Mini-review

Immunotherapy for renal cell cancer

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Introduction

In the West, renal cell cancer accounts for 2% of all cancers. Over 4000 new cases are currently reported annually in the UK1 and a worldwide mortality in excess of 100,000 deaths is predicted in the year 2000.2 The incidence of this tumour is increasing. For example, there has been a 38% increase between 1974 and 1990 in the USA.3

The treatment of renal cell cancer has radically changed in the last decade, and these changes have brought significant hope to patients with this malignancy. Chemotherapy and hormonal treatments are given for metastatic renal cell cancer, but response rates are low at 5–10%, and the duration of response is measured in months. In contrast, immunotherapy is relatively successful with responses in up to 40% of patients. The importance of immunotherapy responses is that they may be durable.

Oestrogens induce renal cancers in Syrian Golden hamsters, and this effect is abrogated by progestagens.4 On the basis of this observation, a trial of medroxyprogesterone acetate was initiated in the 1960s which showed a 27% response rate of 9 months median duration.5 Unfortunately however, this excellent result, a tribute to positive publication, has never been repeated, and progestagens have largely been abandoned as first-line therapy for advanced renal cell cancer.

In a similar fashion, chemotherapy has little effect in renal cell carcinoma. In phase II trials, even with the following wind of positive reporting, no single cytotoxic agent has produced a response rate exceeding 10%. An overall response rate of just 6% is reported in a review of 4093 patients enrolled into 83 chemotherapy trials between 1983 and 1993.6 This failure of chemotherapy could be explained by the high levels of expression of the multi-drug resistance gene in renal tumours.7 Two highly homologous genes, MDR1 and MDR2, located on the long arm of chromosome 7, encode for drug resistance. Transfection studies have demonstrated that MDR1 is responsible for the multi-drug resistance phenotype, although the function of MDR2 in humans is uncertain. High levels of the MDR1 protein, a 170 kDa cell-surface ATP-binding efflux pump, are found in cells of the proximal renal tubules8 and the majority of renal cell cancers are most closely related to these cells immunophenotypically. A correlation has been found between P-glycoprotein expression and progression-free survival in renal cell cancer.9 Although inhibitors of the P-glycoprotein calcium efflux pump do not potentiate the effects of chemotherapy, this may be because dosages sufficient to have a biological effect are not possible in humans because of toxicity.5,10

Against this background of the failure of conventional treatments for renal cell cancer, the successes of immunotherapy have been welcomed by oncologists and embraced by their patients. This article reviews the current status of immunotherapy for renal cell cancers.

Theoretical basis of immunotherapy

Immunotherapy for cancer dates back to the turn of the century to the use of Cooley’s toxins. There are clinical features of renal cell cancer that point to the importance of the host immune system in controlling
tumour growth, and the most significant is the observation of the spontaneous regression of metastatic disease that occurs more frequently in renal cell cancer than in any other solid tumour.\textsuperscript{11–14}

The mechanisms of host vs. tumour immune surveillance, as originally proposed in 1970 by Burnet\textsuperscript{15} remain controversial and ill-defined. T lymphocytes and natural-killer cells are currently thought to be the most significant mediators of this process. The activation of tumour-specific cytotoxic T lymphocytes requires the initial presentation of tumour antigens by antigen-presenting cells, usually dendritic cells or macrophages, in conjunction with MHC class II molecules and co-stimulatory molecules such as the intercellular adhesion molecules B7 and ICAM 1. In this model, class II (HLA-DR) restricted tumour antigens stimulate Th1 helper cells, which release numerous cytokines including IL-2. These cytokines activate cytotoxic T cells that recognize tumour antigens on tumour cells in conjunction with MHC class I antigens (HLA-A, B, C). The cytotoxic T-cell-mediated killing is therefore MHC-restricted.

MHC class I antigens are expressed in normal kidney tissues, renal cell cancer cell lines and both renal cell cancer primary tumours and metastases. In contrast neither normal kidney cells nor renal cell cancers express MHC class II antigens, but these are expressed following induction with inflammatory cytokines, including IFN\textsubscript{a} in models such as autoimmune nephritis and in renal allograft rejection.

