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Phase I Study of Low-Dose Cyclophosphamide and Recombinant Interleukin-2 for the Treatment of Advanced Cancer

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Summary: We conducted a phase I study of low-dose cyclophosphamide and recombinant interleukin-2 (rIL-2) in 66 patients with advanced cancer resistant to standard therapy. All patients were evaluable for toxicity and 46 patients were evaluable for antitumor response. Patients evaluable for antitumor response included 23 with malignant melanoma, 10 with renal cell carcinoma, 4 with colon cancer, and 9 with various other solid tumors. All patients received i.v. cyclophosphamide (350 mg/m^2) on day 1 followed by rIL-2 via 15 min i.v. infusion on days 4–8 and 11–15. The doses of rIL-2 ranged from 6.0 to 36.0 × 10^6 IU/m^2. Each treatment cycle consisted of 21 days and a total of 113 cycles was administered. The number of treatment cycles administered per patient ranged from 1 to 8. The dose-limiting toxicities associated with rIL-2 included altered mental status, arthralgias, diarrhea, fatigue, fever, hypotension, nausea/vomiting, and peripheral edema. Tweleve patients (18%) were removed from the study secondary to toxicity. Among the evaluable patients, 2 (4%) (malignant melanoma, renal cell carcinoma) developed a partial remission, 13 (29%) maintained stable disease, and 31 (67%) developed progressive disease. We conclude that the combination of low-dose cyclophosphamide and rIL-2 is tolerable in most patients but our data do not suggest an improved response rate for the combination vs. rIL-2 alone. **Key Words:** Interleukin-2—Cyclophosphamide—Phase I study—Malignant melanoma—Renal cell carcinoma.

Interleukin-2 (IL-2) is a cytokine secreted by activated T-lymphocytes. It has a broad range of biological activities in vivo and in vitro including stimulation of T-lymphocyte proliferation, generation of lymphokine-activated killer (LAK) cells, enhancement of natural killer cell cytotoxicity, activation of monocytes, and stimulation of secretion of interferon-γ, tumor necrosis factor, and lymphotoxin (1,2). IL-2 causes regression of tumors both with and without concomitant administration of LAK cells in animals and humans (3,4).

Side effects such as hypotension, renal failure, fluid accumulation, and myocarditis complicated the clinical trials of high-dose recombinant IL-2 (rIL-2) and were life threatening in some patients. Some recent clinical trials focused on methods to

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reduce rIL-2 toxicity without compromising antitumor activity. The strategies used include schedule and dose manipulation, coadministration of corticosteroids, and elimination of the costly and labor-intensive adoptive transfer of LAK cells (5–7). Other investigators have shown selective depression of suppressor T-lymphocyte activity by low-dose cyclophosphamide (8). Theoretically, this could lead to enhanced IL-2-induced LAK and T-helper cell activity in vivo (9).

Possibly to optimize the antitumor effects of rIL-2 while maintaining acceptable toxicity, Mitchell et al. treated outpatients with low-dose cyclophosphamide prior to rIL-2 (10). A total of 27 metastatic melanoma patients received 350 mg/m^2 of cyclophosphamide i.v. on day 1 followed by 21.6 $\times$ 10^6 IU/m^2 of rIL-2 by i.v. bolus on days 4–8 and 11–15. Six patients (25%) developed objective responses (one complete, five partial remissions) and an additional eight patients developed minor responses. LAK cell activity measured by in vitro cytolytic activity of patient lymphoid cells against cultured melanoma cells was detectable in all 6 responding patients and 11 nonresponders. Toxicity was moderate and only one patient stopped treatment secondary to rIL-2-induced side effects.

We now report our experience using a similar regimen of low-dose cyclophosphamide prior to escalating doses of rIL-2 in 66 patients with advanced cancer. In addition to malignant melanoma, we included patients with other disseminated and/or incurable solid tumors.

