Recent developments in the treatment of renal cell carcinoma

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Abstract: Renal cell carcinoma (RCC) management has been imbued with new interest, in large part due to the recent success of new treatment options for advanced and metastatic disease. This has also been accompanied by less generally well known advances in the understanding of the molecular characterizations of subtypes of RCC with potential to lead to new therapeutic options. Additionally, the urologic oncology community is focusing on nephron-sparing surgical approaches with limited surgery if possible, and in conjunction with interventional radiologists, on ablative procedures for incidentally determined small renal masses. This report reviews some of the new biologic findings of adenocarcinoma of the kidney, and reviews the new therapeutics which continue to change the landscape for treatment of RCC.

Keywords: Renal cell carcinoma, targeted therapy, immunotherapy, molecular subtypes

Introduction

Renal cell carcinoma (RCC) management has been imbued with new interest, in large part due to the recent success of new treatment options for advanced and metastatic disease. This has also been accompanied by less generally well known advances in the understanding of the molecular characterizations of subtypes of RCC with potential to lead to new therapeutic options. Additionally, the urologic oncology community is focusing on nephron-sparing surgical approaches with limited surgery if possible, and in conjunction with interventional radiologists, on ablative procedures for incidentally determined small renal masses. This report will review some of the new biologic findings of adenocarcinoma of the kidney, and will review the new therapeutics which continue to change the landscape for treatment of RCC.

Renal cell carcinoma subtypes and biology

Several classification systems of RCC have been published, with the most recent being in 2004, from the World Health Organization, which combined features from workshops in 1996 and 1997 that evaluated molecular and clinical features defining various subtypes (Table 1) [Eble *et al.* 2004; Sircar and Tamboli, 2012].

The most common subtype of RCC is clear cell RCC, which comprises about 75% of RCCs in surgical series [Cheville *et al.* 2003]. Although this subtype is usually definitively recognized histologically, its molecular profile is quite variable [Takahashi *et al.* 2006]. This may explain in part the variable clinical course observed among patients with clear cell RCC. A meta-analysis of gene expression profiles has been presented which seems to define a variant subgroup of clear cell RCC, suggesting two molecularly distinct types [Haake *et al.* 2013]. A proposed correlation of these two profiles with the clinical outcome of patients entered into the National Cancer Institute (NCI) sponsored, Eastern Cooperative Oncology Group (ECOG)-led intergroup adjuvant trial (E2805) will evaluate their potential clinical utility (Haas and Rathmell, personal communication 2013).

Clear cell RCC is associated with loss of function of the *vhl* (von Hippel–Lindau) gene which codes for a tumor suppressor gene. This loss of gene function leads to elevated levels of hypoxia inducible factors and increases in vascular endothelial growth factor (VEGF), which facilitates tumor-associated angiogenesis [Kaelin, 2012]. The elucidation of this biology led to the interest in and success of treatment of RCC with the plethora of

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Table 1. Classification of renal tumors: World Health Organization 2004.

Most common phenotypes
Malignant tumors: adenocarcinoma like
Clear cell renal cell carcinoma
Multilocular cystic clear cell renal cell carcinoma
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Xp11 translocation carcinomas
Malignant tumors :transitional cell like
Collecting duct of Bellini
Renal medullary carcinoma
Mucinous and spindle cell carcinoma
Unclassified renal cell carcinoma
Benign tumors
Renal oncocytoma
Papillary adenoma
Angiomyolipoma
Metanephric tumors

Adapted from Eble *et al*. [2004] and Sircar and Tamboli [2012].

antiangiogenesis therapeutics that are now in clinical practice and in clinical trials.

The other best characterized subtypes of RCC are papillary and chromophobe. Papillary RCC, about 15% of surgical series, but less in metastatic series, has been divided histologically into types I and II, with different histologic appearances, reflecting different biologic behaviors. Type I papillary RCC is considered to be clinically less aggressive than type II, and distinct molecular and cytogenetic profiles have been delineated for the two types [Delahunt *et al*. 2001]. Sporadic type I papillary RCC is often indolent and less likely to metastasize. However, when it does metastasize, it is often associated with lymph node metastases and a prolonged course.

Chromophobe RCC accounts for about 5% of RCC. It has a distinct molecular profile and is usually an indolent disease, with less frequent development of metastasis than clear cell RCC. When it does metastasize, the liver is often involved, and resection of metastases is often the initial management.

Translocation RCC was initially described in pediatric renal tumors, but it is now being recognized more frequently in young adults as well. This subtype of RCC is characterized by a genetic translocation of Xp11.2, leading to gene fusions of transcription factor E3. However, it has not been demonstrated to be hereditary. Immunohistochemistry can detect transcription factor E3 (TFE3), a result of the translocation [Argani *et al*. 2003]. Translocation Xp RCC comprises 50% of pediatric RCCs, and it is now more frequently recognized in younger adults with sporadic RCC [Komai *et al*. 2009; Geller *et al*. 2012]. The clinical characteristics are variable, with a subgroup that can be cured by aggressive surgery [Ehrlich *et al*. 2012], and another group with metastatic disease that behaves similarly to adult metastatic RCC [Malouf *et al*. 2011]. This subtype may be responsive to the antiangiogenesis agents [Malouf *et al*. 2010]. A clinical trial led by the Children's Oncology Group is in development to prospectively assess responsiveness to antiangiogenesis therapy to test this observation (Geller, personal communication 2013).

