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Pharmacogenetics of sunitinib in metastatic renal cell carcinoma

Diekstra, M.H.M.; Diekstra M.H.M.

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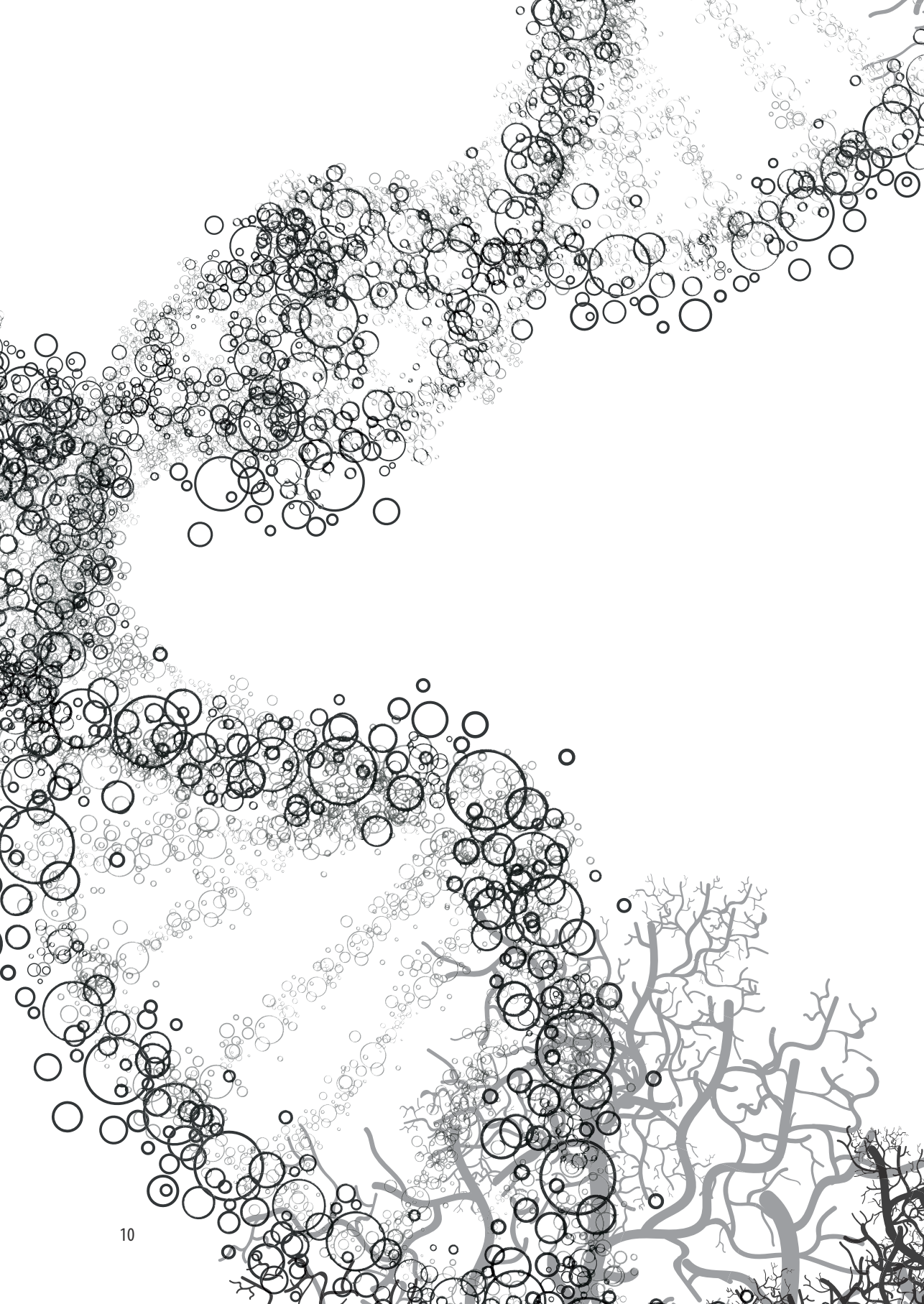
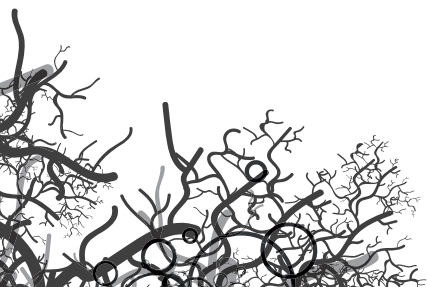


