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# Treatment of Advanced Renal Cell Cancer With Sequential Intravenous Recombinant Interleukin-2 and Subcutaneous α-Interferon

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**Starting from *in vitro* studies suggesting synergistic antitumour activity against renal cell cancer (RCC) of recombinant interleukin-2 (rIL-2) and α-interferon (IFN), a phase II trial was initiated to test the clinical activity of this combination. The two cytokines were administered sequentially, with the aim of reducing the risk of additive toxicity and enhancing the immunological reaction against the tumour. The original treatment schedule consisted of rIL-2 18 × $10^6$ U/$m^2$/day by continuous intravenous infusion for 120 h days 1–5, and α-IFN 2b, at a flat dose of 9 × $10^6$ U by subcutaneous or intramuscular injection thrice in a week, from day 8 to 28. Treatment was planned to be continued for six or more 28-day cycles, depending on clinical response. 12 patients were treated according to this schedule; as some cardiovascular toxicity was experienced in this set of patients, 11 further patients were treated with half-dose rIL-2 (i.e. 9 × $10^6$ U/$m^2$/day). 17 out of 23 enrolled patients completed at least one cycle of treatment and were evaluated for response. We observed six major responses [one complete response (CR) + five partial responses (PR)] for an objective response rate of 35% [95% confidence interval (CI) 17–59%]. 5 additional patients achieved stabilisation of disease; one of them reached CR after surgical extirpation of a lung mass. Sites of response included lung, nodes and bone. Duration of response is 12+ months for CR; 17, 16, 12+, 9 and 9 months for PRs. Median survival is 16 months. Response was not significantly different between full-dose and half-dose rIL-2. Considering stable disease (SD) as responses, there seemed to be a higher chance of response for patients with smaller tumour burden ($P = 0.032$). The toxicity of rIL-2 treatment, mainly cardiovascular, was substantial; 9 patients experienced severe cardiotoxicity, consisting of major arrhythmias, myocardial ischaemia, reduction of ejection fraction measured with heart radionuclide scan, and were excluded from continuing treatment. Other rIL-2-related toxicities forcing exclusion from the study were severe thrombocytopenia (1 case), and generalised exfoliative dermatitis requiring steroids (1 case). Otherwise, treatment was well tolerated; rIL-2-related toxicities promptly recovered after rIL-2 discontinuation in the majority of cases, and no treatment-related deaths were reported. The half-dose rIL-2 regimen was significantly less toxic in terms of hypotension ($P = 0.014$), fever ($P = 0.014$), oliguria ($P = 0.042$), serum creatinine elevation ($P = 0.009$) and prothrombin time elongation ($P = 0.038$). α-IFN was not related to major toxicities. We conclude that the sequential administration of rIL-2 by continuous intravenous infusion and α-IFN by intramuscular or subcutaneous injection following our treatment schedule is feasible and active in RCC. Recombinant IL-2 9×$10^6$ U/$m^2$/day seems to be equally effective and less toxic than 18 × $10^6$ U/$m^2$/day. However, cardiovascular toxicity remains a major problem; particularly, arrhythmias and ischaemic events are poorly predictable and preventable.**

**Key words: interleukin-2, α-interferon, renal cell cancer, immunotherapy, cytokines**
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## INTRODUCTION

METASTATIC RENAL cell carcinoma (RCC) has a poor prognosis, with a 42–82% mortality at 1 year and 96% at 3 years [1]. Currently, α-interferon (IFN) is the most widely accepted treatment for metastatic RCC; an overview of multiple clinical trials with α-IFN has shown a response rate of 13% [2], but complete responses are rare and duration of response is generally short.

More recently, an interesting advance seems to have been obtained with the introduction of recombinant interleukin (rIL)-2. When given at high doses, both by bolus intravenous (i.v.) injection and by constant i.v. infusion, with or without lymphokine activated killer (LAK) cells, it seems to be capable of producing response rates even higher than 30% [3,4]; a similar response rate has been observed in a small trial with lower doses as well [5]. Interestingly, a number of responses are long-lasting and may result in significant prolongation of survival in a subset of patients.

It has been suggested that α-IFN could be helpful in the treatment of advanced RCC in combination with other biological response modifiers (BRM), and particularly with rIL-2 [6]. The mechanisms of α-IFN action in RCC are not well known, but direct cytostatic activity is likely to be induced together with an enhancement of the immune response against the tumour [7]. It has been reported that α-IFN is able to enhance the cytotoxic activity of natural killer (NK) cells [8]. Moreover, α-IFN, enhancing the expression of tumour-associated antigens, could make tumour cells more susceptible to recognition by rIL-2 activated lymphocytes (LAK cells) [9]. Preclinical data suggest that the combination of rIL-2 and α-IFN causes synergistic antitumour activity [10]. A trial combining rIL-2 and α-IFN by bolus injection showed a response rate ranging from 22 to 38% for RCC, depending on the dose level of both drugs; however, toxicity of this schedule was relevant [11]. Many trials have been performed or are currently ongoing to investigate the clinical effects and toxicity of the combination of rIL-2 + α-IFN, most of them adopting a concurrent rather than a sequential schedule of administration [12, 13]. Sequential administration of rIL-2 by continuous infusion and α-IFN was experienced in a few RCC patients treated at our Institution as a salvage therapy after treatment with rIL-2 ± LAK cells. This regimen proved to be well tolerated and only moderately toxic. Furthermore, sequential administration of rIL-2 and α-IFN might have some immunological advantages; α-IFN could enhance the expression of class I antigens on tumour cell surface and augment the potential for specific recognition and lysis by rIL-2-activated effector cells [14].