Sarcomatoid dedifferentiation of RCC is not a specific histologic subtype, but can occur mixed with any RCC subtype and is often associated with more aggressive clinical behavior and rapid recurrence, even if initially confined to the kidney [Cheville *et al*. 2004]. There has been some success with treatment with chemotherapy (unlike in other subtypes) due to the high degree of cells in cell division [Nanus *et al*. 2004; Haas, 2009], and there have been some long-term (>5 years) survivors following chemotherapy for metastatic disease [Dutcher and Nanus, 2011]. Recently, targeted therapy has

been evaluated in patients with tumors having sarcomatoid elements. The Cleveland Clinic group reported objective responses with VEGF-targeted therapies, including partial responses [Golshayan *et al*. 2009]. The partial responses occurred in patients with primarily clear cell histology and with less than 20% sarcomatoid features [Golshayan *et al*. 2009]. Michaelson and colleagues reported responses, including brief partial responses, for the combination of sunitinib and gemcitabine in patients with high-grade RCC or poor risk features, including some with sarcomatoid elements [Michaelson *et al*. 2010, 2013]. This regimen is being prospectively evaluated in a randomized trial through ECOG (E1808) [ClinicalTrials.gov identifier: NCT01164228].

Medullary RCC has been recognized as a rare and distinct renal tumor and is a component of the nephropathy of sickle cell disease [Davis *et al*. 1995]. It is observed in patients with sickle trait, more often than those with homozygous SS disease, and it is seen in young patients, usually with widely metastatic disease [Davis *et al*. 1995; Swartz *et al*. 2002; Yang *et al*. 2004]. Although various chemotherapy regimens have been evaluated, usually in small series or case reports, the outcome is usually very poor [Swartz *et al*. 2002; Hakimi *et al*. 2007].

Hereditary renal cell carcinoma syndromes

Clear cell RCC is the subtype observed in the hereditary von Hippel–Lindau syndrome, an autosomal dominant condition, with germline mutations in the *vhl* gene. In this syndrome, approximately 40% of those affected will develop RCC [Latif *et al*. 1993; Kaelin, 2007], usually manifested by multiple small tumors developing in both kidneys.

Hereditary papillary RCC is associated with a germline mutation of the *c-met* proto-oncogene, which codes for a cell surface protein for hepatocyte growth factor [Zbar *et al*. 1994; Lubensky *et al*. 1999].

Birt–Hogg–Dube syndrome (BHD) is an autosomal dominant disorder in which benign cutaneous tumors and pulmonary cysts with risk of spontaneous pneumothorax are observed. There is also an elevated risk of benign and malignant renal tumors. The syndrome is a result of mutation of the folliculin gene, *FLCN*, at 17p11.2 [Schmidt *et al*. 2001; Pavlovich *et al*. 2002; Khoo *et al*. 2002; Adley *et al*. 2006]. One copy of the mutation leads to cutaneous and pulmonary lesions, but most renal tumors have two copies [Schmidt *et al*. 2001; Pavlovich *et al*. 2002; Khoo *et al*. 2002]. Both chromophobe RCC and oncocytoma have been associated with BHD [Vira and Linehan, 2007; Yusenko, 2010].

Additional familial RCC syndromes have been associated with abnormalities in metabolic pathways, such as the fumarate hydratase enzyme mutations which are associated with hereditary leiomyomatosis and renal cell cancer syndrome [Linehan and Ricketts, 2013; Sudarshan *et al*. 2007]. These mutations are autosomal dominant, usually define an aggressive disease, and require close monitoring in family members carrying the mutations. Another metabolic enzyme mutation of succinate dehydrogenase B was initially reported in familial pheochromocytoma and familial paraganglioma [Astuti *et al*. 2001]. This mutation and syndrome has now been reported to include subjects with renal cell cancers [Vanharanta *et al*. 2004].

Although we are learning more about specific molecular abnormalities in subtypes of RCC, subtype-specific therapies are not yet identified. Therefore, we currently group patients into clear cell, non-clear cell and others (transitional cell carcinoma, collecting duct, medullary and sarcomatoid dedifferentiation). Ongoing research and clinical trials are likely to further delineate targets and targeted therapies for the variety of forms of RCC.

Overview of therapy for renal cell carcinoma

Until the past 8–10 years, immunotherapy was the major therapeutic option for patients with RCC. As that modality became more refined, it was observed that patients with clear cell RCC are the most likely to respond to immunotherapy with a proportion achieving durable complete responses [Fyfe *et al*. 1996; Pyrhonen *et al*. 1999; Upton *et al*. 2005; McDermott *et al*. 2010; Hawkins *et al*. 2012]. In fact, approximately 60–70% of the 10–15% who are complete responders appear to be cured after long-term follow up [Fyfe *et al*. 1996; McDermott *et al*. 2010]. That being said, the last decade of clinical research into therapy of RCC has demonstrated that the antiangiogenesis approach is applicable to a larger number of patients with RCC, including those with non-clear cell RCC. These agents, described below, have shown clinical benefit (partial responses and stable disease) in 60–70% of patients, with extension of median survival for responders, offering

new options to those not eligible for or responsive to immunotherapy.

