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New Insights into the Management of Renal Cell Cancer

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Abstract
Kidney cancer is composed of several bio-histological entities. The most frequent type, clear-cell carcinoma, is not homogenous regarding gene mutations or transcriptomic profiles, but the biologic classifications are not yet mature. Therefore, biologically driven strategies of treatment have not yet been developed in the clinical setting. The choice of first-line agent currently depends on the prognostic criteria published by Motzer et al. [J Clin Oncol 1999;17:2530–2540] and recently by Heng et al. [J Clin Oncol 2009;27:5794–5799], with anti-vascular endothelial growth factor (VEGF) therapies for good- or intermediate-prognosis groups and anti-mammalian target of rapamycin (mTOR) for poor-risk patients. In the past years, biological changes leading to resistance to targeted agents have been widely investigated. Discoveries resulted in the development of second-generation VEGF receptor tyrosine kinase inhibitors, characterized by an improved potency and selectivity. Besides, co-inhibition of signalling pathways mediating resistance to anti-VEGF are being developed targeting fibroblast growth factor and c-Met. Dual mTOR/phosphatidylinositol 3-kinase inhibitors have greater efficacy than rapalogs in preclinical models and are being investigated in early clinical trials. In conclusion, the changing landscape in the biology and treatment of kidney cancer offers new opportunities for clinicians to treat patients, but, due to relatively high costs, the use of targeted therapies will likely be strongly controlled by health authorities.

Introduction

Renal cell carcinoma (RCC) afflicts nearly 88,400 patients each year in Europe, making it the 10th most common malignancy [1]. RCC represents 90% of renal tumors, of which 75% are clear-cell carcinomas (ccRCC) and 25% non-clear-cell carcinomas (papillary, chromophobe, oncocytoma) [2]. Well-established risk factors for RCC are age, cigarette smoking, obesity, arterial hypertension and renal disease (acquired renal cysts disease, dialyses) [1]. However, these risk factors are associated with only a small increase in the relative risk. Conversely, rare genetic syndromes are associated with a high risk of renal cancer, but only account for 2–3% of total RCCs [3]. The management of RCC requires systemic therapy in approximately 25–30% of patients with advanced disease

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at diagnosis [4]. Moreover, 20% of patients undergoing nephrectomy will relapse and develop metastatic RCC (mRCC) during follow-up, raising the question of adjuvant therapies in the management of local RCCs.

Genetics of Clear-Cell Renal Cell Carcinoma

Four major hereditary forms of renal cancer have been related to the following genes: von Hippel-Lindau (VHL), hepatocyte growth factor receptor (c-Met), fumarate hydratase (FH), and Birt-Hogg-Dubé [5]. Technological advances in genome-wide analysis have supported identifying underlying biological determinants of sporadic ccRCC. It appeared that the tumor suppressor VHL was inactivated in nearly 90% of sporadic ccRCC tumors [6]. The protein encoded by VHL, pVHL, is the recognition component of a multiprotein complex that degrades hypoxia-inducible factor (HIF) subunits HIF-1α and HIF-2α under normoxic conditions. VHL inactivation leads to constitutive HIF-α activity and promotes tumor growth by enhancing angiogenesis and cell proliferation. Strikingly, a proportion of tumors from current smokers lack VHL alterations and may represent a biologically distinct clinical entity from inactivated cases [7]. Germline mutations inactivating FH or succinate dehydrogenase (SDH) genes are very rare in familial RCC, but nicely illustrate how metabolic changes within the cells (accumulation of fumarate or succinate secondary to FH or SDH deficiency, respectively) inhibit the VHL-mediated hydroxylation of HIF-α, resulting in HIF accumulation [3].

Systematic exome sequencing of 101 ccRCCs revealed mutations in the histone-modifying genes SETD2 (SET domain containing 2), KDM5C (lysine (K)-specific demethylase 5C) and KDM6A (lysine (K)-specific demethylase 6A), and the tumor suppressor neurofibromin 2 (NF2) [8]. Each of these genes was mutated in approximately 2% of samples. More recently, the exome sequencing of 227 ccRCC identified a second major cancer gene in ccRCC, the SWitch/Sucrose NonFermentable (SWI/SNF) chromatin remodelling complex gene polybromo 1 (PBRM1), that was present in 41% of primary tumors [9]. These genes further define the genetic and molecular architecture of ccRCC. It is remarkable that PBRM1, like the majority of the other non-VHL mutated cancer genes identified in ccRCC, is involved in chromatin regulation. It opens important areas of future renal cancer research in understanding the contribution of PBRM1 mutation to the carcinogenesis of ccRCC as well as exploiting SWI/SNF complex as a therapeutic target [10].

Drug Availability in England – The Health Economics and Politics of Drugs for Advanced Renal Cancer

With newer therapies, the median overall survival (OS) duration in mRCC is about 26 months or even more. This raises the question of treatment cost. In 2008, the high cost associated with sunitinib led to an initial rejection of reimbursement from the National Institute for Health and Clinical Excellence (NICE), a decision-maker in the UK, which generated heated debate among concerned physicians [13]. Although NICE reversed its decision on sunitinib as first-line therapy for patients with mRCC in early 2009, discussions about targeted agents and the economic burden of RCC resulted in the development of several related economic studies.

Decisions of public health authorities are based on cost considerations. In 2005, the US Medicare payments for patients with RCC who were treated with targeted therapy was more than 3-fold higher than those for patients who did not receive targeted therapy (USD 64,082 vs. 18,912) [14]. However, this extra cost has to be balanced with the effectiveness of the drug. Cost-effectiveness studies use the comparison of the incremental cost-effectiveness ratios (ICERs) measured in terms of quality-adjusted life years (QALY) gained by the treatment. ICER associated with targeted therapies in RCC showed a wide range across studies: USD 49,959–272,418 per QALY [15]. The two studies which concluded that sunitinib was cost-effective were sponsored by the pharmaceutical companies that manufacture the agent [16], whereas in two recent studies, sunitinib was associated with ICERs of USD 113,645–144,232, which does not equate to cost-effectiveness if we consider a willingness to pay of USD 50,000–100,000 per QALY [15, 17]. However, another aspect to consider is that survival data extrapolated from pivotal trials are used in QALY estimations and these phase 3 clinical trials did not demonstrate OS advantage mainly because patients in the control arm received targeted therapies at progression. With this in mind, sunitinib is now considered by NICE as a cost-effective first-line targeted therapy.