## PATIENTS AND METHODS

*Patient eligibility*

From December 1990 to December 1992 23 patients were included in the study. Patients' characteristics are summarised in Table 1.

Patients affected by histologically confirmed progressive unresectable or metastatic RCC were eligible for participation in this study provided the following criteria were met: Karnofsky performance status ≥ 80; white blood cell (WBC) count ≥ $4 \times 10^9$/l, platelet count > $100 \times 10^9$/l, haematocrit ≥ 30%; serum bilirubin, creatinine, prothrombin and partial thromboplastin time (PTT) within normal range. Exclusion criteria included significant history or current evidence of severe cardiovascular disease; contra-indications to the use of pressor agents; need of corticosteroids for concomitant disease; serious active infection requiring antibiotic treatment; metastases in the central nervous system; previous treatment with rIL-2; major surgery within 4 weeks prior to study entry; cytotoxic chemotherapeutic drugs, radiation therapy, α-IFN therapy or immunotherapy other than rIL-2 within 6 weeks prior to study entry; prior malignancies (excluding basalioma or carcinoma *in situ* of the uterine cervix); concurrent second primary malignancy; major organ allografts; pregnancy or lactation; presence of hepatitis B surface antigen, anti-hepatitis C antibodies or anti-HIV antibodies.

*Table 1. Patients' characteristics*

| | No. of patients |
|---|---|
| Total no. of patients enrolled | 23 |
| Sex (male, female) | 17:6 |
| Age (years) | |
| Median | 58 |
| Range | 35–69 |
| Previous treatment | |
| Surgery (nephrectomy) | 23 |
| Radiation therapy | 5 |
| Chemotherapy | 1 |
| Hormonal therapy | 0 |
| Immunotherapy | 4 (with α-IFN alone) |
| Sites of disease | |
| Local relapse | 3 |
| Lung | 14 |
| Nodes | 10 |
| Liver | 4 |
| Bone | 4 |
| Pelvis—retroperitoneal masses | 2 |
| Contralateral kidney | 1 |
| Pancreas | 1 |
| Median number of metastatic sites | 2 (1–5) |
| Evidence of progressive disease | 23 patients |

Total tumour burden was calculated as the sum of the products of the two longest perpendicular diameters of each measurable lesion [15].

Within 2 weeks prior to the first rIL-2 course, functional assessment and complete tumour evaluation were performed. A signed informed consent was obtained from each patient before starting the therapy.

*Study design*

This study was an open, non randomised phase II trial. The original treatment schedule consisted of rIL-2 at a dose of 18 MU/m$^2$/day by continuous i.v. infusion days 1–5, i.e. for 120 consecutive hours, and α-IFN, at a flat dose of $9 \times 10^6$ U by subcutaneous (s.c.) or intramuscular (i.m.) injection thrice in a week, from day 8 to day 28. The final dose of $9 \times 10^6$ U of α-IFN was reached escalating from 3 to 6 to $9 \times 10^6$ U in the first three injections. Recombinant IL-2 was resumed on day 29. Because of the toxicity experienced in the first 12 patients, subsequent patients were treated with half-dose rIL-2 (9 MU/m$^2$/day). Patients that completed one rIL-2 infusion cycle followed by a period of 3 weeks of α-IFN therapy were evaluated for response and survival. Every three cycles (or at withdrawal of treatment for any reason) patients underwent full tumour evaluation. Further treatment cycles over the six planned were offered to responder patients and to patients with stable disease, until best response.

*Therapy*

Recombinant IL-2 (Proleukin) was supplied by EuroCetus BV (Amsterdam, The Netherlands). Its specific activity was 18 MU (equivalent to $3 \times 10^6$ Cetus units) per milligram of protein. The lyophilised product was reconstituted with 1.2 ml of sterile water, so that 0.1 ml of the solution gave 1.8 MU. One half of each daily dose of rIL-2 was diluted in 500 ml of 5% glucose and water for administration as a continuous infusion through a central venous line using a volumetric pump IVAC. Patients

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were given prophylactic teicoplanin 400 mg i.v. 1 h before placement of central vein catheter.

