Eur J Cancer, Vol. 29A, No. 5, pp. 724–728, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00
Pergamon Press Ltd

Combination of Interleukin-2 and Gamma Interferon in Metastatic Renal Cell Carcinoma

Bernard Escudier, Françoise Farace, Eric Angevin, Frédéric Triebel, Sami Antoun, Bernard Leclercq, Maud Brandely, Alain Aboudaram, Gérard Nitenberg and Thierry Hercend

The use of high-dose interleukin-2 (IL2), alone or in association with lymphokine activated killer cells in patients with metastatic renal cell carcinoma (MRCC) results in a 20–25% response rate. However, the toxicity of IL2 is substantial and despite many clinical trials, response rates initially reported have not been improved. The aim of this study was to evaluate a combination of IL2 and gamma interferon (IFN) in MRCC with respect to both efficacy and tolerance. IL2 was given by continuous intravenous infusion at a daily dose of 24 × 10^6 U/m^2 for 2 consecutive days during 5 consecutive weeks. Gamma IFN was given subcutaneously at a daily dose of 5 × 10^6 U/m^2 on the same days as IL2. 33 patients with MRCC entered the study. Clinical responses were comparable with other published series: 7 patients (21%) achieved partial response, 13 (39%) were stable and 13 had progression, despite therapy. Immunological profile observed with this regimen showed a major increase in natural killer cells which became the predominant lymphocyte population at the end of the therapy. Tolerance was good with 92.5% of the planned doses actually received by the patients. This was reflected by an early discharge from the hospital in 95% of the cycles, increasing acceptability of the regimen by the patients.
Eur J Cancer, Vol. 29A, No. 5, pp. 724–728, 1993.

INTRODUCTION

THE USE of high-dose interleukin-2 (IL2) alone or associated to lymphokine activated killer cells (LAK) in patients with metastatic renal cell carcinoma (MRCC) leads to a 20–25% response rate [1–3]. Despite many clinical trials there has been no significant improvement since the first report from Rosenberg *et al.* [4] with respect to response rate. West [5] first proposed to modify the initial high-dose schedule with a 24-h continuous infusion instead of a bolus three times a day. This approach is widely employed because it leads to similar clinical results and decreased toxicity. Using lower doses of IL2, both toxicity and efficacy decrease [6]. Thus, trials combining cytokines have been proposed.

According to acceptable efficacy of alpha interferon (IFN) alone in MRCC [7–10], this cytokine has first been tested in association with IL2 [11, 12]. Initial clinical results were encouraging but no significant difference was found in efficacy and the toxicity of this combination appeared very high [11].

Gamma IFN also gives objective responses when used alone in MRCC [13, 14]. Moreover, gamma IFN enhances efficacy of immunotherapy with IL2 in murine models [15]. Based on these points, we have designed a clinical trial combining IL2 and gamma IFN in patients with MRCC. Because previous studies have shown that most of the threatening IL2 toxic side-effects are observed during the second half of the usual 5 days of treatment [1–5], we have used a new schedule where IL2 is given for only 2 days for 5 consecutive weeks.

Correspondence to B. Escudier.
B. Escudier, S. Antoun, B. Leclercq and G. Nitenberg are at the Service de Réanimation; F. Farace, E. Angevin, F. Triebel and T. Hercend are at the Laboratoire d'Hémato-Immunologie; A. Aboudaram is at the Service de Radiodiagnostic, Institut Gustave Roussy, 35 rue Camille Desmoulins, 94805 Villejuif; and M. Brandely is at the Roussel UCLAF, 102 route de Noisy, 93230 Romainville, France.
Revised 4 Aug. 1992; accepted 15 Sep. 1992.

PATIENTS AND METHODS

Patients

33 patients entered the study from April 1989 to May 1990. All patients had histologically proven metastatic renal cell adenocarcinoma with clinically measurable disease. All patients fulfilled the following eligibility criteria: age < 70, performance status ≤ 2, white blood cell (WBC) count ≥ 3000/mm^3, platelet count ≥ 100 000/mm^3, creatinine ≤ 140 μmol/l, liver enzymes < 3 times normal value, absence of brain metastases, of cardiac dysfunction or respiratory failure and of active systemic infection. All patients were required to give informed consent prior to participation in the study. Characteristics of the 33 patients are summarised in Table 1.

