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Predictive and prognostic biomarkers of targeted agents and modern immunotherapy in renal cell carcinoma

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ABSTRACT

In the past decade, several agents targeting angiogenesis and signal transduction pathways have replaced the use of cytokines as standard of care treatment for metastatic renal cell carcinoma (RCC) after showing improved clinical benefit and survival. Currently, several novel immunotherapy agents targeting the programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) pathways are being tested in metastatic RCC and are bound to revolutionise the management of this disease. However, the success of both antiangiogenic drugs and new immunotherapy agents still depends on our ability to select patients most likely to respond to treatment. This article will review the current available evidence on prognostic and predictive biomarkers of response to signal transduction pathways-targeted agents and modern immunotherapy in metastatic RCC.

INTRODUCTION

Prior to the advent of antiangiogenic drugs and tyrosine-kinase inhibitors (TKIs), immunotherapy with cytokines such as interferon-α (IFN-α) and interleukin 2 (IL-2) was considered standard of care treatment for metastatic renal cell carcinoma (RCC). In the past decade, several agents targeting angiogenesis and signal transduction pathways such as pazopanib, sunitinib, temsirolimus, axitinib or everolimus have replaced the use of cytokines after showing improved clinical benefit and survival in randomised prospective clinical trials.[1–5] However, TKIs rarely cause durable tumour regressions and most patients will ultimately experience disease progression despite an initial period of response. For decades, the experience with high-dose IL-2 served as proof of concept that immunotherapy can result in durable complete responses in a small subgroup of patients with metastatic RCC. This has led to the development of novel immunotherapy agents such as programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) inhibitors and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) inhibitors, which are currently being tested in metastatic RCC and several other solid tumours. However, the success of TKIs and new immunotherapy agents still depends on our ability to select patients most likely to respond to treatment and prevent unnecessary toxicity and cost in those unlikely to benefit. For that reason, remarkable efforts are being made to identify biomarkers that may predict response to these new agents. This article will review the current available evidence on prognostic and predictive biomarkers of response to signal transduction pathways-targeted agents and modern immunotherapy in metastatic clear cell RCC.

PREDICTIVE AND PROGNOSTIC BIOMARKERS OF SIGNAL TRANSDUCTION PATHWAYS-TARGETED AGENTS

Clear cell RCC is intrinsically highly resistant to conventional cytotoxic agents. As a result, research on possible treatments of this disease has focused on histopathological and genetic abnormalities that might serve as targets for treatment other than the process of mitosis. It has been well established that RCC are hypervascularised tumours with an abundance of abnormal blood vessels, which makes angiogenesis an attractive target for treatment.

In the past decade, studies have demonstrated that a loss in the Von Hippel-Lindau (*VHL*) tumour suppressor gene, on chromosome 3p25, plays a pivotal role in this process of angiogenesis in RCC.[6] In normal healthy tissue, *VHL* causes proteolysis of hypoxia inducible factor 1 α (HIF-1α), but in RCC a lack of *VHL* leads to an increase in HIF-1α,[7] which consequently leads to an increased transcription of genes involved in angiogenesis and tumourigenesis, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). In addition to the increased transcription of

growth factor genes, *VHL* loss also leads to the direct activation of the phosphatidylinositol 3 kinase (PI3-K)/AKT/mammalian target of rapamycin (mTOR) pathway, a signalling transduction pathway that promotes tumour survival and growth.[8] These insights into the pathogenesis of RCC have led to the development of several drugs with proven clinical benefit, such as sunitinib, sorafenib, axitinib and pazopanib, which preferentially target the VEGF pathway, and temsirolimus and everolimus, which act as mTOR inhibitors.

On the basis of their mode of action, namely targeting angiogenesis, predictive biomarkers of response could be linked to the alterations these drugs cause in soluble angiogenic factors (ie, soluble VEGF, angiopoietins) or transcript levels of the targeted genes. With regard to the mTOR inhibitors, genetic abnormalities in this pathway may serve as biomarkers. Alternatively, baseline patient characteristics or treatment-induced changes in clinical parameters could provide clinicians with important tools for treatment selection and modification.

Prognostic and predictive biomarkers of response to angiogenesis inhibitors

Tumour angiogenesis is mainly driven by VEGF, a potent endothelial cell mitogen. The VEGF family comprises multiple isoforms, produced by alternative splicing from an eight-exon VEGF gene.[9] Three receptors for VEGF have been identified, namely VEGF receptors (VEGFR) 1 and 3. VEGFR1 and VEGFR2 are expressed on endothelial cells, whereas expression of VEGFR3 is limited to lymphangiocytes. The VEGF/epidermal growth factor (EGF)-R2 interaction has mainly been shown to play a pivotal role in tumour angiogenesis. On stimulation of VEGFR2, intracellular tyrosine-kinase residues become phosphorylated, resulting in the downstream activation of protein kinase C, RAS and ERK, as well as PI3-K/AKT/mTOR, ultimately leading to endothelial proliferation.[10]

Regulation of $VEGF_A$ and VEGFR2 is complex, and a large number of contributing factors have been identified. Various cytokines such as tumour necrosis factor α (TNF-α), transforming growth factor β (TGF-β) and EGF have been shown to modify both $VEGF_A$ and VEGFR2 transcription; however, the most important regulator in RCC appears to be HIF-1α, as mentioned earlier. Several VEGF pathway inhibitors have been approved for the treatment of metastatic RCC, including sunitinib,[2] bevacizumab,[11] pazopanib[1] and axitinib.[4] In the search for predictive and prognostic biomarkers for VEGF-targeting compounds, a variety of markers have been explored. Numerous clinical and molecular markers, including carbonic anhydrase-9, VEGF and HIF, have been investigated as potential prognostic and predictive biomarkers. So far, only the Memorial Sloan Kettering Cancer Center (MSKCC) and the Heng prognostic models have been validated as prognostic tools and are included in the most relevant international guidelines such as the European Association of Urology guidelines on RCC[12] and the National Comprehensive Cancer Network Clinical Practice Guidelines in Kidney Cancer.[13] However, no molecular marker has so far been shown to improve the prognostic accuracy of these prognostic scores, and their use is therefore not recommended in routine practice.

