Subcutaneous interleukin-2, interferon alpha-2b and 5-fluorouracil in metastatic renal cell carcinoma as second-line treatment after failure of previous immunotherapy: a phase II trial

A Ravaud*,1, N Trufflardier1, JM Ferrière2, M Debled1, J Palussière3, L Cany1, R Gaston45, S Mathoulin-Pélissier6 and BN Bui1

1Department of Medicine, Institut Bergonie, Bordeaux, France; 2Department of Urology, University Hospital, Bordeaux, France; 3Department of Radiology, Institut Bergonie, Bordeaux, France; 4Department of Surgery, Institut Bergonie, Bordeaux, France; 5Department of Urology, Clinique Saint Augustin, Bordeaux, France; 6Department of Biostatistics, Institut Bergonie, Bordeaux, France

The association of interleukin-2 (IL-2), interferon alpha-2a (IFNα), 5-fluorouracil (5-FU) has been reported to induce response in metastatic renal cell carcinoma (MRCC). This study evaluated IL-2, IFNα and 5FU as second-line treatment after failure under immunotherapy. A total of 35 patients received IL-2, at 9 × 10⁶ IU m⁻², once or t.i.d, 5 days a week, every other week. Interferon alpha was administered at 6 MUL, TIW along with IL-2 every week. 5-Fluorouracil was given at 750 mg m⁻² day⁻¹ on days 1–5 every 4 weeks. One cycle lasted 8 weeks. All patients were evaluable for response and toxicity. There were two objective responses (5.7%) and 14 stable diseases (40%). Survival was 14 months. In all, 17 patients experienced grade 3 toxicity. The predictive factor for progression to second-line immunotherapy was the results of first-line immunotherapy, and performance status, delay from primary tumour to metastases and response or stabilisation to chemo-immunotherapy for survival. IL-2, IFNα and 5-FU induce low objective response but stabilisation in patients with MRCC having failed with immunotherapy, and may be considered only in selected patients on performance status, stabilisation or response after first-line immunotherapy and interval from their primary tumour to metastases.

British Journal of Cancer (2003) 89, 2213–2218. doi:10.1038/sj.bjc.6601419 www.bjcancer.com © 2003 Cancer Research UK

Keywords: immunotherapy; interleukin-2; interferon alpha; 5-fluorouracil; renal cell carcinoma; second-line treatment

The prognosis of patients with metastatic renal cell carcinoma (MRCC) remains poor with an estimated 5-year survival of 0–20% (Linehan et al, 2001). Interferon alpha (IFNα) and interleukin-2 (IL-2) have shown objective responses in 10–25% of patients (Rosenberg et al, 1989; Atzpodien et al, 1993; Buter et al, 1993; Fyfe et al, 1995; Savage and Muss, 1995; Yang and Rosenberg, 1997; Négrer et al, 1998) and long-lasting responders (Fyfe et al, 1996). More recently, IFNα has been shown to prolong survival compared to immunotherapy (Medical Research Council Renal Cancer Collaborators, 1999), although progression occurred in most patients.

When this study was designed, no effective second-line treatment arising from chemotherapy (Yagoda et al, 1995) or cellular therapy (Figlin et al, 1998) was available. Only one study in 13 patients with MRCC treated with s.c. IL-2 after failure under IFNα reported four partial responses (Lissoni et al, 1992).