All patients underwent staging evaluation including computed tomography scans of the brain, the chest and the abdomen, and technetium pyrophosphate bone scan. Radiological evaluation for determination of tumour measurements was repeated 4 and 8 weeks after completion of therapy. All radiological tests were reviewed for response by a radiologist.

Protocol

IL2 and gamma IFN were obtained from the Roussel UCLAF Company (France). IL2 was given by continuous intravenous infusion at a daily dose of 24 × 10^6 U/m^2 for 2 consecutive days. This 2-day treatment was repeated for 5 consecutive weeks. Gamma IFN was given subcutaneously at a daily dose of 5 × 10^6 U/m^2 on the same days as IL2.

After clinical evaluation, patients with progressive disease (PD) or stable disease (SD) did not receive further IL2.

Toxicity grading and dose-modification schema

Toxicity was graded according to the WHO criteria. Treatment was discontinued for toxicity > grade 2, and reinstituted the next week only if toxicity was less than grade 2. Moreover, for each new cycle, patients had to fulfill the following biological

Table 1. Patients' characteristics

Male/female	23/10
Age (years)	
mean	52.1 ± 10.3
Range	19–68
Performance status	
ECOG 0/1	28/5
Prior therapy	
Nephrectomy	27
Surgery of metastases	2
Radiotherapy	6
Hormonotherapy	7
Chemotherapy	9
Sites of disease	
Pulmonary	24
Hepatic	5
Bone	7
Nodes	18
Other	3
Time from initial diagnosis	
< 1 year	15
> 1 year	18

criteria: bilirubin < 20 μmol/l, creatinine < 160 μmol/l, liver enzymes < 3 NV, WBC count ≥ 3000/mm^3 and platelet count ≥ 100 000/mm^3.

When grade 4 toxicity occurred, IL2 was restarted, when possible, at 50% of the initial dose.

Supportive care

All the patients were hospitalised in the intensive care unit. A central venous catheter was inserted in each patient before starting the treatment and was kept in as long as necessary for intravenous therapy. No prophylactic antibiotherapy was given. Heart rate was monitored continuously and blood pressure was checked every 15 min. Patients systematically received aspirin (1 g every 6 h) and paracetamol (1 g every 6 h) to prevent excessive fever. Loperamide was usually given for diarrhoea and chlorpropamide for nausea. Hypotension with systolic blood pressure less than 80 mmHg was first treated with macromolecular fluid infusion. In case of persistent hypotension, dopamine (5 to 20 μg/kg/min) was used; IL2 was stopped if hypotension persisted despite dopamine.

Immunological studies

Heparinised blood samples (20 ml) were drawn from patients on day 1 (prior IL2/gamma IFN administration) and on day 5 (2 days after the end of IL2 infusion) of each cycle. Dual immunofluorescence was performed by incubating 100 μl of blood for 10 min at room temperature with saturating amounts of monoclonal antibodies. The antibodies (anti-CD3, -CD25, -CD56) were purchased commercially from Coulter (Hialeah, Florida, U.S.A.). Red cells were lysed automatically using a Q-PREP system (Coultronics, Hialeah, Florida, U.S.A.). The whole cell suspension was analysed by flow cytometry on an EPICS Elite analyser (Coultronics). The lymphocyte subset was delineated using a forward angle light scatter signal and the logarithmic amplification of the 90° light scatter signal. For the cytotoxicity assays, peripheral blood mononuclear cells (PBMC) were isolated on ficoll hypaque density gradient, washed three times and assayed the same day. Cytotoxicity was assessed in a standard 3 h ^{51}Cr release assay as previously described [17]. Two target cell lines (K562, Daudi) were usually tested. Effector cells were assayed at six effector to target ratios from 40/1 to 1/1 with 3×10^3 labelled targets per well. Results were expressed as the mean of triplicate cultures. Lytic units (LU 30%/10^6 cells) calculated according to Pross *et al.* [18] are defined as the number of 10^6 effector cells required to mediate 30% lysis at 3×10^3 targets.

