Adjuvant therapy of renal cell carcinoma: patient selection and therapeutic options

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Accepted for publication 29 April 2005

KEYWORDS

adjuvant therapy, carbonic anhydrase IX, heat shock proteins, monoclonal antibodies, renal cell carcinoma, tumour-associated antigen

INTRODUCTION

Cancer of the kidney or renal pelvis is estimated to account for 36 160 new cases and 12 660 deaths in the USA in 2005, and over 100 000 deaths worldwide [1,2]. RCC is the most lethal of the urological cancers, with >40% of patients dying from their cancer, compared to the ≈20% mortality rates associated with prostate and bladder cancers [1]. About 70% of patients present with localized or locally advanced disease and are potentially curable by nephrectomy alone [3,4]. However, recurrence rates are high for patients with locally aggressive tumours, at 35–65% depending on pathological stage, Fuhrman nuclear grade and Eastern Cooperative Oncology Group performance status (ECOG-PS) [5].

Historically, studies showed that the presence of lymph node metastases was the most reliable predictor of a poor outcome in locally advanced RCC. Patients with lymph node involvement are reported to have a 5-year survival rate of 10–25% [3,6,7]. Therefore, studies have been undertaken to determine whether adjuvant therapy offers a benefit in terms of disease-free survival (DFS) and overall survival in patients at risk of relapse after surgical resection for RCC. Postoperative adjuvant therapies, e.g. radiation, hormonal therapy, and nonspecific cytokine immunotherapy, have thus far had no clinical benefit for patients with resected high-risk locally advanced RCC (Table 1) [8–13,14]. Nevertheless, the period after resection seems to offer a special immunological opportunity. Residual tumour mass is at its lowest and the host immune system has been relieved of suppression related to tumour bulk. Furthermore, significant achievements in the basic sciences have led to a greater knowledge of the underlying molecular genetics of RCC, which hold promise for increased sophistication in attempts to individualise patient prognostication and future treatment strategies.

SELECTION OF HIGH-RISK PATIENTS

The TNM staging system is currently the most extensively used, but new comprehensive staging methods have emerged from the University of California-Los Angeles (UCLA) and other institutions in an attempt to improve prognostication, by combining other pathological and clinical variables [15]. The UCLA Integrated Staging System (UISS) was developed to better stratify patients into prognostic categories, using statistical tools that accurately define the probability of survival of an individual patient [7]. The initial UISS contained five groups based on TNM stage, Fuhrman grade, and ECOG-PS. Projected 2- and 5-year survival for patients in UISS groups I to V are 96% and 94%, 89% and 67%, 66% and 39%, 42% and 23%, and 9% and 0%, respectively. The UISS has been subsequently modified into a simplified system, based on separate stratification of patients with metastatic and nonmetastatic disease into low-, intermediate- and high-risk groups (Fig. 1) [16]. Nonmetastatic high-risk patients had a high systemic failure rate, suggesting a high risk of occult metastasis at diagnosis (i.e. lesions not detected by current imaging methods). This concept is supported in that nonmetastatic high-risk patients had a similar survival to metastatic low-risk patients. This risk group stratification provides a clinically useful system for predicting postoperative outcome, and a unique tool for risk assignment and outcome analysis to help determine follow-up regimens and eligibility for clinical trials.

HISTORICAL PERSPECTIVE

The concept of systemically treating patients at high risk of micrometastatic disease after surgical resection of the primary tumour has been explored in many different cancers, including breast, lung and colon. There are several reasons why the concept of adjuvant therapy in RCC has not been explored fully and prospectively, including the paucity of fully studied systemic treatments for metastatic RCC, the high toxicity profile of available agents, and that the incidence of RCC is not as high as that of other cancers, making accrual to large randomized trials difficult.

Radiotherapy has been studied to determine whether the risk of local relapse could be decreased after radical nephrectomy. There were no differences in relapse rate or survival in a prospective study of radiotherapy vs observation in 72 patients who had nephrectomy for stages II and III RCC [13,14]. In addition, treatments were associated with a significant amount of morbidity to abdominal organs.

Hormonal agents such as medroxyprogesterone acetate (MPA) block glucocorticoid receptors on some renal tumour cells. Patients with metastatic RCC have had occasional responses to MPA and this has provided a rationale to test MPA in the adjuvant setting. In a prospective randomized trial of adjuvant MPA after radical nephrectomy, 136 patients received either 500 g MPA (three times a week) for 1 year or observation after radical nephrectomy. After a median follow-up of 5 years, a third of patients relapsed after a median disease-free interval of 17 months [8]. There were no significant differences in relapses between the adjuvant group and the observation group (33% vs 34%, respectively).

