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CHAPTER 9

General discussion and future perspectives
INTRODUCTION

Since the last decade, the treatment of metastatic renal cell carcinoma (mRCC) has undergone a head-spinning progress from cytokine-based immunotherapies (such as interleukin-2 and interferon-alpha) to therapies targeting a variety of signaling pathways including tyrosine kinase inhibitors (TKIs), mechanistic target of rapamycin (mTOR) inhibitors as well as checkpoint inhibitors.\(^1\) Sunitinib, an oral TKI, has been used as first-line treatment for patients with mRCC after it was approved by the FDA in 2006\(^2\), because the median progression-free survival (PFS) has been improved significantly from 5 months with interferon-alpha to 11 months with sunitinib.\(^3\) However, the patient’s response to sunitinib treatment with regard to efficacy and toxicity shows large variation and a considerable proportion of patients do not experience a positive response.\(^4, 5\) Therefore, remarkable effort are being made to detect biomarkers in order to predict sunitinib response and to optimize sunitinib treatment. So far, only the Memorial Sloan-Kettering Cancer Center (MSKCC) risk system\(^5\) and the subsequently improved Heng prognostic risk criteria\(^6\) have been validated as prognostic tools and are included in the clinical guidelines. The inter-individual variations remains within patients in the same prognostic group. In addition, it is not clear which patient characteristics render an individual patient at the risk for sunitinib-induced toxicity.

With the important role being played by pharmacogenetics in personalized medicine, it is thought that sunitinib response is, at least partly, a heritable trait. Progress has been made through several explorative studies regarding the assessment of potential single nucleotide polymorphisms (SNPs) in sunitinib treatment in the last decade.\(^7\) Still, consensus has not been reached due to the large heterogeneity among executed studies\(^8\), and, no genetic biomarker has been implemented into clinical practice. Accordingly, the aim of this thesis was to improve our understanding of the genetic variants underlying sunitinib efficacy and toxicity in mRCC patients (Chapter 3 to 7). A focus is placed on the validation of genetic associations reported in literature by our group and others as well as on the discovery of novel associations. This could ultimately contribute to a priori prediction of sunitinib response and thereby minimizing incidence of undesired side effects. In this chapter, the most important findings in this thesis are described, followed by a discussion of factors preventing the utility of genetic variants from clinical practice. Furthermore, we elaborate on further research opportunities and present a future perspective.
MAIN FINDINGS

It is well known that for certain drugs efficacy and toxicity vary greatly among different ethnicities. Out of 167 drugs approved by the FDA between 2008 and 2013, 21% reported ethnic differences in pharmacokinetics, safety, efficacy, or pharmacogenomics in the label. Consequently, the inter-individual sunitinib response could be explained partly by ethnicity.

In Chapter 2, we systematically collected available published data and performed a meta-analysis to compare sunitinib efficacy and toxicity in Asian and Caucasian mRCC patients. We found that Asian patients had similar sunitinib efficacy, but burdened a higher incidence of all grades hand-foot syndrome, > grade 2 fatigue, > grade 2 hand-foot syndrome and > grade 2 thrombocytopenia, in comparison to Caucasian patients. In addition, we observed a wider range of objective response rate in patients from real-world clinical practice than those participating in clinical trials and expanded access program. 

A potential explanation of the observed differences was given regarding the genetic factors such as ABCG2 rs2231142, CYP3A5*3 and CYP3A4*22, together with non-genetic factors such as body surface area, sunitinib metabolism-related cooking habit, proportion of second-line use caused by different reimbursement policies among countries.

In Chapter 3, we interrogated eight SNPs in genes CYP3A4, NR1I2, POR, IL8, IL13, IL4-R and MET, which were identified as prognostic or predictive biomarkers of TKIs treatment between 2011 and 2013, to evaluate their genetic associations with sunitinib outcomes in a cohort of 374 mRCC patients. Though initial results were not confirmed, we found that T allele in IL8 rs1126647 was associated with an increased risk of hypertension and T allele in IL13 rs1800925 with an increased risk of leukopenia and any toxicity > grade 2. So far, the function of SNPs in IL8 and IL13 remains unknown. Xu et al. speculated that T-allele of IL8 rs1126647 was related to a higher IL8 expression, whereas Hacking et al. reported an opposite effect on T-allele of IL8 rs1126647. Also, how and to what extent the IL8 and IL13 proteins influence the VEGF pathway and further affect sunitinib treatment outcomes is still unclear. Given the results produced in further validation studies and better understanding of these interleukins’ function, the findings hold the potential to provide insight into novel therapeutic targets.