METHODS

Patient Selection

Eligibility criteria for entry of patients in this study included histologically confirmed diagnosis of cancer refractory to standard therapy or for which no effective therapy was available, age 18 years or older, measurable tumor by physical examination or noninvasive imaging studies, Karnofsky performance status of 70% or greater, minimum life expectancy of 3 months, absence of known brain metastases, and no treatment with chemotherapy, hormonal therapy, or immunotherapy within 3 weeks prior to study entry. Required baseline laboratory parameters included total white blood cell (WBC) count $\geq$ 3,500/μl, platelet count $\geq$ 100,000/μl, prothrombin time $<$ 1.3 times control, serum creatinine $<$ 2.0 mg/dl, serum bilirubin $<$ 1.5 mg/dl, and SGOT $<$ 1.5 times upper limit of normal. Exclusion criteria included a history of significant cardiac disease, active infection, seizure disorder, active vasculitis, pregnancy, or lactation.

Study Design

The CETUS Corporation (Emeryville, CA, U.S.A.) supplied rIL-2 (specific activity of 18 $\times$ 10^6 IU/mg). All patients received cyclophosphamide, 350 mg/m^2 by i.v. bolus on day 1 and rIL-2 i.v. over 15 min on days 4–8 and 11–15. Days 16–20 were a rest period and a treatment cycle lasted 21 days. rIL-2 dose escalation began at 18 $\times$ 10^6 IU/m^2 and the dose levels included 12, 18, 21, 27, and 36 $\times$ 10^6 IU/m^2. Intrapatient escalation to successive dose levels was allowed after each cycle of therapy if toxicity during the previous cycle did not exceed grade 2 using Southwest Oncology Group (SWOG) criteria. Patients received indomethacin, 50 mg orally 30 min before and 3 h after rIL-2 infusion. Patients with rigors received meperidine, 25–50 mg i.v. as needed. All treatments were initiated in the outpatient clinic at the Arizona Cancer Center. The study procedures and consent form used in this clinical trial were approved by the University of Arizona Human Subjects Committee. All patients gave informed consent prior to study entry.

Dose reductions for grade 1 or 2 toxicity were not required but were allowed at the discretion of the clinical investigator. Grade 3 toxicity required a 50% dose reduction or reduction to the previously tolerated dose level for patients receiving greater than 18 $\times$ 10^6 IU/m^2. Recurrent grade 3 toxicity after dose reduction or any grade 4 toxicity required removal of the patient from the study. Patients were removed from the study if disease progression occurred after completion of three treatment cycles.

Evaluation for Safety and Efficacy

Patients were followed with biweekly assessments of performance status, complete blood count (CBC), and serum chemistries (SMA-20). Assessment for antitumor response was performed after every three cycles of therapy. A complete remission indicated complete disappearance of all evidence of disease for a minimum of 4 weeks. A partial remission indicated a 50% decrease in the sum of the products of the perpendicular diameters of all

measurable lesions for a minimum of 4 weeks without the development of new lesions. Stable disease consisted of any response less than a partial response or tumor progression less than that defining progressive disease. Progressive disease indicated an unequivocal increase of 25% in any measurable lesion or the appearance of new lesions.

RESULTS

Table 1 contains the clinical characteristic of the 66 patients entered into this trial. There were 36 men and 30 women with a mean age of 51 years. Prior therapy included chemotherapy (61%), radiotherapy (20%), surgery (93%), immunotherapy (32%), and tumor vaccine (3%). Malignant melanoma, renal cell carcinoma, and colon cancer represented 74% of the malignancies treated. The most frequent sites of metastatic disease were lung, liver, skin, and/or lymph nodes and bone. All patients were evaluable for toxicity and 46 were evaluable for response. Twenty patients were unevaluable for response for the following reasons: rIL-2 toxicity necessitating early discontinuation of therapy, eight patients; patient refused further treatment, four patients; early death from progressive tumor, three patients; declining performance status secondary to progressive disease, two patients; protocol violation, one patient; incomplete data, one patient; and pre-existing brain metastases discovered after treatment with rIL-2, one patient.

TABLE 1. *Patient characteristics*

No. of patients treated	66
No. of evaluble patients	46
Male	36
Female	30
Mean age (years)	51
Range	15–75
Prior therapy	
Chemotherapy	40
Radiation	13
Surgery	56
Immunotherapy	21
Tumor vaccine	2
Tumor type	
Malignant melanoma	26
Renal cell carcinoma	16
Colon	7
Lung	3
Unknown primary	3
Gastric	2
Pancreatic	2
Leiomyosarcoma	2
Other[a]	5
Sites of metastasis	
Lung	37
Skin and/or lymph nodes	20
Liver	29
Bone	10
Adrenal	5
Intra-abdominal mass	7
Other[b]	10

[a] Breast, 1; ovarian, 1; carcinoid, 1; rectal, 1; nares, 1.
[b] Retroperitoneum, 2; small bowel, 3; spleen, 2; kidney, 1; ovary, 1; brain, 1.