Tumour cells express tumour antigens that are present on the cell surface by MHC class I or class II molecules, and are capable of eliciting tumour-specific immune responses. These immune responses are mediated by CD8+ cytotoxic T lymphocytes and the responses may be further amplified by cytokines secreted by CD4+ helper cells, such

as interleukin-2 and interferon-γ. A rationale therefore exists to immunise patients against antigens derived from tumour cells, either alone or combined with hormones, cytokines or immune adjuvants, such as BCG to further enhance responses.

In a prospective randomized trial, 43 patients were randomly allocated to either adjuvant hormonal immunotherapy or hormonal therapy after nephrectomy. Immunotherapy consisted of autologous irradiated tumour cells admixed with BCG administered intradermally and endolymphatically. At a median follow-up of 30 months, although there was a trend for a longer DFS in patients with stages I-III RCC with localized disease, and longer survival in those with metastatic disease ($P < 0.07$), they were not statistically significant [17]. Another prospective study of 120 patients randomized to either an untreated group or a group receiving autologous irradiated tumour cells mixed with BCG after radical nephrectomy for RCC (pT1–3b pN0 or pN+) was reported [9]. At a median follow-up of 61 months, there was no statistically significant difference in either 5-year DFS (63% for treated and 72% for control patients) or 5-year overall survival (69% and 78%, respectively).

Three trials examined the effects of interferon-α as an adjuvant therapy after complete surgical resection, compared with observation [18]. None of these trials showed a statistically significant improvement in endpoints such as the time to relapse or overall survival. A randomized phase III ECOG/Intergroup trial of adjuvant interferon-α after complete resection of locally extensive RCC (pT3–4a and/or pN+ disease) showed that adjuvant treatment did not contribute to survival or relapse-free survival in this group of patients [10]. A multicentre, prospective, randomized, controlled phase III Cytokine Working Group trial assessed high-dose bolus interleukin-2 after surgery in patients with high-risk RCC, which included resected locally advanced (T3b-4 or N1-3) or metastatic (M1) RCC and no previous systemic therapy. This study concluded that although one course of high-dose bolus interleukin-2 was feasible, there was no improvement in DFS over the observation group [11].

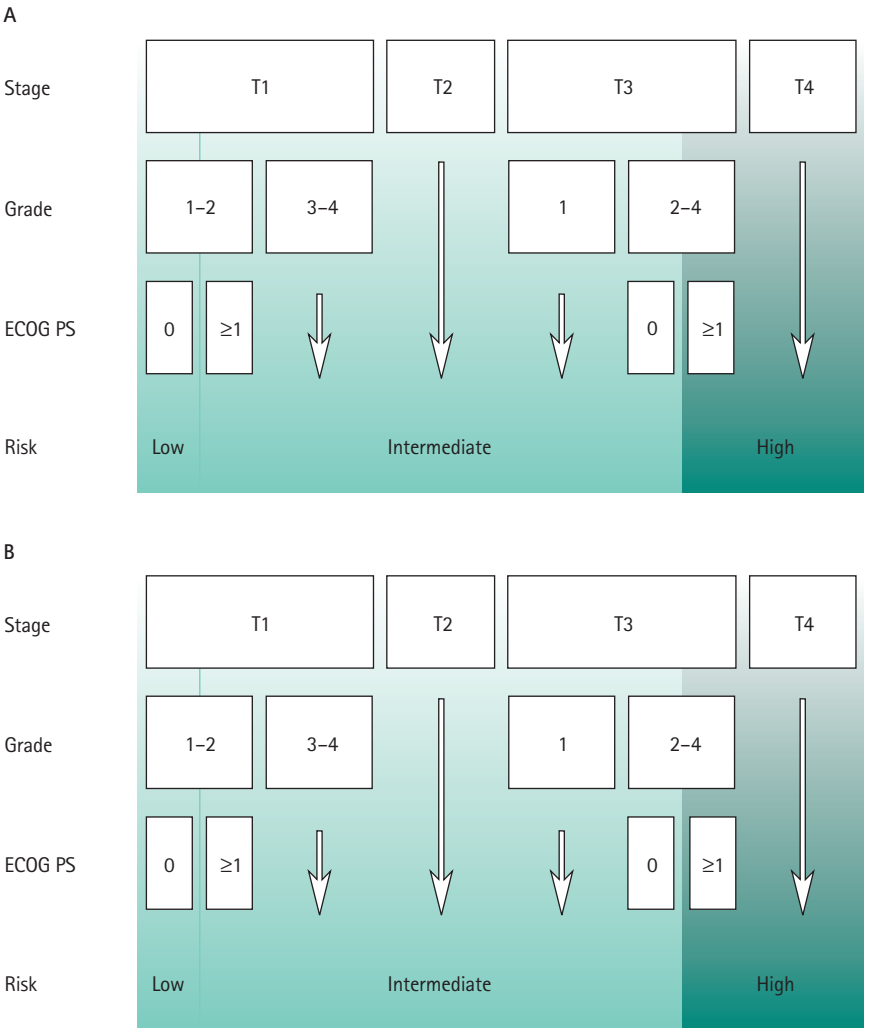
A recent multicentre, randomized phase III trial of individually prepared autologous tumour-derived product (aTL) given in the adjuvant setting after nephrectomy for patients with stage pT2–3b pN0–3 M0 RCC, according to the 1993 UICC classification, appeared to show some benefit [12]. At 60 and 70 months of follow-up, the hazard ratios (95% CI) for tumour progression were 1.58 (1.05–2.37) and 1.59 (1.07–2.36), respectively, in favour of the vaccine group ($P = 0.02$, log-rank test). Progression-free survival rates at