Based on preclinical data suggesting a synergism between IL-2 and 5-fluorouracil (5-FU) on one hand and IFNα and 5-FU on the other, added to the well-known synergism of the association of IL-2 and IFNα (Cameron et al, 1988), the combination of IL-2, IFNα and 5-FU has been investigated in renal cell carcinoma. In animal experiments, IL-2 potentiates the antitumour activity of various cytotoxic drugs including 5-FU (Gauny et al, 1989; Kawano et al, 1994; Lee et al, 1994). Modulation of 5-FU with IFNα has been more extensively studied. IFNα induces thymidine phosphorylase, enhancing the conversion of 5-FU to the active 5-fluorodeoxyuridine monophosphate (FdUMP) inducing the depletion of thymidine triphosphate pools and DNA breakpoint, leading so far to an increase of the cytotoxicity of 5-FU (Wadler et al, 1990; Morita and Tokue, 1999). Moreover, IFNα inhibits the intracellular uptake of thymidine (Pfeffer and Tamm, 1984), and thymidilate synthase (Elias and Sandoval, 1989). At the beginning of the 1990s, clinical trials reported an increased response rate when 5-FU was added to IL-2 and IFNα (Atzpodien et al, 1993; Hofmockel et al, 1996; Joffe et al, 1996; Ellerhorst et al, 1997). Groups using an identical schedule as Atzpodien et al showed the response rate as ranging from 16 to 48% (Atzpodien et al, 1993; Hofmockel et al, 1996; Joffe et al, 1996; Ellerhorst et al, 1997).

Owing to the lack of a validated second-line treatment after immunotherapy in MRCC, the possibility that a second-line chemo-immunotherapy might prevent progression under previous immunotherapy was tested. Based on the reported higher response rate when 5-FU was added to IL-2 and IFNα, this study was designed to test the ability of the association of IL-2, IFNα and 5-FU to induce an objective response or at least stabilization while patients had progressed under IFNα and/or IL-2.
PATIENTS AND METHODS

Patients

Eligible patients had histologically proven renal cell carcinoma with progressive metastatic disease after a previous immune therapy with IFNα and/or IL-2. Patients were adults less than 75 years of age, who had a Karnofsky performance status > 70%. Patients were required to have measurable metastatic disease. They were not to have received either immunotherapy or radiotherapy in the previous 4 weeks. Adequate organ functions were required without cardiac, respiratory, hepatic, renalologic or psychiatric disorders. They had normal blood cell counts, normal bilirubin level, creatinine concentrations less than 180 mmol/l⁻¹, normal cardiac function and a life expectancy of at least 3 months. Patients with severe infection, known positivity of human immunodeficiency virus test or chronic hepatitis were excluded, as were patients on corticosteroids. Patients did not have history of an organ allograft or other malignancies. Pregnant or lactating women were also excluded.

The trial was approved by the CCPPRB in Bordeaux according to the French law. The study was conducted according to the principles of Good Clinical Practice.

Pretreatment evaluation

In addition, clinical history and physical examination were recorded for all patients. Preinclusion staging included cerebral, thoracic, abdominal CT scans and a bone scan. Written informed consent was obtained before inclusion in the trial.

Treatment plan (Table 1)

Interleukin-2 (Proleukin; Chiron Therapeutics, Suresnes, France) was given subcutaneously at a dose of 9 x 10⁶ IU/m²², twice on days 1 and 2, once a day on days 3–5 and every other week for 8 weeks. Interferon alpha (Introna; Schering Plough, Levallois-Perret, France) was administered at a dose of 6 x 10⁶ IU, three times a week, during weeks with IL-2. 5-Fluorouracil was delivered in the first week. 5-FU was administered at a continuous infusion at 750 mg/m²² day⁻¹ for five consecutive days every 4 weeks, starting with IL-2 and IFNα in the first week. Each time an objective response or a stable disease occurred, an additional identical course of treatment was given after 1 week's rest.

Evaluation of treatment

Evaluation of tumour response, including thoracic and abdominal CT-scan and a bone scan, was performed every 8 weeks of treatment. The World Health Organization (WHO) criteria were used to determine tumour response (Miller et al, 1981). Complete response (CR) was defined as the complete disappearance of all measurable and evaluable tumour sites for at least 4 weeks. The duration of CR was calculated from the first date of documentation of CR to the date of the first evaluation of disease progression. Partial response (PR) was considered to be a ≥50% decrease in the sum of products of the greatest perpendicular diameters lasting for at least 4 weeks, with no increase in known lesions and without appearance of any new lesions. When the evaluation showed a <50% decrease in lesions or a <25% increase, patients were considered to have a stable disease (SD). The duration of PR and SD was calculated from the first day of treatment. Progressive disease (PD) was considered to be when any lesion increased by ≥25% or when a new lesion appeared. The results of the successive bone scans were considered as PD in the case of appearance of new spots, stable if not, and complete regression only if all spots disappeared. Patients who presented with a CR, PR or SD were evaluated every 2–3 months during the first year and then every 4–6 months.