Encoding the key sunitinib targeting receptor VEGFR1, a variety of SNPs in VEGFR1 gene (especially rs9582036 and rs9554320) have been explored for their predictive value in sunitinib treatment. However, no consistent results for their associations with PFS and
overall survival (OS) could be reached from the current publications. In Chapter 4, the associations of two SNPs with PFS and OS were assessed among three independent cohorts from The Netherlands (SUTOX), Spain (SOGUG) and USA (CCF).

Analogous to published data, our study also showed contradicting results. In the CCF cohort, patients with the variant genotype of rs9582036 or rs9554320 had a significantly shorter median PFS compared with the major-allele carriers. But associations with OS and PFS from initial studies were failed to reproduce in either SUTOX or SOGUG cohort. Based on the combination of our data and published results, a meta-analysis showed that the association of both SNPs with PFS or OS did not reach the threshold for statistical significance. Our conclusion is that VEGFR1 rs9582036 and rs9554320 are not suitable as biomarkers for prediction of efficacy in mRCC patients receiving sunitinib treatment.

Recently, we reported the association of CYP3A4 rs4646437 with sunitinib-induced hypertension. However, the mechanism of the relationship is unclear because the lack of functional data of CYP3A4 rs4646437. Thereby, we have further explored the role of this genetic variant for pharmacokinetics of sunitinib-treated patient (Chapter 5). In a set of 144 sunitinib-treated patients, the A-allele carriers of CYP3A4 rs4646437 had an average 29.3% and 18.9% higher clearance of sunitinib and SU12662, respectively, compared to patients with wild-type genotype. The findings suggest that the A-allele of CYP3A4 rs4646437 might be associated with an increased CYP3A4 enzyme activity. Due to the longer half-life of SU12662 as well as the relatively smaller effect of CYP3A4 rs4646437 on clearance of SU12662, A-allele carriers rather than patients with GG-genotype had a higher accumulation of SU12662, which contributed to the increased risk of sunitinib-induced hypertension.

The direction of the effect of the A-allele in CYP3A4 rs4646437 is in line with that of CYP3A5 rs776746, which was reported in a comparable cohort consisting of 114 sunitinib-treated patients. In analog to this, both CYP3A5 rs776746 and CYP3A4 rs4646437 have been independently associated with sunitinib-induced hypertension in patients with clear cell mRCC (cc-mRCC). Due to the fact that the CYP3A4 and CYP3A5 genes lie in close proximity (136 kb) to one another on chromosome 7 (chr. 7q22.1), the associations of CYP3A4 rs4646437 with clearance and hypertension might be actually due to CYP3A5 rs776746. It has been suggested that CYP3A4 rs4646437 is in high linkage disequilibrium (LD) to CYP3A5 rs776746. Therefore, we simultaneously analysed the associations of
both SNPs with sunitinib clearance in a post hoc analysis in Chapter 5 and with hypertension in Chapter 6. Our results suggest that CYP3A5 rs776746, rather than CYP3A4 rs4646437 is the causal variant in the associations with clearance and hypertension. However, owing to the ethnic differences in the degree of LD, this conclusion is restricted to the Caucasian population (high LD) rather than an African population, in which the LD between two SNPs is relatively low and thereby the impact of CYP3A4 rs4646437 may not be ignored.

