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Second Line Therapy With Low-dose Subcutaneous Interleukin-2 Alone in Advanced Renal Cancer Patients Resistant to Interferon-alpha

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Interleukin-2(IL-2), given subcutaneously with interferon-alpha, induces clinical results similar to those achieved with intravenous administration in advanced renal cancer but with lower toxicity. This study was performed to investigate the efficacy of IL-2 subcutaneous therapy alone in advanced renal cancer patients pretreated with interferon-2 alpha. The study included 13 evaluable patients, 6 of whom had visceral metastasis sites. The cycle consisted of IL-2 at 9 × 10^6 IU/m^2 twice daily for 2 days, followed by 1.8 × 10^6 IU/m^2 every 12 h for 5 days/week for 6 weeks. Clinical responses were: partial response: 4(31%); stable disease: 7(54%), progressive disease: 2(15%). The median duration of response was 9^+ months (range 6^+–12^+). Toxicity was low in all patients, and in particular no important cardiovascular side-effect was seen. The results of this study show that IL-2 subcutaneous therapy alone is an effective and well tolerated treatment in advanced renal cancer patients progressed under interferon-alpha therapy.
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INTRODUCTION

RENAL CARCINOMA is one of the solid neoplasms most responsive to immunotherapy with interleukin-2 (IL-2) [1–4]. Similar response rates of 30% have been found with IL-2 intravenous administration, either as a bolus [1], or as a 24-h continuous infusion [2]. The role of the concomitant administration of LAK cells, which had been considered as essential to obtain tumour regressions in the preliminary clinical studies, has appeared to be not fundamental in successive studies [3, 4] and, at present, it is known that IL-2 may be active also when it is given intravenously alone. More recently, IL-2, given subcutaneously in association with interferon-alpha, has been proven to induce a percentage of tumour regressions comparable to that reported with the intravenous route of administration [5], but with a lower toxicity; in particular, the cardiovascular side-effects are very moderate with IL-2 subcutaneous therapy, which may be proposed as a home therapy [5]. At present, however, it is still unknown whether IL-2 subcutaneous therapy may be active in the treatment of advanced renal cancer also when it is given alone without a concomitant injection of interferon-alpha, which has been considered to enhance IL-2 efficacy by stimulating class I histocompatibility antigen expression on tumour cells [6].

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The present study was performed to evaluate the efficacy of a subcutaneous immunotherapy with IL-2 alone, as a second line treatment in advanced renal cancer patients, who did not respond to a first line therapy with interferon-alpha with or without vinblastine.

PATIENTS AND METHODS

The study included 14 consecutive advanced renal cancer patients (M/F:9/5), followed at Radiotherapy Division of Monza Hospital. Their median age was 59 years (range 24–73). All patients were pretreated with a first line therapy, consisting of interferon-alpha plus vinblastine, and all of them progressed under the therapy. Interferon was given intramuscularly at a dose of 18 × 10^6 U three times/week, and vinblastine was injected intravenously at a dose of 0.1 mg/kg body weight every 21 days. The median duration of therapy was 7 months (range 3–10). Eligibility criteria to be admitted to IL-2 subcutaneous therapy were, as follows: histologically proven advanced renal adenocarcinoma, measurable lesions, progression in response to a first line therapy consisting of interferon plus vinblastine for at least 3 months, life expectancy greater than 3 months. Patients with second neoplasms, brain metastases or important cardiovascular diseases were not included in the study. Dominant metastatis sites were soft tissues in 3 patients, bone in 5 and visceral lesions in the remaining 6 patients (lung: 4; lung plus liver: 2).