In the case of the following toxicities, rIL-2 infusion was planned to be interrupted until resolution: any grade III WHO toxicity (grade II for serum creatinine); significant arrhythmia [e.g. supraventricular tachyarrhythmia, multifocal or consecutive or frequent (> 10/min) premature ventricular beats; RT phenomenon]; suspicion of myocardial ischaemia, prolongation of prothrombin time (PT) ≥ 3 s over baseline or of PTT ≥ 10 s over baseline; sepsis, dyspnoea at rest, weight gain > 10% of baseline; fever > 40°C lasting for more than 4 consecutive hours. Recombinant IL-2 infusion had to be discontinued and patients went off protocol in the following cases: documented myocardial ischaemia or cardiac heart failure; significant arrhythmia not promptly reversible after rIL-2 interruption and adequate antiarrhytmic therapy; any grade IV toxicity; serum creatinine or bilirubin that failed to return to grade I toxicity or better after rIL-2 interruption.

During rIL-2 infusion, patients were hospitalised in a general medicine ward, and were carefully monitored with regard to vital signs (pulse rate, blood pressure, temperature) every 2 h or less, and with at least daily measurement of body weight and diuresis. Additional monitoring included complete blood counts, haematochemical parameters and autoantibodies titres (anti-thyroid, anti-nucleus, anti-mytocondria, anti-smooth muscle cells, anti-gastric parietal cells antibodies). Immunological tests were performed as described elsewhere [16]. No patients were managed in an intensive care unit. During rIL-2 administration, patients received paracetamol (500 mg orally every 4 h) or indomethacin to ameliorate fever. Ranitidine (300 mg) or famotidine (40 mg) + misoprostol (400 μg twice daily) were given as ulcer prophylaxis. Allopurinol was added to prevent hyperuricaemia; metochlopramide was given orally or parenterally to manage nausea and/or vomiting and loperamide was administered in case of diarrhoea. Hypotension was managed by 20% human albumin i.v. infusions and, when severe, with discontinuation of rIL-2 and administration of dopamine as a pressor agent at the dose of 5 μg/kg/min.

α-IFN2b (Intron-A) was supplied by Schering-Plough (Milan, Italy). The product ready for injection was administered s.c. or i.m. After the first injection, the subsequent doses were self-administered by the patients in a home-setting, with weekly determination of main laboratory parameters (complete blood count, hepatic and renal function). If there was any grade III toxicity, interruption of α-IFN was planned until return to grade I or better; reduction of dose to $3 \times 10^6$ U was applied if there were persistence of toxicity.

*Response criteria*

Response and toxicity were recorded following WHO recommendations [17]. Complete response (CR) was defined as complete resolution of all disease sustained for at least two determinations that were separated by at least 1 month. Partial response (PR) was defined as ≥50% regression in the sum of the products of cross-sectional diameters of all measurable lesions, or an estimated ≥50% decrease of non-measurable lesions such as bone metastases; duration of response was defined from the initiation of treatment. Progressive disease (PD) was defined as a ≥ 25% increase in the sum of the products of cross-sectional diameters of all measurable tumours and/or by the appearance of any new lesions. Stable disease (SD) was defined as anything less than a PR and not progressive disease.

*Data management and statistics*

The original protocol proposed to include at least 14 patients in order to test the null hypothesis at a 95% confidence interval (CI) for a response rate of 20%. At each time point, an overall assessment was summarised by attributing to a patient the best response observed during the study period as evaluated by the tumour assessments, the start of the best response and the duration of the best response. Differences in toxicity and activity between the full-dose and the half-dose regimen were analysed with Fisher's exact test, two-tailed. Survival curves were generated using the Kaplan–Meier method [18].

## RESULTS

Of 23 eligible patients, 17 (74%) completed the first cycle of therapy and were evaluated for response. Among the excluded patients, 2 (9%) experienced cardiac toxicity (ischaemia, supraventricular paroxysmal tachycardia) during the first rIL-2 infusion, with complete recovery after rIL-2 withdrawal; 1 patient (4%) did not complete the first cycle for PT elongation > 3 s over baseline and did not start the second cycle for early progression; another patient (4%) did not complete the first cycle for severe thrombocytopenia (< 10,000/μl) after rIL-2 infusion; 2 patients (9%) were not evaluable because of major protocol violation, although later evaluation suggested that disease was stable in one of them.

4 patients (17%) completed only two cycles of therapy; 12 patients (52%) underwent more than three cycles (maximum 10). Overall, 81 rIL-2 infusion cycles were administered. Seventy-six (94%) infusion cycles were at least 72 h long. Depending on the toxicity observed in the first 12 patients, subsequent patients were treated with half-dose rIL-2.

We observed six major responses (one CR and 5 PR), so that the response rate for all evaluable patients was 35% (95% CI, 17–59%). 5 additional patients achieved SD. Details of responders' and SD patients' characteristics are presented in Table 2. No significant difference in response rates was observed between full-dose and half-dose rIL-2 (4/7 versus 2/10 evaluable patients, $P = 0.28$). Objective responses were observed in lung, bone and nodes.