Clinical-related biomarkers

In 2009, Heng *et al*[14] published a landmark retrospective study looking at baseline features predictive of survival in untreated patients with metastatic RCC treated in seven American and Canadian cancer centres. Overall, 645 patients were included, of whom 396, 200 and 49 patients were treated with sunitinib, sorafenib and bevacizumab, respectively. Pretreatment factors independently associated with a shorter overall survival (OS) on multivariate analysis were: haemoglobin < the lower limit of normal (p<0.0001), corrected calcium > the upper limit of normal (ULN; p=0.0006), Karnofsky performance status (PS) <80% (p<0.0001), time from diagnosis to treatment <1 year (p=0.01), neutrophils>ULN (p<0.0001) and platelets>ULN (p=0.01).[14] Patients were classified into three risk categories: the favourable-risk group (no prognostic factors; n=133), in which median OS was not reached and 2-year OS was 75%; the intermediate-risk group (1–2 prognostic factors; n=301), in which median OS was 27 months and 2-year OS was 53%; and the poor-risk group (3–6 prognostic factors; n=152), in which median OS was 8.8 months and 2-year OS was 7% (log-rank p<0.0001).[14] This prognostic model, called the International Metastatic RCC Database Consortium (IMRDC, also called the Heng criteria), was the first to validate the use of the components of the MSKCC[15] prognostic model previously described for cytokines with the addition of platelet and neutrophil counts in patients treated with VEGF pathway inhibitors. The prognostic role of the IMRDC model was then externally validated in a cohort of 1028 patients treated worldwide with sunitinib, sorafenib, pazopanib, axitinib or bevacizumab, enabling us to stratify patients by risk in future clinical trials and to counsel patients about prognosis.[16]

Mechanism-based adverse events (AEs)

Mechanism-based AEs are treatment-related toxicity events that indicate on-target effects of a targeted agent and its inhibition of a given pathway. Several clinical side effects of VEGFR inhibitors such as hypertension and hypothyroidism have been investigated as potential predictive biomarkers of treatment efficacy.

Although side effects between the VEGF targeting agents differ, several class-effect toxicities are frequent for all approved drugs and usually occur within the first weeks of treatment. Hypertension developing during treatment with sunitinib has been reported to be both a predictive marker of response and a prognostic factor of improved survival. The first evidence that treatment-related hypertension was related to improved

outcome in metastatic RCC was described with the anti-VEGF monoclonal antibody bevacizumab.[17] Forty-three consecutive patients with metastatic RCC were treated with bevacizumab and their blood pressures were monitored. Interestingly, patients who developed hypertension had a higher response rate (RR) to treatment (p=0.005) and a longer median time-to-disease progression (8.1 months, 95% CI 5.3 to 11.3, vs 4.2 months, 95% CI 2.6 to 5.6, p=0.036).[17] These results were then validated in the phase III trial of bevacizumab plus IFN-α versus IFN-α alone in patients with metastatic RCC. Patients on bevacizumab plus IFN-α who developed grade 2 hypertension had a significantly greater progression-free survival (PFS, 13.2 months, 95% CI 10.6 to 15.5, vs 8.0 months, 95% CI 5.9 to 8.6, p=0.001) and OS (41.6 months, 95% CI 26.3 to 55.1 vs 16.2 months, 95% CI 14.2 to 18.7, p=0.001) compared with patients who did not develop hypertension.[11]

Rini *et al*[18] investigated the relationship between hypertension and sunitinib antitumour efficacy in a retrospective analysis including pooled data from 544 patients with metastatic RCC in the four prospective multinational clinical trials. Similarly, the appearance of treatment-induced hypertension defined by an increase to 140 mm Hg or higher of systolic pressure was related to better outcomes than those without hypertension: objective RR 54.8% vs 8.7%; median PFS 12.5 months, 95% CI 10.9 to 13.7 vs 2.5 months, 95% CI 2.3 to 3.8; and median OS 30.9 months, 95% CI 27.9 to 33.7 vs 7.2 months, 95% CI 5.6 to 10.7, p<0.001 for all).[18] Similar data have been found for axitinib,[19 20] sorafenib[19] and tivozanib,[21] but for pazopanib no data are yet available. In the phase III trial comparing the efficacy of axitinib versus sorafenib as second-line therapy for metastatic RCC, the occurrence of a diastolic blood pressure of ≥90 mm Hg was related to a longer median OS: 20.7 months (95% CI 18.4 to 24.6) versus 12.9 months (95% CI 10.1 to 20.4) in the axitinib group (p=0.01), and 20.2 months (95% CI 17.1 to 32.0) versus 14.8 months (95% CI 12.0 to 17.7) in the sorafenib group (p=0.002).[19]

Regarding treatment-induced hypothyroidism, the results are conflicting. Several small and often exploratory trials have suggested a potential predictive role of hypothyroidism, especially with sunitinib and sorafenib. To better understand the relationship between TKI-induced hypothyroidism and treatment efficacy, Nearchou *et al*[22] performed a meta-analysis of 11 retrospective and prospective studies encompassing 500 patients treated with sunitinib or sorafenib for metastatic RCC.[22] No statistically significant difference in PFS was observed between patients with acquired hypothyroidism during sunitinib and those without hypothyroidism (HR 0.82; 95% CI 0.59 to 1.13, p=0.22; 6 studies, 250 patients). For studies that included patients treated with either sunitinib or sorafenib, PFS was significantly longer in patients with acquired hypothyroidism (HR 0.59; 95% CI 0.42 to 0.84, p=0.003; 3 studies; 205 patients). Similarly, OS was longer in patients who developed hypothyroidism during sunitinib therapy compared with patients who did not (HR 0.52, 95% CI 0.31 to 0.87, p=0.01; 4 studies, 147 patients). However, these data should be interpreted with caution since the results were mostly influenced by a positive association by the retrospective studies.[22]

Other mechanism-based AEs secondary to VEGRF inhibitors have been studied as potential biomarkers of treatment efficacy. In an updated analysis of the pooled data from four prospective trials with sunitinib mentioned earlier, hand-foot syndrome (HFS), fatigue, neutropenia and thrombocytopenia were analysed.[23] Importantly, neutropenia was significantly associated with longer PFS and OS (p=0.013 and p=0.0122, respectively), and HFS with longer OS (p=0.0218). In a multivariate analysis, hypertension, neutropenia and HFS remained as independent statistically significant prognostic factors of OS.[23]

Circulating cytokines and angiogenic factors (CAFs)

Given the ease of its detection, serum levels of circulating CAFs are one of the most tested biomarkers with VEGF pathway inhibitors. One of these trials that addressed biomarkers was a randomised, double-blind, placebo-controlled phase III trial of sorafenib as second-line treatment in metastatic clear cell RCC, the TARGET trial.[24] Here, plasma samples were collected from all 903 eligible patients during screening. A preplanned multivariate analysis, including baseline VEGF levels, Eastern Oncology Cooperative Group (ECOG) PS 0 vs 1 or 2 and MSKCC score (low vs intermediate), demonstrated that higher than median VEGF concentrations (131 pg/mL) were an independent prognostic factor for OS (p=0.0145) but not PFS (p=0.625) in patients treated with sorafenib. In addition, an exploratory predictive analysis was performed using the 75th centile (254 pg/mL) to differentiate between $VEGF_{low}$ and $VEGF_{high}$ groups. Using this stratification, the authors found an increased benefit in terms of PFS for $VEGF_{high}$ patients versus $VEGF_{low}$ patients treated with sorafenib (HR 0.27, 95% CI 0.15 to 0.460 vs HR 0.58, 95% CI 0.43 to 0.78, p=0.020).