Antiangiogenesis therapy of advanced and metastatic renal cell carcinoma

VEGF mediates neoangiogenesis in a variety of circumstances, and in tumors such neovascularization allows nourishment and growth of the tumor [Folkman, 1971]. Blocking VEGF activity, and therefore inhibiting the production of blood vessels, impairs tumor growth and produces a state of reduced or stable disease. The mammalian target of rapamycin (mTOR) is a key mediator of tissue growth, proliferation and angiogenesis, and its inhibition can also lead to reduced growth and stabilization, particularly in growing tissues such as tumors [Luan *et al.* 2002]. RCC, a vascular tumor resistant to standard chemotherapy, has thus become a model for effectiveness of antiangiogenesis and mTOR inhibitory therapy. Additionally, VEGF has been shown to be immunosuppressive, and thus inhibiting its effect may also enhance antitumor immunity [Gabrilovich *et al.* 1998, 1999; Finke *et al.* 2008].

Seven drugs have been approved for the treatment of RCC within the past 8 years. Four agents directly inhibit the VEGF receptor (VEGFR) thus blocking angiogenesis, two agents inhibit mTOR, and one is an antibody that binds directly with VEGF and prevents it engaging with its receptor. All of these agents also have other targets, which explains some of the toxicities and possibly additive antitumor effects as well as activity in a variety of other malignancies. All have demonstrated improvement in progression-free survival (PFS) in patients with RCC, in randomized clinical trials. Additionally, the two groups, anti-VEGF and mTOR inhibitors, have class-specific toxicities that vary in intensity based on binding affinity, and on intrapatient variability. These include, for the anti-VEGF agents, hypertension, fatigue, diarrhea and hand–foot syndrome, and for the mTOR inhibitors, mucositis, hyperglycemia, hyperlipidemia and rarely interstitial pneumonitis. Clear activity of these agents was demonstrated early, in a variety of phase II trials, and all agents were approved based on the results of phase III randomized clinical trials. An overview of the pivotal trials that led to their approval is described below (Table 2).

December 2005: sorafenib

This was the first antiangiogenesis agent approved for treatment of metastatic clear cell RCC, and RCC was its first approved indication. This agent inhibits VEGFR tyrosine kinase (VEGRF TKI) and platelet-derived growth factor receptor (PDGFR), which supports the pericytes of new blood vessels. It inhibits multiple other targets, of potential importance in other tumor types (Flt 3, RAF and others) [Wilhelm *et al.* 2004]. The pivotal trial compared sorafenib with placebo in patients who had received treatment and whose condition had progressed within 8 months of completing immunotherapy with interferon or interleukin 2 (IL2) [Escudier *et al.* 2007]. A treatment crossover to sorafenib was allowed for patients in the placebo group after progression. The median PFS was significantly better for the sorafenib group (167 days *versus* 84 days, hazard ratio (HR) 0.44) [Escudier *et al.* 2007]. There was a benefit in terms of survival but it was not statistically significant, in part attributed to treatment of patients with progressive disease on placebo with sorafenib (crossover effect).

January 2006: sunitinib

Closely following the approval of sorafenib was accelerated approval of sunitinib, based initially on two single-arm phase II studies in patients with cytokine refractory RCC [Motzer *et al.* 2006]. In February 2007, regular approval was granted based on a randomized phase III trial comparing sunitinib with interferon α in patients with clear cell RCC who had had received no prior therapy [Motzer *et al.* 2007b]. This trial demonstrated a significant improvement in median PFS in favor of sunitinib [47 weeks *versus* 22 weeks, HR 0.42] [Motzer *et al.* 2007b].

Immediately following regulatory approval and prior to commercial availability, both sorafenib and sunitinib were made available by expanded access protocols in North America and Europe. These studies enrolled more than 5000 patients on each agent and provided a considerable amount of safety data and experience with the use of these agents, leading to improved management of side effects [Stadler *et al.* 2010; Beck *et al.* 2011].

May 2007: temsirolimus

The mTOR inhibitor, temsirolimus, was the next agent approved for advanced RCC, based on a randomized phase III trial that compared it with interferon or with the combination of temsirolimus plus interferon, in patients with RCC who were previously untreated and had poor risk features of their disease [Hudes *et al.* 2007]. This patient population

Table 2. US Food and Drug Administration approved targeted therapy for renal cell carcinoma.