The patient who achieved a CR is alive and disease-free after 1 year. Of the 5 patients who achieved a PR, 2 presented with bone metastases. In one case, the ischiopubic branch was involved, causing extreme pain requiring opioid therapy. After three courses of treatment, pain gradually decreased until complete recovery and partial recalcification on X-ray examination was subsequently observed. The patient progressed after 16 months from the start of therapy in sites other than the ischiopubic branch. In the other case, vertebral metastases with spinal chord compression were present. After three cycles, we observed a computed tomography (CT) scan imaging of partial regression of vertebral involvement with no evidence of spinal canal invasion and disappearance of neurological symptoms. The patient is still in PR and symptom-free 12 months after starting the therapy. 2 of the patients classified as SD achieved a reduction of tumour mass of about 30%. One of them, affected by lung involvement (tumour area 5 $cm^2$) underwent surgical eradication after 10 months from the start of treatment, and is still alive and disease-free; the other was affected by contralateral kidney involvement and progressed 6 months after starting the therapy. All the 6 non-responder patients were affected by extensive disease at the start of treatment.

The duration of response is shown in Table 2. Briefly, duration of CR is 12+ months and durations of PR are 17, 16, 12+, 9 and

*Table 2. Characteristics of responders and stable disease patients*

| Age (years) | Sex | Sites of disease (tumour area) | No. of cycles | Total dose of r-IL2 (MU) | Total dose of α-IFN (MU) | Best response | Duration (months at January 1993) |
|---|---|---|---|---|---|---|---|
| 67 | M | Lung (4 $cm^2$) | 6 | 858.825 | 432 | CR | 12+ |
| 58 | M | Lung-liver (40.24 $cm^2$) | 8 | 468 | 600 | PR | 17 |
| 52 | F | Bone (ischio-pubic branch) | 8 | 474 | 600 | PR | 16 |
| 53 | F | Bone (vertebral) | 6 | 330 | 450 | PR | 12+ |
| 63 | F | Lung (5 $cm^2$) | 5 | 337.5 | 288 | PR | 9 |
| 43 | M | Lung, nodes (52 $cm^2$) | 10 | 768 | 750 | PR | 9 |
| 68 | M | Perirenal fat (1.5 $cm^2$) | 3 | 330.75 | 72 | SD | 22+ |
| 62 | M | Lung (5 $cm^2$) | 6 | 395 | 450 | SD (minor response; surgical eradication) | 12+ |
| 56 | F | Local relapse, lung (29.4 $cm^2$) | 1 | 56.497 | 72 | SD | 4 |
| 48 | M | Lung, nodes (40 $cm^2$) | 4 | 337.5 | 288 | SD | 8 |
| 61 | M | Contralateral kidney (4 $cm^2$) | 4 | 240 | 288 | SD | 6 |

M, male; F, female; CR, complete response; PR, partial response; SD, stable disease.

9 months. Stabilisation of disease lasted 22+, 8, 6 and 4 months. The patient whose SD was surgically converted to CR 10 months after the start of treatment is not included. Median follow-up is 15 months. Estimated median survival according Kaplan–Meier's method is 16 months. The survival curve for evaluable patients is shown in Figure 1.

We investigated whether there was any correlation between responses and laboratory parameters. The patient who achieved a CR had a remarkable eosinophilia (maximum 4292 × $10^9$/l), and so did 2 PR patients, although a similar value (3267 × $10^9$/l) was also found in 1 non-responder patient. Eosinophilia seemed more relevant in patients treated with the combination therapy than in our previous series of patients treated with rIL-2 alone. Lymphopenia was found in all patients during rIL-2 infusion (median peripheral blood lymphocyte count reduced to 10% of baseline value). Although the CR patient had a minimum of 0.99 × $10^9$/l, lymphocytic count did not clearly correlate to clinical response. We also found variable results in auto-antibodies titres' variations: 2 PR patients and 1 SD patient developed hypothyroidism that required substitutive hormone therapy; 1 PD patient presented an elevated anti-thyroid antibodies titre, without clinical evidence of hypothyroidism. Tumour surface < 50 $cm^2$ seems to be related to better response ($P = 0.032$).

