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Expert Review of Molecular Diagnostics

ISSN: 1473-7159 (Print) 1744-8352 (Online) Journal homepage: informahealthcare.com/journals/iero20

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To cite this article: Meta H. M. Diekstra, Jesse J. Swen, Hans Gelderblom & Henk-Jan Guchelaar (2016) A decade of pharmacogenomics research on tyrosine kinase inhibitors in metastatic renal cell cancer: a systematic review, Expert Review of Molecular Diagnostics, 16:5, 605-618, DOI: 10.1586/14737159.2016.1148601

To link to this article: https://doi.org/10.1586/14737159.2016.1148601

EXPERT REVIEW OF MOLECULAR DIAGNOSTICS, 2016
VOL. 16, NO. 5, 605–618
http://dx.doi.org/10.1586/14737159.2016.1148601

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ORIGINAL RESEARCH

OPEN ACCESS

A decade of pharmacogenomics research on tyrosine kinase inhibitors in metastatic renal cell cancer: a systematic review

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ABSTRACT

Objective: The individual response to targeted tyrosine kinase inhibitors (TKIs) in the treatment of metastatic renal cell cancer (mRCC) is highly variable. Outlined in this article are findings on potential biomarkers for TKI treatment outcome in mRCC and an evaluation of the status of clinical implementation.
Methods: Articles were selected by two independent reviewers using a systematic search in five medical databases on renal cell carcinoma, TKIs, and pharmacogenetics.
Results: Many researchers have focused on predictive biomarkers for treatment outcome of targeted therapies in mRCC patients. Attempts to explain differences in efficacy and toxicity of TKIs by use of genetic variants in genes related to the pharmacokinetics and pharmacodynamics of the drug have been successful.
Conclusion: Most findings on potential biomarkers have not been validated and therefore biomarker testing to guide choice of therapy and dose in mRCC is not yet feasible.

ARTICLE HISTORY
Received 1 December 2015
Accepted 27 January 2016
Published online
16 February 2016

KEYWORDS
pharmacogenetics; pharmacogenomics; tyrosine kinase inhibitor; metastatic renal cell cancer; single nucleotide polymorphism; biomarker; toxicity; efficacy

Using pharmacogenomics in our endeavor to personalize mRCC treatment

Renal cell cancer (RCC) represents ~3% of cancer in adults (approximately 170,000 people worldwide per year). RCC typically occurs in adults aged 50–70 years and is more common in men than in women. For many years, cytokine therapies (interleukin-2 and interferon-alpha) were the only available treatment options with limited efficacy and considerable toxicity [1,2]. In the last decade, multiple targeted agents for the treatment of metastatic RCC (mRCC) have become available, namely: the multitargeted oral tyrosine kinase inhibitors (TKIs) with antiangiogenic activity. Of the first generation TKIs, sorafenib was approved for the treatment of mRCC in 2005, followed by sunitinib (2006) and pazopanib (2009). The first-generation TKIs target the vascular endothelial growth factor (VEGF) pathway by inhibition of the vascular endothelial growth factor receptors (VEGFR-1, 2, and 3) but also inhibit many other targets. Sorafenib, sunitinib, and pazopanib all target the platelet-derived growth factor receptor (PDGFR) α and/or β and the c-Kit protein (c-Kit). Additionally, sunitinib and sorafenib inhibit FMS-like tyrosine kinase 3 (Flt-3) and glial cell line-derived neurotrophic factor receptor (REarranged during Transfection) tyrosine kinases. Furthermore, sunitinib inhibits colony-stimulating factor 1 receptor, and sorafenib specifically inhibits the RAF kinases (C-RAF and B-RAF) [3–6].

Starting 2012, the second-generation TKIs emerged with an improved potency and selectivity, such as axitinib. Many of these second-generation TKIs were assessed for the treatment of mRCC, such as tivozanib, cediranib, and cabozantinib [2–5,7,8]. Axitinib predominantly blocks the VEGF receptors, with a 50–450 times higher potency than the first-generation TKIs and less off-target activity, which could explain the larger therapeutic window and fewer side effects [2,7].

Increased exposure to sunitinib is associated with improved survival outcomes but also an increased risk for adverse events [3,5]. Sunitinib revealed an improvement in progression-free survival (PFS) and objective response rate compared to interferon-alpha ($P < 0.001$) and showed a tendency towards an improved overall survival (OS) ($P = 0.051$) [3]. For several years, sunitinib has been considered the first-choice treatment for mRCC. However, based on the results of the PISCES trial (a randomized, controlled, double-blind, cross-over trial assessing treatment preference for pazopanib versus sunitinib in patients with metastatic renal cell carcinoma) and the larger COMPARZ trial (a phase 3 study of pazopanib versus sunitinib in the treatment of locally advanced and/or metastatic renal cell carcinoma)[9,10] indicating noninferiority for pazopanib with regard to toxicity, patient preference, and quality of life, both pazopanib and sunitinib are prescribed as first-line treatment for mRCC [9,10].

The individual response to TKIs is highly variable in mRCC patients: some experience severe toxicities for which dose reductions or even cessation of therapy is needed, while others show no response at all. Previous studies identified single nucleotide polymorphisms (SNPs) in genes encoding enzymes or transporters related to pharmacokinetics (PK) and pharmacodynamics (PD) of TKIs which have been associated with toxicity and efficacy of TKIs [11,12]. Also cytokines and antiangiogenic factors have been studied as potential predictors for TKI treatment outcome in mRCC patients.

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Supplemental data for this article can be accessed here.

Ultimately, biomarker testing prior to start of therapy may result in providing the individual patient the most effective treatment with the least possible side effects.

Several review articles on pharmacogenomics in mRCC have been published [11–14], but only one with a systematic approach [14]. In our current systematic review article, we strictly focus on the predictive value of biomarkers (as opposed to the prognostic value), especially SNPs (pharmacogenetic biomarkers) and only in TKI treatment. Here, we outline the findings from currently available pharmacogenomics studies on potential predictive biomarkers for mRCC treatment outcome. In addition, we evaluate the quality of the selected studies, clarify the status on implementation of these predictive biomarkers into the clinic, and give recommendations as to what the focus of attention should be in future studies.

Methods

Protocol

A systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [15]. Prior to the literature search, a systematic review protocol was prepared following the PRISMA-P 17-item checklist (Supplementary document 1) [15] and registered at the international prospective register of systematic reviews (PROSPERO; CRD42015016509) [16].

Systematic literature search

Together with an experienced librarian, we performed a systematic search in 5 medical databases: MEDLINE, EMBASE (OVID version), Web of Science, The Cochrane Library, and Cochrane Central Register of Controlled Trials (CENTRAL). This search comprised three topics: renal cell carcinoma, TKIs (e.g. sunitinib, sorafenib), and pharmacogenetics (Supplementary document 2). All databases were searched for keywords on these items taking into account the terminological and technical differences between these databases. Various synonyms and related terms for all subjects were used. The search was performed on 14 January 2015. Results were limited to articles in the English language and from the year 2004 onwards. Meeting abstracts were excluded. From our initial search up to the moment of our final selection of articles, new studies may have appeared. To cover articles published after 14 January 2015, the initial search was repeated in MEDLINE on 31 May 2015.

Selection process and data extraction

Articles were first selected by title, then by abstract, and finally relevant data were extracted systematically from included full text articles. Selection of included articles was performed by two independent reviewers (MD, JS). In case of disagreement, a third reviewer (HJG) made the final decision. References were eligible when genetic biomarkers (i.e. SNPs, genetic variants, gene/protein expression) were investigated in relation to TKI treatment and mRCC. Review articles, preclinical studies (animal models and *in vitro* studies), phase I or II clinical trials, and papers discussing pathology, prognosis, tumor biology, or somatic mutations (e.g. Von Hippel-Lindau mutations) were excluded. Accordingly, articles were excluded if nontreatment-related biomarkers were studied or if no TKI response was assessed, because these generally consider prognostic biomarkers.

