Optimization of sunitinib treatment in metastatic renal cell carcinoma : pharmacogenetic evidence and challenges

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CHAPTER 1

General introduction

INTRODUCTION

Epidemiology and risk factors of renal cell carcinoma

Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for nearly 2% of all cancer diagnoses and cancer deaths.[1] Worldwide, the incidence of RCC is increasing annually and varies per region, with generally higher incidence rates in developed countries than in developing countries.[2] In the European Union, ~84,000 new cases and ~35,000 death were recorded in 2012.[3] RCC predominantly occurs in males, with an incidence of 1.6 to 2.0 times higher in males than that in females.[4] The median age at diagnosis of the disease is 64 years.[5]

Major risk factors for RCC include cigarette smoking, obesity and hypertension.[6] Several other factors have been associated with an increased risk, for example, use of alcohol as well as certain disease (chronic kidney disease and kidney transplantation) and medicines (such as phenacetin, diuretics and calcium channel blockers), but the quantification of these effects on national incidence rates remains unclear.[7] Based on the candidate-gene approaches, some rare genetic and hereditary factors including germline mutations in *BAP1, FLCN, FH, MET, PTEN, SDHB, SDHC, SDHD, TSC1, TSC2* and *VHL* genes have been identified for their associations with the risk of RCC, although some of which have been reported in irregular RCC cases and do not show consistent associations.[8]

Classification and stage of renal cell carcinoma

RCC consists of a heterogeneous group of cancer with distinct genetic and molecular variabilities. According to the International Society of Urological Pathology classification scheme of renal tumor, there are more than 10 subtypes of RCC mainly defined by their histology. Clear cell RCC (ccRCC) is the most common subtype of RCC, accounting for approximately 70-75% of all RCC. Less common are the non-clear cell subtypes: papillary RCC (10-16%), chromophobe RCC (7%) and other rare subtypes (such as collecting duct RCC, multilocular cystic RCC, medullary carcinoma, neuroblastoma-associated RCC and mucinous tubular and spindle-cell carcinoma, etc.)[9] (**Figure 1**). Rarely, unclassified RCC is identified when a tumor does not fit any of the other categories. Due to the predominance of clear cell histology and distinct treatment responses between clear cell and non-clear cell RCC subtypes, the thesis is mainly focused on ccRCC.

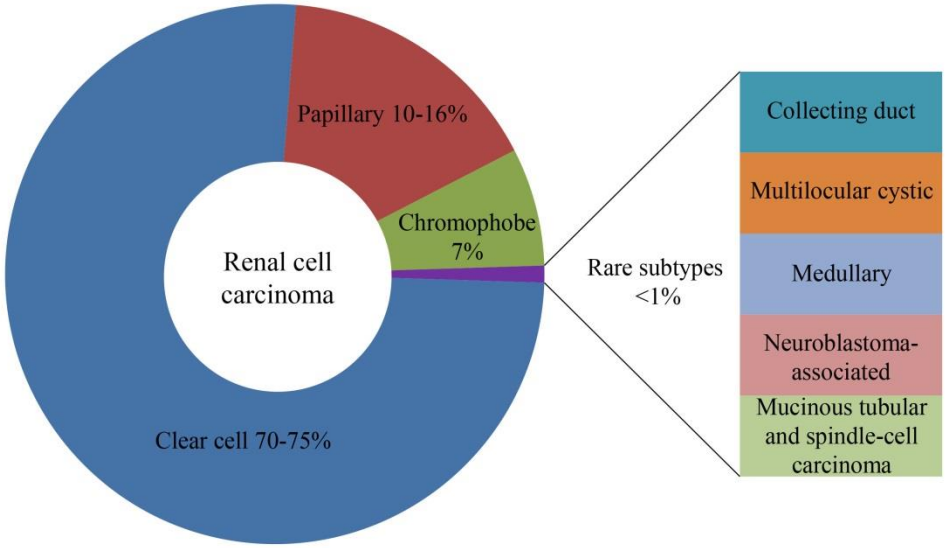

Figure 1 Histological classification of renal cell carcinoma

According to the size of tumor and the extent of tumor spread, RCC can be categorized into four stages. In stage I and II, tumor is localized in the kidney, with tumor size of < 7cm and > 7 cm respectively. In stage III, tumor has spread into a major vein or surrounding lymph nodes. When RCC reaches stage IV (also known as metastatic RCC (mRCC)), tumor has advanced to other organs.[10]

