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Tolerance and Effectiveness of Recombinant Interleukin-2 (r-met Hu IL-2 [ala-125]) and Lymphokine-activated Killer Cells in Patients with Metastatic Solid Tumors

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Abstract—*The tolerance of a recombinant human interleukin-2 (rIL-2, r-met Hu IL-2 [ala-125], Ortho Pharmaceutical) and its antitumor effectiveness in combination with lymphokine-activated killer (LAK) cells was examined in 26 patients with metastatic solid tumors, including 14 renal cell carcinomas, seven melanomas, three extragonadal germ cell tumors refractory to chemotherapy and two colon carcinomas. rIL-2 was administered as a bolus at 30,000 U/kg every 8 h on days 1–5 and days 12–19. Leukapheresis was done on days 8–12, lymphocytes were incubated with rIL-2 for 3 or 4 days in vitro and reinfused on days 12, 13 and 15. The mean number of reinfused cells was 5.1×10^{10} per patient. IL-2 dose and schedule were adjusted according to toxicity. Patients received a median of 100% (range 87–100%) of planned rIL-2 on days 1–5 and a median of 71% (range 13–100%) on days 12–19. Capillary leak syndrome with hypotension and impaired renal function and CNS toxicity were the major reasons for dose modification. Patients with renal cell carcinoma, most of whom underwent a prior nephrectomy, had a reduced tolerance to rIL-2. Partial responses were documented in three renal cell carcinomas and one melanoma. The median response duration was 5.5 (range 1–6) months.*

INTRODUCTION

INTERLEUKIN-2 (IL-2) is a secretory product of activated T-helper cells. Among other cellular effects it induces *in vitro* and *in vivo* the activation of a heterogeneous population of non-specific cytotoxic lymphocytes (lymphokine activated killer cells or LAK cells) which can directly lyze tumor cells *in vitro* [1]. An antitumor effect from IL-2 and IL-2 and LAK cells has also been shown in mouse models *in vivo*, and in some model systems the combination of IL-2 and LAK was more efficient than IL-2 alone [2, 3]. In clinical trials based on experience with these model systems, Rosenberg *et al.* have demonstrated an antitumor effect of recombinant IL-2 and LAK cells in renal cell carcinoma, melanoma and non-Hodgkin's lymphoma [4, 5]. The aim of our cooperative study conducted in two centers (Brussels and Zürich) was to examine the clinical tolerance of a slightly modified, water soluble recombinant human IL-2 (rIL-2, r-met Hu IL-2 [ala-125], Ortho Pharmaceutical) and the effectiveness of its use in combination with *in vitro* activated LAK cells in a phase I/II study.

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MATERIALS AND METHODS

Patient selection

Criteria for inclusion in the study were bidimensionally measurable metastatic solid tumor, age 18–65, performance status of 0–2 [6], and an interval between any previous and study treatment of at least 4 weeks. Patients with brain metastasis, significant concomitant disease, abnormalities of routine laboratory findings (>125% of upper normal range, or positive hepatitis B-antigen and HIV-1 serology were excluded from participation in the study. All protocols were approved by the institutional review board of each hospital. The procedures, along with the potential risks and benefits, were explained in detail to patients and family members, and written consent was obtained.

Treatment schedule, lymphocyte culture technique and examinations

rIL-2 (recombinant methionyl human interleukin-2 [alanine-125]) was supplied by Ortho Pharmaceutical, Rariton, NJ, U.S.A. The compound differs from the natural sequence human IL-2 in that approx. 95% of the molecules have an additional methionyl residue bound to alanine at position one. In addition, rIL-2 contains an alanine in place of cysteine at position 125 and is not glycosylated. The compound is water soluble without the addition of a detergent. Patients received rIL-2 at 30,000 U/kg as bolus infusion over 10–15 min every 8 h on days 1–5 and 12–19. Adjustments of dose were made according to clinical toxicity. Leukapheresis was performed daily on days 8–12 through a double lumen central venous or large peripheral catheter. Eight to 16 l of blood were processed each day using a Fenwal CS-3000 blood cell separator (or a Dideco: Brussels, patients 1–9) and ACD-A solution as anticoagulant. Mononuclear cells were separated by Ficoll/Hypaque density gradient centrifugation, washed twice in Hank's buffer and then incubated for 3 or 4 days at 1.5×10^6 (Brussels) or 2.5×10^6 (Zürich) cell/ml in roller bottles with RPMI 1640 medium supplemented by 2% L-glutamine, 50 μg/ml gentamycin, 2% human AB serum and 1500 U/ml rIL-2. The cells were washed twice and retransfused on days 12, 13 and 15 over 30 min. Aliquots of the cell suspension were obtained for Gram stain and culturing. LAK cell functions were tested in a chromium release assay using K562 and Daudi (Zürich) or Raji (Brussels) cells as target cells. Patients were observed daily for signs of toxicity. Antitumor activity was assessed by clinical and radiological examination on day 22 and then at monthly intervals.

