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Interleukin-2 Therapy for Refractory and Relapsing Lymphomas

Jean-Marc Tourani, Vincent Levy, Josette Briere, Rafaël Levy, Chris Franks and Jean-Marie Andrieu

Recombinant interleukin-2 (rIL-2) has been reported to be active in metastatic renal cell carcinoma and malignant melanoma. The purpose of this trial was to determine the efficacy and toxicity of rIL-2 administered in continuous infusion in patients with Hodgkin's disease (HD) and non-Hodgkin lymphoma (NHL). 21 patients with HD (4 patients), diffuse large-cell NHL (7) or low-grade NHL (10) in failure or relapse after multiple-conventional treatments were included in this trial. rIL-2 therapy consisted of an induction period of two cycles separated by 3 weeks of rest, and, in the absence of progressive disease or undue toxicity, a maintenance period of 4 monthly cycles. Each induction cycle comprised the continuous infusion of rIL-2: 18 × 10^6 IU/m^2 per day on days 1–5 and days 12–16. Each maintenance cycle comprised the continuous infusion of rIL-2: 18 × 10^6 IU/m^2 per day on days 1–5. Among the 21 treated patients, 5 (all of those with low-grade NHL) responded to the induction phase (1 complete response, 4 partial responses) and 2 patients had a mixed response. Conversely, no response was observed in patients with HD or large-cell NHL. The median duration of response was 4 months. rIL-2 administered as a continuous infusion was well tolerated and most patients received the full dosage, and management did not require intensive care. During the induction period, 2 patients experienced grade III cardiovascular or renal toxicity. During the maintenance period, rIL-2 had to be interrupted in 1 patient because of a myocardial infarction. This trial confirms the inefficacy of rIL-2 for the treatment of large-cell NHL and HD. Conversely, in low-grade NHL, rIL-2 activity needs to be explored by further studies. rIL-2 may have a place in the early phase of the disease, when the immune system is not compromised, as an adjuvant treatment in residual disease in order to improve the duration of response.
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INTRODUCTION

RECOMBINANT INTERLEUKIN-2 (rIL-2) has been first used in clinical trials in 1984 [1]. Since then, rIL-2, whether in bolus injections [2, 3] or in continuous infusions [4, 5] whether or not associated with lymphokine-activated killer cell (LAK) infusions, has been used for the treatment of solid tumours and particularly in metastatic renal cell carcinoma and malignant melanoma. With or without LAK infusions, rIL-2 can achieve a response rate of about 25% in metastatic renal cell carcinoma and 20% in malignant melanoma. Malignant lymphomas have sometimes been treated with rIL-2. However, histology is often not documented and the response rate cannot, at the present time, be determined. The purpose of this study is to determine the efficacy and toxicity of rIL-2 (18 × 10^6 IU/m^2 per day continuous infusion) in 21 patients suffering from refractory or relapsing lymphoma.

PATIENTS AND METHODS

Eligibility criteria and initial evaluation

21 patients were included in the study; 4 with Hodgkin's disease (HD) and 7 with large cell non-Hodgkin lymphoma (NHL) who failed to respond to second, third or fourth line therapy (refractory disease) or who experienced a second or third relapse. 10 patients with low-grade NHL were also treated. These patients were also relapsing or in failure after a second, third or fourth line therapy. All were free of treatment for at

least 30 days, were younger than 65, and had a Karnofsky performance status greater than 70. None presented severe disease incompatible with the treatment. All had a white blood cell count >3 10^9/l with platelets >50 10^9/l and haemoglobin >100 g/l.

Pretreatment evaluation included a complete physical examination with blood cell count, erythrocyte sedimentation rate (ESR), renal and hepatic chemistries, haemostasis evaluation, lactic acid dehydrogenase (LDH), chest X-ray, thoracic and abdominal computed tomography (CT), bone marrow biopsy, electrocardiogram and echocardiography.

Treatment

After being hooked up to a central line, patients were treated in an intensive care oncology–haematology unit, with sequential induction and maintenance therapy.