CHAPTER 1

General introduction



Renal cell carcinoma: epidemiology, pathology and risk factors

The most common type of kidney cancer (>90%) is renal cell carcinoma (RCC) which comprises about 2-3% of all malignancies in adults.[1,2] RCC typically occurs in people aged between 60 and 70 years old and is more common in men than in women.[1-3] In 2013, worldwide 350,000 people were diagnosed with RCC with more than 140,000 deaths. In the Netherlands 2,327 new cases were diagnosed in that year.[3,4] The age-adjusted incidence of RCC seems to have increased in the last couple of years with a higher incidence in developed countries compared to developing countries. The higher RCC incidence could be explained by enhanced use of imaging techniques such as ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI).[2,3] In most cases tumors are small and non-metastatic at first presentation, but approximately 17% of RCCs are metastasized at first diagnosis. Preferred sites of metastasis are lung, liver, bone, brain, and only rarely adrenal glands or the contralateral kidney.[1-3]

RCC is a heterogeneous tumor encompassing a wide variety of tumor types with clear cell RCC as the most common histological subtype (≈75%). Less common are the non-clear cell types: papillary type I (5%), papillary type II (10%), chromophobes (7%), medullary, collecting duct, and translocation renal tumors (**Figure 1**).[1-3] Moreover, multiple subtypes can be distinguished within a tumor. Non-clear cell subtypes have a poor prognosis in contrast to clear cell RCCs that show improved survival outcomes.[5]

Risk factors for RCC are smoking, obesity, hypertension or hereditary causes.[1-3] A mutation in the von Hippel-Lindau (*VHL*) gene is the most frequent hereditary cause (>80% of the clear cell RCCs). This causes a dysfunction of the VHL protein which prevents VHL to form a complex with hypoxia inducible factor α (HIF-α) so that HIF-α cannot be destroyed by proteasome degradation. Consequently, HIF-α accumulates in the cell inducing the secretion of proangiogenic factors, including the vascular endothelial growth factor (VEGF), that allow tumors to grow easily through oxygen supply by blood vessel formation.[1,3,6]

RCC treatment options in the era of targeted therapy

First and foremost, before any treatment is being considered and if RCC is discovered at an early stage, surgical removal is the standard of care with the aim to cure the disease, provided that the patient has a good performance status. Minimally invasive techniques such as nephron sparing surgery (partial nephrectomy), exposure to extreme temperatures (cryoablation or radiofrequency ablation) are used rather than radical nephrectomy (removal of the entire kidney) to prevent chronic kidney disease and other morbidity.[1-3] Elderly patients with a poor prognosis or comorbidity and diagnosed with (localized) RCC with a tumor size <40 mm, should be under active surveillance by regularly imaging. Especially in asymptomatic patients, this period of observation before starting treatment is essential. In about 20% of the cases, small renal tumors may turn

out to be benign; slowly growing and self-limiting tumors, and only in 1 to 2% of the cases metastatic disease was reported.[1-3]

