Dose optimization of oral targeted therapies in oncology

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Voor Hanneke en René

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General introduction

Cancer is a leading cause of morbidity and mortality [1,2]. In 2012, there were worldwide approximately 14 million patients diagnosed with cancer and 8.2 million cancer related deaths [1,2]. Considering the Netherlands, there were more than 100,000 patients diagnosed with cancer in 2014. In addition, over 42,000 patients died of cancer in that year, making it the leading cause of mortality in the Netherlands [3,4]. Once diagnosed, there are three types of treatment modalities including surgery, radiotherapy and systemic therapy which can be used both in a (neo)adjuvant and palliative setting.

In the last two decades, the systemic treatment options with anticancer drugs in oncology have changed remarkably. With the increased understanding of cancer pathophysiology, the treatment of several cancers has shifted from the use of nonspecific chemotherapy aimed at killing all dividing cells, towards more specific treatment with (oral) targeted therapies and immunotherapy. Targeted drugs inhibit the growth of cancer by interfering with specific target molecules involved in the growth, activation and differentiation of cancer cells and therefore act more specific when compared to conventional therapies.

Tyrosine kinases are such specific molecules and these proteins have become an important target for anticancer drug design. Tyrosine kinases transfer phosphate from adenosine-5'-triphosate (ATP) to tyrosine residues on cellular proteins which activates signal-transduction pathways [5]. Insights into the dysregulation of these pathways in cancer cells led to the development of tyrosine kinase inhibitors (TKIs). TKIs compete with ATP for the ATP-binding pocket of tyrosine kinases that are mutated or overexpressed in some cancer cells and hereby block the dysregulated signal-transduction pathways critical for the growth, activation, differentiation and death of (cancer) cells [5]. With the introduction of the first TKI imatinib in 2001, a whole new era of rationally designed TKIs has emerged. Since then, 22 other TKIs have been registered by the European Medicines Agency for the treatment of different types of both solid as well as hematological cancers [6].

Another important target for anticancer drug design is the mammalian target of rapamycin (mTOR), a serine-threonine kinase that is a key signaling molecule in the phosphatidylinositol 3-kinase (PI3K)/Akt pathway [7]. This pathway is involved in the regulation of growth, proliferation, metabolism, survival and angiogenesis of cells and dysregulated in cancer. Everolimus and temsirolimus are examples of oral targeted therapies that specifically inhibit mTOR and that are used in oncology.

In recent years, the clinical pharmacology of oral targeted therapies including TKIs and everolimus has been studied extensively [8-10]. Despite the large variability in pharmacokinetics (PK) between patients, all TKIs, as well as everolimus, are registered at a fixed oral dose. This results in large differences in exposure between patients. As evidence for a relationship between drug exposure and treatment outcome is growing for TKIs, fixed dosing could potentially result in sub- or supratherapeutic drug exposure with decreased therapeutic effects in some patients or an increased incidence and severity of toxicity in others [11-13]. It can be hypothesized that dose individualization could improve clinical outcomes. Interestingly, within transplantation medicine dose individualization of everolimus is already the standard of care [14]. However, in the field of oncology dose individualization of everolimus is largely unexplored.

A better understanding of the underlying causes of inter-patient variability in drug exposure of oral targeted therapies is warranted. Also, strategies should be developed to easily monitor treatment with these drugs in clinical practice. Moreover, studies that investigate the feasibility of dose individualization strategies such as therapeutic drug monitoring (TDM) are needed. Therefore, the aim of this thesis is to investigate and develop dose optimization strategies of oral targeted therapies used in oncology, in particular for the TKIs pazopanib and sunitinib and the mTOR inhibitor everolimus.

In **chapter 2** a systematic overview is given of current knowledge and evidence for individualized dosing of TKIs that are used for the treatment of solid tumors. Different criteria should be met to make dose individualization for a drug of potential interest. This chapter evaluates whether TKIs meet these criteria, with an emphasis on the primary requirement; a proven drug exposure-response relationship.

A technical prerequisite for dose individualization of a drug, is the availability of a quantitative bio-analytical assay to measure drug levels and to monitor therapy in clinical practice. In **chapter 3** the development and validation of a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) assay to simultaneously detect six TKIs including pazopanib and sunitinib and two active metabolites in human serum is described.

Monitoring of drug levels in serum or plasma as described in this assay makes blood sampling by venapuncture necessary. This has several disadvantages, including its invasive character, the requirement for patients to come to the clinic and the need for trained personnel. In **chapter 4** we investigate the feasibility of dried blood spot (DBS) sampling as a simple, flexible and more patient friendly alternative for the monitoring of pazopanib therapy.

In **chapter 5** we describe the feasibility of TDM to optimize the dosing of pazopanib. With the use of TDM, dosing can be individualized after steady-state PK has been reached.

Another approach for dose individualization could be the use of a noninvasive phenotyping probe. With this probe, drug exposure is predicted before initiation of therapy. In **chapter 6** midazolam is evaluated as a potential phenotyping probe for CYP3A4 activity to predict sunitinib exposure in patients with cancer.

Patients with gastrointestinal stromal tumors (GIST) often have an altered anatomy of the gastrointestinal tract due to either resection of the primary tumor or subsequent surgery for recurrence and/or metastasis. This could influence drug absorption and thus lead to differences in drug exposure between patients. In **chapter 7** the effect of different gastrointestinal resections on sunitinib exposure in patients with GIST is investigated.

Everolimus is a promising drug for the treatment of different solid tumors such as breast cancer and metastatic renal cell carcinoma. However, many patients are in need of dose interruptions, reductions and treatment discontinuation due to toxicity of this drug. In contrast to transplantation medicine where everolimus' dosing is based on TDM, a fixed oral and high dose of 10 mg is used in oncology. In **chapter 8** the correlation between everolimus exposure and toxicity and its population pharmacokinetics in patients with thyroid cancer is evaluated.

This thesis ends with the general discussion and future perspectives in **chapter 9**.

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2

Individualized dosing of tyrosine kinase inhibitors – are we there yet?

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ABSTRACT Tyrosine kinase inhibitors (TKIs) are registered at a fixed oral dose, despite their large variability in pharmacokinetics (PK). Given that the evidence for a relation between drug exposure and treatment outcome is growing, this one-dose-fits-all approach can unintentionally lead to under- and overexposure. Dose individualization could lower this variability and thereby beneficially effect treatment outcome. In this article, we explore whether TKIs used for solid tumors meet the criteria for dose individualization. Despite limitations such as retrospective analysis, current data suggest that the following C_{trough} levels could be used: imatinib 1100 ng/mL, sunitinib when continuously dosed 37.5 ng/mL, intermittent 50 ng/mL and pazopanib 20 µg/mL. A comprehensive review of the literature also shows that prospective trials investigating the influence of dose individualization on treatment outcome are warranted.

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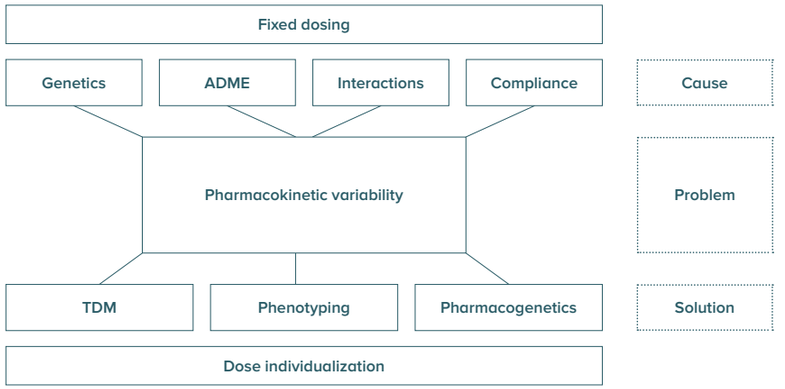
Introduction

With the increased understanding of cancer pathophysiology, tyrosine kinases have become important targets for anticancer drug design. Tyrosine kinases activate signal-transduction pathways that are crucial for growth, activation, differentiation, and death of cells [1]. Insights into dysregulation of these pathways in cancer led to the development of tyrosine kinase inhibitors (TKIs). With the introduction of TKIs, a new category of rationally designed targeted anticancer agents has emerged.

Fixed dosing is usually a good option for drugs with a broad therapeutic window, small inter-patient variability in exposure, and limited toxicity [2]. However, most TKIs show a large variability in their exposure (pharmacokinetics; PK) and treatment outcome (pharmacodynamics; PD). Different causes for variability in PK are summarized in Figure 1. In addition, the evidence for a relation between drug exposure and response for TKIs is growing fast [3-7]. Consequently, fixed dosing could potentially result in sub- or supratherapeutic exposure with decreased therapeutic effects in some patients or increased incidence and severity of toxicity in others.

Figure 1 Variability of tyrosine kinase inhibitor pharmacokinetics



Abbreviation: ADME, absorption, distribution, metabolism, and excretion.

Several studies have focused on reducing the inter-patient variability in exposure by dose individualization [8-11]. Some general criteria for dose individualization include: repeated administration, no easier assessable biomarkers to determine the response (e.g. blood pressure or rash), an available quantitative bioanalytical assay, and a validated dose-adaptation strategy. Dose proportional PK is helpful for the development of such strategies [12]. All these criteria are in general applicable to TKIs. However, the most important criteria that should be met to prove the added value of dose individualization are a narrow therapeutic window and a proven exposure-response relation [12]. A narrow therapeutic window is applicable for all anticancer agents, including TKIs. Moreover, it is important that variability in PK within patients (intra-patient) is small compared with the variability between patients (inter-patient) [12]. In this review, we evaluate whether TKIs used for the treatment of solid tumors meet the criteria necessary for dose individualization. We emphasize the evidence for exposure-response relations and the inter- and intra-patient variability in PK.

Search

A PubMed search was performed using different synonyms of the keywords 'pharmacokinetics' and 'variability', and the names of the individual TKIs registered by the European Medicines Agency (EMA) up until February 2014 (Table 1). In addition, reference lists were screened for other relevant studies and registration information from the EMA and U.S. Food and Drug Administration (FDA) was used. Results were limited to studies in humans and English full-text articles published until the 24th of February 2014. An overview of PK properties of the selected TKIs is shown in Table 2. Evidence for correlations between exposure-efficacy and exposure-toxicity is summarized in Tables 3 and 4, respectively. Table 5 describes the inter- and intra-patient variability in PK.