Survival duration was evaluated from the start of treatment to the date of the last contact or the date of death. Progression-free survival was calculated from the start of treatment to the date of last follow-up or the date of progression.

Toxicities encountered were classified according to the WHO grading system.

Statistical analysis

The primary end point was the response rate. The secondary end points were stabilisation rate, prolonged stabilisation rate (at least a period following chemo-immunotherapy as long as two cycles of treatment: 2 x [2 x 8 weeks] = 32 weeks or 8 months), overall survival, toxicity and prognostic factors for progression under second-line chemo-immunotherapy and overall survival.

The trial was conducted according to the two-stage Gehan design (Gehan, 1961). As first-line treatment, IFNα and IL-2 have shown objective responses in 18% of patients included into trials that we conducted (Négrier et al, 1998; Ravaud et al, 1994); we planned to detect a response rate ≥10%. We assessed the response rate after 29 patients had been recruited to have a 95% chance of detecting at least one response when the actual response rate was ≥10%. If at least one response occurred in the first 29 patients, we planned to increase the number of patients to assess the response rate with 5% precision (i.e., one response justified the inclusion of four more patients: at least 33 patients for the study).

To study prognostic factors, patients presenting response and stabilisation were pooled. Progressive disease was considered in the case of progression of the disease at tumour evaluation performed at 8 weeks. The following potential clinical prognostic parameters were analysed: gender, time from primary tumor to occurrence of metastases (<12 months vs ≥12 months), type of first-line immunotherapy (IL-2 vs IFNα alone), response to first-line immunotherapy (PD vs objective response or stabilisation), number of sites before second-line treatment (1 vs >1) and general status (Karnofsky >90% vs <90%). The association between response and stabilisation after second-line immunotherapy was assessed using the χ² test and survival distribution was estimated using the Kaplan–Meier method (Kaplan and Meier, 1958). The relationship between survival and parameters was analysed with the log-rank test (Mantel, 1966). Parameters that were significantly associated with survival at a P-value of <0.10 were included in a forward stepwise Cox model (Mantel, 1966).

RESULTS

Patient characteristics

From September 1994 to April 2000, 35 patients with MRCC were entered into the trial. All patients were evaluable for response and for toxicity and their main characteristics are outlined in Table 2. Most patients had an ambulatory performance status: 21 patients (60%) had a Karnofsky performance status ≥90%, while 14 (40%) patients had a Karnofsky performance status <90%. The time from diagnosis of renal cell carcinoma to the occurrence of metastases was less than 12 months in 25 patients (71.4%). All
patients had a prior nephrectomy. The median delay from diagnosis of metastatic disease to first immunotherapy was 3.4 months (range: 0 – 29 months). For the first-line immunotherapy, 21 patients were treated with IFN, nine with IL-2 and five with the association of IFN and IL-2. Six patients (17.2%) showed an objective response (1 CR and 5 PR), while 18 (51.4%) had a SD. In a median time of 9 months (range: 2 – 77 months), patients were included in this study. At the time of second-line treatment, the metastatic disease was localised in the lung (27 patients), mediastinal or abdominal lymph nodes (17 patients), bone (six patients), liver (four patients) and at the nephrectomy site (three patients). In all, 26 patients (74%) had at least two tumour sites at the time of second-line treatment initiation.

Administration of treatment and toxicity

During cycles, the median dose given to patients was 100% for each drug. The main toxicities (Table 3) were decrease in performance status (28 patients, 80%), fever (24 patients, 69%), nausea/vomiting (17 patients, 49%), diarrhoea (12 patients, 34%), cutaneous erythema (11 patients, 31%), hypotension (eight patients, 23%) and haematological disturbances (11 patients, 31%). In all, 17 patients (49%) had treatment-related grade 3 toxicity; 27 grade 3 events were reported: fever (six patients, 17%), nausea/vomiting (four patients, 11%), decrease in performance status, hypotension (three patients, 9%), diarrhoea, cardiotoxicity, mucositis (two patients) and skin erythema, anaemia, neutropenia and hypothyroidism (one patient). Nevertheless, none had grade 4 toxicity or died within the treatment course.