Pathophysiology of renal cell carcinoma

Angiogenesis pathway

Nearly half a century ago, Judah Folkman suggested that tumor growth is dependent on angiogenesis.[11] Given the highly vascularized characteristic of RCC, angiogenesis is regarded to be particularly important to RCC compared to other types of tumors. Hereafter, an increasing number of researchers have tried to untangle the process that modifies angiogenesis of RCC. One crucial finding is the discovery of the mutation in von Hippel–Lindau (*VHL*) gene, which is seen in up to 70% of ccRCC.[12] It is noted that the absence of functional *VHL* protein (pVHL) acts as an essential role in upregulation of the hypoxia pathway via the hypoxia inducible factors (HIFs).[12] In addition, recent studies suggest another pathway, the phosphoinositide 3-kinase (PI3K) together with its downstream

mammalian target of rapamycin (mTOR), enhancing the HIF transcription.[13] The aberrant accumulation of HIF in turn amplifies a variety of downstream angiogenetic genes such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), multidrug resistance pump (MDR-1), and erythropoietin (EPO), which contribute to the angiogenesis and tumor growth (**Figure 2**).[14]

Checkpoint pathway

T cells play an important role in immune response when tumor cells are detected as antigen by the immune system. Either eradication of tumor cells or promotion of their proliferation is mediated by several regulatory receptors that express on the surface of T cells and act as immune checkpoints.[15] Some T-cell receptors, such as CD28, positively regulate T cells leading to tumor destruction. The others, such as programed cell death protein 1(PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), negatively regulate T cells after binding to their ligands (PD-L1 and B7.1/B7.2, respectively), resulting in prolonged tumor survival.[15] Interestingly, CTLA-4 and PD-1can be expressed by various types of tumor cells including RCC,[16-18] which is regarded as a mechanism for how tumor cells escape immunity (**Figure 2**).

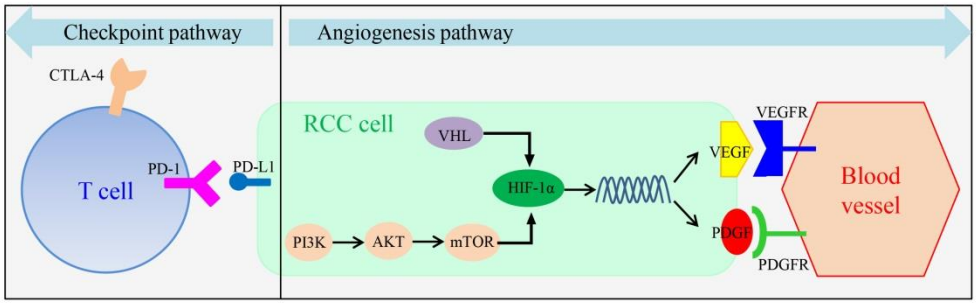

Figure 2 Pathophysiology of renal cell carcinoma

Treatment for renal cell carcinoma

Partial or radical nephrectomy is the curative treatment for patients with localized RCC. In some cases, surgery is still a part of treatment if the patient has a good performance status and the tumor has only spread to one removable organ.[19] Otherwise, systemic therapy is the only remaining option for patients with metastatic RCC.

Unlike other tumor types, RCC does not respond to conventional chemotherapeutic agents.[19] For decades, only cytokine-based treatment such as interleukin-2 and interferon-alpha was available, but the outcome for patients with mRCC was disappointing.[19] Based on their significant improvements in overall survival (OS) and/or progression-free survival (PFS), therapies targeting angiogenesis have been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) including tyrosine kinase inhibitors (TKIs, such as sorafenib, sunitinib, pazopanib, axitinib, cabozantinib and lenvatinib), mTOR inhibitors (temsirolimus and everolimus), and the VEGF monoclonal antibody bevacizumab.[20] Recently, an improved understanding of how tumor cells evade anti-tumor response and how T-cell can be modulated by activating and inhibitory receptors shed light on the development of the targeting immunotherapy paradigm. The immune checkpoint inhibitors have emerged as promising RCC treatments, such as PD-1 inhibitor (pembrolizumab and nivolumab), PD-L1 inhibitor (atezolizumab, avelumab and durvalumab), and CTLA-4 inhibitor (ipilimumab and tremelimumab).[21] Besides, ongoing trials are likely to result in a broad scope of treatment options for mRCC patients, including agents targeting angiogenesis pathway and checkpoint pathway, as well as the combination of both. **Figure 3** shows the evolution of treatment for mRCC patients.

