Emerging role of tyrosine kinase inhibitors in the treatment of advanced renal cell cancer: a review

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Advanced and metastatic renal cell cancer (RCC) is resistant to conventional chemotherapy. Only a very small number of patients survive long term after immunotherapy. However, any effect of interleukin-2 (IL-2) and/or interferon on median overall survival is small, and treatment-associated toxicities may be severe. The disease is therefore an area of high unmet medical need. Activation of the VEGF and EGF/RAS/RAF/MAP kinase pathways is frequent in solid tumours such as RCC. Such activation is implicated in tumour angiogenesis and proliferation, VEGF and EGF receptors and molecules (such as RAF kinase) involved in downstream signalling are therefore potential appropriate targets for drug therapy. Several antibodies and low molecular weight tyrosine kinase inhibitors (TKIs) have completed phase II clinical trials. Phase II studies of multitargeted agents, which include inhibition of VEGFR tyrosine kinase in their repertoire (sorafenib, sunitinib and AG 013736), show clear second-line activity in metastatic RCC. The same is true of the anti-VEGF antibody, bevacizumab. In a randomised phase III comparison against placebo in pretreated patients, sorafenib doubled median progression free survival (24 versus 12 weeks). Studies now in progress will determine whether benefits seen second-line will also be evident first-line, and whether the activity of novel agents can be increased by combining them with each other, with cytokines, or with chemotherapy.

Key words: bevacizumab, interleukin-2, interferon, renal cell cancer, sorafenib, sunitinib, tyrosine kinase inhibitors

Introduction

In solid tumours such as those of lung and colon, the addition of certain novel targeted agents to conventional treatment (either monoclonal antibodies or small molecule inhibitors of tyrosine kinases) has been shown to prolong survival when compared with standard treatment alone [1–3]. There is now hope that patients with advanced and metastatic renal cell cancer, a condition which has proven stubbornly resistant to therapy, will benefit from a similar advance.

This review focuses on the potential of tyrosine kinase inhibitors (TKIs) in the treatment of renal cell cancer (RCC), alone and in combination with other agents. To put the TKIs in context, we also describe monoclonal antibody inhibitors of the pathways targeted by the TKIs, and their potential use in combination therapy.

Epidemiology and natural history

About 3% of all adult malignancies are RCC [4, 5]. Each year in Europe, approximately 40 000 patients are newly diagnosed with RCC, and almost 20 000 people die of the disease. In the United States in 2003, RCC accounted for 31 000 people diagnosed with the disease, 12 000 deaths, and was the 10th most common cause of cancer mortality in men [5]. Renal cell cancers generally arise in the epithelium of the proximal tubule [5,6]. Approximately 80% are of clear cell histology and 15% are papillary.

RCC occurs predominantly in patients over the age of 50 and is more common in men than women (21 000 cases compared with 14 000 in the USA in 2003) [7]. RCC is associated with risk factors such as: cigarette smoking; occupational exposure to agents such as asbestos and petroleum products; obesity and hypertension; acquired cystic disease of the kidney; and genetic factors [8]. Structural alterations in the short arm of chromosome 3 (3p) are associated with both hereditary and sporadic (non-hereditary) RCC [4]. Nearly 40% of patients with mutations of the von Hippel–Lindau (VHL) gene develop clear cell RCC [4, 5, 8]. The VHL gene is a tumour suppressor gene [5, 6, 8, 9]. In addition, the altered form of this protein in VHL syndrome might enhance the expression of vascular endothelial growth factor, thereby promoting vascularisation and further contribute to disease progression [4, 5, 8].

RCC is often asymptomatic (or associated with non-specific symptoms) until disease is extensive, with one-third to one-half of RCC patients presenting with locally advanced or stage IV...
disease, on diagnosis [4, 5]. It is increasingly common for RCC to be diagnosed incidentally as a result of ultrasound or other forms of scanning for other medical reasons.

Early detection and treatment of RCC is associated with an improved outcome. In its early stages, the disease can be successfully treated by radical nephrectomy or even nephron-sparing surgery [4, 5, 8, 10]. However, many patients present or develop metastases. Ten to 25% of patients have lymph node involvement and about 5% of patients have inferior vena cava involvement [4]. Around two thirds of patients with stage I or II disease are alive at 5 years. However, 5-year survival for stage III patients (with involvement of the renal vein or inferior vena cava and/or regional nodes) is little over 40%, and survival falls to lower than 11% for patients with locally advanced disease and/or distant metastases [4, 5, 10].

current treatment

local treatment

Potentially curative radical or partial nephrectomy is appropriate for stage I and II patients and a proportion of stage III disease. Partial nephrectomy is usually only performed in patients with either small pT1a tumours (<4 cm in size) isolated to one kidney, in small bilateral tumours, or in patients whose kidney function is impaired [4]. Patients with T1 renal carcinoma who have undergone partial nephrectomy have similar outcome to those patients treated with radical nephrectomy, i.e. a local recurrence rate of 2.7%–5.8%, with a 90% disease-free survival rate at 5 and 10 years [11]. However, patients with >T1a lesions do not experience such favourable results with partial nephrectomy.

classic surgical treatment options for metastatic disease

Unfortunately, 20%–40% of patients treated surgically with curative intent subsequently develop metastases and 25%–30% of patients have metastatic disease (usually multifocal) at diagnosis [4, 5, 8, 10].