Response to treatment and survival

The median follow-up was 14 months. At the evaluation performed after 8 weeks of treatment, two patients (5.7%; 95% CI: 0.07 – 19.15%) had achieved an objective response, with one CR obtained after an immediate subsequent mediastinal radiotherapy. The duration of response was 6 and 56 months. In all, 14 patients (40%; 95% CI: 23.8 – 57.9%) had SD for a median time of 4 months (range: 2 – 16 months), including four patients (11.4%) with SD >8 months, while 19 showed disease progression. The sites of response were lung and lymph nodes for both patients. The median survival of all patients was 14 months (95% CI: 10.4 – 17.7 months) (Figure 1). Patients who showed stabilisation or an objective response had a median survival of 22 months (95% CI: 10.2 – 33.8 months), while those with a PD had a median survival of 9 months (95% CI: 4.7 – 13.3 months).

Table 2 Characteristics of patients

| Characteristics                          | No. | %     |
|-----------------------------------------|-----|-------|
| Eligible patients                       | 35  |       |
| Patients assessable for toxicity        | 35  | 100   |
| for response                            | 35  | 100   |
| Men/women                              | 27/8| 77/23 |
| Age (years) median (range)              | 62 (25 – 75) |
| Performance status (Karnofsky)          |     |       |
| 100%                                    | 7   | 20    |
| 90%                                     | 14  | 40    |
| 80%                                     | 12  | 34.3  |
| 70%                                     | 2   | 5.7   |
| Time from diagnosis to first metastases |     |       |
| <12 months                              | 25  | 71.4  |
| ≥12 months                              | 10  | 28.6  |
| Prior nephrectomy                       | 35  | 100   |

Table 3 Toxicity

| Toxicity                          | No. (%) |
|-----------------------------------|---------|
| Decrease in performance status    | 8 (23)  |
| Fever                             | 1 (3)   |
| Diarrhoea                         | 2 (6)   |
| Nausea/vomiting                   | 5 (14)  |
| Local skin disorders              | 5 (14)  |
| General skin disorders            | 5 (14)  |
| Hypotension                       | 1 (3)   |
| Mucositis                         | 3 (9)   |
| Cardiotoxicity                    | 0       |
| Neurological                      | 0       |
| Psychiatric                       | 0       |
| Infection                         | 0       |
| Weight gain                       | 1 (3)   |
| Haematological                    | 5 (14)  |
| Increase in transaminases         | 2 (6)   |
| Hypercreatininaemia               | 2 (6)   |
| Others                            | 0       |

Figure 1 Overall survival.
Predictive factors for progression to second-line immunotherapy

In univariate analysis, only the results of primary immunotherapy: stabilisation or objective response vs progression were significantly predictive for PD under second-line immunotherapy treatment ($\chi^2, P: 0.026$). Neither gender, time from primary tumour to occurrence of metastases ($<12$ months vs $\geq 12$ months), type of first-line immunotherapy (IL-2-based treatment vs IFNz alone), number of sites before second-line treatment ($1$ vs $>1$) nor general status (Karnofsky $\geq 90$ vs $<90\%$) reached statistical significance.

Predictive factors for survival following second-line immunotherapy

In univariate analysis, factors significantly ($P<0.05$) associated with better survival were objective response or stabilisation after second-line treatment ($P<0.001$) and general status at the time of second-line treatment ($P<0.01$). Neither the type of first-line immunotherapy nor the number of sites at second-line treatment was predictive of outcome.

Parameters showing an association with survival in univariate analysis with a degree of significance $<0.10$ were included in a forward stepwise Cox multivariate analysis. General performance status (Karnofsky $\geq 90\%$ vs $<90\%$) ($P: 0.003$), time from primary tumour to metastases ($P: 0.033$) and response or stabilisation to second-line treatment ($P: 0.038$) were considered as independent factors predictive of survival (Table 4).