Sunitinib treatment

Sunitinib is an oral TKI inhibiting VEGF receptors 1, 2, and 3, PDGF receptor α and β, KIT, Fms-like tyrosine kinase 3 receptor (FLT3) and the receptor encoded by the ret proto-oncogene (RET). It was approved by the FDA in 2006 and has been became the first-line treatment for mRCC patients because the median PFS has been improved considerably from 5 months with interferon-alpha to 11 months with sunitinib.[22] The standard treatment schedule of sunitinib is a fixed daily dose of 50mg for 4 weeks followed by a 2-week rest period. There is possibility for continues dosing regimen during rest period and dose modification due to tolerance and toxicity.[23] Sunitinib is predominately metabolized by the cytochrome P450 enzyme *CYP3A4* to its active metabolite, SU12662, which could be further metabolized to inactive metabolite SU14335 by *CYP3A4*. 61% of total drug is eliminated in feces, and 16% through urine.[23]

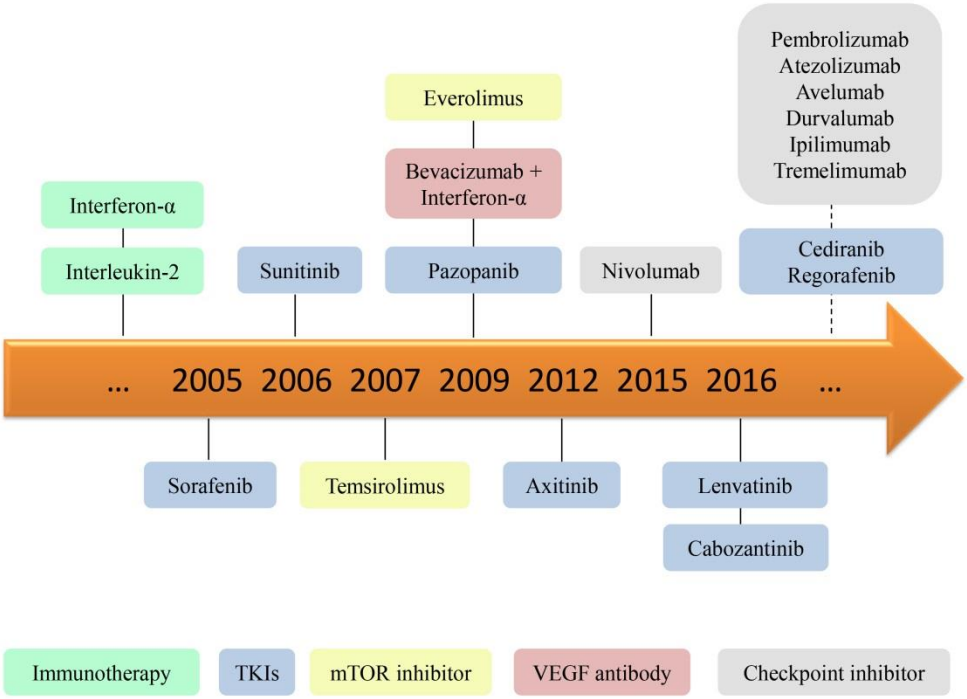

Figure 3 Treatments approved by FDA and EMA for mRCC patients and novel therapies under clinical trials.