Axitinib

Correlation between exposure and efficacy

Recently, a study that used pooled data of 168 patients with metastatic renal cell carcinoma (mRCC) showed that patients with an area under curve $(AUC)_{0\text{-}24} \geq 300$ ng·hr/mL after 4 weeks of treatment had significantly ($P = 0.003$) longer progression-free survival (PFS) and significant ($P < 0.001$) longer overall survival (OS) compared with patients with an $AUC_{0\text{-}24} < 300$ ng·hr/mL [13]. Moreover, with every 100 ng·hr/mL increase in $AUC_{0\text{-}24}$, a 1.5-fold increase in probability of partial response (PR) was found ($P < 0.001$) [13]. In another study, 49 patients with mRCC were grouped into four quartiles based on their day 1, 1-2 hour post-dose axitinib levels. Patients in the third quartile ($C_{1\text{-}2}$ 45.4 - 56.4 ng/mL and $AUC_{0\text{-}12}$ 154-620 ng·hr/mL) showed the best 5-year clinical outcome with longer OS, PFS, and higher overall response rate (ORR) [14]. The better outcomes in the third quartile compared with the fourth quartile were explained by the higher incidence of grade ≥ 3 toxicities leading to early discontinuation and interruptions in the fourth quartile. Another pooled analysis found a median OS of 69 weeks for patients with an $AUC_{ss} \leq 605$ ng·hr/mL versus 88 weeks for patients with an $AUC_{ss} > 605$ ng·hr/mL, but this difference was not significant ($P > 0.05$)

[15]. However, this analysis did show that patients with diastolic blood pressure (dBP) ≥ 90 mmHg had longer OS compared with patients with dBP < 90 mmHg, which was also shown in other analyses [13,16-19].

A double-blind placebo-controlled randomized phase II study prospectively evaluated the effect of axitinib dose titration on treatment outcome in 203 patients with mRCC [20]. Patients started with axitinib 5 mg twice daily (BID) for 4 weeks. Patients with BP ≤ 150/90 mmHg, no grade 3/4 axitinib-related toxicities, no dose reductions, and ≤ 2 anti-hypertensive treatments, were randomized to receive axitinib 5 mg BID plus dose titration up to a total of 10 mg axitinib BID or dose titration with placebo. Patients not eligible for titration continued with axitinib ≤ 5 mg BID. Patients who were eligible for dose titration showed two times lower axitinib exposures compared with patients not eligible (AUC_{0-24} 176 versus 432 ng·hr/mL). Furthermore, the axitinib dose titration group showed significantly ($P = 0.019$) more objective responses compared with the placebo titration group. Patients not eligible for titration (those with initial higher initial axitinib exposure) had comparable objective responses to the axitinib dose titration group. This demonstrates a positive relation between axitinib exposure and response, although there was no difference in PFS or OS between the axitinib and placebo dose titration arm.

Correlation between exposure and toxicity

In the before-mentioned study, patients eligible for titration had over two times lower axitinib exposures compared with patients not eligible for dose titration because of dose-limiting toxicities (DLT), including hypertension, suggestive of a correlation between exposure and toxicity [20]. However, in a PK-PD analysis on axitinib-related BP increase, the correlation between exposure and dBP change was only weak (r^2 values < 0.10) [13,15]. Therefore, dBP could be useful as a predictive biomarker to optimize axitinib therapy. However, dBP is potentially also not merely a reflection of higher axitinib exposure. Therefore, the most adequate biomarker (drug exposure or BP) needs to be established. Thyroid-stimulating hormone changes have also been suggested as a biomarker of axitinib exposure [21,22]. The axitinib drug approval report from the FDA states that pooled exposure-safety analysis from three phase II trials and a pivotal phase III trial, showed a significant ($P < 0.001$) exposure dependent increase in hypertension, proteinuria, fatigue, and diarrhea [23]. However, an analysis of 128 patients with metastatic colorectal cancer (mCRC) did not find any correlation ($P > 0.05$) [18].

Inter- and intra-patient variability in exposure

Axitinib shows large inter-patient variability in PK with coefficients of variation (CV%) ranging from 17% to 94% for the AUC and 17% to 113% for the apparent oral clearance (CI/F) [21,22,24-26]. The intra-patient variability is modest, with CV% values for C_{trough} and CI/F of 20-22 CV% and for AUC of 20-33 CV% [25,27]. Population PK analysis found that age, ethnicity, and body weight could partly explain inter-patient variability, although effect

Table 1 Overview of indications and targets of TKIs for the treatment of solid tumors

TKI	Indication	Targets	REF
Axitinib	mRCC	VEGFR 1-3	[259]
Dabrafenib	melanoma	BRAF	[260]
Erlotinib	NSCLC, pancreatic cancer	EGFR	[261]
Gefitinib	NSCLC	EGFR	[262]
Imatinib	ALL, CEL, DFSP, CML, GIST, HES, MDS/MPD	Bcr-Abl, cKIT, PDGFRα,β	[263]
Lapatinib	HER2+ breast cancer	EGFR, HER2	[264]
Pazopanib	mRCC, STS	cKIT, PDGFRα,β, VEGFR 1-3	[163]
Regorafenib	CRC, GIST	BRAF, cKIT, PDGFRα,β, RAF, RET, TEK, VEGFR 1-3	[265]
Sorafenib	HCC, mRCC	cKIT, FLT3, PDGFRβ RAF-kinases, VEGFR 1-3	[266]
Sunitinib	GIST, mRCC, pNET	cKIT, CSFR, FLT3, PDGFRα,β, RET, VEGFR 1-3	[267]
Vandetanib	MTC	EGFR, RET, VEGFR 2	[268]
Vemurafenib	melanoma	BRAF	[269]

Abbreviations: ALL, acute lymphoblastic leukemia; Bcr-Abl, fusion protein; BRAF, B-rapidly accelerated fibrosarcoma oncoprotein; CEL, chronic eosinophilic leukemia; c-KIT, mast/stem cell growth factor receptor; CML, chronic myeloid leukemia; CRC, colorectal cancer; CSFR, colony stimulating factor receptor; DFSP, dermatofibrosarcoma protuberans; EGFR, epidermal growth factor receptor; FLT3, FMS-like tyrosine kinase 3; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; HER2+, human epidermal growth factor receptor mutation positive; HES, hypereosinophilic syndrome; MDS/MPD, myelodysplastic/myeloproliferative diseases; mRCC, metastatic renal cell carcinoma; MTC, medullary thyroid cancer; NSCLC, non-small cell lung cancer; PDGFR, platelet derived growth factor receptor; pNET, pancreatic neuroendocrine tumor; RAF, receptor accessory factor; RET, rearranged during transfection; STS, soft tissue sarcoma; VEGFR, vascular endothelial growth factor receptor.

Table 2 Pharmacokinetic parameters of the TKIs

TKI	Dosage	Bioavailability	T_{max} (hr)	Protein binding	T½ (hr)	REF
Axitinib	5 mg BID	58%	2-6	99%	2-5	[21,24,27]
Dabrafenib	150 mg BID	95%	2	>99%	8	[29]
Erlotinib	100-150 mg QD	59%	3	95%	36	[51,270]
Gefitinib	250 mg QD	59%	3-7	90%	48	[271]
Imatinib	400-800 mg QD	98%	2-4	95%	18	[263]
Lapatinib	1000-1500 mg QD	N/A	3-4	99%	24	[272]
Pazopanib	800 mg QD	14-39%	2-4	98.8%	31	[163,273]
Regorafenib	160 mg QD: 3/1	N/A	3-4	>99%	20-40	[174]
Sorafenib	400 mg BID	N/A	3	>99%	25-48	[274]
Sunitinib	50 mg QD: 4/2, 37.5 mg QD	N/A	6-12	~95%	40-60	[267]
Vandetanib	300 mg QD	N/A	6	93%	480	[275]
Vemurafenib	960 mg BID	N/A	4	>99%	57	[255]

Abbreviations: 3/1, three weeks on therapy followed by 1 week off therapy; 4/2, four weeks on therapy followed by 2 weeks off therapy; BID, twice daily; N/A, not available; QD, once daily.

