Novel treatment strategies in clear-cell metastatic renal cell carcinoma

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Metastatic renal-cell carcinoma (mRCC) is highly resistant to cytotoxic agents or hormones and is currently mainly treated with cytokine-based therapy. Transient responses and moderate survival advantages have been achieved in a subset of patients with these aspecific biological response modifiers. Side-effects are considerable, especially with high-dose interleukin (IL)-2. Efforts made in the field of specific immunotherapy have focused on optimization of dendritic cell vaccination and on administration of monoclonal antibodies, either cold (unconjugated) or hot (radioactively labeled). Furthermore, allogeneic bone marrow transplantation is able to induce remissions but, regrettably, is related to substantial morbidity and mortality. Neutralization of the biological activity of some immunosuppressive cytokines produced by RCC (IL-6 and tumor necrosis factor-α) with monoclonal antibodies is currently under investigation. Insights gained into the processes and pathways underlying carcinogenesis have led to the development of new treatment strategies. These treatments can be used for clear cell RCC, since they focus on blocking gene products that are upregulated by mutations in the von Hippel–Lindau gene. Specific strategies include anti-vascular endothelial growth factor monoclonal antibody (bevacizumab) or inhibition of its receptor kinases (oral SU11248 or PTK787), or targeting the Raf kinase pathway (by BAY 43-9006) or the mammalian target of rapamycin (mTOR) pathway (by CCI-779). Early clinical results are promising, but their place in the treatment of RCC has to be determined. *Anti-Cancer Drugs* **16:709–717**

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Introduction

Renal cell carcinoma (RCC) is the third most common urological cancer after prostate and bladder cancer, with an incidence of approximately 5–10 per 100 000. About 70–80% of patients present with localized disease; however, up to half of these patients will eventually develop metastases [1]. The median survival of patients with metastatic RCC (mRCC) is 6–12 months, with a 5-year survival of only 9% [2].

mRCC is considered to be non-responsive to hormonal therapy. Most chemotherapeutic agents fail, probably as a consequence of overexpression of the multidrug-resistance (MDR) gene. In clinical trials some therapeutic benefit has been seen with vinblastine, 5-fluoruracil, gemcitabine and capecitabine [3].

Up to now, mRCC has been treated with cytokine-based therapy. Prior to the start of cytokine treatment, a radical nephrectomy should be performed in patients with a good performance status and candidates for cytokine therapy. A combined analysis of two randomized studies showed a median survival benefit of 6 months when nephrectomy was performed in good/intermediate prognosis patients prior to interferon (IFN)-α [4,5].

IFN-α has several mechanisms of action. First, it stimulates the lytic capacity of natural killer cells. Second, it augments the expression of HLA class I on tumor cells, therefore enhancing the recognition by cytotoxic T cells [6]. In addition to a role in the tumor immune response it has direct anti-proliferative effects and serves in low doses as an inhibitor of angiogenesis [7]. IFN-α results in several months of prolongation of overall survival in a small, but significant, number of patients. Response rates vary between 8 and 26%, with complete responses in 2–7%. Partial responses have a median duration of 10 months [8,9].

Interleukin (IL)-2 serves as a crucial growth factor and activator of the cellular immune response, which is considered to play a central role in tumor rejection. Median overall survival with IL-2 treatment is similar to IFN-α. High-dose bolus IL-2 is associated with approximately 6% complete responses, frequently of long duration [10–18]. The median duration of a partial

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response is 12–19 months, although in some studies the median duration of the complete response has not yet been reached. IL-2-based therapy is accompanied with more acute side-effects in comparison with IFN-α, especially high-dose i.v. IL-2. Combination strategies of IFN-α and IL-2 induce higher response rates, but these have not resulted in a survival benefit [19,20].

It is becoming increasingly clear that the subtype of RCC will predict the response on cytokine therapy. The majority (approximately 85%) of RCCs are of the clear cell type [6]. This subtype is considered to be the most sensitive to systemic therapy, especially in the case of IL-2-based regimens [20]. Although cytokine-based immunotherapy is up to now the best option for systemic treatment, it will induce transient responses in a minority of patients and only a few patients achieve long-term survival [21].