Human recombinant IL-2 was supplied by Euro-Cetus (Amsterdam, Holland). According to the doses proposed by Atzpodien *et al.* [5], IL-2 was given subcutaneously at a dose of

Table 1. Clinical data and response to IL-2 subcutaneous therapy in 13 evaluable advanced renal carcinoma

Cases	Sex	Age	P.S.	Metastasis sites	Clinical response*	Response duration (months)	Sites of response	Sites of progression	Survival (months)
1	F	55	70	Lung, bone	PD	—	—	Lung	8
2	M	60	80	Lung, liver	PR	12^+	Lung, liver	—	12^+
3	F	68	100	Bone	SD	8^+	—	—	8^+
4	M	59	70	Lung	PD	—	—	Lung	7
5	M	67	80	Soft tissues	PR	10^+	Soft tissues	—	10^+
6	F	52	90	Bone, soft tissues	SD	8	—	Soft tissues	9^+
7	M	24	100	Soft tissues	PR	8^+	Soft tissues	—	8^+
8	M	49	90	Bone	SD	7^+	—	—	7^+
9	F	73	70	Soft tissues	SD	5	—	Soft tissues	7^+
10	M	64	70	Liver, lung	SD	4	—	Liver	5
11	M	52	80	Lung, bone	PR	6^+	Lung	—	6^+
12	F	51	90	Bone	SD	4^+	—	—	4^+
13	M	59	100	Lung	SD	3^+	—	—	3^+

*PR: partial response; SD: stable disease; PD: progressive disease.

9×10^6 IU/m^2 every 12 h for 2 days, as an induction phase, followed by 1.8×10^6 IU/m^2 twice daily for 5 consecutive days/week for 6 weeks, corresponding to one IL-2 cycle. IL-2 was injected into different parts of the abdominal wall. The rationale for the induction phase with higher doses of IL-2 was related to the results previously reported in the literature [1–5], suggesting that the immune activation and IL-2 receptor expression are IL-2 dose-dependent phenomenons. On the contrary, in respect to the schedule shown by Atzpodien *et al.* [5], we decided to inject IL-2 alone without interferon, because of the progression of patients during its previous administration. IL-2 immunotherapy was started at least 1 month after the progression during interferon and vinblastine therapy. Clinical response and toxicity were evaluated according to WHO criteria, by repeating radiological examinations every month during IL-2 cycles, then every 10 weeks during the maintenance phase. Liver metastases were evaluated by CT scan. Complete response (CR) was defined as complete resolution of all clinically evaluable disease for at least 1 month; partial response (PR) as at least 50% reduction in the sum of the products of the longest perpendicular diameters of measurable lesions for at least one month; stable disease (SD) as no objective response or progression greater than 25% in the sum of the products of the longest perpendicular diameters of lesions for at least 3 months; progressive disease (PD) as an increase of at least 25% in the sum of the products of the longest perpendicular diameters of all measurable lesions, or the appearance of new lesions. In patients with response or SD, a second cycle of IL-2 was given after 28 day rest period. After that, patients with response or SD underwent a maintenance period, consisting of IL-2 at 1.8×10^6 IU/m^2 twice daily for 6 consecutive days every month, until progression or toxicity.

Routine laboratory tests included blood cell count with differential count, creatinine, glucose, bilirubin, electrolytes, proteins, hepatic and cardiac enzymes. They were repeated every week during IL-2 administration, then every month during the follow-up. Electrocardiogram and echocardiogram were performed before and after each IL-2 cycle. For immune detections, venous blood samples were collected during the morning before, and at days 3, 7, 14, 21 and 42 of the first IL-2 cycle. In each sample, T lymphocytes (CD3), T helper (CD4), T suppressor/cytotoxic (CD8), T suppressor ($CD8^+$ $CD57^+$), T cytotoxic ($CD8^+$ $CD57^-$), IL-2 receptor positive lymphocytes (CD25) and natural killer (NK) cells (CD16) were measured with an immunofluorescence analysis by FACS and monoclonal antibodies supplied by Becton Dickinson (Milan, Italy). In the same samples, we have also measured serum levels of soluble IL-2 receptors (SIL-2R) with an enzyme immunoassay, by using commercial kits (T Cell Sciences, Cambridge, Massachusetts); normal values obtained in our laboratory (95% confidence limits) were <480 U/ml.