Table 3 Correlations between exposure and efficacy of TKIs

TKI	Tumor type	N	PK parameter	Outcome	Correlation	Significance	REF
Axitinib	mRCC	168	AUC_{0-24} ≥ versus < 300 ng·hr/mL	OS	37.4 versus 15.8 months	*P* < 0.001	[13]
			AUC_{0-24}	PFS	13.8 versus 7.4 months	*P* = 0.003	
				PR	1.5 fold increase in probability of a PR for every 100 ng·hr/mL increase in AUC_{0-24}	*P* < 0.001	
		49	C_{1-2}: 45.2-56.4 ng/mL AUC_{0-12}: 154-620 ng·hr/mL	OS	NR versus 20.3-27.7 months	N/A	[14]
				PFS	28.3 versus 7.5-11.8 months	N/A	
				ORR	81.8% versus 16.7-53.8%	N/A	
		109	AUC_{ss} ≥ versus < 605 ng·hr/mL	OS	88 versus 69 weeks	*P* > 0.05	[15]
		112	Dose titration versus no titration	ORR	54% versus 34%	*P* = 0.019	[20]
Erlotinib	NSCLC	56	C_{trough} C_{trough} ≥ versus < 4.6 nmol/mL	OR	5.22 versus 4.00 versus 3.44 nmol/mL for PR, SD and PD respectively	*P* > 0.05	[33]
				OS	HR: 1.424 (95%-CI: 0.677-2.996)	*P* = 0.351	
				PFS	HR: 1.765 (95%-CI: 0.852-3.657)	*P* = 0.127	
		16	Ratio C_{trough} D8/D2 > median versus < median	PFS	11.2 versus 5.6 months	*P* = 0.044	[34]
	HNSCC	18	C_{trough} C_{trough}	OS	OS was related to magnitude C_{trough} of OSI-420	*P* = 0.019	[35]
				TTP	TTP was related to magnitude C_{trough} of erlotinib and OSI-420	*P* = 0.042 and 0.036	
		42	C_{trough} OSI-420	OS	HR: 1.387 (95%-CI: 1.135-1.695)	*P* = 0.0014	[36]
		47	C_{5-10} erlotinib and OSI-420	OS	HR: 1.054 (95%-CI: 1.008-1.103) and 1.422 (95%-CI: 1.166-1.735)	*P* = 0.021 *P* = 0.0005	
Gefitinib	NSCLC	44	Ratio C_{trough} D8/D3 < versus ≥ 1.587	PFS	HR: 0.452 (95%-CI: 0.237-0.862)	*P* = 0.0158	[74]
		30	C_{trough} ≥ versus < 200 ng/mL	OS	14.6 versus 4.7 months	*P* = 0.007	[75]
	HNSCC	20	C_{trough}	Response	1,117 versus 520 ng/mL for patients with PR + SD versus PD	*P* = 0.0103	[76]
Imatinib	GIST	73	C_{trough} ≥ versus < 1,110 ng/mL	TTP	> 30 versus 11.3 months	*P* = 0.0029	[4]
	GIST KIT exon 11	39	C_{trough} ≥ versus < 1,110 ng/mL	OOBR	100% versus 67%	*P* = 0.001	
	GIST	38	AUC_{0-24} unbound	CR + PR	2.6 fold increase in probability of CR+PR for every doubling of unbound AUC_{0-24}	*P* = 0.026	[102]
Pazopanib	mRCC	10	C_{trough} ≥ versus < 15 µg/mL	PR + SD	83% versus 0%	N/A	[163]
	NPC	19	AUC_{0-24}	reduction v2	ΔT v2 decreased linear with AUC_{0-24} (r = 0.54)	*P* = 0.021	[164]
	HCC	17	C_{trough} > 20 µg/mL	decrease K_{trans}	Δ K_{trans} decreased most with C_{trough} > 20 µg/mL	N/A	[165]
	mRCC	205	C_{trough} > versus ≤ 20.6 µg/mL	PFS	49.4 versus 20.3 weeks	*P* = 0.0041	[7]
				RR	45% versus 18%	*P* < 0.0001	
				tumor shrinkage	37.8% versus 8.8%	*P* < 0.0001	
Sorafenib	melanoma	27	AUC_{max} ≥ versus < 100 µg·hr/mL	tumor control	86% versus 50%	*P* = 0.04	[177]
				PR+SD	80% versus 33%	*P* = 0.02	
				PFS	21 versus 10 weeks	*P* = 0.005	
	HCC	36	C_{max} ≥ versus < 4.78 µg/mL	OS	12.0 versus 6.5 months	*P* = 0.0824	[178]
Sunitinib	mRCC	146	AUC_{0-24} ≥ versus < 800 ng·hr/mL	TTP	TTP increased with increasing AUC_{0-24}	*P* = 0.001	[5]
				OS	OS increased with increasing AUC_{0-24}	*P* = 0.010	
				ORR	ORR increased with increasing AUC_{0-24}	*P* < 0.001	
				SD	SD increased with increasing AUC_{0-24}	*P* = 0.002	
	GIST	278	AUC_{0-24} ≥ versus < 600 ng·hr/mL	TTP	TTP increased with increasing AUC_{0-24}	*P* = 0.001	[5]
				OS	OS increased with increasing AUC_{0-24}	*P* = 0.001	
				ORR	ORR increased with increasing AUC_{0-24}	*P* = 0.06	
				SD	SD increased with increasing AUC_{0-24}	*P* < 0.001	
	solid		C_{trough} 50-100 ng/mL	target inhibition	a C_{trough} 50-100 ng/mL is the minimum plasma concentration required to inhibit Flk-1/KDR and PDGFRβ	N/A	[215]
Vemurafenib	N/A	N/A	C_{trough}	PFS	HR: 0.653 (95%-CI: 0.503-0.848)	*P* = 0.0014	[255]
	melanoma	403	Low, medium and high AUC_{0-12}	tumor growth	22% versus 11% versus 9% respectively	N/A	[256]

Abbreviations: AUC, area under the concentration-time curve; C_{1-2}, concentration 1-2 hours post-dose; C_{5-10}, concentration 5-10 hours post-dose; CR, complete response; dBP, diastolic blood pressure; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell cancer; K_{trans}, volume transfer coefficient: an indicator of vascular response; PFS, progression free survival; mRCC, metastatic renal cell carcinoma; mRCC, metastatic renal cell carcinoma; N/A, not available; NPC, nasopharyngeal carcinoma; NR, not reached; NSCLC, non-small cell lung carcinoma; OR, objective response; ORR, objective response rate; OS, overall survival; OSI-420, active metabolite erlotinib; OOBR, overall objective benefit rate (complete response + partial response + stable disease); PD, progressive disease; PR, partial response; RR, response rate; SD, stable disease; TTP, time to progression; V, varying.

sizes were small, making dose adjustment based on these covariates unnecessary [13,28].

Dose individualization
The above-mentioned individualization study shows that titration based on toxicity facilitates optimization of plasma exposure and is associated with a greater proportion of patients with mRCC achieving a response. Therefore, toxicity-driven dose adjustment is beneficial to optimize and individualize axitinib therapy [20].

Conclusion
Axitinib has substantional inter-patient, with relatively modest intra-patient PK variability. Several studies showed a clear exposure-response relation and BP also seems a potential biomarker to select patients in need of dose adjustment. Surprisingly, conflicting data are presented on the correlation between exposure and BP. Therefore, the most adequate biomarker (drug exposure or BP) needs to be established. However, the current available data from the axitinib dose titration trial provide evidence for a toxicity-driven individualized axitinib dosing approach.

Dabrafenib
Correlation between exposure and efficacy and toxicity
There are currently no data that explore the relation between dabrafenib exposure and efficacy or toxicity.

Inter- and intra-patient variability in exposure
The inter-patient variability in PK is large, with CV% for AUC, C_{trough}, and Cl/F of 38-68%, 119% and 58%, respectively [29,30]. Weight, age, and gender were not considered clinically relevant in explaining the large inter-patient variability [29,31,32]. No data on intra-patient variability are available.

Dose individualization
There are currently no studies investigating dose individualization strategies for dabrafenib.

Conclusion
Dabrafenib shows high inter-patient variability in exposure. However, data regarding the intra-patient variability are lacking and, most importantly, there are no proven correlations between drug exposure and response. These main prerequisites need to be met before dose individualization of dabrafenib can be considered.

Erlotinib
Correlation between exposure and efficacy
A study in 56 patients with stage IV non-small cell lung cancer (NSCLC) showed that C_{trough} levels after 7 days of therapy were 5.22 nmol/mL in patients with PR, 4.00 nmol/mL in patients with stable disease (SD), and 3.44 nmol/mL in patients with progressive disease (PD), although, statistically, this was not significantly different ($P > 0.05$) [33]. In addition, the cut-off value of 4.6 nmol/mL for C_{trough} associated with skin toxicity (patients with skin toxicity had better treatment outcome) could not predict OS ($P = 0.351$) and PFS ($P = 0.127$) [33]. In another phase II study in 19 patients with NSCLC, C_{trough} levels were measured on day 2 and 8 of treatment [34]. The C_{trough} day 8:C_{trough} day 2 ratio represented the accumulation of erlotinib over time. A larger ratio was considered to reflect low metabolism and thereby higher erlotinib exposure. In this analysis, a higher ratio was associated with longer PFS ($P = 0.004$). However, an effect of this ratio on OS could not be shown. Although erlotinib is not registered for the treatment of head and neck squamous cell cancer (HNSCC), two studies showed a correlation in this patient population. In a phase II study in 18 patients with HNSCC, time to progression (TTP) was related to C_{trough} levels of erlotinib ($P = 0.042$) and its active metabolite OSI-420 ($P = 0.036$) [35]. A correlation with OS was only found for OSI-420 C_{trough} levels ($P = 0.019$). Another study in patients with HNSCC evaluated three sampling windows; C_{trough} window (20-25 hours post-dose, n = 42), C_{max} window (2-5 hours post-dose, n = 77) or $C_{5\text{-}10}$ (5-10 hours post-dose, n = 47]. The median $C_{5\text{-}10}$ of both erlotinib and OSI-420 ($P = 0.021$ and $P = 0.0005$), as well as C_{trough} of OSI-420 ($P = 0.0014$) predicted improved OS [36].

Correlation between exposure and toxicity
Besides the correlation between erlotinib exposure and efficacy, several studies have reported on associations between the occurrence and severity of rash and clinical outcome. In a phase II study in 57 patients with NSCLC, the median OS for patients with ≥ grade 2 rash was 19.6 months versus 8.5 for grade 1 rash, and 1.5 months for patients without rash [37]. Comparable results were shown in other trials [33,35,36,38-45]. Surprisingly, in the studies that showed correlations between PK and treatment outcome and/or toxicity and treatment outcome, PK parameters were not always related to toxicity [33-36]. This indicates that skin toxicity is not merely a reflection of high erlotinib exposure. The largest analysis performed to determine the correlation between exposure and toxicity is that of the pivotal BR.21 trial in 339 patients with NSCLC. In this analysis, a correlation between $AUC_{0\text{-}24}$ and C_{max} and rash was demonstrated. However, because of a large overlap in PK parameters between patients with and without toxicity, the correlation was considered not relevant [46]. Several smaller analyses have also shown correlations between