Supportive therapy

Patients received acetaminophen and novaminsulfone (Zürich) or indomethacine (Brussels) for fever, pethidine for rigors, ranitidine to prevent gastric irritation, and furosemide for the management of fluid retention. Epinephrine or dobutamine and/or low dose dopamine were given for the management of refractory hypotension and/or oliguria.

Assessment of toxicity and tumor response

Clinical toxicity and alteration of laboratory parameters were documented and graded daily and antitumor effect was evaluated according to established criteria as complete response, partial response, no change or progressive disease [6].

RESULTS

Patient summary

The main patient characteristics including patients' identification numbers are summarized in Table 1. Twelve of 14 patients with renal cell carcinoma had undergone tumor nephrectomy 1 month to 5 years before study entry but none of them had any form of previous systemic therapy. Some of the patients with other tumors had either chemotherapy and/or radiotherapy prior to study entry.

Toxic effects

All patients developed systemic symptoms including fever over 38.5°C, chills, rigors and malaise. Other clinical toxicities graded according to WHO criteria are summarized in Table 2. All patients had desquamative erythema of the skin, hoarseness and glossitis. The latter was the most disabling subjective side-effect for many patients. Loss of appetite and nausea were the major signs of gastrointestinal toxicity. All patients had sinus tachycardia. In addition two had atrial fibrillation and three unifocal PVCs. All patients developed a capillary leak syndrome with interstitial fluid retention, dyspnea, hypotension and decreased urinary output. The average weight gain was 12% (6–23%) of body weight at study entry. The maximal body weight was reached on day 18 (Fig. 1). At least 14 patients experienced some degree of central nervous system toxicity with cognitive impairment predominantly towards the end of the third week of treatment, a side-effect not included in the CNS toxicity grading scale. Twenty-one patients required vasopressors because of decrease in urinary output and/or refractory hypotension. Three patients developed an adult respiratory distress syndrome requiring intubation. In two of these sepsis contributed to the pulmonary toxicity (2B, *Staphylococcus aureus;* 3Z, Gram-negative bacteria from a contaminated commercially obtained buffer solution). Nine additional patients required antibiotic therapy because of Gram-positive organisms documented in blood cultures.

Changes in laboratory parameters

Figures 2–8 summarize changes of major laboratory parameters during IL-2 therapy. All patients had a rebound lymphocytosis with a maximum on day 8 (Fig. 2). A steady decline in hemoglobin and platelet count was observed (Figs 3 and 4). A dramatic increase in eosinophils occurred in all patients towards the end of hospitalization (Fig. 5). Acute phase reactants (Fig. 6) and serum creatinine increased concordant with rIL-2 treatment. The increase in creatinine was more pronounced in patients with renal cell carcinoma than in other patients ($p < 0.05$, t-test for peak values, Fig. 7). Liver function test showed an increase of bilirubin (Fig. 8), transaminases (Fig. 9) and alkaline phosphatase (Fig. 10). Serum albumin (Fig. 11) and serum cholesterol (Fig. 12) were transiently reduced during treatment.