Results were reported as means (S.E.). Data were statistically analysed by χ^2 test, Student's *t*-test, and analysis of variance, as appropriate. Patients were considered as evaluable when they received at least one IL-2 cycle.

RESULTS

Evaluable patients were 13/14. One patient with bone lesions refused the therapy after the first week for personal reasons, despite the lack of important toxicity. Clinical data of evaluable patients and response to therapy are reported in Table 1. No patient had a CR. A PR was achieved in 4/13 (31%) patients, with a median duration of 9^+ months (range 6^+–12^+); one responder patient had lung and liver as metastasis sites, while the other 3 had soft tissue lesions as sites of response. A SD was obtained in 7 patients (54%), with a median duration of 4 months (range 3^+–8^+), whereas 2 patients only (15%) rapidly progressed during the first IL-2 cycle. Toxicity was mild in all patients and, in particular, no important ischaemic or arrhythmic cardiac disorder was seen. The main toxicities observed in evaluable patients are reported in Table 2. Fever higher than 38°C was seen in 11/13 patients, but it was limited to the first 2 days of the induction phase, during which IL-2 was injected at higher doses; no patient showed fever higher than 38°C during the other days of the cycle. Transaminases and gamma-glutamyl transferase increased in all patients; transaminases became within the normal range after the first 2–3 weeks of therapy, while

Table 2. Main toxicities observed during IL-2 subcutaneous therapy in 13 evaluable advanced renal cancer patients

Toxicity	*n*	%
Fever >38°C	11/13	85
Increase in transaminases and/or gamma-GT	13/13	100
Hypotension grade 1	1/13	8
Nausea/vomiting grade 1–2	5/13	38
Diarrhoea grade 1	2/13	15
Pruritus	4/13	31
Asthenia	2/13	15
Anorexia	3/13	23
Thrombocytopenia grade 1	2/13	15
Hyperthyroidism	2/13	15
Local induration	4/13	31

gamma-glutamyl transferase mean levels remained significantly higher than those seen before until the end of the IL-2 cycle. No significant difference in total bilirubin mean levels was seen before and during IL-2 therapy, while mean levels of conjugated bilirubin significantly decreased during IL-2 administration [mean (S.E.)] (0.7(0.1) vs. 0.2(0.1) mg: 100 ml; $P < 0.05$). Total cholesterol mean levels rapidly decreased during IL-2 therapy, and the lowest mean levels were reached during weeks 1–2 of therapy ($P < 0.001$ vs. before). Hyperthyroidism, with increase in both total and free thyroid hormones, was seen in 2/13 patients, without, however, important clinical signs, while no patient had hypothyroidism during IL-2 therapy. Persistent local induration at the injection sites was seen in 4/13 patients. Performance status improved in all patients with response or SD, and all patients referred a better tolerability to IL-2 with respect to that presented by themselves during the previous treatment with interferon. The induction phase was performed in hospital; after that, patients remained hospitalised for 3–7 days (median duration of hospitalisation: 4 days), they then continued the treatment at home, and all patients referred as a positive experience to participate directly to the management of their disease.

As far as immune changes are concerned, peripheral blood lymphocyte, CD3, CD4, CD8, NK and CD25 cell mean number significantly decreased at day 3 of therapy with respect to the values seen before. After the third day of therapy, the mean number of lymphocytes and of their subsets significantly increased (see Fig. 1), as well as NK and CD25 cells (see Fig. 2), with a peak on the second week of therapy. No significant difference was seen between responder and nonresponder patients in the highest mean values of lymphocytes, CD3, CD4, CD8, NK and CD25 cells observed during IL-2 injection, as shown in Table 3, while eosinophil mean peak was significantly higher in responder than in nonresponder patients ($P < 0.05$). Eosinophilia greater than 20% was seen in 11/11 patients with response or SD, and in none of the progressed patients. T suppressor cell mean number significantly decreased during IL-2 therapy (23(3) vs. 41(5)/mm^3; $P < 0.05$); the decrease was more evident in responder than in nonresponder patients, without, however, any significant difference (−58(4) vs. −41(5)%). An evident increase in SIL-2R mean concentrations was seen in 13/13 patients, and the highest mean levels were seen on the second week of therapy, as illustrated in Fig. 3. Moreover, peak mean values of SIL-2R were significantly higher