DISCUSSION

The RR obtained in this study after a second-line immunotherapy-based treatment remains low (two out of 35 patients) despite one prolonged complete remission (>56 months). Since the start of the study, only two other studies have examined this theme (Paolorossi et al, 1995; Escudier et al, 1999). A large study in 113 patients confirmed the low response (four patients, 3.5%) and stabilisation (13 patients, 11.5%) rates, following a switch from IFNz to IL-2 and from IL-2 to IFNz after failure of the first-line cytokine therapy (Escudier et al, 1999). In the former study (Escudier et al, 1999), one of the patients received the association of both cytokines after one had already failed. In another study (Paolorossi et al, 1995) following the initial work of Lissoni et al (1992), 15 patients received IFNz and vinblastine after failure under IL-2. Two patients showed an objective response and five had stabilisation (Paolorossi et al, 1995).

Nevertheless, the occurrence of stabilisation in these circumstances, second-line treatment in patients, especially while they are still in a good general performance status (Karnofsky $\geq 90\%$) and when no alternative second-line treatment for renal cell carcinoma is available other than inclusion in clinical trials, could be considered to be of clinical interest. Furthermore, in this study, those patients with stabilisation or an objective response had a prolonged median survival of 22 months even after a second-line treatment. Nevertheless, spontaneous slow progression and/or long stabilisation of renal cell carcinoma without any antitumoral treatment may affect interpretation of survival outside compared phase III clinical trials. For this reason, it was considered useful when making decisions to point out predictive factors for outcome following a second-line immunotherapy-based treatment. This study shows that the most clinical significant predictive factor for no progressive disease at 8 weeks under second-line chemotherapy is the efficacy of first-line immunotherapy, which has not been assessed until now. Only one study reported that only patients with SD or transient responders with first-line cytokine treatment were responders (three out of four responders among 113 treated patients) (Escudier et al, 1999). In our study, parameters favourably affecting survival were a good general performance status at initiation of second-line treatment (Karnofsky $\geq 90\%$), the delay from primary tumour to metastases (>12 months) and the response to second-line treatment. Previous studies on prognostic factors for survival in MRCC, especially those carried out in patients under immunotherapy, showed both general performance status and delay from primary tumour to metastases to affect survival significantly (Palmer et al, 1992; Négrier et al, 2002). As the efficacy of second-line immunotherapy-based treatment was significantly correlated to the efficacy of the first-line treatment and as the second-line treatment had a significant impact on survival, it would have been helpful to study the correlation of survival to the first-line treatment, but the study was not designed for this purpose.

While a second-line immunotherapy-based treatment may be considered in selected patients, this study does not provide sufficient evidence that the association of IL-2, IFNz and 5-FU and the schedule used are a standard. This protocol is closely related to the schedule designed for other clinical research trials with IL-2, IFNz and ± 5-FU within the framework of the French Immunotherapy Group (Ravaud et al, 1998; Négrier et al, 2000). These large studies showed an unexpectedly low response rate (Ravaud et al, 1998; Négrier et al, 2000) like the present study, compared to more promising results obtained by others (Atzpodien et al, 1993; Hofmockel et al, 1996; Joffe et al, 1996; Ellerhorst et al, 1997; Tourani et al, 1998; Allen et al, 2000; Dutcher et al, 2000; Elias et al, 2000; van Herpen et al, 2000).

Toxicities encountered by patients during this study were moderate as expected with no grade 4 WHO toxicity and 49% of grade 3. Although the study was performed with second-line treatment, the toxicity profile did not show any differences compared to trials performed with first-line treatment (Ravaud et al, 1998; Négrier et al, 2000).

In conclusion, we achieved 5.7% of objective response and 40% of stabilisation including 11.4% prolonged stabilisation >8 months with IL-2, IFNz, 5-FU in patients with renal cell carcinoma in whom previous first-line immunotherapy failed. Therefore, the clinical benefit has to be considered as limited. Nevertheless, second-line immunotherapy may be considered only for selected patients who show either stabilisation or an objective response at evaluation of first-line immunotherapy, who have a good general status and a delay from the primary tumour to metastasis longer than 12 months. The recommended protocol therefore requires further evaluation.

