Immunotherapy with concurrent subcutaneous GM-CSF, low-dose IL-2 and IFN-α in patients with progressive metastatic renal cell carcinoma

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The purpose of the study was to determine toxicity, efficacy and immunologic effects of concurrent subcutaneous injections of low-dose interleukin-2 (LD-IL-2), granulocyte–monocyte colony-stimulating factor (GM-CSF) and interferon-α 2b (IFNα) in progressive metastatic renal cell carcinoma. In a multicentre phase II study, 59 evaluable patients received two to six cycles of subcutaneous IL-2 (4 mIU m⁻²), GM-CSF (2.5 μg kg⁻¹) and IFNα (5 mIU flat⁻¹) for 12 days per 3 weeks with evaluation after every two cycles. Cycles were repeated in responding or stable patients. Data were analysed after a median of 30 months follow-up (range 16–48 months). In 42 patients, the immunologic response was studied and related to response and survival. The main toxicity were flu-like symptoms, malaise and transient liver enzyme elevations, necessitating IL-2 reduction to 2 mIU m⁻² in 29 patients, which should be considered the maximal tolerable dose. The response was 24% (eight out of 34, three complete response (CR), five partial response (PR)) in patients with metachronic metastases and 12% (three out of 25, 2CR, 1PR) in patients with synchronous metastases. Overall response was 19% (11 out of 59). Median survival was 9.5 months. All tested patients showed expansion and/or activation of lymphocytes, T cells and subsets, NK cells, eosinophils and monocytes. Pretreatment HLA-DR levels on monocytes and number of CD4⁺, CD8⁺ T cells, but not of NK or B cells, correlated with prolonged survival. Immunotherapy with concurrent subcutaneous GM-CSF, LD-IL-2 and IFNα has limited toxicity, can be given as outpatient treatment and can induce durable CR. Response and survival with this form of immunotherapy seem to be more dependent on expansion/activation of T cells than of NK cells.

Keywords: renal cell carcinoma; immunotherapy; multicentre phase II study

Metastatic renal cell carcinoma (mRCC) is insensitive to chemotherapy and only moderately sensitive to radiotherapy. As it is considered to be one of the most immunogenic tumours, immunotherapy has been studied extensively (Bukowski, 1999; Motzer et al., 2000). Currently, high-dose interleukin-2 (HD-IL-2)-based immunotherapy seems most promising as treatment for mRCC. Long-lasting complete responses (CRs) are induced in 5–10% of the patients (Fyfe et al., 1995; Bukowski, 1997; Negrier et al., 1998). Unfortunately HD-IL-2 therapy is rather toxic, requiring intensive care treatment in a considerable number of patients. A less toxic treatment, generally used in Europe as single-agent therapy, is subcutaneous (s.c.) interferon-α 2b (IFNα) (Fossa, 2000). The combination of HD-IL-2 and IFNα did not induce more responses than either of the single-agent treatments alone (Bukowski, 1997; Negrier et al., 1998).

The mechanism of action of HD-IL-2 therapy in mRCC is not exactly known. The therapeutic effect can be exerted by either activated T cells or activated NK cells. In mRCC patients several immunologic defects have been described, comprising insufficient antigen-presenting cell numbers, suppressed function of dendritic cells (DCs) as well as T-cell function defects (Troy et al., 1998; Bukowski, 1999).

We combined s.c. low-dose IL-2 (LD-IL-2) with IFNα and granulocyte–monocyte colony-stimulating factor (GM-CSF) to activate all limbs of the immune system and to avoid the toxicity of intravenous (i.v.) HD-IL-2. These three cytokines together stimulate all cells known to be involved in the induction of antitumour responses: IL-2 stimulates T cells (Kolitz et al., 1987; Thompson et al., 1988), IFNα induces better effector cell functions and a higher expression of adhesion molecules and MHC class I on tumour cells (Knop, 1990; Luft et al., 1998), while GM-CSF gives

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proliferation and differentiation of DC (Ragnhammar, 1996). Therefore, the combination may correct most defects in the immune system.