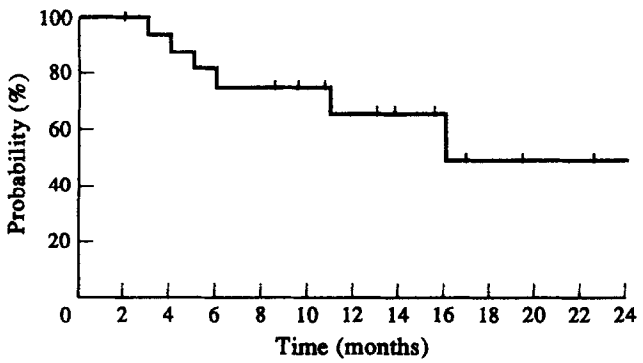


**Figure 1. Survival curve for evaluable patients**

The rIL-2-related toxicities observed in this trial were similar to those expected, and are shown in detail in Table 3. Virtually all patients experienced a flu-like syndrome including malaise and fever within a few hours of starting treatment. Skin toxicity included mild to medium grade erythrodermia in the majority of cases, but in 1 patient it assumed the form of severe erythrodermia with desquamation and complete resolution 48 h after termination of rIL-2 infusion. In 11 patients (48%) the treatment was interrupted due to severe toxic effects, mainly related to rIL-2. 5 of the first 12 patients treated with 18 MU/$m^2$/day rIL-2 went off protocol for cardiovascular toxicity [electrocardiogram (ECG) signs of myocardial ischaemia, major arrhythmias, hypotension requiring pressor agents], and another patient went off protocol for haematological toxicity (grade IV thrombocytopenia). Because of such serious toxicities, the subsequent patients were treated with half-dose rIL-2 (9 MU/$m^2$/day). Again, 3 out of 11 patients treated went off protocol due to cardiac toxicity (ECG signs of myocardial ischaemia), and a fourth patient went off protocol due to severe exfoliative derma-

*Table 3. Toxicity related to rIL-2*

| Type of toxicity | No. of patients | | | No. of cycles | | |
|---|---|---|---|---|---|---|
| | Total (%) $n=23$ | 18 MU/m²/day $n=12$ | 9 MU/m²/day $n=11$ | Total $n=81$ | 18 MU/m²/day $n=46$ | 9 MU/m²/day $n=35$ |
| Fever | 22(96%) | 11(92%) | 11(100%) | 74(91%) | 41(89%) | 33(94%) |
| Nausea/vomiting | 10(43%) | 6(50%) | 4(36%) | 18(22%) | 10(22%) | 8(23%) |
| Diarrhoea | 10(43%) | 4(33%) | 6(55%) | 14(17%) | 6(13%) | 8(23%) |
| Hypotension grade 3–4 | 12(52%) | 8(67%) | 4(36%) | 22(27%) | 17(37%)* | 5(14%)* |
| ECG ischaemia | 3(13%) | 1(8%) | 2(18%) | 3( 4%) | 1( 2%) | 2( 6%) |
| Major arrhythmias | 4(17%) | 2(17%) | 2(18%) | 4( 5%) | 2( 4%) | 2( 6%) |
| Reduction of LVEF | 1( 4%) | 1(8%) | 0 | 1( 1%) | 1( 2%) | 0 |
| Cutaneous | 16(70%) | 6(50%) | 10(91%) | 55(68%) | 27(59%) | 28(80%) |
| Clinical hypothyroidism | 3(13%) | 2(17%) | 1( 9%) | 3( 4%) | 2( 4%) | 1( 3%) |
| Weight gain >5% base | 3(13%) | 1(8%) | 2(18%) | 4( 5%) | 1( 2%) | 3( 9%) |
| Oliguria(<800 ml/day) | 14(61%) | 9(75%) | 5(45%) | 25(31%) | 18(39%) | 7(20%) |
| S-Creatinine (>2 mg/dl) | 6(26%) | 6(50%)* | 0* | 10(12%) | 10(22%)* | 0* |
| AST>50 U/l | 10(43%) | 4(33%) | 6(55%) | 18(22%) | 6(13%)* | 12(34%)* |
| T>3 s over base | 5(22%) | 5(42%)* | 0* | 7( 9%) | 7(15%)* | 0* |
| Platelets<100 000/μl | 4(17%) | 2(17%) | 2(18%) | 7( 9%) | 3( 7%) | 4(11%) |
| Eosinophils>600/μl | 14(61%) | 6(50%) | 8(73%) | 22(27%) | 9(20%) | 13(37%) |

*$P<0.05$, Fisher's exact test two-tailed. LVEF, left ventricular ejection fraction; AST, aspartate aminotransferase; PT, prothrombin time.

titis requiring corticosteroids, that did not resolve promptly after rIL-2 withdrawal. Another patient had a serum creatine kinase elevation while on α-IFN 3 weeks after the first, half-dose rIL-2 infusion had been completed without evidence of toxicity. The patient was asymptomatic and there were no ECG signs; however, a myocardial radionuclide scan demonstrated cardiac damage. Treatment was interrupted and we could not determine whether toxicity was specifically attributable to rIL-2 or α-IFN. Complete resolution of the toxicity occurred in 9/11 patients, while in 2 patients cardiac abnormalities persisted after rIL-2 infusion withdrawal, even if asymptomatic (Table 4).