Table 4 Univariate and multivariate stepwise Cox model analysis of survival for all patients

| Prognostic factor | Univariate | Multivariate |
|------------------|------------|-------------|
|                  | HR         | 95% CI      | P            |
| Objective response and stabilisation to second–line immunotherapy with IL-2, IFNz and 5-FU | 0.006 | 2.43 | 1.05–5.6 | 0.038 |
| General performance status (Karnofsky $\geq 90\%$ vs $<90\%$) | 0.007 | 3.72 | 1.55–8.91 | 0.003 |
| Time interval from primary tumour to occurrence of metastases ($\leq 12$ vs $>12$ months) | 0.07 | 2.71 | 1.08–6.78 | 0.033 |
ACKNOWLEDGEMENTS

We are indebted to the nurses of the department of medical oncology at Institut Bergonié who provided the

REFERENCES

Allen MJ, Vaughan M, Webb A, Johnston S, Savage P, Eisen T, Bate S, Moreau J, Ahles KA, Grunenfelder ME (2008) Protracted venous infusion 5-fluorouracil in combination with subcutaneous interleukin-2 and alpha-interferon in patients with metastatic renal cell cancer: a phase II study. Br J Cancer 83: 980 – 985

Atzpodien J, Kirchner H, Hainninen EL, Korfer A, Fenner M, Menzel T, Deckert M, Franzke A, Jonas U, Poliwoda H (1993) European studies of interleukin-2 in metastatic renal cell carcinoma. Semin Oncol 20: Suppl 9): 22 – 26

Buter J, Sleijfer DT, van der Graaf WT, de Vries EG, Willems PH, Mulder NH (1993) A progress report on the outpatient treatment of patients with advanced renal cell carcinoma using subcutaneous recombinant interleukin-2. Semin Oncol 20: 16 – 21

Cameron RB, McIntosh JK, Rosenberg SA (1988) Synergistic antitumor effects of combination immunotherapy with recombinant interleukin-2 and a recombinant hybrid alpha-interferon in the treatment of established murine hepatic metastases. Cancer Res 48: 5810 – 5817

Dutcher JP, Logan T, Gordon M, Sozman J, Weiss G, Margolin K, Passe T, Mier J, Lotze M, Clark J, Atkins M (2000) Phase II trial of interleukin-2, interferon alpha, and 5-fluorouracil in metastatic renal cell cancer: a cytokine working group study. Clin Cancer Res 6 3442 – 3450

Elías L, Dew D, Figlin RA, Flangan RC, Thompson ME, Triozzi PL, Belt RJ, Wood Jr DP, Rivkin SE, David E (2000) Infusional interleukin-2 and 5-fluorouracil with subcutaneous interferon alpha for the treatment of patients with advanced renal cell carcinoma: a southwest oncology group Phase II study. Cancer 89: 597 – 603

Elías L, Sandoval JM (1989) Interferon effects upon fluorouracil metabolism by HL-60 cells. Biochem Biophys Res Commun 163: 867 – 874

Ellerhorst JA, Sella A, Amato RJ, Tu SM, Millikan RE, Finn LD, Banks M, Logothetis CJ (1997) Phase II trial of 5-fluorouracil, interferon-z and continuous infusion interferon-alpha-2 for patients with metastatic renal cell carcinoma. Cancer 80: 2128 – 2132

Escudier B, Chevreau C, Lasser C, Douillard JY, Ravaud A, Fabbrro M, Caty A, Rossi JF, Viens P, Bergerat JP, Savary J, Negrier S (1999) Cytokines in metastatic renal cell carcinoma: is it useful to switch to interleukin-2 or interferon after failure of a first treatment?. Groupe Francais d’Immunothe´rapie. J Clin Oncol 17: 2039 – 2143

Félix R, Thompson J, Roudet C, Lange P, Belldegrun A (1998) Multi-center randomized placebo controlled phase III trial of CD8(+) tumor infiltrating lymphocyte therapy (CD8(+)TIL)/recombinant interleukin-2 (IL-2) in metastatic renal cell carcinoma (MRCC). Proc Am Soc Clin Oncol 17: 1225 (abstract)