After a phase I trial to determine the maximum tolerable dose (MTD) of the combination of these cytokines (de Gast et al, 2000), we started a multicentre phase II trial in the Netherlands with the MTD. We here report the results of that phase II study in 63 patients receiving daily s.c. injections of LD-IL-2, IFNz and GM-CSF for 12 days per 3 weeks. Apart from toxicity and efficacy, an important part of the study was to determine activation and expansion of immune cells in peripheral blood and to investigate the correlation of these parameters with response to therapy and survival.

PATIENTS AND METHODS

Patients

All patients entered into the trial, approved by the local ethical committees, had biopsy or cytologic proven progressive mRCC and had signed informed consent before therapy. Eligibility criteria further included age above 18 years, WHO performance score 0 for the ability to give informed consent, adequate bone marrow function (leucocytes > 4.0 x 10^9/l, platelets > 100 x 10^9/l) and adequate renal (creatinine < 180 µmol/l^2) and liver function (transaminases, alkaline phosphatase < 5 x upper limit of normal (ULN), in case of liver metastases, normal bilirubin).

Exclusion criteria included serious cardiac, vascular, pulmonary or metabolic disease, pregnancy or lactation, systemic immunosuppressive treatment, including systemic steroids, previous or present autoimmune disease, aHIV antibodies and presence of brain metastases unless solitary and removed. Previous immuno therapy with IFNz was allowed but had to be stopped at least 2 months before the start. Previous radiotherapy for bone metastasis was allowed. Patients with only bone metastasis were excluded. Characteristics of the 63 patients are listed in Table 1. In all, 38 patients had metachronic metastases (metastases that developed some time after nephrectomy) and 25 patients had their primary tumour still in situ.

A majority of patients had lung metastases and two or more sites of metastases.

| Table 1 | Characteristics of the 63 eligible patients |
|-------------|------------------------------------------|
| Age (years) | Median 58, Range 31–75 |
| WHO performance status | 0 36, 1 23, 2 4 |
| Male/female | 44/19 |
| Synchronic metastases | 25 |
| Metachronic metastases | 38 |
| Sites of disease | Soft tissue only 3, Lung metastasis (± soft tissue) 36, Bone+others 19, Liver± others 9, Adrenal+lung 2 |
| No. of metastatic sites | 1 23, 2 25, >2 15 |

Treatment

Low-dose interleukin-2 (Aldesleukin, Proleukin® 4 mIU m^-2, Chiron BV Amsterdam, the Netherlands), GM-CSF (Molgramostim, Leukomax® 2.5 µg kg^-1, provided by Schering-Plough, Maars sen, the Netherlands) and IFNz (Intron-A®, 5 mIU fixed dose, Schering-Plough, Maarsen, the Netherlands) were given as daily s.c. injections for 12 days in, respectively, abdominal wall, left leg and right leg every day at different places. Immunotherapy was initiated in the first cycle under controlled conditions in the hospital and continued at home after 2–3 days when the drug dosage was deemed safe for home administration. The remainder of the first cycle and following cycles (every 3 weeks) was completely given as outpatient treatment.

Dose modification

Interleukin-2 dose was reduced to 2 mIU m^-2 if grade 4 fever with hypotension, persistent severe malaise, diuretics insensitive weight gain >5% or grade 3 liver enzyme elevations did occur. Granulocyte–monocyte colony-stimulating factor had to be reduced by 50% if allergic symptoms persisted despite adequate treatment with antihistaminics. On the basis of leucocyte numbers in the blood after 7 days, GM-CSF was stopped immediately (if leucocytes > 30 x 10^9/l on day 7), stopped after 9 days (if 25–30 x 10^9/l), or continued for the full 12 days (if < 25 x 10^9/l on day 7) in order to prevent excessive leucocytosis with eosinophilia. In case of any grade 3 CTC toxicity except fever and flu-like syndrome, medication had to be interrupted until resolution of that toxicity and the most probable causative agent reduced to 50% in the next cycle.