Patients with locally advanced or metastatic disease may benefit from surgery with partial removal of the tumour, which improves response to immunotherapy [4, 12]. Metastectomy can assist in the palliative treatment of stage IV patients, with a limited number of metastatic sites. Although RCC is relatively radioresistant, patients with metastases (especially of the brain and bone) may also gain symptomatic relief from palliative radiotherapy. The role of autologous tumour vaccines requires further investigation and is discussed later in this review [13].

role of immunotherapy

Prognosis following the diagnosis of metastatic disease is poor and median survival is in the range of 6–12 months. Since the most common clear cell type of RCC is an immunogenic tumour (i.e. modulation of the immune system may help control the disease) [8], the main focus of systemic treatment to date has been immunotherapy with interferon and interleukin-2, alone or in combination.

Interferons belong to a family of cytokines that have antiproliferative and immunomodulatory activities. Interferon-α has multiple immunological effects, including: the induction of histocompatibility complex antigen expression; enhanced natural killer cell activity; and enhanced expression of the tumour necrosis factor-related apoptosis-inducing ligand [8]. Interferon-γ possesses anti-proliferating, antiviral and immunomodulating capabilities, whilst the cytokine interleukin-2’s (IL-2) major anti-tumour effect is probably through lymphocyte activation [8].

First-line cytokine therapy could be regarded as standard of care in the USA and probably also in Europe, although enthusiasm for it is less. This position may be supported by the most recent data from the French Immunotherapy Intergroup’s PERCY Quattro trial [14]. This phase III study showed no significant improvement in median progression-free survival (PFS) or overall survival (OS) with use of cytokines, alone or in combination, when compared with a medroxyprogesterone acetate (MPA) control. Survival was 14.9 months with MPA, 15.2 months with interferon, 15.3 months with subcutaneous IL-2, 16.8 with interferon plus IL-2. Three-year survival in all groups was around 20% and 5-year survival was 10%.

However, this study was restricted to untreated patients with more than one metastatic site and a Karnofsky score ≥80 and to patients with an intermediate prognosis for response to cytokine treatment (i.e. a 5%–25% probability of response, a 25%–70% probability of failure and a median survival time of 13 months) [14], and there may continue to be a role for intensive cytokine treatment in selected patients with good prognosis and favourable metastatic sites.

Two earlier studies showed significantly longer OS with interferon. In the MRC Renal Cancer Collaborators’ trial [15], median OS was 8.5 months with interferon compared with 6 months with MPA. In the trial conducted by Pyrhonen et al. [16], median OS was 15.7 months with interferon plus vinblastine compared with 8.2 months with vinblastine alone. Both studies also showed that interferon was associated with longer PFS, of 3–4 months compared with 2–3 months.

Interferon is typically given at a dose of 9–10 MIU subcutaneously three times a week. Overall response rates (RR) of 15% have been reported, but, in the recent PERCY Quattro trial, the RR to monotherapy with interferon alpha at 4 months was only 4% [14]. Among the 122 patients randomised to interferon, there were 78 grade 3/4 toxic events. Taking trials overall, flu-like symptoms occur in more than 90% of patients treated with interferon, nausea and vomiting in 85% and neurological toxicity in 30%–65%.

According to the range of reports, high-dose bolus IL-2 has RRs reaching 15%, and this treatment is favoured by some because a proportion of the 5% of patients who enjoy a CR survive long term [17]. High-dose IL-2 was the only treatment approved by the US Food and Drug Administration for the treatment of RCC until very recently. However, the toxicity of high-dose IL-2 is substantial, to the extent that patients may need the services of an intensive care unit, depending on the chosen schedule. Intermediate dose IL-2 delivered by continuous infusion and low-dose subcutaneous IL-2 also have appreciable toxicity.

The combination of interferon with IL-2 (with the possible addition of 5-fluorouracil) appears to be somewhat more active than monotherapy, and a median overall survival (OS) of
2 years has been reported [18]. Combining the two cytokines, IL-2 and interferon-α, yielded higher RRs than the respective monotherapies. However, survival rates were not significantly improved whilst the toxicity with the combination was higher [8, 19].

As the response to immunotherapy can be variable, factors that could predict the response to these therapies would be helpful. New biomarkers that have been identified in RCC patients may provide important information on the prognosis and response to treatment. Small studies with RCC patients showed that an increased proliferating cell nuclear antigen [20], Ki-67 [21], silver staining nucleolar organising regions [22], and argyrophilic nucleolar proteins [23] correlated with poor survival [24]. Other markers that might prove to be important prognostic factors include markers relating to cellular signal transduction, transcription, apoptosis, cell adhesion, cytoskeletal regulation, angiogenesis, immune regulation and tumour suppression [24]. Carbonic anhydrase IX (CA IX), which appears to be induced in some tumour types but absent in most normal tissues, is an important RCC marker, and CA IX expression correlates with IL-2 treatment [24–31]. The classification of biomarkers in RCC may allow for better identification of which patients would benefit the most from certain treatment, such as IL-2. Immunological markers [HLA, tumour infiltrating lymphocytes (TIL) and tumoral immune complexes expression] will also allow for more specific selection of immunotherapy treatments [32–39].