New Agents in Renal Cancer

Before the era of targeted therapies, cytokine therapy with interferon and interleukin-2 were the main treatment, with little benefit in terms of progression-free survival (PFS) and OS. Since 2005, progress in systemic therapy has led to the approval of many new agents by the US Food and Drug Administration (FDA) and European

Medicines Agency. These drugs are tyrosine kinase inhibitors (TKI) of the vascular endothelial growth factor receptors (VEGFR), monoclonal antibodies directed against the ligand vascular endothelial growth factor A (VEGF-A) and mammalian target of rapamycin (mTOR) inhibitors.

Angiogenesis Inhibitors: Current Applications

Sunitinib and sorafenib are oral TKI that inhibit VEGFR and platelet-derived growth factor receptor (PDGFR). In previously untreated mRCC, a phase 3 trial of sunitinib demonstrated a benefit in terms of PFS (11 vs. 5 months, $p < 0.0001$) and response rates by investigator assessment (37 vs. 9%, $p < 0.001$) compared with interferon [18]. And in second-line treatment, after failure of cytokines, sorafenib was superior to placebo in the TARGET trial [19]. Then bevacizumab, a humanized antibody directed against VEGF-A, in combination with interferon alpha became an alternative to sunitinib in previously untreated RCCs. Two phase 3 trials demonstrated that bevacizumab + interferon was superior to interferon alone in terms of PFS and response rate [20, 21]. Recently, in a phase 3 randomized study, pazopanib, an oral TKI anti-VEGFR, PDGFR and c-Kit, showed improved PFS against placebo (9.2 vs. 4.2 months, $p < 0.0001$) [22]. Presented this year at ASCO, the PISCES trial showed that 70% of patient preferred pazopanib over sunitinib in the first-line setting due to fatigue decrease and improved quality of life [23]. The COMPARZ phase 3 trial comparing first-line pazopanib with sunitinib has completed patient enrolment and it is hoped that the results of this study will provide an answer as to whether pazopanib is as effective as sunitinib with less toxicity.

Sunitinib, sorafenib, pazopanib and bevacizumab resulted in a complete change in the treatment paradigm, but, ultimately, patients developed resistance and the rate of complete response was low. Guidelines recommend the use of VEGF inhibitors as first-line therapy in patients with good and intermediate risk [24].

Overcoming Resistance to Angiogenesis Inhibitors

Many strategies are being developed to overcome resistance to anti-VEGF therapies in mRCC [25]. Already available in the clinical setting, second-generation VEGF inhibitors, with more potency and selectivity against VEGFR-1, -2 and -3, have been developed. One of them, axitinib, has shown in vitro a VEGFR-2 inhibition potency 40 times greater than sunitinib [26]. In the recent phase 3 AXIS trial, axitinib was shown to be effective as second-line therapy compared with sorafenib [27]. As 54% of patients had been previously treated with sunitinib, a preplanned subgroup analysis was conducted in this population and showed a benefit of axitinib in sunitinib-refractory patients. In January 2012, the FDA has approved axitinib in this indication. Interestingly, axitinib is administered with a dose escalation from 5 mg BID to 10 mg bid in the absence of hypertension (blood pressure >150/90 mm Hg). Indeed, dose escalation allowed increasing drug exposure, which could be another way to overcome resistance to anti-VEGF TKI [28]. More recently, axitinib phase II data in the first-line setting demonstrated impressive results with a PFS of 14.5 months (95% CI 11.5–17.4), which was even longer (22.5 months) in patients with diastolic blood pressure >90 mm Hg. In this trial, patients with no grade 3–4 adverse event and blood pressure ≤150/90 mm Hg were randomized between axitinib dose escalation versus flat dose [29]. This comparison will provide further insight into the clinical benefits of dose escalation in metastatic RCC.

Tivozanib, another second-generation TKI with potent pan-VEGFR inhibitory activity, is under development for mRCC and has shown promising activity in ccRCC in phase 2 trials [30]. Tivozanib was compared with sorafenib in a randomized phase 3 trial in 517 VEGF therapy-naïve mRCC patients. Tivozanib demonstrated a statistically significant improvement in PFS with a median PFS of 11.9 months compared with 9.1 months for sorafenib (HR = 0.80, 95% CI 0.64–0.99; $p = 0.042$) [31].

Another strategy to overcome the resistance to VEGF therapy is to block the pathways that become hyperactivated in resistant mRCC. For instance, fibroblast growth factor (FGF) has been implicated in cell lines that developed acquired resistance to VEGF therapy and the authors demonstrated that simultaneous blockade of FGF and VEGF pathways restored antitumor efficacy [32]. Dual inhibitors of VEGFR-1, -2, and -3, and FGFR-1, -2 and -3 (together with PDGFR) are being developed in clinical trials. In a phase 1/2 trial involving 31 mRCC patients previously treated with VEGF or mTOR inhibitors, dovitinib, an oral TKI anti-FGFR and -VEGFR, was associated with 4 partial responses and 13 patients experienced stable disease [33]. Dovitinib is currently being evaluated in a phase 3 study (the GOLD trial) compared with sorafenib, in third-line mRCC after one anti-VEGF and one anti-mTOR treatment.

Signalling through the hepatocyte growth factor (HGF) and its receptor c-Met is the subject of active research in kidney cancer. This pathway mediates motility, proliferation and differentiation of the cells, and cooperates with VEGF to induce tumor invasion and vascularization [34]. Preclinical studies indicate that c-Met is fre-

Table 1. Histological subtypes of renal cancer correspond to specific genetic alterations

	Clear-cell carcinoma	Papillary type 1	Papillary type 2	Chromophobe	Oncocytome
Frequency	75%	5%	10%	5%	5%
Hereditary gene	VHL SDH chromosome 3 translocations	c-Met	FH	BHD	BHD
Sporadic gene	VHL (92%) [6, 11] PBRM1 (41%) [9] c-Myc [11]	c-Met (13%)	unknown	BHD (11%) [12]	unknown

BHD = Birt-Hogg-Dubé; c-Met = hepatocyte growth factor receptor; FH = fumarate hydratase; PBRM1 = chromatin remodelling complex gene polybromo 1; VHL = von Hippel-Lindau; SDH = succinate dehydrogenase.

quently amplified in mRCC [35] and could play a role in resistance to antiangiogenic therapies. RCC tumors resistant to sunitinib have a greater amount of HGF than sunitinib-sensitive tumors, especially in endothelial cells associated with the tumors [36]. Moreover, the combination of a c-Met inhibitor with sunitinib restored the antitumor efficacy in sunitinib-resistant cell lines [36]. Several compounds are in development. Cabozantinib is a dual TKI of c-Met and VEGFR2 that showed improved efficacy relative to VEGFR2-only inhibitors in preclinical models [37]. Cabozantinib has been tested in a double-blind randomized discontinuation trial in patients with solid tumors. After 12 weeks of treatment with cabozantinib, patients with stable disease were randomized to either cabozantinib or placebo. The study showed, in patients with metastatic castration-resistant prostate cancer, 84% tumor shrinkage and 86% bone scan partial or complete responses at 6 weeks [38]. Antitumor activity has also been observed in preclinical studies with RCC treated with cabozantinib; therefore, further evaluation of this agent in mRCC patients is warranted.