Agent, date approved	Study design, patient population	Primary endpoint	Results
Sorafenib December 2005	Randomized phase III, comparison to placebo; one prior therapy - cytokine	PFS	Median PFS 167 days *versus* 84 days HR 0.44 (0.35–0.55)
Sunitinib Accelerated approval January 2006	Two phase II studies in cytokine refractory patients	RR	34%; 36.5%
Regular approval February 2007	Randomized phase III, compared with IFN; no prior therapy	PFS	Median PFS 47 weeks *versus* 22 weeks HR 0.42 (0.32–0.54)
Temsirolimus May 2007	Randomized phase III, comparison to IFN; no prior therapy; poor risk features	OS	Median OS 10.9 months *versus* 7.3 mo HR 0.73 (0.58–0.92)
Everolimus March 2009	Randomized phase III compared with placebo; progression after prior TKI	PFS	Median PFS 4.9 months *versus* 1.9 months HR 0.33 (0.25–0.43)
Bevacizumab July 2009	Randomized phase III comparing bevacizumab + IFN with IFN alone No prior therapy	PFS	Median PFS 8.5 months *versus* 5.2 months (Rini) HR 0.71 (0.61–0.83) Median PFS 10.2 months *versus* 5.4 months (Escudier) HR 0.60 (0.49–0.72) OS 18 months and 19.8 months
Pazopanib October 2009	Randomized phase III comparison to placebo; prior cytokine or no prior therapy	PFS	Median PFS 9.2 months *versus* 4.2 months, all patients HR 0.46 (0.34–0.62) Median PFS 11.1 months *versus* 2.8 months, no prior therapy HR 0.4 (0.27–0.60) Median PFS 7.4 months versus 4.2 months, prior cytokine HR 0.54 (0.35–0.84)
Axitinib January 2012	Phase III randomized, compared with sorafenib; at least one prior therapy	PFS	Median PFS 6.7 months *versus* 4.7 months, all patients HR 0.66 (0.54–0.81), all patients OS 20.1 months axitinib; 19.2 months sorafenib HR 0.969

HR, hazard ratio; IFN, interferon; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase.

was chosen based on phase II data showing clinical benefit in this population compared with historical data with interferon [Atkins *et al.* 2004]. Completion of the phase III trial was prolonged due to the selective patient population, but the result was positive, demonstrating a statistically significant survival benefit for temsirolimus compared with interferon (median overall survival 10.9 months *versus* 7.3 months, HR 0.73). There was a statistically significant benefit for PFS for both temsirolimus-containing arms compared with interferon. The combination arm was the most toxic, with additive toxicities from the two drugs, and the use of this combination has been abandoned because it did not offer benefit over the single agent. A retrospective evaluation of patients with non-clear cell RCC in the phase III study (10%) confirmed the significant survival benefit from temsirolimus compared with interferon in this subset (11.6 months *versus* 4.3 months) [Dutcher *et al.* 2009].

March 2009: everolimus

This is an oral mTOR inhibitor that was approved for the treatment of RCC following progression on a VEGFR TKI, based on a randomized study

comparing everolimus with placebo. Patients could have had more than one prior therapy to enroll in this trial. There was a statistically significant PFS benefit compared with placebo (4.9 months *versus* 1.9 months, HR 0.33) and patients who received placebo could be treated with everolimus upon progression [Motzer *et al.* 2008, 2010].

July 2009: bevacizumab plus interferon

Bevacizumab is a monoclonal antibody that binds to VEGF and prevents it interacting with its receptor. Initial activity in metastatic RCC was shown in a study evaluating two doses of bevacizumab and a placebo-treated control group [Yang *et al.* 2003]. Two randomized phase III clinical trials compared the combination of bevacizumab plus interferon with interferon alone in previously untreated patients with metastatic RCC [Escudier *et al.* 2007; Rini *et al.* 2008, 2010]. There was no bevacizumab-alone arm in either trial. Both studies showed statistically significant PFS benefit for the bevacizumab -containing arm (Rini: 8.5 months *versus* 5.2 months, HR 0.71; Escudier: 10.2 months *versus* 5.4 months, HR 0.63) and both allowed continuation of bevacizumab in the event that interferon was discontinued due to toxicity while response was ongoing. All arms of these studies demonstrated comparable prolonged survival (Rini: median 18 months *versus* 17.4 months; Escudier: median 19.8 months for both) which, when compared with historical controls for intermediate-risk RCC, is double that of prior published data (nearly 20 months in these studies, compared with 10 months historically). The prolongation of survival was attributed to the availability and use of subsequent treatment options after completion of study treatment. This has in fact been noted in most subsequent RCC trials.

October 2009: pazopanib

This agent, also a VEGFR TKI, was developed with more specific VEGFR binding affinity, including receptors 1, 2 and 3. Data from a randomized phase III trial in advanced RCC, conducted primarily in Europe, demonstrated a statistically significant overall median PFS difference compared with placebo (9.2 months *versus* 4.2 months, HR 0.46) [Sternberg *et al.* 2010]. In the treatment-naïve population, the median PFS was 11.2 months *versus* 2.8 months, HR 0.40, and in the cytokine pretreated patients, median PFS was 7.4 months *versus* 4.2 months for placebo, HR 0.54 [Sternberg *et al.* 2010].