TABLE 1 Randomized trials of adjuvant therapy after nephrectomy for high-risk localized RCC

Treatment [reference]	Outcome
Radiation vs observation [13,14]	Survival (26 months): 50% vs 62% (NS)
MPA vs observation [8]	Relapse rate: 32.7% vs 33.9% (NS)
Tumour cells + BCG vs observation [9]	5-year DFS: 63% vs 72% (NS)
Interferon-α vs observation [10]	Median survival: 5.1 vs 7.4 years (NS)
High dose interleukin-2 vs observation [11]	2-year DFS: 19.5 vs 36 months (NS)
Tumour cell vaccine vs observation [12]	5-year PFS: 77.4% vs 67.8% ($P = 0.02$)

PFS, progression-free survival; NS, not significant.

FIG. 1. ***A,*** *N0M0 patients after nephrectomy assigned into risk groups. Progress from top to bottom using 1997 American Joint Committee on Cancer (AJCC) T stage, Fuhrman nuclear grade, and ECOG PS.* ***B,*** *For N1, N2 or M1 patients, starting with the 1997 AJCC TNM stage.*



60 and 70 months were 77% and 72%, respectively, in the vaccine group, and 68% and 59%, respectively, in the control group. The vaccine was well tolerated, with only 12 adverse events associated with the treatment. However, some methodological issues should be considered, which include the number of patients lost after the randomization step (174/553, 32%), the imbalance of this loss (99 from aTL, 75 from placebo), and the absence of tabulation of overall survival.

CURRENT APPROACHES

Heat-shock proteins (HSPs) are ubiquitous molecules found in all living cells that protect the cell when injury occurs and take part in helping the cell to build resistance to subsequent stress [19]. The function of these proteins is to assist newly synthesized polypeptides to fold into their functional conformations, and they have been likened to 'chaperones', ensuring that cellular proteins are guided to the correct location. They help to facilitate the transport of proteins from one compartment to another inside the cell and are thought to play a role in the presentation of peptides on the cell surface to help the immune system recognize diseased cells. In tumour cells HSPs can inhibit apoptosis, but they can also act as classic foreign antigens and elicit an immune response, leading to cell destruction.

The complex formed between HSPs and their respective target peptides has been shown to be highly immunogenic. The striking feature about purified HSP-peptide complexes is their ability to programme relevant immune pathways necessary to target cancers and infectious agents. They have been shown to activate CD8+ and CD4+ lymphocytes, induce an innate immune response, including natural-killer cell activation and cytokine secretion, and induce maturation of dendritic cells [20]. The immunogenic and potential antitumour effects of HSPs were first elucidated in early cancer vaccine studies. Mice vaccinated with attenuated tumour cells were conferred specific protection against future establishment of similar live tumour cells [21]. The success of tumour-specific vaccination was attributed to HSPs through fractionation experiments with tumour cell lysates, in which protective immunity was detected in every fraction containing HSPs [22]. In addition, HSPs only elicited immunity to the tumours from which they were purified. Furthermore, treatment with HSP-peptide complexes resulted in deceleration of tumour growth, stabilization of tumour size, and tumour shrinkage in mice with large, established tumours. The most striking display of the antitumour activity of HSPs occurred in mice with minimal residual disease, which led to long-lasting protection from recurrence and allowed the animals to reach their normal life spans [23].