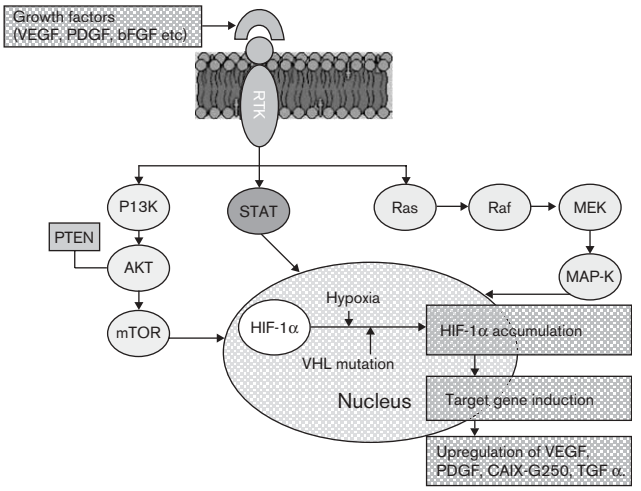
Although much research has focused on developing more specific immune therapeutic strategies, recent progress in the understanding of several pathways and processes underlying carcinogenesis and the crucial role of angiogenesis has led to the development of new i.v. and oral compounds. Since these compounds interfere with the biological activity of genes upregulated by von Hippel–Lindau (VHL) mutations, a frequently occurring event in RCC, clinical studies to investigate their role in RCC have been initiated. The mechanisms of action and the clinical results of these new compounds will be discussed.

RCC and angiogenesis

Clear cell RCC is correlated with mutations in the VHL gene. These mutations have been found in over 75% of sporadic clear cell RCCs [22]. Several studies have identified the VHL mutation as an early event in the pathogenesis of clear cell RCC [23]. VHL is a tumor suppressor gene that encodes for a 213-amino-acid protein (pVHL), which interacts with HIF-1α. After binding of pVHL to HIF-1α, this complex mediates ubiquitination-mediated, oxygen-dependent proteosomal degradation of HIF-1α (Fig. 1). Hypoxia or mutated pVHL leads to accumulation of HIF-1α and binding to HIF-1β [24]. This complex increases the transcription of hypoxia-inducible genes encoding several growth factors. These include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), erythropoietin, transforming growth factor (TGF)-α [25,26] and the cellular membrane antigen carbonic anhydrase (CA) IX [27,28] (Fig. 1). CA IX, also known as the G250 protein, is an isoenzyme of the carbonic anhydrase family and plays a role in the pH regulation of the cell [29].

VEGF, PDGF and bFGF play a central role in angiogenesis and proliferation [30]. The downstream receptor tyrosine kinases will be activated after binding of specific receptors, eventually leading to cell proliferation, survival and angiogenesis. In this way VEGF overexpression contributes to the typical hypervascular histology of clear cell RCC. In addition, TGF-α is a strong mitogen for the tumor cell itself and, of interest, both TGF-α as well as its receptor epidermal growth factor receptor (EGFR) are commonly overexpressed by RCC, thus creating an autocrine loop [31,32]. The overexpression of the transmembrane CA IX/G250 leads to acidification of the tumor environment, promoting cell growth [30,33]

Fig. 1



pVHL (protein encoded by the VHL gene) is a tumor suppressor that under normoxic circumstance will bind HIF-1α to form a complex. This complex will be ubiquitinated and subsequently degraded in the proteasomes. In conditions of defective/mutated pVHL or hypoxia this interaction is dysfunctional resulting in accumulation HIF-1α. Subsequently, HIF-1α translocates to the nucleus and dimerizes with HIF-1β, resulting in the transcription of several hypoxia-inducible genes including various growth factors such as VEGF, PDGF, bFGF, erythropoietin and TGF-α. These growth factors bind to their specific transmembrane receptors on endothelial and/or tumor cells activating the receptor tyrosine kinase (RTK) resulting in proliferation and stimulation of various cell functions.

Angiogenesis is crucial for the growth and spread of cancer cells, and VEGF has been identified as the most important angiogenic cytokine [22,34]. Many different tumor cells overexpress VEGF-A, which after binding to its receptor promotes endothelial cell migration, division and survival, thus stimulating angiogenesis. Angiogenesis seems to be a promising target for specific therapy with minimal toxicities, since this is an infrequent event in normal adult tissues.

Anti-angiogenesis

The preliminary clinical results of bevacuzimab, SU11248, thalidomide, neovastat and PTK787/ZK222584 will be discussed below.