Zurita *et al* conducted a CAF profiling analysis in 69 patients with metastatic RCC treated in a randomised study of sorafenib alone or sorafenib with IFN-α. Several CAFs were assessed at baseline and on treatment, including interleukins, macrophage colony-stimulating factor-1 (M-CSF), E-selectin, EGF, TGF-β, osteopontin, carbonic anhydrase-9, $VEGF_A$ and soluble VEGFR2. On univariate analyses, 14 of these factors correlated with PFS. However, on multivariate analysis, only IL-5, M-CSF and EGF showed independent prognostic value.[25] The authors also searched for markers that identified groups of patients who experienced different degrees of benefit from sorafenib versus sorafenib+IFN-α. The only significant treatment-by-factor interactions for the 52 baseline CAFs analysed were for osteopontin and VEGF (p for

interaction 0.004 and 0.01, respectively) where low expression of either biomarker predicted superior PFS with sorafenib plus IFN-α as compared with sorafenib alone.[25]

The largest evaluation of the CAF profile published so far was performed with data from the phase II and III clinical trials of pazopanib in metastatic RCC.[26] The authors used a three-step approach for screening, confirmation and validation of prospective CAF biomarkers. Initially, potential CAFs were screened in 129 patients who had the greatest or least tumour shrinkage in the phase II trial of 215 patients treated with pazopanib. The candidate CAFs positively related to tumour response and PFS identified from this screening were then confirmed with an independent analytical platform in the whole phase II trial population (215 patients). Confirmatory analyses identified associations of low levels of IL-6, IL-8, osteopontin and hepatocyte growth factor (HGF) with continuous tumour shrinkage and PFS in patients treated with pazopanib ($p<0.05$ for all). These markers were then validated in 344 patients from a randomised, placebo-controlled, phase III clinical study of pazopanib. In the validation set, it was confirmed that patients treated with pazopanib who had high concentrations of IL-8 ($p=0.006$), osteopontin ($p=0.0004$), HGF ($p=0.010$) and TIMP-1 ($p=0.006$) had shorter PFS than did those with low concentrations. These factors were stronger prognostic markers than were standard clinical classifications such as ECOG PS, MSKCC model and Heng criteria).[26]

Single-nucleotide polymorphisms (SNPs)

Several studies have explored SNP of factors along the VEGF pathway. Lambrechts *et al* assessed the correlation of SNPs in the VEGF pathway with PFS and OS in patients treated with bevacizumab in the AVOREN trial. Exploration of a total of 138 SNPs revealed variants in VEGFR1 that were predictive of poor PFS with bevacizumab (HR 1.81, 95% CI 1.08 to 3.05, p=0.033) but not of OS (HR 0.91, 95% CI 0.45 to 1.82, p=0.78). Specifically, an SNP at rs7993418 located in the tyrosine-kinase domain of VEGFR1 was implicated.[27]

SNPs in the VEGF pathway and related factors have also been analysed with the use of pazopanib therapy. A panel of 27 functional SNPs within 13 genes were evaluated in 397 patients with RCC treated with pazopanib across three clinical trials. Three SNPs in IL-8 and HIF-1α and five SNPs in HIF-1α, NR1I2 and $VEGF_A$ showed a significant association with PFS and RR ($p\leq0.05$), respectively. For instance, RR were reduced in patients with the $VEGF_A$ 1498CC genotype as compared with the 1498TT genotype (33% vs 51%, $p\leq0.05$).[28]

In a study by Kim *et al*, a panel of candidate VEGF and VEGFR2 SNPs were evaluated for associations with clinical outcome in 63 patients receiving sunitinib for metastatic RCC. No single VEGF or VEGFR SNPs were found to correlate with clinical outcome. However, the combination of VEGF SNP 936 and VEGFR2 SNP 889 was associated with OS after adjustment for the prognostic risk group ($p=0.03$).[29] A similar recent study comparing two widely used dosing schedules of sunitinib (50 mg once daily 4-week-on/2-week-off, vs 37.5 mg once daily continuous dosing), examined germline SNPs in $VEGF_A$ and VEGFR3 genes.[30] Again, no association could be detected between the presence of SNPs and time-to-progression, PFS or OS. A prognostic value for the angiogenic protein angiopoietin-2 was established, however.

Similar negative results have been described with axitinib and sorafenib. In the phase III trial comparing second-line axitinib versus sorafenib in patients with previously treated metastatic RCC, analyses of associations between germline SNPs and outcomes were conducted. Fifteen SNPs in $VEGF_A$, VEGFR1, VEGFR2 and HIF-1α were analysed. In axitinib-treated patients, $VEGF_A$ rs699947 and rs833061 correlated with longer OS (27.0 vs 13.4 months, HR 0.39, p=0.015). In sorafenib-treated patients, VEGFR2 rs2071559 was associated with longer OS (26.8 vs 13.8 months; HR 0.41, p=0.030). However, on multivariate analyses, no SNP predicted axitinib efficacy, but VEGFR2 rs2071559 predicted PFS ($p=0.0053$) and OS ($p=0.0027$) for sorafenib. The authors conclude that sensitivity/specificity limitations in the study preclude the use of VEGFR2 rs2071559 for selecting individual patients for sorafenib treatment.[31]

Other genetic and analytical factors

Since the vast majority of RCC are driven by a mutation in the *VHL* gene, Choueiri *et al*[32] examined whether or not the *VHL* mutation status predicted response in 123 patients with metastatic RCC treated with sunitinib, axitinib, sorafenib or bevacizumab. In a multivariate analysis, patients with wild-type *VHL* demonstrated an RR of 31%, whereas patients with a loss of function mutation in *VHL* (frame shift, nonsense, splice or in-frame deletions/insertions, but not missense mutations), demonstrated a response of 52% ($p=0.04$). Remarkably, these data have not been confirmed in subsequent trials. Tumour samples obtained in a phase II trial that assessed the safety and efficacy of pazopanib in locally recurrent or metastatic RCC (the VEG102616 trial) were examined for *VHL* status. Here, no correlation between any of the markers and overall RR or PFS could be established.[33] Similar data were found for patients treated with sorafenib in a large phase III trial.[34] Unfortunately, VHL mutational status was only available for 134/712 patients in this trial. Conclusive trials establishing the predictive value for VHL are still awaited.

In parallel to the VHL status, several studies have examined the predictive value of expression levels of HIF-1α. In the study by Choueiri *et al*[33] mentioned earlier, expression levels of HIF-1α were predictive of response to pazopanib. In a study examining several circulating proteins as a biomarker for efficacy in first-line treatment with sunitinib, baseline expression levels of HIF-1α, as determined by immunohistochemistry (IHC)

low versus high, did prove to be of prognostic significance.[30] Patients whose tumours expressed high levels of HIF-1α at baseline had a significantly longer PFS of 42.0, weeks (95% CI 31.0 to 56.3) when compared with patients with low levels of expression, who displayed a PFS of 30.4 weeks (95% CI 22.2 to 43.9; HR 1.55, p=0.034).