TKI	Tumor type	N	PK parameter	Outcome	Correlation	Significance	REF
Axitinib	mRCC	73	AUC_{0-24}	hypertension, grade 3-4 toxicity, dose reductions and ≤ 2 AH-treatments	432 versus 176 ng·hr/mL for patients with and without toxicity	N/A	[20]
	solid	10	AUC_{0-12}	ΔTSH level	ΔTSH increased linear with AUC_{0-12} (r = 0.72 and r = 0.80)	*P* = 0.018 *P* = 0.005	[21,22]
	mRCC	233	AUC_{0-24}	hypertension, proteinuria, fatigue and diarrhea	probability for toxicities was AUC_{0-24} dependent	*P* < 0.001	[23]
	mCRC	128	AUC_{ss}	diarrhea, fatigue and hypertension	no correlation	*P* > 0.05	[18]
Erlotinib	NSCLC	339	AUC_{0-24} and C_{max}	rash	severity of rash increased with AUC_{0-24} and C_{max} (r = 0.14 and r = 0.13)	*P* = 0.01 *P* = 0.02	[46]
		84	C_{trough}	grade 3-4 toxicities	incidence of grade 3/4 toxicities increased with C_{trough}	*P* = 0.007	[45]
			C_{trough} ≥ versus < 1.21 µg/mL	grade ≥ 2 rash	OR: 2.83 (95%-CI: 1.10-7.29)	*P* = 0.031	
				grade ≥ 2 diarrhea	OR: 3.79 (95%-CI: 1.09-13.2)	*P* = 0.037	
			C_{trough}	ILD	~1000 versus ~3300 ng/mL for patients with and without ILD	*P* = 0.014	
		28	AUC_{0-24}	rash	54.2 and 59.1 vs 36.2 µg·hr/mL for patients with grade 2 and 3 or grade 1 rash	*P* = 0.046	[47]
			C_{max}	rash	1.99 and 1.86 vs 1.29 µg/mL for patients with grade 2 and 3 or grade 1 rash	*P* = 0.044	
	brain	46	AUC_{0-24}	skin toxicity	severity of skin toxicity increased with AUC_{0-24} probability for skin toxicity was AUC_{0-24} dependent	*P* = 0.06 N/A	[48]
	HNSCC	42	AUC_{0-24}	skin toxicity	severity of skin toxicity increased with AUC_{0-24} probability for skin toxicity was AUC_{0-24} dependent	*P* = 0.014 N/A	[49]
	NSCLC, HNSCC and ovarian	80	AUC_{0-24}	grade ≥ 2 rash	1.18 fold increase in probability of grade ≥ 2 rash for every 10 µg·hr/mL increase in AUC_{0-24}	*P* = 0.082	[50]
			C_{trough}	grade ≥ 2 rash	1.75-fold increase in probability of grade ≥ 2 rash for every 1 µg/mL increase in C_{trough}	*P* = 0.040	
	solid	40	AUC_{0-24}	skin toxicity	18 versus 11.8 µg·hr/mL for patients with and without skin toxicity	*P* = 0.02	[51]
Gefitinib	NSCLC	30	C_{trough} ≥ versus < 200 ng/mL	incidence skin toxicity	85.7% versus 42.9%	*P* = 0.043	[75]
	solid	27	C_{trough}	≥ grade 1 diarrhea	probability for ≥ grade 1 diarrhea was C_{trough} dependent	*P* < 0.05	[80]
Imatinib	GIST	38	AUC_{0-24}	toxicity	2.2 fold increase in probability of toxicity for every doubling of the AUC_{0-24}	*P* < 0.001	[102]
	GIST	30	AUC_{0-24} unbound	% decrease in ANC	Δ ANC decreased linear with AUC_{0-24} (r = 0.56)	*P* < 0.001	[103]
	CML	351	C_{trough} > 1,170 versus < 647 ng/mL	fluid retention, rash, myalgia and anemia	76% versus 53%, 51% versus 32%, 30% versus 20% and 20% versus 8% respectively	N/A	[3]
	CML	240	C_{trough} > 3180 ng/mL	grade 3-4 neutropenia, rash, diarrhea, myalgia and edema	32% versus 17%, 35% vs 12%, 35% versus 17%, 27% versus 17% and 22% versus 5% respectively	N/A	[104]
Pazopanib	solid	54	C_{trough} ≥ versus < 15 µg/mL	hypertension	77% versus 39%	N/A	[163]
	solid	31	AUC_{0-24}	DLT	896 versus 367 µg·h/mL for patients with and without DLT	*P* = 0.039	[167]
					incidence of DLT increased linear with AUC_{0-24} (r = 0.595)	*P* = 0.001	
			C_{trough}	DLT	38.8 versus 29.6 µg/mL for patients with and without DLT	*P* = 0.040	
			C_{trough}	grade 2-3 hypertension	43.7 versus 29.4 µg/mL for patients with grade 2/3 hypertension and normotensive patients	*P* = 0.004	
		22	AUC_{0-72}	sBP	magnitude and duration of elevation in sBP greater for patients with AUC_{0-72} of 1,840 versus 786 µg·h/mL	N/A	[168]
	mRCC	205	C_{trough} 12.6-46 µg/mL	diarrhoea, hair colour change, ALT increase, HFS and stomatitis	≥ 2 fold increase in incidence of toxicities with increase of C_{trough}	N/A	[6]
			C_{trough}	HFS	occurrence and severity increased with C_{trough}	*P* < 0.001	[169]
Sorafenib	solid	72	AUC_{0-12}	grade 3-4 toxicities	61.9 versus 53 µg·hr/mL for patients with and without grade 3-4 toxicities	*P* = 0.017	[179]

Table 4 [Continued]

TKI	Tumor type	N	PK parameter	Outcome	Correlation	Significance	REF
Sorafenib			AUC_{0-12}	HFSR	high AUC_{0-12} was associated with the occurrence of HFSR	$P = 0.03$	
	melanoma	27	AUC_{0-12}	≥ grade 2 hypertension	82 versus 54 µg·hr/mL for patients with and without hypertension	$P = 0.02$	[177]
				HFSR	76 versus 61 µg·hr/mL for patients with and without HFSR	$P = 0.0008$	
	solid		AUC_{cum}	≥ grade 3 toxicity	OR: 1.07 (95%-CI: 1.01-1.12)	$P = 0.037$	[180]
	RCC and HCC	52	C_{trough}	≥ grade 2 HFS	C_{trough} lower for patients with grade 0-1 HFS versus patients with ≥ grade 2 HFS	$P = 0.0045$	[178]
				≥ grade 2 hypertension	C_{trough} lower for patients with grade 0-1 hypertension versus patients with ≥ grade 2 hypertension	$P = 0.0453$	
	prostate and NSCLC	96	AUC_{0-12}	rash grade	severity of rash increased with AUC_{0-12}	$P = 0.02$	[181]
	HCC	17	AUC_{0-12}	DLT	106.4 versus 56.7 µg·hr/mL for patients with and without DLT	$P = 0.09$	[182]
	NSCLC	42	C_{trough} ≥ versus < median	grade 2-3 diarrhea	patients with C_{trough} > median were more likely to develop diarrhea	$P = 0.04$	[183]
	solid	22	C_{trough}	grade 3 toxicity	7.6 versus 4.4 µg/mL for patients with and without grade 3 toxicity	$P = 0.0083$	[184]
Sunitinib	solid	28	C_{trough} > 100 ng/mL	DLT	most patients with DLT had C_{trough} > 100 ng/mL	N/A	[216]
	mRCC	19	C_{trough} ≥ versus < 90 ng/mL	grade ≥ 2 thrombocytopenia	100% versus 55.6%	$P = 0.033$	[217]
				grade ≥ 2 hypertension	90% versus 22.2%	$P = 0.0055$	
	solid, GIST and mRCC	443	AUC_{0-24}	fatigue	positive correlation between AUC_{0-24} and incidence of fatigue	N/A	[5]
			AUC_{cum28}	ANC	Δ ANC decreased linear with AUC_{cum28} (r = -0.40)	N/A	
			C_{trough}	dBP	Δ dBP increased linear with C_{trough} (r = 0.29)	N/A	
	solid	24	C_{trough} ≥ versus < 180 ng/mL	QTc	15.4 versus 9.6 msec.	N/A	[218]
	pNET, GIST and mRCC	52	Cl/F	grade 3 toxicity	34.4 versus 41.4 L/hr for patients with and without grade 3 toxicity	$P = 0.025$	[219]
			C_{trough}	fatigue	positive correlation between C_{trough} and occurrence of fatigue	$P = 0.007$	
Vandetanib	MTC	223	C_{trough}	grade ≥ 2 diarrhea	positive correlation between C_{trough} and probability of diarrhea	$P = 0.025$	[244]
				grade ≥ 2 fatigue	positive correlation between C_{trough} and probability of fatigue	$P = 0.02$	
Vemurafenib	N/A	N/A	C_{trough}	SCC	positive correlation between C_{trough} and risk of SCC	$P < 0.0001$	[255]
	melanoma	132	C_{trough}	QTc-interval prolongation	positive correlation between C_{trough} QTc-interval prolongation	$P < 0.0001$	[256]

Abbreviations: AH, antihypertensive; ANC, absolute neutrophil count; AUCcum28, 28-day cumulative AUC; dBP, diastolic blood pressure; CML, chronic myeloid leukemia; DLT, dose limiting toxicities; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell cancer; HFS, hand-foot syndrome; HFSR, hand foot skin reactions; ILD, interstitial lung disease; mCRC, metastatic colorectal cancer; mRCC, metastatic renal cell carcinoma; MTC, medullary thyroid cancer; N/A, not available; NSCLC, non-small cell lung carcinoma; p-NET, pancreatic neuroendocrine tumor; sBP, systolic blood pressure; SCC, squamous cell carcinomas; THS, thyroid stimulating hormone

AUC_{0-24}, C_{trough}, C_{max}, and grade 3/4 toxicities, skin toxicity, rash, and diarrhea in NSCLC, HNSCC, ovarian cancer, and brain tumors, as shown in Table 4 [45,47-50].

Inter- and intra-patient variability in exposure

The inter-patient variability in C_{trough}, AUC, and CI/F is 38-76%, 18-156%, and 10-129%, respectively [40,42,47,51-72]. The European Public Assessment Report (EPAR) of erlotinib reports an intra-patient AUC variability of 16-24 CV% measured in healthy volunteers.

Dose individualization

A phase II trial investigated the feasibility of toxicity-driven dosing to a maximal level of tolerable target rash (TR) that required symptomatic treatment with minocycline [73]. Only 21% of the patients who ultimately experienced a TR developed this under dose escalation, whereas most patients experienced the TR under the standard dose of 150 mg once daily (QD). In addition, no increase in anticancer activity was observed in the dose-escalated group.

Table 5 PK inter- and intra-patient variability of TKIs

TKI	Inter-patient variability (CV%)			Intra-patient variability (CV%)			
	C_{trough}	AUC_a	Cl/F (L/hr)	C_{trough}	AUC_a	Cl/F (L/hr)	Ref
Axitinib	N/A	17-113%	17-113%	20-22%	20-33%	20-22%	[21,22,24-26]
Dabrafenib	119%	59%	59%	N/A	N/A	N/A	[29,30]
Erlotinib	38-76%	10-129%	10-129%	N/A	16-24%	N/A	[40,42,47,51-72]
Gefitinib	14-166%	79-90%	79-90%	2-49%	14%	N/A	[75,77,78,81-98]
Imatinib	25-64%	17-88%	17-88%	15-27%	12%	N/A	[4,105-123]
Lapatinib	55-97%	48%	48%	N/A	30-36%	N/A	[147-162]
Pazopanib	11-90%	N/A	N/A	N/A	N/A	N/A	[158,163,165,167,168,170,171]
Regorafenib	57%	N/A	N/A	N/A	34%	N/A	[172,174-176]
Sorafenib	25-104%	13-80%	13-80%	N/A	31-47%	N/A	[92,177-180,184-210]
Sunitinib	34-59%	28-46%	28-46%	N/A	N/A	N/A	[209,216,220-236]
Vandetanib	20-56%	8-55%	8-55%	N/A	8%	N/A	[245-253]
Vemurafenib	N/A	32-54%	32-54%	N/A	N/A	N/A	[255-258]

Abbreviations: %CV, coefficient of variation; AUC, area under the concentration time curve; Cl/F, apparent oral clearance; C_{trough}, minimum plasma concentration level; N/A, not available. [a]AUC_∞ following a single dose or AUC over the dosing interval at steady state.