Extracted data were first author, year of publication, demographics (age, sample size, sex, ethnicity), sample material and origin (i.e. germline DNA from blood, serum, or other), methods, tumor type, drug information (i.e. drug name, treatment line, dose), evaluated biomarkers, endpoints, and statistical approach. Articles resulting from the secondary search on 31 May 2015 were subject to an identical selection process. Using cross-references of the included articles from our systematic search, relevant articles were added to our final selection.

Clinical validity (the robustness of the statistical association between a pharmacogenetic variant and outcome of drug therapy) and clinical utility (a test's health-care value) of the included articles was evaluated in a descriptive manner [17,18].

Results

Search results

Our primary search identified 968 articles (Supplementary document 2). The secondary search identified 30 additional articles. After selection by title, abstract, and full text, a total of 54 articles were available for evaluation in this review. Figure 1 represents a flowchart on the study selection process. The extracted data from the included articles are presented in Table 1 and Supplementary document 3.

SNPs associated with TKI treatment outcome in mRCC patients

From 2007 up till now, several genetic variants have been assessed for their possible association with the treatment outcome of sunitinib, pazopanib, sorafenib, or axitinib. These SNPs have been selected according to the candidate gene approach based on the knowledge on PK and PD of the drug. The reported findings in this field are outlined below and summarized in Table 1 and Figure 2 [19–44].

Pharmacogenetics to predict sunitinib toxicity

The first pharmacogenetic study on sunitinib tested for associations of genetic polymorphisms with toxicity endpoints ($P < 0.05$) in a cohort of 219 patients with mRCC (n = 159), gastrointestinal stromal tumor (GIST) (n = 50), or other tumors (n = 10). Van Erp *et al*. observed an increased risk for leukopenia in *CYP1A1* rs1048943, and *FLT3* rs1933437 and absence of CAG in the *NR1I3* haplotype (rs2307424, rs2307418, rs4073054). *CYP1A1* rs1048943 was associated with mucosal inflammation, and presence of TTT in *ABCB1* (rs1045642, rs1128503, rs2032582) was associated with HFS. Presence of the TT copy in *ABCG2* (rs55930652, rs2622604) and *VEGFR2* rs2305948 was associated with any toxicity >grade 2 [19].

Later, in a group of 95 mRCC patients, Garcia-Donas *et al*. reported that the *CYP3A5* A-allele (*CYP3A5*1*) of rs776746 was

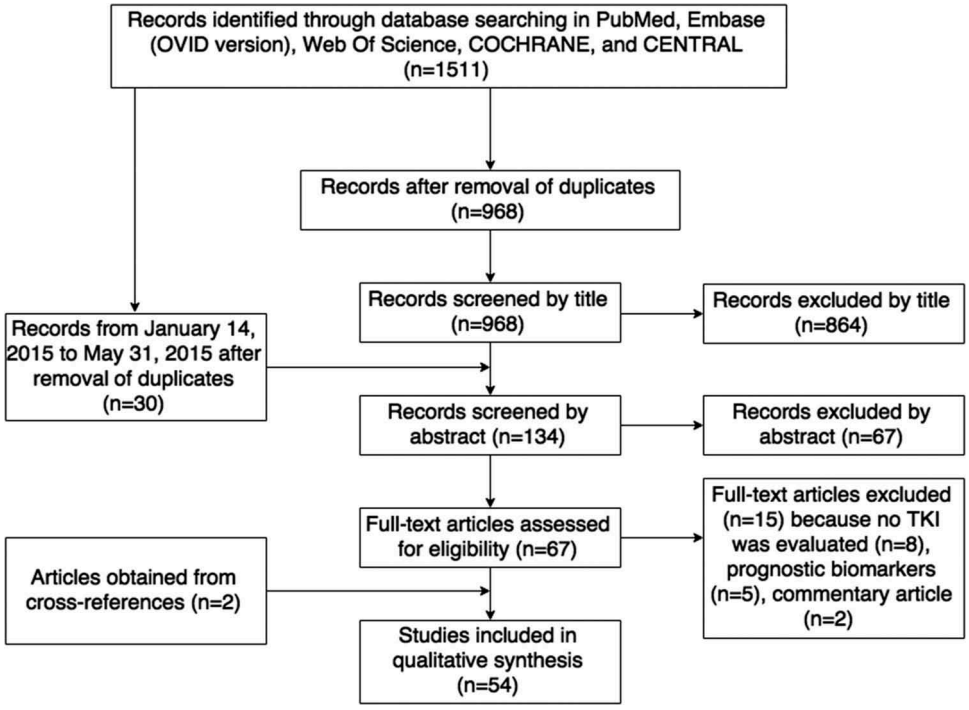

Figure 1. Flowchart of study selection process. Our original search on the 14th of January 2015 resulted in a total of 968 articles (Supplementary document 2). For the first selection by title, there was a disagreement between the two independent reviewers on a number of 114 titles (12%). After consensus was reached with the last review author (HJG), this resulted in a number of 104 articles which was complemented with 33 additional articles of the search from January 14 to May 31, 2015. A total of 134 articles was available for the second selection step based on abstract reading. Consensus had to be reached for 20 of the 134 abstracts (15%) and resulted in exclusion of 67 articles. Consequently, a number of 67 articles underwent full text review and data extraction. Finally, 15 articles were excluded for data extraction because no treatment was evaluated (n = 8), prognostic biomarkers were investigated (n = 5) or these were no original articles but commentaries (n = 2) and using cross-references, we included two additional studies [37, 69]. Ultimately, a total of 54 articles was included in our systematic review.

associated with dose reductions due to toxicity (adjusted $P < 0.05$) [20]. In a study of Beuselinck *et al*., an increased time-to-dose reduction was seen for the TT genotype patients of rs1128503 and rs2032582 in *ABCB1* compared to C-allele and G-allele carriers, respectively [21].

Mizuno *et al*. [22,23] observed a case of severe hematological toxicity in an RCC patient with the AA genotype of rs2231142 in *ABCG2*. This prompted a study in 5 patients (1 variant homozygous, 3 heterozygous, and 1 wild-type homozygous) in which they observed an elevated sunitinib exposure for AA carriers [22]. The variant allele A is common in Asians (~30%), but rare in Caucasians (~10%) and African Americans (~5%). In a subsequent study with 19 Japanese patients, only 1 patient had the AA genotype of rs2231142 in *ABCG2* [23]. It was reported that this SNP was associated with an increase in systemic exposure to sunitinib, possibly causing thrombocytopenia and hypertension [23]. Miura *et al*. [24] followed with a case report in 2014 describing a patient with mRCC who developed severe grade 3 and 4 adverse events on sunitinib, namely fever, thrombocytopenia, transaminase elevation, hypoxia, and pulmonary edema. Again, the AA genotype of rs2231142 in *ABCG2* was suggested to explain these toxicities in Asians.

Hypertension is a common adverse effect of sunitinib. Eechoute *et al*. identified genetic biomarkers by using the pathway approach using data from 255 patients with clear cell mRCC (n = 167), non-clear cell mRCC (n = 22), an unknown type of RCC (n = 4), GIST (n = 53), or another tumor type (n = 9) [25]. An ACG haplotype in *VEGF-A* (rs699947, rs833061, rs2010963) was associated with elevations in systolic blood pressure (SBP) and mean arterial pressure (MAP). The same haplotype in *VEGF-A* and presence of the C-allele of rs2070744 in *eNOS* showed a tendency to develop grade 3 hypertension [25]. Similarly, Kim *et al*. [26] evaluated 63 sunitinib-treated mRCC patients for the association of SNPs in *VEGF* or *VEGF-R* with hypertension and clinical outcome. The GG genotype of SNP rs2010963 in *VEGF* was associated with a greater chance for the occurrence and duration of hypertension [26].