Few studies have analysed the potential role of circulating cell-free DNA (cfDNA) as predictive biomarkers to antiangiogenic drugs. cfDNA is thought to originate from tumours, through necrosis, apoptosis or cell lysis of tumour cells, and could represent an easily accessible and non-invasive biomarker.[35] Feng *et al*[36] conducted a study to determine whether plasma circulating cfDNA levels could predict treatment efficacy in patients with metastatic RCC treated with sorafenib. Eighteen patients with treatment-naïve or cytokines-only treated metastatic RCC were selected. Patients received 400 mg of sorafenib orally twice daily and were followed up weekly. CT scans were performed at baseline, with follow-up scans obtained at 6-week intervals for the first 24 weeks (or until disease progression), and every 8 weeks thereafter. Plasma cfDNA levels were quantified by quantitative real-time PCR at six different time points: before treatment, 4, 8, 12, 16 and 24 weeks. Treatment response was assessed as per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria. Interestingly, baseline cfDNA levels could not predict remission, stable disease and progression (p=0.27, p=0.073, respectively). However, a decrease in cfDNA levels was observed in patients with partial response to sorafenib, whereas the levels increased in patients with stable disease or progressive disease. Moreover, a significantly lower plasma cfDNA level, measured from 8 to 24 weeks, was found in patients with remission or stable disease than in those with progression (p<0.05). Increasing levels of plasma cfDNA during the course of treatment indicated a poor outcome. For predicting progression, cfDNA levels at 8 weeks yielded a sensitivity of 66.7% and a specificity of 100%. The authors conclude that cfDNA may play a potential role in monitoring the efficacy of sorafenib treatment.[36]

Prognostic and predictive biomarkers of response to mTOR inhibitors

The PI3-K/AKT/mTOR pathway plays an important role in the pathogenesis of many tumours. In RCC, the loss of the tumour suppressor gene *VHL* leads to the direct activation of the PI3-K/AKT/mTOR pathway.[37] Furthermore, the mTOR pathway controls the synthesis of key proteins needed for tumour and endothelial cell proliferation.[38] Parallel to VEGF, mTOR is strongly associated with HIF-1α. This is illustrated by the detection of decreased HIF-1α transcription induced by administering rapamycin.[39] The importance of the mTOR pathway in RCC has led to the development of everolimus and temsirolimus. Both drugs are allosteric inhibitors of the mTOR complex 1, one of two main multiprotein complexes that nucleates mTOR, and have demonstrated a clear benefit in patients with metastatic RCC.[3 5] In comparison to the VEGFR inhibitors, there are currently very limited data regarding potential predictors of clinical efficacy to mTOR inhibitors.

Mechanism-based AEs

Several mTOR inhibitors-related mechanism-based AEs have been analysed as potential predictive and prognostic biomarkers. Non-infectious pneumonitis (NIP) is a class effect of mTOR inhibitors and has been described with everolimus and temsirolimus. White *et al* were the first to assess the relationship between NIP and outcome in 274 patients treated with everolimus. NIP was diagnosed in 13.5% of patients, but no differences in PFS were seen between patients with radiographic findings consistent with pneumonitis and patients without NIP (p=0.96).[40] However, in another retrospective study of 46 patients treated with either everolimus or temsirolimus, the occurrence of NIP (30%) was related to an increased response of target lesions by RECIST criteria (mean change of tumour long axis size: −2.9% vs +4.3%, p=0.002).[41] In the largest study published hitherto, Atkinson *et al* addressed the role of NIP as a potential biomarker of mTOR inhibitors. In a retrospective review of 310 patients with metastatic RCC treated with temsirolimus or everolimus, NIP occurred in 6% of temsirolimus-treated patients and 23% of everolimus-treated patients. Interestingly, patients who developed NIP had a significantly longer median time on treatment (4.1 months, 95% CI 2.53 to 6.97 vs 2 months, 95% CI 1.84 to 2.37, p=0.035) and longer median OS (15.4 months, 95% CI 11.51 to not reached vs 7.4 months, 95% CI 5.99 to 9.54, p<0.001). On multivariate analysis, NIP remained an independent predictor of improved OS (HR 0.32, 95% CI 0.2 to 0.5, p<0.001).[42]

Few small studies have looked at the predictive role of increases in serum cholesterol, triglyceride and glucose, a common AE with mTOR inhibitors. Lee *et al* examined serum metabolic changes in a phase III trial in which patients with metastatic RCC with an intermediate-risk or poor-risk classification were randomised to temsirolimus or IFN-α. Greater increases in serum cholesterol levels from baseline in patients treated with temsirolimus were significantly associated with longer PFS (HR 0.81, p<0.0001) and OS (HR 0.77, p<0.0001).[43] Interestingly, temsirolimus-related hypertriglyceridaemia and hyperglycaemia were not associated with improved clinical outcomes.

Lactate dehydrogenase (LDH)

LDH is an enzyme involved in anaerobic glycolysis and regulated by the PI3-K/AKT/mTOR containing complex 1 pathway as well as tumour hypoxia/necrosis,[44] which provides a rationale for its study as a potential biomarker of mTOR inhibition. High serum LDH levels are associated with poor prognosis in patients with metastatic RCC and in several other tumours.[15] Increased LDH had previously been incorporated as a negative factor in the MSKCC prognostic model but was then

dropped from the Heng prognostic model. Nevertheless, neither of these prognostic models have been validated with the use of mTOR inhibitors.

Armstrong *et al* evaluated pretreatment and post-treatment serum LDH in 404 poor-risk patients with RCC treated with temsirolimus or INF-α in a phase III randomised trial. On multivariate analysis, elevated LDH was independently associated with an increased risk of death (HR 2.81, 95% CI 2.01 to 3.94, p<0.001). Temsirolimus was found to significantly improve OS in those patients with an elevated LDH (6.9 vs 4.2 months, p<0.002) as compared with INF-α. Conversely, temsirolimus did not prolong OS in those patients with a normal LDH (11.7 vs 10.4 months, p=0.514). The authors conclude that serum LDH is a prognostic and a predictive biomarker of OS in poor-risk patients with RCC treated with temsirolimus.[44]

PREDICTIVE AND PROGNOSTIC BIOMARKERS OF MODERN IMMUNOTHERAPY AGENTS

The ideal immunological biomarker is one that can be measured easily from body fluids, such as blood; is quantitative, allowing patient stratification based on magnitude of response; and reflects the mechanism of action of the agent studied or the direct effect of immunity on cancer.[45] Biomarkers of immunotherapy may include baseline tumour profiling (gene expression, immunophenotyping and localisation of immune infiltrates, cancer genetics), baseline patient's genetic profile, baseline peripheral blood markers (gene expression, composition of immune cells, proteomics, soluble factors), and on-treatment changes in peripheral blood and tumour microenvironment (gene expression, composition of immune cells, proteomics, receptor occupancy). Nevertheless, identifying biomarkers of cancer immunotherapy is challenging given the dynamic adaptative nature of immunity, the multiple immune checkpoints involved in T cell regulation, and the molecular heterogeneity of the tumour and immune compartment.