AMG102, a monoclonal antibody directed against HGF, has demonstrated modest activity in mRCC [39]. Furthermore, the combination of AMG102 with bevacizumab or motesanib appears to be safe and feasible and should be evaluated in metastatic renal tumors [40].

Beside its role in angiogenesis, c-Met is directly implicated in the carcinogenesis of papillary renal tumors. Germline mutations of c-Met are involved in the hereditary papillary RCC syndrome, and somatic mutations have been found in 13% of sporadic type 1 papillary RCC (table 1) [41, 42]. Foretinib (XL880), a c-Met and VEGFR2 inhibitor, showed antitumor activity in papillary tumors in phase 1 and phase 2 trials and could be further developed [43, 44].

mTOR Inhibitors: Current Limits and Promising Second-Generation Agents

First-generation mTOR inhibitors derive from rapamycin (rapalogs) and selectively inhibit mTOR complex 1 (mTORC1) (fig. 1). They have demonstrated antitumor activity in mRCC. In a randomized phase 3 trial, temsirolimus, an intravenous inhibitor of mTORC1, was associated with prolonged survival compared with interferon alpha in previously untreated poor-risk patients (median OS 10.9 vs. 7.3 months, $p = 0.0069$). Everolimus, an oral inhibitor of mTORC1, was tested in patients with mRCC previously treated with anti-VEGF TKI (sunitinib or sorafenib or both) in the RECORD-1 phase 3 trial [45]. The results showed improved PFS with everolimus relative to placebo (5.5 vs. 1.9 months, $p < 0.001$). Both compounds resulted in tumor stabilization and rarely in objective responses (response rate with temsirolimus: 8.6% and everolimus: 1.8%) [45]. The lack of objective responses could be due to the biological limits of these first-generation mTOR inhibitors. Rapalogs are potent inhibitors of mTORC1 but neither mTORC2 nor phosphatidylinositol 3-kinase (PI3K) (fig. 1) [46]. After treatment with rapamycin, tumor cells exhibit hyperactivation of PI3K, notably through feedback loops containing insulin-like growth factor 1 receptor [47]. Moreover, mTORC2 directly phosphorylates Akt, a downstream component of the PI3K pathway [48]. PI3K activation favors proliferation and angiogenesis in the tumors and leads to mTOR inhibitor resistance (fig. 1) [49].