Induction period. The first induction cycle comprised the continuous infusion of rIL-2 (Eurocetus) at the dose of 18 MIU/m^2 per day days 1–5 (120 h) and days 12–16 (108 h). After 3 weeks of rest, a second induction cycle was performed under the same conditions.

Maintenance period. After the two induction cycles and 3-week rests, patients underwent maintenance therapy. Four maintenance cycles were performed separated by 3-week rests. Each maintenance cycle consisted of a 5-day (120 h) continuous infusion of 18 MIU/m^2 per day (days 1–5) of rIL-2.

Before each continuous infusion of rIL-2 a complete evaluation was performed including a physical examination with weight measurement, blood cell count, ESR, LDH, haemostasis, hepatic and renal chemistries, blood calcium and phosphorous levels, chest X-ray and electrocardiogram.

During rIL-2 administration, vital signs were regularly monitored: blood pressure every 2 h, temperature every 4 h, urine output every 6 h and weight every 12 h. Physical examination, blood cell count and renal chemistries were performed daily. Hepatic chemistries, blood calcium and phosphorous levels were controlled at day 1, 3 and 5 of each cycle. All patients were put on fluid restriction (≤11/24 h). Renal (serum creatinine ≥150 μmol/l) and cardiovascular (systolic blood pressure <85 mmHg) toxicities were managed with dopamine (3 or 10 μg/kg/min). No steroid therapy was authorised, in order to control general symptoms.

In case of disease progression or severe toxicity (WHO grade IV) treatment was interrupted either during the induction period or the maintenance period.

In case of grade III toxicity, IL-2 therapy was transiently interrupted and or administered at half-dosage.

Criteria of response

A complete response (CR) was defined by the disappearance of all clinical, radiological and histological signs of the disease. Partial response (PR) was a 50% or more decrease of measurable lesions without appearance of any new lesions, a mixed response (MR) was a complete or partial decrease of some measurable lesions with a minor decrease or stabilisation of other lesions without appearance of any new lesions and stable disease (SD) was an increase or a decrease in the tumour size less than 25% or a stabilisation of measurable lesions. Progressive disease (PD) was an increase in tumour size greater than 25%, or the appearance of new lesions.

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RESULTS

Patients' characteristics

From June 1988 to June 1990, 21 patients with HD or NHL were included in this study. There were 7 men and 14 women with a median age of 39 years (range 19–65).

4 patients presented with refractory HD clinical stage II (2 cases), III (1 case), IV (1 case) (Ann Arbor classification). Among the 17 patients with refractory (11 cases) or relapsing (6 cases) NHL, 7 presented with a large-cell lymphoma (Working Formulation 1982) [6] stage IV and 10 patients had a low-grade NHL stage IV. Among these low-grade lymphomas, there were 7 follicular small-cleaved cell lymphomas, 2 small lymphocytic lymphomas and 1 follicular mixed lymphoma (Table 1).

Treatment administration

Induction. Among the 21 patients included in this study, 6 received a single induction cycle (100% of the theoretical dose) because of a progressive disease under treatment. For the 15 remaining patients, the total dose administered for the two induction cycles was 100% in 10 patients and 97% in 3 other patients. In 2 patients, the doses administered were 75% and 64% because of a grade III toxicity leading to a transient interruption and a dose reduction (Table 1).

Maintenance. 7 responding patients (CR, PR, MR, all presenting with low-grade NHL) underwent the maintenance therapy. Among these, 4 received the four maintenance cycles at 100% of the theoretical dose and 3 patients only received two maintenance cycles because of a grade IV toxicity (1 patient) or for personal reasons (2 patients).

Response to treatment

Induction. After the induction period, 1 patient was in CR, 4 in PR, 2 in MR; 1 had SD and 13 PD (Table 1). The response to therapy was related to the histological type of lymphoma. The 4 patients with HD and the 7 patients with diffuse large-cell NHL experienced progressive disease during or at the end of the induction period. Conversely, among the 10 patients with low-grade NHL, 5 had a response (1 CR, 4 PR), 2 a MR, 1 SD and 2 a progression.