Table 1. Patient characteristics

No.	Dg	PS	Age	Sex	Site of metastasis	Tr
1Z	R	2	42	m	Pleura, left lung	N
7Z	R	0	43	m	Lungs	N
9Z	R	1	47	f	Mediastinum, lungs	N
10Z	R	1	51	m	Mediastinum, pleura and lung	N
12Z	R	1	46	m	Retroperitoneum, mediastinum, pleura, lungs	N
13Z	R	1	27	f	Retroperitoneum, mediastinum, lungs	N
14Z	R	0	63	f	Lungs, mediastinum	N
15Z	R	0	65	m	Lungs	N
2B	R	1	57	m	Lungs, bone	N
3B	R	1	42	m	Kidney (primary tumor)	—
6B	R	1	21	m	Adrenal gland, bone	N
7B	R	1	59	m	Lungs, bone	N
9B	R	0	56	f	Lungs, bone	—
12B	R	0	49	f	Lungs	N
2 + 5Z	M*	0	43	m	Skin, axillary lymph nodes	S
6Z	M	1	46	m	Duodenum, left adrenal gland	S
8Z	M	2	30	f	Skin, retroperitoneum, mediastinum, ovaries	S
1B	M	0	31	f	Skin, liver, kidney	S/C
4B	M	1	37	f	Liver	S
5B	M	1	65	m	Lymph node	S/X
10B	M	0	43	f	Liver	S/C
3Z	G	0	47	m	Retroperitoneum, lungs	C
4Z	G	1	35	m	Retroperitoneum	C/X
11Z	G	0	58	m	Mediastinum, pleura, lungs	S/C
8B	CC	0	50	f	Lungs	S/C
11B	CC	1	48	f	Liver	S

*Retreatment of patient No. 2Z.
B: Institut Jules Bordet, Brussels; C: chemotherapy; CC: colon cancer; G: extragonadal germ cell tumor; M: melanoma; N: nephrectomy; PS: performance status; R: renal cell cancer; S: surgery; Tr: treatment before IL-2/LAK therapy; X: radiation therapy; Z: University Hospital, Zürich.

Table 2. Toxicity grades

Grade	Skin	GI	Heart	Lung	Renal	BP	CNS*
I	5	3	20	2	13	12	0
II	17	17	5	5	12	4	0
III	5	7	1	13	1	7	1
IV	—	—	1	2	—	2	1

GI: gastrointestinal toxicity; BP: hypotension and degree of oliguria; CNS: central nervous system toxicity.
*Thirteen patients developed some degree of cognitive impairment, one patient each disabling nightmares and severe psychosis with suicide attempt.

LAK cell generation and rIL-2 dose modifications

Leukapheresis was well tolerated. Because of rebound lymphocytosis the efficiency of cell separation was best on day 8 and declined thereafter despite the processing of increasing volumes of blood. Hematocrit and contamination with granulocytes were below 5% (Zürich) or 15% (Brussels). Overall cell recovery was of 50% (Zürich) and 30% (Brussels). The average viability of cells at re-transfusion was 89% (STD 5%). The mean number of viable re-transfused cells was 5.1×10^{10} per patient (STD 2.6×10^{6}). Table 3 states the actual number of LAK cells re-transfused and the actual dose of rIL-2 given for each course. Patients received a median of 100% (range 87–100%) of the planned rIL-2 during days 1–5 and a median of 71% (range

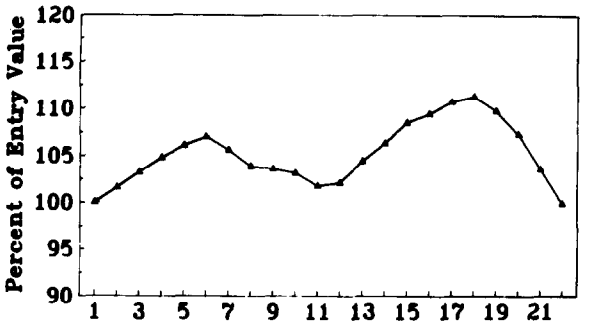

Fig. 1. Weight.

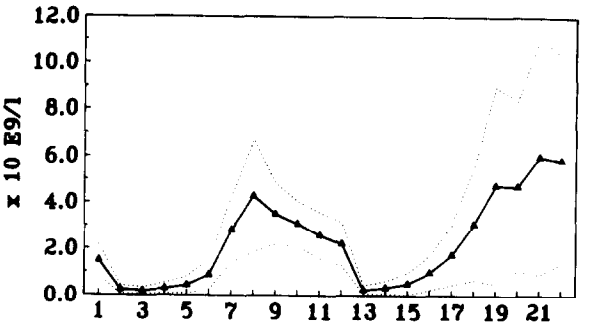

Fig. 2. Lymphocytes.