Supportive measures

Prior to the start of immunotherapy, patients received 1/2 l of saline (NaCl 0.9%) intravenously and during immunotherapy Acetaminophen 1 g with the injections, 1 g at the start of chills and saline (NaCl 0.9%) intravenously and during immunotherapy. Metoclopramide was used to treat or prevent nausea. No systemic steroids were allowed unless absolutely necessary and NSAIDs were avoided in order not to suppress macrophage function.

Evaluations

Prior to treatment, intravenous contrast enhanced computer tomography (CT) scans of chest, abdomen and pelvis were made, magnetic resonance imaging (MRI) of the brain and radioactive technetium scan of the bone was performed in addition to physical examination (PE) to determine the extent of the disease. In addition, an electrocardiogram and blood tests for haematology, liver and renal function and an HIV antibody test were performed. Complete blood counts, differential WBC count, platelet count, liver and renal function tests were performed at start of treatment, after 1 week and at the end of immunotherapy (after 12 days) and after 3 weeks (before the next cycle). In addition, the absolute numbers of T cells (CD3, CD4, CD8), NK cells (CD3-CD16 + 56 + ), monocytes (CD14 + ) and B cells (CD19 +) and of the activated cells (double staining with HLA-DR) were determined before treatment, at day 12 and day 23 with monoclonal antibodies on the FACS scan flow cytometer as described before (de Gast et al, 2000). Soluble IL2-receptor (sIL2R, Eurogenetics, Tessonderlo, Belgium) and sCD8 (T cell Diagnostics, Cambridge MA, USA) were assayed by ELISA as described before (de Gast et al, 2000).

Response evaluation

Physical examination, CT scans and MRI of the brain were repeated after two cycles in week 7. Metastatic disease was
quantified as the sum of products of perpendicular diameters of marker lesions. All measurable lesions on CT scans were used as marker lesions and up to 10 subcutaneous nodules were used as such. Responses were defined as CR: disappearance of all measurable disease; as partial response (PR): 50% or more reduction in measurable disease with no new lesions; as stable disease (SD): less than 25% increase or less than 50% decrease in measurable disease and no new lesions; or as progressive disease (PD): 25% or more increase in measurable disease or the appearance of new lesions, or nonreturn for evaluation because of deteriorating clinical condition. Responses must have been confirmed by another post-treatment evaluation 6 weeks later.

In case of PD, immunotherapy was stopped and palliative radiotherapy considered. In case of SD, another two cycles were given. In patients with regression, cycles were continued with a response evaluation after every two cycles. If CR was reached, two consolidation cycles were given. In patients with the primary tumour in situ, nephrectomy was performed and cycles with immunotherapy given within 6 weeks after recovery with response evaluation after every 2 cycles.

Statistics
Survival curves were constructed using the Kaplan–Meier technique using the initiation of immunotherapy as starting point. Levels of circulating cells in peripheral blood and of cytokines, at various time points and in various groups, were compared by student’s t-test. P-values < 0.05 were considered significant.

RESULTS
Patients
In all, 63 patients started treatment. Four patients stopped treatment within 7 days because of rapidly PD (2), hypercalcaemia (1) or cardiac arrhythmia and decompensation (1). As shown in Table 2, eight of the 34 evaluable patients with metachronous metastases achieved remission (24%), three CR, five PR and 13 showed SD (38%). Four patients received IFNz for metastatic disease prior to treatment with concurrent immunotherapy. In two patients, a SD was reached, but no responses were seen. One CR patient relapsed in the bone after 8 months and died after 31 months. The two other CR patients are both in continued CR status, concomitant HLA-DR expression on lymphocytes and number of eosinophils. To test the activation immunologic parameters in peripheral blood. The parameters that were tested were lineage markers for T cells, B cells, NK cells and monocytes and number of eosinophils. To test the activation status, concomitant HLA-DR expression on lymphocytes and