In the cytokines era, few clinical and laboratory factors were identified and prospectively validated as being predictive or prognostic for IFN-α and IL-2. These included clear cell histology,[46 47] ECOG PS,[48 49] MSKCC prognostic group,[46 48 50 51] number of metastatic sites,[46 49 50 52] C reactive protein[49 52] and neutrophils levels.[49 51 52] However, many of these factors derived from small retrospective studies which provided inconsistent and sometimes contradictory data and failed to efficiently identify all responders. Nevertheless, these factors are relevant as they offer an insight into potential biomarkers for modern immunotherapy such as anti-PD-1/PD-L1 agents and CTLA-4 inhibitors.

Prognostic and predictive biomarkers of response to PD-1 and PD-L1 inhibitors

PD-1 is a key immune checkpoint molecule implicated in T cell-mediated immunosuppression and immune tolerance. PD-1 is physiologically expressed in monocytes, activated T cells and B cells. PD-1 binds to two ligands, PD-L1 and PD-L2. Its major ligand PD-L1, also known as B7-H1, is mainly expressed by haematopoietic cells, including T and B cells, macrophages and dendritic cells, and by vascular endothelial cells. The interaction between PD-1 and PD-L1 negatively regulates activated T cell effector functions and leads to immune suppression and tolerance. PD-L1 is also aberrantly expressed in several solid tumours, including RCC, as an adaptative mechanism which promotes an immunosuppressive tumour microenvironment, ultimately leading to tumour immune tolerance. Consequently, novel immune modulating agents, such as antibodies blocking PD-1 and PD-L1 have the potential to enhance the host own immune response and trigger antitumour immunity.

PD-L1 is overexpressed in up to 30% of RCC tumours, in primary and metastatic sites of disease.[53 54] Moreover, PD-L1 overexpression has been demonstrated to correlate with advanced tumour stage, higher Fuhrman grade, the presence of necrosis, sarcomatoid differentiation and poorer survival in several case series, suggesting a prognostic role of PD-L1 expression.[53 55–57] This is probably due to its immunosuppressive role. Recently, a subanalysis from the randomised COMPARZ study comparing sunitinib and pazopanib as a first-line therapy for patients with metastatic RCC showed that increased PD-L1 expression in tumour cells is associated with a significantly shorter OS, further supporting the role as a prognostic biomarker of PD-L1 expression.[58] Consequently, various PD-1 and PD-L1 inhibitors are currently being tested in RCC and are at different stages of clinical development. Most of these ongoing clinical trials include an exploratory biomarker subanalysis to try to identify predictive biomarkers of response to PD-1/PD-L1 inhibition such as PD-L1 expression and characterisation of tumour-infiltrating immune cells (TIIC). However, their role as potential predictive biomarkers of benefit to the PD-1/PD-L1 blockade remains controversial and is still under investigation in the ongoing clinical trials.

PD-L1 expression by tumour cells or TIIC

Expression of PD-L1 by tumour cells or TIIC is currently the most studied factor as a potential predictive biomarker of response to PD-1/PD-L1 blockade. The current available evidence on PD-L1 expression as a biomarker comes mainly from exploratory analysis in phase I and II trials and has not yet been validated in a prospective randomised fashion. Therefore, it should be treated as hypothesis generating. Table 1 summarises the available evidence on PD-L1 expression as a predictive biomarker of PD-1/PD-L1 blockade in RCC.

The first-in-human dose escalation phase I trial of the PD-1 inhibitor nivolumab in solid malignancies was the first study to assess the relationship between pretreatment PD-L1 immunohistochemical expression in

Table 1 Selected studies on the role of PD-L1 expression as a predictive biomarker of response to PD-1/PD-L1 inhibitors in clinical trials

Phase	Description	Clinicaltrials.gov	Immunohistochemical cut-off point	PD-L1 status	RR (%)	Median OS (months, 95% CI)
Phase I*	Nivolumab in refractory solid tumours[59]	NCT00441337	≥5% of tumour cells membrane staining	+	3/4 (75)†	
				–	0/5 (0)	
Phase I*	Nivolumab in advanced solid tumours[60]	NCT00730639	≥5% of tumour cells membrane staining	+	9/25 (36)‡	
				–	1/17 (0)	
Phase I	Nivolumab in metastatic RCC[62 63]	NCT01358721	≥5% of tumour cells membrane staining	+	4/18 (22)	NR
				–	3/38 (8)	23.4 (13.1 to 33.3)
Phase II	Nivolumab in metastatic RCC[64 65]	NCT01354431	≥5% of tumour cells membrane staining	+	9/29 (31)	29.9 (13.4 to NR)
				–	14/78 (18)	18.2 (12.7 to 27.2)
Phase I	Nivolumab plus ipilimumab in metastatic RCC[67]	NCT01472081	≥1% of tumour cells membrane staining	+	8/16 (50)	
				–	11/20 (55)	
			≥5% of tumour cells membrane staining	+	1/4 (25)	
				–	18/32 (56.3)	
Phase I	Nivolumab plus sunitinib metastatic RCC[68]	NCT01472081	≥1% of tumour cells membrane staining	+	6/15 (40)	
				–	9/14 (63.3)	
			≥5% of tumour cells membrane staining	+	1/5 (20)	
				–	14/24 (58.3)	
Phase I	Nivolumab plus pazopanib in metastatic RCC[68]	NCT01472081	≥1% of tumour cells membrane staining	+	3/7 (42.9)	
				–	5/10 (50)	
			≥5% of tumour cells membrane staining	+	1/2 (50)	
				–	7/15 (46.7)	
Phase I	Atezolizumab in metastatic RCC[69]	NCT01375842	≥1% of TIIC membrane staining	+	7/35 (20)§	
				–	2/21 (10)§	
Phase III	Nivolumab vs everolimus in metastatic RCC[66]	NCT01668784	≥1% of tumour cells membrane staining	+	94/370 (25)§	21.8 (16.5 to 28.1)
				–	276/370 (75)§	27.4 (21.4 to NR)
			≥5% of tumour cells membrane staining	+	44/370 (11)§	21.9 (14.0 to NR)
				–	326/370 (89)§	24.6 (21.4 to NR)

*Including metastatic melanoma, colorectal cancer, castrate-resistant prostate cancer, non-small cell lung cancer and/or RCC.
†Including one RCC case among responders.
‡Including two RCC cases among responders.
§Nivolumab arm only.
NR, not reached; OS, overall survival; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; RCC, renal cell carcinoma; RR, response rate; TIIC, tumour infiltrating immune cells.

tumour cells and response to treatment.[59] Tumours were considered PD-L1 positive if >5% of tumour cells showed positive membrane staining. Three of the four PD-L1-positive patients (including 1 RCC case) were associated with objective response, whereas none of the five patients with PD-L1-negative tumours showed tumour regression (p=0.04). In another phase I trial of nivolumab in advanced solid tumours, 9 of the 25 PD-L1-positive tumours (including two RCC cases) experienced objective response but none of the 17 PD-L1-negative tumours patients showed tumour regression (p=0.006).[60] PD-L1 expression by tumour cells and TIIC was assessed for the first time in a biomarker subanalysis of the latter phase I trial.[61] Importantly, PD-L1 expression in tumour cells (n=41) was significantly correlated with increased objective RR and with clinical benefit (defined as an objective response or a stable disease lasting at least 6 months) (p=0.02 and p=0.005, respectively). Conversely, PD-L1 expression in infiltrating immune cells (n=41) was found to significantly correlate with clinical benefit (p=0.03) but not with RR (p=0.14).