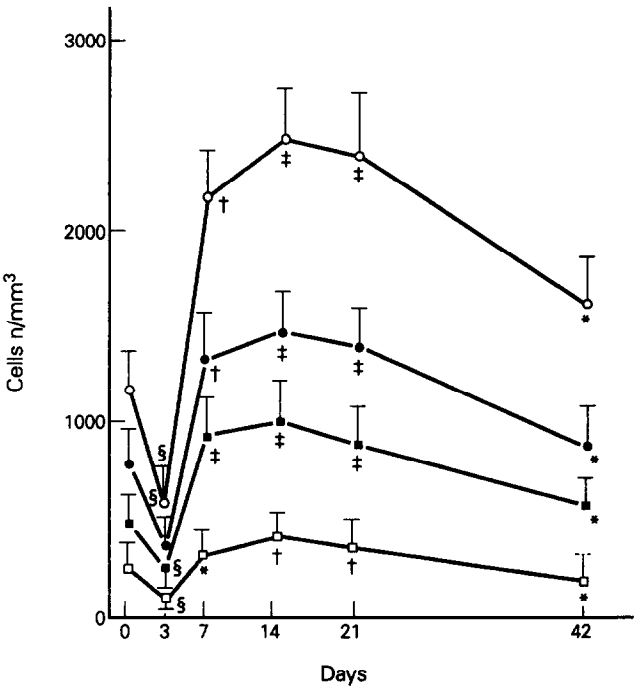

Fig. 1. Changes in lymphocyte, T lymphocyte, CD4 and CD8 cell mean number, evaluated by ANOVA, during IL-2 subcutaneous therapy in 13 advanced renal cancer patients. Mean (S.E.). ○ Lymphocytes, ● T lymphocytes (CD3), ■ T helper lymphocytes (CD4), □ T suppressor–cytotoxic lymphocytes (CD8). *$P < 0.05$ vs. before, †$P < 0.01$ vs. before, ‡ $P < 0.001$ vs. before, §$P < 0.005$ vs. before.

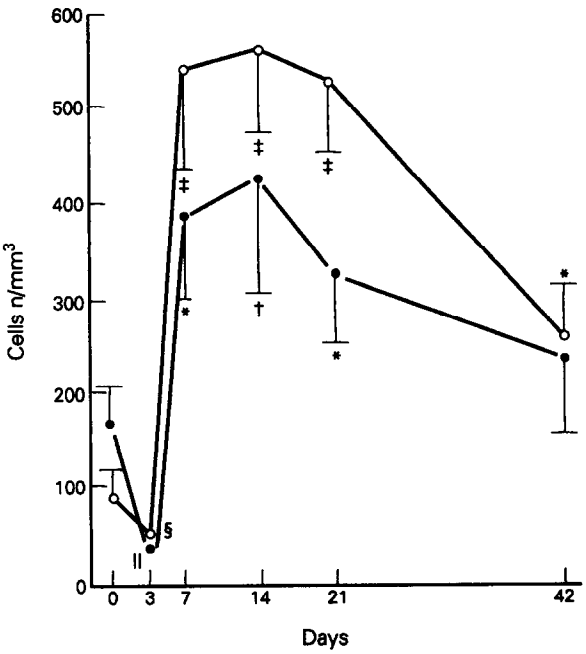

Fig. 2. Changes in NK and CD25 positive cell mean number, evaluated by ANOVA, during IL-2 subcutaneous therapy in 13 advanced cancer patients. Mean (S.E.). ○ NK cells, ● CD25-positive cells. *$P < 0.05$ vs. before, †$P < 0.01$ vs. before, ‡$P < 0.005$ vs. before, §$P < 0.05$ vs. before, ‖$P < 0.01$ vs. before.