Sunitinib-induced toxicities in relation to pharmacogenetic determinants were studied by Kim *et al*. [27] in a number of 65 Korean mRCC patients. Compared to C-allele carriers, the AA genotype of rs2231142 in *ABCG2* had an increased risk for grade 3 or 4 thrombocytopenia, neutropenia, and HFS [27]. In addition, a case report of Takayoshi *et al*. [28] reported a 65-year-old woman with mRCC who showed severe toxicities and was found to have the TTT haplotype on *ABCB1* (rs1045642, rs1128503, rs2032582). It was thought that the severe toxicities as observed in this patient could be associated with a high sunitinib exposure, probably explained by the *ABCB1* genetic polymorphism [28].

Teo *et al*. [29] reported an association analysis in a Chinese population of 25 patients to investigate the effect of SNPs on both PK and clinical outcome (i.e. toxicity and response). The variant CC genotype of rs1045642 in *ABCB1* was associated with an increased risk for rash and mucositis as compared to T-allele carriers ($P < 0.05$). No association with sunitinib treatment outcome was observed for rs776746 in *CYP3A5* [29].

In order to verify the value of earlier reported associations, Diekstra *et al*. [30] pooled patients from exploratory studies in

Table 1. Reported SNP associations ($P \leq 0.05$) with TKI treatment outcome in mRCC from 2009 to 2015.

First Author, year	TKI	No. of patients	Ethnicity	Tumor type	Associated SNPs	Study endpoint	Effect size	95% CI	*P* value
van Erp *et al.*, 2009 [19]	SUN	219	White: 93.6%	mRCC: n = 159, GIST: n = 50, other: n = 10	CYP1A1 rs1048943	Leukopenia	OR:6.24	1.20–32.42	$P = 0.029$
					FLT3 rs1933437		OR:0.36	0.17–0.77	$P = 0.008$
					NR1I3 (rs2307424, rs2307418, rs4073054) CAG haplotype		OR:1.74	1.02–2.96	$P = 0.041$
					VEGFR2 rs2305948	Any toxicity >grade 2	OR:2.39	1.02–5.60	$P = 0.046$
					ABCG2 (rs55930652, rs2622604)		OR:0.38	0.17–0.83	$P = 0.016$
					CYP1A1 rs1048943	Mucosal inflammation	OR:4.03	1.24–13.09	$P = 0.021$
					ABCB1 (rs1045642, rs1128503, rs2032582) TTT haplotype	HFS	OR:0.39	0.16–0.94	$P = 0.035$
Garcia-Donas *et al.*, 2011 [20]	SUN	89 (efficacy)	Mostly European	CC	VEGFR3 rs307826	PFS	HR:3.57	1.75–7.30	$P = 0.0079$
					VEGFR3 rs307821		HR:3.31	1.64–6.68	$P = 0.014$
		95 (toxicity)			CYP3A5 rs776746	Dose reductions due to toxicity	HR: 3.75	1.67–8.41	$P = 0.022$
Beuselinck *et al.*, 2014 [21]	SUN	96	95% CAU	CC = 92% (For efficacy analysis: only CC patients were considered)	ABCB1 rs1125803	Time-to-dose-reduction	–	–	$P = 0.012$
					ABCB1 rs2032582	(TTDR)	–	–	$P = 0.024$
Mizuno *et al.*, 2010 [22]	SUN	5	JAP	RCC (subtype not mentioned)	ABCG2 rs2231142	Exposure and toxicity	–	–	$P < 0.05$
Mizuno *et al.*, 2012 [23]	SUN	19	JAP	RCC (subtype not specified)	ABCG2 rs2231142	Dose-adjusted AUC	–	–	$P = 0.02$
Miura *et al.*, 2014 [24]	SUN	1	JAP	CC with sarcomatoid components	ABCG2 rs2231142	Grade 3 and 4 toxicity	–	–	–
Eechoute *et al.*, 2012 [25]	SUN	255	91% CAU	CC: n = 167 non-clear cell: n = 22, unknown RCC: n = 4, GIST: n = 53, other: n = 9	VEGFA (rs699947, rs833061, rs2010963)	SBP	–	–	$P = 0.014$
						MAP	–	–	$P = 0.036$
					ACG haplotype	Grade 3 hypertension	OR:0.59	0.34–1.03	$P = 0.031$
					eNOS rs2070744	Grade 3 hypertension	OR:2.62	1.08–6.35	$P = 0.045$
Kim *et al.*, 2012 [26]	SUN	63	CAU	CC	VEGFA rs2010963	Prevalence of hypertension	OR:13.62	3.71–50.04	$P = 0.05$
					VEGFA rs2010963	Duration of hypertension	21%	12%–29%	$P = 0.02$
					VEGFA rs3025039 + *VEGF-R2* rs2305948	OS	HR:3.18	1.12–8.99	$P = 0.03$
Kim *et al.*, 2013 [27]	SUN	65	Asian (Korea)	CC = 93.8%	ABCG2 rs2231142	Grade 3 or 4 thrombocytopenia	OR:9.90	1.16-infinity	$P = 0.04$
						Grade 3 or 4 neutropenia	OR:18.20	1.49–222.09	$P = 0.02$
						Grade 3 or 4 HFS	OR:28.46	2.22–364.94	$P = 0.01$
Takayoshi *et al.*, 2015 [28]	SUN	1	Probably JAP	CC	ABCB1 (rs1045642, rs1128503, rs2032582)	Severe toxicity	–	–	–
Teo *et al.*, 2015 [29]	SUN	25	CHI = 86.1% (other Malay or Indian)	RCC (subtype not specified)	ABCB1 rs1045642	Sunitinib exposure	76.81 vs 56.55 ng/ml		$P = 0.03$
						All-grade rash	RR:3.00	1.17–7.67	$P < 0.05$
						All-grade mucositis	RR:1.60	1.10–2.34	$P < 0.05$
						Disease progression	RR:4.57	1.08–19.42	$P = 0.05$
Diekstra *et al.*, 2015 [30]	SUN	333	96% = white	CC	CYP3A5 rs776746	Dose reduction	OR:2.0	1.0–4.0	$P = 0.039$
					ABCB1 (rs1128503, rs2032582, rs1045642)	PFS	HR:1.9	1.3–2.6	$P = 0.000275$

(*Continued*)

Table 1. (Continued).