Table 2  Response evaluation

| Response | Metachronous metastases | Synchronous metastases | Total |
|----------|-------------------------|------------------------|-------|
|          | With Nx* | Without Nx |       |       |
| CR       | 3 (24%)  | 2          | 0     | 5     |
| PR       | 5        | 1          | 0     | 6     |
| SD       | 13 (1sCR)| 9          | 0     | 22    |
| PD       | 13       | 2          | 11    | 26    |
|          | 34       | 14         | 11    | 59    |

*Nx = nephrectomy, CR = complete remission; PR = partial remission; SD = stable disease; PD = progressive disease; sCR = surgical CR.

because of rapidly progressive disease (Table 2). Two patients achieved CR after nephrectomy, of whom one got a relapse of his lung and pleural lesions after 4 months and one is still in continued CR for 15+ months. One patient with lung and adrenal metastasis achieved a PR. The response rate in the whole group was 11 of 59 (19%) with five CR and six PR. Three of the five CR patients are in maintained remission (15+, 40+, 40+ months). Survival of all patients entering the study (n = 63) is shown in Figure 1.

Toxicity
All patients developed flu-like symptoms with fever, chills, malaise and anorexia, which was only partly alleviated by Acetaminophen (Table 3). Interleukin-2 was reduced to 2 mlU m⁻² in eight patients with grade 4 fever with hypotension on the first day. As a result of persistent fatigue grade 3, IL-2 was reduced in another 15 patients after 1 week. In addition, IL-2 was reduced in the second cycle because of grade 3 liver function disturbances (only enzyme elevations) in five patients and because of grade 3 creatinine elevation in one. So in total, IL-2 was reduced from 4 to 2 mlU m⁻² in 29 out of 59 patients, who received at least 1 full cycle of combined immunotherapy. A further reduction to 1 mlU m⁻² of IL-2 was necessary in two patients because of persistent grade 3 fatigue. Allergic reactions grade 3 (angioedema) occurred in two patients, rapidly reacting to antiallergics and stopping of GM-CSF. One patient had cardiac arrhythmia and decapsulation after 3 days, necessitating stopping of all treatment.

Leucocytosis with eosinophilia was seen in all patients during immunotherapy (median 22 nl⁻¹, range 15–37 nl⁻¹). Granulocyte–monocyte colony-stimulating factor was stopped because of excessive leucocytosis in only two patients. None of the patients died of treatment-related toxicity or needed hospital admission.

Immunologic evaluation
From all patients treated in the Antoni van Leeuwenhoek Hospital, Amsterdam (n = 42) blood samples were taken during and after therapy to assess the effects of combined immunotherapy on immunologic parameters in peripheral blood. The parameters that were tested were lineage markers for T cells, B cells, NK cells and monocytes and number of eosinophils. To test the activation status, concomitant HLA-DR expression on lymphocytes and
monocytes and sIL-2R and sCD8 levels in serum were examined. All markers, except the number of B cells, showed a significant increase on day +12 of immunotherapy, but not the number of B cells (CD19).

Toxicity (NCI-CTC 2.0)

Table 3

| Grade | Pre | d+12 | d+23 |
|-------|-----|------|------|
| CD4* | 130 ± 34 | 56 ± 8 | 56 ± 8 | P = 0.05 |
| CD8* | 2689 ± 374 | 1777 ± 122 | 1777 ± 122 | P = 0.02 |
| CD3* | 1897 ± 350 | 1215 ± 119 | 1215 ± 119 | P = 0.04 |
| CD4 | 1058 ± 132 | 652 ± 82 | 652 ± 82 | P = 0.01 |
| CD8 | 606 ± 190 | 249 ± 31 | 249 ± 31 | P = 0.03 |
| NK | 459 ± 69 | 359 ± 53 | 359 ± 53 | NS |
| sIL-2R | 15 049 ± 2323 | 9447 ± 1170 | 9447 ± 1170 | P = 0.05 |
| sCD8 | 889 ± 155 | 641 ± 92 | 641 ± 92 | NS |