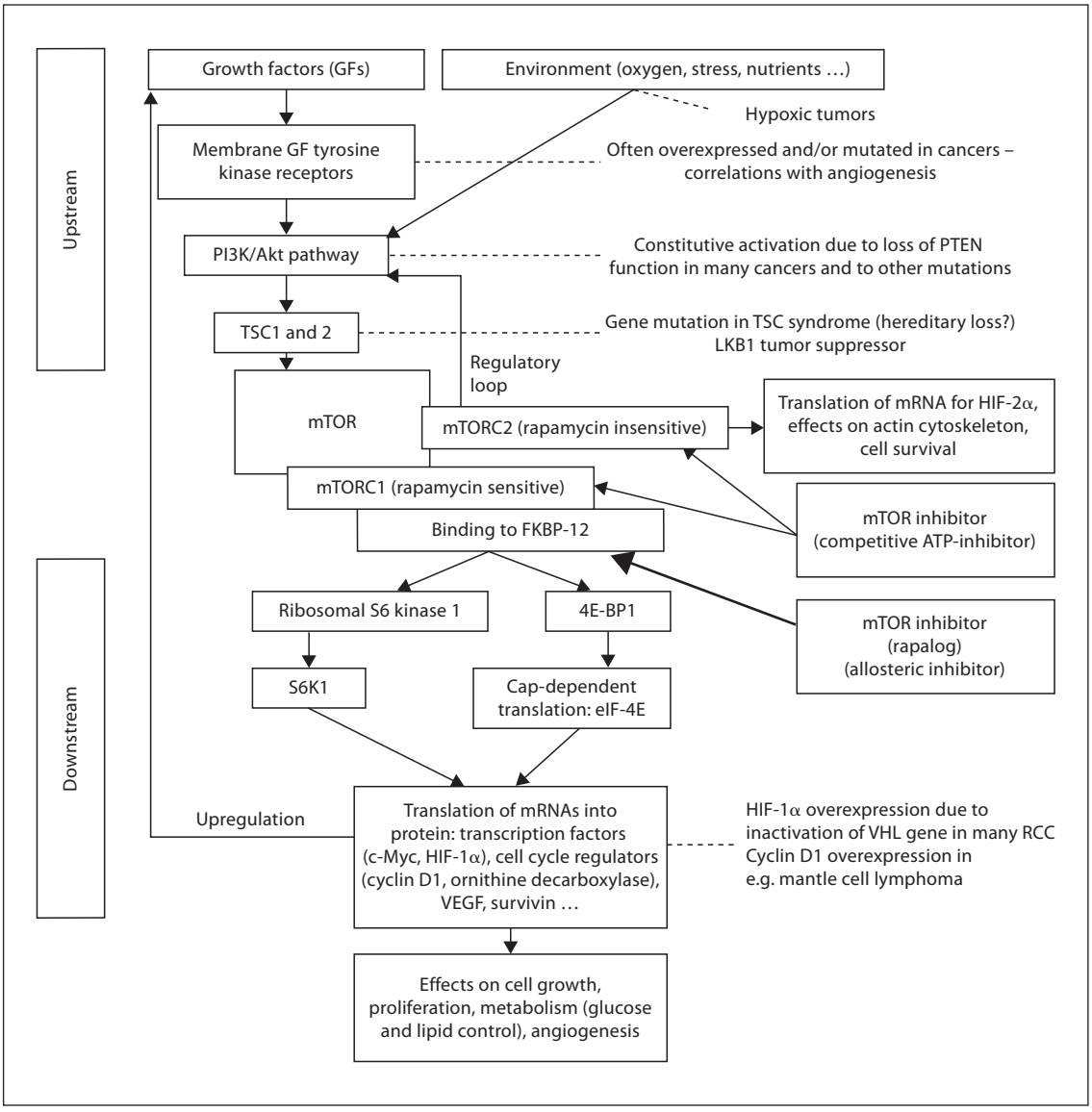


Fig. 1. Mammalian target of rapamycin (mTOR) is a major intracellular pathway upregulated in renal cell carcinoma and targeted by therapeutic agents. 4E-BP1 = 4E-binding protein 1; eIF-4E = eukaryotic initiation factor-4E; FKBP-12 = FK506-binding protein 12; GF = growth factors; HIF = hypoxia-inducible factor; LKB1 (or STK11) = serine-threonine kinase 11; mRNA = messenger ribonucleic acid; mTORC1 and 2 = mammalian target of rapamycin complex 1 and 2; PI3K = phosphatidylinositol 3-kinase; PTEN = phosphatase and tensin homolog; RCC = renal cell carcinoma; S6K1 = protein S6 kinase 1; TSC1 and 2 = tuberous sclerosis protein 1 and 2; VHL = von Hippel-Lindau.

Second-generation mTOR inhibitors are small-molecule mimetics of adenosine triphosphate that target the mTOR kinase domain; they have also entered clinical trials. These second-generation kinase inhibitors could inhibit mTOR, PI3K and other PI3K-related protein kinases. In RCC xenografts, a dual PI3K/mTOR inhibitor was shown to inhibit tumor cell proliferation more effectively than rapamycin [50]. NVP-BEZ235 and GDC-0980 are PI3K/mTORC1/mTORC2 inhibitors that recently entered into phase 2 development in patients with mRCC previously treated with antiangiogenic therapy.

Sequential Treatment Strategies for Patients with Advanced Renal Cell Carcinoma

Several questions about the optimal sequence of treatment in patients with mRCC have yet to be answered. For instance, will patients gain enhanced clinical benefit from a second targeted therapy if it is initiated before disease progression on first-line therapy? The ongoing EVERSUN trial was designed to address this question by evaluating the effect of alternating treatment with everolimus and sunitinib in patients with mRCC in the absence of disease progression. A second question is whether response to specific targeted therapies can be predicted in individual patients? Two ongoing clinical trials sponsored by the PREDICT Consortium are focused on the identification of predictive biomarkers for response to everolimus (E-PREDICT trial) and sunitinib (S-PREDICT trial). In these studies, paired pretreatment biopsies and on-treatment nephrectomy specimens from patients with previously untreated mRCC will be collected for use in molecular analyses and integration with clinical efficacy data [51].

New Approaches in Surgical Management of Renal Cancer

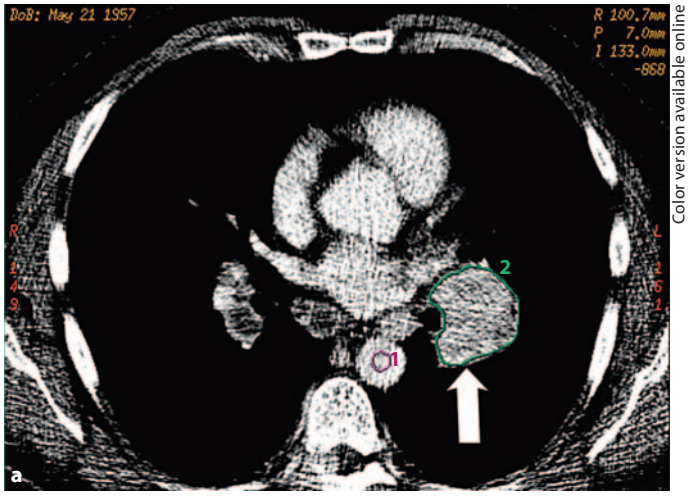
At the time of interferon, radical nephrectomy was associated with survival benefit in a combined analysis of two phase 3 trials [52]. By extension, debulking surgery is considered as an option in patients receiving TKI, particularly in patients presenting with a solitary metastasis [53]. Data for nephrectomy status of three phase 3 clinical trials comparing a targeted therapy with interferon alpha provide contradictory information concerning the therapeutic impact of surgery. Temsirolimus showed a survival benefit in patients that were not nephrectomized compared with the interferon arm (n = 138), whereas no survival advantage was found in patients who had undergone prior nephrectomy (n = 278) [54]. Similarly, the survival improvement of bevacizumab + interferon alpha over interferon alpha was significant in patients that were not nephrectomized (n = 112; HR 0.65, p = 0.04) [20]. The trend in survival benefit of sunitinib over interferon alpha was similar in patients with prior nephrectomy (n = 674) and without nephrectomy (n = 76) [18]. Whilst one could conclude that prior nephrectomy does not appear to be essential for the benefit from targeted therapy with either VEGFR or mTOR inhibitor therapies, these trials were not designed to specifically address this question. Dedicated studies are ongoing, with two phase 3 trials: the CARMENA trial, comparing isolated sunitinib versus primary nephrectomy followed by sunitinib, and the SURTIME trial (trial identifier: EORTC 30073), investigating optimal nephrectomy timing (immediate vs. deferred) with sunitinib therapy.

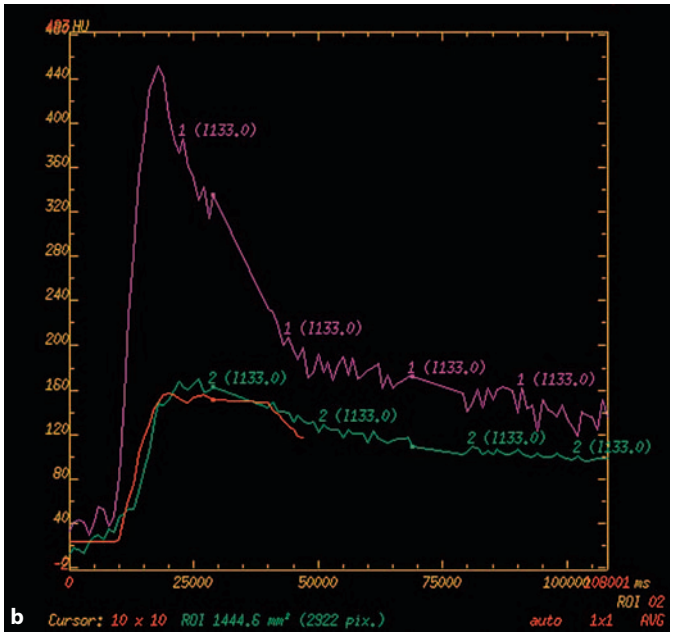
At the time of interferon, metastases surgery was essential to obtain longer survival. A retrospective study in 101 patients reported long recurrence-free survival after metastasectomy (14% at 45 months and 7% at 60 months) [55] and a recent retrospective study of 887 patients confirmed that complete resection of multiple metastases was associated with a prolongation of survival [56]. In a third study, the survival benefit associated with metastasectomy in 44 patients was present across various risk categories [57]. The breakthrough of targeted therapies in the management of RCC raised the question of the place of metastases surgery. Currently, a limited number of retrospective studies indicate that metastasectomy is safe and feasible after targeted therapies, essentially sunitinib [57]. However, the type and timing of perioperative treatments must be addressed in dedicated clinical trials based on the half-life of targeted drugs.