In case of metastases or if surgery is not deemed feasible, treatment options will be more palliative than curative. Radiotherapy can offer pain relief in bone or brain metastases and chemotherapy has little or no effect.[1-3] The current drug arsenal against metastatic renal cell carcinoma (mRCC) is large and continues to expand. For a long time cytokines (interleukin-2 and interferon-alpha) were the only available treatment options with limited efficacy and substantial side effects. Since 2005 the number of drugs against kidney cancer has been growing with the arrival of targeted therapies that act on small proteins present in signal transduction pathways in and around the tumor. Targeted therapies that act in the tumor cell and inhibit the mammalian target of rapamycin (mTOR) are the so-called mTOR inhibitors everolimus and temsirolimus and cause a decreased expression of HIF-α. Temsirolimus is used in patients with a poor prognosis but is less and less prescribed, while everolimus is indicated as second-line treatment after VEGF-targeted therapy for good or intermediate prognosis. An alternative treatment option is the monoclonal antibody bevacizumab used in combination with interferon-alpha, which functions in between endothelial cells and tumor cells targeting VEGF and preventing it from binding to its receptor. Especially the (multi)targeted tyrosine kinase inhibitors (TKIs) showed to be very successful and target receptors on the endothelial cell with an anti-angiogenic effect (**Figure 1**).

Sorafenib was the first TKI approved for the treatment of RCC.[7] Sunitinib entered the market in 2006. In the pivotal phase III trial of sunitinib in mRCC, it was shown that sunitinib significantly improves progression-free survival (PFS) and objective response rate compared to the former standard choice of therapy interferon-alpha ($P<0.001$) and an almost significant improvement in overall survival (OS) was seen for sunitinib ($P=0.051$).[8] Later, in 2009, pazopanib was approved for mRCC treatment and meanwhile many more TKIs were being investigated, such as axitinib.[9,10] The targeted therapies as mentioned above have been proven effective in clear cell RCC. For non-clear cell RCC however, less is known about the efficacy of anti-VEGF therapies and mTOR-inhibitors.[5]

Sunitinib pharmacology

The work described in this thesis focuses on sunitinib. Sunitinib is administered orally, most often given as 50 mg in a 4 week on/2 week off schedule. Dose reduction can be applied in steps of 12.5 mg where a continuous regimen of 37.5 mg is also applied. The main pharmacodynamic targets of sunitinib are VEGF-receptors type 1, 2 and 3, platelet-derived growth factor receptors (PDGFRs) α and β, Fms-related tyrosine kinase 3 (FLT3), and cytokine receptor Kit (c-KIT).[8] Sunitinib is mainly metabolized by cytochrome P450 (CYP)3A4 to its active metabolite SU12662, and inactive metabolites. Both sunitinib and