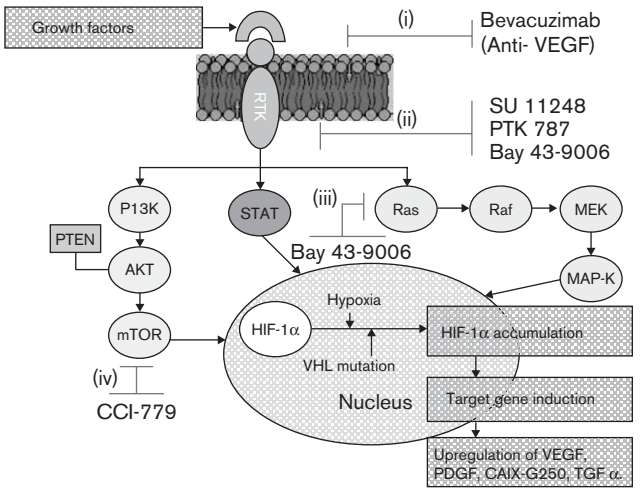
Bevacizumab (avastin)

Bevacizumab is a recombinant humanized monoclonal antibody which binds and neutralizes VEGF (Fig. 2) [35]. In a phase II study in 116 patients with progressive clear cell RCC, the effectivity of bevacizumab 3 ($n = 37$) or 10 mg/kg ($n = 39$) was compared against placebo ($n = 40$). Patients treated in the high-dose group had a significantly longer progression-free survival. The probability of being progression-free at 8 months was 14% in the low- and high-dose group combined versus 5% in the placebo group. A total of four patients achieved a partial response (10%) with a maximum duration of 2 years. The main toxicities observed were hypertension and asymptomatic proteinuria. No differences in overall survival between the two treatment arms were observed, probably due to the fact that patients who had received placebo were given access to bevacizumab after unblinding [36]. At the moment two phase III studies are ongoing in which previously untreated patients with mRCC are being treated with either IFN-α together with 10 mg/kg bevacizumab i.v. (every 2 weeks) or IFN-α together with placebo [22,37].

SU11248

SU11248 is a novel, oral, small-molecule, multi-targeted receptor tyrosine kinase inhibitor. It has anti-tumor and anti-angiogenic activity via targeting and inhibiting the VEGFR, PDGFR, KIT and FLT3R tyrosine kinase (Fig. 2) [38,39]. SU11248 has been investigated in a multicenter phase II study in 63 cytokine-refractory mRCC patients. This study revealed a promising response rate of 33%, much higher than response rates seen with any other experimental treatment in RCC. Of interest is that an additional large proportion of patients (37%) had disease stabilization for more than 3 months, resulting in a 'clinical benefit ratio' of 70% of the patients (objective response + stable disease). In contrast to cytotoxic drugs, which actually destroy tumor cells, resulting in tumor shrinkage, kinase inhibitors inhibit tumor growth. For this reason, stable disease should also be considered a clinical response. The most frequently observed treatment-related adverse events were fatigue, diarrhea, nausea, dyspepsia, stomatitis (grade 2 and 3) and bone marrow abnormalities, with grade 4 neutropenia in 2%. A phase III study is currently being conducted in previously untreated patients with mRCC comparing IFN-α with SU11248, both as a single agent.

Fig. 2



These pathways can be inhibited at several steps. (i) Depletion of VEGF by monoclonal antibody (e.g. bevacizumab). (ii) Inhibition of receptor tyrosine kinases: VEGFR and PDGFR by PTK787, SU11248 and BAY 43-900 (EGFR by GW-572016, OSI-774, ZD1839, not shown). (iii) Inhibition of the Ras/Raf pathway (by BAY 43-9006). (iv) Inhibition of mTOR (by CCI-779).

Thalidomide

Thalidomide inhibits angiogenesis by downregulating VEGF. Aside from anti-angiogenic properties, thalidomide has multiple other anti-tumor effects, such as reduction of tumor necrosis factor (TNF)-α levels, induction of apoptosis, and modulation of natural killer and T cell activity [22]. In nine single-agent phase II studies, no complete responses were observed with variable percentages of partial responses (0–7%). Observed side-effects were constipation, lethargy, neuropathy and thromboembolic complications [22,40]. In 30 mRCC patients, combination therapy of thalidomide with low-dose IFN-α yielded a response rate of 21% [41]. In a phase III trial 353 previously untreated patients were randomly assigned to IFN-α alone or to IFN-α combined with thalidomide. Comparisons made between both study arms revealed no differences in response rates, time to progression or overall survival [22]. However, toxicity profiles were significantly worse in the combination group, being fatigue, myelosuppression and thrombotic events.