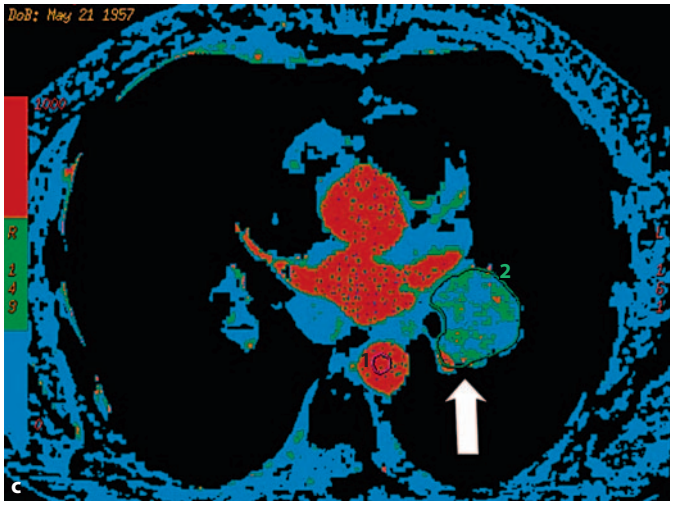
Defining Response – New Challenges in Oncological Imaging

In clinical trials evaluating cytotoxic therapy, an objective response is often determined using the Response Evaluation Criteria in Solid Tumors (RECIST) [58]. Despite its widespread use, RECIST has a limited ability to reflect the activity of recently developed targeted therapies. Response rates with TKI range from 47 to 10% [59–61] and with anti-mTOR from 0 to10% [45, 54]. As these agents inhibit tumor growth by decreasing tumor perfusion rather than by a direct cytotoxic effect on tumor cells, tumor size may not be significantly reduced despite good antitumor activity [62].

A new cut-off criteria based on modified RECIST criteria was proposed both for sunitinib and everolimus. A relative reduction of 10% in the sum of the largest tumor diameters appears to be a reliable threshold for identifying patients with mRCC who are benefiting from antiangiogenic treatment with sunitinib and for guiding the oncologist's decision [63]. Patients reach this threshold earlier than the RECIST –30% threshold, allowing a quicker evaluation of treatment efficacy. Similarly, a study of everolimus, based on the patients from the RECORD-1 study, demonstrated that a relative reduction of 5% in the sum of







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longest diameter of target tumors was a better predictor of PFS benefit than the classical 30% reduction used with RECIST [64]. This optimized response threshold may be useful in the evaluation of antiangiogenic therapy in clinical trials and as a guide in treatment decisions in the clinical setting in conjunction with other patient- and disease-related parameters. Some studies tried to adapt the Choi criteria, defined for evaluating response in gastrointestinal stromal tumors treated with imatinib, to the evaluation of mRCC under antiangiogenic therapies. These criteria are based on changes in size *or* tumor density (necrosis) measured with computed tomography (CT). However, these criteria do not seem to correlate to survival data [65].

New techniques of functional imaging do not detect changes in size, but rather a physiological characteristic, and could reveal changes in response to treatment which arise earlier [66, 67]. A 'dynamic contrast-enhanced' (DCE) or 'perfusion' acquisition can be incorporated into the regular CT follow-up of patients easily and with no additional cost (fig. 2). In two phase 3 trials involving 51 patients treated for mRCC with antiangiogenic drugs (sorafenib n = 10, sunitinib n = 22), placebo (n = 12) or interferon (n = 7), microvascular parameters of a metastatic target (tumor blood flow and tumor blood volume) were measured using DCE-CT before and after treatment, based on dynamic enhancement before and after injection of iodinated contrast medium. The results were compared with the RECIST response rates, as well as PFS and OS [62]. Among the patients receiving antiangiogenic drugs, baseline microvascular parameters were higher in responders than in nonresponders but did not correlate with PFS or OS. After the first cycle of treatment, there was a significant decrease in tumor blood flow and tumor blood volume in patients receiving antiangiogenic treatment, while no change occurred in patients receiving placebo or interferon [62]. On the other hand, the study of

Fig. 2. Perfusion imaging using dynamic contrast-enhanced computed tomography (DCE-CT). **a** The perfusion acquisition is performed at low radiation dose by a repeated acquisition over time during iodine contrast injection at a selected metastatic site, in this case a pulmonary metastasis (arrow). A region of interest is drawn on the aorta (circle 1) and tumor (circle 2). **b** An enhancement density curve over time is obtained in the aorta (curve 1) and in the tumor (curve 2) from the regions of interest. A mathematical model is applied to calculate the perfusion parameters. **c** The value of a chosen perfusion parameter can be calculated in each pixel of the image and represented as a 'parametric map', in which values are color-coded – here, a parametric map of tumor blood flow. The tumor (arrow) shows a heterogeneous vascularization.

Lamuraglia et al. [68], which utilized DCE-ultrasound to study the response of RCC metastases to sorafenib after 3 and 6 weeks of treatment, found that microvascular parameters were predictive of the response of early-stage tumors, showing that PFS duration was prolonged in patients which they defined as 'good responders' compared with those they defined as 'poor responders'.

Concluding Remarks

Recent biological discoveries provide new insights into RCC pathogenesis. Multiple pathways, such as angiogenesis, metabolism and, more recently, chromatin remodelling, have been shown to be involved in the carcinogenesis of RCCs. Genetic alterations refine the classification of RCCs and could be used as predictive markers of response to treatment. Current therapeutic development remains very active, mainly focusing on reversing acquired resistance to antiangiogenic drugs. Activation of alternative pathways (FGF, c-Met, angiopoietin receptors) is frequently involved in resistance to anti-VEGF therapies and could be targeted by specific agents. New anti-mTOR compounds that properly inhibit both mTORC-1 and -2 are currently under investigation in the clinical setting with the hope of improved antitumor activity.