As the efficacy of the vaccine became more apparent in these animal models, more of the mechanisms of the vaccine began to emerge and investigators renewed their search for a clear definition of the vaccine itself. The vaccine consists of HSP-peptide complexes isolated from a patient's tumour. After vaccination, antigenic tumour peptides are processed and presented on the surface of potent antigen-presenting cells of the immune system, such as macrophages and dendritic cells, which results in a much more potent antitumour immune response than that generated by expression of the same antigens by the tumour cell itself. The advantage of autologous HSP technology is its high specificity for the tumours of individual patients, and its capability of circumventing the immune tolerance of various tumours. Another advantage is that the complex presents a vast array of the antigenic peptides from the tumour, thereby having the greatest chance to elicit a specific antitumour immune response. The hypothesis as to why they are effective in cancer is related to the fact that tumour immunity is largely mediated by T cells that recognize a complex of a MHC class I molecule and a peptide [20]. Several lines of evidence indicate the concept that HSPs isolated from tumour cells are bound to low molecular weight antigenic peptides, which in turn activate a T-cell response in the immunized host [24].

The first autologous HSP vaccine introduced in clinical trials was HSP-peptide complex 96 (HSPPC-96, Oncophage), produced from surgically resected cancer tissue and formulated for intradermal or s.c. injection [20]. After resection, the cancer tissue is frozen and shipped to a central laboratory for vaccine production. The technology requires ≈7 g of tumour tissue to produce sufficient vaccine. The initial trials began in 1995 with a phase I pilot study that enrolled patients with metastatic cancer that had been refractory to previous therapies. After these initial efforts showed that using the vaccine was feasible and well tolerated, nine phase I or II clinical studies were done to further establish the safety profile of the vaccine, characterize immune responses, identify the most practical and effective dose and route of administration, and document its clinical activity. Adverse effects have generally been characterized as mild and transient, and usually have been limited to injection-site reactions or low-grade fevers.

In a phase II study of HSPPC-96 for patients with metastatic RCC, Assikis *et al.* [25] reported on their results in 61 patients. As part of the protocol, the study also assessed the activity of additional s.c. interleukin-2 for patients whose cancer progressed while they were receiving the vaccine. The vaccination treatment schedule consisted of 25 μg of HSPPC-96 intradermal injections at weekly intervals in weeks 1–4, followed by every 2 weeks until progression. At 10 weeks after the start of treatment and at 8-week intervals thereafter patients were evaluated for progression. Of the 61 patients who received at least one dose of the vaccine, two had a partial remission, one had a complete remission (remaining disease-free after 2.5 years) and 18 had stable disease. Of those whose disease progressed, seven of 16 responded clinically to the addition of interleukin-2 by achieving disease stability. The median progression-free survival for the entire group was 18 weeks, while for those treated with vaccine and interleukin-2, it was 25 weeks. Two years after initiating the vaccine, 30% of all patients were alive; there was no significant or unexpected toxicity.

A randomized phase III clinical trial evaluating this approach as an adjuvant therapy for nonmetastatic kidney cancer at high risk of recurrence after nephrectomy was launched at 145 clinical sites in the USA, Canada, Europe, and Israel. The trial has enrolled ≈650 patients, randomized to undergo surgical removal of the primary tumours followed by outpatient treatment with HSPPC-96 or surgery only. Future trials are expected to also focus on how the efficacy of HSP-peptide vaccines may be enhanced, possibly through the concomitant administration of other more conventional cytostatic or immunomodulatory agents.

G250 MONOCLONAL ANTIBODY (MAB) THERAPY

Tumours in laboratory animals will regress after treatment with serum derived from

immunocompetent animals immunised with tumour antigens [26]. The pooled serum used in early studies consisted of antibodies directed against antigens expressed by a given tumour, as well as other non-tumour-specific antibodies and proteins. These experiments showed that antibodies generated against tumours could inhibit tumour cell growth and induce tumour cell death. The concept of selective tumour targeting with antibodies is based on the avid interaction between the antibody and an antigen that is expressed specifically on malignant cells. This specificity can be used to guide toxic substances or radionuclides to the tumour. Alternatively, effector functions such as antibody-dependent cellular cytotoxicity (ADCC), which are intrinsically present in antibodies, may lead to tumour cell death.