In the first RCC-specific phase I trial of nivolumab, 91 patients with previously treated and untreated metastatic RCC received nivolumab at three possible dosages (0.3, 2 or 10 mg/kg).[62] Interestingly, RR was significantly higher in PD-L1-positive tumours (22%, 4/18) than in PD-L1-negative tumour (8%, 3/38), suggesting a potential role of PD-L1 expression as a predictive biomarker.[62] However, this study showed, for the first time, that PD-L1-negative tumours can also derive clinical benefit from an anti-PD-1 blockade, challenging the use of PD-L1 expression as sole inclusion criteria for future prospective randomised trials. In an updated analysis of this study, median OS and 2 years OS rate were significantly longer in the PD-L1-positive tumour group than in the PD-L1-negative group (not reached vs 23.4 months, 95% CI 13.1 to 33.3, p not given and 64%

(95% CI 37% to 82%) vs 48% (95% CI 30% to 64%), p not given, respectively).[63]

In the randomised blinded phase II trial of nivolumab, 168 patients with previously treated metastatic RCC were randomised to receive nivolumab at 0.3, 2 or 10 mg/kg. As an exploratory end point, efficacy parameters were assessed according to PD-L1 expression status. PD-L1 expression was measured in archival tumour tissue by IHC and positivity was defined by membrane staining of ≥5% of tumour cells. A cut-off of ≥1% was also assessed.[64] Median OS, median PFS and RR were similar across the three different dosages. Using the ≥5% cut-off, 29 of the 107 evaluable patients were considered PD-L1 positive (27%) and 78 (73%) were PD-L1 negative. Median PFS was 4.9 months (95% CI 1.4 to 7.8) in the PD-L1-positive group versus 2.9 months (95% CI 2.1 to 4.2) in the PD-L1-negative group (p not shown). RR was higher in the PD-L1-positive group than in the PD-L1-negative group (31% vs 18%, p not shown). Median OS was 29.9 months (95% CI 13.4 to not reached) in the PD-L1-positive group and 18.2 months (95% CI 12.7 to 27.2) in the PD-L1-negative group (p not shown).[65] When a cut-off of ≥1% was used to define PD-L1 positivity, median PFS, RR and OS were similar in both groups.[64]

Motzer *et al*[66] recently published the first phase III randomised trial of nivolumab in metastatic RCC. The study randomised 821 patients with metastatic RCC previously treated with one or two regimens of antiangiogenic therapies to receive 3 mg/kg of nivolumab or 10 mg/day of everolimus. Membrane PD-L1 tumour expression was included as a secondary end point and was assessed in tumour cells using two different exploratory cut-offs (1% and 5%). Among all the randomised patients, 92% had quantifiable tumour PD-L1 expression in pretreatment samples (756/821). In total, 24% of patients with quantifiable PD-L1 expression (181/756) had ≥1% PD-L1 expression, whereas 76% were considered PD-L1 negative (575/756). Among patients with ≥1% PD-L1 expression, the median OS was 21.8 months (95% CI 16.5 to 28.1) in the nivolumab arm and 18.8 months (95% CI 11.9 to 19.9) in the everolimus arm (HR 0.79, 95% CI 0.53 to 1.17, p not shown). Among patients with <1% PD-L1 expression, the median OS was 27.4 months (95% CI 21.4 to not estimable) in the nivolumab arm and 21.2 months (95% CI 17.7 to 26.2) in the everolimus arm (HR 0.77; 95% CI 0.60 to 0.97, p not shown). Similar results were observed using the 5% PD-L1 expression cut-off point. However, the interpretation of these data is limited by the small numbers of patients with 5% or greater expression (85/756).[66] A benefit with nivolumab over everolimus was therefore seen regardless of PD-L1 expression. Interestingly, median OS was consistently lower in the PD-L1-positive group irrespective of the treatment arm. These findings support the negative prognostic role of PD-L1 expression previously mentioned but do not support the role of PD-L1 expression as a predictive marker of response to PD-L1 blockade.

Combination studies of nivolumab have also assessed the potential role of PD-L1 expression as a marker of response but have provided conflicting data so far. In the phase I trial of nivolumab plus ipilimumab in metastatic RCC, an exploratory analysis with a retrospective collection of samples evaluated two different IHC cut-off points (1% or 5% in tumour cells membrane staining) for PD-L1 expression. Interestingly, RRs ranged from 25% to 56.3% and were similar across PD-L1-positive and PD-L1-negative tumours regardless of the used cut-off point.[67] Similarly, the phase I trial of nivolumab in combination with sunitinib or pazopanib also provided equivalent RRs regardless of the IHC cut-off point, and the PD-L1 status was similar across the sunitinib and pazopanib groups (52% vs 45%, respectively).[68]

Available data on the role of PD-L1 expression with PD-L1 inhibitors are more limited given the earlier stage of drug development as compared with anti-PD-1 agents. The phase I study of atezolizumab (also known as MPDL3280A), an engineered anti-PD-L1 monoclonal antibody in metastatic solid tumours, included 69 patients with metastatic RCC. PD-L1 expression was evaluated in the TIIC. Using a cut-off point of ≥1%, 35 of the 56 evaluable patients were considered PD-L1 positive and 21 of the 56 were PD-L1 negative. Interestingly, RR was twofold increased in the PD-L1-positive group compared with the PD-L1-negative group (20% vs 10%, respectively, p not shown),[69] indicating a potential relationship between PD-L1 expression in TIIC and antitumour response to atezolizumab. PFS was also longer in the PD-L1-positive group (24 vs 20 weeks, p not shown).[69] In a biomarker exploratory subanalysis of the study, PD-L1 tumour expression at baseline was compared with on-treatment PD-L1 expression using paired serial biopsies in 26 patients including four RCC cases.[70] Interestingly, upregulation of PD-L1 in on-treatment biopsies as compared with baseline expression was significantly associated with RR, suggesting that adaptative changes of PD-L1 expression during treatment have a role as a dynamic on-treatment predictive biomarker.[70]

Taken together, these preliminary findings suggest that PD-L1 expression in tumour cells or in a tumour microenvironment might be considered a prognostic factor. However, its role as a predictive biomarker of response to PD-1 or PD-L1 blockade remains controversial in view of the recent results from the phase III trial of nivolumab. Despite the fact that PD-L1 expression increases the probability to benefit from PD-1/PD-L1 inhibitors, it fails to identify all responders as many PD-L1-negative tumours do benefit from this therapy. Furthermore, some patients with PD-L1-positive tumours do not respond to treatment. Clinical benefit among PD-L1-negative patients, the evidence of intratumoral heterogeneity of PD-L1 expression and the weak correlation seen between PD-L1 expression in primary and metastatic sites[54 71] question the role of PD-L1 expression as a robust predictive biomarker and selection criteria for anti-PD-1/PD-L1 clinical trials.