With the advent of immune checkpoint inhibitors as potential new drugs for mRCC and the fact that TKIs interact with immune responses, we performed an exploratory study in Chapter 7, aiming to investigate whether polymorphisms of genes involving in immune checkpoints are related to the clinical outcome of cc-mRCC patients treated with sunitinib. This chapter was designed to explore the potential association of sunitinib outcomes with genetic variability in checkpoint-related genes PDCD1, CD274 and CTLA-4 in a large discovery cohort followed by a validation in an independent cohort as well as a meta-analysis. In the discovery cohort of 550 cc-mRCC patients, CTLA-4 rs231775 A>G and CD274 rs7866740 C>G showed significant associations with OS. In the validation cohort of 138 cc-mRCC patients, the associations of both SNPs with OS did not meet the significance threshold of p<0.05. After pooling all the data together, CTLA-4 rs231775 showed a significant association with OS in the multivariable analysis. Patients with the GG genotype showed an increased OS compared to those with the GA or AA genotype. However, no significant results were found in the genetic association with PFS. To provide possible explanations for our interesting finding that CTLA-4 rs231775 was associated with OS, we utilized data from GTEx and OncoLnc. In GTEx, a significantly decreased mRNA expression of the CTLA-4 gene was observed in the GG genotype compared to the AA and AG genotypes in testis. By OncoLnc, mRNA expression levels were linked to the survival data from 537 cc-RCC patients from The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA-KIRC) collection. It was revealed that patients with lower CTLA-4 mRNA expression (lower 50 percentile) had a longer OS. Combining all findings, the G-allele of CTLA-4 rs231775 is indeed associated with lower CTLA-4 mRNA expression, and lower mRNA expression links to longer OS, which is consistent with our results. Interestingly, Song et al. have reported that in patients with advanced non-small cell lung cancer, G-allele carriers of CTLA-4 rs231775 experienced a significantly longer OS.
than those with an AA genotype after correction for many covariates among which was treatment. Together with our findings, it is strongly suggested that the CTLA-4 rs231775 is more of influence on the biological behavior of the disease than with the effects of treatment, implying the prognostic role of this polymorphism.

**CHALLENGES PREVENTING UTILITY OF GENETIC VARIANTS FROM CLINICAL PRACTICE**

The pharmacogenetic studies in mRCC patients so far give important clues on the genetic component of inter-individual variabilities in response to sunitinib treatment and hold the potential to improve our ability of a personalized therapy. Yet, consensus on clinical utility remains missing due to several reasons. Chapter 8 describes a brief answer to the question “what do we need to make genetic biomarker guided treatment for renal cell carcinoma a reality”\(^ {40}\). Hereby, a focus is placed on a detailed discussion of challenges preventing the implementation of genetic variants from clinical practice, followed by potential solutions.

**Part I: retrospective data**

**Heterogeneity of patient characteristics**

Of note, the data from most pharmacogenetic studies were collected retrospectively from various centers across the world and patients were not enrolled in a designated clinical trial. Use of observational data may have the disadvantage of a high heterogeneity among patients. For example, sunitinib has a standard dose regime, but dose adjustment is needed for more than 30% of patients over the whole treatment period.\(^ {41}\) Although both efficacy and toxicity are dose-related, there is no data including dose adjustment as covariate to correct multivariate analyses. Moreover, to reach a sufficient sample size, patient data are collected over a large timeframe. It is likely that clinical practice (i.e. toxicity management) has evolved in the course of time. Unlike efficacy (which is evaluated as time-to-event format), toxicity is usually dichotomized according to grades, rather than taking time-point of occurrence into account. Besides, prior treatment can influence the efficacy of subsequent therapy, and therefore, proportions of patients with prior treatment before sunitinib, especially other TKIs, should not be omitted either (discussed in Chapter 4). Furthermore, source of DNA is also of vital importance. Compared to germline DNA, DNA
isolated from tumor can be biased by intra-tumor heterogeneity, as for instance, the loss of heterozygosity occurred in \textit{CYP2D6} locus in breast cancer.\textsuperscript{42}