G250 refers to a mAb that was raised > 10 years ago by immunising mice with human RCC homogenates [27]. The RCC-associated transmembrane protein designated G250 has since proven to be identical to tumour-associated protein MN or carbonic anhydrase IX (CA IX) [28], a tumour-associated antigen first identified in HeLa cells, and expressed in cervical cancer, using murine mAb M75 [29]. The normal function of CA IX is to catalyse the reversible conversion of carbon dioxide and water to carbonic acid, playing a role in the intra- and extracellular pH regulation of cells [30]. It has been implicated as an intrinsic marker of hypoxia, as well as a prognostic marker for several types of human tumours [31]. CA IX over-expression may be mediated by hypoxia-inducible factor-α (HIF-α) and contribute to an acidic tumour microenvironment, to help cancer progression and metastasis [31,32]. Up-regulation of HIF-α is a common feature in von Hippel-Lindau gene alterations in clear cell RCC, and is significantly associated with an up-regulation of vascular endothelial growth factor (VEGF), which promotes angiogenesis [33]. Studies confirm that CA IX expression in normal tissue is extremely limited, mainly to gastrointestinal tract, gallbladder and pancreatic ducts [30]. However, it is expressed in numerous malignancies including cervical, uterine, breast, lung, oesophageal, gastric, biliary tree, colorectal, bladder, and skin cancers [30,34].

cG250 (WX-G250, Rencarex®) is a chimeric monoclonal IgG1 antibody that binds to CA IX, which is found on 95% of clear cell RCC, but not on normal kidney tissue. Administration of unmanipulated cold antibody may lead to the recruitment of effector cells or complement activation, resulting in cell death. Although cG250 had little ADCC *in vitro*, a phase II study was conducted in which cG250 was administered weekly by i.v. infusion to patients with advanced progressive RCC [35]. Thirty-six patients received 12 infusions of 50 mg of cG250 per dose. There was a tumour response and/or disease stabilization in nine of 32 evaluable patients (28%), with a median survival of 16 months and a 2-year survival rate of 39% during the follow-up.

Potent and sustained immune effector activity was reported with cG250 and cytokines *in vitro* [36], which suggested that combined immunotherapy with cG250 and cytokines such as interleukin-2 might be advantageous in the treatment of RCC. Indeed, subsequent trials are focusing on the combination of cG250 with biological response modifiers. In one trial, the effects of cG250 (20 mg of cG250 per dose) and low-dose interleukin-2 s.c. (1.8 MIU daily interrupted by bi-weekly pulses of 5.4 MIU for 3 days) in metastatic RCC are being studied [37]. In 30 evaluable patients with metastatic RCC, administration of 50 mg cG250 over 12 weeks had an overall clinical benefit rate (objective response or stable disease of at least 6 months) of 25% and a median survival time of 22 months in patients with metastatic RCC. In another trial, the effects of cG250 combined with interferon-α are being studied [38]. Patients received weekly 20 mg of cG250 for 3 months combined with interferon-α-2a s.c. (3 MIU, three times per week). Patients showing a response to treatment were offered continuation of cG250 treatment for 6 weeks. Of 26 evaluable patients, 42% showed tumour response and/or disease stabilization. Survival data are currently being collected.

The good safety profile, combined with the observed clinical benefits and enhanced long-term survival of patients, have led to the rationale of using cG250 as an adjuvant drug in a placebo-controlled phase III trial. In 2004, a global, randomized, double-blind phase III study termed ARISER (Adjuvant Rencarex Immunotherapy trial to Study Efficacy in nonmetastasized RCC) was started to evaluate cG250 vs placebo as an adjuvant treatment in 612 patients with nonmetastatic clear cell RCC at high risk of relapse after nephrectomy and lymphadenectomy.

THE FUTURE OF ADJUVANT THERAPY

Previous attempts to predict progression and survival have relied on traditional clinical variables, as previously described [14]. While comprehensive integrated staging systems have led to the availability of improved diagnostic and prognostic information for patients with RCC, the incorporation of molecular biomarkers into future staging systems is expected to completely revolutionise the approach to diagnosis and prognosis. More recently, methods based on gene arrays, which screen for differential expression of thousands of genes, have identified very many new, potentially important prognostic markers [39]. The evaluation of protein expression in a high-throughput tissue array is a natural extension of the efforts in molecular staging. Sections of the microarray provide targets for parallel *in situ* detection of DNA, RNA and protein, and allow the rapid analysis of hundreds of molecular markers, which can be correlated with clinical data with respect to disease progression, treatment response and survival.