Conclusion

Erlotinib shows large inter-patient variability and, although based on limited data, the intra-patient variability appears small. Some studies have shown exposure-efficacy and exposure-toxicity relations. Rash is often suggested as a potential early biomarker to select patients in need for dose adjustment, although dosing to rash did not improve clinical activity. Furthermore, in studies that showed correlations between PK and treatment outcome and/or toxicity and treatment outcome, PK parameters were not always related to toxicity. In our opinion, it is unlikely that rash can be used to individualize erlotinib therapy because dosing to rash did not demonstrate improved treatment outcomes.

Gefitinib

Correlation between exposure and efficacy

Similar to erlotinib, a study in 44 patients with NSCLC measured C_{trough} levels [74]. A high C_{trough} day 8:C_{trough} day 3 ratio was associated with better PFS ($P = 0.0158$), although individual C_{trough} levels were not related to longer PFS. Furthermore, no correlation with OS was found. A prospective study in 30 patients with NSCLC showed that patients with high gefitinib exposure ($C_{trough} \geq 200$ ng/mL) had longer OS ($P = 0.007$) compared with patients with low exposure ($C_{trough} < 200$ ng/mL) [75]. Additionally, the patients with wild type epidermal growth factor receptor (EGFR) appeared to be more sensitive to higher exposure levels with longer survival (~2 months longer median OS) compared with the other patients. Finally, in a dose escalation to skin toxicity study with 20 patients with HNSCC, C_{trough} levels for patients with disease control (PR + SD) were higher compared with patients with PD (1117 versus 520 ng/mL, $P = 0.0103$) [76].

Correlation between exposure and toxicity

Different phase I studies explored a possible relation between gefitinib plasma concentrations and skin- and gastrointestinal toxicity [77-79]. Zhao et al. showed that patients with high gefitinib exposure ($C_{trough} \geq 200$ ng/mL) experienced more rash ($P = 0.043$) compared with patients with low exposure ($C_{trough} < 200$ ng/mL [75]. The incidence of gastrointestinal toxicity was not found to differ between the two groups [75]. However, in the population PK analysis of Li et al., gefitinib C_{trough} level was a significant predictor for the incidence of ≥ grade 1 diarrhea ($P < 0.05$) [80].

Inter- and intra-patient variability in exposure

Gefitinib shows large inter-patient variability in AUC (31-112%), Cl/F (79-90%) and C_{trough} (14-166%) [75,77,78,81-98]. The intra-patient variability for C_{trough} is 2-49% [77,91]. A phase I study designed to determine the intra-patient variability, showed a two-fold variability in AUC within subjects, whereas the variability between patients was 15-fold [85]. Population PK studies indicated that gender, age, bodyweight, ethnicity, or creatinine clearance cannot explain the large inter-patient variability [99].

Dose individualization

There are three dose individualization studies published for gefitinib; two phenotyping studies and one toxicity-driven dosing study [76,80,100].

Given that cytochrome P450, family 3, sub-family A (CYP3A) is the principal enzyme that metabolizes gefitinib, variability in its activity might be an explanation of PK variability. The first phenotyping study showed that midazolam oral clearance as a measure of CYP3A activity accounted for 37% of the inter-patient variability in gefitinib oral clearance [80]. Furthermore, midazolam clearance was strongly associated with both gefitinib clearance ($r^2 = 0.68$) and gefitinib C_{trough} ($r^2 = 0.58$). Therefore, midazolam could be used to identify those patients at risk for under- or overdosing, respectively. The second phenotyping study showed a borderline significant correlation between midazolam and gefitinib AUC [100]. In a dose escalation study in patients with HNSCC, the gefitinib dose was escalated from 500 to 750 mg in those patients without grade 2 skin toxicity [76]. In the preplanned analysis of patients with and without ≥ grade 2 skin toxicity, there was no difference observed in treatment benefit.

Conclusion

The intra-patient variability in gefitinib PK appears small compared with the large inter-patient variability. Further investigation to determine the exact correlation between gefitinib exposure and treatment benefit is required, because the two studies that showed a correlation were performed in small cohorts. Once this has been established and after prospective validation, dose individualization seems a reasonable option to improve treatment efficacy and prevent underdosing.

Imatinib

Correlation between exposure and efficacy

The most convincing evidence for a correlation in solid tumors comes from a retrospective analysis of a phase II trial including 73 patients with gastrointestinal stromal tumors (GIST). This analysis showed that patients with C_{trough} levels < 1100 ng/mL after 29 days of therapy, had shorter TTP (11.3 months) compared with patients with C_{trough} levels above this concentration (> 30 months, $P = 0.0029$) [4]. Patients with low exposure also showed a trend towards a lower overall objective benefit rate (OOBR; CR + PR + SD). These findings suggest that a minimal concentration of imatinib is necessary to achieve and maintain clinical response in patients with GIST. A prospective population PK study on imatinib C_{trough} levels observed a decrease in imatinib exposure of approximately 30% after 3 months of therapy [101]. Therefore, measuring levels should be time-point specific and repeated after 3 months of therapy. Widmer et al. similarly demonstrated the importance of sufficient drug exposure to achieve and maintain therapeutic responses with the use of PK-PD data from 38 patients with GIST [102]. However, this analysis suggested that it is unbound imatinib exposure, rather than total imatinib exposure, which is associated with response.

Correlation between exposure and toxicity

Widmer et al. also showed that the occurrence and number of adverse effects were associated with both imatinib total and free plasma concentrations ($P < 0.001$) in patients with GIST [102]. A phase III trial in patients with GIST showed that hematologic toxicity (% decrease in ANC and platelets) was also correlated with unbound imatinib $AUC_{0\text{-}24}$ at steady-state ($P < 0.001$) [103]. Larson et al. showed that the discontinuation rate of imatinib resulting from toxicity was higher in patients with high C_{trough} levels (> 1170 ng/mL) compared with patients with low C_{trough} levels (≤ 1170 ng/mL) [3]. Another study showed that high C_{trough} levels (Q4, C_{trough} > 3180 ng/mL) were associated with the frequency of all-grade and grade 3/4 neutropenia, anemia, and leukopenia observed within the first 3 months of therapy and, to a lesser extent, all-grade thrombocytopenia. For non-hematologic toxicities, C_{trough} levels were associated with the frequency of all-grade rash, edema, nausea, diarrhea, vomiting, arthralgia, myalgia, and extremity pain within the first 3 months of therapy [104].

Inter- and intra-patient variability in exposure

Imatinib shows large inter-patient variability in AUC (21-66%) and C_{trough} (25-64%) [4,105-121]. There are four studies that report both the intra- and inter-patient variability in C_{trough}; these ranged from 19% to 27% versus from 37% to 47%, respectively [117,119,121,122]. A fifth study showed an intra-patient variability in AUC of 12.4% versus 11.6% for the inter-patient variability [123]. In different population PK analysis, body weight, age, sex, disease diagnosis, plasma α1-acid glycoprotein, albumin, granulocyte count, white blood cells (WBC), hemoglobin (Hb), and major gastrectomy were found to explain a certain part of the inter-patient variability, but dose adjustment based on these covariates was not considered necessary [103,119,124-132].

Dose individualization

Although several retrospective studies are in support of dose individualization, the results of the first prospective trials assessing the influence on treatment outcome are awaited. There are ongoing trials aiming to establish the optimal use of therapeutic drug monitoring (TDM) for imatinib in chronic myeloid leukemia (CML; ISRCTN 31181395) and two studies to determine whether dose adjustments to reach a target exposure will improve treatment outcome in GIST patients (NCT01031628) and CML (NCT01827930). Meanwhile, several case reports underscore the value of dose individualization of imatinib [133-135].

Conclusion

We consider imatinib the TKI with currently the most evidence available to justify the measurement of C_{trough} levels. There is a clear correlation

between exposure and efficacy with C_{trough} levels > 1000-1100 ng/mL associated with better treatment outcome. Moreover, the intra-patient variability is small compared with the inter-patient variability. However, prospective trials investigating the influence of dose individualization on treatment outcome are awaited. Currently, TDM is already applied by some clinicians, although it is not part of routine clinical practice yet [136-143]. If measurement takes place, this should be time-point specific and repeated every 3 months because patients with GIST show a decrease in exposure over time [101].

Lapatinib

Correlation between exposure and efficacy

The only suggestion for a correlation comes from the first phase I trial in which most responders had a C_{trough} level within the 0.3-0.6 µg/mL range [144].

Correlation between exposure and toxicity

Another phase I study reported that the frequency and severity of rash seemed to be related to AUC_{0-24}, C_{max}, and C_{trough} rather than the dose [145]. The FDA approval report states that a relation between lapatinib concentrations and prolonged QTc-interval is possible, although convincing evidence is lacking [146].

Inter- and intra-patient variability in exposure

The CV% in AUC and C_{trough} ranged from 42% to 117% and 55% to 97%, respectively [147-159,145,160-162]. The only data considering intra-patient variability are reported in the EPAR and is estimated to be 30-36% for AUC_{0-24} [161]. Sex, weight, ethnicity, or age could not explain the inter-patient variability in PK [161].

Dose individualization

There are currently no studies considering individualization strategies for lapatinib.

Conclusion

In theory, lapatinib meets many of the criteria for dose individualization. Moreover, the inter-patient variability is relatively large compared with the intra-patient variability. However, evidence for a correlation between lapatinib exposure and treatment benefit or toxicity is lacking. Currently, there is insufficient evidence to support dose individualization of lapatinib.

Pazopanib

Correlation between exposure and efficacy

Several smaller studies with pazopanib have shown a threshold for efficacy of approximately 20 µg/mL [163-165]. The most convincing evidence for this threshold comes from a retrospective PK analysis of a phase II trial in 205 patients with mRCC [7,166]. Patients with a C_{trough} > 20.6 µg/mL after 4 weeks of pazopanib 800 mg QD, showed significantly longer PFS ($P = 0.0041$) [7]. In addition, the RR as well as the mean percentage tumor shrinkage was improved in patients with C_{trough} levels > 20.6 µg/mL ($P < 0.0001$) [7].