First Author, year	TKI	No. of patients	Ethnicity	Tumor type	Associated SNPs	Study endpoint	Effect size	95% CI	*P* value
van der Veldt *et al*., 2011 [31]	SUN	136	CAU: 95.6%	CC	CYP3A5 rs776746	PFS	HR:0.27	0.08–0.89	$P = 0.032$
					NR1I3 (rs2307424, rs2307418, rs4073054) CAT haplotype		HR:1.76	1.11–2.79	$P = 0.017$
					ABCB1 (rs1045642, rs1128503, rs2032582) TCG haplotype		HR:0.52	0.29–0.95	$P = 0.033$
Scartozzi *et al*., 2013 [32]	SUN	84	CAU (Italy)	CC = 92%	VEGFA rs833061	PFS	HR:0.71	–	$P = 0.0197$
						OS	HR:0.69		$P = 0.0011$
					VEGFA rs2010963	PFS	HR:0.19	–	$P = 0.0201$
					VEGFR3 rs6877011	PFS; OS	HR:0.35; HR:0.39	–; –	$P < 0.0001$; $P < 0.0001$
Motzer *et al*., 2014 [33]	SUN	202	88% CAU	mRCC	VEGFA rs699947 VEGFA rs1570360 VEGFR3 rs448012 VEGFR3 rs307821 VEGFR3 rs307826	PFS, OS, objective response or time-to-tumor progression	No significant correlations		
Beuselinck *et al*., 2013 [34]	SUN	88	94% CAU	CC	ABCB1 rs1128503	PFS	HR:0.464	0.234–0.918	$P = 0.027$
					NR1I3 rs4073054		HR:1.864	1.082–3.210	$P = 0.025$
					VEGF-R3 rs307821		HR:1.981	1.060–3.702	$P = 0.032$
					FGF-R2 rs2981582		HR:2.669	1.094–6.511	$P = 0.031$
					NR1I2 rs2276707		HR:2.978	1.012–8.761	$P = 0.047$
					ABCB1 rs1128503	OS	HR:0.415	0.193–0.894	$P = 0.025$
					NR1I3 rs4073054		HR:1.927	1.046–3.549	$P = 0.035$
					VEGF-R3 rs307821		HR:2.265	1.202–4.268	$P = 0.011$
					NR1I3 rs2307424		HR:1.913	1.006–3.636	$P = 0.048$
					VEGF-R3 rs307826		HR:2.223	1.187–4.163	$P = 0.013$
Beuselinck *et al*., 2014 [35]	SUN	91	93% CAU	CC: n = 87, papillary: n = 4	VEGF-R1 rs9582036	Response Rate (RR)	0% vs. 46%		$P = 0.028$
						OS	HR:0.249	0.078–0.799	$P = 0.008$
					VEGF-R1 rs9554320	PFS	HR:2.713	1.321–5.575	$P = 0.005$
Mizuno *et al*., 2014 [36]	SUN	19	JAP	RCC (subtype not specified)	ABCG2 rs2231142	Oral clearance (CL/F)	Improvement of PK model: ΔOFV = 8.59 ($P = 0.003$)		
Diekstra *et al*., 2014 [37]	SUN	114		RCC: n = 69, neuro-endocrine tumors: n = 14, GIST: n = 8, other solid tumor type: n = 23	CYP3A4 rs35599367	Sunitinib clearance	−22.5%	–	$P = 0.01$
					CYP3A5 rs776746	SU12662 clearance	+28.0%	–	$P = 0.04$
					ABCB1 rs2032582	Sunitinib clearance	+18.0%	–	$P = 0.02$
Xu *et al*., 2010 [38]	PAZ	116 (exploratory) 130 (replication)	White	RCC (subtype not specified)	UGT1A1 rs8175347 (*28) TA7 homozygotes	Bilirubin levels	OR:13.1	5.3–32.2	$P = 4.5 \times 10^{-5}$
Xu *et al*., 2011 [39]	PAZ	115 (exploratory) and 128 (replication)	White	RCC (subtype not specified)	HFE rs2858996+rs707889 (in strong LD)	ALT levels	OR:39.7	2.2–703.7	$P = 0.006$
Xu *et al*., 2011 [40]	PAZ	397	White:84%	RCC (subtype not specified)	IL8 rs1126647	PFS	HR:1.8	1.2–2.7	$P = 0.009$
					HIF1A rs11549467		HR:1.8	1.1–3.1	$P = 0.03$
					HIF1A rs11549467	Response rate (RR)	RR:30% vs. 43%		$P = 0.02$
					NR1I2 rs3814055		RR:37% vs. 50%		$P = 0.03$
					VEGF-A rs833061		RR:33% vs. 51%		$P = 0.02$

(*Continued*)

Table 1. (Continued).

First Author, year	TKI	No. of patients	Ethnicity	Tumor type	Associated SNPs	Study endpoint	Effect size	95% CI	*P* value
Motzer *et al*., 2013 [41]	SUN or PAZ	719 (SUN: n = 350, PAZ: n = 369)	Europe, North America, Asia, Australia	CC	UGT1A1 rs8175347 and rs4148323 (*28,*37 and *60)	Bilirubin levels on sunitinib	OR:4.51	1.26–16.11	$P = 0.024$
						Bilirubin levels on pazopanib	OR:3.65	1.31–10.16	$P = 0.012$
Xu *et al*., 2015 [42]	PAZ or SUN	3 substudies: (1) 186 pts (PAZ) (2) 690 pts (353 PAZ + 337 SUN) (3) 88 (SUN)	White = 87%, 62%, 98%	Not specified for study 1 + 2, for study 3: CC	IL8 rs1126647	OS	HR:1.32	1.15–1.52	$P = 8.8 \times 10^{-5}$
Tsuchiya *et al*., 2013 [43]	SOR	33	JAP	RCC (subtype not specified)	ABCC2 rs717620	High-grade skin rash	35 vs. 0%	–	$P = 0.032$
					HLA-A*24		–	–	$P = 0.049$
Escudier *et al*., 2015 [44]	AXI and SOR	305 (AXI: n = 159, SOR: n = 146)	94.1% = white	CC component	VEGF-R2 rs2071559	PFS sorafenib	2.223	1.268–3.897	$P = 0.0053$
						OS sorafenib	2.584	1.390–4.803	$P = 0.0027$

ABCB1: ATP-binding cassette member B1; ABCG2: ATP-binding cassette member G2; ALT: alanine aminotransferase; AST: aspartate aminotransferase; AXI: axitinib; CAU: Caucasian; CC: clear cell renal cell carcinoma; CHI: Chinese; GIST: gastrointestinal stromal tumor; JAP: Japanese; ΔOFV: delta Objective Function Value; OS: overall survival; PAZ: pazopanib; PFS: progression-free survival; PK: pharmacokinetic; SOR: sorafenib; SUN: sunitinib.

The Netherlands, Spain, and the United States [19,20,25,26,31], resulting in a sample size of 333 mRCC patients. Data confirmed the association of *CYP3A5*1* with the need for dose reductions ($P < 0.05$) as presented earlier by Garcia-Donas *et al*. [20,30].

Pharmacogenetics to predict sunitinib efficacy

In a follow-up paper to van Erp *et al*. [19,31], the same group tested a comparable set of SNPs for associations with sunitinib efficacy. In a population of 136 mRCC patients, they observed that *CYP3A5*1*, presence of the TCG copy in *ABCB1* (rs1045642, rs1128503, rs2032582), or absence of the CAT copy in *NR1I3* (rs2307424, rs2307418, rs4073054) were associated with an improved PFS. Carriers of the favorable genetic profile (at least an A-allele of *CYP3A5*, a TCG copy in *ABCB1*, or a missing CAT copy in *NR1I3*) showed an improved PFS and OS compared to the disadvantageous genetic profile carriers [31].

With regard to SNPs in *VEGF* and *VEGF-R*, Garcia-Donas *et al*. reported SNPs rs307826 and rs307821 in *VEGF-R3* to be associated with a decrease in PFS in a study of 89 mRCC patients [20]. Kim *et al*. [26] showed that carriers of the combination of CC and GG genotypes of rs3025039 in *VEGF-A* and rs2305948 in *VEGF-R2*, respectively, showed a worse OS compared to noncarriers [26]. In another study of *VEGF* and *VEGF-R* SNPs in a group of 84 mRCC patients, Scartozzi *et al*. [32] report that carriers of the favorable SNP genotypes of rs833061, rs6877011, rs2010963, and rs699947 showed a better overall response rate compared to the unfavorable genotype carriers [32]. By using a similar approach with a slightly different SNP set, Motzer *et al*. [33] report conflicting results. They could not identify any significant associations of SNPs in *VEGF-A* or *VEGF-R3* with efficacy outcomes in sunitinib treatment (PFS, OS, objective response, or time-to-tumor progression) [33].

Beuselinck *et al*. assessed 16 SNPs in 10 genes for their relation with efficacy endpoints in 88 mRCC patients treated with sunitinib [34]. SNP rs1128503 in *ABCB1*, rs4073054 in *NR1I3*, and rs307821 in *VEGF-R3* were associated with PFS and OS. For PFS only, rs2981582 in *FGF-R2* and rs2276707 in *NR1I2* showed an association. For OS only, rs2307424 in *NR1I3* and rs307826 in *VEGF-R3* were associated ($P < 0.05$) [34]. In a separate study on 91 subjects, the CC genotypes of rs9582036 in *VEGF-R1* had a lower response rate and a decreased survival (PFS and OS) compared to A-allele carriers [35]. Furthermore, a decreased PFS was observed for the AA genotype of rs9554320 in *VEGF-R1* compared to C-allele carriers [35]. Teo *et al*. [29] reported that the CC genotype of rs1045642 in *ABCB1* was associated with progression of the disease as compared to T-allele carriers ($P < 0.05$) [29] in 25 Chinese patients.