Maintenance therapy. The 7 responding patients underwent two maintenance cycles. Upon completion of these two cycles, the response to treatment remained unchanged (1 CR, 4 PR, 2 MR). Among the 4 patients who benefitted from two other maintenance cycles, the 3 patients in PR did not change their response status and the patient with CR showed progressive disease.

Duration of response. The duration of response was 16 weeks (range 9–21+) after its occurrence and 29 weeks (20–36+) after the onset of treatment.

Toxicity

Toxicity was evaluable in all 21 patients (36 cycles) during the induction period and in 7 patients (22 cycles) during the maintenance therapy. Toxicities are summarised in Table 2.

Table 1. Characteristics of patients

	Initial status				Induction			Maintenance (response)	
	Histology	Previous treatment	Pre IL-2r status	Stage	No. cycles	Dosage	Response	Two cycles	Four cycles
HD	2	4CT+RT	Refractory	IV	2	100%	PD	–	–
HD	2	3CT+RT	Refractory	II	1	100%	PD	–	–
HD	2	3CT+RT	Refractory	III	2	100%	PD	–	–
HD	3	2CT	Refractory	II	2	64%	PD	–	–
NHL	DLC	2CT	Refractory	IV	1	100%	PD	–	–
NHL	DLC	4CT+RT	Refractory	IV	1	100%	PD	–	–
NHL	DLC	2CT+RT	Relapse	IV	2	100%	PD	–	–
NHL	DLC	2CT+RT	Relapse	IV	2	97%	PD	–	–
NHL	DLC	2CT+RT	Refractory	IV	1	100%	PD	–	–
NHL	DLC	2CT+RT	Refractory	IV	1	100%	PD	–	–
NHL	DLC	2CT+RT+ABMT	Refractory	IV	2	100%	PD	–	–
NHL	FSC	4CT+RT	Relapse	IV	2	97%	CR	CR	PD
NHL	FSC	2CT+RT	Relapse	IV	2	100%	MR	MR	–
NHL	FM	2CT	Refractory	IV	2	100%	PR	PR	–
NHL	FSC	3CT	Refractory	IV	2	75%	PD	–	–
NHL	FSC	2CT	Relapse	IV	2	97%	PR	PR	PR
NHL	FSC	2CT	Refractory	IV	2	100%	MR	MR	–
NHL	FSC	4CT+RT	Refractory	IV	1	100%	PD	–	–
NHL	SL	3CT	Refractory	IV	2	100%	PR	PR	PR
NHL	SL	2CT	Refractory	IV	2	100%	SD	–	–
NHL	FSC	2CT	Relapse	IV	2	100%	PR	PR	PR

HD = Hodgkin's disease, NHL = non-Hodgkin lymphoma, DCL = diffuse large-cell lymphoma, FSC = follicular small-cleaved cell lymphoma, FM = follicular mixed lymphoma, SL = small lymphocytic lymphoma, 2CT, 3CT, 4CT = second, third and fourth-line chemotherapy, RT = radiotherapy, ABMT = autologous bone marrow transplantation, CR = complete response, PR = partial response, MR = mixed response, SD = stable disease, PD = progressive disease.

Table 2. Toxicity of treatment

Grade (WHO)	Induction (n = 21, 36 cycles)			Maintenance (n = 7, 22 cycles)		
	II	III	IV	II	III	IV
Fever/chills	3(14)	16(76)	–	1(5)	2(10)	–
Hypotension	4(19)	1(5)	–	1(5)	–	–
Blood nitrogen	2(10)	1(5)	–	1(5)	–	–
Rash	10(47)	1(5)	–	1(5)	–	–
Nausea/vomiting	2(10)	1(5)	–	1(5)	–	–
Myocardial infarction	–	1(5)	–	–	–	1(5)
Disorientation	–	–	–	–	–	–
Sepsis	1(5)	–	–	1(5)	–	–
Dyspnoea	2(10)	–	–	–	–	–
Cholestasis	1(5)	4(19)	–	1(5)	1(5)	–
Anaemia	6(29)	–	–	1(5)	–	–
Thrombocytopenia	4(19)	3(14)	–	1(5)	–	–

No. (%).