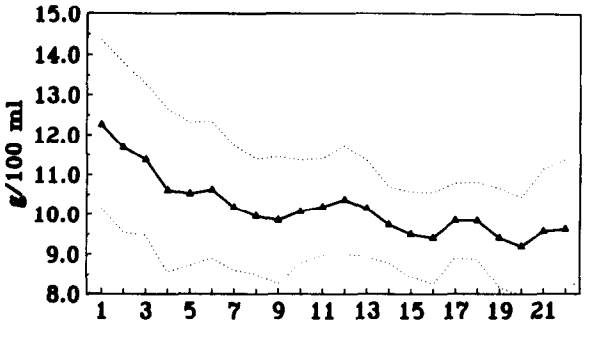

Fig. 3. Hemoglobin.

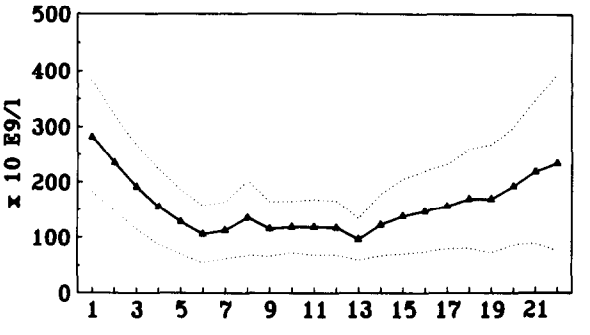

Fig. 4. Platelets.

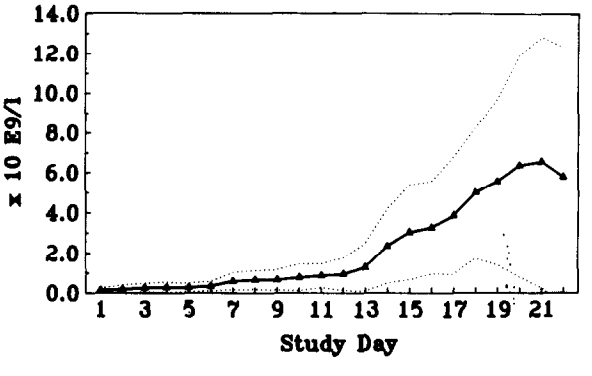

Fig. 5. Eosinophils.

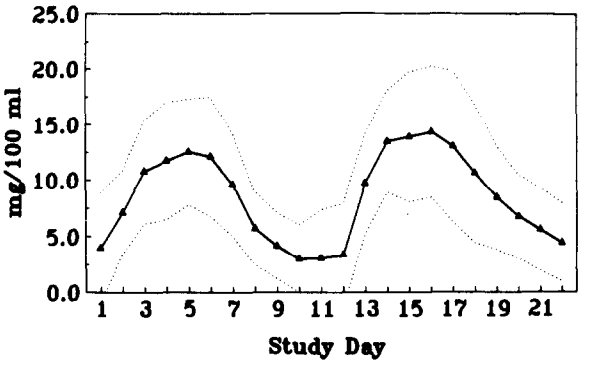

Fig. 6. C-Reactive protein.

Figs 1–6. Changes in body weight and hematological parameters during IL-2/LAK treatment. Mean (—▲—) and standard deviation (·····) of 27 courses of treatment.

13–100%) on days 12–19 resulting in a median total of 82% (range 46–100%). Hypotension with impaired renal function and central nervous system toxicity were the major reasons leading to dose modification. Of the cumulative dose of 1.17×10^6 U/kg (equivalent to approx. 46×10^6 U/m^2) planned to be administered during treatment, patients with renal cell carcinoma tolerated an average dose of 0.84×10^6 U/kg (equivalent to 32.8×10^6 U/m^2), the other patients 1.04×10^6 U/kg (equivalent to 38.3×10^6 U/m^2).

Antitumor effect

Objective partial responses were seen in three patients with renal cell carcinoma and one patient with melanoma (Table 4). In the patients with renal cell carcinoma (Nos 9Z, 10Z and 2B), maximal response was documented 1–3 months after completion of therapy and response durations were 5, 6 and 6 months respectively. In the patient with melanoma (No. 2Z) maximal response was seen on discharge with the disappearance of all skin lesions and the reduction in size of a nodal metastasis and response duration was 1 month. This patient had a mixed response when he was retreated upon relapse (No. 5Z).