In the aforementioned study of Diekstra *et al*., presence of CGT in the *ABCB1* haplotype (rs1128503, rs2032582, rs1045642) was confirmed to be associated with an improved PFS ($P < 0.001$) as previously observed by van der Veldt *et al*. [30,31].

Pharmacogenetics to predict sunitinib PK

To investigate the effect of genetic variants on the PK of sunitinib, Mizuno *et al*. performed a population PK study in a set of 19 patients which identified the AA genotype of

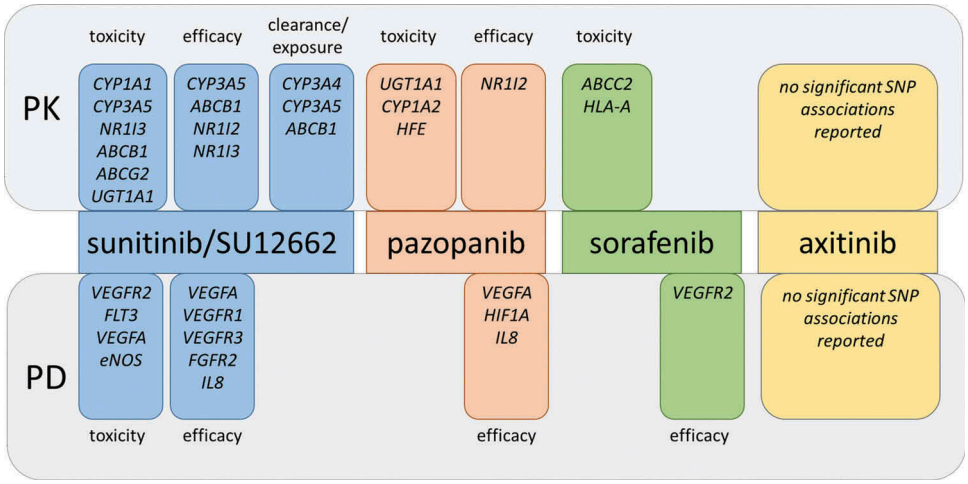

Figure 2. Main results on associations of SNPs involved in pharmacokinetics or pharmacodynamics of TKIs with clinical treatment outcome in mRCC.
An increased risk for common sunitinib toxicities was seen for SNPs in *CYP1A1, NR1I3, ABCB1* and *ABCG2*. Furthermore, *CYP3A5*1* was associated with dose reductions due to toxicity and an increased time-to-dose-reduction (TTDR) was seen for *ABCB1* SNPs. Associations with the clearance of sunitinib and/or SU12662 were observed for SNPs in *CYP3A5, ABCB1* and *CYP3A4*. A higher exposure to sunitinib and progressive disease was reported for rs1045642 in *ABCB1* in Chinese patients. For efficacy, *CYP3A5*1* and SNPs in *ABCB1, NR1I2* and *NR1I3* were associated with PFS. Associations of *CYP3A5*1* with the need for dose reductions and of the *ABCB1* haplotype with PFS were confirmed in a larger study [30]. Regarding pharmacodynamics, SNPs in *VEGFR2, FLT3, VEGF-A* and *eNOS* were associated with sunitinib toxicities and with hypertension in particular. Associations with either response rate, PFS and/or OS were observed for SNPs in *VEGF-A, VEGF-R1, VEGF-R3* and *FGF-R2* [19-44]. For pazopanib, only articles from the group of Xu et al. were included in our search. The main findings were that *UGT1A1*28, UGT1A1*60* and *CYP1A2* (rs762551) were associated with bilirubin levels and SNPs in *HFE* were associated with increased ALT levels. Additionally, SNPs in *IL8, HIF1A, NR1I2* and *VEGFA* were associated with either PFS or response rate. For patients using either sunitinib or pazopanib, *UGT1A1* SNP genotypes *28, *37 and *6 showed an increased risk for hyperbilirubinemia and rs1126647 in *IL8* was associated with OS. Only two pharmacogenetic studies on sorafenib were found in which genetic polymorphisms in *ABCC2* and *HLA-A* were associated with high-grade skin rash and rs2071559 in *VEGF-R2* was associated with PFS and OS. For axitinib, no associations were reported [40-44].

rs2231142 in *ABCG2* to be a predictor for increased clearance of sunitinib [36]. In the study of Teo *et al*. [29], including 25 patients of Chinese origin, the variant CC genotype of rs1045642 in *ABCB1* was associated with a higher exposure to sunitinib as compared to T-allele carriers ($P < 0.05$) [29]. Diekstra *et al*. [37] tested a selection of the earlier associated SNPs related to the PK of sunitinib for possible association with clearance of sunitinib and/or its active metabolite (SU12662). Fourteen SNPs were tested in a sample of 114 patients with RCC (n = 69), neuro-endocrine tumors (n = 14), GIST (n = 8), or another solid tumor type (n = 23). A 28% increase in clearance of SU12662 was observed for the AG genotype of rs776746 in *CYP3A5*. Furthermore, the TT variant genotype of rs2032582 in *ABCB1* showed an 18% increase in clearance of sunitinib ($P < 0.05$). For T-allele carriers of rs35599367 in *CYP3A4* (*CYP3A4*22*), a 22.5% decreased clearance was observed ($P < 0.01$) [37].

Pharmacogenetics to predict pazopanib toxicity

Fewer studies are available on pharmacogenetics of pazopanib, since pazopanib was approved 3 years after sunitinib. Pazopanib and sunitinib share most common toxicities, although for pazopanib hepatotoxicity with elevations of alanine transaminase levels in particular is a common adverse effect [38]. In 2010, Xu *et al*. [38] investigated 28 SNPs for their relation with levels of alanine aminotransferase (ALT) and bilirubin in pazopanib-treated mRCC patients from an exploratory cohort (n = 116) and a replication cohort (n = 130). In the exploratory cohort, SNPs rs8175347 (*UGT1A1*28*), rs4148323 (*UGT1A1*60*), and rs762551 in *CYP1A2* were associated with bilirubin levels, and none of the SNPs showed an association with ALT levels. In the replication cohort, only the TA-repeat polymorphism of *UGT1A1*28* was associated with hyperbilirubinemia. The TA7 homozygote patients developed more hyperbilirubinemia compared to other genotypes, probably due to a reduced expression of UGT1A1. It was suggested that this is not likely to affect pazopanib exposure nor toxicities, since pazopanib is not glucuronized by UGT1A1 [38]. In roughly the same patient cohorts (n = 115 explorative and n = 128 for replication), Xu *et al*. studied 9308 SNPs in 282 candidate genes. For rs2858996 and rs707889 in *HFE*, which are in strong linkage disequilibrium, the TT genotypes were associated with increased ALT levels [39].

Pharmacogenetics to predict pazopanib efficacy

In a subsequent study, Xu *et al*. [40] investigated a slightly different set of 27 SNPs in candidate genes (SNPs in *IL-8* and *HIF1A*) to test whether these are related to pazopanib efficacy. In a cohort of 397 RCC patients, a decreased PFS was observed for TT variant carriers of rs1126647 in *IL8* compared to wild-type AA. A worse PFS and response rate were observed for AG carriers of rs11549467 in *HIF1A* compared to wild-type GG. A reduced response rate was also observed for TT carriers of rs3814055 in *NR1I2* compared to wild-type CC and for wild-type CC of rs833061 in *VEGFA* compared to TT genotypes [40].