In addition to anti-angiogenic effects, thalidomide has been shown to reduce IL-6 and C-reactive protein concentrations in mRCC patients. Since high levels of IL-6 have been correlated with resistance to IL-2-based immunotherapy, combination strategies of IL-2 and thalidomide may yield better clinical responses. The addition of thalidomide in four IL-2-refractory mRCC patients resulted in disease stabilization and partial responses. The treatment regimen was well tolerated without increased IL-2-related toxicity [42]. Further investigation is warranted.

Neovastat

Neovastat (AE-941) is a naturally occurring agent obtained by homogenization and purification of shark cartilage. Research has revealed that this compound has an inhibiting function in several VEGF-dependent processes through competitive binding with VEGFR2

and also promotes endothelial cell apoptosis. Although this agent had shown promising results in a phase II trial [43,44], no survival benefit could be detected in a large multicenter phase III trial conducted in more than 300 patients refractory to first-line immunotherapy [45,46].

PTK787/ZK222584 (vatalanib)

PTK787/ZK222584 is an oral selective inhibitor of VEGFR and PDGFR tyrosine kinases, without interfering with kinases from other enzyme families (Fig. 2) [47]. *In vitro* and *in vivo* experiments have shown VEGF-related inhibition of endothelial cell proliferation, survival and migration by PTK 787 [37,48]. In a phase I/II trial 42 patients with mRCC were evaluable after treatment with PTK 787. Partial responses were observed in two patients (5%), minor responses (as defined by 25–50% shrinkage) in six patients (15%) and 60% of the patients had stable disease. The median time to progression was 5.3 months, the median estimated survival was 21.5 months and the 1-year survival was 67%. Most common adverse events (all grade 1 or 2) were nausea, fatigue and vomiting [49]. Phase III studies are currently ongoing.

Raf kinase inhibition

The Raf kinase pathway

Another pathway of importance in mRCC is the Ras/Raf/MEK/ERK pathway. This complex pathway, with multiple kinases and transcription factors, can be activated or inactivated by protein phosphorylation. Constitutive activation of the Ras/Raf/MEK/ERK pathway due to mutations or genes in this pathway may play a key role in oncogenesis. Downstream signaling by this cascade leads to activation of the Ras and Raf proteins (Fig. 1). Ras proteins play principal roles in regulating normal cell proliferation and programmed cell death [50,51]. Subsequently, Raf relays the extracellular signal from the receptor/Ras complex to a cascade of cytosolic kinases by the activation of MEKs and ERKs (extracellular signal-regulated kinases) [50–52] The activated ERKs result in upregulation of several genes and nuclear transcription factors, consequently regulating cell growth and cell cycling. Activating mutations of the Ras and Raf family of oncogenes have been demonstrated in different solid tumors, including RCC [50,51,53,54], making this pathway a potential target for mRCC treatment.

BAY 43-9006 (sorafenib)

BAY 43-9006 is a specific oral inhibitor of Raf-1 kinase belonging to a class of bis-aryl ureas. Preclinical studies have demonstrated promising anti-tumor effects with this compound in human xenograft models [55]. In addition to the effects on Raf, BAY 43-9006 inhibits the receptor kinases of VEGFR2 and VEGFR3, FLT-3, PDGFR, and c-KIT (Fig. 2) [54,56]. A phase II study in 41 RCC patients with oral BAY 43-9006 resulted in 30% of patients with disease stabilization and 40% of patients with objective responses. Another phase II study with BAY 43-9006 was performed in 397 patients with a variety of advanced refractory solid tumors. After a 12-week treatment period, patients with stable disease were randomly assigned to BAY 43-9006 or placebo. In this study, 89 mRCC patients were evaluable for response, resulting in objective responses in 37 patients (42%), stable disease in 45 patients (50%) and progressive disease in seven patients (7%). This suggests a meaningful activity of this compound in mRCC [57]. Toxicity profile was mild (mainly grade 1 and 2) including skin rash, hand–foot syndrome, fatigue, anorexia, hypertension and diarrhea. A randomized placebo-controlled phase III trial of BAY 43-9006 is currently being performed in mRCC patients refractory to cytokine treatment.