During the induction period, the rIL-2 dose had to be diminished in 2 patients because of a grade III toxicity (renal and cardiovascular).

Among the 7 patients who underwent the maintenance therapy, rIL-2 had to be interrupted in 1 patient because of a grade IV cardiac toxicity (myocardial infarction). The other severe toxicities (grade ≥II) were fever (n = 19), rash (n = 11) and haematological toxicity (n = 9). None of the patients experienced a gain in body weight in excess of 5% under therapy.

DISCUSSION

Recombinant interleukin-2 was first used in clinical trials in 1984 [1]. Since then, rIL-2 therapy has been used for the treatment of tumour with a bad prognosis and particularly in metastatic renal cell carcinoma and malignant melanoma. With or without LAK infusions, rIL-2 therapy can achieve a response rate of about 25% in metastatic renal cell cancer and 20% in malignant melanoma [2–5].

A careful review of the literature since 1985 [2, 4, 7–12] shows that 44 patients with NHL or HD have been treated with rIL-2 either alone or in association with LAK cell infusions or recombinant interferon. Response to treatment was evaluable in 42 (NHL:38/HD:4). 11 patients among the 38 with NHL (29%) experienced an objective response. Unfortunately, only Allison *et al.* [12] have mentioned the histological type of the 7 lymphomas in their study, and they observed a partial response in 2 patients with low-grade NHL and in 1 patient with intermediate-grade NHL. Among the 4 evaluable patients with HD, a partial response was achieved in only 1 patient.

Our study seems to confirm the inefficacy of rIL-2 for the treatment of refractory or relapsing intermediate-grade NHL or HD (no response in 11 patients). Conversely, among the 10 patients with low-grade NHL, 7 presented a tumoural response (1 CR, 4 PR, 2 MR) with a median duration of response of 4 months.

The antitumoural activity of rIL-2 in renal carcinoma and

malignant melanoma is likely to be related to the activation of the immune system [13] and particularly to the major histocompatibility complex (MHC)-restricted [14, 15] and non-restricted [16] cellular cytotoxicity. In the lymphoproliferative disorders of B-cell origin, there may be additional mechanisms of rIL-2 activity. The fact that about 75% of chronic lymphoid leukaemia cases and 60% of NHL express the CD25 antigen (TAC antigen/IL-2 receptor) on 20–80% of tumoral cells [17] raises the possibility that rIL-2 may act directly upon these tumoural cells. At the cellular level, rIL-2 may act directly on these tumoral B-cells with a negative control of malignant proliferation or indirectly by modulating oncoprotein or growth factor expressions which may be responsible, at least in part, for oncogenesis in these malignant proliferations [18–20]. Biological investigations must be developed in order to understand the mechanisms of activity of rIL-2 in such lymphoid system pathology.

The treatment of disseminated low-grade NHL stage IV is very controversial. Oral chlorambucil may achieve a CR in 40 to 60% of patients with a median relapse-free survival of 18–36 months. Combined chemotherapy—cyclophosphamide, vincristine, prednisone (CVP); cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP); bleomycin, doxorubicin, cyclophosphamide, vincristine, prednisone (BACOP)—improve the CR rate (65–85%) but without noticeable benefit for survival [21]. The combination of intensive chemotherapy and total lymphoid irradiation can lead to a CR of 78% at 4 years [22]. At the present time, the place of intensive chemotherapy with autologous or allogenic bone marrow transplantation is restricted to young patients presenting a disease with bad prognosis after achievement of CR [23, 24]. In these trials, the number of patients is too small and the follow-up too short to judge any benefit. On the other hand, recombinant interferon-alpha (rIFN-α) has been used in refractory or relapsed low-grade NHL and gives a response rate ranging from 40% to 55% with a median duration of response of around 8 months [25, 26].