Significantly less toxicity was observed with the half-dose regimen in terms of the number of days with grade ≥ III hypotension ($P = 0.014$), the number of days with fever ≥ 38.5°C ($P = 0.014$), the number of days with diuresis < 800 ml ($P = 0.042$), the number of cycles with grade ≥ III hypotension ($P = 0.043$), the number of cycles with elevation of serum creatinine > 2 mg/dl ($P = 0.004$) and the number of cycles with prolongation of PT > 3 s with respect to baseline ($P = 0.038$).

α-IFN administration was interrupted for fever (less than 39°C) and subjective intolerance only in 2 cases. In 1 patient, α-IFN was not administered due to toxicity related to previous

*Table 4. Causes of interruption of treatment due to toxicity related to rIL-2*

| Age | Sex | No. of rIL-2 infusions | Reason for interruption of treatment | Resolution after rIL-2 interruption |
|---|---|---|---|---|
| 50 | M | 1 | SVPT and fever>40°C after 84 h infusion | 1 min (SVPT) and 1 h (fever) |
| 44 | M | 1 | ECG signs of myocardial ischaemia | — |
| 35 | M | 3 | Diminishing of ejection fraction at cardiac radionuclide scan | — |
| 58 | M | 3 | Hypotension grade IV + AVB grade II 12 h after start of third IL-2 cycle | 8 h |
| 58 | M | 1 | Hypotension grade IV + tachycardia (CF>220/min) 12 h after start of third IL-2 cycle | 10 h |
| 66 | M | 1 | Thrombocytopenia(<10 000/μl) | 1 week |
| 56 | F | 1 | ECG signs of ischaemia, hypotension grade III, anuria | 24 h |
| 66 | F | 2 | ECG ischaemia + persistent sinus tachycardia (CF = 160/min) | 1 h and 20 min |
| 60 | M | 2 | Frequent VEB and ECG signs of ischaemia after 48 h of the second IL-2 infusion cycle | 24 h |
| 66 | M | 1 | CK elevation and signs of myocardial damage at cardiac radionuclide scan | 1 week (CK elevated) |
| 61 | M | 4 | Extremely severe exfoliative dermatitis requiring steroids | — |

M, male; F, female; SVPT, supraventricular paroxysmal tachycardia; AVB, atrial-ventricular block; CF, cardiac frequency; VEB, ventricular ectopic beats; CK, serum creatinine kinase.

rIL-2 infusion (thrombocytopenia), and in another patient it had to be administered at lower doses ($6 \times 10^6$ U thrice/week) due to elevation of transaminase related to previous rIL-2 infusion.

There were no treatment-related deaths. Furthermore, we did not observe major signs of vascular leakage syndrome, such as relevant weight gain, prolonged anuria, peripheral and pulmonary oedema. Hypotension which needed pressor agents (grade IV toxicity) occurred in only 2 patients, with complete resolution of toxicity in both of them after rIL-2 interruption.

## DISCUSSION

Recombinant IL-2 alone has proved to be effective in advanced RCC, with a 18% response rate in 60 consecutive patients treated by Rosenberg [19], and the goal for ongoing studies is to determine the optimal schedule of administration of this cytokine. Experimental evidence of synergistic effects of rIL-2 and α-IFN and preliminary clinical trials using rIL-2 in combination with α-IFN suggested a superior antitumour activity in comparison with each agent given alone [11]. On this basis, several studies have been performed in order to test the clinical activity of the association of these two biological response modifiers, with extremely variable response rates [13, 20–23]. Various dosage schedules were adopted by these authors, all consisting of concurrent rIL-2 and α-IFN administration. Recombinant IL-2 has also been given by s.c. injection, together with α-IFN, with a preliminary response rate of 29% and sensibly lower toxicity [22]. We performed a clinical trial based on sequential, rather than concurrent, administration of the two BRM, with the aim of utilising the theoretical α-IFN-induced upregulation of major histocompatibility complex (MHC) class I antigens on the surface of tumour cells [14]. This might increase susceptibility of tumour cells to lysis by specific cytotoxic T-lymphocytes other than LAK cells activated by rIL-2. We believe that the i.v. route is a better choice in order to achieve the best antitumoral response, based on the lack of large randomised studies with the s.c. route, and on the fact that the outpatient setting of rIL-2 administration does not permit an accurate side-effect evaluation, with particular regard to cardiac toxicity. In fact, we documented left ventricular dysfunction, detectable only by radionuclide ventriculography, in patients treated with rIL-2 in whom ECG and enzymes remained normal [24], and the s.c. route itself is not free from major toxicity, as demonstrated by a toxic death in one trial [23]. Furthermore, in our limited experience of 9 patients treated with s.c. rIL-2 administration (at lower doses than those reported in other trials) we did not observe major responses (data not shown).

This study demonstrates the feasibility of our treatment schedule with a promising high response rate (35% CR + PR). As eligibility criteria included a proved progressive disease, the stabilisation of disease might be considered a positive clinical result, that might significantly improve survival. In this trial, we obtained stable disease in 5 cases, and 1 case is still stable after 22 months. Indeed, some cases of SD on imaging could actually only be the scar of previous active disease [25].