**The interferons**

In the field of oncology, immunotherapy really began with the use of partially purified interferons derived from leukocytes (IFN\textsubscript{x}), fibroblasts (IFN\textsubscript{b}) and lymphocytes (IFN\textsubscript{c}). The first report documenting responses in advanced renal cell cancer with partially-purified leukocyte interferon appeared in 1983.\textsuperscript{19} Subsequent trials have described responses using both non-recombinant and recombinant interferons. Similar response rates are achieved with the different recombinant IFN\textsubscript{x}s available. Combining data from 29 studies of IFN\textsubscript{x} involving over 1000 patients, the objective response rate was 12%,\textsuperscript{20} but the complete remission rate was only 2% and the median response duration of those patients achieving a complete response, just 8 months.

The optimal dosage schedule for IFN\textsubscript{x} remains controversial. Initially, in a classical oncology investigatory approach, phase I studies examined the highest treatment dosages possible. The interferons given in dosages of up to 100 000 mega U, were profoundly toxic. In the mid 1980s, investigators backed off from high-dosage schedules, with the realization that maximal tolerable dosages did not equate with efficacy. Although laboratory studies have shown a dose response to IFN\textsubscript{x} with maximal responses at 18 mega U, these findings have not been reproduced in patients with cancer. Currently, the majority of patients are treated with dosages of 3–10 mega U subcutaneously 3 times weekly. Responses are often slow to develop, and are seen most frequently in patients with a good performance status, who have undergone a nephrectomy and who have pulmonary metastases. It remains unknown whether IFN\textsubscript{x} has an impact upon survival in metastatic renal cell cancer. At one single institution, the median survival of 159 patients with metastatic renal cell cancer who were treated with rhIFN\textsubscript{x}2a was 11.4 months, but the 5-year survival was only 3%. Multivariate analysis of this data set for survival outcome confirmed that good performance status and prior nephrectomy were independent good prognostic factors.\textsuperscript{21}

A retrospective prognostic factor analysis of patients treated with IFN\textsubscript{x} or with chemotherapy in various EORTC clinical trials was published in 1994.\textsuperscript{22} The median survival for good prognosis patients receiving IFN\textsubscript{x} was double that of similar patients treated with chemotherapy. However, there was no difference in the duration of survival for patients with moderate or poor risk disease. A randomized multicentre comparison of IFN\textsubscript{x} (10 mega U subcutaneously three times a week) and medroxy progesterone acetate (300 mg od orally) expressed following induction with inflammatory cytokines, including IFN\textsubscript{a} in models such as autoimmune nephritis and in renal allograft rejection.

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The mechanism of anti-tumour activity for interferon remains unclear. In vitro, interferons have a direct antiproliferative action on renal cell tumour cell lines. This anti-proliferative activity is thought to be due to interferon-induced expression of an RNA-dependent tyrosine kinase that phosphorlates the z subunit of eIF-2, a translation initiation factor.\textsuperscript{24} In addition, interferons stimulate host lymphocytes and macrophages and upregulate MHC class I antigen expression. A third possible mechanism of action that has been proposed for interferon is as an anti-angiogenesis agent. Resistance to interferon therapy may in part be due to the development of neutralizing antibodies.\textsuperscript{25}

IFN\textsubscript{a} has greater in vitro activity against renal cell carcinoma lines than either IFN\textsubscript{x} or IFN\textsubscript{b}. However, laboratory activity does not match clinical data, and similar clinical results are achieved to those with other interferons, with a cumulative response rate of 12% in 234 patients treated in 10 phase I/II trials.\textsuperscript{26} Response rates for IFN\textsubscript{a} are lower in patients with bulky disease who have not undergone nephrectomy.\textsuperscript{27}
Interleukin-2 and LAK cells

Non-MHC-restricted tumour-cell killing by natural-killer lymphocytes was first described in 1980 for murine and human tumours.\textsuperscript{16,17} In vitro cytotoxicity of these natural-killer lymphocytes was found to be enhanced by prior exposure to interleukin through the generation of lymphokine-activated killer (LAK) cells.\textsuperscript{18} This technique formed the basis for a novel approach to tumour immunotherapy called adoptive immunotherapy using autologous peripheral blood mononuclear cells stimulated ex vivo with IL-2.

