loyola University Medical Center; Bruce A. Lowe, MD, Oregon Health Sciences University; Thomas E. Ahlering, MD, University of California, Irvine; L. Michael Glode, MD, University of Colorado Health Sciences Center; Doug Orr, MD, Texas Oncology, PA; Michael O. Koch, MD, Vanderbilt University Medical Center; Gary Spitzer, MD, Latter Day Saints Hospital; Timothy M. Lestingi, MD, Lutheran General Hospital; Judd Moul, MD, and Raymond A. Costabile, MD, Walter Reed Army Medical Center; Michael T. Lotze, MD, University of Pittsburgh Medical Center; John W. Smith II, MD, and David C. Smith, MD, University of Michigan Medical Center; Robert Dreicer, MD, University of Iowa/Hospital and Clinics; Joel Picus, MD, Barnard Cancer Center; Julie A. Ellerhorst, MD, The University of Texas M.D. Anderson Cancer Center; Mark A. Goepel, MD, University of Essen; Gerhard Zöller, MD, University of Göttingen, Medical School; Stephan Peter, MD, Städtische-Kliniken Darmstadt; W. Schultze-Seemann, MD, University Clinics Freiburg; Günter Gastl, MD, Albert-Ludwigs-Universitat Freiburg; Urs E. Studer, MD, University of Berne; Christoph Rochlitz, MD, Kantonsspital Basel; Georg Bartzch, MD, University of Innsbruck; Sylvie Negrier, MD, Centre Léon Berard Lyon; Bernard Escudier, MD, Institut Gustave-Roussy Villejiuf; and Jean Pierre Bergerat, MD, Hopital Civil Strasbourg.

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