Correlation between exposure and toxicity

The first suggestion for a correlation between pazopanib exposure and toxicity comes from the same first phase I study [163]. Twenty out of 26 patients (77%) with C_{trough} levels ≥ 15 µg/mL on day 22 developed hypertension, whereas only 11 out of 28 patients (39%) with C_{trough} levels < 15 µg/mL did so [163]. In a phase I trial in children, patients with DLT had a significantly larger AUC_{0-24} and C_{trough} compared with those without (896 versus 367 µg·hr/mL, $P < 0.039$ and 38.8 versus 29.6 µg/mL, $P < 0.040$, respectively) [167]. Moreover, a significant relation between BP and C_{trough} was identified. In patients with drug-related grade 2 or 3 hypertension after a median of two cycles, mean C_{trough} was 43.7 µg/mL versus 29.4 µg/mL in normotensive patients ($P < 0.004$) [167].

In a food interaction study with pazopanib, the incidence of elevated systolic blood pressure (≥ 140 mmHg) was found to be similar in both fed and fasted conditions. However, the magnitude and duration of elevated BP were greater when the drug was administered with a meal, correlating with an increased AUC_{0-24} [168].

The most convincing evidence comes from analysis of the before mentioned 205 patients with mRCC included in a phase II trial [6,166]. This analysis showed that the incidence of different pazopanib-induced toxicities seemed to be concentration dependent; there was a more than twofold increase in the incidence of diarrhea, hair color change, ALT increase, hand-foot syndrome (HFS), and stomatitis when C_{trough} after 4 weeks of treatment increased from 12.6 to 46 µg/mL. Additionally, the occurrence and severity of HFS was also correlated with higher week 4 C_{trough} levels ($P < 0.001$) [169].

Inter- and intra-patient variability in exposure

Pazopanib shows large inter-patient variability in PK with values ranging from 11% to 67% for C_{trough} and from 19% to 76% for AUC [158,163,165,167, 168,170,171]. Data considering the intra-patient variability are lacking thus far. Our own unpublished results indicate that the intra-patient variability

is relatively large, possibly because of the large effect of food on the low and variable bio-availability of pazopanib.

Dose individualization
Different studies are currently investigating the feasibility of TDM for pazopanib. We investigated the feasibility of TDM to reach a target exposure within a predefined window. There is also a study designed to reach a target pazopanib C_{trough} 20 > µg/mL by TDM. Outcomes of these studies are awaited.

Conclusion
In our opinion, a C_{trough} level above 20 µg/mL should be targeted in clinical practice to prevent underdosing and unjustified discontinuation of pazopanib treatment. Given that our results show a relatively large intra-patient compared with inter-patient variability, measuring C_{trough} levels should be performed under standardized conditions to make interpretation possible. The described saturated absorption of pazopanib might be challenging for dose adjustment, although we hypothesize that dividing the daily dose or the administration with food might overcome this problem [163]. Given that pazopanib exposure has been correlated with hypertension, BP could be a potential valuable biomarker.

Regorafenib
Correlation between exposure and efficacy and toxicity
There are no data available that report on PK-PD relations. Both FDA and EMA approval reports state that this will be investigated post-marketing [172,173].

Inter- and intra-patient variability in exposure
The inter-patient variability in PK is relatively large, with CV% for AUC and C_{trough} of 43-88% and 57%, respectively [172,174-176]. The reported intra-patient variability in AUC is 34% [175]. No significant or clinically relevant influence of weight, age or gender, race, or bilirubin on PK parameters could be shown [173].

Dose individualization
There are no studies that investigate dose individualization strategies.

Conclusion
In theory, regorafenib meets many of the criteria for dose individualization. Moreover, the inter-patient variability is relatively large compared with the intra-patient variability, although its dose-limited absorption might be challenging [172-174]. However, most importantly, there are currently no data that show a correlation between regorafenib exposure and treatment benefit or toxicity. Therefore, there is currently insufficient evidence to support dose individualization of regorafenib therapy.

Sorafenib
Correlation between exposure and efficacy
Although sorafenib is not registered for this indication, the first PK-PD analysis was performed in 27 melanoma patients. Patients with high sorafenib exposure ($AUC_{ss} \geq 100$ µg·hr/mL) showed higher tumor control ($P = 0.04$), tumor response (PR and SD) ($P = 0.02$) and longer PFS ($P = 0.005$) [177]. Another analysis showed that patients with hepatocellular carcinoma (HCC) with high exposure ($C_{max} \geq 4.78$ µg/mL) had a trend ($P = 0.0824$) towards longer OS compared with patients below this threshold [178].

Correlation between exposure and toxicity
The first suggestion for a relation between sorafenib exposure and toxicity comes from a phase I trial and different later studies have also reported this observation [177-184]. In a retrospective analysis of 83 patients treated with sorafenib at a dose of 200-400 mg BID, patients with severe toxicity (grade 3-4 adverse events) had significantly higher sorafenib exposure than that observed in the remaining patients (61.9 versus 53 µg·hr/mL, $P = 0.017$) [179]. Additionally, a high AUC_{0-12} on day 30 of treatment was significantly ($P = 0.03$) associated with the occurrence of hand food skin reaction (HFSR).

In the aforementioned study, sorafenib median AUC_{0-12} after 1 month was greater in patients with grade ≥ 2 hypertension compared with those with normal BP (82 versus 54 µg·hr/mL, $P = 0.02$) and patients with grade ≥ 2 HFSR compared with those without HFSR (76 versus 61 µg·hr/mL, $P = 0.0008$). However, no correlations were observed for other toxicities, such as diarrhea, anorexia, allergic, and nonallergic skin rash [177]. Another analysis showed that increased AUC_{cum} was associated with any grade ≥ 3 toxicity ($P = 0.037$) [180]. The opposed AUC_{cum} threshold acquired by simulation that predicted a toxicity of grade ≥ 3 was 3161 µg·hr/mL.

A PK-PD analysis by Fukudo et al. showed that steady-state C_{trough} in patients with grade ≥ 2 HFSR ($P = 0.0045$) and hypertension ($P = 0.0453$) were larger than in patients with < grade 2 adverse events. The proposed C_{trough} threshold for grade ≥ 2 HFSR and grade ≥ 2 hypertension were estimated to be 5.78 µg/mL and 4.78 µg/mL, respectively [178]. Another study showed that the severity of rash increased ($P = 0.02$) with increasing AUC_{0-12} [181]. Additionally, Mir et al. showed that patients who experienced a DLT during the first 4 weeks of treatment had higher AUC_{0-12} (106.4 versus 56.7 µg·hr/mL, $P = 0.09$) [182].

Inter- and intra-patient variability in exposure
Sorafenib exhibits high variability in C_{trough} (25-104%), AUC (12-117%) and CI/F (13-80%) compared with modest intra-patient variability in AUC (31-47%) [92,177-180,184-210]. Gender is suggested to be a covariate of significant influence on sorafenib PK, whereas bodyweight could only explain a clinically non-relevant part of the inter-patient variability [180,211].

Dose individualization
There are no studies that investigated sorafenib dose individualization strategies.

Conclusion
It can be concluded that the inter-patient variability of sorafenib is relatively large compared with the intra-patient variability. The dose-limited absorption of this drug might be challenging for dose individualization [212]. Further research to determine the exact correlation between sorafenib exposure and treatment benefit is required. Similar to imatinib, it seems that sorafenib exposure decreases after 3-4 months of treatment [177-179,213]. This might have relevant clinical implications in patients with initial clinical benefit who develop subsequent progression. Dose escalation in these patients could be supported by measuring plasma concentration levels, although routine application of TDM for sorafenib is currently not justified.

Sunitinib
Correlation between exposure and efficacy
The most convincing evidence for a correlation between exposure and treatment response in humans comes from a PK-PD analysis by Houk et al. This analysis showed that patients with mRCC (n = 169), GIST (n = 401), or solid tumors (n = 69) and a sunitinib $AUC_{ss} \geq 800$, 600, and 700 ng·hr/mL, respectively, had longer TTP and better OS [5]. Extrapolation of these sunitinib AUCs would correspond with sunitinib + SU12661 C_{trough} levels of 36.4, 24.6, and 30.5 ng/mL respectively, which are close to the concentrations (50-100 ng/mL) found in preclinical in vivo research [214,215]. Additionally, there was a significant relation ($P < 0.001$) between exposure and the probability of a PR or CR in patients with mRCC. Finally, a relation between the probability of SD and sunitinib exposure was demonstrated for patients with mRCC ($P = 0.002$) and GIST ($P < 0.001$) [5]. Sunitinib is also continuously dosed as 37.5 mg QD in patients with pancreatic neuroendocrine tumors (pNET) and sometimes those with GIST. For this indication, it is reasonable to use a lower target for C_{trough} that corresponds with this lower dose. Given that sunitinib shows dose proportional PK, a realistic recommendation is a target sunitinib + SU12661 C_{trough} of > 37.5 ng/mL.

Correlation between exposure and toxicity
The first phase I trial in 28 patients treated with sunitinib showed that the occurrence of DLTs was associated with total sunitinib trough levels > 100 ng/mL [216]. In an explorative study in 19 patients with mRCC, those with high sunitinib exposure ($AUC_{0\text{-}24}$ > 2600 ng·hr/mL and C_{trough} > 90 ng/mL) experienced more grade ≥ 2 thrombocytopenia ($P = 0.033$) and hypertension ($P = 0.0055$) compared with patients with low sunitinib exposure [217]. The meta analysis by Houk et al. showed a positive relation between total AUC and the incidence of fatigue; a negative relation between absolute neutrophil count (ANC) and AUC_{cum} after 28 days; and a positive relation between total C_{trough} level and dBP changes [5]. A PK-PD analysis in 24 patients showed that changes in QTc interval correlated with sunitinib exposure AUC, and C_{trough} [218]. In a recently published phenotyping study, patients with any type of grade 3 toxicity had a significantly lower clearance of sunitinib than patients without grade 3 toxicities (34.4 versus 41.4 L/hr, $P = 0.025$) [219]. Additionally, total C_{trough} levels were positively correlated with the occurrence of fatigue ($P = 0.007$) [219].

Inter- and intra-patient variability in exposure
The reported inter-patient variability is large for C_{trough} (34-59%), AUC (13-49%) and CI/F (26-46%) [209,216,220-236]. Data on intra-patient variability are lacking. A population PK analysis showed that tumor type, race, gender, body weight, and Eastern Cooperative Oncology Group (ECOG) score could explain some of the inter-patient PK variability, although dose adjustment based on these covariates is not advised [237].

Dose individualization
Two phenotyping studies with midazolam have been conducted [214, 219]. The first study showed that midazolam exposure was highly correlated with both sunitinib and total sunitinib $AUC_{0\text{-}24}$, as well as with C_{trough} levels and that CYP3A4-activity explained a large proportion of the inter-patient variability in sunitinib PK [214]. The second phenotyping study found a significant, although weak correlation between the 1'OH-midazolam:midazolam ratio and sunitinib clearance [219].