Molecular tumour markers are expected to have an enormous impact on the future diagnosis, prognostication and selection of therapeutic targets for RCC. Currently, markers relating to tumour proliferation, growth, angiogenesis, and loss of cell adhesion, among others, are being evaluated for their prognostic potential [14]. Much attention has recently been paid to CA IX. A study at UCLA using survival-tree analysis determined that a threshold of 85% CA IX staining provided the most accurate prediction of survival [40]. Low CA IX staining was an independent prognostic indicator of poor survival in patients with metastatic RCC. Furthermore, complete responders (8%) to interleukin-2 immunotherapy were those with high CA IX (>85%) staining of the primary tumour. These findings were corroborated by a recent study from the Dana Farber Cancer Institute, which showed that high CA IX expression was significantly associated with improved response to interleukin-2 [41]. A recent study also showed a close correlation between HIF-1α expression and CA IX expression in clear cell RCC, whereas there was no relationship between HIF-2α expression and CA IX [42]. It remains unclear whether the expression of CA IX in RCC is a function of von Hippel-Lindau gene mutation, tumour hypoxia, or a combination of the two. However, CA IX

reflects significant changes in tumour biology, which may be useful to predict the clinical outcome and identify high-risk patients in need of adjuvant immunotherapy and CA IX-targeted therapies.

Current efforts at UCLA are to integrate molecular information from tissue microarrays into the UISS to generate a Molecular Integrated Staging System. A custom tissue microarray was constructed using clear cell RCC from 318 patients, representing all stages of localized and metastatic RCC [43]. Immunohistochemical staining was performed for Ki-67, p53, gelsolin, CA IX, CA XII, PTEN, EpCAM, and vimentin. These markers were selected based on previous reports linking them to the development of malignancies. Increased staining for Ki-67, p53, vimentin, and gelsolin correlated with worse survival, whereas the inverse was seen for CA IX, PTEN, CA XII, and EpCAM. Only metastatic status ($M \geq 1$ or $N > 0$), gelsolin, p53, and metastatic status/CA IX remained significant predictors of survival in a multivariate analysis, and were used to create a prognostic model (marker model). Using a similar approach, a prognostic model was constructed using a combination of clinical variables and marker data (clinical/marker model). CA IX, vimentin, and p53 were statistically significant predictors of survival, independent of the clinical variables, T-stage, metastatic status, ECOG-PS, and grade in a multivariate analysis. Both nomograms were calibrated, using bootstrap bias-corrected estimates, to be accurate to within 10% of the actual 2- and 4-year survival rates. Furthermore, prognostic systems based on protein expression profiles for clear cell RCC were shown to perform better than standard clinical predictors such as TNM stage, histological grade, and ECOG-PS. The predictive accuracy of the marker model for RCC was comparable to the UISS, and the clinical/marker model was significantly more accurate than the UISS. Although these nomograms are useful for visualizing predictive models, they need to be tested on independent populations of patients before being applied to patient care.

CONCLUSIONS

Optimal adjuvant therapy for resected RCC remains to be defined and the evaluation of adjuvant therapies will require properly controlled and adequately powered randomized trials. Recent randomized trials evaluating interferon-α and high-dose interleukin-2 as adjuvant therapy have shown no clinical benefit. Autologous tumour cell vaccine appears to be promising in this setting, but many patients were lost after the randomization step and overall survival was not reported in that trial. HSPPC-96 and WX-G250 are currently two adjuvant trials that are ongoing, which may shed more light on this subject. Recent advances have paved the way for developments that may enhance early diagnosis, better predict tumour prognosis, and improve survival for patients with RCC. Future adjuvant studies will undoubtedly incorporate cytokine regimens and new treatment strategies, e.g. tumour vaccines, anti-angiogenesis agents, and small-molecule inhibitors. Until more conclusive data are available, observation alone continues to remain the standard of care.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **MPA**, medroxyprogesterone acetate; **DFS**, disease-free survival; **ECOG–PS**, Eastern Cooperative Oncology Group performance status; **UCLA**, University of California-Los Angeles; **UISS**, UCLA Integrated Staging System; **aTL**, autologous tumour-derived product; **HSP**, heat-shock protein; **mAb**, monoclonal antibody; **ADCC**, antibody-dependent cellular cytotoxicity; **CA IX**, carbonic anhydrase IX; **HIF-α**, hypoxia-inducible factor-α.