\textbf{Ethnicity}

As already described in \textbf{Chapter 2}, there are differences in sunitinib toxicity in Asian and Caucasian mRCC patients, which may be at least partly explained by the genetic diversities among populations owing to the varying minor allele frequency (MAF). One example is \textit{ABCG2} rs2231142, A-allele carriers of which has been associated with higher sunitinib exposure in Japanese\textsuperscript{43, 44} and French\textsuperscript{45} patients. As exposure has been related to the incidence of toxicity and A-allele is more common in Asian (30\%) than in Caucasian (10\%), the ethnic difference in MAF of this SNP might be an underlying cause of higher sunitinib toxicity in Asian patients.\textsuperscript{46} Similarly, \textit{CYP3A5} rs776746 also have different MAF in Asian and Caucasian population, contributing to the altered toxicity incidence.\textsuperscript{10} In addition to diversity in MAF, varying LD among populations should also be taken into account (\textbf{Chapter 5 and 6}). It has been suggested that \textit{CYP3A4} rs4646437 is in high LD to \textit{CYP3A5} rs776746 (\(r^2\) from 0.781 to 0.913), based on a population consisting of Caucasian and African-American persons.\textsuperscript{32} Thus, it is reasonable to hypothesize that \textit{CYP3A5} rs776746 is the causal genetic variant, as its function has been well-studied. However, the degree of LD is different in Asian (Dˈ=1, \(r^2\)=0.558) and African population (Dˈ=0.557, \(r^2\)=0.273).\textsuperscript{47} As a result, the impact of \textit{CYP3A4} rs4646437 should not be ignored when testing genetic associations of both variants to a trait in these populations. Bearing this in mind, our results in this thesis might not be directly translated into another ethnicity without validation.

\textbf{Missing data}

To some extent, missing observations of clinical variables or missing genotypes because of low call rate are unavoidable in pharmacogenetic studies. How to deal with missing value demands caution. Omission could lead to reduced power and cause bias in interpretation of results. Another way to handle missing value is by imputation, which has been used for clinical variables earlier (\textbf{Chapter 4 and 7}). With the arrival of large reference panels such as the 1000 Genomes Project,\textsuperscript{48} imputation is also an optimal tool for those non-genotyped SNPs in genome wide association studies (\textbf{Chapter 7}). Still, imputation in clinical data may have several pitfalls when dealing with non-normally distributed variables (such as binary or categorical variables), data are missing not at random, or computational problem
occurs.\textsuperscript{49} Truly, the higher proportion of missing data will result in an increased impact of these pitfalls on the reliability of imputation. Therefore, when dealing with missing data, it may be important to take this into account. In Chapter 7, imputation for clinical observation was implemented in 16% and 10% patients in the discovery and validation cohort, respectively, which could be considered negligible. With regard to genotyping imputation, the inherent uncertainty of haplotype estimated from 1000 Genomes Project (due to the low-coverage sequencing used) would be a challenge.\textsuperscript{50} In addition, caution is also needed when analyzing rare imputed SNPs, as a small number of rare allele carriers may lead to false positives.\textsuperscript{50} In the discovery analyses of Chapter 7, we observed an association of \textit{CD274} rs7866740 with OS, but failed confirmation in the final analysis, probably it was because \textit{CD274} rs7866740 was imputed and the allele frequency was relatively small.

\textbf{Part II: causality}

Even though the genetic variants in most pharmacogenetic studies were selected based on a drug-related candidate gene approach, whether a genetic association with treatment outcome is caused by the genetic variant itself or another variant in LD with the associated variant is not sure. For example, both \textit{CYP3A5} rs776746 and \textit{CYP3A4} rs4646437 have been independently associated with sunitinib-induced hypertension in cc-mRCC patients.\textsuperscript{8, 28} Due to the high LD of both SNPs exists in Caucasian population, the causal genetic variant of the above mentioned association is actually \textit{CYP3A5} rs776746 (Chapter 6). For those newly identified genetic variants without high LD with other known functional variants, how to interpret the association findings is of challenging. Usually, the potential explanations are often informally described in a picture using arrows pointing in the assumed direction of causality step by step from polymorphisms to genes to proteins to classical phenotypes. However, functional characterization of some genetic variants in pharmacogenetic studies (including the Chapter 3 in this thesis) is absent. Nowadays, with the advent of large-scale “post-genomics” networks consisting of quantitative trait locus mapping, it becomes relatively simple to identify causality between traits and genetic variation. In Chapter 7, the Genotype-Tissue Expression\textsuperscript{36} and The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA-KIRC) collection\textsuperscript{38} were employed to build up the relationship between polymorphism and gene expression as well as survival data,
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aiming to explain the association of \textit{CTLA-4} rs231775 and OS in cc-mRCC patients receiving sunitinib treatment.