Inhibition of the mammalian target of rapamycin (mTOR)

mTOR

Another intracellular pathway of relevance in mRCC is mTOR (Fig. 1). Rapamycin does not directly inhibit mTOR, but binds to FK-binding protein-12 and then interacts with mTOR, thereby inhibiting its function [58]. mTOR is a serine/threonine kinase and is involved in many critical cell cycle functions [59]. mTOR acts in the downstream signaling of phosphatidylinositol 3-kinase and the Akt pathway. Akt activity is inhibited by pTEN, a tumor suppressor gene [58]. Essentially, mTOR regulates signal transduction pathways involving the coupling of growth stimuli to cell cycle progression, cell proliferation, survival and mobility, and angiogenesis [60]. Moreover, mTOR activity results in increased HIF-1α activity, which, as outlined above, plays an important role in the pathogenesis of RCC [61]. For these reasons mTOR is an interesting target for therapeutic intervention in RCC [59].

Cell cycle inhibitor (CCI)-779 (temsirolimus)

CCI-779 is a specific inhibitor of mTOR with proven anti-tumor effects both *in vitro* and *in vivo* [62,63] (Fig. 2). Of interest, pTEN-deficient or mutated tumors are especially sensitive to CCI-779, suggesting pTEN status is of predictive value for response to CCI-779 therapy [64,65]. In phase I studies, CCI-779 displayed reversible adverse events such as acne, rashes, mucositis/stomatitits, asthenia and nausea [66]. In a phase II study of 111 patients with advanced refractory RCC, objective responses were observed in 7% and minor responses in 26% [67–69].

In a phase I single-arm, dose-escalating study the combination of CCI-779 with IFN-α was generally well tolerated in 71 patients with mRCC. Grade 3–4 related side-effects were rash, asthenia, mucositis, and several hematological and biochemical blood abnormalities. Evaluation of clinical responses revealed partial responses in eight patients (11%) and stable disease in 21 patients

(30%). In the 39 patients treated at the maximum tolerated dose (15 mg/week, i.v.) partial responses were observed in three patients (8%) and stable disease in eight patients (21%). Median time to progression was 9.1 months [70]. A phase III trial comparing IFN-α monotherapy, CCI-779 monotherapy or combination treatment in poor-risk mRCC patients is currently ongoing.

Inhibition of EGFR

EGFR in RCC

TGF-α is upregulated by VHL mutation in RCC and promotes epithelial proliferation through interaction with EGFR [31]. Since both TGF-α and its receptor are overexpressed in RCC, compounds interfering in this pathway have been investigated. However, up to now, the clinical results of several compounds interfering with the EGFR pathway have been disappointing [71,72].

Erlotinib and bevacizumab

The combination of anti-VEGF activity together with inhibition of EGFR appears an interesting approach. This can be achieved by combining erlotinib (an EGFR tyrosine kinase inhibitor) with bevacizumab (Fig. 2). In a recently performed phase II study, mRCC patients were treated with the combination of oral erlotinib and i.v. bevacizumab. A 25% partial response rate was observed. The most common observed toxicities (grade 3 or 4) were hypertension, diarrhea, rash, and nausea [73]. Further investigation is warranted.

Developments in specific immunotherapy

Dendritic cells (DCs)

Renal tumors may evade the cellular immune response by decreasing the expression of MHC antigens and tumor epitopes, by downregulating costimulating molecules, and by secreting immunosuppressive cytokines (IL-6, IL-10 and TGF-β) [74]. With the increased knowledge of tumor immunology, the recognition of immunogenic tumor antigens and the development of monoclonal antibodies, new treatment options with increased specificity and subsequently fewer side-effects can be explored. DCs have been identified as the most potent antigen-presenting cells (APCs) of the immune system [74].

DC vaccinations have been shown to be safe and to have low toxicity in several clinical trials. These vaccinations have, however, yielded only little clinical benefit [75–77]. Vaccinations of DCs pulsed with tumor lysate have been used in clinical trials in order to induce tumor immunogenicity [75,76]. In these rather small series, objective responses have been observed, with some durable responses. On the whole, DC vaccinations have not led to a significant improvement in clinical outcome. Future research should be focused on suppressing regulatory T cells, improving maturation strategies and combining DC vaccinations with cytokine therapy [78].

Tumor-associated antigen (TAA) G250/CA IX

TAAs are antigens with restricted expression in tumor cells or antigens which are expressed in normal tissues, but brought to a higher degree expression by tumor cells. G250 is a cell membrane protein expressed on 85% of all RCC types and on virtually all of the clear cell subtype. Moreover, there is no detectable expression on normal kidney tissue [79]. Recently, a G250-derived peptide has been identified, which can be recognized by both cytotoxic T lymphocytes and by T helper (T_h) lymphocytes [80].