Clinical response

Complete response (CR) was defined as the disappearance of all tumour. Partial response (PR) was defined as > 50% reduction in the sum of the products of the largest perpendicular diameters in all measurable lesions without any new lesion or lesion increasing in size; progressive disease (PD) as an increase > 25% in the same sum of products, or the appearance of one or more new lesions; and stable disease (SD) as less than 25% change in measurable lesions without any new lesion.

Duration of the response was assessed from the time of occurrence of the response to the time when progression was first noticed. Survival time started on the first day of treatment.

RESULTS

Administration of treatment and toxicity

33 patients were enrolled in this protocol. Among these, 5 and 3, respectively, had their treatment either definitively or transiently interrupted. The 25 remaining patients received 100% of the planned doses. Together, total doses of 92.5% of the scheduled doses for both IL2 and gamma IFN.

Side-effects are listed in Table 2, and severe toxicities (grade 3 and 4) are emphasised in Table 3. Reasons for definitive interruptions were acute renal failure in 2 patients (patient 4 after the fourth cycle; patient 17 after the first cycle), unusual pemphigoid-like severe skin reaction (patient 5), cirrhotic decompensation (patient 27) and bronchospasm with refractory hypotension (patient 29). Temporary interruption was due to hypotension in 3 patients. Overall, hypotension was the main

Table 2. Toxicity and side-effects

	Number*	Percentage
Fever	33	100
Rash	30	91
Pruritus	22	67
Nausea/vomiting	32	97
Diarrhoea	32	97
Weight gain > 5%	2	6
Hypotension		
Requiring fluid	22	67
Requiring pressors	19	58
Atrial fibrillation	1	3
Dyspnea at rest	2	6
Septicemia	4	12
Somnolence	5	15
Disorientation	4	12
Thrombocytopenia		
< 100 000	5	15
< 50 000	1	3
Anaemia requiring transfusion	3	9
Creatinine > 200 μm/l	19	58
Bilirubin increase	0	0
ASAT increase (> 2* normal)	10	33

*Number of patients experiencing at least one episode of toxic effect.

Table 3. Severe toxicities (grades 3 and 4)

	Grade 3 (*n*)	Grade 4 (*n*)	Total (%)
Hypotension	2	18	61
Atrial fibrillation	1	0	3
Fever	1	2	9
Nausea/vomiting	3	0	9
Diarrhoea	1	0	3
Disorientation	0	1	3
Respiratory distress	2	0	6
Skin lesions	3	1	12
Infection	1	4	15
Thrombocytopenia	0	1	3
ALAT increase	4	0	12
Creatinin increase	1	1	6

toxicity, occurring in 58% of the cycles (67% of the patients) and requiring vasopressors in 13% of the cycles (58% of the patients). Severe thrombocytopenia (< 50 000) occurred only once (patient 6).

A high infection rate has been reported during IL2 treatment, and prophylactic antibiotics are recommended [19]. Although we do not give antibiotic prophylaxis, we only observed 4 septicaemic episodes (all with *Staphylococcus aureus*). Septicaemia was treated with antibiotics; central venous catheter could be maintained in 3 cases (and subsequently used again), and IL2 had to be stopped in only 1 patient (patient 4).

Overall, it is important to point out that patients were able to leave the hospital within 2 h after the end of IL2 in 95% of the cycles.

Response to treatment

All the patients treated are included in the evaluation although 2 of them received less than 40% of the dose. Of the 33 patients, 7 (21%) had a PR, 13 (39%) a SD, and 13 (39%) had PD. None of the 6 non-nephrectomised patients had PR. Response rate did non-significantly vary according to age of patients, sedimentation rate, delay between primary tumour and metastases, and presence of extrathoracic metastases (Table 4). Thus, there was no significant difference in known prognostic factors [23] between responding and non-responding patients.

Duration of PR and survival cannot readily be interpreted because PR patients are further treated in an additional protocol by a combination of IL2 and lymphokine activated natural killer (LANAK) cells. Note, however, that overall survival appears different in patients who responded to therapy compared with those who did not respond, since the former are all alive with a median follow-up of 18 months while only 16% of the progressive patients are still alive 11 months after the beginning of therapy (data not shown).