Pharmacogenetics to predict clinical outcome of sunitinib and pazopanib

In some of the included studies, a combined genetic association analyses was performed on patients who are either treated with sunitinib or pazopanib. Motzer *et al*. [41] investigated the *UGT1A1* polymorphisms as reported by Xu *et al*. [38] in 719

patients from the COMPARZ trial: a phase III, randomized, clinical trial comparing pazopanib versus sunitinib in mRCC treatment [10,41]. Subjects with an expected reduction in function of UGT1A1 based on the SNP genotypes of rs8175347 and rs4148323 (*28, *37, and *6) showed increased levels of bilirubin at baseline and had a higher risk for developing hyperbilirubinemia on treatment with either sunitinib or pazopanib. None of the UGT1A1 SNPs showed an association with elevated ALT levels [41]. More recently, Xu *et al*. [42] examined three separate patient cohorts of 186 patients on pazopanib in the first cohort, 690 patients using either pazopanib (n = 353) or sunitinib (n = 337) in the second cohort, and the third cohort comprised 88 patients on sunitinib. The variant T allele of SNP rs1126647 in *IL8* was associated with an inferior OS in every separate cohort ($P < 0.05$). In a meta-analysis of the association results in the combined data set of 964 patients, this SNP was also associated with a worse OS [42].

Pharmacogenetics to predict clinical outcome of sorafenib or axitinib

Few studies are available on pharmacogenetics of sorafenib in mRCC and axitinib. In a study of 55 Japanese mRCC patients, Tsuchiya *et al*. [43] reported that the CC genotype of rs717620 in *ABCC2* compared to the CT genotype and the *HLA-A*24* allele were both associated with an increased risk for sorafenib-induced high-grade skin rash.

In 2015, Escudier *et al*. [44] reported a set of 305 mRCC patients (n = 159 axitinib and n = 146 sorafenib), in which 15 SNPs were investigated in *VEGF-A, VEGF-R1, VEGF-R2*, and *HIF-1A* for the association with blood pressure or efficacy outcomes. It was observed that no significant association was reported for any of the SNPs with treatment outcome on axitinib. For sorafenib treatment, it was found that SNP rs2071559 in *VEGF-R2* was associated with PFS and OS ($P < 0.01$), but the sensitivity/specificity analysis on this SNP showed a result of <80%. Therefore, it cannot be considered as a predictive SNP for survival outcome on sorafenib in mRCC [44].

Other associated biomarkers with TKI treatment outcome in mRCC patients

In addition to SNPs as potential biomarkers, many other biomarkers including histological subtypes, molecular subtypes, protein expression, immuno-expression, and microRNA were evaluated for potential association with TKI treatment outcome. Results are presented in Supplementary document 3 [45–72].

Histological and molecular subtypes in sunitinib treatment

In a case report by Choueiri *et al*. [45], a 49-year-old woman initially diagnosed with clear cell mRCC was later confirmed to have an Xp11.2 translocation RCC histological subtype which generally involves the *TFE3* gene. This patient developed severe HFS on sorafenib and, therefore, switched to sunitinib on which she showed a partial response followed by a durable response for more than 2 years [45]. This could indicate that the Xp11.2 translocation histological subtype is a biomarker that predicts a more favorable efficacy and toxicity profile on sunitinib compared to sorafenib.

In a recent communication, Beuselinck *et al*. [46] showed that molecular subtypes of clear cell mRCC can predict the response to sunitinib with regard to response rate, PFS, and OS. These tumor subtypes are characterized based on chromosome copy-number aberrations, methylation status, and gene mutations in von Hippel–Lindau and the polybromo-1 protein (PBRM1) [46].

Protein expression and immunoexpression in sunitinib treatment

With regard to protein expression, Paule *et al*. [47] investigated molecular biomarkers in primary tumors of 23 mRCC patients. Herein, levels of VEGF soluble isoforms $VEGF_{121}$ and $VEGF_{165}$ were associated with sunitinib response, and the ratio of $VEGF_{121}/VEGF_{165}$ was associated with prognosis. Gruenwald *et al*. [48] investigated another type of soluble markers and reported circulating endothelial cells (CEC) to be elevated within 28 days after start of sunitinib treatment in patients with a PFS above the median [48]. Later, in 2012, expression levels of the chemokine receptor CXCR4 were associated with objective response rate and survival on sunitinib as communicated by D'Alterio *et al*. [49]. In a report of Garcia-Donas *et al*. [50], protein expression was investigated in 67 sunitinib-treated mRCC patients. A high expression of HIF2A and PDGFRB proteins showed an improved response to sunitinib, and VEGFR3 expression was associated with PFS. Furthermore, expression of HIF2A and VEGF-A or EGLN3 mRNA content were associated with OS [50]. Minardi *et al*. [51] showed that VEGF expression was correlated with distant metastasis-free survival (DMFS) and OS in mRCC patients, but showed no association with regard to sunitinib treatment [51]. In a set of 50 Japanese RCC patients, Sato *et al*. [52] investigated the Extracellular Matrix MetalloPRoteinase Inducer (EMMPRIN), a cell-surface glycoprotein that belongs to the immunoglobulin superfamily encoded by a gene localized to 19p13.3. High expression of EMMPRIN was seen for sunitinib-treated patients and sunitinib-resistant cells [52]. In the above-mentioned study of Motzer *et al*. [33], baseline levels of angiopoietin-2 and matrix metalloproteinase were associated with tumor response, and HIF-1α expression was associated with PFS [33]. The expression of phosphorylated VEGF-R2 was associated with PFS and OS in mRCC patients by Del Puerto-Nevado *et al*. [53]. Gambini *et al*. [54] reported a case of a 60-year-old man with complete remission after 1 year on sunitinib. For this patient, a significant expression of neuron-specific enolase was observed together with the presence of pancreatic metastases. The hypothesis is that these neuroendocrine markers could predict the response to TKIs [54].

Considering immunoexpression, Mikami *et al*. [55] studied a subset of 25 Japanese mRCC patients on sunitinib. Herein, a high expression of the cancer stem cell marker CD44 was associated with a poor treatment outcome and responsible for developing sunitinib resistance. Also, the combination of high expression of TNF-α and CD44 was associated with a decreased PFS [55]. Dornbusch *et al*. [56] evaluated the immunoexpression of tumor protein markers in 42 patients with mRCC. They concluded that HIF-1a, CA9, Ki67, CD31,

pVEGFR1, VEGFR1 and -2, pPDGFR-a and -b were associated with the response to sunitinib ($P < 0.05$) [56].

Micro RNA in sunitinib treatment

Some studies focused on microRNA (miRNA) as a possible predictor for sunitinib treatment outcome. miRNAs are non-coding RNAs that regulate gene expression [57]. Gámez-Pozo *et al*. [57] associated the expression of miRNA combinations (e.g. hsa-miR-141, hsa-miR-31, hsa-miR-125a-5p) in peripheral blood of RCC patients with poor or prolonged response to sunitinib [57]. Also, Berkers *et al*. [58] showed that miRNA was related to the response to sunitinib as tested in 20 mRCC patients: a significantly lower expression of miRNA-141 was observed for poor responders [58]. On miRNA expression of miRNA, Prior *et al*. [59] identified miRNA-942, miR-628-5p, miR-133a, and miR-484 as predictors for sunitinib efficacy (i.e. time to progression, OS, and resistance). It was postulated that high levels of miRNA-942 will stimulate the secretion of matrix metalloproteinase-9 and VEGF and thereby effectuate resistance to sunitinib [59].

Pazopanib

Other than SNPs, our literature search identified no associations of biomarkers with pazopanib treatment outcome in mRCC. Choueiri *et al*. [60] studied biomarkers along the VHL/HIF-1a/HIF-2a axis but reported no significant associations with pazopanib activity in patients with advanced clear-cell RCC [60].

Sorafenib

Mean VEGF plasma levels showed a significant increase in response to sorafenib, while plasma proteins VEGF-R2 and tissue inhibitor of metalloproteinase 1 (TIMP-1) were decreased in response to sorafenib as was observed by Peña *et al*. [61]. Feng *et al*. [62] showed that high levels of circulating cell-free DNA (cfDNA) were associated with a poor response to sorafenib in 18 Chinese mRCC patients. Aziz *et al*. [63] reported that a high microvessel area was associated with a better response on sorafenib; smaller primary tumors were observed ($P = 0.005$) [63]. Another study showed that miRNA-30a is predictive for sorafenib efficacy [64]. Zheng *et al*. [64] explain that miRNA-30a inhibits its targeted gene Beclin-1, and consequently, the cytotoxic potential of sorafenib will increase [64].