Fyfe G, Fisher RJ, Rosenberg SA, Snol M, Parkinson DR, Louie AC (1995) Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 13: 688 – 696

Fyfe GA, Fisher RJ, Rosenberg SA, Snol M, Parkinson DR, Louie AC (1996) Long-term response data for 255 patients with metastatic renal cell carcinoma treated with high-dose recombinant interleukin-2 therapy. J Clin Oncol 14: 2410 – 2411

Gauny S, Zimmerman RJ, Winkelhake JL (1989) Combination therapies using interleukin-2 and chemotherapeutics in murine tumors. Proc Am Assoc Cancer Res 30: 372 (abstract 1475)

Gehan EA (1961) The determination of the number of patients required in a regimen of interleukin-2, interferon-2-z and 5-fluorouracil. J Urol 156: 18 – 21

Joffe JR, Banks RE, Forbes MA, Hallam S, Jenkins A, Patel PM, Hall GD, Velikova G, Adams J, Crossley A, Johnson PW, Whitcher JT, Selby PJ (1996) Phase II study of interleukin-2, interferon-2-z and 5-fluorouracil in advanced renal carcinoma: clinical data and laboratory evidence of protease activation. Br J Urol 77: 638 – 649

Kaplan EL, Meier P (1958) Non-parametric estimation from incomplete observations. Am Stat Assoc 53: 457 – 481

Kawano Y, Kubota T, Watanabe M, Fujita S, Kuo T, Kawamoto K, Sakai N, Yasui N, Fujii T, Teramoto T, Yamada Y, Kitajima M (1994) Synergistic antitumor activity of interleukin-2, mitomycin C and 5-fluorouracil against colon cancer. Proc Am Assoc Cancer Res 35: 323 (abstract 1920)

Lee M, Pierce A, Mahaffey W, Specht S, Stemmler N, Katoh A (1994) Interleukin-2 in neoadjuvant therapy potentiates inhibitory activity of 5-fluorouracil, leucovorin and interleukin-2. Anticancer Drugs 5: 239 – 243

Linehan WM, Zbar B, Bates SE, Zelefsky MJ, Yang JC (2001) Cancer of the kidney and ureter. In: Cancer: Principles & Practice in Oncology 6th edn, DeVita VT, Hellmann S, Rosenberg SA (eds) pp 1362 – 1383. Philadelphia: Lippincott Williams Wilkins

Lissoni P, Barni S, Ardivizio A, Crispino S, Paolorossi F, Archilli C, Vagli M, Tancini G (1992) Second line therapy with low-dose subcutaneous interleukin-2 alone in advanced renal cancer patients resistant to interferon-alpha. Eur J Cancer 28: 92 – 96

Mantel N (1966) Evaluation of survival data and two new rank order statistics arising in consideration. Cancer Chemother Rep 50: 163 – 170

Medical Research Council Renal Cancer Collaborators (1999) Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Lancet 353: 14 – 17

Miller AB, Hoogstraten B, Staquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 207 – 214

Morita T, Tokue A (1999) Biomodulation of 5-fluorouracil by interferon-alpha in human renal carcinoma cells: relationship to the expression of thymidine phosphorylase. Cancer Chemother Pharmacol 44: 91 – 96

Negrier S, Caty A, Lesimple T, Douillard JY, Escudier B, Rossi JF, Viens P, Gomez F (2000) Treatment of patients with metastatic renal cell carcinoma with a combination of subcutaneous interleukin-2 and interferon alfa with and without fluorouracil. J Clin Oncol 18: 4009 – 4015

Negrier S, Escudier B, Gomez F, Douillard JY, Ravaud A, Chevreau C, Buclon M, Perol D, Lasser C (2002) Prognostic factors of survival and rapid progression in 782 patients with metastatic renal carcinomas treated with cytokines: a report from the Groupe Francais d’Immunothe´rapie. Ann Oncol 13: 1460 – 1468