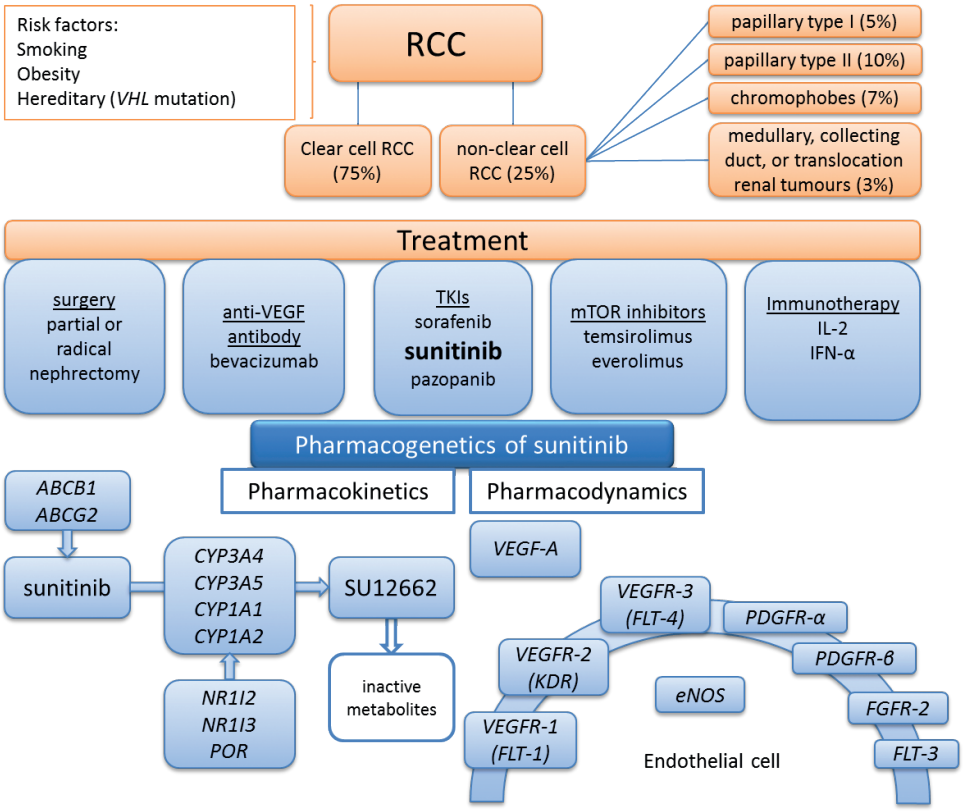


Figure 1: Overview of RCC risk factors, tumor types, and treatment options with a focus on sunitinib and pharmacogenetics of sunitinib in mRCC.

SU12662 embody the total amount of active drug in plasma.[11-13] Furthermore, CYP3A5, CYP1A1 and CYP1A2 could be involved in sunitinib metabolism. The pregnane X receptor (NR1I2), the constitutive androstane receptor (NR1I3) and P450 oxidoreductase (POR) can influence CYP3A4 expression or activity.[14,15] Other important modulators of sunitinib pharmacokinetics (PK) are the efflux transporters ATP-binding cassette (ABCB1) and the breast cancer resistance protein (ABCG2).[16] Patients show a high variability in PK of sunitinib and SU12662 and respond very differently to drugs with large differences in toxicity and efficacy.[12,13,17-19] Most common side effects are fatigue, hand-foot syndrome (HFS), hypertension, mucositis, hair or skin discoloration and hypothyroidism. About 30% of RCC patients on sunitinib required a dose reduction for any of these adverse events.[13,17-19]

Despite successful advances in RCC treatment since the advent of targeted therapy, we have not yet reached our goal. Interpatient variability in response is large and the great difficulty is that we cannot predict an individual's response to sunitinib, which is highly essential in order to give patients an optimally effective and safe treatment and to efficiently use the available drug arsenal without adjustments afterwards. For that reason, we need specific biomarkers that can predict the individual treatment response with regard to efficacy and toxicity of sunitinib.

Pharmacogenetics of sunitinib

It is plausible that genetic variability in genes involved in the PK or pharmacodynamics (PD) of a drug are important determinants of a person's response to the drug with respect to adverse events and efficacy. Pharmacogenetics thoroughly explores this hypothesis. Previous pharmacogenetic studies used the candidate gene approach to select small genetic variations, namely Single Nucleotide Polymorphisms (SNPs), located in genes thought to be related to PK and PD of sunitinib. PK related genetic variants in genes coding for CYP enzymes, drug efflux transporters or influencers of CYP3A activity have been identified to be associated with toxicity or efficacy of sunitinib in mRCC.[17-19,20-21] SNPs in *CYP1A1*, *CYP3A5*, *ABCB1*, *ABCG2*, and *NR1I3* showed an association directly with toxicity or with dose reductions due to toxicity.[17,18] Furthermore, SNPs in *CYP3A5*, *ABCB1, NR1I2* and *NR1I3* have been associated with PFS on sunitinib.[19] Regarding PD, SNPs associated with sunitinib toxicity or efficacy were located in genes encoding receptors on the endothelial cells or endothelial nitric oxide synthase (eNOS). SNPs in *VEGF-A*, *VEGFR-1*, *VEGFR-2 VEGFR-3, FLT3,* and *eNOS* have been associated with adverse events (particularly hypertension), PFS or OS on sunitinib (**Figure 1**).[19,20,21] Yet, no genetic biomarker has been implemented for clinical use and little is known about the mechanisms underlying possible SNP effects. Therefore, the objective of this thesis is to investigate whether pharmacogenetic testing of SNPs prior to treatment initiation may possibly predict the individual response to sunitinib in mRCC and therefore benefit the patient's wellbeing.