Potential biomarkers from analyses including patients using different therapies

Some of the included articles evaluate more types of TKIs in the same analysis or in combination with other therapies in mRCC. In a cohort of 282 mRCC patients, Doberstein *et al*. showed that the expression of the L1 cell adhesion molecule was predictive both for OS and for therapy resistance in mRCC treatment with either sunitinib, rapamycin, or cisplatin [65]. Furuya *et al*. [66] studied 66 Japanese patients (26 received IFN-alpha and 21 patients received concomitant sorafenib as a second-line treatment) in which significantly higher serum levels of the IFN-alpha receptor messenger RNA were seen for patients with a better response and longer OS on IFN-alpha treatment with or without the addition of sorafenib [66]. Sharpe *et al*. [67] observed a significant reduction in vessel density (CD31), phospho-S6K expression, PDL-1 expression, and FOXP3 expression, but also a significant increase in cytoplasmic FGF-2, MET receptor expression in vessels, Fuhrman tumor grade, and Ki-67 in 85 patients who use either sunitinib or pazopanib. In addition, progression occurred later in patients with high levels of Ki-67 and CD31 [67].

Yamada *et al*. [68] studied 27 cytokines as possible predictive biomarkers for TKI treatment in 13 mRCC patients treated with sunitinib (n = 8), axitinib (n = 4), or sorafenib (n = 1). The plasma granulocyte macrophage colony-stimulating factor level was higher in patients who showed a partial response compared to patients with stable disease or progressive disease ($P < 0.05$). Although not significant, higher IL-6 levels were observed in patients with progressive disease ($P = 0.141$) [68]. Interestingly, Yamada *et al*. refer to a paper of Tran *et al*. [69] who describe that for patients receiving pazopanib, high IL-6 was associated with an improved PFS compared to placebo ($P = 0.009$) [69].

Peters *et al*. [70] examined epigenetic biomarkers, namely the methylation status in primary tumor tissues in a cohort of 18 patients treated with either sunitinib (n = 12), sorafenib (n = 4), axitinib (n = 1), or bevacizumab (n = 1), as a first-line treatment. A decreased PFS and OS were observed for the hyper methylated status of *CST6* and *LAD1*. For the prediction of therapy failure on first-line therapy, sensitivity and specificity were determined and resulted in a specificity of 1.0 and a sensitivity of 0.73 for *LAD1* methylation [70]. Dubrowinskaja *et al*. [71] of the same research group studied the same patient cohort and showed that a higher methylation status of the Neurofilament Heavy polypeptid gene was related to a decrease in OS [71].

Finally, Choudhury *et al*. [72] investigated 48 patients on molecular subtypes of clear-cell RCC for their association with response to either sunitinib or pazopanib as a first-, second-, or third-line treatment. Using a multigene assay (8 genes: *CXCL5, EFNA5, EMCN, LAMB3, PLG, PRAME, RARRES1, SLC6A19*), clinical prognostic subtypes were identified, which were shown to be predictive for response and survival on TKI treatment [72].

Discussion

This systematic review outlines the available literature on associations of SNPs and other biomarkers with clinical treatment outcome of TKIs in mRCC. The most promising biomarkers on predicting TKI outcome are related to PK (genes encoding CYP450 enzymes, CYP450 regulators, or drug efflux transporters) and PD (genes encoding VEGF or VEGF receptors and interleukins) [19–44]. For sunitinib, SNPs in *CYP1A1, CYP3A5, CYP3A4, NR1I2, NR1I3, ABCB1, ABCG2, VEGF-A, VEGF-R1, VEGFR2, VEGF-R3, FGF-R2, FLT3, eNOS, UGT1A1*, and *IL8* were related to toxicity, efficacy, clearance, or drug exposure. Moreover, the potential of SNPs in *CYP3A5* and *ABCB1* to predict sunitinib related outcomes was confirmed in a large set of patients. With regard to pazopanib, SNPs in *UGT1A1, CYP1A2*, and *HFE* were associated with either bilirubin levels or ALT levels, and SNPs in *IL8, HIF1A, NR1I2*, and *VEGFA* were associated with efficacy outcomes on pazopanib. The

association of rs1126647 in *IL8* with OS was confirmed in two independent data sets including patients treated with either sunitinib or pazopanib [40,42]. Sorafenib toxicity and efficacy were related to genetic polymorphisms in *ABCC2, HLA-A*, and the only large study (n > 300) available on sorafenib treatment showed rs2071559 in *VEGF-R2* was related to efficacy. For axitinib, no SNP associations were reported [19–44]. Other reported biomarkers include proteins (e.g. VEGF), epigenetic markers (miRNA or methylation), and histological or molecular subtypes [45–72]. Clinical implementation of these biomarkers remains a difficult issue, since most studies present nonvalidated findings from retrospective cohort studies, effect sizes are rather small, heterogeneity between studies is large, sample sizes are limited, and above all, no consensus has been reached on the interpretation of these findings in terms of clinical utility on guiding individual dosing regimens of TKIs [17,18].

Our systematic approach minimizes the risk that we have missed relevant articles. Moreover, we only focused on TKIs (excluding other mRCC treatments) to ensure that similar mechanisms and drug targets are compared. A meta-analysis is generally preferred to easily summarize the findings of all studies and subsequently value the clinical utility of associated biomarkers. However, in this case, a meta-analysis would be misjudging because of the large heterogeneity in study design, sample sizes, ethnicities, study endpoints, tested SNPs, evaluated treatments, and statistical approach. Sample sizes range from 1 to 451, in which the number of tested SNPs varied between 1 and 6852 SNPs [22,39]. Furthermore, publication bias has very likely occurred and obscures a clear conclusion from a meta-analysis. In this review, we compared study results on the drugs of interest, sample sizes, ethnicity differences, and effect sizes with confidence intervals and *P*-values. One of the limitations in the studies in this field is that biomarkers are typically selected with a significance threshold value of $P < 0.05$. However, often a large number of hypotheses are tested in the same data set, increasing the chance for false-positive associations [73]. Another important issue is the difference in allele frequencies of SNPs among ethnicities. For example, SNP allele frequencies in *CYP3A5* and *ABCB1* differ between Caucasian and Asian patients. Furthermore, co-medication during TKI treatment and total exposure to the TKI was not taken into account in most studies, while drug–drug interactions may be especially important with regard to the effects on PK and PD. These matters have an important impact on the clinical validity and should be taken into account before assessing the possibilities on clinical implementation.