the role of chemotherapy

In metastatic RCC, single-agent chemotherapy has a very low response rate and no impact on survival. In the past, chemotherapy has mostly involved either 5-fluorouracil (5-FU) or vinblastine, but paclitaxel, carboplatin, ifosfamide, gemcitabine and doxorubicin have also been utilized. The RR with ‘classical’ agents such as fluoropyrimidines or vinblastin is in the range of 7%–12%. In part this seems to be due to multidrug resistance conferred by RCC cells’ high expression of p-glycoprotein [4, 8]. When 5-FU is combined with interferon α a RR of 19% has been observed [4]. Weekly gemcitabine combined with continuous infusion 5-FU has produced a response rate of 17% in RCC [4, 40].

Cytotoxic agents have so far offered limited help in this disease. However, Table 1 provides a brief overview of selected newer studies with single agent and combination therapy for the treatment of RCC, which appears more promising [5, 41–48].

other systemic approaches

Hormonal treatment with tamoxifen and toremifene has a similar low response rate as seen with the earlier chemotherapy trials. The role of hormonal manipulation in this tumour type is dubious. Hormones have been commonly used for treatment of ‘control groups’ in randomised trials.

CCI-779 is a macroline antibiotic (a derivative of rapamycin), which indirectly downregulates specific mRNAs causing G1 arrest [49]. In a phase II randomized trial, using three different dose levels (25 mg, 75 mg or 250 mg weekly), an objective response rate of 7% and a 26% minor response rate was observed in a total of 111 patients. Median survival was 15 months and median time to progression was 5.8 months [50]. Combination trials have also commenced with CCI-779 in RCC. A phase I trial with CCI-779 (at 5 mg dose increments per level from 5 mg to 25 mg) in combination with interferon-α showed the combination to have favourable results in terms of efficacy and safety [51]. The maximum tolerated dose was selected as 15 mg CCI-779 plus 6 million units interferon-α [51]. A phase III trial is underway, comparing this CCI-779 +

Table 1. Overview of recent studies with single agents and combination chemotherapy in RCC

<table>
<thead>
<tr>
<th>Agent/s</th>
<th>Mechanism of action</th>
<th>Number of evaluable patients</th>
<th>Number of partial responses</th>
<th>Stable disease</th>
<th>Median duration of response</th>
</tr>
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<tbody>
<tr>
<td>Single agent</td>
<td></td>
<td></td>
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<tr>
<td>ABT-510 [43]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Favourable antitumour activity observed in phase IB trials. Study ongoing in RCC</td>
</tr>
<tr>
<td>Thalidomide [44]</td>
<td>Immunomodulator antiangiogenic</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>12.2 months</td>
</tr>
<tr>
<td>Combination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dose thalidomide + IFN-α [5, 45]</td>
<td>Immunomodulator antiangiogenic Immunotherapy</td>
<td>14</td>
<td>21%</td>
<td>7</td>
<td>17.4 months</td>
</tr>
<tr>
<td>Thalidomide + IL-2 [5, 45]</td>
<td>Immunomodulator antiangiogenic Immunotherapy</td>
<td>36</td>
<td>42%</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>5-FU + Thalidomide + IFN-α and IL-2 (FUNIL) [46]</td>
<td>Immunomodulator antiangiogenic</td>
<td>7</td>
<td>14%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Capecitabine + IFN-α [47]</td>
<td>Immunotherapy, fluoropyrimidine carbamate, inhibits DNA</td>
<td>24</td>
<td>25%</td>
<td>33%</td>
<td>134 days</td>
</tr>
<tr>
<td>Gemcitabine + Capecitabine [48]</td>
<td>Immunotherapy fluoropyrimidine carbamate, inhibits DNA</td>
<td>16</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>

*Median survival.
IFN-α, Interferon-alpha.
interferon-α versus CCI-779 versus interferon-α as a first-line therapy in poor risk metastatic RCC patients.

Various forms of vaccine therapy and non-myeloablative stem cell transplantation are among other experimental approaches under investigation in RCC. Currently cancer vaccines differ from vaccines such as flu vaccines, in that they are therapeutic rather than preventative. Cancer vaccines contain the antigen found on the cancer cells. They are injected with the appropriate booster (i.e. cytokines, heat shock proteins) and are designed to stimulate the immune system to mount an immune response to that specific cancer [5, 52].

The most extensively studied are the modified autologous tumour vaccines. However, a randomised trial with adjuvant use of an autologous tumour vaccine containing bacillus Calmette–Guérin (BCG), in patients undergoing nephrectomy for RCC, found that the BCG containing vaccine did not decrease the rate of recurrence [8]. Autologous vaccines are being assessed with cytokine therapy. Activity was observed in a phase II trial in 61 patients who received heat-shock protein–peptide complex vaccine (HSPPC-96) and additional IL-2. In this study one complete response, two partial responses and 18 patients with stable diseases were observed. No adverse events were reported. Thirty per cent of patients were alive 2 years after the initiation of the vaccine. Based on these findings, a phase III trial is underway with HSPPC-96 [5, 53]. Other antigen targets for the development of kidney cancer vaccines include: prostate specific membrane antigen (which is expressed on the tumour vasculature of renal cell carcinoma), MAGE-3 (expressed in a high percentage of kidney cancer) and HER-2/neu (overexpressed in renal cell carcinomas) [8].