DISCUSSION

This report summarizes the clinical experience of two centers with a phase I/II study of a slightly

modified IL-2 (rIL-2, r-met Hu IL-2 [ala-125], Ortho Pharmaceutical) and *in vitro* activated LAK cells in the treatment of metastatic solid tumors. The treatment schedule and LAK cell activation was similar to that initially described by Rosenberg *et al.* using IL-2 from another source [2, 3]. rIL-2-related clinical toxicity observed in our trial was considerable, though comparable to that of other reports using bolus administration of other recombinant IL-2 preparations [4, 5, 7]. The capillary leak syndrome with associated hypotension and impairment of renal function necessitated the use of vasopressors in 73% of our patients, a proportion similar to that observed by others [7]. Transitory impairment of renal function was more pronounced in patients with renal cell carcinoma than in other patients, presumably due to impaired baseline renal function because of nephrectomy or tumor involvement of the kidney [8]. CNS toxicity was manifested by cognitive changes and confusion. This form of CNS toxicity which cannot be well graded according to the current WHO criteria was also observed by others [9]. Refractory hypotension with reduced urinary output and confusion were the major dose limiting toxicities. Because of toxicity, rIL-2 scheduled to be given at 30,000 U/kg every 8 h had to be reduced in dose and some doses omitted completely during the last week on study, especially in patients with renal cell carcinoma. Dose reduction became necessary in most patients towards the last few days of treatment indicating the cumulative nature of rIL-2 toxicity. The average cumulative dose of rIL-2 given in our study was 0.94×10^6 U/kg (equivalent to 35×10^6 U/m^2) which represents in our experience the maximal tolerated dose for bolus administration in this schedule. This is somewhat over half of the average cumulative dose others were able to administer using a different recombinant IL-2 preparation [5, 7].

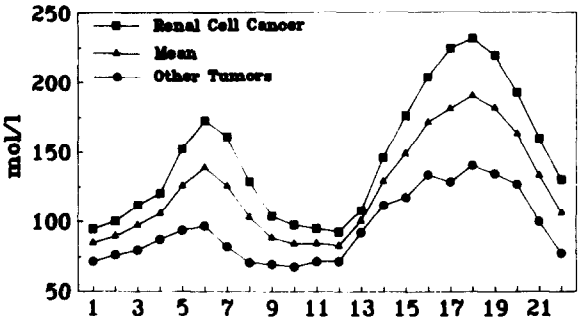

Fig. 7. Changes in serum creatinine during IL-2/LAK treatment. Mean values of 25 courses of treatment and comparison between 14 patients with renal cell carcinoma (—■—) and 11 other patients (—●—). Two patients with other tumors were excluded from analysis because of septic shock augmenting and prolonging renal impairment.

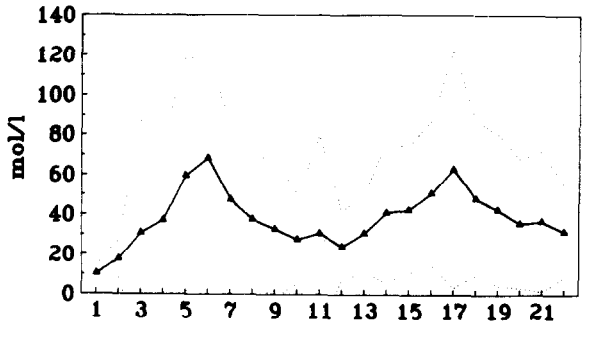

Fig. 8. Total bilirubin.

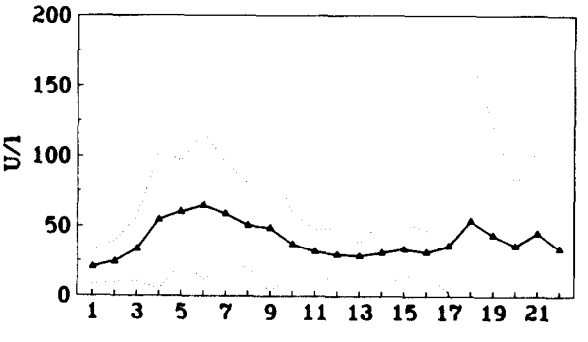

Fig. 9. SGOT.