2012: axitinib

This is a VEGFR-specific TKI, interacting with all three VEGF receptors. This agent was approved based on a randomized trial comparing axitinib with sorafenib in patients whose condition had progressed after one or more prior therapies, including anti-VEGFR TKIs. This agent demonstrated significant overall improvement in median PFS compared with the control arm (6.7 months *versus* 4.7 months, HR 0.665) [Rini *et al.* 2011; Motzer *et al.* 2013b]. An overall survival analysis has recently been published, showing a similar outcome for each arm: axitinib 20.1 months and sorafenib 19.2 months, HR 0.969 [Motzer *et al.* 2013b].

Combinations of targeted therapies in renal cell carcinoma

A logical extension of the demonstrated activity of these agents was the evaluation of combinations of two antiangiogenesis drugs or antiangiogenesis drugs with cytokines or with mTOR inhibitors. This was not as straight forward a process as was initially expected, and in fact unusual toxicities were uncovered. In the phase I evaluation of the combination of temsirolimus and interferon, based on preclinical data showing synergistic benefit, there were necessary dose reductions to allow tolerable administration [Motzer *et al.* 2007a]. When this combination, at lower than standard doses, was utilized in the phase III trial, it was no better than temsirolimus alone and more toxic [Hudes *et al.* 2007].

In attempts at dual inhibition of angiogenesis, studies of combinations with bevacizumab and sunitinib or sorafenib were also problematic. The combination of bevacizumab with sunitinib led to toxic vascular effects, including renal failure and microangiopathic hemolytic anemia [Feldman *et al.* 2009]. The combination of bevacizumab with sorafenib required dose reductions of both drugs for safe administration [Sosman *et al.* 2008]. A phase I/II clinical trial demonstrated the ability to safely combine temsirolimus with bevacizumab, and suggested an enhanced effect [Merchan *et al.* 2007]. A phase I study also determined safe doses for the combination of sorafenib and temsirolimus [Patnaik *et al.* 2007].

Based on these phase I/II data, combinations including sorafenib, temsirolimus and bevacizumab were incorporated into an NCI-sponsored clinical trial conducted by ECOG, E2804, which

Table 3. ECOG BeST Trial, randomized phase II trial of single agent bevacizumab and three combination regimens: advanced RCC, no prior therapy.

Regimen/dose/schedule	Patients eligible and treated (*n*)	Response	Median PFS	Median survival, HR compared with A
A. Bevacizumab alone; 10 mg/kg every 2 weeks	86	12% PR 50% SD	8.7 months	~24 months HR –
B. Temsirolimus 25 mg weekly; bevacizumab 10 mg/kg every 2 weeks	81	28% PR 51% SD	7.3 months	~24 months HR 1.00
C. Bevacizumab 5 mg/kg every 2 weeks; sorafenib 200 mg twice daily, 5 days weekly	87	30% PR 41% SD	11.3 months	~24 months HR 1.01
D. Temsirolimus 25 mg weekly; sorafenib 200 mg twice daily	86	26% PR 44% SD	7.7 months	~24 months HR 0.98

From McDermott *et al*. [2013b].
ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; SD, stable disease.

evaluated combination therapies compared with a single agent 'control' arm (Table 3). The four arms of this study were: arm A, bevacizumab alone at 10 mg/kg every 2 weeks; arm B, temsirolimus 25 mg/dose weekly with bevacizumab 10 mg/kg every 2 weeks; arm C, bevacizumab 5 mg/kg every 2 weeks with sorafenib, 200 mg twice daily, for 5 days weekly; arm D, temsirolimus 25 mg weekly plus sorafenib 200 mg twice daily. The results were reported at the Genitourinary American Society of Clinical Oncology (ASCO) meeting, in 2013, and demonstrated no significant difference in PFS among the four arms: arm A, 8.7 months; arm B, 7.3 months; arm C, 11.3 months; arm D, 7.7 months [McDermott *et al*. 2013b]. In terms of toxicity, grade 3 and 4 toxicity was half as much for single agent bevacizumab compared with any of the three combination arms (grade 3: 36% *versus* 67%; grade 4: 1% *versus* 7%, 13%, 15%). However, there were two deaths in the bevacizumab-alone arm. Although the response rates were higher in the combination arms, they showed no improvement in PFS. Similarly, although arm C had an insignificantly longer PFS, it had lower dose intensity and was not well tolerated as a regimen. Overall survival was identical among the four arms. Based on this study, none of these combinations was recommended over single agent bevacizumab [McDermott *et al*. 2013b]

In contrast, the combination of bevacizumab and interferon was reasonably well tolerated, and in the two studies leading to the approval of this regimen, there was a higher response rate and PFS with the combination arm compared with interferon alone in both studies [Escudier *et al*. 2007; Rini *et al*. 2008]. More recently, a phase II study of the combination of bevacizumab and IL2 has shown tolerability with a median PFS of 11.2 months compared with a median PFS of 4.2 months with IL2 alone in a previous study [McDermott *et al*. 2005; Dandamudi *et al*. 2010]. This is also longer than the PFS of 8.5 months for bevacizumab alone in a randomized phase II study in previously untreated patients [Bukowski *et al*. 2007]. The PFS achieved with the combination of bevacizumab plus IL2 PFS is similar to that observed in the combination arms of the two bevacizumab plus interferon studies described above, but with a shorter exposure to cytokines [Escudier *et al*. 2007; Rini *et al*. 2008; Dandamudi *et al*. 2010].