Aim and outline of thesis

The general aim of this thesis was to study the predictive ability of germline pharmacogenetic markers on toxicity and efficacy outcomes of sunitinib in mRCC patients. This research is part of a European collaborative project on "TArgeted therapy in Renal cell cancer: GEnetic and Tumor related biomarkers for response and toxicity" (EuroTARGET) funded by the European Commission under the Seventh Framework Programme (FP7).[22]

Chapter 2 systematically reviews studies on SNPs (pharmacogenetics) but also gene expression or epigenetic biomarkers (pharmacogenomics) as predictors of TKI treatment

outcome in mRCC containing studies from 2005 to 2015. The quality of included studies is discussed and the prospect for clinical implementation of study results or the need for follow-up studies.

Previously, SNPs have been associated with toxicity or efficacy outcomes on sunitinib, but it is not clear whether these SNPs are also causally related to PK of sunitinib.[17-19] In **Chapter 3** it is studied whether previously associated SNPs located in genes involved in the metabolism or absorption of sunitinib influence the clearance of sunitinib and its active metabolite SU12662.

In **Chapter 4** a pooled dataset is formed by including patients from previous pharmacogenetics analyses on sunitinib performed in the United States, Spain and the Netherlands.[17-19,20,21] The aim of this study is to investigate whether SNPs associated with toxicity and efficacy on sunitinib in earlier exploratory studies could be confirmed in a larger group of 333 mRCC patients. To this end, 22 significantly associated SNPs ($P<0.05$) from previous exploratory studies are selected and retested in this study for association with sunitinib treatment outcomes.

At the American Society of Clinical Oncology (ASCO) annual meeting in 2012, Urun *et al*. reported that SNP rs4646437G>A in *CYP3A4* had a protective effect on sunitinib related toxicity as shown in a cohort of 159 mRCC patients.[23] In **Chapter 5** the relationship between rs4646437 in *CYP3A4* and sunitinib toxicity or efficacy is further investigated in a large cohort of mRCC patients.

A set of relatively new SNPs (reported from 2011 onwards) in *CYP3A4*, *NR1I2*, *POR*, *IL8*, *IL4-R*, *IL13*, *HIF1A* and *MET* were reported as potential prognostic or predictive markers for TKI treatment outcome.[24-30] With an exploratory nature, **Chapter 6** evaluates whether these SNPs play a role in sunitinib toxicity or efficacy outcomes in a large cohort of sunitinib treated mRCC patients.

In **Chapter 7** SNP rs34231037 in *VEGFR-2* (*KDR*) is analyzed in a large series of clear cell mRCC patients treated with sunitinib for the possible effect on response and PFS. This study objective was based on a Genome Wide Association Study (GWAS) of Maitland *et al*. in which this SNP was identified as a possible predictor for variations of soluble VEGFR2 (sVEGFR2) in the blood and sVEGFR2 levels at baseline and after 4 weeks of pazopanib treatment.[31]

A better understanding of the relationships between sunitinib exposure, the pharmacological response, and the clinical outcomes is vital to find predictive biomarkers for treatment outcome. **Chapter 8** describes a nonlinear mixed-effects population PK/PD model that is built by means of blood sampling data of sunitinib treated patients diagnosed with either mRCC or colorectal carcinoma (CRC) and a group of 12 healthy volunteers.[32] The aim of this study is to evaluate the predictive performance of this model including PK parameters, blood pressure, sVEGFR-2, sVEGFR-3, and SNP genotypes.

Subsequently, the applicability of the model to predict clinical outcomes on sunitinib treatment is explored.

Due to the rapid developments in genetic testing technologies, possibilities to test more than one SNP at a time become easily accessible. In **Chapter 9** about 1 million SNPs are tested for the association with sunitinib toxicity or efficacy in a GWAS that allows less biased hypothesis-free testing for associations as compared to the candidate gene approach used in the aforementioned chapters.

This thesis closes with a general discussion and future perspective on the topic in **Chapter 10** and a summary written in English and Dutch in **Chapter 11**.

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