Another form of immunotherapy research for RCC is allogeneic stem-cell or bone marrow transplantation. In a trial with 19 patients with refractory RCC, three patients experienced a complete response and seven patients had a partial response [4]. However, further assessments of this therapy will be needed as it is associated with significant toxicities and even treatment-related deaths.

Novel agents targeted at growth receptors and intracellular signalling, which have already scored successes in both solid and haematological tumours, are proving to be of greatest current interest.

**tyrosine kinases and their inhibitors**

**epidermal growth factor**

Activation of receptors by growth factors plays a key role in driving tumour cell replication. The epidermal growth factor receptor (EGFR) is part of a family of closely related cell surface receptors, i.e. EGFR (HER1 or erbB1), erbB2 (HER2/neu), erbB3 (HER3) and erbB4 (HER4) [54]. This family of EGFRs are also known as type I receptor tyrosine kinases or erbB. These receptors are transmembrane glycoproteins with an extracellular ligand-binding domain and an intracellular tyrosine kinase domain (Figure 1). The receptors are inactive as single units (or monomers) but form active pairs (or dimers) when bound by a ligand, i.e. when the extracellular domain is activated by epidermal growth factor (EGF) and other ligands such as amphiregulin, transforming growth factor-α (TGF-α), heparin-binding EGF, betacellulin, epiregulin and neuregulin G2β [55]. The dimer may consist of two of the same monomers (i.e. an EGFR may pair with another EGFR) thus forming a homodimer, or two non-identical receptors may pair together forming a heterodimer (i.e. an EGFR may pair with HER2/neu). Dimer formation promotes activation of the internal tyrosine kinase domain, thus catalysing protein phosphorylation [54, 55–58]. This then activates the next step in the pathway within the cell cytoplasm, with the activation of the ras protein, which then triggers a cascade of phosphorylations that activate mitogen-activated protein kinase (MAPK). This signalling cascade results ultimately in the signal passing into the nucleus,

![Diagrammatic structure of the members of the EGFR family](image)

**Figure 1.** Diagrammatic structure of the members of the EGFR family (or type I receptor tyrosine kinases, or erbB) [54–61]. EGF, epidermal growth factor; TGF-α, transforming growth factor-α; HB-EGF, heparin-binding EGF.
where the cyclins (in particular cyclin D) and cyclin-dependent kinases become associated and cause activation of cell division [56–58].

Thus, non-physiological activation of EGFR can lead to uncontrolled cell division and ultimately a growing tumour mass (Figure 2). However, the activation of EGFR not only leads to the intracellular activation of the ras pathway leading to cell division, but also to the activation of other intracellular pathways, which result in inhibition of apoptosis, stimulation of angiogenesis and promotion of metastases/invasion [56–60]. These latter cascades include the phosphatidylinositol 3-kinase and the downstream protein-serine/threonine kinase (PI3K/Akt) pathways and the Jak/Stat pathway (the stress-activated protein kinase pathway, with protein kinase C and Jak/Stat) [57].

Increased EGFR-mediated signalling in cancer cells may block apoptosis (the homeostatic process of destroying damaged, old or mutated cells), thus allowing abnormal cells to carry on dividing [57]. Similarly, the increased EGFR-mediated signalling in cancer cells stimulates the production of angiogenic factors such as vascular endothelial growth factor, which is described in further detail later. The activation of EGFR also seems to promote the invasion of neighbouring tissues by the tumour cells, in particular the vascular endothelium, leading to metastases [57, 60].

EGFR is often overexpressed in human tumours, thus there is a good rationale for trying to inhibit the EGFR for providing advances in cancer treatment [57]. Currently, there are two approaches to the inhibition of EGFRs, namely monoclonal antibodies and small molecule inhibitors of the EGFR tyrosine kinase enzyme (Figure 3).

The receptor is expressed in 50%–90% of RCCs [55]. In many human tumours, overexpression of EGFR is associated with poor outcome, and anti-EGFR agents can be given at doses which inhibit receptor signalling [57].

Tyrosine kinase inhibitors (TKIs) are small molecules that act inside the cell, competing with adenosine triphosphate (ATP) for binding to the catalytic tyrosine kinase domain. This blocks initiation of downstream signalling. Because TKIs target abnormalities specific to tumour cells, there is hope that they will be free from the generalised toxicities associated with cytotoxic chemotherapy.

vascular endothelial growth factor

Vascular endothelial growth factor [VEGF, also known as vascular permeability factor (VPF) and VEGF-A] a member of the platelet-derived growth factor (PDGF) family, which are involved in the development of new vasculature from adjacent host blood vessels to allow for the transfer of oxygen and nutrition from the blood to the new cells that have been formed [61]. New blood vessels are necessary for tumours to survive, grow and metastasise [62]. Angiogenesis is the growth of new microvasculature from existing blood vessels, this occurs in wound healing, menstrual cycle and tumours [63, 64]. There are various angiogenic factors that play a critical role in angiogenesis. Endothelium-specific growth factors include the VEGF family, the angiotropin and the ephrin families [62].