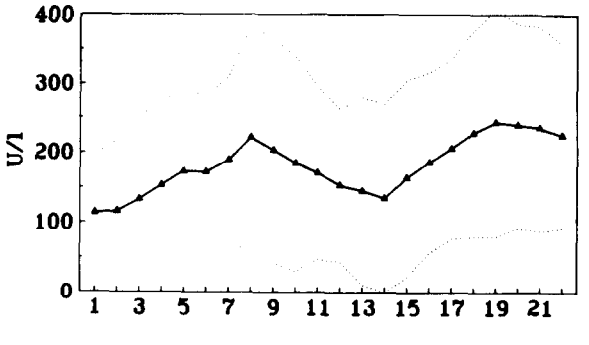

Fig. 10. Alkaline phosphatase.

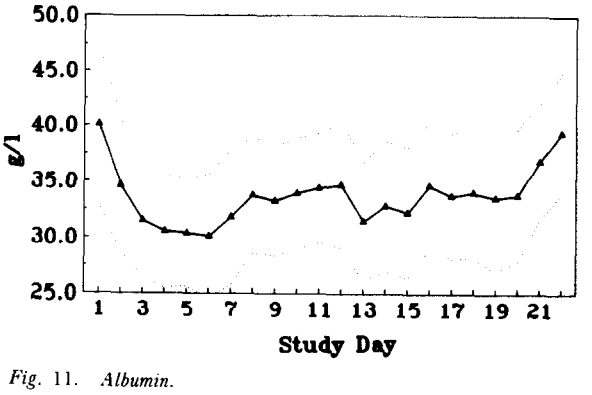

Fig. 11. Albumin.

Figs 8–11. Changes in parameters of liver function during IL-2/LAK treatment. Mean (—▲—) and standard deviation (·····) of 27 courses.

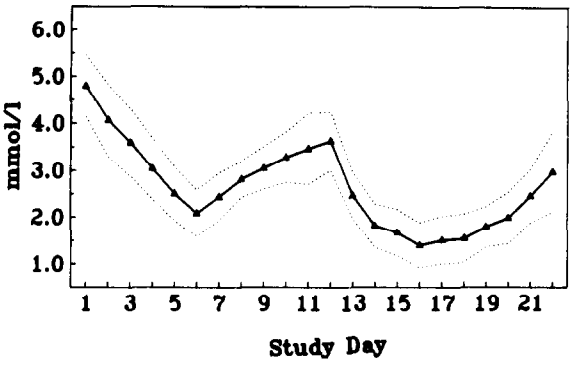

Fig. 12. Changes in serum cholesterol during IL-2/LAK treatment of patients treated in Zürich. Mean (—▲—) and standard deviation (·····) of 15 courses.

The antitumor effect of rIL-2 and LAK cells observed in our study is comparable to that reported by others and confirms the activity of this treatment on renal cell carcinoma and melanoma. In renal cell carcinoma our response rate was 21%, in melanoma 13%. The overall response rates in renal cell carcinomas to IL-2 with or without *in vitro* activated cells reported from larger series range from 14 to 23% [5, 7, 10, 11], response rates in melanoma from 19 to 26% [5, 12]. However, in contrast to other reports, no complete responses were observed in our study and responses did not last more than 6 months.

Considerably longer responses were seen by investigators who observed complete responses and/or retreated responding renal cell carcinoma patients with a second cycle of IL-2/LAK [5, 7]. Long lasting responses also have been observed in a series of patients with melanoma treated continuously