The role of PD-L1 expression as a biomarker is also limited by the lack of standard IHC cut-off points to define positivity and by the lack of evidence as to whether PD-L1 expression should be better assessed in tumour cells, TIIC or both. It is also relevant to mention that most studies use archival tissue samples to assess PD-L1 expression. However, owing to the dynamic nature of the immune system and the effect of time and prior treatment lines, the PD-L1 status in the archival sample may not be representative of the current PD-L1 status at study entry, which may be many years after the biopsy was taken. Consequently, current randomised phase III clinical trials of PD-1/PD-L1 inhibitors are not restricting patient selection to PD-L1-positive cases but are recruiting PD-L1-negative patients. Standardisation in IHC cut-off points and use of baseline fresh tissue biopsy in phase III trial are therefore needed in order to better understand the real role of PD-L1 expression as a biomarker of response to PD-1/PD-L1 inhibitors. Our capability to identify these and other robust biomarkers that reliably predict clinical response to PD-1/PD-L1 blockade will be vital for efficiently identifying patients most likely to respond. Finally, the increasing knowledge gained over the past years with PD-1 and PD-L1 inhibitors in different solid tumours has actually provided contradictory data with regard to the predictive role of PD-L1 expression. Therefore, it is likely that the relationship between PD-L1 expression and outcomes after PD-1/PD-L1 blockade is tumour-specific and even histology-dependent.

Other immune-related biomarkers

Several other immune-related factors are currently being studied as potential predictive biomarkers of response to PD-1/PD-L1 inhibitors. However, none of them have so far been validated and they should be considered as hypothesis-generating. In an exploratory biomarker analysis of the nivolumab phase I trial (including 2 RCC cases), immune microenvironmental factors such as PD-L2 expression by tumour cells or tumour infiltrating T cells, the CD4:CD8 ratio, the presence of CD20+ B cells, tumour necrosis and lymphoid aggregates were analysed in relation to treatment outcome but failed to show any correlation with response to treatment.[61] A prospective exploratory biomarker analysis of the phase I trial of three dosages of nivolumab in metastatic RCC assessed several immune-related factors such as serum chemokines, tumour T cell infiltrates, gene expression profiling and T cell receptor (TCR) repertoire, at baseline and after cycle 2. Interestingly, higher tumour infiltration by CD3+ and CD8+ T cells at baseline significantly correlated with higher tumour regression.[62] Similarly, responding patients showed a significant increase in CD3+ and CD8+ tumour infiltrating T cells between baseline and on-treatment as compared with non-responding patients, suggesting tumour infiltrating T cells as a potential adaptative predictive biomarker.[62] Moreover, several other changes in immune biomarkers indicating an adaptative antitumour immune activity (increased serum markers of IFN-γ activation, increased tumour gene expression favouring lymphoid and myeloid tumour infiltration, increased baseline tumour T cell frequency) were also correlated with better clinical outcomes.[63]

The biomarker subanalysis of the phase I study of atezolizumab in advanced solid tumours, including metastatic RCC, conducted an messenger RNA expression assay of 90 different immune profile genes (T cell markers, cytokines, chemokines, immune regulation and cell population markers) using pretreatment paraffin-embedded tissue from 96 patients.[70] In addition, 23 patients had paired baseline and on-treatment samples. Interestingly, higher expression at baseline of several cytotoxic Th1 T cell markers such as IFN-γ, granzyme-A, CD8a and EOMES in tumour tissue was correlated with greater response to atezolizumab. Similarly, responding tumours showed an increasing Th1-dominant immune infiltrate, whereas non-responders showed a minimal tumour CD8+ T cell infiltration and an absence of T cell activation measured by granzyme-A and perforin expression.[70] Taken together, these findings suggest that signs of immune competency in pretreatment tumour samples in the form of infiltration by inflammatory signature T cell markers are a predictive marker of response to PD-L1 inhibitors. These observations are consistent with what has already been described for metastatic melanoma where patients who have the so-called 'tumour inflamed' gene signature, including T cell markers and pro-inflammatory chemokines, have significantly improved responses to immunotherapy.[72]

Clinical-related biomarkers

Few clinical-related factors have been studied as potential biomarkers of survival to PD-1/PD-L1 inhibitors. Given the validated prognostic value of the MSKCC prognostic classification with cytokines, its potential role has also been studied with new immunotherapy agents. In the phase I study of atezolizumab in metastatic RCC, more responses were seen in patients with the MSKCC poor prognostic group (n=15), both in the whole group (RR of 27%, 8% and 20% for the poor, intermediate and favourable groups, respectively) and when only looking at PD-L1-positive patients (n=7) (RR of 57%, 13% and 0% for the poor, intermediate and favourable groups, respectively). However, in view of the reduced number of patients, it is not possible to draw any meaningful conclusions.[69] Conversely, in the phase II trial of nivolumab, median OS was significantly longer in the MSKCC favourable prognostic group (n=56) (not reached, 95% CI 24.9 to not reached) compared with the MSKCC intermediate (n=70) and poor groups (n=42) (20.3 months, 95% CI 13.4 to not reached and 12.5 months, 95% CI 8.1 to 18.6, respectively, p not shown), suggesting that the MSKCC risk group might also have a role as a prognostic biomarker with PD-1/

PD-L1 inhibitors.[64 73] Similarly, the number of prior systemic regimens was also found to be a prognostic marker because patients who had received only one previous treatment (n=50) had a significantly longer median OS (not reached, 95% CI 19.8 to not reached) as compared with patients who had received two or more prior regimens (n=118) (18.7 months, 95% CI 13.4 to 26, p not shown).[64 73]