Figure 2. Simplified illustration of the activation of EGFR and tyrosine kinases and their key role in various biochemical pathways and cellular functions [54–61].
Other factors, which are non-endothelial cell specific, include the cytokines, proteinases, adhesion and junctional molecules [62]. VEGF has been extensively studied and is among the most potent endothelial cell-specific angiogenic factor causing endothelial cell proliferation, migration and tube formation and induces vascular hyperpermeability [64–67]. VEGF is secreted by a number of tumour cells and is elevated in RCC. In RCC, VEGF expression correlates with tumour vascularity and may be a significant predictor of outcome [62, 68]. Endothelial cells of tumour vessels have, amongst others, an enhanced expression of two receptors for VEGF, namely VEGFR-1 and VEGFR-2, hence making these potential targets of inhibition for treatments [3, 4]. VEGF increases the expression of tissue factor and thrombomodulin in endothelial cells and thus can affect the hemostasis [64, 69, 70]. VEGF is regulated by several growth factors (i.e. MAPKs and P13K) and oncogenes, at multiple levels [71].

Use of targeted agents to bind VEGF protein or inhibit VEGFR tyrosine kinase, therefore, has a strong rationale in RCC and several small molecule inhibitors of VEGFR TKs have shown substantial clinical activity (see below).

tyrosine kinases in RCC

Among the first TKIs developed were the inhibitors of EGFR tyrosine kinase gefitinib (Iressa) and erlotinib (Tarceva). While the inhibitory activity of these agents is relatively specific, a further group of TKIs has multiple kinase targets. Notable among them in the context of RCC is sorafenib, which targets VEGFR-2 and -3, platelet derived growth factor receptor (PDGFR), B-RAF, C-RAF, c-kit and Flt-3. A pivotal phase III study of sorafenib in RCC is described below. Sorafenib is also being investigated in phase III trials in hepatocellular carcinoma and (in combination with carboplatin and paclitaxel) in metastatic melanoma. Also relevant to RCC are SU 11248 (sunitinib, Sutent), which targets VEGFR-2, PDGFR, c-kit and Flt-3; imatinib (Glivec), which targets c-kit and PDGFR; and AG 013736, which targets both VEGFR-2 and PDGFR. The activity against VEGF/VEGFR, conferring anti-angiogenic potential, is common to both bevacizumab and the majority of TKIs considered.

monoclonal antibodies in renal cell cancer

anti-EGFR agents

Motzer et al. [74] reported no overall responses and an unimpressive median time to progression among 35 patients treated with the EGFR antibody cetuximab (Erbitux). Following
VEGFR-2 and PDGFR-proliferation, and also inhibits receptor tyrosine kinases such as RAS/RAF/MEK/ERK signalling pathway leading to cell cancer. Sorafenib is a potent inhibitor of RAF-1, a key enzyme in the pathway leading to cell proliferation, and also inhibits receptor tyrosine kinases such as RAS/RAF/MEK/ERK signalling pathway leading to cell cancer. Sorafenib is the only agent which also has evidence of achieving partial responses up to 40% of patients. However, the phase II trials of three TKIs tyrosine kinase inhibitors in renal cell cancer (sorafenib, sunitinib and AG 013736) suggest that the agents have activity in the treatment of second-line RCC, achieving partial responses up to 40% of patients. However, sorafenib is the only agent which also has evidence of second-line single-agent efficacy in a randomised phase III.

sorafenib

Sorafenib is a potent inhibitor of RAF-1, a key enzyme in the RAS/RAF/MEK/ERK signalling pathway leading to cell proliferation, and also inhibits receptor tyrosine kinases such as VEGFR-2 and PDGFR-β involved in angiogenesis [79]. This novel dual-targeted TKI shows broad activity in tumour xenografts and significantly inhibits neovascularisation [79, 80]. Preclinical study of sorafenib showed dose-dependent inhibition of growth in a murine renal adenocarcinoma (RENCA) model. In a phase I trial, the dose of sorafenib was escalated from 50 mg to 800 mg b.i.d. [81]. Dose-limiting skin toxicity was seen at 600 mg and diarrhoea and asthenia at 800 mg. Biological activity, evident as decreased phosphorylation of ERK in peripheral blood lymphocytes, was seen at 200 mg and upwards, supporting the preclinical evidence of efficacy noted above. Given these phase I data, 400 mg b.i.d. was the dose chosen for further clinical trials. In contrast to sunitinib, dosing of sorafenib is continuous.

Following the phase I study suggesting evidence of activity in RCC, Ratain et al. [82] undertook a phase II trial with a randomised discontinuation design. In this study, 70% of the 202 RCC patients were free of progression at 12 weeks. Those randomised to continue receiving the drug, rather than placebo, experienced significant further benefit: 50% of sorafenib patients were free of progression at 24 weeks, compared with 18% of patients on placebo ($P = 0.008$). Median progression-free survival, following randomisation, was 23 weeks with sorafenib and 6 weeks with placebo ($P = 0.0001$). Toxicities, including hand–foot syndrome, rash, diarrhoea and hypertension, were manageable and reversible.

the phase III TARGETs trial

The multinational TARGETs (Treatment Approaches in Renal cell cancer Global Evaluation) Trial randomised patients with histologically or cytologically confirmed unresectable or metastatic clear cell RCC to either sorafenib 400 mg b.i.d. or placebo with best supportive care [83, 84]. Patients had failed one prior systemic therapy (predominantly with a cytokine) and were of Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1. Groups were well balanced for age, sex, performance status (PS), number of metastatic sites, proportion of patients with liver or lung involvement, proportion with nephrectomy (more than 90% in both groups) and Motzer risk category (low in 50% and intermediate in 50%).