Table 3. LAK cell retransfusion and dose modification

No.	Dg	LAK cells ($\times 10^{10}$)	rIL-2 given	Omitted doses	Main reason(s)
9B	R	1.6	90%	4	RDF
1Z	R	8.8	85%	6	CNS, RDF
9Z	R	5.6	85%	2	RDF, hypotension
10Z	R	8.5	82%	2	RDF, CNS
12Z	R	8.3	77%	3	CNS, RDF
13Z	R	6.4	77%	6	CNS, hypotension
3B	R	2.7	72%	5	RDF
6B	R	1.9	71%	4	RDF
14Z	R	5.9	68%	10	CNS, hypotension
7Z	R	5.9	67%	1	RDF, hypotension
2B	R	1.5	64%	14	Coma, RDF, ARDS
12B	R	3.7	64%	14	Lung edema
15Z	R	4.2	59%	15	Sepsis, hypotension
7B	R	3.8	46%	21	ARDS
2Z	M	5.7	100%	0	
5Z*	M	7.7	100%	0	
6Z	M	7.2	100%	0	
4B	M	1.7	100%	0	
8Z	M	7.5	96%	1	CNS
1B	M	0.6	92%	3	Patient refusal
5B	M	1.5	85%	6	Lung edema
10B	M	6.3	82%	7	CNS
4Z	G	9.5	100%	0	
11Z	G	7.6	81%	0	Hypotension, CNS
3Z	G	5.1	51%	19	Sepsis
8B	CC	2.1	92%	3	Lung edema
11B	CC	4.7	74%	10	RDF, lung edema
Mean		5.1	68%	5.8	
Median		5.7	71%	4.0	
Range		0.5–9.5	46–100%	0–21	

*Retreatment of patient No. 2.

ARDS: adult respiratory distress syndrome; CNS: central nervous system toxicity; RDF: renal dysfunction with high creatinine and oliguria. For further abbreviations see Table 1.

Table 4. Antitumor effect

No.	Dg	Tumor mass (cm^2)		Response	Duration
		Pre-	Post-treatment		
1Z	R	144.0	144.0	NC	
7Z	R	3.1	3.4	NC	
9Z	R	68.9	33.6	PR	6 months
10Z	R	42.5	12.3	PR	5 months
12Z	R	35.2	20.9	NC	
13Z	R	7.7	7.1	NC	
14Z	R	28.8	25.0	NC	
15Z	R	10.4	10.4	NC	
2B	R	19.6	2.8	PR	6 months
3B	R	78.0	79.2	NC	
6B	R	16.0	23.5	PD	
7B	R	10.2	13.0	PD	
9B	R	86.2	86.7	NC	
12B	R	0.8	0.8, new lesion	PD	
2Z	M	7.7	1.2	PR	1 month
5Z*	M	12.5	6.6†	PD	
6Z	M	58.4	83.0	PD	
8Z	M	67.0	57.3, new lesion	PD	
1B	M	68.8	135.7	NC	
4B	M	27.4	36.2	PD	
5B	M	208.0	238.0	NC	
10B	M	64.8	76.2	NC	
3Z	G	55.8	63.6, new lesion	PD	
4Z	G	25.2	20.0	NC	
11Z	G	63.6	64.8, new lesion	PD	
8B	CC	20.6	20.2, new lesion	PD	
11B	CC	22.3	21.8	NC	
Total:		partial remission in 4/26 patients			(=15.3%)

*Retreatment of patient No. 2.
†A lymph node metastasis showed >25% increase of size.
NC: no change; PD: progressive disease; PR: partial remission. For further abbreviations see Table 1.

with lower doses of IL-2 in combination with cyclophosphamide [13]. Together, these observations indicate that more than one cycle of IL-2 treatment or continued IL-2 administration may be needed to achieve a prolongation of the beneficial antitumor effect for the patient. Not enough is known yet about the spectrum of activity of IL-2 in other tumors and such investigations will need to be expanded further. In our series, no antitumor effect was seen in patients with germ cell tumors refractory to chemotherapy and patients with colon carcinoma.

We do not yet know of any parameters that predict with certainty which patients with renal cell carcinoma or melanoma would respond to IL-2, although for renal cell carcinoma it has been suggested that pulmonary metastasis might respond better than extrapulmonary metastasis [7]. In our series, tumor response did not correlate with the height of rebound lymphocytosis, the number of LAK cells re-transfused or the dose of IL-2 administered.

The acute toxicity associated with this form of IL-2 treatment and the complexity of such a regimen using *in vitro* activation of lymphocytes warrant investigations into alternative ways to effectively administer IL-2. Also, major efforts will have to focus on improving the antitumor efficacy by reaching higher response rates and longer response durations. This might be accomplished by the combination of IL-2 with other agents such as low dose cyclophosphamide [13], monoclonal antibodies [14] or other biologicals such as interferon-alpha [15] and by using prolonged low dose treatment regimens such as have been found effective in the treatment of hairy cell leukemia with interferon-alpha. To achieve this goal, continued coordinated efforts and responsible clinical investigations will be necessary.

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