Immunological results

Immunological responses were evaluated on days 1 and 5 of each cycle. Absolute counts of lymphocytes increased progressively during the treatment, each IL2/gamma IFN cycle inducing a lymphocytic rebound greater than those of the previous cycles. For the last two rebounds (day 5 of the fourth and fifth cycle), the mean of absolute counts of CD56+ lymphocytes were significantly ($P = 0.005$) superior to that of CD3+ lymphocytes (Fig. 1). Among the 28 patients who had complete immunological monitoring, CD56+ lymphocytes represented at day 5 of the last cycle the major lymphocyte population in 18 patients, CD3+ were predominant in 7 patients whereas the two populations were equivalent in the 5 remaining patients. Dual immunofluorescence staining performed to better characterise T and natural killer (NK) subpopulations have shown that T and NK cells had conventional phenotypes throughout the treatment, i.e. CD3+CD56- and CD3-CD56+, respectively (data not shown). Double-positive CD3+CD56+ cells were rare and not modified by the treatment, i.e. 3.9 ± 3.1% before starting therapy (1/1) and 2.0 ± 2.8% at the last lymphocytic rebound (5/5). CD25+ cells progressively increased during treatment. The alpha chain of the IL2 receptor was expressed by a minor fraction of CD3+ lymphocytes, whereas it was generally not detectable on NK cells (data not shown). The cytotoxic potential detected against K562 and DAUDI target cells increased progressively and significantly ($P < 0.001$) during treatment (Fig. 2).

Immunological results were analysed according to the clinical response (Table 5). Comparisons were performed on day 5 of the fourth and fifth cycles (4/5 and 5/5) between PR patients and non-PR patients. Among all the parameters studied (total lymphocytes, CD3+, CD25+, CD56+ subsets, cytolytic activity against K562 and Daudi), the only significant difference was observed on CD25+ lymphocytes at 4/5: 0.72 ± 0.46 (10^9/l) for non-PR patients ($n = 20$) and 1.25 ± 0.52 (10^9/l) for PR patients ($n = 6$) ($P = 0.025$).

DISCUSSION

The present report describes the results of the combined administration of IL2 and gamma IFN for the treatment of MRCC. This association was stimulated by (i) the synergistic antitumour effects of IL2 and gamma IFN in murine models [15, 20] and (ii) the demonstration that IL2 and gamma IFN have individual antitumour activity in patients with MRCC [1, 3, 11, 13, 14]. Response rates with gamma IFN alone in short therapy (less than 3 months) were between 6 and 10% in reported series [13, 21, 22]. Aulitzky [14] reported a 30% response rate, but with a median time of therapy of 10 months.

Overall, the objective response rate in this series of patients is 21%. These results are comparable with other published series of MRCC treated with IL2 [1–3] which range from 17 to 35%. Thus, there is no evident benefit due to the addition of gamma IFN.

The immunological changes observed with this regimen were a progressive increase in the number of circulating NK lymphocytes accompanied by a substantially enhanced cytotoxicity against two conventional target cell lines at the end of therapy. Such high counts of NK cells were not detected during classical 5-day IL2 schedules [24]. We found a significant difference in the numbers of CD25+ cells between responding and non-responding patients at day 5 of the fourth cycle. Whether CD25+ lymphocyte numbers correlate with clinical response needs further confirmation on a larger population of MRCC patients treated with this IL2 schedule. Recently, Blay [25] reported a correlation between clinical response to IL2 and tumour necrosis factor level, a finding which also has to be confirmed in further studies.