Table 4 Immunologic evaluation and response

| Cell type | Responders | SD | PD | Resp. vs PD |
|-----------|------------|----|----|-------------|
| CD4*DR*   | 111 ± 32   | 113 ± 40 | 57 ± 8 | P = 0.05 |
| CD4       | 870 ± 169  | 658 ± 47 | 696 ± 98 | NS |
| CD8*DR*   | 208 ± 123  | 76 ± 10 | 52 ± 16 | NS |
| CD8       | 625 ± 301  | 236 ± 22 | 215 ± 34 | NS |
| CD14      | 734 ± 93   | 791 ± 80 | 780 ± 72 | NS |
| HLA-DR level | 1059 ± 167 | 722 ± 84 | 556 ± 77 | P = 0.01 |

Table 5 Immunologic evaluation and survival

| Cell type | Long survival | Short survival | P-value |
|-----------|---------------|---------------|---------|
| CD4*DR*   | Pre*          | 130 ± 34      | 56 ± 8  | P = 0.05 |
| CD3       | Post          | 2689 ± 374    | 1777 ± 122 | P = 0.02 |
| CD4       | Post          | 1897 ± 350    | 1215 ± 119 | P = 0.04 |
| CD8       | Post          | 1058 ± 132    | 652 ± 82  | P = 0.01 |
| NK        | Post          | 606 ± 190     | 249 ± 31  | P = 0.03 |
| sIL-2R    | Post          | 459 ± 69      | 359 ± 53  | NS |
| sCD8      | Post          | 15 049 ± 2323 | 9447 ± 1170 | P = 0.05 |

DISCUSSION

As RCC is known for its resistance against chemotherapy and for its immunogenic properties, cytokines have been used with various successes to treat this disease. The cytokines used in the current immunotherapy protocol, LD-IL-2, IFN-z and GM-CSF, have been reported to induce antitumour activity to a greater or lesser extent (Sarna et al., 1987; Figlin et al., 1988; Vogelzang et al., 1993; Schiller et al., 1996; Bukowski, 2000; Fossa, 2000; Westermann et al., 2001). The combination of these three cytokines given simultaneously is based on the idea that expansion and activation of antigen-presenting cells (by GM-CSF), T cells (LD-IL-2 and GM-CSF) and effector cells (by LD-IL-2, GM-CSF, IFN-z), as well as an increase in immunosensitivity of the tumour cells (by IFN-z) is required for an optimal effect. Concurrent immunotherapy consisted of daily s.c. injections of 4 mIU m⁻² IL-2, 2.5 µg kg⁻¹ GM-CSF and 5 mIU fixed dose IFN-z for 12 days per 3 weeks, a dose determined as the MTD in a phase I study (de Gast et al., 2000). However, in this phase II study IL-2 reduction to 2 mIU m⁻² was necessary in 29 of the 59 patients because of grade 4 fever with hypotension (eight), severe fatigue (15), grade 3 liver enzyme elevations (five) or grade 3 renal function impairment (one). Therefore, we have to conclude that the MTD of this combination is IL-2 2 mIU m⁻², GM-CSF 2.5 µg kg⁻¹ and IFN-z 5 mIU fixed dose.

