Pharmacogenomics

What do we need to make genetic biomarker-guided treatment for renal cell carcinoma a reality?

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Renal cell carcinoma (RCC) is the most common type of kidney cancer, in more than 80% caused by a *VHL* gene mutation that allows tumor growth through secretion of proangiogenic factors. Worldwide, 350,000 persons are diagnosed with RCC each year and this number is increasing. Roughly 17% of patients present with metastases at first diagnosis and have a poor prognosis. The vast majority of patients have a clear cell RCC histological subtype as opposed to nonclear cells with a worse prognosis. Surgical removal is the best option in an early stage of the disease, but may not always be possible because of a poor performance status or metastases [1].

Since 2005, the era of targeted therapy has arisen, antiangiogenic therapy has significantly improved the treatment outcome of patients with RCC. The first choice of treatment in metastatic RCC (mRCC) is systemic therapy with a tyrosine kinase inhibitor (TKI) that acts on VEGF receptors among other tyrosine kinases. Sorafenib and sunitinib were the first TKIs that showed improved patient outcomes compared with the former cytokines IL-2 and IFN-α [2]. The pivotal Phase III trial of sunitinib showed an improvement in progression-free survival and objective response rate (p < 0.001) and a trend toward a better overall survival (p = 0.051) compared with IFN-α [2]. The mTOR inhibitors, everolimus and temsirolimus, are given to patients with a poor prog-

nosis. Bevacizumab, a VEGF inhibitor and monoclonal antibody, is commonly used in combination with an mTOR inhibitor. Later, TKIs pazopanib and axitinib were approved for mRCC treatment, and pazopanib is currently prescribed in the same extent as sunitinib [3]. New game changers in the field for second-line treatment are the TKIs lenvatinib, cabozantinib and the monoclonal antibody nivolumab. These drugs show an improved efficacy compared with everolimus. Especially, the arrival of nivolumab (US FDA approved for mRCC in October 2015) may well mean that we are facing a new era of 'targeted immunotherapy'. Nivolumab is a programmed death-1 checkpoint inhibitor that releases the brakes on T cells activating tumor immune surveillance [4–6].

With the expanding drug arsenal in mRCC, each with its own drug-specific pharmacokinetics (PK) and pharmacodynamics (PD) profile, the possibility of opting for the most effective treatment for a specific individual has grown. However, despite successful advances in RCC treatment, we are not yet able to give patients an optimally effective and safe treatment without adjustments afterwards. There is a large variability in individual response to TKIs but also to nivolumab, and there is a lack of biomarkers that predict treatment outcome. Some germline pharmacogenetic markers seem promising to elucidate differences in efficacy and toxicity in patients receiving

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targeted therapy. Candidate gene studies interrogating SNPs located in genes related to the PK and PD of the drug of interest have been performed [7–10]. For sunitinib, the most interesting findings were SNPs in genes involved in PK encoding the CYP3A5 enzyme and the efflux transporter ATP-binding cassette ABCB1. SNPs in *CYP3A5* and *ABCB1* were confirmed to be associated with sunitinib treatment outcome in a large pooled sample set of 333 mRCC patients [9]. These results are supported by the effect of these SNPs in *CYP3A5* and *ABCB1* on clearance of sunitinib and its active metabolite [10]. Related to PD, SNPs in *VEGFR1* are associated with progression-free survival and overall survival on sunitinib, and this was validated in independent cohorts [11]. For pazopanib, SNPs in *UGT1A1* and *IL8* show the highest evidence for association with toxicity and survival, respectively [12–14].

> "Although the need for biomarkers is urgent, we should not rush on implementation without valid evidence."

Still, the selection of SNPs in candidate gene studies is restricted to our current knowledge on PK and PD. About a decade ago, pharmacogenetic investigational methods were reinforced with the arrival of genome-wide association studies. In a genome-wide association study about 1 million common genetic variants can be tested for association with drug outcomes and thereby provides an unbiased approach as compared with the candidate gene studies. Such a study has been set up for sunitinib and pazopanib as a part of the European collaborative project on EuroTARGET [15]. Entering the era of next generation sequencing, future studies including also rare genetic variants (minor allele frequency <1%) are an interesting prospective in our search for genetic biomarkers. Especially, because rare genetic variants in *CYP3A4*, the most important drug metabolizing enzymes, have an effect on functional variability [16].