Data considering TDM as an approach to individualize sunitinib therapy are limited to case reports and conference abstracts [238-242]. However, all reports show the feasibility of TDM as an approach to achieve optimal C_{trough} plasma concentrations.

Conclusion
In our opinion, sunitinib is, after imatinib, the TKI with the most evidence available to support dose individualization. There is an evident correlation between sunitinib exposure and efficacy as well as toxicity and the report-

ed inter-patient variability is large. In addition, different reports have shown the feasibility of TDM to achieve an optimal target sunitinib exposure. However, prospective trials assessing treatment outcome with dose individualization are warranted. Alternative biomarkers for dose individualization could be phenotyping CYP3A(4) activity, although this also needs prospective validation. Although it is not yet part of routine clinical practice, we believe that a drug level-based dose adjustment with a target C_{trough} level of > 50 ng/mL for intermittent dosing and > 37.5 ng/mL for continuous dosing is justified.

Vandetanib

Correlation between exposure and efficacy

In the phase III study in 226 patients with medullary thyroid cancer (MTC) treated with 300 mg vandetanib QD, no evidence was found for a correlation between C_{trough} levels at day 56 and PFS [243,244].

Correlation between exposure and toxicity

Significant relations were identified between exposure and diarrhea and fatigue, but not for hypertension and rash [274]. In addition, the QTc-interval prolongation was concentration dependent [244].

Inter- and intra-patient variability in exposure

The first phase I trial with vandetanib in solid tumors showed inter-patient variability in exposure of 44-99% [245]. Inter-patient variability in AUC has also been reported by other studies in both healthy subjects as well as in patients with different types of cancer, ranging from 8% to 59% [245-253]. Intra-subject variability in vandetanib exposure was found to be small; AUC of 8-10% and C_{max} of 11% [253]. The EPAR describes weight as a clinically non-relevant covariate. Race, gender, and age showed no effect on vandetanib PK [254].

Dose individualization

There are no studies investigating dose individualization strategies.

Conclusion

The intra-patient variability in vandetanib PK is small compared with the described inter-patient variability, although some reported inter-patient variability is also not large. Most importantly, evidence for an exposure-response relation is lacking and the evidence for a correlation with toxicity is marginal. Given that vandetanib is an EGFR inhibitor, rash might be a relevant early biomarker, although no correlations have yet been observed. There is currently insufficient evidence to support dose individualization of vandetanib therapy.

Vemurafenib

Correlation between exposure and efficacy

In a phase III study in patients with B-rapidly accelerated fibrosarcoma oncoprotein (BRAF) mutant melanoma, a statistically significant ($P = 0.0014$) relation between C_{trough} and PFS was shown [255]. The population PK-PD analysis reported in the EPAR showed that patients with low exposure had more increase in tumor size compared with the medium and high exposure group, suggestive of a correlation [256].

Correlation between exposure and toxicity

Analysis of the pivotal phase III trial also showed a relation between C_{trough} and the risk of developing squamous cell carcinomas ($P < 0.0001$) [255]. Exposure-QTc response analysis showed that vemurafenib prolonged the QTc interval in a concentration dependent manner ($P < 0.0001$). However, no major changes (i.e., >20 ms) in the mean QTc interval were detected and, therefore, the clinical relevance of this observation should be considered [256].

Inter- and intra-patient variability in exposure

The reported inter-patient variability in vemurafenib AUC ranged from 28% to 52% [255-258]. There are no data available considering the intra-patient variability. Covariates including baseline total bilirubin, AST and ALT, baseline creatinine clearance, age, gender, race, bodyweight, height, or body mass index had no influence on vemurafenib PK.

Dose individualization

There are no studies investigating dose individualization of vemurafenib.

Conclusion

In theory, vemurafenib meets many of the criteria for dose individualization. However, although the inter-patient variability is large, data considering the intra-patient variability are unreported. Moreover, there is only marginal evidence for a correlation between vemurafenib exposure and treatment benefit or toxicity. Therefore, there is currently insufficient evidence to support dose individualization of vemurafenib therapy.

Concluding remarks

Compared with conventional chemotherapy, TKIs are generally less toxic and have the advantage of oral administration. Although convenient to patients, oral administration might have the potential disadvantage of introducing variability in drug exposure between and within patients. Review of the literature shows that there is increasing evidence that treatment outcome of TKIs is related to their exposure. The current available

data suggest that a target C_{trough} level of > 1100 ng/mL, > 50 ng/mL, > 37.5 ng/mL, and > 20 µg/mL could be used for imatinib, sunitinib 50 mg 4/2, sunitinib 37.5 mg continuously, and pazopanib, respectively. For axitinib, dose adjustment should be toxicity driven.

An important limitation is that most exposure-response correlations are defined by retrospective analysis. Therefore, the effect of drug levels on treatment outcome is still lacking for most TKIs. In addition, studies are generally small, except those with axitinib, imatinib, pazopanib, and sunitinib. More attention should be paid to exposure-response relations during drug development, which would facilitate dose individualization and treatment optimization right after registration of a drug. Surprisingly, neither the time a drug is used nor the potential for dose individualization seems to be a predictor for the amount of data available on exposure-response relations. Most importantly, prospective studies investigating the clinical feasibility of dose individualization with treatment benefit as the primary outcome are awaited.

Nevertheless, monitoring C_{trough} levels of at least imatinib, sunitinib, and pazopanib might be indicated in clinical practice, for example in cases of extreme or unexpected toxicity, a lack of clinical benefit, suspected PK drug-drug interactions, in patients with a major gastrectomy or in suspected therapy nonadherence, to support clinical decision making. A difficulty for drug-level monitoring is the reported high, or sometimes unknown, intra-patient variability of some TKIs, which can depend on the individual physicochemical properties of the TKI (e.g. low oral bioavailability).

Challenges for dose individualization are the facilities required (e.g. equipment and trained personnel for the determination of TKI plasma concentrations). However, PK samples are readily transferable and there are multiple laboratories available that can measure the drug concentrations of TKIs. Another challenge encountered is that some exposure-efficacy/toxicity relations are based on AUCs, which are patient unfriendly and time consuming to measure. Effort should be made to determine surrogate PK markers (C_{trough} or limited sampling) that show a good correlation with the AUC to make TDM feasible for the clinical practice.

Obviously, drug exposure is not the sole determinant of clinical outcome in patients with cancer. PD factors and patient- or tumor-specific characteristics also contribute to the efficacy of TKIs [20]. For different reasons, such as unnecessary toxicity, treatment delay, de novo inefficacy but also costs, it is crucial to identify those patients who are most likely to respond to TKI therapy. After selecting the most effective drug for a specific tumor type, dose individualization could further help to optimize the individual treatment benefit-risk ratio, with the highest possible efficacy and the lowest possible toxicity of therapy.

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3

A validated assay for the simultaneous quantification of six tyrosine kinase inhibitors and two active metabolites in human serum using liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT A sensitive, sophisticated and practical bioanalytical assay for the simultaneous determination of six tyrosine kinase inhibitors (imatinib, sunitinib, nilotinib, dasatinib, pazopanib, regorafenib) and two active metabolites (N-desmethyl imatinib and N-desethyl sunitinib) was developed and validated. For the quantitative assay, a mixture of three stable isotopes as internal standards was added to human serum, standards and controls. Thereafter, samples were pre-treated using protein precipitation with methanol. The supernatant was diluted with water and injected into an ultra pressure liquid chromatographic system with an Acquity TQ tandem mass spectrometry detector. The compounds were separated on an Acquity BEH C18 analytical column (100 mm × 2.1 mm ID, 1.7 µm particle size) and eluted with a linear gradient system. The ions were detected in the multiple reaction monitoring mode. The lower limit of quantification and the linearity of all compounds generously met with the concentrations that are to be expected in clinical practice. The developed bioanalytical assay can be used for guiding TKI therapy in daily clinical practice as well as for investigator-initiated research.

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Introduction

In recent years, multiple tyrosine kinase inhibitors (TKIs) have been approved as monotherapy for cancer treatment such as in renal cell cancer, gastro intestinal stromal tumors and leukemia, and numerous others are under investigation. Since these targeted anticancer compounds specifically inhibit cellular processes that are deregulated in various types of tumor cells, they were initially considered to be less toxic than conventional chemotherapy. However, it appears that similar to conventional chemotherapy, dose interruptions or reductions due to adverse effects are necessary in a large number of patients which indicates that TKIs have a narrow therapeutic window [1-4]. TKIs show large inter-patient variability in pharmacokinetics, which results in highly variable plasma concentrations and consequently drug exposure [5-9].

For several TKIs an indication for the optimal drug exposure (therapeutic window) has been derived from retrospective data-analysis [10-16]. The highly variable drug exposure will result in exposure levels outside the therapeutic window in a considerable number of patients. This might explain, at least in part, the toxicity and suboptimal response seen in some individuals.

Therapeutic drug monitoring (TDM) comprises the measurements, interpretation and adjustment of therapy in order to reach exposure levels within the target range. TKIs have most of the characteristics that are required for TDM, such as a narrow therapeutic window, large inter-patient variability compared to intra-patient variability and the chronic use until disease progression [17]. Therefore, TDM might be a very promising tool for this new class of drugs in order to improve treatment benefit by reducing toxicity and increasing efficacy.

To support clinical pharmacological studies and to address observations (toxicities and inefficacies) in daily clinical practice, it was essential to develop and validate a quantitative bioanalytical assay in which the mostly used TKIs can be quantified. We hereby present an assay in which imatinib, N-desmethyl imatinib, sunitinib, N-desethyl sunitinib, nilotinib, dasatinib, pazopanib and regorafenib can be determined simultaneously. The described validations were performed according to the FDA guidelines for bioanalytical method validation [18].

Material and methods

Chemicals and reagents

Imatinib mesylate and N-desmethyl imatinib mesylate were kindly provided by Novartis International Pharmaceuticals (Cork, Ireland). Nilotinib base and the isotope $^{13}C_3{}^{15}N$ nilotinib hydrochloride were kindly provided by Novartis Pharma A.G. (Basel, Switzerland). Sunitinib maleate and N-desethyl sunitinib were kindly provided by Pfizer Inc. (Groton, USA). Regorafenib and pazopanib hydrochloride were obtained from Axon Medchem (Groningen, The Netherlands). Dasatinib base was obtained from LC laboratories (Woburn, MA, USA). The isotopes $^{2}H8$ dasatinib base and $^{13}C^{2}H_3$ pazopanib hydrochloride were obtained from Alsachim (Strasbourg, France). DMSO (Uvasol), methanol (Lichrosolv), formic acid (Emsure) and ammonium acetate were obtained from Merck (Darmstadt, Germany). Methanol absolute (HPLC supra-gradient) was purchased from Biosolve (Valkenswaard, the Netherlands). Millipore quality water was used.