Table 3. Changes in immune parameters in responder and in nonresponder advanced renal cancer patients during IL-2 subcutaneous therapy

Cases	n	Maximum values [$\bar{x}$(SE)] on study (n/mm³) Lymphocytes	CD3	CD4	CD8	CD25	CD16	Eosinophils
Responder patients	4	2475(389)	1427(208)	1058(262)	427(89)	481(53)	579(142)	1553(276)*
Nonresponder patients	9	2100(363)	1309(332)	912(285)	362(94)	432(68)	597(187)	619 (92)

*$P < 0.05$ vs. nonresponder patients.

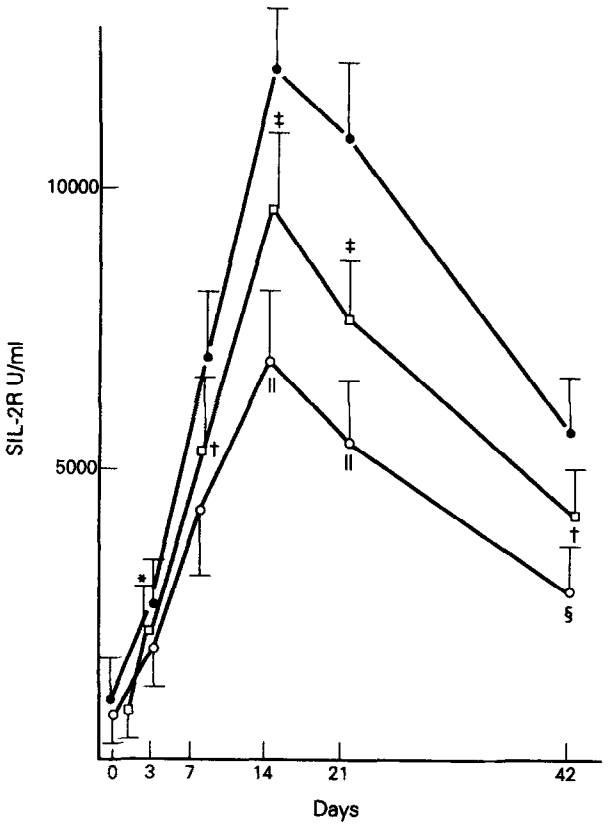

Fig. 3. Changes in SIL-2R serum mean concentrations, evaluated by ANOVA, during IL-2 subcutaneous therapy in 13 advanced cancer patients in relation to their clinical response. Mean (S.E.). ■ Overall patients ($n = 13$), ● nonresponders ($n = 9$), ○ responders ($n = 4$). *$P < 0.05$ vs. before, †$P < 0.01$ vs. before, ‡$P < 0.001$ vs. before, §$P < 0.05$ vs. nonreponders, ‖$P < 0.01$ vs. nonresponders.

in nonresponder than in responder patients ($P < 0.01$), whereas no difference was seen in CD25 cells, as shown in Table 3.

DISCUSSION

The results of this study show that IL-2 subcutaneous monotherapy is an effective and tolerated treatment in advanced cancer patients progressed during a first line therapy with interferon and vinblastine, with a response rate comparable to that reported by Atzpodien *et al.* [5] with IL-2 plus interferon. Even though these results are preliminary to draw definitive conclusions about the survival time, it seems that objective tumour regressions tend to persist for several months; therefore, it is probable that tumour regression induced by IL-2 may be associated with an increased survival time.