Ongoing combination studies of targeted therapies in renal cell carcinoma

There are two recently reported randomized studies of an mTOR inhibitor (one each with temsirolimus and everolimus) combined with bevacizumab, based on phase I/II data suggesting additive benefit [Merchan *et al*. 2007; Hainsworth *et al*. 2010], in comparison with the approved combination of bevacizumab plus interferon. These reports describe similarity in terms of PFS and overall survival for mTOR plus bevacizumab compared with interferon plus bevacizumab [Rini *et al*. 2012; Ravaud *et al*. 2012, 2013]. The dose of interferon used in both studies was 9 MU three times per week. Further evaluation of comparative toxicity and tolerability is ongoing.

Preclinical data have demonstrated that elevated levels of VEGF are immunosuppressive [Gabriliovich *et al.* 1998] and that anti-VEGF strategies can enhance immune activity and perhaps enhance immunotherapy [Gabrilovich *et al.* 1999; Finke *et al.* 2008]. Enhanced immune activity by anti-VEGF strategies include improved function of dendritic cells, and decreases in regulatory T-cell and myeloid-derived suppressor cell numbers [Gabrilovich *et al.* 1999; Finke *et al.* 2008]. Immunological data were not collected on initial trials of the combinations of bevacizumab and interferon [Rini *et al.* 2008; Escudier *et al.* 2007], but ongoing research is further evaluating this concept. A study of IL2 and bevacizumab has completed accrual, and in this study, effects on immunological activation are being evaluated and we await reports [Dandamudi *et al.* 2010]. An additional trial of IL2 and axitinib is being initiated which will include evaluation of immunologic status and enhancement of immune activation with the combination (Fishman, personal communication 2013).

Combination therapies are also being explored in non-clear cell subtypes of RCC. In patients with sarcomatoid elements, building on the results of the combination of doxorubicin and gemcitabine, described above [Nanus *et al.* 2004; Haas *et al.* 2009; Dutcher and Nanus, 2011], a combination of gemcitabine with sunitinib has been reported [Michelson *et al.* 2013]. This is being further explored by ECOG, comparing the combination with sunitinib alone (E1808). It is not yet clear if this combination will produce the durable complete responses seen in a few patients with the chemotherapy regimen [Dutcher and Nanus, 2011]. In papillary RCC, there is an ongoing Southwest Oncology Group (SWOG) phase II clinical trial of erlotinib (epidermal growth factor receptor inhibitor) in combination with ARQ197 (c-*met* inhibitor) *versus* ARQ197 alone (S1107) [ClinicalTrials.gov identifier: NCT01688973]. This is based on preclinical data demonstrating the overexpression of the *met* oncogene in papillary RCC [Zbar *et al.* 1994] and a report of activity of erlotinib in papillary RCC [Gordon *et al.* 2009].

Adjuvant therapy for renal cell carcinoma

Despite the clinical activity of cytokines and targeted therapies in advanced and metastatic RCC, the identification of effective adjuvant therapy remains elusive. A large intergroup randomized clinical trial of interferon *versus* postsurgical monitoring (standard of care) did not demonstrate a benefit of early treatment following nephrectomy compared with treatment of recurrent disease [Messing *et al.* 2003]. A similar multicenter randomized trial conducted in Italy with interferon *versus* monitoring yielded the same results [Pizzocaro *et al.* 2001]. A smaller trial of high-dose IL2 demonstrated no beneficial effect of early treatment *versus* treating at time of recurrence [Clark *et al.* 2003].

Currently, there are three adjuvant trials in which anti-VEGFR TKI therapy is compared with placebo and one trial of an mTOR inhibitor compared with placebo, that are still in follow up or accrual, and are awaiting unblinding. The ASSURE trial, sponsored by the NCI through the US Cooperative Oncology Groups with ECOG in the lead, was initiated in 2005, and is comparing 1 year of treatment with sunitinib with placebo or 1 year of treatment with sorafenib with placebo (E2805) [ClinicalTrials.gov identifier: NCT00326898]. At this time, all patients in this trial have completed treatment, and follow up is ongoing, in the eighth year of the study, still in a blinded fashion. Similarly, the adjuvant study in the UK has just completed accrual, which utilized sorafenib for 1 year *versus* sorafenib for 3 years *versus* placebo (SOURCE, Medical Research Council). It is too early to report an outcome at this time. There are two additional adjuvant trials that are still in the patient accrual phase. One is global and commercially sponsored, evaluating pazopanib *versus* placebo for ` year. The second is an NCI-sponsored study through the North American Cooperative Groups, evaluating everolimus *versus* placebo therapy for 1 year (S0931) [ClinicalTrials.gov identifier: NCT01120249]. Additionally, two post-metastectomy 'adjuvant' trials are ongoing. One is using pazopanib *versus* placebo and is ongoing through ECOG (E2810) [ClinicalTrials.gov identifier: NCT01575548]. The second is a multicenter randomized phase II trial of sorafenib *versus* best supportive care being conducted in Italy [ClinicalTrials.gov identifier: NCT01444807]. Data from all of these trials will evolve over the next several years.