The survival curves generated for eligible patients indicate a 1-year survival of 64% and median survival of 16 months. This could suggest a real benefit in terms of survival for the combination treatment, compared with our previous series of 20 patients treated with the West protocol (median survival 11.7 months). The sites of major responses included lung, nodes and bone, although bone metastases regression is unfrequently reported. Furthermore, the achievement of SD after treatment has made possible a complete surgical eradication of tumour mass (pulmonary metastasis) in 1 case. Even if not evaluable as a CR to treatment, this case certainly represents a major clinical success. On the whole, response to treatment could be related to the tumour size at the start of the treatment. In fact, our CR patient had a basal tumour area of 4 $cm^2$ while all PD patients had extensive disease (> 27 $cm^2$) at the start of treatment. We obtained good responses in patients with lung metastases, as reported previously [26], but it is noteworthy that our treatment has also proved to be effective in bone and node metastases.

In our series, tolerance to treatment has been an important factor in allowing high amounts of rIL-2 and α-IFN to be given: in fact response seemed to be related to the total amount of rIL-2 received, as we observed major responses in patients who received a total dose of at least 330 MU rIL-2 and 290 MU α-IFN. The analysis of the results obtained with the two rIL-2 dosages shows that the higher dose regimen had 4/12 major responses and 6/12 failures for toxicity, while the half-dose regimen had 2/11 major responses and 5/11 failures for toxicity. This suggests that the first schedule could be more effective, while the second is equally toxic as regards major side-effects, particularly cardiovascular. The toxicity observed in this trial is qualitatively similar to those reported previously [27–30]. We did not observe any treatment-related death, and therapy was substantially well tolerated. Treatment toxicity was mainly due to cardiovascular events, both related to the known capillary leakage syndrome, such as grade III–IV hypotension, and to direct cardiac toxicity, such as major arrhythmias. Overall, cardiovascular toxicity remains a major problem; it was responsible for exclusion from the study for 8 patients. However, in our experience, we did not have to administer any course in an intensive care unit, inasmuch as we did not document any severe complication of the capillary leakage syndrome, such as pulmonary oedema, myocardial infarction, acute renal failure, prolonged anuria. A careful monitoring of vital parameters, pharmacological prophylaxis of side-effects and the prompt institution of adequate therapy during each treatment cycle may account for this favourable clinical course.

However, we did not observe any major toxicity related to α-IFN; in only 2 cases was α-IFN administration interrupted due to subjective intolerance. Therefore, the addition of α-IFN to rIL-2 following this schedule has not augmented treatment toxicity with respect to rIL-2 alone. Furthermore, its use seems to potentiate rIL-2 immunological effects, as shown by our *in vitro* studies that will be published elsewhere. Sequential rather than concurrent administration of the two drugs may determine a better recovery from rIL-2-related toxicity prior to the start of α-IFN administration.

We conclude that sequential therapy with continuous i.v. infusion rIL-2 and s.c. α-IFN is feasible and effective. Although there is currently increasing interest in alternative routes of rIL-2 administration, namely the s.c. route, we emphasise the relevant response rate obtained with this protocol, with limited and manageable toxicity, and limited periods of hospitalisation, as α-IFN administration can be performed in an outpatient setting.

1. Linehan WM, Shipley WV, Longo DL. Cancer of the kidney and ureter. In De Vita, VT jr, Hellman S, Rosenberg SA, eds. *Cancer. Principles and Practice of Oncology*, 3rd edition. Philadelphia, J.B. Lippincott, 1989, 979–1007.
2. Buzaid AC, Todd MB. Therapeutic options in renal cell carcinoma. *Semin Oncol* 1989, **16** (suppl. 1), 12–19.
3. Rosenberg SA, Lotze MT, Mule JJ. New approaches to the