This peptide may thus be a potential tool for peptide-based DC vaccination. Currently, a phase I/II clinical trial is being carried out in our department to investigate the ability of this G250 peptide to activate cytotoxic T lymphocytes and to induce clinical responses.

Monoclonal antibodies against G250/CA IX

The concept of selective tumor targeting with antibodies is based on the avid interaction between an antibody and a TAA. TAAs can be employed to guide monoclonal antibodies to the tumor in order to selectively ablate tumor cells [81]. G250 meets the crucial requirements for this guided therapy, since it is homogeneously expressed in tumors and has restricted expression in normal tissue [81].

Radioimmunotherapy targeting G250 has been under thorough investigation in mRCC. Clinical studies performed in our institution demonstrated that ^{131}I-labeled chimeric G250 accumulates in RCC and that up to approximately 60 mCi/m^2 can be administered safely [82]. A partial response was observed in two out of eight patients receiving high activity doses. In a phase I/II trial, 29 patients were treated with two high doses of ^{131}I-labeled WX-G250 over a period of 3 months. Although no objective responses were seen, 25% showed disease stabilization for a duration of 6 months [83].

Furthermore, the administration of unconjugated monoclonal antibodies in RCC has been studied. The rationale for this treatment is that the antibody itself mediates cytotoxicity by antibody-dependent cell cytotoxicity, activation of complement and through specific binding of the antigen. In a multicenter phase II trial, weekly administrations of i.v. WX-G250 in 36 patients with mRCC were safe and well tolerated. One complete response, 10 durable disease stabilizations and a median survival of 15 months were observed, suggesting that WX-G250 has the ability to modulate the natural history of mRCC [84,85].

Monoclonal antibodies against IL-6

Tumor growth may be promoted by various cytokines, including IL-6. IL-6 is produced by the majority of RCCs and it is essential to the proliferation of RCC cell lines, thus creating an autocrine loop [86,87]. The physiological action of IL-6 is complex, producing both pro-inflammatory and anti-inflammatory responses, dependent on the environment [88,89]. IL-6 inhibits the differentiation and maturation of dendritic cells [90]. Furthermore, IL-6 activates T_h2 and inhibits T_h1 lymphocytes, thereby stimulating the humoral immunity and inhibiting cellular immunity [91]. The majority of patients with metastatic RCC have high plasma levels of IL-6, which are associated with paraneoplastic symptoms and appear to be a prognostic factor [89,92]. High IL-6 levels are negatively correlated with treatment benefits of IL-2 therapy [93].

The rationale behind treating RCC patients with anti-IL-6 antibodies is the disruption of the autocrine loop, the treatment of paraneoplastic symptoms and the abortion of IL-6-regulated immune suppression. In one trial, three predominantly immunotherapy-resistant patients were treated with a monoclonal IL-6 antibody. The treatment was non-toxic, all patients experienced an increase in performance status and significant reductions in C-reactive protein levels were observed [94]. Currently, a multicenter phase I/II trial with a chimeric anti-IL-6 monoclonal antibody is being performed.

Allogeneic stem cell transplantation (allo-SCT)

A new and effective form of adoptive cellular immunotherapy against various forms of hematological malignancies is allo-SCT with or without donor lymphocyte infusions (DLIs). Allo-SCT for tumor treatment is based on graft versus host disease (GVHD) caused by T cell reactivity against non-self minor histocompatibility antigens. Safety, feasibility and clinical results of allo-SCT after non-myeloablative chemotherapy were evaluated in six studies with mRCC patients [95–100]. Response rates varied between 0 and 56%, and were accompanied by substantial toxicity as a consequence of GVHD [101]. Transplantation-related mortality of up to 33% was observed. Ongoing clinical studies are investigating the use of T cell-depleted transplants with delayed DLIs in order to facilitate graft take while preventing GVHD [102].

Monoclonal antibodies against TNF-α (infliximab)

Levels of TNF-α are frequently raised in patients with mRCC. TNF-α is thought to play an important role in the pathogenesis by autocrine and paracrine stimulation. Normal levels of TNF-α are predictive of a good prognosis in untreated patients [103]. Moreover, TNF-α probably plays a role in paraneoplastic symptoms. Blocking TNF-α with monoclonal antibody may suppress the effects of this cytokine. Infliximab, a chimeric monoclonal TNF-α antibody, is being used in several diseases such as rheumatoid arthritis and Crohn's disease to ameliorate inflammation [104].