Current status of high-dose interleukin 2

IL2 remains a major and important treatment for advanced RCC, and efforts are ongoing to enhance the response rate and expand the percentage of patients achieving durable complete responses. McDermott and colleagues reported the SELECT trial in which selection by clinical

features resulted in the identification of a group of patients with a doubling of the historical response rate (clear cell, performance status 0) [McDermott *et al*. 2010]. Additional biological parameters that may predict immune responsiveness and therapeutic benefit are under investigation. Enhanced ability to select for responders to IL2 has also been reported by a group in the UK, with a doubling of response rate and increased numbers of complete responders [Hawkins *et al*. 2012]. The next goal in IL2 therapy is to enhance response rate and potentiate response durability. This is being explored in an ongoing clinical trial with the use of axitinib to enhance response rate followed by IL2 to prolong response duration (Mayer Fishman, personal communication 2013). An evaluation of synergy in immunologic activation by the two agents will be performed. Another new direction, based on a clearer understanding of the patterns of immune cellular activation with IL2, is to attempt to separate induction of cytotoxic T cells and mature dendritic cells, while diminishing induction of suppressive cells in the immune system. Laboratory data suggest that this is possible, utilizing intermittent pulses of IL2 to control the expansion of different activated cell populations in the immune response [Finkelstein *et al*. 2010; Coventry and Ashdown, 2012]. An intermittent IL2 schedule of five doses weekly for 4 weeks was investigated clinically with concurrent immune cell monitoring in a single institution study. This study reports an improvement in dendritic cell activity with clinical activity similar to or improved over historical reports [Finkelstein *et al*. 2010]. This is a direction well worth further investigation.

Emerging immunotherapy

Further elucidation of the subtleties of the immune system has defined the impact of regulatory mechanisms which control the degree and duration of ongoing immune activation, a mechanism to prevent or reduce autoimmunity [Salomon and Bluestone, 2001; Saito and Yamasaki, 2003]. Checkpoint inhibitors control immune cellular activation, by blocking costimulatory signals at specific points of immune activation [Salomon and Bluestone, 2001]. This has led to investigations of antitumor cytotoxicity by blocking these regulatory mechanisms [Leach *et al*. 1996].

Subsequent clinical trials have demonstrated antitumor activity in humans with new agents that inhibit cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), an inhibitory signal of early immune activation that controls costimulatory interaction by dendritic cells and T cells (ipilimumab, tremelimumab) [Ribas, 2012; Pardoll, 2012]. Clinical trials of this approach demonstrated major responses in melanoma, some of extended duration, and this has led to the development and approval of ipilimumab for the treatment of metastatic melanoma [Hodi *et al*. 2010]. Yang and colleagues reported activity of ipilimumab in renal cell cancer, with durable responses, including in patients who had not responded to IL2 [Yang *et al*. 2007]. However, associated with the clinical activity of anti-CTLA4 inhibitors are significant autoimmune toxicities, some quite severe in nature, requiring careful patient monitoring and sometimes intervention with corticosteroids or anti-tumor necrosis factor antibodies [Yang *et al*. 2007; Hodi *et al*. 2010]. There are currently numerous ongoing clinical trials of anti-CTLA4 antibodies in patients with other advanced malignancies, some not traditionally thought of as immune-responsive diseases [ClinicalTrials.gov identifiers: NCT01498978, NCT01331525, NCT01285609].

Another site of immune checkpoint inhibition is at the programmed cell death 1 (PD-1) receptor, which is more selective and has been demonstrated at the tumor cell–T-cell interface [Blank *et al*. 2004; Okazaki and Honjo, 2007]. PD-1 ligands (PD-L1, PD-L2) are present on tumor cells and bind to PD-1, causing inhibition of a more localized immune response at the tumor site [Ribas, 2012; Sznol and Chen, 2013]. Recently, phase I trials have demonstrated clinical antitumor activity with the use of this checkpoint inhibitor and with its ligand [Topalian *et al*. 2012; Brahmer *et al*. 2012]. Subsequent follow up has demonstrated durability of some responses in a variety of tumor types, including melanoma and RCC, some lasting more than 1 year [McDermott *et al*. 2013a]. Further development is ongoing, including combination studies [Sznol and Chen, 2013; Wolchok *et al*. 2013].

Emerging vaccine trials in renal cell cancer

IMA901

Peptide vaccines have been in development for at least two decades, and some progress has occurred over that period of time. IMA901 is a peptide vaccine being developed for the treatment of patients

who are HLA-A-02 positive with RCC. It targets 9 HLA class I and 1 HLA class II binding peptides that have been determined to be overexpressed in RCC. In the phase II trial, the ability to generate an immune response to two or more of these peptides appeared to have an impact on lengthening survival [Walter *et al*. 2012. 2013]. A phase III randomized clinical trial comparing IMA901 plus sunitinib with sunitinib alone has completed patient accrual [ClinicalTrials.gov identifier: NCT01265901]. The first interim analysis of overall survival (primary endpoint) is expected to be presented in the first half of 2014. IMA901 has been granted orphan drug designation by the US Food and Drug Administration.