The apparent efficacy of rIL-2 in low-grade NHL reported in this trial requires confirmation by other studies. The place of IL-2 therapy in this broad spectrum of treatment remains to be determined. Most types of treatment (except perhaps high-dose chemotherapy with bone marrow transplantation) give a high rate of relapses within the first 6 years [27]. The evaluation of antitumoural activity of rIL-2 in low-grade NHL needs further study. Recombinant IL-2 with or without rIFN-α, might be an adjuvant therapy of residual disease at an early stage when the immune system is not compromised. This may improve the duration of response since after conventional treatment, despite a high response rate, more than half of the patients relapse within 2–6 years. In addition, in spite of the good tolerance of rIL-2 administered as a continuous infusion, in this chronic disease, the subcutaneous administration of rIL-2 described by Atzpodien *et al.* [7] can be envisaged because of its long-term good tolerance.

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Growth Factor Requirements of Human Colorectal Tumour Cells: Relations to Cellular Differentiation

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Human colorectal tumour lines that exhibit different degrees of differentiation were studied to define their growth requirements. The poorly differentiated cell lines SW620, SW480, SW48 and SW837 proliferated in Dulbecco's modified Eagle's medium without exogenously added growth factors. In contrast, the moderately differentiated cell lines SW1222, HT29, PC/JW and LS174T proliferated only in medium supplemented with growth factor. SW1222 and HT29 cells required transferrin for growth, which was improved by other growth-promoting factors including epidermal growth factor (SW1222) and sodium selenite (HT29). PC/JW and LS174T required both insulin and transferrin for optimal growth. The tumour cell lines could be passaged continuously in serum-free medium supplemented with growth factor and in some cases they grew better than in serum-supplemented medium. The serum-free growth conditions should prove useful for studies on differentiation in colorectal cell lines and their interactions with growth factors.
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INTRODUCTION

THE PROLIFERATION of cells is regulated by a complex interplay of growth-stimulating and inhibitory factors, including polypeptide growth factors and extracellular matrix proteins [1–6]. Many tumour cells in culture exhibit a marked reduction in their requirement for exogenous growth factors and an increased ability to produce growth factors that act in an autocrine or paracrine fashion [1–3, 7]. Hence, defining the particular growth factor requirements of different cell types may help understanding the normal pattern of growth control, differentiation and subsequent changes during tumour progression.

Colorectal cancer is, overall, the second most frequent cancer in the developed world [8]. New approaches are needed to improve its prevention and treatment and these are most likely to come from a better understanding of the fundamental cell and molecular biology of normal and abnormal colorectal epithelium [9]. As it is difficult to grow normal colorectal epithelium in culture [10], long-term cultures of tumour cells provide a model system to study their dependence upon exogenously added growth-promoting factors.

The study presented here defines the growth factor requirements of several human colorectal tumour cell lines of different degrees of differentiation within a particular range of passages (Table 1). The results show that poorly differentiated human colorectal cell lines can be distinguished from moderately differentiated cell lines in their ability to proliferate in serum-free medium.

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MATERIALS AND METHODS

Cells

Cell lines used for these experiments were SW620, SW837, SW480, SW48, SW1222 [11]; HT29 [12]; LS174T [13] and PC/JW [14]. Cell lines SW480 and SW620 were derived from the same patient; SW480 was derived from a colon adenocarcinoma whereas SW620 was from a lymph node metastasis. For particular cell characteristics and passages used for this study, see Table 1.

Media

The cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Gibco, Europe), glutamine 2 mmol/l, penicillin 100 U/ml, streptomycin 100 μg/ml (DMEM-10). Several media, namely Ham's F12, RPMI-1640, McCoy's 5A, MCDB104, Waymouth's and DMEM (all from Gibco Europe), were tested on the cell lines for growth in different concentrations of FCS (10%, 5%, 2% and 0).

The serum-free conditions [15] were studied according to the method of Murakami and Masui [16]. Briefly, the media were supplemented with insulin (2 μg/ml), transferrin (2 μg/ml), hydrocortisone (10 ng/ml), epidermal growth factor (EGF: