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The Out-Patient Use of Recombinant Human Interleukin-2 and Interferon Alfa-2b in Advanced Malignancies

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We studied the safety, tolerance, and clinical effects of the combined administration of subcutaneous recombinant human interleukin-2 and interferon alfa-2b in 54 patients with advanced cancer, for whom no effective standard therapy was available. Treatment courses consisted of a 2-day interleukin-2 pulse (14.4-18 million units (MU) m²/day), followed by 3.6 up to 4.8 MU/m²/day, 5 days per week, over 6 consecutive weeks and interferon alfa-2b at 3 up to 6 MU/m², administered two-three times weekly for 6 weeks. Overall, patients received more than 90% of the projected dose of interleukin-2 and interferon alfa-2b, respectively. Of 54 evaluable patients (32 renal cell cancer, 12 melanoma, eight colorectal cancer, one B-cell lymphoma, one Hodgkin's disease), four complete responses occurred in patients with renal cell carcinoma, and a greater than 50% reduction in tumour size (partial response) in six renal cell carcinoma patients and one melanoma patient. Moreover, 21 patients (13 renal carcinoma) had stable disease. The median duration of response was 19 months (range 16-22 months) in complete responders. Clinical responses were associated with a mean peripheral blood eosinophil count of more than 1,000/μL ($P < 0.05$ versus non-responders). Systemic toxicities included fever, chills, nausea, anorexia, and hypotension limited to WHO grades I and II in more than 80% of patients treated. No treatment-related deaths occurred. This combination of subcutaneously administered recombinant interleukin-2 and interferon alfa-2b has significantly diminished the side effects normally observed with high-dose intravenous recombinant interleukin-2, which requires admission to hospital. It has been shown to induce objective tumour regression in out-patients with progressive metastatic renal cell carcinoma and malignant melanoma.
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INTRODUCTION

OBJECTIVE REMISSIONS have been reported in patients with metastatic cancer who received high-dose intravenous (i.v.) bolus recombinant interleukin-2 (rIL-2), in conjunction with autologous lymphokine-activated killer cells (LAK) [1]. This was the first time that therapeutic activation of host immune defence mechanisms induced regression of established human malignancies. As a result of these initial observations, numerous clinical studies have been carried out to enhance further the clinical efficacy of rIL-2-based cancer immunotherapy. These trials have investigated the use of i.v. rIL-2 alone, in combination with LAK, or with tumour infiltrating lymphocytes (TIL), thus exploiting broad cell-mediated cytotoxic reactivity against various human tumours [2-6]. Promising results have been reported in patients with metastatic renal cell carcinoma and malignant melanoma [1-6]. However, the severity of adverse reactions (life-threatening fluid retention, hypotension and pulmonary oedema) has primarily limited this therapeutic approach to the in-patient and/or intensive care setting, depending on the schedule used [5, 7, 8].

Previously, rIL-2 has been given i.v. by 8-hour bolus [1, 2, 4], or by continuous infusion [3], with comparable therapeutic results. Significant differences were observed in systemic toxicity, since most bolus-treated patients received therapy in intensive care, whereas the majority of the continuous infusion-treated patients received therapy in the standard oncology ward. Rarely, other routes of administration have been evaluated, with the prolonged systemic application of rIL-2 at lower doses [9, 10]. More recent studies have demonstrated evidence of synergism between rIL-2 and recombinant alpha interferon in a variety of experimental models [11]. Recombinant interferon (rIFN) alfa-2b alone has shown some therapeutic efficacy in patients with renal cell carcinoma and malignant melanoma [12-14], but little clinical information has been reported using the combination of rIL-2 and recombinant alpha interferon [15-17].

In the present clinical trial, we developed a subcutaneous rIL-2 schedule, in combination with rIFN alfa-2b, in order to evaluate the possibility of reducing drug-induced toxicity, whilst retaining optimal treatment intensity and therapeutic efficacy in patients with advanced progressive cancer. A total of 54 out-patients were treated. The safety, tolerance, and clinical results are summarized in this paper.

METHODS

Patients

Fifty-four patients with progressive metastatic cancer were

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treated on this protocol. Thirty-two had renal cell carcinoma, 12 malignant melanoma, eight colorectal cancer, one patient B-cell lymphoma and one Hodgkin's disease. All patients had histologically confirmed tumours, and clinically evaluable progressive disease, which was refractory to available standard therapy. Prior anti-neoplastic treatment of renal cell carcinoma patients included surgery (n = 32), chemotherapy (n = 4), radiotherapy (n = 9), hormonal therapy (n = 6), and immunotherapy (n = 5) (Table 1).

Table 1. Subcutaneous rIL-2 plus recombinant interferon (rIFN) alfa-2b in metastatic renal cell cancer: patient characteristics

Sex	
Male	19
Female	13
Age	
Median	52 years
Range	23-69 years
Pretreatment therapy	
Surgery	32
Chemotherapy	4
Radiotherapy	9
Hormones	6
Immunotherapy	5

The eligibility criteria included an expected survival of greater than 3 months, Karnofsky performance status of ≥ 70%, adequate organ function as defined by white blood count ≥ 3500 μL, platelet count ≥ 100,000/μL, haematocrit ≥ 28%, a creatinine clearance ≥ 60 ml/min, absence of congestive heart failure, coronary artery disease, or serious cardiac arrhythmias, and a forced expiratory volume of more than 2 litres in 1 second (or ≥ 75% of that predicted for height and age). Patients were excluded when there was evidence of central nervous system disease, a history of seizure disorders, or serious active infections, including positivity for human immunodeficiency virus or hepatitis B surface antigen. Patients receiving corticosteroids were not eligible. No prior chemotherapy or immunomodulatory therapy was permitted during the prior 4 weeks. The concomitant use of prostaglandin E2 synthesis inhibitors was not allowed. Signed informed consent was obtained from each patient before any treatment was administered.

Recombinant interleukin-2 and interferon rIFN alfa-2b

rIL-2 was provided by EuroCetus (Amsterdam, The Netherlands). This material has a specific activity of approximately 18 million international units (MU) (equivalent to 3 million Cetus units)/mg of protein.

rIFN alfa-2b was supplied by Essex Pharma (München, Germany). Biological activity was measured at 1 MU per 6 μg of recombinant interferon rIFN alfa-2b.

Treatment plan and patient evaluation

All patients were treated with subcutaneous rIL-2 and rIFN alfa-2b. Recombinant interleukin-2 and interferon rIFN alfa-2b were both self-administered on an out-patient basis. Patients received a 2-day IL-2 pulse of 14.4 to 18 MU/m²/day, followed by 3.6-4.8 MU/m²/day, 5 days per week, over 6 consecutive weeks, and interferon rIFN alfa-2b at 3 up to 6 MU/m², administered two to three times weekly for 6 weeks. Treatment courses were repeated unless progression of disease occurred.

Re-evaluation of the patient's tumour status was performed at 10-week intervals. A complete remission was defined as the disappearance of all clinical and laboratory signs of disease for a minimum of 4 weeks, a partial remission as a minimum of 50% reduction in the sum of the products of the greatest perpendicular diameters of measurable lesions without an increase in size of any lesion, or the appearance of new lesions, stable disease as less than a partial response in the absence of disease progression for at least 8 weeks, and disease progression as an increase of at least 25% in the sum of the products of the longest perpendicular diameters of measurable lesions, or the development of new lesions.

Evaluation of patient sera

To quantitate serum levels of anti-IL-2 and anti-alpha interferon IgG antibodies, an indirect enzyme-linked immunosorbent assay (ELISA) was used as reported previously [18].

For the detection of the potential presence of neutralizing activity against rIL-2, patient sera were heat inactivated (52°C, 30 minutes), and subsequently tested for inhibition of mouse cytotoxic T-lymphocytes employing a ^{3}H-thymidine proliferation assay. A neutralizing titre > 100, defined as a fold reduction of IL-2 induced proliferation times the final serum dilution, was considered positive [19].

Haematological studies

The haematological effects of rIL-2 were evaluated, using differential blood counts, obtained from all patients at weekly intervals.

Statistical analyses

Statistical significance was assessed using t-test and paired t-test analyses.

RESULTS

In the present clinical trial, 54 patients received a total of 81 courses of therapy. More than 90% of the projected total doses of rIL-2 and rIFN alfa-2b, respectively, were given to patients.

Treatment response

Of the 54 evaluable patients, four had a complete response and seven partial remissions. Objective tumour regressions were observed in renal cell carcinoma (n = 10; objective remission rate 31%, complete response 13%), and malignant melanoma (n = 1; objective remission rate 8%) (Table 2). The responses occurred in lungs, liver, lymph nodes, bone and pleural metastases. No partial or complete remissions were seen in patients with colorectal cancer, B-cell lymphoma, or Hodgkin's disease (Table 2). In all renal cell carcinoma patients who responded to treatment, objective tumour remissions occurred during the first treatment cycle. The median response duration was 19+ months (range, 16 to 22 months) in patients achieving complete tumour regression. All four complete responders are off therapy and continue to be disease free.