The SNPs tested in most of the included studies were selected according to the candidate gene approach. On PK, the results show that SNPs in genes coding for CYP enzymes, CYP regulators, and drug efflux transporters (ABCB1, ABCC2, and ABCG2) seem to play an important role in sunitinib treatment outcome, especially *CYP3A5* and *ABCB1* SNPs [19–21,29,31,34,37]. In some of the articles on sunitinib, the intermediate endpoints clearance and exposure were investigated and confirmed the hypotheses that SNPs in *CYP3A4, CYP3A5*, and *ABCB1* influence drug exposure [22,29,36,37] (Figure 2). Sunitinib and pazopanib are metabolized by CYP3A4 and probably CYP3A5 but are not known to be metabolized by UGT1A1 [38,41,74]. Pazopanib inhibits UGT1A1 *in vitro*. In pazopanib-treated patients, SNPs in *UGT1A1* may cause a reduced expression of UGT1A1 and, together with the inhibitory effect of pazopanib, would result in hyperbilirubinemia. For sunitinib, however, this enhanced effect on inhibition does not apply [38,41]. Since sunitinib and pazopanib are both metabolized by the same CYP enzymes and are substrates for ABCB1 and ABCG2, one could expect to find the same SNPs in these PK-related genes to be related to pazopanib outcomes. Only for an SNP in *NR1I2*, a relation with response rate on pazopanib was observed [40]. The articles in this review have not tested pazopanib toxicity outcomes for these SNPs related to PK. However, the effects on total drug exposure resulting from changes in CYP enzyme activity may be very different for sunitinib and pazopanib. Including both sunitinib- and pazopanib-treated patients in this type of analyses is not very reasonable [41,42], since sunitinib is converted to an active metabolite with similar activity and potency and a longer half-life, while pazopanib only has less active metabolites. In addition, the extent of absorption for pazopanib and sunitinib may be very different (due to substrate specificity, for example), and its role in the total PK of the TKI is unclear. Changes in genes encoding efflux transporters can, therefore, be more important for sunitinib than for pazopanib. Regarding pharmacodynamics of sunitinib, SNPs in *eNOS, IL8, VEGF-A*, and *VEGF-R1,2,3* present promising biomarkers for toxicity or survival on sunitinib [19,20,25,26,32,34,35]. However, results on *VEGF-A* and *VEGF-R1,2* or *3* genes are contradictory for both toxicity and efficacy outcomes since also negative associations were reported [30,33]. In a recent analysis of our group, we associated SNPs in *IL8* and *IL13* with sunitinib toxicities (hypertension, leukopenia, and any toxicity >grade 2) and referred to the findings of Xu *et al*. [40,42,75]. Here, it was carefully hypothesized that IL8 and VEGF can be related in their effect on sunitinib-induced toxicities [75]. For sorafenib, only one study with a significant sample size is available that reported an SNP in *VEGF-R2* to be a potential predictor for efficacy (PFS and OS) [44]. This effect was explained by a change in the promoter, subsequently altering transcription factor-binding and thereby the expression of VEGF-R2, but no clear downstream effect was presented [44]. In addition to SNPs, other types of biomarkers are potentially important. Results on epigenetics (miRNA and DNA methylation status) may influence gene expression and SNP effects, and results on protein markers can provide useful insight into mechanisms involved in SNP effects [45–72]. The associated SNPs and other biomarkers are different between TKIs, and therefore, we cannot assume a class effect pharmacogenetics in mRCC. While many targets, metabolizing enzymes, and drug transporters of the TKIs are comparable, significant differences in mechanisms of action can still exist.

A randomized clinical trial (RCT) is considered the hallmark of evidence to show utility for a biomarker in a clinical setting. However, an RCT is not critical for clinical application. It has already been proven that genetic substudies of RCTs in cancer are sufficient evidence to implement pharmacogenetic testing as is illustrated by testing *EGFR* for erlotinib and *(K)RAS* for cetuximab and panitumumab [76]. Funakoshi *et al*. [14]

assessed the level of evidence (LOE) of associated biomarkers by arranging the articles into different categories [77,78]. Categories A and B represent the highest LOE with prospective RCT addressing biomarker questions and prospective studies not primarily designed to address biomarker questions. Categories C and D represent the lowest LOE with prospective and retrospective observational studies [14,77,78]. In this field, these categories would be a false premise since no randomized studies and few prospective studies (category A or B) are available on pharmacogenetic biomarker studies in mRCC, which means only category C and D studies with a low LOE are left.

Gillis *et al*. [18] mention that both clinical validity and clinical utility should be considered in translating the findings into a practical method to improve clinical practice. There is a lack of consensus on the definition of clinical utility. However, Gillis *et al*. are confident that validation of the associated biomarker in an independent replication cohort is the best evidence of clinical utility [18]. Janssens *et al*. consider a pharmacogenetic test useful when it is 'sufficiently predictive for clinically important phenotypes, contributes to improved health outcomes, and is economically feasible to implement in community-based practice settings' [17]. Clinical application is very likely for the reported SNP associations of *CYP3A5* and *ABCB1*, which reflect a considerably high predictive value since these SNPs have been associated with sunitinib PK [37], associations on clinical outcome are confirmed [20,30,31], and these findings are further supported by the exposure–effect relationship on sunitinib treatment [7]. However, no genetic biomarkers for clinical outcome on TKIs in mRCC have been implemented yet in any of the innovative guidelines such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) [79,80].

In conclusion, a decade of pharmacogenomics research on TKIs in mRCC patients has revealed *CYP3A5*1* and *ABCB1* as the most promising biomarkers for sunitinib treatment outcome. Implementation of pre-emptive pharmacogenetic testing will become likely when confirmed in independent cohorts preferably in prospective studies.

In the near future, results from genome-wide association studies from discovery and validation sets are expected. Many more SNPs in different genes will be investigated in the genome-wide association studies as compared to the candidate–gene approach that is used in most of the currently available studies. This could mean that earlier discovered SNPs will be affirmed, but these results may also identify additional genetic variants as biomarkers for outcome of TKI treatment in renal cell cancer. Additionally, we think that it is essential to further study both immunological and angiogenic factors (both protein expression and genetic polymorphisms) on prognosis and anti-VEGF treatment outcome in mRCC. Besides the effect on VEGF inhibition, sunitinib and other TKIs may influence the immune system, but we need a better understanding on the mechanisms involved [42,69,75]. Anti-angiogenic therapy may result in a further induction of the immunosuppressive environment of the RCC tumor [81]. Future findings may indicate that we need a combination therapy to treat both the immunogenic and pro-angiogenic character of RCC. Utilizing the pharmacogenetic profile of the patient may optimize TKI selection. Currently, the drug arsenal for mRCC is growing with new available TKIs or TKIs in development, and new promising immune-based therapies for RCC treatment are on their way, such as anti-programmed death-ligand 1 (PD-L1) [82]. Recent findings showed that in some patients, the efficacy of anti-angiogenic therapy might improve by PD-L1 blockade. However, as Liu *et al*. mention: 'The top priority and challenge is the selection of patients who are likely to respond to anti-PD-L1 therapy' [81].

Within 5 years from now, we expect that some of the presented biomarkers here will be validated in replication cohorts and prospective validation studies with a longitudinal observation in time and a systematic data collection. These can be designed as noninterventional because blood samples (DNA) can be collected during routine monitoring visits of the patients. In retrospect, it will be investigated whether the determined genotypes of the patients have been a prediction for the outcome of TKI treatment. Two large prospective, nonrandomized clinical trials on precision medicine in oncology have already been set up as presented at the 2015 American Society of Clinical Oncology (ASCO) annual meeting: ASCO's Targeted Agent and Profiling Utilization Registry (TAPUR) study and the National Cancer Institute (NCI) Molecular Analysis for Therapy Choice (NCI-MATCH) [83,84]. In these trials, more than 20 different targeted therapies will be investigated including sunitinib, and these drugs will be made freely available. For the NCI-MATCH study, a number of 3000 patients is anticipated. Patients are treated with a drug based on the molecular profile of the tumor, and important clinical outcomes (e.g. overall response rate and PFS) will be recorded to see how patients can benefit from these drugs [83,84].

Financial & competing interests disclosure

MHM Diekstra was supported by the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 259939. The authors thank Jan Schoones (Walaeus Library, LUMC, Leiden, The Netherlands) for his assistance on the database search. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Key issues

- The response to tyrosine kinase inhibitors is highly variable in patients with metastatic renal cell carcinoma.
- To date, most studies selected single nucleotide polymorphisms according to the candidate gene approach based on the knowledge on PK and PD of the drug.
- Promising biomarkers for predictive genotyping of sunitinib and pazopanib are genetic variants in *ABCB1, CYP3A5, UGT1A1*, and *IL8*.
- Results on *VEGF-A* and *VEGF-R1,2* or *3* genes are contradictory for both toxicity and efficacy outcomes in sunitinib.
- Information on nongenetic biomarkers should be involved in future research on SNPs to increase the predictive value.
- Future research efforts should be focused on immune-related biomarkers.
- Genome-wide association results from discovery and replication sets are expected to shed more light on predicting TKI response.

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