The rIL-2 administration and antitumor response data are tabulated in Table 2. The number of cyclophosphamide/rIL-2 cycles in evaluable patients ranged from 1 to 8. The dose of rIL-2 ranged from 6.0 to 36.0 $\times$ 10^6 IU/m^2. No patient in this study developed a complete response. Among the 46 evaluable patients, 2 (4%) developed a partial remission, 13 (29%) had stable disease, and 31 (67%) developed progressive disease.

One partial remission occurred in a 49-year-old female with renal cell carcinoma with a 5.2 $\times$ 4.3 cm right lower lobe pulmonary metastasis and a large left retroperitoneal mass. She received seven cycles of cyclophosphamide/rIL-2 with complete resolution of her lung metastasis and stabilization of the retroperitoneal mass. Her disease remained stable for 18 weeks before the retroperitoneal mass increased in size. She was considered a partial response as the retroperitoneal mass could not be accurately measured by computed tomography (CT) scan and thus was classified as evaluable only. The other partial response occurred in a 46-year-old male with malignant melanoma with multiple skin and lymph node metastases involving the left lower extremity. He received five cycles of therapy with partial regression of his skin nodules after the first cycle before multiple new skin metastases developed. Both patients subsequently died of progressive disease.

There was no consistent relationship between rIL-2 dose and grade 1 or 2 toxicities (Table 3). Grade 3 and 4 toxicities were rarely seen at 18 $\times$ 10^6 IU/m^2. Grade 3 and 4 fatigue/decreased performance status, fever/chills, and elevated bilirubin were slightly more common at 27 $\times$ 10^6 IU/m^2. The most common toxicities requiring dose reduction were altered mental status, arthralgias, diarrhea, fatigue, fever, hypotension, nausea/vomiting, and peripheral edema. Among evaluable patients, the median administered dose of rIL-2 was 21.0 $\times$ 10^6 IU/m^2. Of the 69 cycles delivered at this dose level, 64% were complete and did not require dose reduc-

TABLE 2. *rIL-2 administration and antitumor response*

Tumor type	No. of patients	Median no. of rIL-2 cycles (range)	Median rIL-2 dose[a] (range)	CR[b]	PR[b]	SD[b]	PD[b]
Malignant melanoma	23	2 (1–6)	21.0 (12.0–36.0)	0	1	3	19
Renal cell	10	3 (1.5–7)	21.0 (6.0–32.4)	0	1	5	4
Colon	4	2.75 (1–8)	21.0 (11.4–36.0)	0	0	4	0
Other[c]	9	2 (1.5–3.5)	21.0 (12.0–27.0)	0	0	1	8

[a] $IU/m^2 \times 10^6$.
[b] PD, progressive disease; SD, stable disease; PR, partial remission; CR, complete remission.
[c] Unknown primary, 2; ovarian, 1; lung, 1; leiomyosarcoma, 1; rectal, 1; pancreatic, 2; carcinoid, 1.

tions. Fifty-two percent (11/21) of patients given rIL-2 at $>21.0 \times 10^6$ IU/m^2 required dose reduction for toxicity. Forty-two percent (25/57) of patients given rIL-2 at $\leq 21.0 \times 10^6$ IU/m^2 required dose reduction for toxicity.

DISCUSSION

With the advent of recombinant DNA technology, large quantities of immune modulating substances became available for testing in cancer patients. rIL-2 is a potent biological response modifier that demonstrated antitumor activity in animal models and human clinical trials (3–14). Since the initial trials by Rosenberg et al. utilizing high-dose rIL-2 with LAK cells, other investigators have studied rIL-2 with or without conventional agents in the outpatient setting.