The primary end point was overall survival. Patients receiving sorafenib had a 39% improvement in survival compared with placebo. The median survival for the placebo group was 14.7 months, whilst the median survival for the patients receiving sorafenib has not yet been reached and follow-up for survival analysis continues. However, at the pre-planned interim analysis conducted among the 903 patients randomised before the data cut-off point of 31 May 2005, it was found that the median progression-free survival in the sorafenib group was 5.5 months, compared with 2.8 months in patients assigned to placebo (hazard ratio (S/P) = 0.51). Progression-free survival was based on investigator assessment [84].

This benefit in progression-free survival was seen in all subsets, including those defined by age, Motzer prognostic score, prior treatment and site of metastasis. Seventy-six per cent of sorafenib patients had some degree of tumour shrinkage, compared with 25% of placebo patients [82]. Among patients in whom the drug was effective, tumour shrinkage was generally seen within 4 months.

The objective responses by investigator assessment included <1% complete response, 10% partial response and 78% stable disease in patients receiving sorafenib, compared with 0%, 2% and 53%, respectively, in the placebo group. Progressive disease was observed in 12% of the sorafenib group compared with 37% of the placebo group. Missing data was 4% and 8% in the sorafenib and placebo groups, respectively [84].

According to a recent toxicity update, drug-related adverse events were greater in the sorafenib group than among placebo-treated patients: hypertension of any grade 17% versus 2%; diarrhoea 43% versus 13%; hand–foot skin reaction 30% versus 6%. However, there was no significant difference between...
sorafenib and placebo groups in the incidence of fatigue (any grade: 37% versus 28%) and there was no appreciable grade 3/4 haematological toxicity associated with active treatment [84].

Because the duration of progression-free survival with sorafenib was twice that seen with placebo, the phase III study has been modified to allow crossover from placebo to sorafenib, which will have considerable implications for the intent-to-treat survival analysis of the trial.

An ancillary study used Doppler ultrasonography to measure perfusion of metastatic renal cancer in 30 patients, with the aim of determining whether early changes in tumour vascularisation predict progression-free survival and overall survival [85]. Data suggest that changes in vascularity are seen as early as 3 weeks and that such changes predict extended progression-free survival and overall survival, serving as a proof of concept for the antiangiogenic properties of the agent. Sorafenib has been approved for the 1st and 2nd treatment of patients with RCC in December 2005 by the FDA.

A randomised phase II study in first-line RCC is now entering patients, with a target accrual of 160. Patients are stratified according to high, intermediate or low Motzer score and randomised to either sorafenib 400 mg b.i.d. or interferon 9 MIUs three times a week. The primary end point is progression-free survival, and the hope is that the 5-month progression-free survival seen with interferon can be extended to 9 months. Patients randomised to interferon can cross over to sorafenib on progression. Patients who progress on 400 mg sorafenib will have their dose increased to 600 mg, to determine the validity of anecdotal evidence that raising the dose can improve activity.

specific settings, including adjuvant

In addition to the trials discussed above, there are studies of single-agent sorafenib in particular clinical settings such as brain metastasis, in patients with impaired renal or hepatic function, and in patients with metastatic non-clear cell RCC. However, particular interest is focused on the drug’s potential as an adjuvant. SORCE is a randomised, double-blind phase III trial of sorafenib versus placebo in patients with resected primary RCC with no residual disease who are nevertheless at intermediate or high risk of relapse (Leibovich score 3–8). The Leibovich score is a scoring algorithm to predict cancer specific survival rates. The scoring is done on symptoms [i.e. in RCC: nephrectomy (+2), metastases to the bone (+2) or liver (+4), metastases in multiple simultaneous sites (+2), metastases at nephrectomy (+1) or within 2 years of nephrectomy (+3), complete resection of all metastatic sites (–5), tumour thrombus level I to IV (+3), and the primary pathological features of nuclear grade 4 (+3) and histological tumour necrosis (+2)] [86]. The cancer specific survival rates at 1 year were 85.1% (for scores of –5 to –1), 72.1% (for scores of 0–2), 58.8% (for scores of 3–6), 39.0% (for scores of 7–8), and 25.1% (for scores of 9 or more) [86]. In this phase III trial, patients will be randomised in the ratio 2:3:3 to sorafenib 400 mg b.i.d. for 3 years, placebo for 3 years, or sorafenib for 1 year followed by placebo for 3. The primary end point is metastasis-free survival, and a secondary end point is time to death from RCC. The study is powered to detect an improvement in 3-year metastasis-free survival from 64% to 71% following an average 2 years’ treatment with sorafenib.