At the NCI in the United States, Rosenberg demonstrated that IL-2 alone and in combination with LAK cells decreased the size of pulmonary and hepatic metastases.\textsuperscript{26} In mouse tumour models, LAK cells with IL-2 were more effective than IL-2 alone, and the combination, but not IL-2 alone, was active in immunodeficient mice. The best responses in mice were achieved using the maximal tolerated dosages of both LAK cells and IL-2. Since very high doses of IL-2 were necessary to generate LAK cells in vivo, ex-vivo expansion was advocated. These findings in animal models were the basis for a series of clinical studies which combined ex-vivo generated autologous LAK cells and maximal doses of IL-2.\textsuperscript{29}

These phase I studies demonstrated activity of LAK cells with IL-2, but also profound toxicity, with the informal suggestion that the majority of patients treated required ICU care because of the multisystem complications of vascular leak syndrome. Now, because mouse studies had shown that IL-2 alone was active in immunocompetent mice and responses had been seen among some patients who were given IL-2 alone, a randomized comparative study of IL-2 with or without LAK cells resulted, which showed identical response rates.\textsuperscript{30}

Ten trials of bolus high-dose intravenous IL-2 have been published which have enrolled a total of 537 patients. The combined objective response rate is 19%, with a median response duration of 22 months.\textsuperscript{31} However, severe toxicity was associated with the treatment, due to vascular leak syndrome from bolus injections. The treatment-related mortality with bolus IL-2 is 4%.\textsuperscript{32}

In the late 1980s, it began to emerge that the importance of the complete response to IL-2 was the durability of that response, which was in contrast to any other treatment of renal cell cancer. This realization led to a persistence with IL-2, and investigations of ways of reducing the toxicity of bolus therapy. The first step in this path was the investigation of the effects of continuous infusions of IL-2. A summary of trials in 422 patients showed an objective response rate of 14%.\textsuperscript{33}

The next phase of development for IL-2 occurred in the early 1990s. Although preclinical data had suggested that there was a dose response for IL-2, this had not been demonstrated in early clinical trials. In an attempt to reduce further the toxicity of high-dose IL-2 therapy, lower-dose schedules using subcutaneous bolus treatment were introduced. Six trials of low-dose out-patient IL-2 have been completed, and the overall response rate in 104 patients is 20%.\textsuperscript{31} A randomized phase III comparison of high- and low-dose IL-2 confirmed that the high-dose schedule was more cardiotoxic, and was also associated with thrombocytopenia and more infectious episodes. There was no statistically significant difference in the response rates or complete remission rates between the high- and low-dose arms.\textsuperscript{34}

As a result of these trials, treatment with IL-2 has been transformed and moved to an out-patient setting, where it can be given with little or no grade III/IV toxicity.

Combination immunotherapy

After the development of safe out-patient low-dose single-agent immunotherapy schedules for both IFN\textsubscript{a} and IL-2, investigators sought to develop combination immunotherapy. In animal models, the partnership between IFN\textsubscript{a}-induced upregulation of MHC expression and IL-2-induced T-lymphocyte and NK-cell activation leads to a synergy of immunological and anti-tumour effects.\textsuperscript{35–37} Initial studies in humans were encouraging,\textsuperscript{38} however, subsequent prospective randomized studies suggested that the proportion of responses with the combination immunotherapy was no higher than with IL-2 alone.\textsuperscript{39–41}

Eventually a randomized study was organized, and the French Immunotherapy Group of the French Federation of Cancer Centres have recently reported the interim results of their CRECY study. They randomly assigned 425 patients to receive one of three immunotherapy schedules: continuous intravenous IL-2 infusion; subcutaneous IFN\textsubscript{a}; or a combination of continuous infusional IL-2 and subcutaneous IFN\textsubscript{a}. Results, reported so far only in abstract form, have shown that the combination therapy was superior to either single agent as assessed by response rate and event-free survival at one year, although the toxic death rate for the combination arm was reported as 3.6% compared to 0.7% for IFN\textsubscript{a} alone and 7.9% for IL-2 alone.\textsuperscript{42}

Chemoinmunotherapy

Efforts have been made to assess whether there is an advantage in combining chemotherapy and immunotherapy. Initially, interferon was combined with 5-fluorouracil, as there is biochemical synergy, and response rates of 35% resulted.\textsuperscript{43} More ambitious
chemoimmunotherapy regimens including both IFNα and IL-2 were devised, and in an initial trial, 35 patients with metastatic renal cell cancers were treated with alternating cycles of combination immunotherapy with IL-2 and IFNα and chemoimmunotherapy with bolus 5-fluorouracil and IL-2. The regimen was administered on an out-patient basis. The overall response rate was 48%, and was without severe toxicity. These results have been reproduced by one group but not by others.