Prognostic and predictive biomarkers of response to CTLA-4 inhibitors

CTLA-4 is a key immune checkpoint receptor expressed by T cells which acts as a negative regulator of early immune responses. CTLA-4 binds to B7.1 and B7.2 ligands, which are expressed on the surface of antigen presenting cells. Its activation results in the downregulation of T cells proliferation and cytokines production leading to immunosuppression and immune tolerance. Ipilimumab and tremelimumab are the two monoclonal antibodies inhibiting CTLA-4, which have so far been studied in patients with metastatic RCC. However, their clinical development in RCC is at an earlier stage as compared with PD-1/PD-L1 inhibitors, and therefore the available evidence on prognostic and predictive biomarkers is more limited. Interestingly, the onset of autoimmune-mediated AEs with CTLA-4 inhibitors has consistently been described as the main potential biomarker of tumour response.[74 75]

The phase II trial of ipilimumab in advanced solid tumours included 198 patients, of which 61 had metastatic RCC.[74] Patients were assessed for clinical response to treatment and onset of autoimmune-mediated AEs. All RCC cases were either IL-2 refractory or IL-2 ineligible. Patients with a previous history of autoimmune diseases were excluded. The study comprised two treatment cohorts: cohort I received ipilimumab at 3 mg/kg with subsequent cycles of 1 mg/kg every 3 weeks and cohort II was treated with 3 mg/kg every 3 weeks for all doses. In the whole group, enterocolitis was the most frequent autoimmune-mediated AE seen with ipilimumab (18%), but autoimmune hypophysitis (7%), dermatitis (4%), arthritis (2%) and uveitis (1%) were also observed. Enterocolitis was defined as the clinical scenario of sudden-onset diarrhoea; no alternative aetiology was identified, and response to steroid therapy and/or presence of endoscopic and histopathological findings are suggestive of enterocolitis. The incidence of enterocolitis among patients with RCC was 20% in both treatment cohorts. Interestingly, the onset of enterocolitis was significantly associated with RR in the whole group as well as in the RCC cohort. The overall RR for the evaluable 189 patients was 14% compared with 36% (14/39) for the assessable patients with enterocolitis (n=39). The RR for patients with RCC with enterocolitis (n=17) was 35% compared with 2% in patients who did not develop enterocolitis (n=44) (p=0.001).[74] No temporal relationship was evidenced between the onset of enterocolitis and the onset of tumour regression.

A subgroup analysis of the previous phase II trial of ipilimumab evaluated the onset of other autoimmune-related AEs in the cohort of 61 patients with metastatic RCC.[75] Thirty-three per cent of patients experienced a grade 3 or 4 immune-mediated AE. Interestingly, the onset of other autoimmune AEs such as hypophysitis, dermatitis or arthritis was also significantly correlated with tumour response to ipilimumab. The RR among patients with significant autoimmune AE (n=20) was 30% as compared with 0% in patients free of autoimmune toxicity (n=41) (p=0.0007).[75] Taken together, these findings suggest that autoimmune-related toxicity such as enterocolitis or hypophysitis could represent a sign of adaptative antitumour immune activity triggered by CTLA-4 blockade and a surrogate marker of drug efficacy. This is consistent with what has already been described with CTLA-4 inhibitors in metastatic melanoma.[76] The underlying mechanism linking ipilimumab-induced autoimmunity and tumour response remains unclear. The evidence that graft-versus-host-related enterocolitis following an allogeneic peripheral blood stem cell transplantation is associated with tumour regression in patients with RCC[77] has led to the hypothesis that ipilimumab-induced autoimmunity could be due to a T cell-mediated graft-versus-host-like response. However, the potential life-threatening risk of autoimmune toxicity challenges its use as a valid biomarker of response to CTLA-4 blockade. Of note, the mortality rate among patients who developed enterocolitis was 5% as compared with only 1% among all treated patients. Moreover, the incidence of perforation or colectomy in the whole group study was significantly higher in patients being treated for RCC than in patients with melanoma (6.6% vs 0.7%, p=0.03).[74] A larger experience with ipilimumab in RCC is therefore needed to adequately balance the risk of life-threatening immune-related AE against the potential benefits that can be obtained with CTLA-4 inhibitors.

EXPERT COMMENTARY

Since 2005, the advent of targeted therapies such as sunitinib, pazopanib and everolimus has revolutionised the systemic treatment of metastatic RCC. However, many patients present with primary resistance and never benefit from treatment, whereas most responders ultimately develop progressive disease. The studies cited above suggest that certain mechanism-based toxicities such as hypertension or hypothyroidism with antiangiogenic therapies or hypercholesterolaemia with mTOR inhibitors may serve as a surrogate marker of pharmacodynamic effect and may be used as predictive and prognostic biomarkers. However, most of these biomarkers do not help patient selection as they are assessed after starting treatment. Moreover, most targeted therapies for metastatic RCC were approved on the basis of clinical trials of an unselected population and not on the basis of a molecularly stratified biomarker-guided approach. Therefore, despite the widespread use of targeted

therapies in RCC, robust predictive biomarkers to help clinicians identify responding patients and minimise toxicity are still lacking and remain an unmet need.

Regarding modern immunotherapy agents, the identification of predictive and prognostic biomarkers has so far shown some promising preliminary results but remains at a very early and immature stage. The studies cited above indicate that there are various clinical and immune-related biomarkers which are relevant and significantly correlate with tumour regression and survival with PD-1/PD-L1 and CTLA4 inhibitors. However, much of the published evidence is based on retrospective or small prospective studies and has provided inconsistent results for many biomarkers. Most accepted biomarkers are mainly clinical-related and no cancer immunological biomarkers have been validated until now. The characterisation of on-treatment lymphocytes subsets with PD-1/PD-L1 inhibitors is indeed encouraging and can potentially help identify responders, but it cannot be used to guide patient selection as these are assessed after starting treatment. Similarly, although baseline tissue biomarkers are promising, their validation for drug investigation has been limited by tumour heterogeneity, the use of archival tissue and the lack of standardisation in immunohistochemical cut-off points. Moreover, PD-L1 expression, which is the most studied immune-related biomarker so far, has been shown to be of prognostic value but not a robust predictor of treatment response. On the other hand, baseline genetic and peripheral blood biomarkers such as circulating tumours cfDNA and microRNA in serum/plasma are more easily and objectively measured and may therefore hold the key for future biomarkers.

FIVE-YEAR VIEW

The management of metastatic RCC continues to evolve as newer therapies are proving to be beneficial. Decades of research have convincingly shown RCC tumours to be immunogenic and development of more potent strategies beyond cytokines has been enthusiastically welcomed. These immuno-oncology (IO) therapies remain a very promising area of new drug development and are proving valuable in many different tumour types (lung, melanoma, bladder, etc). We envision a new treatment paradigm over the next 5 years that incorporates biomarker-specific IO therapies integrated with what is now considered traditional VEGF-targeted therapy. With the recent advent of technologies, namely next-generation sequencing, we might be able to quantitatively and qualitatively identify nucleic acid biomarkers in plasma and serum. These genetic biomarkers probably hold the key to overcoming intratumoural heterogeneity and finally identifying robust, predictive and prognostic biomarkers.

KEY ISSUES

Optimising and integrating new therapies into the current treatment algorithm for management of RCC.

Competing interests None declared.

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