Clinical trials of rIL-2 without LAK cells yielded mixed results. Atkins et al. treated 17 patients with various advanced malignancies with rIL-2 alone and observed no antitumor activity (12). Sosman et al. treated 25 patients with renal cell carcinoma, malignant melanoma, and non-Hodgkin's lymphoma with up to 6 months of rIL-2 and observed only 1 partial response (13). Lindemann et al. treated 29 patients with rIL-2 after low-dose i.v. cyclophosphamide and reported a 10% response rate (14). In a more promising study, Mitchell et al. reported a response rate of 25% in 27 malignant

TABLE 3. *Toxicity data*

Toxicity	Grade 1–2 toxicity (% of pts.), rIL-2 dose ($\times 10^6$ IU/m^2)			Grade 3–4 toxicity (% of pts.), rIL-2 dose ($\times 10^6$ IU/m^2)		
	18.0 (n = 20)	21.0 (n = 37)	27.0 (n = 21)	18.0 (n = 20)	21.0 (n = 37)	27.0 (n = 21)
Fatigue/↓ performance status	75	81	57	8	19	38
Hypotension	10	51	19	4	22	10
Edema	20	32	38	4	3	10
Altered mental status	30	27	24	4	5	10
Arthralgias	10	22	19	0	8	5
Myalgias	0	14	5	0	5	5
Nausea/vomiting	40	95	47	0	0	0
Fever/chills	45	89	52	4	5	19
Diarrhea	20	22	19	4	3	0
Dyspnea	10	14	5	4	3	5
Diaphoresis	5	11	0	0	0	0
↑ Bilirubin	0	0	10	0	0	10
↑ SGOT	0	11	0	0	3	0
↑ Creatinine	10	5	10	0	3	0
Hematologic	5	0	24	0	0	5

n, no. of patients.

melanoma patients treated with low-dose i.v. cyclophosphamide and rIL-2 (8).

We used a similar outpatient regimen as that by Mitchell et al. in 46 evaluable patients with disseminated malignancy and observed only 2 partial remissions. The procedures in the two trials were similar although we included patients with tumors other than malignant melanoma and we tested varying doses of rIL-2. Our patients were slightly older (average age of 51 vs. 44 years) and increasing age has been associated with decreased tolerance to rIL-2, especially renal insufficiency (1,17). However, there is no convincing evidence supporting decreased antitumor responsiveness to rIL-2 in elderly patients. The median number of cycles of cyclophosphamide/rIL-2 given per patient in the two trials (2.4 vs. 3.0) was comparable and the median dose of rIL-2 per cycle (21.0 vs. 21.6 $\times$ 10^6 IU/m^2) was essentially identical. Other inherent and unmeasurable differences between such diverse patient populations including underlying immune status and tumor burden may have influenced the toxicities observed and treatment outcome.

In contrast to Mitchell et al., we found this regimen to be moderately toxic. Side effects severe enough to mandate withdrawal from our study occurred in 12 patients (18%). In Mitchell's study, only one patient withdrew secondary to rIL-2-induced side effects. Toxicity also resulted in rIL-2 dose reduction in 26 patients (39%) in our study. Examination of Mitchell's data reveals a comparable percentage of dose reductions (41%). The range of toxic side effects we observed was similar to those previously described (15–17). However, unlike other investigators, we did not observe any episodes of myocarditis, myocardial ischemia, or acute myocardial infarction in our patients (18).

In summary, pretreatment with low-dose cyclophosphamide prior to the administration of rIL-2 did not enhance antitumor efficacy vs. that previously reported with rIL-2 alone. In addition, the regimen was moderately toxic. We consider 21.0 $\times$ 10^6 IU/m^2 to be the maximally tolerated dose because higher doses resulted in more grade 3 and 4 toxicities and more frequent dose reductions. It should be emphasized that our objective was to administer cyclophosphamide/rIL-2 in an outpatient setting without hospitalization. Despite the disappointing results of our trial, the two partial remissions in patients with tumors unresponsive to standard therapy (malignant melanoma, renal cell carcinoma) warrant further investigation of rIL-2. Clearly, new strategies of rIL-2 administration to optimize antitumor activity are needed. Approaches that merit investigation include coadministration of rIL-2 with new cytotoxic agents or other biological response modifiers, maneuvers to increase IL-2 reeptors in vivo, and further attempts to define the optimal schedule for rIL-2 administration. Only through well-designed clinical trials will rIL-2 find a place among the therapeutic armamentarium of practicing oncologists.

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