It seems unjustified that none of the aforementioned genetic variants have been implemented into clinical practice, but several barriers keep this from happening [17]. One of the difficulties is the large heterogeneity among pharmacogenetic studies: patients have different ethnicities with corresponding allele frequencies; studies may be underpowered; and tested end points can vary in definitions and may not have been collected with the intention to perform pharmacogenetic association analyses. Changes in clinical practice owing to the experience gained in RCC treatment, such as earlier dose reductions or altered dosing schedules to prevent severe adverse events, may alter treatment outcomes. Pharmacogenetic studies investigating somatic DNA

(instead of germline DNA) can be biased by intratumor heterogeneity, in other words, mutations in the tumor result in a different genetic profile and probably cause adaptive resistance to targeted treatment. The detection of ctDNA may be used to monitor dynamic changes and help individualize treatment. And what about 'negative' findings with p-values above 0.05? These are often not published and therefore considered as nonexisting which can blur our vision. Furthermore, findings from these studies have either not been validated in independent cohorts or are merely confirmed in small cohorts based on retrospective data. As a rule, prospective validation in an independent cohort is vital to bring SNP testing to the clinic. Yet, this has not stopped *KRAS* mutation status to be applied as a biomarker for response to anti-EGFR treatment in colorectal cancer [17].

Although the need for biomarkers is urgent, we should not rush on implementation without valid evidence. One of the main barriers that withhold clinical use is that no consensus has been reached on a clear definition for clinical utility of pharmacogenetic testing to guide individual dosing regimens of targeted therapies. In general, clinical utility means that there is evidence that the test will improve patients' treatment outcome. But what exactly this evidence is remains unclear. In order to make progress, we first need to make sure that we have universally applicable requirements so that we can waive the debate on clinical utility. The indisputable proof to use a pharmacogenetics test should be based on a critical evaluation of available studies on the genetic variant of interest with an assessment on sample size, ethnicity, end point definitions, effect sizes, significance thresholds and the level of evidence regarding validation of findings in prospective studies. Then and there, we can concentrate on successful use of genetic markers by all healthcare professionals and facilitate ready-to-use recommendations. The Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group already take this responsibility in providing clinical guidelines on genotyping results [18,19]. As a final point, pharmacogenetic testing is not easily accepted for uptake in clinical practice, which could be accomplished by active support of pharmaceutical companies and patient groups [17].

In the meantime, while we are not yet able to predict, we are able to adjust. Therapeutic drug monitoring (TDM) provides an accurate and feasible option to guide treatment with targeted therapies in mRCC using measured drug concentrations in blood to determine PK/PD parameters, and adjust the dose if needed. Several criteria need to be fulfilled to apply TDM; the drug has a narrow therapeutic window;

there is a relation between drug concentration and effect; there is a high interindividual variability in PK; the dose cannot be optimized by clinical observation; there is no clear relation between dose and effect; and the intrapatient variability is less than the interpatient variability [10,20]. In addition to TDM, ongoing studies are aimed at finding a synergistic antitumor effect within the current drug arsenal against mRCC. New drug combinations or sequences targeting both the angiogenic and immunogenic characters of the tumor possibly improve patient outcomes.

In short, we know what hurdles need to be overcome before genetic biomarker-guided treatment becomes a reality. We need to provide scientific evidence, demonstrate the clinical relevance, develop guidelines with recommendations to direct clinical use of pharmacogenetic testing and then need to make sure that the use of pharmacogenetic tests is widely accepted [17–19]. Efforts for broad testing of genetic variants and vali-

dation studies have already been established. We have high expectations of the results on genetic markers for TKI treatment and the development of new successful drugs or drug combinations. Not only will we move forward in pharmacogenetic research and our endeavor to find genetic markers, these novel markers will also help brighten up the treatment of RCC patients by using a more personalized approach.

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