The most interesting aspect of this regimen appears to be its good tolerance with actually received doses of 92.5% of the planned doses. Hypotension was the most common toxicity (61%), but requirement to vasopressors was infrequent (13% of cycles). This frequency of hypotensive episodes falls in the same range as those reported by Rosenberg *et al.* with 78% of the 652

Table 4. Response of patients

Patient	Age	Sex	Sites	Time (months) K/mets	SR	Response Status	Response Duration
1	53	F	Lu	72	15	SD	15
2	52	M	Lu	1	35	PRO	
3	49	M	Lu, 1°	0	12	SD	3
4	61	M	Lu	4	45	PR	16+
5	58	M	Lu, Bo	105	20	SD	10
6	56	M	Lu, Ln	14	8	SD	5
7	52	F	Ki, Ln	84	16	PRO	
8	56	M	Lu	112	45	PR	16+
9	44	M	Lu, Med	35	4	PR	14
10	54	M	Lo, Bo, Pl	0	90	SD	6
11	58	M	Lu, Bo, Ln	0	140	PRO	
12	35	M	Lu, Pl	30	10	PD	6
13	42	M	Lu, Ln, Lo	0	86	PRO	
14	19	F	Lo, Ln	8	120	PRO	
15	46	F	Lu, Pl, Ln	5	22	PRO	
16	57	F	Lu	0	106	PR	16+
17	57	M	Lu, Bo	40	90	SD	2
18	52	M	Lu	0	20	SD	6
19	25	F	Li	0	85	PRO	
20	51	F	Lu	24	100	PRO	
21	55	M	Li, Lo	0	33	SD	1
22	47	M	Lu, Lo	6	22	PRO	
23	55	M	Lu	30	32	SD	2
24	49	M	Lu, Med, Li	102	32	PR	12+
25	52	M	Lu, Li, Bo	0	74	PRO	
26	67	F	Lu	5	4	SD	5
27	64	F	Lu, Li	0	38	PRO	
28	56	M	Bo, soft tissue	0	6	PR	14+
29	56	M	Lu	44	105	PRO	
30	67	M	Lu, Ln, Bo	30	4	PR	12+
31	63	M	Lu, Ln	44	36	SD	4
32	53	M	Lu, Med, Ad	24	140	SD	2
33	54	F	Lu, Bo, Ln	0	24	PRO	

mets, Metastases; SR: sedimentation rate; Lu, lung; Med, mediastinum; Li, Liver; Bo, bone; Ln, lymph node; Lo, local; Ki, controlateral kidney; 1°, initial tumour; Ad, adrenal; Pl, pleura; PR, partial response; SD, stable disease; PRO, progression.

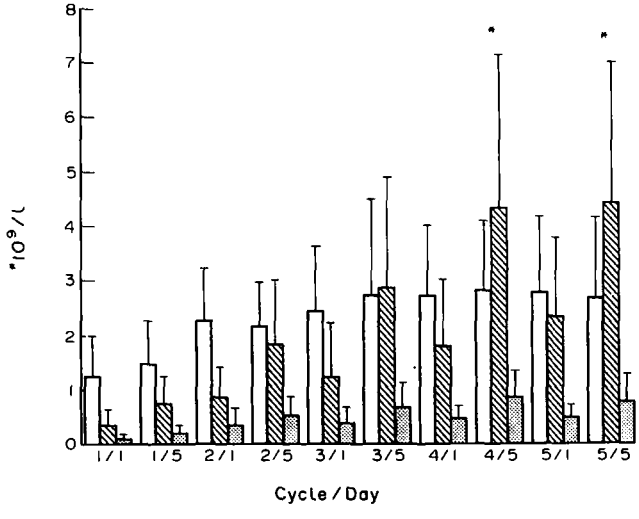

Fig. 1. Phenotypic analysis of lymphocyte subsets in 33 MRCC patients. Immunofluorescence stainings with anti-CD3, anti-CD56 (NKH1) and anti-CD25 monoclonal antibodies were performed at day 1 (immediately prior to the IL2 infusion) and day 5 of each cycle. □ CD3+, □ CD56+, □ CD25+ lymphocytes. *P = 0.005 between CD56+ and CD3+ counts.

patients [11] or Margolin *et al.* with 74% of 93 patients [26]. The other severe toxicities, such as neuropsychiatric disorders and respiratory distress, were very rare. Note, however, that we have observed two types of toxicity unusual with IL2 alone: early acute renal failure (especially in the patient where it occurred after the first cycle), and a pemphigoid-like eruption which has been previously described in detail [27]. Renal failure may have