In conclusion, the immunotherapy protocol, LD-IL-2, IFN-z and GM-CSF given simultaneously, resulted in an overall response rate of 19%, with values at the end of immunotherapy (day 12), five more markers were found. Total number of lymphocytes, of CD3, CD4 and CD8 T cells and levels of sIL-2R, but not of NK cells or levels of sCD8, correlated with survival.
Our trial is the first in which markers for response and survival of IL-2-based therapies are reported for mRCC. In a study reported by Westermann et al (2001), IL-2 and IFN-α were added sequentially to GM-CSF in doses comparable to our scheme. Although they also found a general increase in lymphocytes, the number of treated patients was too small to distinguish between responder groups. In a trial with LD-IL-2 i.v., 25 patients were treated and no correlation with response could be found for any of the lymphocyte populations tested (Favrot et al, 1990). In several other studies in which the combination of IL-2 and IFN-α was used, the trials were too small to determine a correlation with response to treatment (Bukowski, 1997). However, in a large trial with HD-IL-2 therapy for metastatic melanoma, responding patients were found to have a significant higher maximum lymphocyte count immediately after therapy (Phan et al, 2001). Notably, in that trial only total lymphocyte numbers were determined and no differentiation was made between T-cell and NK-cell numbers. This indicates that patients responding to immunotherapy may be identified by analysis of peripheral blood samples. The correlation between immunological markers and survival should first be confirmed in a prospective study. As our series was limited, a multivariate analysis was not possible to study whether the immune parameters added anything over the use of known clinical parameters determining the presence of low, intermediate or high risk for survival (Zisman et al, 2002).

Immunotherapy protocols are thought to be most effective if smaller amounts of tumour are present, as indicated by the patient groups that respond best to cytokine immunotherapy, for example, good performance status, prior nephrectomy, lung metastases only, few metastatic sites (Mani et al, 1995). This might indicate that this protocol could be more effective in a perioperative setting in which nearly all tumour can be removed. In a perioperative immunotherapy protocol, it is also possible to look at the effects of immunotherapy at the site of the tumour and to relate those results with the effects in peripheral blood. Especially the activation and attraction to the tumour site of DCs and T cells, in relation to the effects on peripheral blood, may give important insight into the kinetics of cells of the immune system. Such a trial, which is currently ongoing in our hospital, may give us more understanding in how the antitumor effect is achieved and may enable us to further improve upon cytokine immunotherapy.

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REFERENCES

Bukowski RM (1997) Natural history and therapy of metastatic renal cell carcinoma: the role of interleukin-2. Cancer 80: 1198 – 1220
Bukowski RM (1999) Immunotherapy in renal cell carcinoma. Oncology (Huntingt) 13: 801 – 810
Bukowski RM (2000) Cytokine combinations: therapeutic use in patients with advanced renal cell carcinoma. Semin Oncol 27: 204 – 212
de Gast GC, Klumpen HJ, Vyth-Dreese FA, Kersten MJ, Verra NC, Sein J, Batchelor D, Nooijen WJ, Schornagel JH (2000) Phase I trial of combined immunotherapy with subcutaneous granulocyte macrophage colony-stimulating factor, low-dose interleukin 2, and interferon alpha in progressive metastatic melanoma and renal cell carcinoma. Clin Cancer Res 6: 1267 – 1272
Favrot MC, Combaret V, Negrè S, Philip I, Thiéss P, Freydel C, Bijmann JT, Franks CR, Mercatello A, Philip T (1990) Functional and immunophenotypic modifications induced by interleukin-2 did not predict response to therapy in patients with renal cell carcinoma. J Biol Response Mod 9: 167 – 177
Figlin RA, deKernion JB, Mukamel E, Palleroni AV, Itri LM, Sarna GP (1988) Recombinant interferon α-2a in metastatic renal cell carcinoma: assessment of antitumor activity and anti-interferon antibody formation. J Clin Oncol 6: 1604 – 1610
Flanigan RC, Salmon SE, Blumenstein BA, Bearman SI, Roy V, McGrath PC, Catonfr JR, Munshi N, Crawford ED (2001) Nephrectomy followed by interferon α-2b compared with interferon α-2b alone for metastatic renal-cell cancer. N Engl J Med 345: 1655 – 1659
Fossa SD (2000) Interferon in metastatic renal cell carcinoma. *Semin Oncol* 27: 187–193

Fyfe G, Fisher RI, Rosenberg SA, Szol 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