It is known that IL-2-induced tumour regressions are mediated by host biological response [1–5], whereas it remains to be established which immune change may be considered as predictive of the clinical response. According to the results reported by Favrot *et al.* [7] and by Atzpodien *et al.* [8], immunophenotypic lymphocyte modifications induced by IL-2 do not seem to correlate to the clinical response and do not predict response to therapy. On the contrary, as previously observed by Atzpodien *et al.* [5], eosinophil increase would represent an unfavourable biological event. Moreover, this study would suggest that an exaggerated increase in SIL-2R may be negatively correlated to the clinical efficacy of IL-2; this finding might depend on the capacity of SIL-2R of binding IL-2 [9], even though with a low affinity, and competing for IL-2 with IL-2 cell surface receptors, with a following reduced IL-2 bioavailability to activate host antitimour immune response. However, the low number of patients does not allow us to draw define conclusions about the relation between immune changes and clinical response. The relatively high response rate, obtained by ourselves with low-dose IL-2 alone in comparison to other reports [1–4], might depend on the selection of patients or, alternatively, on different effects on some immune parameters influencing the antitumour response, such as SIL-2R levels themselves, which increase could be more pronounced with higher doses of IL-2 or with the association with other cytokines. In particular, the role of a concomitant administration of interferon needs to be further defined. In the present study, we decided to give IL-2 alone either because patients previously progressed under interferon therapy, or because the interferon-induced enchancement of IL-2 efficacy has still to be better demonstrated. Interferon has been shown to increase IL-2 activity by stimulating histocompatibility antigen expression on tumour cells [6]; however, other studies would suggest that tumour cells are more sensitive to the cytotoxic action when their histocompatibility antigen expression is low [10].

In conclusion, this study shows that low-dose IL-2 subcutaneous administration is also effective in advanced renal cancer when it is given alone. Randomised studies are needed to establish whether a co-administration of interferon may potentiate IL-2.

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Comparison of Two Carboplatin-containing Regimens with Standard Chemotherapy for Small Cell Lung Cancer in a Randomised Phase II Study

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The EORTC Lung Cancer Cooperative group performed a randomised phase II study in patients with small cell lung cancer comparing the standard cyclophosphamide/doxorubicin/etoposide (CDE) regimen with two regimens containing the new and active cisplatin derivative, carboplatin, 400 mg/m² in combination with ifosfamide, a drug without important myelotoxicity, at a dose of 5 g/m² (IMP) or the non-myelotoxic drug vincristine twice 2 mg (VP). Of 178 evaluable patients, 63 received CDE [30 limited disease (LD), 33 extensive disease (ED)], 55 received IMP (22 LD, 33 ED) and 60 (26 LD, 34 ED) were treated with VP. The response duration was not statistically different: CDE 31 weeks, IMP 29 weeks and VP 21 weeks. The time to progression after CDE was 28 weeks, IMP 24 weeks and VP 17 weeks. This was significantly shorter after VP than after CDE ($P = 0.017$). The 60% response rate of the VP combination was low compared with CDE (83%) and IMP (77%). Toxicity of all three regimens was acceptable, and dose reduction for myelosuppression was necessary in only a minority of the patients. We conclude from this study that the combination of carboplatin, at the maximally tolerated dose of 400 mg/m², in combination with ifosfamide 5 g/m², is an active regimen with efficacy comparable with the standard CDE regimen.
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INTRODUCTION

AFTER THE introduction of chemotherapy for small cell lung cancer (SCLC) more than two decades ago, the initially impressive results [1] have unfortunately not resulted in a cure for many patients. The results of (chemo)therapy have reached a plateau in the last 10 years. One way to progress from this is the introduction of new active agents into existing treatment protocols.

One of the most promising agents in phase I and II studies is the cisplatin derivative carboplatin [2, 3]. Its 60% response rate in previously untreated extensive disease patients is high [2]. Compared to cisplatin it is less nephro, oto and neurotoxic and also less emetogenic. Dose limiting toxicity was myelosuppression, especially thrombocytopenia, at a dose of 400 mg/m² in a 4-weekly schedule [4]. If combined at this dose with the myelotoxic drug teniposide unacceptable myelotoxicity was seen [5].

In order to incorporate carboplatin in combination chemotherapy regimens at the maximum tolerated dose (400 mg/m²),