The study will collect fixed tumour and blood samples from all centres and fresh frozen tumour from selected centres to investigate biological predictors of survival such as VHL, VEGFR2, FGF2, B-RAF, MEK and ERK. It will also attempt to corroborate the Leibovich risk model.

A neoadjuvant study has also been suggested. Patients with metastatic disease and a primary RCC still in place would receive a course of sorafenib prior to nephrectomy. Following surgery, patients would be treated with IL2 and interferon.

expanded access programme

In Europe, an expanded access phase III programme in 10 countries will further assess the safety of sorafenib in RCC. Eligible patients are those who have failed prior systemic therapy, who have not tolerated it or who are deemed unsuitable. Limited efficacy data will also be collected. Patients will be of ECOG PS 0–2 and have complete healing of any surgical wound.

combinations

A phase I study evaluated the combination of sorafenib with bevacizumab in patients with advanced solid tumours and we can expect to see this combination under investigation in RCC patients [87].

SU 11248 (sunitinib, sutent)

Sunitinib is an oral oxindole TK inhibitor, with selective multi-targeted inhibition of VEGFR-2 and PDGFR-β, KIT and FLT-3 tyrosine kinases [61]. Since expression of both VEGF and PDGF receptors is upregulated in clear cell RCC, it was logical to assess the potential of the novel agent in this disease. Sutent is also being studied in breast cancer and in GIST following failure of imatinib.

Preclinical studies of sunitinib showed selective in vivo inhibition of VEGFR-2 and PDGFR-β phosphorylation, in vitro inhibition of endothelial cell and fibroblast proliferation, and anti-tumour effects on mouse xenografts [88]. In phase I trials with single agent of sunitinib [89–91], the dose-limiting toxicity was fatigue. Other toxicities included diarrhea, nausea, low blood counts and rash. Partial responses were observed in renal cell, GIST and other tumours. The recommended dose for the phase II studies was 50 mg daily on a ’4 weeks on and 2 weeks off’ schedule.

Phase II studies now demonstrate that sunitinib is active in the second-line treatment of patients with RCC [92].

phase II trials

The two independent, single arm, phase II trials used a 50 mg dose of sunitinib given daily for 4 weeks, followed by 2 weeks off treatment giving a 6-week cycle [92]. Previous studies showed that hypertension is seen above 75 mg and that asthenia increases with time on treatment (and may be a more limiting factor than bevacizumab or sorafenib).

The first trial involved 63 patients with RCC who had failed cytokine therapy. Histology was clear cell in 87% of cases.
Response was assessed by RECIST criteria every one to two cycles. While no patient showed a complete response, 25 patients (40%) had a partial response as assessed by investigators and a further 18 patients (28%) had stable disease for 3 months or longer. The median time to partial response was 2.3 months, median response duration 12.3 months and median time to progression 8.7 months. Median overall survival was 16.4 months.

The second trial involved 106 patients, all with clear cell histology and all with radiologically documented progression on interferon, IL-2 or their combination. The partial response rate to sunitinib in 105 patients (one patient with seminoma was excluded from the analysis) was 45% (43 patients) and the complete response rate 1% (one patient), with an overall response rate of 44% (46 patients). Disease stabilisation occurred in 23% (24 patients). The preliminary time to response was 2.3 months, the duration of response was 9.9 months and time to progression was 8.1 months. Median overall survival has not been reached.

In the two sunitinib phase II trials, grade 2/3 fatigue was seen in 38% and 22% of patients, diarrhoea in 24% and 16%, and nausea in 13% and 19%.

Motzer and colleagues concluded that the drug was relatively well tolerated and noted that several responding patients had been on treatment for more than 2 years. Furthermore, in these two consecutively phase II trials, sunitinib appeared to have substantial antitumour activity as second-line therapy for metastatic RCC patients and the antitumour activity seen in trial 2 supported those of trial 1 [92].

**phase III**

A phase III trial will randomise patients with no prior systemic therapy to sunitinib on a ‘4 weeks on 2 weeks off’ (4/2) schedule or interferon-α administered t.i.w. with progression-free survival, overall survival and overall response rate as end points. Target accrual is 690 patients [93].

**other trials with sunitinib**

Sunitinib will also be evaluated in bevacizumab-refractory RCC. Additional studies in RCC will assess sunitinib in combination with gefitinib, interferon, bevacizumab, gemcitabine or capecitabine. Sunitinib will also be assessed as an adjuvant and neoadjuvant treatment in RCC.

An open-labelled, multicentre renal effect study will randomise to either sunitinib continuous dose schedule, sunitinib 4/2 schedule or sunitinib 4/2 schedule + IFN-α. The primary end point is time to progression and the secondary end points are response rate, overall survival and quality of life.

**AG 013736**

AG 013736 is also a VEGFR and PDGFR TKI and, like sunitinib, has demonstrated antitumour activity in metastatic renal cell carcinoma [94].

In a multicentre phase II study, 52 patients (87% with clear cell histology) were given 5 mg b.i.d. AG 013736 for 4 weeks. A partial response was seen in 46% of patients and stable disease in a further 40%. When data were reported at the 2005 meeting of ASCO, median time to progression had not been reached after 12–18 months follow-up and 21 patients in partial response remained on treatment.