\section*{Part III: statistical issues} \label{part_iii}

\subsection*{Sample size} \label{sample_size}

Due to the fact that the prevalence of RCC is not as high as that of other common cancers, the majority of pharmacogenetic studies in sunitinib treated mRCC patients were conducted in relatively small cohorts ranging from 5 to 374 patients.\cite{7} Generally, research findings from underpowered studies are less likely to be true, which could be one reason of the failed validation.\cite{51} Despite our cohort is one of the largest mRCC cohort available for pharmacogenetic studies, the number of patients may be considered relatively small when patients are divided into different groups according to their genotype (especially when minor allele frequency is low) or clinical drug response (such as tumor progression, death and especially severe toxicity with low incidence), which could influence the statistical power. Therefore, sufficient sample size is the most important determinant to provide enough power to detect robust effect of a genetic variant. However, large sample size is not always feasible in pharmacogenetic studies. An alternative option to increase the sample size and statistical power in an investigation is to pool data from several studies into a meta-analysis. As presented in \textit{Chapter 4 and 7} of this thesis, this approach has been used to explore the true effect of genetic variants of \textit{VEGFR1} and \textit{CTLA-4} polymorphisms. In discovery studies, \textit{VEGFR1} rs9582036 and rs9554320 were regarded as promising genetic predictors for sunitinib efficacy,\cite{25} while subsequent validation studies have showed conflicting results.\cite{24,26} Followed by new validations in three small cohorts, a meta-analysis was conducted and finally indicated that the predictive role of both SNPs was limited (\textit{Chapter 4}).\cite{27} With regards to \textit{CTLA-4} polymorphism, the meta-analysis confirmed the conclusion in discovery study that \textit{CTLA-4} rs231775, but not \textit{CD274} rs7866740 was a prognostic biomarker for patients with mRCC (\textit{Chapter 7}). Even though meta-analysis has been successfully applied to reach a sufficient sample size and help resolve inconsistencies, the limitations such as the heterogeneity of included studies leading to erroneous inferences, should not be ignored.\cite{52} In addition, owing to the publication bias in studies with negative results, conclusions in the meta-analysis should be given in a suggestive way.
Chapter 9

Diversities in statistical analysis

It is true that genetic variants alone cannot completely explain inter-individual variations in drug responses. Demographic and clinical characteristics of patients are important to include for analyses in pharmacogenetic association studies. Indeed, several studies linked different non-genetic factors to sunitinib outcome in mRCC patients. For example, the MSKCC risk system\(^5\) or the Heng prognostic risk criteria\(^6\) or some specific factors included in these criteria are considered as the essential covariates when efficacy (PFS or OS) is evaluated as endpoint in the genetic association studies (discussed in Chapter 4). However, with regards to toxicity, there is no definite factor related to causality of a specific adverse effect. In this case, factors with significant associations in univariate analyses are treated as covariates. Yet, these unrecognized factors might dilute or obscure true associations. As a rule, multivariate analyses are more powerful than univariate genetic analyses, and therefore, varying covariates used in genetic association studies should not be forgotten as a part of problems leading to the reproductive failure in the validation study. However, it is unrealistic to use an uniform statistical methodology in such analyses due to the fact that most genetic association studies are performed using retrospective data, which are not as informative as data collected from perspective studies.