Negrier S, Escudier B, Lasser C, Douillard JY, Savary J, Chevreau C, Ravaud A, Mercatello A, Peny J, Mousseau M, Philip T, Tursz T (1998) Interleukin-2, interferon or both in 425 patients with metastatic renal cell cancer: results of a multicenter randomized controlled trial. N Engl J Med 338: 1272 – 1278

Palmer PA, Pinke J, Philip T, Negrier S, Atzpodien J, Kirchner H, Oskam R, Franks CR (1992) Prognostic factors for survival in patients with advanced renal cell carcinoma treated with recombinant interleukin-2. Ann Oncol 3: 475 – 480

Paolorossi F, Villa S, Barni S, Tancini G, Andres M, Lissoni P (1995) Second-line therapy with interferon-alpha plus vinblastine in metastatic renal cell cancer patients progressed under interleukin-2 subcutaneous immunotherapy. J Urol 153: 651 – 654

Pfeffer LM, Tamm I (1984) Interferon inhibition of thymidine incorporation into DNA through effects on thymidine transport and uptake. J Cell Physiol 121: 431 – 436

Ravaud A, Audhy B, Gomez F, Escudier B, Lesimple T, Chevreau C, Douillard JY, Caty A, Geoffrois L, Ferrero JM, Linassier C, Drevon M, Negrier S (1998) Subcutaneous interleukin-2, interferon alfa-2a, and continuous infusion of fluorouracil in metastatic renal cell carcinoma: a multicenter phase II trial. J Clin Oncol 16: 2728 – 2732

Ravaud A, Negrier S, Cany L, Merrouche Y, Le Guillou M, Blay JY, Clavel M, Gaston R, Oskam R, Philip T (1994) Subcutaneous low-dose recombinant interleukin-2 and alpha-interferon in patients with metastatic renal cell carcinoma. Br J Cancer 69: 1111 – 1114

Rosenberg SA, Lotze MT, Yang JC, Linehan WM, Seipp C, Calabro S, Karp SE, Levy RM, Steinberg S, White DE (1989) Combination therapy with interleukin-2 and alpha-interferon for the treatment of patients with advanced cancer. J Clin Oncol 7: 1863 – 1874

© 2003 Cancer Research UK

British Journal of Cancer (2003) 89 (12), 2213 – 2218
Savage PD, Muss HB (1995) Renal cell cancer. In Biological Therapy of Cancer De Vita VT, Hellman S, Rosenberg SA (eds) pp 373–387. Philadelphia: JB Lippincott.

Tourani JM, Pfister C, Berdah JF, Benhammouda A, Salze P, Monnier A, Paule B, Guillet P, Chretien Y, Brewer Y, Di Palma M, Untereiner M, Malaurie E, Tadrist Z, Pavlovitch JM, Hauteville D, Mejean A, Azagury M, Mayeur D, Lucas V, Krakowski I, Larregain-Fournier D, Abourachid H, Andrieu JM, Chastang C (1998) Outpatient treatment with subcutaneous interleukin-2 and interferon alfa administration in combination with fluorouracil in patients with metastatic renal cell carcinoma: results of a sequential nonrandomized phase II study. J Clin Oncol 16: 2505–2513

van Herpen CM, Jansen RL, Kruit WH, Hoekman K, Groenewegen G, Osanto S, de Mulder PH (2000) Immunochemotherapy with interleukin-2, interferon-alpha and 5-fluorouracil for progressive metastatic renal cell carcinoma: a multicenter phase II study. Dutch Immunotherapy Working Party. Br J Cancer 82: 772–776

Wadler S, Westo R, Weinberg V, Thompson D, Schwartz EL (1990) Interaction of fluorouracil and interferon in human colon cancer cell lines: cytotoxic and cytokinetic effects. Cancer Res 50: 5735–5739

Yagoda A, Abi-Rached B, Petrylak D (1995) Chemotherapy for advanced renal-cell carcinoma: 1983–1993. Semin Oncol 22: 42–60

Yang JC, Rosenberg SA (1997) An ongoing prospective randomized comparison of interleukin-2 regimens for the treatment of metastatic renal cell cancer. Cancer J Sci Am 3: S79–S84