Cellular adoptive immunotherapy

There has been further progress in the field of adoptive immunotherapy in the 1990s, mainly involving studies of the effects of tumour-infiltrating lymphocytes (TILs), which are lymphocytes extracted from tumour biopsies. LAK cell activity is thought to be NK-cell-mediated, but TIL are cells that express both helper (CD4+) and cytotoxic (CD8+) T-cell markers. These TIL cells have anti-tumour activity presumably because they recognize, and are specific for, tumour antigens. Treatment with TIL cells involves initial harvesting and then expansion by ex vivo culture in the presence of IL-2. After short-term culture, tumour cells have been depleted while the TIL cells proliferate. These autologous TIL cells are reinfused with IL-2 into patients. In murine lung and liver metastasis models, TIL cells are 50–100 times more potent than LAK cells. Phase I/II trials of TIL therapy for metastatic renal cell carcinoma have reported response rates of 13% in 31 patients and 33% in 48 patients. At UCLA, the response rates, median response duration and 3-year survival rates were higher in 58 patients treated with TIL/IL-2 than in patients who received IL-2 alone or in combination with IFNα. The benefits of TIL/IL-2 therapy over IL-2 alone are currently being investigated in a randomized study.

Autolymphocyte therapy

Autolymphocyte therapy (ALT) aims to activate memory T cells by ex-vivo expansion of peripheral blood mononuclear cells with supernatants of anti-CD3 monoclonal-antibody-activated autologous peripheral blood lymphocytes and IL-2. Anti-CD3 only activates T cells previously exposed to antigen, and clonal expansion of these memory T cells is driven by IL-2. Prior to re-infusion, the cells are treated with cimetidine and irradiated to destroy suppressor T cells. In a randomized trial that enrolled 90 patients, the addition of ALT to cimetidine produced a survival advantage compared with cimetidine alone, and this result has been confirmed in other centres. The place of ALT in the clinical management of metastatic renal cell cancer will have to await results from a randomized comparison with IL-2.

Vaccination programmes

Cancer cell vaccination therapy involves the treatment of patients with tumour cells that have been genetically modified to enhance the host immune response against established residual tumour masses. The protocols usually involve the isolation and culture of tumour cells from biopsies, followed by the transfection of established tumour cell lines in culture. The transfected autologous tumour cells are then irradiated prior to re-injection. For renal cell cancers, transfection with cytokine genes has been advocated to induce a tumour-specific immune response. One early clinical trial randomized patients with localized disease to nephrectomy or nephrectomy followed by immunization with irradiated autologous tumour cells plus Bacillus Calmette-Guérin. Most of the vaccinated patients developed a delayed hypersensitivity response to the tumour cells, although no difference in survival was detected. In a randomized trial in locally-advanced renal cell cancer, patients were observed without treatment or vaccinated with autologous tumour cells that had been incubated with Newcastle disease virus, irradiated and then mixed with IL-2. In this study, there was an improvement in disease-free survival in the vaccinated patients, but this did not reach statistical significance. Autologous tumour cells transfected with the GM-CSF gene were used to vaccinate 16 patients with advanced renal cell carcinoma and again T-cell-mediated delayed hypersensitivity was observed, although only one patient had an objective partial response.

Conclusion

Immunotherapy is the most effective of the current therapies available for metastatic renal cell cancer. Patients with a good performance status, a long disease-free interval, and metastases in favourable sites have the best prognosis, and are most likely to benefit from immunotherapy. Patients with none of these clinical features have a poor chance of response and should not be subjected to the prospect of treatment-related toxicity without the hope of benefit from therapy. The results of MRC study confirm the benefits of immunotherapy over other treatment modalities in metastatic renal cell cancers. However, which immunotherapy regimen is optimal remains controversial, although the CRECY study results favour the combination of IL-2 and IFNα given in low dosages subcutaneously. Future investigations
of new cytokines and combination chemo-immunotherapy offer hope in this condition.

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References