Multiple testing

Multiple testing is an important issue particularly in the field of genetic association studies. In a total, our cohort has been tested for more than 60 SNPs from genes in pharmacokinetic and pharmacodynamic pathways in several studies so far.\(^8, 19, 27, 28\) Owing to the fact that multiple testing creates false positive results, correction is therefore suggested. Bonferroni correction is the most common correction method, the result of which is to offer a corrected significance threshold to be reached through dividing 0.05 by number of test. However, SNPs in this thesis were selected based on a strong prior study hypothesis or by tagging principle, and therefore, were not entirely independent. As a conservative test, Bonferroni correction will increase the type II error (the chances of false-negative results) and may fail to notice real existing differences.\(^53\) Moreover, it has been reasoned that correction for multiple testing is not necessary when there is a strong basis for expecting the results to be true.\(^53\) Furthermore, an inflated type I error due to multiple comparisons is better solved by a validation study than by a Bonferroni correction (Chapter 3, 4 and 7).
Effect size and confidence interval instead of p-value

Mostly, scientific journals and studies weigh more on statistical significance (based on $p$-value) than on effect size and confidence interval. However, an estimation of the effect size and the confidence interval are far more important in pharmacogenetic quantitative studies and a shift in emphasis from hypothesis testing ($p$-value) to estimation (effect size) has been promoted. Several examples were given in this thesis. In Chapter 5, the A-allele carriers of $CYP3A4$ rs4646437 had an average 29.3% higher clearance of sunitinib with a $p$-value of 0.006, compared to patients with wild-type genotype. Although, the estimated effect size of 29.3% is regarded as clinically relevant ($\geq 25\%$) in sunitinib treatment, the 95% confidence interval (8.5% to 53.4%) ranged widely, implying the predictive utility of $CYP3A4$ rs4646437 is still limited. Another example is presented in Chapter 6, in which $CYP3A5$ rs776746 and $CYP3A4$ rs4646437 were analyzed simultaneously with sunitinib-induced hypertension. Even though no SNP reached the significance threshold of 0.05, the odds ratio (OR) of $CYP3A5$ rs776746 changed only marginally while the OR of $CYP3A4$ rs4646437 changed substantially. Consequently, our conclusion was that $CYP3A5$ rs776746, rather than $CYP3A4$ rs4646437 is the causal variant of this genetic association.

FUTURE PERSPECTIVES

Post-candidate gene approach era

Predominantly, the SNPs investigated in this field were selected based on a candidate gene approach, the limitations of which are obvious. That is the gene selection largely relied on prior knowledge. With the arrival of high-throughput, low-cost genotyping platforms, genome wide association study (GWAS) is considered as an unbiased approach as compared to candidate gene approach. In 2014, the first GWAS on TKI pharmacogenetics was performed in 1099 mRCC patients treated by pazopanib or sunitinib, who participated in clinical trials. Genetic variants from $LOXL2$, $ENTPD4$, $UGT1A1$, $ANAPC4$ and $SLC34A2$ were significantly associated with efficacy or toxicity. Hereafter, another GWAS was conducted on sunitinib efficacy in 550 mRCC patients who were enrolled from the European consortium on “TArgeted therapy in Renal cell cancer: GEnetic and Tumor related biomarkers for response and toxicity” (EuroTARGET). The preliminary analyses showed that variant alleles of 9 novel SNPs on chromosome 21 were significantly associated with an inferior PFS ($P<5\times10^{-8}$) with a hazard ratio ranging from 2.3 to 2.4
The follow-up studies of this EuroTARGET project are still ongoing.

As deduced from clinical trials, target total trough level (TTL, sunitinib plus SU12662) is suggested to be in the range of 50-100ng/ml. Still, some patients receiving standard regimen have TTL higher than 100 ng/ml and thereby have a higher risk of toxicity.\(^{57}\) Previous studies with candidate gene approach have investigated the associations of SNPs located in pharmacokinetic (PK) genes with sunitinib PK parameters.\(^{30}\) Yet, no genetic biomarker has been widely validated in large independent cohorts. Earlier, the approach of GWAS has been successfully applied in detecting genetic determinants for warfarin dose.\(^{58}\) Likewise, GWAS could also provide us with better biological understanding of the relationships among genetic variants and variability of sunitinib PK characteristics.

Even though GWAS takes variants from “non-functional” regions into account, it does not provide adequate coverage of rare variations (MAF lower than 5%). The potential solutions include (i) genotype imputation, which is the most economical method of investigating rare non-genotyped variation, but also has the obvious risk (discussed before); (ii) whole exome sequencing (WES), which only focuses on protein-coding regions; (iii) whole genome sequencing (WGS), which provide an even broader range of genetic variation (i.e. insertion, deletion, translocation and copy number variation).