Knop J (1990) Immunologic effects of interferon. *J Invest Dermatol* 95: 725–745

Kolitz JE, Welte K, Wong FY, Holloway K, Merluzzi VJ, Engert A, Bradley EC, Konrad M, Polivka A, Gabriole JL (1987) Expansion of activated T-lymphocytes in patients treated with recombinant interleukin 2. *J Biol Response Mod* 6: 412–429

Luft T, Pang KC, Thomas E, Hertzog P, Hart DN, Trapani J, Cebon J (1998) Type I IFNs enhance the terminal differentiation of dendritic cells. *J Immunol* 161: 1947–1953

Mani S, Todd MB, Katz K, Poo WJ (1995) Prognostic factors for survival in patients with metastatic renal cancer treated with biological response modifiers. *J Urol* 154: 35–40

Mickisch GH, Garin A, van Poppel H, de Prijck L, Sylvester R (2001) Radical nephrectomy plus interferon alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell carcinoma: a randomised trial. *Lancet* 358: 966–970

Motzer RJ, Mazumdar M, Bacik J, Russo P, Berg WJ, Metz EM (2000) Effect of cytokine therapy on survival for patients with advanced renal cell carcinoma. *J Clin Oncol* 18: 1928–1935

Negrier S, Escudier B, Lasset C, Douillard JY, Savary J, Chevreau C, Ravaud A, Mercatello A, Peny J, Mousseau M, Philip T, Tursz T (1998) Reombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma. Groupe Francais d’Immunotherapie. *N Engl J Med* 338: 1272–1278

Negrier S, Maral J, Drevon M, Vinke J, Escudier B, Philip T (2000) Long-term follow-up of patients with metastatic renal cell carcinoma treated with intravenous recombinant interleukin-2 in Europe. *Cancer Sci* 2: 2003 Cancer Research UK

Phan QG, Attia P, Steinberg SM, White DE, Rosenberg SA (2001) Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma. *J Clin Oncol* 19: 3477–3482

Raghammar P (1996) Anti-tumoral effect of GM-CSF with or without cytokines and monoclonal antibodies in solid tumors. *Med Oncol* 13: 167–176

Sarna G, Figlin R, de Kernion J (1987) Interferon in renal cell carcinoma. The UCLA experience. *Cancer* 59: 610–612

Schiller JH, Hank JA, Khorsand M, Storer B, Borchert A, Huseby-Moore K, Burns D, Wesly O, Albertini MR, Wilding G, Sondel PM (1996) Clinical and immunological effects of granulocyte-macrophage colony-stimulating factor coadministered with interleukin 2: a phase IB study. *Clin Cancer Res* 2: 319–330

Thompson JA, Lee DJ, Lindgren CG, Benz LA, Collins C, Levi D, Fefer A (1988) Influence of dose and duration of infusion of interleukin-2 on toxicity and immunomodulation. *J Clin Oncol* 6: 669–678

Troy AJ, Summers KL, Davidson PJ, Atkinson CH, Hart DN (1998) Minimal recruitment and activation of dendritic cells within renal cell carcinoma. *Clin Cancer Res* 4: 585–593

Vogelzang NJ, Lipton A, Figlin RA (1993) Subcutaneous interleukin-2 plus interferon alfa-2a in metastatic renal cancer: an outpatient multicenter trial. *J Clin Oncol* 11: 1809–1816

Westermann J, Reich G, Kopp J, Haus U, Dorken B, Pezzutto A (2001) Granulocyte-macrophage-colony-stimulating-factor plus interleukin-2 plus interferon alphas in the treatment of metastatic renal cell carcinoma: a pilot study. *Cancer Immunol Immunother* 49: 613–620

Zisman A, Pantuck AJ, Wieder J, Chao DH, Dorey F, Said JW, deKernion JB, Figlin RA, Beldegrun AS (2002) Risk group assessment and clinical outcome algorithm to predict the natural history of patients with surgically resected renal cell carcinoma. *J Clin Oncol* 20: 4559–4566