Therapeutic efficacy was not diminished by the emergence of neutralizing antibodies to rIL-2 (n = 4) or recombinant

Table 2. Subcutaneous rIL-2 plus recombinant interferon (rIFN) alfa-2b: clinical responses

Tumour type	Evaluable	CR	PR	SD	PD
Renal cell carcinoma	32	4	6	13	9
Melanoma	12	-	1	3	3
Colon carcinoma	8	-	-	3	5
Lymphoma	2	-	-	2	-
Total	54	4	7	21	17

All patients evaluable completed at least one cycle of treatment. Lymphoma included B-cell lymphoma (n = 1) and Hodgkin's disease (n = 1).
CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease.

interferon rIFN alfa-2b (n = 0), and none of the 54 patients demonstrated serum neutralizing activity during the first treatment course. While neutralizing activity was rarely found, non-neutralizing anti-rIL-2 serum antibodies developed in up to 40% of the patients treated.

Toxicity

The systemic toxicity of subcutaneous rIL-2 and rIFN alfa-2b in this study is summarized in Table 3. A total of 81 cycles were evaluated for treatment-related adverse events. Toxicity was moderate, and hospitalization was not required.

Table 3. Systemic toxicity of subcutaneous rIL-2 and recombinant interferon (rIFN) alfa-2b

Side effect	No. of treatment cycles			
	WHO Grade I	Grade II	Grade III	Grade IV
Fever	2	76	0	0
Anorexia	38	32	1	0
Chills	41	17	0	0
Malaise	53	19	5	0
Nausea/vomiting	36	28	1	0
Hypotension	15	5	0	0
Diarrhoea	18	11	2	0
Liver toxicity	26	7	1	0
Respiratory distress	11	17	2	0
Arrhythmias	0	2	0	0
Peripheral polyneuropathy	3	1	0	0
Alopecia	7	2	0	0

A total of 54 patients, and 81 treatment cycles were evaluated for toxicity.

WHO grade I or II fevers, chills, and malaise were very common and occurred in 78, 58, and 72 of the 81 treatment courses, respectively. Anorexia (grade I/II) was seen in 70 cycles, and was frequently associated with nausea and vomiting (64 treatment courses), and/or diarrhoea (39 treatment courses). No increases in the serum bilirubin were observed in patients without metastic liver disease. However, transient elevations of the serum alkaline phosphatase or serum transaminases occurred in 34 treatment cycles. Hypotension was noted in 20 of the 81 courses, and was mild. Therapy-related respiratory distress was observed in 30 cycles, but was not associated with radiological evidence of pulmonary fluid retention. Cardiac side effects included a left bundle branch block in one patient, and ventricular extrasystole in a second patient. In all patients, systemic toxicity resolved after termination of treatment. Capillary leak-induced fluid retention and weight gain did not occur. No significant haematological toxicity was noted. None of the patients exhibited treatment-related toxicities of the central nervous system.

Of the 54 patients, 32 were evaluated for potential thyroid dysfunction. During the first course, laboratory evidence of hypothyroidism and hyperthyroidism was obtained in four and 15 patients, respectively; seven of the 15 patients presenting with hyperthyroidism had normal levels of serum thyroxine (T4) and tri-iodothyronine (T3), but showed a significant decrease in spontaneous and thyrotrophin-releasing hormone-induced thyrotrophin secretion. Anti-thyroid treatment was required in one patient. Treatment-induced thyroid dysfunction was transient, and usually resolved within 4 weeks after cessation of therapy (data not shown).

At the injection sites, the subcutaneous administration of rIL-2 resulted in transient inflammation and local induration which persisted for up to 2 weeks following treatment. However, none of the patients considered this to be unacceptable.

Haematological effects

Peripheral lymphocytes increased approximately 1.6-fold during the 7-week treatment cycle. Peripheral blood eosinophils increased overall from 248/μL on day 0, to 652/μL after 7 weeks of treatment. A comparison of patient subgroups revealed that a significant expansion of eosinophils ($P < 0.02$) occurred in responding and stable disease patients.

DISCUSSION

In the present clinical trial, we evaluated an out-patient regimen, using combined administration of rIL-2 and rIFN alfa-2b, in patients with advanced malignancy. The biological and therapeutic mechanisms by which IL-2 and alpha interferon mediate tumour regression are not yet fully understood. However, it has been postulated that alpha interferon may augment IL-2-induced killing through activation of cytotoxic lymphocytes, and via enhanced expression of major histocompatibility complex class I antigens on tumour cells [11, 20, 21].

The majority of objective tumour regressions have occurred in patients with progressive metastatic renal cell carcinoma. Previous therapeutic strategies in this tumour type have included hormonal therapy (responses of less than 5%) [22], and chemotherapy (response below 10% for vinblastine and other cytotoxic agents [23]). Although drugs such as interferon have been reported to achieve objective remission rates of between 5% and 27%, the overall major response rate, in patients with metastatic renal cell carcinoma, is approximately 16% [12, 13, 24, 25]. In a clinical study conducted by Rosenberg and colleagues, the systemic administration of rIL-2 by i.v. bolus, using approximately 72 MU/m² daily together

with LAK, resulted in a response rate of 33%, with several complete remissions and a median duration of response of 5+ months [2]. Remission rates of between 0% and 18% have been reported with rIL-2 alone [2, 26, 27]. While demonstrating clinical efficacy, high-dose i.v. bolus rIL-2 has been associated with substantial toxicity and morbidity [1, 2, 4, 7, 8], requiring treatment in intensive care.

When administering rIL-2 as a continuous infusion i.v. at doses of 18 MU/m² daily, West and colleagues have reported reduced rIL-2-related adverse effects [3]. With response rates comparable to those obtained using bolus rIL-2, the safety and tolerability of the treatment was increased to a degree where the majority of patients could receive rIL-2 in the standard oncology ward [28].

In this prospective clinical study, low-dose subcutaneous rIL-2 was used with a standard dose of recombinant interferon rIFN alfa-2b. The overall toxicity of the combination was low, even when compared to the i.v. administration of single agent rIL-2. Furthermore, low-dose rIL-2 combined with recombinant interferon rIFN alfa-2b achieved clinical efficacy in patients with metastatic renal carcinoma (13% complete remission, 18% partial remission), comparable to the most effective rIL-2 regimen available [2]. However, all the patients could be treated as out-patients. Treatment-related toxicity was primarily limited to WHO grades I and II, most patients experiencing flu-like symptoms, e.g., general malaise, fever, and nausea. As in previous studies [29], rIL-2-induced capillary leak syndrome, interstitial oedema, hypoalbuminaemia, hypotension, and weight gain were not observed.

Results from this trial indicate that long-term out-patient rIL-2 and rIFN alfa-2b, as used here, can induce prolonged tumour regressions in patients with metastatic renal cell carcinoma, with a median response duration of 19+ months. The percentage of patients achieving stable disease (41%) further suggests that combination therapy results in patient populations free from tumour progression similar to those reported in short-term i.v. rIL-2 protocols [28]. Sustained follow up is necessary to assess progression-free and overall survival duration in these patients, and also to determine whether the long-term results are comparable to those with i.v. regimens [2, 3, 28].

Since the patients in this study have been treated using an out-patient schedule, with markedly reduced toxicity, this therapeutic regimen appears to have a favourable risk-benefit ratio.

1. Rosenberg SA, Lotze MT, Muul LM *et al.* Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* 1985, **313**, 1485-1492.
2. Rosenberg SA, Lotze MT, Muul LM *et al.* A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 1987, **316**, 889-897.
3. West WH, Tauer KW, Yannelli JR *et al.* Constant-infusion recombinant interleukin-2 in an adoptive immunotherapy of advanced cancer. *N Engl J Med* 1987, **316**, 898-905.
4. Fisher RI, Coltman CA, Doroshow JH *et al.* Metastatic renal cancer treated with interleukin-2 and lymphokine-activated killer cells. *Ann Intern Med* 1988, **108**, 518-523.
5. Rosenberg SA, Lotze MT, Mulé JJ. New approaches to the immunotherapy of cancer using interleukin-2. *Ann Intern Med* 1988, **108**, 853-864.
6. Rosenberg SA, Packard BS, Aebersold PM *et al.* Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *N Engl J Med* 1988, **319**, 1676-1680.
7. Rosenstein M, Ettinghausen SE, Rosenberg SA. Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin-2. *J Immunol* 1986, **137**, 1735-1742.
8. Lee RE, Lotze MT, Skibber JM *et al.* Cardiorespiratory effects of immunotherapy with interleukin-2. *J Clin Oncol* 1989, **7**, 7-20.
9. Kolitz JE, Wong GY, Welte K *et al.* Phase I trial of recombinant interleukin-2 and cyclophosphamide: augmentation of cellular immunity and T-cell mitogenic response with long-term administration of rIL-2. *J Biol Response Mod* 1988, **7**, 457-472.
10. Atzpodien J, Körfer A, Evers P *et al.* Low dose subcutaneous recombinant interleukin-2 in advanced human malignancy: a phase II outpatient study. *Mol Biother* 1989, **2**, 18-26.
11. Atzpodien J, Shimazaki C, Wisniewski D *et al.* Interleukin-2 und Interferon-α in der adoptiven Immuntherapie des Plasmozytoms: Ein experimentelles Modell. In: Lutz D, Heinz R, Nowotny H, Stacher A, eds. *Leukämien und Lymphome.* München, Wien, Baltimore, Urban and Schwarzenberg, 1988, 211-212.
12. Vugrin D, Hood L, Taylor W. Phase II study of human lymphoblastoid interferon in patients with advanced renal cell carcinoma. *Cancer Treat Rep* 1985, **69**, 817-820.
13. Quesada JR, Rios A, Swanson DA. Antitumor activity of recombinant-derived interferon alpha in metastatic renal cell carcinoma. *J Clin Oncol* 1985, **3**, 1522-1528.
14. Legha SS. Interferons in the treatment of malignant melanoma. A review of recent trials. *Cancer* 1986, **57**, 1675-1677.
15. Lee KH, Talpaz M, Rothberg JM *et al.* Concomitant administration of recombinant human interleukin-2 and recombinant interferon α-2a in cancer patients: a phase I study. *J Clin Oncol* 1989, **7**, 1726-1732.
16. Rosenberg SA, Lotze MT, Yang JC *et al.* Combination therapy with interleukin-2 and alpha-interferon for the treatment of patients with advanced cancer. *J Clin Oncol* 1989, **7**, 1863-1874.
17. Atzpodien J, Körfer A, Franks CR, Poliwoda H, Kirchner H. Home therapy with recombinant interleukin-2 and interferon-α2b in advanced human malignancies. *Lancet* 1990, **335**, 1509-1512.
18. Engvall E, Perlman P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971, **8**, 871-874.
19. Gillis S, Ferm MM, Ou W. T cell growth factor: parameters of production and a quantitative microassay for activity. *J Immunol* 1978, **120**, 2027-2032.
20. Spiegel RE. The alpha interferons: clinical overview. *Semin Oncol* 1986, **13**, 207-217.
21. Brunda MJ, LeBellantoni D, Sulich V. In vivo anti-tumor activity of combinations of interferon alpha and interleukin-2 in a murine model. Correlation of efficacy with the induction of cells resembling natural killer cells. *Int J Cancer* 1987, **40**, 3948-3953.
22. Harris DT. Hormonal therapy and chemotherapy of renal cell carcinoma. *Semin Oncol* 1983, **10**, 422-430.
23. Krown SE. Therapeutic options in renal cell carcinoma. *Semin Oncol* 1985, **12**, 13-17.
24. Edsmyr F, Esposits PL, Anderson L. Interferon therapy in disseminated renal cancer. *Radiother Oncol* 1985, **4**, 21-26.
25. Muss HB. Interferon therapy for renal cell carcinoma. *Semin Oncol* 1987, **14**, 36-42.
26. Whitehead RP, Ward DL, Hemingway LL. Effect of subcutaneous recombinant interleukin 2 in patients with disseminated renal cell carcinoma. *Proc Am Soc Clin Oncol* 1987, **6**, 241.
27. Sosman JA, Kohler PC, Hank J. Repetitive weekly cycles of recombinant human interleukin-2: Responses of renal carcinoma with acceptable toxicity. *J Natl Cancer Inst* 1988, **80**, 60-63.
28. Negrier S, Philip T, Stoter G *et al.* Interleukin-2 with or without LAK cells in metastatic renal cell carcinoma: a report of a European multicentre study. *Eur J Cancer Clin Oncol* 1989, **25**, 21-28.
29. Allison MA, Jones SE, McGuffey P. Phase II trial of outpatient interleukin-2 in malignant lymphoma, chronic lymphocytic leukemia, and selected solid tumors. *J Clin Oncol* 1989, **7**, 75-80.