Treatment-related adverse events (including hypertension, stomatitis, fatigue and diarrhoea) were reported in 12% of patients. Grade 3/4 diarrhoea was seen in 8%, hypertension in 15% and fatigue in 8% of patients.

**other TKIs**

**gefitinib**

Drucker et al. [95] reported using gefitinib (Iressa) 500 mg/day in 16 patients with RCC. There were no objective responses; median TTP was 3.7 months and at 4 months, 80% had progressed. Dawson et al. [96] gave patients the same dose of gefitinib with a 5% overall response rate and 8-month median overall survival.

**PTK 787 (vatalanib)**

PTK 787 is a potent inhibitor of VEGFR-2 and also, at higher concentrations, of PDGFR and c-kit [93, 97]. Cell-based assays show that it inhibits endothelial cell proliferation, migration and survival. *In vivo*, PTK787 inhibits growth of a murine renal carcinoma model [98]. Inhibition of microvessel formation in the tumour interior is evident on histology.

A phase I study of once daily PTK 787 (ZK 222584) in 45 patients with RCC assessed five dose levels (300–1500 mg/day), however, no MTD was reached [99]. Based on the data, 1200 mg/day was selected for the dose expansion phase. Forty-one patients received doses ≥1000 mg/day, DLT occurred in two patients: one at 1000 mg/day with grade 3 headache and one at 1500 mg/day with grade 3 hypertension. The most frequent adverse events included: nausea (59%), fatigue (41%), vomiting (41%), diarrhoea (24%) and headache (24%). The measurable response, in 37 evaluable patients, was 14% (seven patients: one partial and six minor) with a median TTP of 5.5 months. Seventeen patients (46%) had stable disease. The 1-year OS was 63.7% [99].

**imatinib (STI-571, Glevec, Gleevec)**

Imatinib has achieved remarkable success in chronic myeloid leukaemia and in gastrointestinal stromal tumours, through its specific targeting of Bcr-Abl kinase and c-kit, respectively. However, this novel agent also inhibits the PDGFR tyrosine kinase, suggesting that its potential activity in RCC should be assessed. That said, results to date (from a phase II study in 12 patients) have been modest [5]. The combination of imatinib with pegylated interferon is being investigated.

**TKIs in combination**

The limited activity demonstrated by single agents and our understanding of the redundancy of signalling pathways within the tumour cell suggest that the greatest clinical benefit will be derived from some form of combination therapy.

**sorafenib plus immunotherapy**

A phase I study of sorafenib in combination with interferon in 12 patients showed no exacerbation of the toxicities seen when the two agents are used alone. Five of the six evaluable patients...
tumour, and brings to the fore the potential importance of end to be cytostatic than cytotoxic brings into question the relevance designing efficacy trials, the fact that novel agents are more likely biological dose as much as maximum tolerated dose. In a fresh approach to clinical trial design.

However, the advent of novel, targeted therapies has required selective in their effects than chemotherapy, and better tolerated. pathways that are specific to tumours and so should be more the case when combining EGFR and VEGFR TKIs.

sorafenib combinations
A phase I/II trial at Vanderbilt University will assess the potential of combining sorafenib and bevacizumab in second-line patients. If promising, this will possibly lead to a randomised phase II of sorafenib plus bevacizumab versus sorafenib alone.

other TKI combinations
Hainsworth et al. [100] have reported a partial response rate of 29% and a median progression-free survival of 11 months in 63 metastatic RCC patients treated with bevacizumab 10 mg/kg plus erlotinib 10 mg. Sixty-eight per cent of the patients included had been treated previously. The authors concluded that the combination had substantial clinical activity and was well tolerated, despite the occurrence of rash and diarrhoea. A randomised phase II of bevacizumab plus or minus erlotinib has been completed.

discussion
Targeted therapies are directed against molecules and signalling pathways that are specific to tumours and so should be more selective in their effects than chemotherapy, and better tolerated. However, the advent of novel, targeted therapies has required a fresh approach to clinical trial design.

The focus of Phase I studies has been identifying optimum biological dose as much as maximum tolerated dose. In designing efficacy trials, the fact that novel agents are more likely to be cytostatic than cytotoxic brings into question the relevance of standard response criteria requiring profound shrinkage of tumour, and brings to the fore the potential importance of end points such as progression-free survival and stabilisation of disease [5].

In Europe, interferon is an accepted standard of care in metastatic renal cell carcinoma. However, randomised studies suggest that treatment improves progression-free survival by only 1 month when compared with control [14, 15]. In a trial conducted predominantly in second-line patients, progression-free survival with sorafenib was 6 months, compared with 3 months in the placebo arm. This is an indication of the results that can be obtained with a TKI, and suggests a likely effect on overall survival.

Tyrosine kinases initiate intracellular signalling pathways that influence cell growth, replication, differentiation and apoptosis. Their pathophysiological role in cancer responded to small molecule TKIs and antibodies affecting the activation of growth receptors by ligands.

TKIs have a generally favourable safety profile. This should allow their use in combination with each other and with traditional classes of agents such as cytotoxics and cytokines. The recent approval of sorafenib for treatment of patients with RCC is reflecting the rapidly changing treatment scenario in this tumour type.

references