immunotherapy of cancer using interleukin 2. *Ann Intern Med* 1988, **108**, 853–864.
4. West WH, Tauer KW, Yannelli JR, *et al.* Constant infusion recombinant interleukin 2 in adoptive immunotherapy of advanced cancer. *N Engl J Med* 1987, **316**, 898–905.
5. Schoof DD, Gramolini BA, Davidson DL, Massaro AF, Wilson RE, Eberlein TJ. Adoptive immunotherapy of human cancer using low-dose interleukin 2 and lymphokine-activated killer cells. *Cancer Res* 1988, **48**, 5007–5010.
6. Goldstein G, Bach JF, Wigzell H, eds. *Immune Regulation by Characterized Polypeptides*. UCLA Symposia on Molecular and Cellular Biology. New Series, Vol.41. New York, Alan R Liss, 1987.
7. Kirkwood JM, Ernstoff MS. Interferons in the treatment of human cancer. *J Clin Oncol* 1984, **2**, 236–253.
8. Herberman RB. Effects of biological response modifiers on effector cells with cytotoxic activity against tumours. *Semin Oncol* 1986, **13**, 195–199.
9. De Maeyer-Guignard J, De Maeyer E. Immunomodulation by interferons: recent developments. In Gaessar I, ed. *Interferon*, vol.VI. New York, Academic Press, 1985, 69–86.
10. Cameron RB, McIntosh JK, Rosenberg SA. Synergistic antitumour effects of combination immunotherapy with recombinant interleukin 2 and a recombinant hybrid alpha-interferon in the treatment of established murine hepatic metastases. *Cancer Res* 1988, **48**, 5810–5817.
11. 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.
12. Figlin R, Abi-Aad AS, Belldegrun A, deKernion JB. The role of interferon and interleukin-2 in the immunotherapeutic approach to renal cell cancer. *Semin Oncol* 1991, **18** (suppl.7), 102–107.
13. Mittelman A, Puccio C, Ahmed T, Zeffren J, Choudhuri A, Arlin Z. A phase II trial of interleukin 2 by continuous infusion and interferon by intramuscular injection in patients with renal cell carcinoma. *Cancer* 1991, **68**, 1699–1702.
14. Beniers AJ, Peelen WP, Debruyne FM, Schalken JA. HLA-class I and -class II expression on renal tumor xenografts and the relation to sensitivity for alpha-IFN, gamma-IFN and TNF. *Int J Cancer* 1991, **48**, 709–716.
15. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
16. Fortis C, Ferrero E, Besana C, *et al.* Recombinant interleukin 2 and lymphokine-activated killer cells in renal cancer patients: I. Phenotypic and functional analysis of the peripheral blood mononuclear cells. *Cancer Immunol Immunother* 1990, **32**, 161–167.
17. WHO. *Handbook for Reporting Results of Cancer Treatment*. WHO Offset Publication No. 48. WHO, Geneva, 1979.
18. Kaplan EL, Meier P. Nonparametric estimation for incomplete observations. *J Am Statist Ass* 1958, **53**, 457–481.
19. Rosenberg SA. Immunotherapy and gene therapy of cancer. *J Clin Oncol* 1992, **10**, 180–199.
20. Stahl M, Wilke HJ, Seeber S, Schmoll HJ. Cytokines and cytotoxic agents in renal cell carcinoma: a review. *Semin Oncol* 1992, **19**, 70–79.
21. Ilson D, Motzer R, Kradin R, *et al.* A phase II trial of recombinant interleukin 2 and alpha-2b interferon in patients with advanced renal cell carcinoma. *J Clin Oncol* 1992, **10**, 1124–1130.
22. Atzpodien J, Korfer A, Menzel T, Poliwoda H, Kirchner H. Home therapy using recombinant human IL-2 and IFN alpha 2B in patients with metastatic renal cell carcinoma. *Semin Oncol* 1991, **18**(suppl.7), 108–112.
23. Sleijfer D, Janssen R, Buter J, *et al.* Phase II study of subcutaneous interleukin 2 in unselected patients with advanced renal cell cancer on an outpatient basis. *J Clin Oncol* 1992, **10**, 1119–1123.
24. Besana C, Benti R, Bruno A, *et al.* Left ventricular disfunction in patients treated with continuous i.v. interleukin-2 infusion for advanced cancer. *Eur J Cancer* 1991, **27** (suppl. 2), A1374.
25. Flaherty LE, Redman BG, Weaver D, *et al.* Surgical resection of residual radiographic abnormalities in patients with metastatic malignant melanoma after therapy with chemotherapy and IL-2 or IL-2 alone. *Proc Annu Meet Am Soc Clin Oncol* 1991, **10**, A1030.
26. Neidhart JA, Anderson SA, Harris JE, *et al.* Vinblastine fails to improve response of renal cancer to interferon alpha-N1: high response rate in patients with pulmonary metastases. *J Clin Oncol* 1991, **9**, 832–836.
27. Textor SC, Margolin K, Blayney D, Carlson J, Doroshow J. Renal, volume, and hormonal changes during therapeutic administration of recombinant interleukin 2 in man. *Am J Med* 1987, **83**, 1055–1061.
28. Ettinghausen SE. Moore JG, White DE, Platanias L, Young NS, Rosenberg SA. Hematologic effects of immunotherapy with lymphokine activated killer cells and recombinant interleukin 2 in cancer patients. *Blood* 1987, **69**, 1654–1660.
29. Gaspari AA, Lotze MT, Rosenberg SA, Stern JB, Katz SI. Dermatologic changes associated with interleukin 2 administration. *JAMA* 1987, **258**, 1624–1629.
30. Denicoff KD, Rubinow DR, Papa MZ, *et al.* The neuropsychiatric effects of treatment with interleukin 2 and lymphokine-activated killer cells. *Ann Intern Med* 1987, **107**, 293–300.

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