**Treatment beyond sunitinib**

The great success of first generation of TKIs (including sorafenib, sunitinib and pazopanib) shed light on development of more potent and selective drugs in this pathway aiming to improve efficacy and tolerability. As a result, axitinib, lenvatinib and cabozantinib have been approved by FDA successively.\(^{59}\) Besides the fast development of angiogenesis inhibitors, an improved understanding of immune checkpoint pathway has promoted the approval of nivolumab, a programmed death-1 (PD-1) inhibitor in 2015 due to its durable remission and manageable toxicity profile.\(^{60}\) Furthermore, ongoing trials are likely to result in addition to these in the near future, for example new TKIs (cediranib and regorafenib), PD-1 inhibitors (pembrolizumab), PD-1 ligand (PD-L1) inhibitor (atezolizumab, durvalumab and avelumab), and cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitor (ipilimumab and tremelimumab).\(^{33}\) In addition to monotherapy, several combinations, such
as sunitinib or pazopanib combined with nivolumab\textsuperscript{61} as well as ipilimumab combined with nivolumab,\textsuperscript{33} have emerged as promising treatments for mRCC patients.

With the significant progress in treatment options for mRCC patient, a new challenge is faced: how to make an optimal choice among these therapies. Currently, sunitinib, pazopanib and bevacizumab plus interferon-alpha are first-line treatment options for treatment-naïve patients with cc-mRCC and a good-to-intermediate prognostic risk score while temsirolimus for cc-mRCC patients with poor prognostic risk score, according to European Association of Urology 2014.\textsuperscript{62} However, after the failure of first-line treatment, what is the best subsequent therapy remains unknown due to the absence of evidence-based sequencing guidelines and biomarkers. In addition, nearly 25% of mRCC patients have intrinsic resistance to treatment during first-line TKI therapy and the remaining who respond initially will eventually display acquire resistance.\textsuperscript{63} Even though several hypotheses have been proposed, the resistance mechanisms are unclear yet. Besides, the lack of ability to promptly capture tumor evolution is the major reason for the failure of systemic treatment because of the difficulties in obtaining repeated tumor biopsy.

**Circulating tumor DNA**

Recently, circulating tumor DNA (ctDNA), serving as a non-invasive “liquid biopsy”, has been suggested as a promising tool to reveal tumor genetic changes, monitor treatment resistance and select subsequent treatment.\textsuperscript{64} Several investigating studies in melanoma,\textsuperscript{65} breast\textsuperscript{66} and lung cancers\textsuperscript{67} have shown a good correlation of ctDNA level with tumor changes and treatment outcome. However, no studies have correlated ctDNA with tumor burden in RCC. In a pilot study, ctDNA was detected in 1 of 4 patients.\textsuperscript{68} Hereafter, Hahn et al. reported a discordance in the type of genomic alteration when comparing the results of NGS from tumor tissue and ctDNA from 19 mRCC patients.\textsuperscript{69} Several reasons are possible for the failure of ctDNA in RCC including the large genetic heterogeneity within in a single lesion as well as the challenge in sensitivity of ctDNA detection. Besides the value of ctDNA in providing guidance for personalized treatment, profiling of ctDNA might provide new targeting points for treatment development. Pal et al.\textsuperscript{70} have assessed and compared the ctDNA profile in a cohort of mRCC patients who received first-line or post first-line treatment. Disparity in genomic alterations in post first-line versus first-line were in \emph{TP53} (49\% vs 24\%), \emph{VHL} (29\% vs 18\%), \emph{NF1} (20\% vs 3\%), \emph{EGFR} (15\% vs 8\%),
and PIK3CA (17% vs 8%), indicating potential mechanism to identify potential actionable targets.  

**CONCLUSION**

The studies in this thesis have provided important evidence for the role of pharmacogenetics to individualize sunitinib treatment in mRCC, described challenges preventing utility of genetic biomarkers in clinical practice, and finally foresaw the development in new technologies which could lead us to a better understanding of genetic signature of tumors and hopefully a better treatment strategy and hence clinical outcome of our patients.
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