Even though the survival of mRCC patients has been improved dramatically by sunitinib, patient's response to sunitinib treatment has large variation. Only 35% of mRCC patients benefit from sunitinib and about 30% of patients need dose reductions due to adverse events of which grades vary among patients.[24] Given the large inter-individual variabilities, it would be helpful to predict the individual treatment outcome at the initiation of therapy to minimize the risk of adverse events of higher grades and to optimize treatment efficacy. In recent years, several single nucleotide polymorphisms (SNPs) in genes involved in the sunitinib pharmacokinetic pathway (such as *CYP3A4, CYP3A5, ABCB1, ABCG2, NR1I3*) and pharmacodynamic pathway (such as *VEGFR-1, 2, 3, FLT3, NOS3*) have been tested as potential genetic biomarkers for sunitinib outcome in mRCC patients.[25] Unfortunately, no biomarkers have been implemented into clinical practice yet due to the lack of successful independent validation in large cohorts.

AIM AND SCOPE OF THIS THESIS

The objective of this thesis is to identify genetic biomarkers for prediction of sunitinib efficacy and toxicity in mRCC patients.

Evidence has emerged that ethnic differences in drug response exist especially in the field of oncology. However, a comprehensive analysis of the ethnic differences in sunitinib outcome in mRCC patients is still lacking. In **Chapter 2**, a systematic literature search and a meta-analysis is performed to compare sunitinib efficacy and toxicity in Asian and Caucasian mRCC patients.

Earlier, a validation study was performed to investigate the association of efficacy and toxicity of sunitinib with 22 SNPs of interest.[26] Recently, additional SNPs (*CYP3A4*, *NR1I2*, *POR*, *IL8*, *IL4-R*, *IL13*, *HIF1A* and *MET*, reported after 2011) have been suggested as prognostic or predictive biomarkers for TKIs treatment.[27-34] In **Chapter 3**, we aim to evaluate whether these novel SNPs can be validated in a large cohort of mRCC patients receiving sunitinib treatment.

Chapter 4 is based on three previous pharmacogenetics analyses by Beuselinck *et al* [35, 36] and Dornbusch *et al* [37], in which *VEGFR1* rs9582036 and rs9554320 were regarded as promising predictors for sunitinib efficacy. Therefore, the aim of this chapter is to assess the role of the *VEGFR1* rs9582036 and rs9554320 with regard to their associations with sunitinib efficacy in 286 sunitinib-treated mRCC patients as well as to perform a meta-analysis of current and published data.

CYP3A4 rs4646437 was reported for its association with sunitinib related toxicity in 2012 American Society of Clinical Oncology annual meeting.[38] Hereafter, the relationship between *CYP3A4* rs4646437 and sunitinib-induced hypertension was explored by Diekstra *et al*.[39] However, the mechanism of the relationship is still unclear because no functional of *CYP3A4* rs4646437 is available. Therefore, **Chapter 5** is designed to investigate the effect of *CYP3A4* rs4646437 on the clearance of both sunitinib and its metabolite SU12662.

Both *CYP3A5* rs776746 and *CYP3A4* rs4646437 were individually reported for their association with sunitinib-induced hypertension in mRCC patients.[26, 39] The *CYP3A4* and *CYP3A5* genes lie in close proximity (136 kb) to one another on chromosome 7 (chr. 7q22.1). Therefore, some effects originally thought to be due to a *CYP3A4* allele may be

actually due to a *CYP3A5* allele. In **Chapter 6**, we explore whether the *CYP3A5* rs776746 or *CYP3A4* rs4646437 or both SNPs are the causal genetic factor in the association with sunitinib-induced hypertension.

The recent discovery of the immune checkpoint pathway has broadened our understanding of mRCC. An increasing number of studies have been reported on the association of expression of immune checkpoints or genetic variants in genes encoding immune checkpoints with treatment outcomes.[40-42] **Chapter 7** is conducted to analyze whether polymorphisms in genes involved in immune checkpoints are related to clinical outcomes of sunitinib-treated patients with mRCC in a large exploratory cohort and in an independent validation cohort.

Till now, hundreds of studies have been done attempting to find a genetic biomarker to predict sunitinib outcomes. Still, none of the previously investigated genetic variants have been implemented into clinical practice. In **Chapter 8**, we give our opinion what we need to make this dream a reality.

This thesis ends with a general discussion and a future outlook in **Chapter 9** and a summary of results in English, Dutch and Chinese is presented in **Chapter 10**.

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