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References

1 Ljungberg B, Campbell SC, Cho HY, Jacqmin D, Lee JE, Weikert S, Kiemeney LA: The epidemiology of renal cell carcinoma. Eur Urol 2011;60:615–621.

2 Lopez-Beltran A, Carrasco JC, Cheng L, Scarpelli M, Kirkali Z, Montironi R: 2009 update on the classification of renal epithelial tumors in adults. Int J Urol 2009;16:432–443.

3 Linehan WM, Srinivasan R, Schmidt LS: The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol 2010;7:277–285.

4 Gupta K, Miller JD, Li JZ, Russell MW, Charbonneau C: Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev 2008;4:193–205.

5 Kluger N, Giraud S, Coupier I, Avril MF, Dereure O, Guillot B, Richard S, Bessis D: Birt-Hogg-Dubé syndrome: clinical and genetic studies of 10 French families. Br J Dermatol 2010;162:527–537.

6 Kaelin WG: The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. Clin Cancer Res 2007;13:680s–684s.

7 Moore LE, Nickerson ML, Brennan P, Toro JR, Jaeger E, Rinsky J, Han SS, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Schmidt LS, Lenz P, Karami S, Linehan WM, Merino M, Chanock S, Boffetta P, Chow WH, Waldman FM, Rothman N: Von Hippel-Lindau (VHL) inactivation in sporadic clear cell renal cancer: associations with germline VHL polymorphisms and etiologic risk factors. PLoS Genet 2011;7:e1002312.

8 Dalgliesh GL, Furge K, Greenman C, Chen L, Bignell G, Butler A, Davies H, Edkins S, Hardy C, Latimer C, Teague J, Andrews J, Barthorpe S, Beare D, Buck G, Campbell PJ, Forbes S, Jia M, Jones D, Knott H, Kok CY, Lau KW, Leroy C, Lin ML, McBride DJ, Maddison M, Maguire S, McLay K, Menzies A, Mironenko T, Mulderrig L, Mudie L, O'Meara S, Pleasance E, Rajasingham A, Shepherd R, Smith R, Stebbings L, Stephens P, Tang G, Tarpey PS, Turrell K, Dykema KJ, Khoo SK, Petillo D, Wondergem B, Anema J, Kahnoski RJ, Teh BT, Stratton MR, Futreal PA: Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 2010;463:360–363.

9 Varela I, Tarpey P, Raine K, Huang D, Ong CK, Stephens P, Davies H, Jones D, Lin M-L, Teague J, Bignell G, Butler A, Cho J, Dalgliesh GL, Galappaththige D, Greenman C, Hardy C, Jia M, Latimer C, Lau KW, Marshall J, McLaren S, Menzies A, Mudie L, Stebbings L, Largaespada DA, Wessels LFA, Richard S, Kahnoski RJ, Anema J, Tuveson DA, Perez-Mancera PA, Mustonen V, Fischer A, Adams DJ, Rust A, Chan-on W, Subimerb C, Dykema K, Furge K, Campbell PJ, Teh BT, Stratton MR, Futreal PA: Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 2011;469:539–542.

10 Wilson BG, Roberts CWM: SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer 2011;11:481–492.

11 Young AC, Craven RA, Cohen D, Taylor C, Booth C, Harnden P, Cairns DA, Astuti D, Gregory W, Maher ER, Knowles MA, Joyce A, Selby PJ, Banks RE: Analysis of VHL gene alterations and their relationship to clinical parameters in sporadic conventional renal cell carcinoma. Clin Cancer Res 2009;15:7582–7592.

12 Gad S, Lefèvre SH, Khoo SK, Giraud S, Vieillefond A, Vasiliu V, Ferlicot S, Molinié V, Denoux Y, Thiounn N, Chrétien Y, Mejean A, Zerbib M, Benoît G, Hervé JM, Allègre G, Bressac-de Paillerets B, Teh BT, Richard S: Mutations in BHD and TP53 genes, but not in HNF1β gene, in a large series of sporadic chromophobe renal cell carcinoma. Br J Cancer 2006;96:336–340.

13 Shih YC, Chien CR, Xu Y, Pan IW, Smith GL, Buchholz TA: Economic burden of renal cell carcinoma: part I – an updated review. Pharmacoeconomics 2011;29:315–329.

14 Shih YC, Chien CR, Xu Y, Pan IW, Smith GL, Buchholz TA: Economic burden of renal cell carcinoma in the US: part II – an updated analysis. Pharmacoeconomics 2011;29:331–341.

15 Thompson Coon J, Hoyle M, Green C, Liu Z, Welch K, Moxham T, Stein K: Bevacizumab, sorafenib tosylate, sunitinib and temsirolimus for renal cell carcinoma: a systematic review and economic evaluation. Health Technol Assess 2010;14:1–208.

16 Purmonen T, Martikainen JA, Soini EJO, Kataja V, Vuorinen RL, Kellokumpu-Lehtinen PL: Economic evaluation of sunitinib malate in second-line treatment of metastatic renal cell carcinoma in Finland. Clin Ther 2008;30:382–392.