AGS-003

Vaccines based on the utilization of dendritic cells to process antigens have been in development for several years. AGS-003 represents truly personalized immunotherapy in which the individual patient's monocytes are leukapheresed and differentiated into dendritic cells, which are then loaded with mRNA from the patient's tumor, to serve as an antigen to generate an immune response. The initial phase II trial with AGS-003 combined with sunitinib was first presented in 2010 [Figlin *et al*. 2010]. Updates have been presented annually, and in a follow-up report at the 2013 Genitourinary ASCO meeting, prolonged overall survival beyond 30 months in 52% of the patients with newly diagnosed, unfavorable risk metastatic RCC was reported [Amin *et al*. 2013]. A phase III clinical trial of AGS-003 plus sunitinib *versus* sunitinib alone is ongoing [ClinicalTrials.gov identifier: NCT01582672].

Emerging targeted agents and new targets

Many of the new drugs in clinical development for RCC are directed toward inhibition of the VEGFR, but many are also directed at additional targets such as PDGFR, *c-kit*, fibroblast growth factor receptor, *ret*, *raf*, *met* and others, each having differing affinities to these targets. Whether they will prove superior to the current anti-VEGFR agents is yet to be determined. Such agents in ongoing clinical trials include tivozanib [Hutson *et al*. 2013; Motzer *et al*. 2013a], cediranib [ClinicalTrials.gov identifier: NCT00423332], lenvatinib [Molina *et al*. 2013], nintedanib [Eisen *et al*. 2013], regorafenib [ClinicalTrials.gov identifier: NCT00664326], ramucirumab (IMC-1121B) [ClinicalTrials.gov identifier: NCT01282463], and aflibercept (E4805) [ClinicalTrials.gov identifier: NCT00357760]. It is too early to report results, and all of these agents are undergoing further clinical evaluation.

One agent that is both an anti-VEGFR-directed drug and also targets the *met* and *ret* oncogenes is cabozantinib, which has recently been approved for metastatic medullary thyroid carcinoma [Nagilla *et al*. 2012]. It has also shown activity in bony metastases in prostate cancer in early reports [Smith *et al*. 2012] and positive results in metastatic renal cell cancer, including in patients who have had prior anti-VEGFR agents and in patients with bone metastases [Choueiri *et al*. 2012]. Further development is ongoing in RCC and several other malignancies.

Another anti-*met* agent undergoing evaluation is ARQ197, a non-adenosine triphosphate competitive inhibitor targeting MET tyrosine kinase [Adjei *et al*. 2011]. As mentioned previously, an ongoing study in RCC is underway in papillary RCC sponsored by the NCI and the cooperative groups (S1107) [ClinicalTrials.gov identifier: NCT01688973]. A recent report evaluating ARQ197 in patients with microphthalmia transcription factor associated tumors, including translocation-associated RCC (Xp translocation), showed limited clinical activity however [Wagner *et al*. 2012]. Further development of anti-*met* agents continues.

Additional potential targets that are being investigated in advanced RCC include angiopoeitin 1, which maintains tumor-associated blood vessels. AMG 386 is a peptibody (a peptide Fc fusion) that inhibits interaction of angiopoietin 1 and angiopoietin 2 with TIE2 (tyrosine kinase with immunoglobulin-like and EGF-like domains 2) [Rini *et al*. 2012]. It has been explored in a clinical trial in combination with sorafenib compared with sorafenib alone. The initial report did not observe an improvement in PFS [Rini *et al*. 2012]. However, this remains an interesting target and other agents are in development.

Several agents are in development which target phosphoinositide 3 kinase (PI3K) or AKT (upstream from mTOR) or are dual PI3K/mTOR inhibitors (BEZ235, GDC-0980, MK2206, among others) [Elfiky *et al*. 2011; Nyfeler *et al*. 2012; Figlin *et al*. 2013; Serova *et al*. 2013] [ClinicalTrials.gov identifiers: NCT01453595, NCT01442090,NCT01239342,NCT01482156]. There may be synergy with the approved mTOR

inhibitors [Elfiky *et al.* 2011; Nyfeler *et al.* 2012]. These are in early clinical trials and there are no results to report as yet.

Summary

The past 10 years have seen a dramatic increase in the development of new agents and approaches to the treatment of advanced RCC. Seven new agents have been approved for this disease in that time frame. Also, with increased interest and research, there is a better understanding of the biology of subtypes of RCC, which is likely to lead to more specific therapies. Additionally, improved understanding of the complexities of the immune system and improved technology are leading to new ideas for immunotherapy. There is considerable optimism regarding both emerging targeted therapies and new approaches to immunotherapy.

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Conflict of interest statement

Dr Dutcher is a consultant and speaker for Prometheus, Pfizer, Novartis, and a member of data safety committees for BMS, Merck.

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