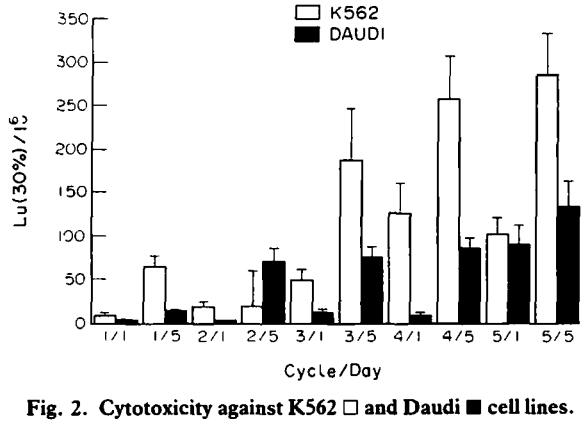

Fig. 2. Cytotoxicity against K562 □ and Daudi ■ cell lines.

Table 5. Immunological results according to the clinical response

Cycle/day	Response		Lympho	CD3+	CD25+	CD56+
1/1	PR	m	1.84	1.29	0.05	0.26
		sd	0.75	0.52	0.04	0.14
	Non-PR	m	1.91	1.20	0.10	0.34
		sd	1.39	0.83	0.07	0.34
4/5	PR	m	8.98	3.63	1.25*	3.93
		sd	2.20	0.99	0.52	0.70
	Non-PR	m	8.02	2.55	0.72	4.44
		sd	4.14	1.32	0.46	3.26
5/5	PR	m	9.12	3.48	1.11	4.42
		sd	3.48	1.57	0.19	1.45
	Non-PR	m	7.81	2.51	0.72	4.39
		sd	3.93	1.47	0.53	2.82

m, Mean; sd, standard deviation.
*$P<0.05$.

been favoured by gamma IFN, since this toxicity was previously reported [28] with this drug alone. Dermatological toxicity may also be increased by gamma IFN as it is with other types of IFN [29]. Nevertheless, possible early discharge from hospital is a very interesting finding, in terms of both comfort and cost of treatment. This last point has led to the development of this new schedule for high-dose IL2 regimen. A trial is presently in progress which will evaluate if the satisfactory response rate observed here with an improved tolerance is maintained using an identical IL2 schedule but without gamma IFN.