17 Chabot I, Rocchi A: How do cost-effectiveness analyses inform reimbursement decisions for oncology medicines in Canada? The example of sunitinib for first-line treatment of metastatic renal cell carcinoma. Value Health 2010;13:837–845.
18 Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, Negrier S, Szczylik C, Pili R, Bjarnason GA, Garcia-del-Muro X, Sosman JA, Solska E, Wilding G, Thompson JA, Kim ST, Chen I, Huang X, Figlin RA: Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. J Clin Oncol 2009;27:3584–3590.
19 Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, Negrier S, Chevreau C, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Anderson S, Hofilena G, Shan M, Pena C, Lathia C, Bukowski RM: Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol 2009; 27:3312–3318.
20 Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Archer L, Atkins JN, Picus J, Czaykowski P, Dutcher J, Small EJ: Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. J Clin Oncol 2010; 28:2137–2143.
21 Escudier B, Bellmunt J, Negrier S, Bajetta E, Melichar B, Bracarda S, Ravaud A, Golding S, Jethwa S, Sneller V: Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. J Clin Oncol 2010;28:2144–2150.
22 Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, Barrios CH, Salman P, Gladkov OA, Kavina A, Zarba JJ, Chen M, McCann L, Pandite L, Roychowdhury DF, Hawkins RE: Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol 2010;28:1061–1068.
23 Escudier BJ, Porta C, Bono P, De Giorgi U, Parikh O, Hawkins RE, Sevin E, Negrier S, Khan S, McCann L, Mehmud F, Cella D: Patient preference between pazopanib (Paz) and sunitinib (Sun): results of a randomized double-blind, placebo-controlled, crossover study in patients with metastatic renal cell carcinoma (mRCC) – PISCES study, NCT 01064310. J Clin Oncol 2012;30(suppl; abstr CRA4502).
24 Heng DYC, Xie W, Regan MM, Warren MA, Golshayan AR, Sahi C, Eigl BJ, Ruether JD, Cheng T, North S, Venner P, Knox JJ, Chi KN, Kollmannsberger C, Mcdermott DF, Oh WK, Atkins MB, Bukowski RM, Rini BI, Choueiri TK: Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. J Clin Oncol 2009; 27:5794–5799.
25 Bergers G, Hanahan D: Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592–603.
26 Hu-Lowe DD, Zou HY, Grazzini ML, Hallin ME, Wickman GR, Amundson K, Chen JH, Rewolinski DA, Yamazaki S, Wu EY, McTigue MA, Murray BW, Kania RS, O'Connor P, Shalinsky DR, Bender SL: Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. Clin Cancer Res 2008; 14:7272–7283.
27 Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, Michaelson MD, Gorbunova VA, Gore ME, Rusakov IG, Negrier S, Ou Y-C, Castellano D, Lim HY, Uemura H, Tarazi J, Cella D, Chen C, Rosbrook B, Kim S, Motzer RJ: Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. Lancet 2011;378: 1931–1939.
28 Rixe O, Dutcher J, Motzer RJ, Wilding G, Stadler WM, Garrett M, Pithavala Y, Kim S, Tarazi J, Rini BI: Diastolic blood pressure (dBP) and pharmacokinetics (PK) as predictors of axitinib efficacy in metastatic renal cell cancer (mRCC). J Clin Oncol 2012; 27(suppl; abstr 5045).
29 Rini BI, Grünwald V, Fishman M, Melichar B, Ueda T, Karlov P, Bair A, Chen Y, Kim S, Jonasch E: Axitinib for first-line metastatic renal cell carcinoma (mRCC): overall efficacy and pharmacokinetic (PK) analyses from a randomized phase II study. J Clin Oncol 2012;30(suppl; abstr 4503).
30 Nosov D, Bhargava P, Esteves WB, Strahs AL, Lipatov ON, Lyulko OO, Anischenko AO, Chacko RT, Doval D, Slichenmyer WJ: Final analysis of the phase II randomized discontinuation trial (RDT) of tivozanib (AV-951) versus placebo in patients with renal cell carcinoma (RCC). J Clin Oncol 2011; 29(suppl; abstr 4550).
31 Motzer RJ, Nosov D, Eisen T, Bondarenko IN, Lesovoy V, Lipatov ON, Tomczak P, Lyulko AA, Alyasova A, Harza M, Kogan M, Alexeev B, Sternberg CN, Szczylik C, Zhang J, Strahs AL, Esteves B, Slichenmyer WJ, Berkenblit A, Hutson TE: Tivozanib versus sorafenib as initial targeted therapy for patients with advanced renal cell carcinoma: results from a phase III randomized, open-label, multicenter trial. J Clin Oncol 2012; 30(suppl; abstr 4501).
32 Casanovas O, Hicklin DJ, Bergers G, Hanahan D: Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 2005;8:299–309.
33 Angevin E, Grunwald V, Ravaud A, Castellano DE, Lin CC, Gschwend JE, Harzstark AL, Chang J, Wang Y, Shi MM, Escudier BJ: A phase II study of dovitinib (TKI258), an FGFR- and VEGFR-inhibitor, in patients with advanced or metastatic renal cell cancer (mRCC). J Clin Oncol 2011;29(suppl; abstr 4551).
34 Qian F, Engst S, Yamaguchi K, Yu P, Won KA, Mock L, Lou T, Tan J, Li C, Tam D, Lougheed J, Yakes FM, Bentzien F, Xu W, Zaks T, Wooster R, Greshock J, Joly AH: Inhibition of tumor cell growth, invasion, and metastasis by EXEL-2880 (XL880, GSK1363089), a novel inhibitor of HGF and VEGF receptor tyrosine kinases. Cancer Res 2009;69:8009–8016.
35 Gibney G, Conrad P, Aziz SA, Camp RL, Schwartz BE, Chen C, Kelly WK, Kluger HM: C-met as a therapeutic target using ARQ 197 in renal cell carcinoma. J Clin Oncol 2011;29(suppl 7; abstr 360).
36 Shojaei F, Lee JH, Simmons BH, Wong A, Esparza CO, Plumlee PA, Feng J, Stewart AE, Hu-Lowe DD, Christensen JG: HGF/c-Met acts as an alternative angiogenic pathway in sunitinib-resistant tumors. Cancer Res 2010; 70:10090–10100.
37 You W-K, Sennino B, Williamson CW, Falcón B, Hashizume H, Yao L-C, Aftab DT, McDonald DM: VEGF and c-Met blockade amplify angiogenesis inhibition in pancreatic islet cancer. Cancer Res 2011;71:4758–4768.
38 Smith DC, Smith MR, Small EJ, Sweeney C, Kurzrock R, Gordon MS, Vogelzang NJ, Scheffold C, Ballinger MD, Hussain M: Phase II study of XL184 in a cohort of patients (pts) with castration-resistant prostate cancer (CRPC) and measurable soft tissue disease. J Clin Oncol. 2011;29(suppl 7; abstr 127).
39 Schöffski P, Garcia JA, Stadler WM, Gil T, Jonasch E, Tagawa ST, Smitt M, Yang X, Oliner KS, Anderson A, Zhu M, Kabbinavar F: A phase II study of the efficacy and safety of AMG 102 in patients with metastatic renal cell carcinoma. BJU Int 2011;108:679–686.
40 Rosen PJ, Sweeney CJ, Park DJ, Beaupre DM, Deng H, Leitch IM, Shubhakar P, Zhu M, Oliner KS, Anderson A, Yee LK: A phase Ib study of AMG 102 in combination with bevacizumab or motesanib in patients with advanced solid tumors. Clin Cancer Res 2010; 16:2677–2687.
41 Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJ, Walther MM, Tsui LC, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Storkel S, Lerman MI, Linehan WM, Zbar B: Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 1997;16:68–73.