Recently, an interesting open, single-arm phase II study of infliximab in patients who had progressed after immunotherapy (IFN-α with/or IL-2) has been presented. Infliximab (5 mg/kg) was administered on weeks 0, 2, 6, 14, 22 and 30. This treatment was continued until progressive disease. Partial responses were observed in three patients (16%) and stable disease in another three patients (16%) of a total 19 evaluable patients. The probability of 1-year survival was 55% [105]. Infliximab administration was safe and non-toxic. Nevertheless, the number of patients studied was rather small and further investigation is warranted.

Discussion

mRCC is considered to be highly resistant to chemo- and radiotherapy. Cytokine-based therapy, although currently considered as first-line treatment, has yielded only moderate clinical responses. IFN-α treatment results in a survival benefit of several months in a small number of patients. Although IL-2-based therapy gives similar median survival rates, high-dose i.v. IL-2 is the only treatment occasionally resulting in sustained complete remissions. Furthermore, cytoreductive nephrectomy prior to immunotherapy has improved the prognosis of patients with a good performance status [33]. Nonetheless, the overall 5-year survival rate for patients with mRCC is still less than 10% and improved treatment strategies are needed.

Specific immunotherapeutic strategies concentrate on the optimization of DC vaccinations and pulsing DCs with immunogenic TAAs, e.g. CA IX/G250. Furthermore, anti-G250 monoclonal antibodies, either unconjugated or radioactively labeled, are currently being investigated in phase II clinical trials. Allogeneic bone marrow transplantation has met with substantial transplant-related mortality and putatively less toxic T cell-depleted transplants are currently being investigated. RCC produces several cytokines, e.g. IL-6 and TNF-α, which are immunosuppressive, related to paraneoplastic symptoms and have autocrine growth properties. Neutralization of the biological activity of these cytokines by monoclonal antibodies is currently under investigation.

Recently gained insights in the pathogenesis of tumorigenesis in general have led to the recognition of several gene products upregulated by VHL gene mutation, a very common event in clear cell RCC. These gene products play an important pathogenetic role and are interesting targets for specific therapeutic interventions. VHL-related gene-products include VEGF (angiogenesis),

TGF-β (growth/survival) and CA IX/G250 (metabolism/pH regulation). Promising treatment strategies target these or other pathways including anti-VEGF antibodies (bevacizumab) and VEGF receptor kinase inhibitors (SU 11248, PTK 787), and/or inhibit the Raf kinase pathway (BAY 43 9006) or inhibit the mTOR pathway (CCI 779). All these compounds have yielded promising early clinical results and all are currently being tested in phase III clinical trials. The following observations have been made since the early results became available. The first observation is that these recognized targets are relevant because objective responses have been seen and early observations show at least an impact on progression-free survival. Whether these early parameters are valid for estimating the true value of these compounds on clinical outcome remains a matter of debate [106]. The second observation is that so far no complete responses have been seen, which does suggest modification of biology, but not cure of this fatal disease. This probably indicates the necessity of long-term treatment. Although long-term application of these compounds seems feasible, our knowledge regarding prolonged treatment in general is limited. The toxicity profile, certainly when compared with chemotherapy and cytokines, is favorable, but still not ideal for long-term treatment. Furthermore, it has to determined if resistance will arise after prolonged exposure.

Finally, the target, angiogenesis, reflects a physiologic pathway with the activation of normal genes and normal receptors. Activation of these genes and receptors leads to overexpression and increased susceptibility, but the link with apoptotic pathways is weak. The lessons from gefitinib in lung cancer underline the relevance of mutations in the receptor tyrosine kinases to render tumor cells sensitive towards inhibition. In RCC this kind of knowledge regarding the EGFR receptor is lacking, but single-agent studies which aim at this target do not point in the direction of a dominant pathway in this disease. However, cross-reactivity with other receptors does play a role.

When reviewing the data available, it seems that we are aiming at a new era for patients with RCC, at least for those with the clear-cell subtype. However, the major challenge still remains the translation of this novel target sensitivity into a way to destroy the stem cell and in this way bring cure within our reach. Future efforts concerning the treatment of mRCC should probably focus on combining the novel targets, immunotherapy and chemotherapy.

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