1. 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.
2. Fisher RI, Coltman CA, Doroshow JH, *et al.* Metastatic renal cancer treated with interleukin-2 and lymphokine activated killer cells. A phase II clinical trial. *Ann Intern Med* 1988, **108**, 518–523.
3. 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 multicenter study. *Eur J Cancer Clin Oncol* 1989, **25**, S21–S28.
4. 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.
5. West WH, Tauer KW, Yanelli JR, *et al.* Constant infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. *N Engl J Med* 1987, **316**, 898–905.
6. Parkinson DR, Fisher RI, Rayner AA, *et al.* Therapy of renal cell cancer carcinoma with interleukin-2 and lymphokine-activated killer cells: phase II experience with a hydrid bolus and continuous infusion interleukin-2 regimen. *J Clin Oncol* 1990, **8**, 1630–1636.
7. Fossa SD, De Garis ST, Heier MS, *et al.* Recombinant interferon alfa-2a with or without vinblastine in metastatic renal cell carcinoma. *Cancer* 1986, **57**, 1700–1704.
8. Buzaid AC, Robertone A, Kisala C, Salmon SE. Phase II study of interferon alfa-2a recombinant (Roferon-A) in metastatic renal cell carcinoma. *J Clin Oncol* 1987, **5**, 1083–1089.
9. Figlin RA, deKernion JB, Mukamel E, *et al.* Recombinant interferon alfa-2a in metastatic renal cell carcinoma: assessment of antitumor activity and anti-interferon antibody formation. *J Clin Oncol* 1988, **6**, 1604–1610.
10. Bergerat JP, Herbrecht R, Dufour P, *et al.* Combination of recombinant interferon alpha2a and vinblastine in advanced renal cell cancer. *Cancer* 1988, **62**, 2320–2324.
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. Atzpodien J, Korfer A, Franks CR, Poliwoda H, Kirchner H. Home therapy with recombinant IL2 and recombinant interferon alpha2b in advanced human malignancies. *Lancet* 1990, **335**, 1509–1512.
13. Garnick MB, Reich SD, Maxwell B, Coval-Goldsmith S, Richie JP. Phase I/II study of recombinant interferon gamma in advanced renal cell carcinoma. *J Urol* 1988, **139**, 251–255.
14. Aulitzky W, Gastl G, Aulitzky WE, *et al.* Successful treatment of metastatic renal cell carcinoma with a biologically active dose of recombinant interferon-gamma. *J Clin Oncol* 1989, **7**, 1875–1884.
15. Dubinett SM, Kurnick JT, Kradin RL. Adoptive immunotherapy of murine pulmonary metastases with interleukin 2 and interferon gamma. *Am J Resp Cell Mol Biol* 1989, **1**, 361–369.
16. Escudier B, Farace F, Droz JP, *et al.* Lymphokine activated natural killer cells may induce shift from partial into complete response in metastatic renal cell carcinoma: an hypothesis. *Proc Am Soc Clin Oncol* 1991, A527.
17. Hercend T, Farace F, Baume D, *et al.* Immunotherapy with lymphokine activated natural killer (LANAK) cells and rIL-2: a feasibility trial in metastatic renal cell carcinoma. *J Biol Res Mod* 1990, **9**, 546–555.
18. Pross HF, Baines MG, Rubin P, *et al.* Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantification of natural killer cell activity. *J Clin Immunol* 1981, **1**, 51–63.
19. Bock SN, Lee RE, Fisher B, *et al.* A prospective randomized trial evaluating prophylactic antibiotics to prevent triple-lumen catheter-related sepsis in patients treated with immunotherapy. *J Clin Oncol* 1990, **8**, 161–169.
20. Iigo M, Masanori S, Tomohide T, Nagahiro S, Hoshi A. *In vivo* antitumour activity of multiple injections of recombinant interleukin-2, alone and in combination with three different types of recombinant interferon, on various synergic murine tumors. *Cancer Res* 1988, **48**, 260–264.
21. Rinehart JJ, Young D, Laforge J, Colburn D, Neidhart J. Phase I/II trial of recombinant gamma-interferon in patients with renal cell carcinoma: immunologic and biologic effects. *J Biol Response Mod* 1987, **6**, 302–312.
22. Quesada JR, Kurzrock R, Sherwin SA, Gutterman JU. Phase II studies of recombinant human interferon gamma in metastatic renal cell carcinoma. *J Biol Response Mod* 1987, **6**, 20–27.
23. Droz JP, Theodore C, Ghosn M, *et al.* Twelve-year experience with chemotherapy in adult metastatic renal cell carcinoma at the Institut Gustave Roussy. *Semin Surg Oncol* 1988, **4**, 97–99.
24. Farace F, Mathiot C, Brandely M, *et al.* Phase I trial of recombinant interleukin-2 (rIL-2): immune activation by rIL-2 alone or following pretreatment with recombinant interferon-gamma. *Clin Exp Immunol* 1990, **82**, 194–199.
25. Blay JY, Favrot MC, Negrier S, *et al.* Correlation between clinical response to IL2 therapy sustaine production of TNF. *Cancer Res* 1990, **50**, 2371–2374.
26. Margolin KA, Rayner AA, Hawkins MJ, *et al.* Interleukin-2 and lymphokine-activated killer cell therapy of solid tumors; analysis of toxicity and management guidelines. *J Clin Oncol* 1989, **7**, 486–498.
27. Guillaume JC, Escudier B, Espagne E, *et al.* Dermatose bulleuse avec dépôts linéaires d'IgA le long de la membrane basale au cours d'un traitement par l'association interféron gamma-interleukine 2. *Ann Dermatol Venereol* 1990, **117**, 899–902.
28. Ault BH, Stapleton FB, Gaber L, *et al.* Acute renal failure during therapy with recombinant human gamma interferon. *N Eng J Med* 1988, **319**, 1397–1400.
29. Ramseur WL, Richards II F, Buggan DB. A case of fatal pemphigus vulgaris in association with beta interferon and interleukin-2 therapy. *Cancer* 1989, **63**, 2005–2007.