42 Schmidt L, Junker K, Nakaigawa N, Kinjerski T, Weirich G, Miller M, Lubensky I, Neumann HP, Brauch H, Decker J, Vocke C, Brown JA, Jenkins R, Richard S, Bergerheim U, Gerrard B, Dean M, Linehan WM, Zbar B: Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene 1999;18:2343–2350.

43 Eder JP, Shapiro GI, Appleman LJ, Zhu AX, Miles D, Keer H, Cancilla B, Chu F, Hitchcock-Bryan S, Sherman L, McCallum S, Heath EI, Boerner SA, LoRusso PM: A phase I study of foretinib, a multi-targeted inhibitor of c-Met and vascular endothelial growth factor receptor 2. Clin Cancer Res 2010;16:3507–3516.

44 Srinivasan R, Linehan W, Vaishampayan U, Logan T, Shankar SM, Sherman LJ, Liu Y, Choueiri TK: A phase II study of two dosing regimens of GSK1363089 (GSK089), a dual MET/VEGFR2 inhibitor, in patients (pts) with papillary renal carcinoma (PRC). In J Clin Oncol 2009;27(suppl; abstr 5103).

45 Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, Kay A, Ravaud A: Phase 3 trial of everolimus for metastatic renal cell carcinoma. Cancer 2010;116:4256–4265.

46 Jacinto E, Loewith R, Schmidt A, Lin S, Rüegg MA, Hall A, Hall MN: Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 2004;6:1122–1128.

47 Tamburini J, Chapuis N, Bardet V, Park S, Sujobert P, Willems L, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D: Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways. Blood 2008;111:379–382.

48 Dancey J: mTOR signaling and drug development in cancer. Nat Rev Clin Oncol 2010;7:209–219.

49 Carew JS, Kelly KR, Nawrocki ST: Mechanisms of mTOR inhibitor resistance in cancer therapy. Targ Oncol 2011;6:17–27.

50 Cho DC, Cohen MB, Panka DJ, Collins M, Ghebremichael M, Atkins MB, Signoretti S, Mier JW: The efficacy of the novel dual PI3-kinase/mTOR inhibitor NVP-BEZ235 compared with rapamycin in renal cell carcinoma. Clin Cancer Res 2010;16:3628–3638.

51 Swanton C, Larkin JM, Gerlinger M, Eklund AC, Howell M, Stamp G, Downward J, Gore M, Futreal PA, Escudier B, Andre F, Albiges L, Beuselinck B, Oudard S, Hoffmann J, Gyorffy B, Torrance CJ, Boehme KA, Volkmer H, Toschi L, Nicke B, Beck M, Szallasi Z: Predictive biomarker discovery through the parallel integration of clinical trial and functional genomics datasets. Genome Med 2010;2:53.

52 Flanigan RC, Mickisch G, Sylvester R, Tangen C, Van Poppel H, Crawford ED: Cytoreductive nephrectomy in patients with metastatic renal cancer: a combined analysis. J Urol 2004;171:1071–1076.

53 Escudier B, Kataja V, on behalf of the ESMO Guidelines Working Group: Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21:v137–v139.

54 Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IGH, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ, Global ARCC Trial: Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356:2271–2281.

55 van der Poel H, Roukema J, Horenblas S, Debruyne F: Metastasectomy in renal cell carcinoma: a multicenter retrospective analysis. Eur Urol 1999;35:197–203.

56 Alt AL, Boorjian SA, Lohse CM, Costello BA, Leibovich BC, Blute ML: Survival after complete surgical resection of multiple metastases from renal cell carcinoma. Cancer 2011;117:2873–2882.

57 Karam JA, Rini BI, Varella L, Garcia JA, Dreicer R, Choueiri TK, Jonasch E, Matin SF, Campbell SC, Wood CG, Tannir NM: Metastasectomy After Targeted Therapy in Patients With Advanced Renal Cell Carcinoma. J Urol 2010;185:439–444.

58 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–216.

59 Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM, TARGET Study Group: Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356:125–134.

60 Gore ME, Szczylik C, Porta C, Bracarda S, Bjarnason GA, Oudard S, Hariharan S, Lee S-H, Haanen J, Castellano D, Vrdoljak E, Schöffski P, Mainwaring P, Nieto A, Yuan J, Bukowski R: Safety and efficacy of sunitinib for metastatic renal-cell carcinoma: an expanded-access trial. Lancet Oncol 2009;10:757–763.

61 Ward JE, Stadler WM: Pazopanib in renal cell carcinoma. Clin Cancer Res 2010;16:5923–5927.

62 Fournier LS, Oudard S, Thiam R, Trinquart L, Banu E, Medioni J, Balvay D, Chatellier G, Frija G, Cuenod CA: Metastatic renal carcinoma: evaluation of antiangiogenic therapy with dynamic contrast-enhanced CT. Radiology 2010;256:511–518.

63 Thiam R, Fournier LS, Trinquart L, Medioni J, Chatellier G, Balvay D, Escudier B, Dromain C, Cuenod CA, Oudard S: Optimizing the size variation threshold for the CT evaluation of response in metastatic renal cell carcinoma treated with sunitinib. Ann Oncol 2010;21:936–941.

64 Thiam R, Cuenod C, Fournier L, Lamuraglia M, Medioni J, Barascout B, Scotté F, Fabre-Guillevin E, Oudard S: Determination of a new RECIST threshold using everolimus treatment in metastatic renal cell carcinoma: evaluation from the RECORD-1 study. Ann Oncol 2010;21(suppl; abstr 4147).

65 Lucidarme O, Lamuraglia M, Escudier B, Ravaud A, Rolland F, Chevreau C, Negrier S, Duclos B, K S, Oudard S: Interest of CHOI and modified CHOI criterion for evaluation of mRCC patients treated with Everolimus. Cancer Imaging 2011;11:S41–S42.

66 d'Assignies G, Couvelard A, Bahrami S, Vullierme MP, Hammel P, Hentic O, Sauvanet A, Bedossa P, Ruszniewski P, Vilgrain V: Pancreatic endocrine tumors: tumor blood flow assessed with perfusion CT reflects angiogenesis and correlates with prognostic factors. Radiology 2009;250:407–416.

67 Padhani AR, Neeman M: Challenges for imaging angiogenesis. Br J Radiol 2001;74:886–890.

68 Lamuraglia M, Escudier B, Chami L, Schwartz B, Leclère J, Roche A, Lassau N: To predict progression-free survival and overall survival in metastatic renal cancer treated with sorafenib: pilot study using dynamic contrast-enhanced Doppler ultrasound. Eur J Cancer 2006;42:2472–2479.