Preparation of stock solutions, calibration standards and quality controls samples

Two independent stock solutions were made for each analyte: imatinib, N-desmethyl imatinib, nilotinib, sunitinib, N-desethyl sunitinib, dasatinib, pazopanib and regorafenib. One stock solution was used for the preparation of calibration standards (CS) and the other for the quality control (QC) samples. All stock solutions were prepared in DMSO and contained 1 mg/mL free base except for pazopanib solution, which contained 2 mg/mL free base.

Stock solutions of different analytes were mixed and diluted with methanol to obtain four QC and CS substock solutions. Subsequently, the substock solutions were combined and further diluted with methanol to prepare the working solution for the CS. The QC samples were prepared by adding combinations of the (sub)stock solutions to blank human serum.

Table 1 Target concentrations of the six TKIs and two metabolites in the calibration standards (CS) and quality control (QC) samples

Sample type	Imatinib	Desmethyl imatinib	Nilotinib	Sunitinib	Desethyl sunitinib	Dasatinib	Pazopanib	Regorafenib
	(mg/L)	(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)
CS zero	0	0	0	0	0	0	0	0
CS 0	0	0	0	0	0	0	0	0
CS 1	0.1	0.1	0.1	2	2	5	1.0	0.1
CS 2	0.5	0.5	0.5	10	10	25	5.0	0.5
CS 3	1.0	1.0	1.0	40	40	100	10	1.0
CS 4	2.0	2.0	2.0	80	80	200	20	2.0
CS 5	3.0	3.0	3.0	160	160	300	30	3.0
CS 6	5.0	5.0	5.0	200	200	500	50	5.0
QC LLOQ	0.1	0.1	0.1	2	2	5	1	0.1
QC low	0.3	0.3	0.3	5	5	15	3	0.3
QC medium	1.5	1.5	1.5	50	50	150	15	1.5
QC high	4.0	4.0	4.0	180	180	400	40	4.0

Abbreviations: LLOQ, lower limit of quantification

The (sub)stock solutions were diluted at least 15-fold. CS were prepared by adding 5 µL of the working solution to 50 µL blank human serum. The target concentrations of the CS and QC samples are listed in Table 1. Stock solutions of the internal standards were also prepared in DMSO at a concentration of approximately 1 mg/mL. The internal standard working solution contained a mixture of the three internal standards in methanol: 1 mg/L ^{2}H8 dasatinib, 5 mg/L $^{13}C^{2}H3$ pazopanib and 5 mg/L $^{13}C3^{15}N$ nilotinib.

All stock, substock and working solutions were stored at -20 °C. The QC samples were prepared in bulk and divided into aliquots of 75 µL in polypropylene vials which were stored at -20 °C. The CS were freshly prepared before each validation run.

Sample pre-treatment

Protein precipitation was used as sample pre-treatment for serum samples. To 50 µL of serum sample, 10 µL internal standard substock and 500 µL methanol were added. After vortex mixing for 3 min, samples were centrifuged at 13,000 × g for 5 min. To 200 µL supernatant, 200 µL water was added and mixed.

Liquid chromatography

The ultra pressure liquid chromatographic (UPLC) system used consisted of a coupled binary solvent manager, sample manager, column heater and mass spectrometry detector (Acquity, UPLC, Waters, Wilford, MA, USA). Chromatographic separation of the eight compounds and three internal standards in this assay was carried out using an Acquity UPLC BEH C18 chromatographic column (100 × 2.1 mm ID, particle size 1.7 µm, Waters) protected with an Acquity UPLC BEH C18 pre-column (5 × 2.1 mm ID, particle size 1.7 µm, Waters).

For the analysis of pazopanib 1 µL and for the other compounds 10 µL was injected onto the column. The compounds were eluted with a linear gradient system at a flow rate of 0.4 mL/min. Mobile phase A consisted of 0.1% (v/v) formic acid and 2 mM ammonium acetate in water and mobile phase B consisted of 0.1% (v/v) formic acid and 2 mM ammonium acetate in methanol. The following linear gradient was used in this assay [time scale (min-min) mobile phase A (%)/mobile phase B (%)]: 0-1 75/25; 1-3 75/25 → 50/50; 3-5 50/50 → 10/90; 5-6 10/90; 6-6.5 10/90 → 75/25; 6.5-7 75/25. The column temperature was maintained at 50 °C and the autosampler temperature at 15 °C. The total run time was 7.0 min.

Mass spectrometry

The LC eluate was directed into a tandem quadruple, atmospheric pressure ionization (API) mass spectrometer (TQ detector, Acquity, Waters, Wilford, MA, USA). The detector was equipped with an electrospray ionization (ESI) source operating in the positive ion mode and configured in multiple reaction monitoring (MRM) mode. The data were acquired and processed using Masslynx™ Sofware (version 4.1, Waters). The general MS settings and analyte specific parameters for the assay are summarized in Table 2.

Table 2 General settings and analyte specific parameters for the analysis of all analytes

General Settings								
Capillary voltage (V)	1000							
Extractor voltage (V)	1							
Source temperature (°C)	150							
Desolvation temperature (°C)	400							
Cone gas flow (L/hr)	50							
Desolvation gas flow (L/hr)	900							
Collision gas flow (mL/min)	0.25							
Analyte specific parameters	**Imatinib**	**Desmethyl imatinib**	**Nilotinib**	**Sunitinib$_a$**	**Desethyl sunitinib$_a$**	**Dasatinib**	**Pazopanib**	**Regorafenib**	**IS-1**	**IS-2**	**IS-3**
Parent mass *(m/z)*	494.1	480.1	530.1	399.2	371.1	488.1	438.1	483.0	496.1	442.1	534.1
Product mass *(m/z)*	393.9	393.9	288.9	282.9	282.9	400.9	357.0	269.9	405.5	361.0	292.9
Cone voltage (V)	45	50	55	40	35	60	50	45	60	40	55
Collision energy (V)	30	30	32	25	25	32	30	35	32	30	30
Dwell time (ms)	31	31	31	31	31	31	31	31	31	31	31
Typical retention time (min)	3.8	3.8	5.0	3.2 - 4.3	2.9 - 4.1	4.1	3.6	5.7	4.0	3.6	5.0

Abbreviations: IS-1, ^{2}H8 dasatinib; IS-2, $^{13}C^{2}H3$ pazopanib; IS-3, $^{13}C3^{15}N$ nilotinib. [a]Diastereomeric *cis/trans* isomers: sunitinib → RT 3.2 and 4.3 and desethyl sunitinib → RT 2.9 and 4.1 are the *trans*- and *cis*-isomers, respectively.

Quantification

The six TKIs and two metabolites were quantified in serum by describing the relationship between the peak area ratio with the internal standard versus the nominal concentration. ^{2}H8 dasatinib,$^{13}C^{2}H_3$ pazopanib and $^{13}C_3{}^{15}$N nilotinib were used as internal standards for their target analytes. ^{2}H8 dasatinib was also found to be a suitable internal standard for the quantification of sunitinib and N-desethyl sunitinib. $^{13}C_3{}^{15}$N nilotinib was used as the internal standard for imatinib, N-desmethyl imatinib and regorafenib. The calibration lines were chosen to cover the clinically relevant range of concentrations that are expected in patients treated with the registered dose.

Validation procedures

The validation of the assay was performed according to the FDA guidelines for validation of bioanalytical assays including linearity, accuracy, precision, selectivity, recovery and stability [18].

The linearity of the assay was assessed by preparing and analyzing 6 non-zero calibration standards in six independent analytical runs. Least squares linear regression analysis was applied to describe the relationship between the peak area ratio with the internal standard versus the nominal concentration. The lowest total bias and the most constant bias across the range were obtained using a weighting factor of $1/\chi$.

The within-run and between-run precision and accuracy were determined. To test the within-run precision, 6 replicates of the QC samples; QC low, QC medium and QC high were analyzed in one analytical run. To test the between-run precision, the QC samples QC LLOQ, QC low, QC medium and QC high were analyzed in six analytical runs on six different days. Precision was expressed as CV values and accuracy as deviations from the nominal concentrations.

Initially the cross-analyte interference was investigated by injecting dilutions of each analyte (TKIs, metabolites and IS) separately.

The selectivity of the assay was tested in six different batches of blank control serum and plasma. The selectivity was analyzed for blank serum, blank plasma, low (~ the LLOQ concentration) and high (~ highest standard) controls of each analyte prepared in duplo in 6 individual batches of blank control serum and plasma. The total recovery, covering sample preparation and matrix effect, was determined six times at two concentration levels for each analyte (~ LLOQ and highest standard) and the internal standards. Carry-over was tested by injecting a blank sample after the highest calibration standard.

The stability of imatinib, desmethyl imatinib, nilotinib, sunitinib, desethyl sunitinib, dasatinib, pazopanib, and regorafenib in serum was tested at ambient temperature and at 2-8 °C after 0, 2, 3 days; 1, 2 weeks; and 1, 2 and 3 months. The long term stability at -20 °C was additionally tested at 9 months. Deviations were calculated against the initial concentrations. Analytes are considered stable in the matrix if the deviation is within ±15%. The stability of the individual stock solutions at -20 °C was determined with n = 6. Analytes were considered to be stable in stock solution if 90-110% of the initial concentration was found. The stability of the individual stock solutions was tested at 12 months.

Results

Method development

Sample pre-treatment

The most simple form of sample pre-treatment was initially tested; protein precipitation. Acetonitrile (ACN) and methanol were tested for protein precipitation. The in vial stability appeared to be less favorable for ACN sample pre-treatment. Additionally, peak symmetry appeared to be less favorably after ACN precipitation. Methanol was therefore selected as the solvent to precipitate proteins and showed high extraction recoveries for all analytes. To improve chromatographic separation water was added to the extract.

Chromatography

Two different combinations of mobile phases were tested; mobile phase 1. A: 0.05% formic acid + 5 mM ammonium acetate in water and B: 100% acetonitrile and mobile phase 2. A: 0.1% formic acid + 2 mM ammonium acetate in water and B: 0.1% formic acid + 2 mM ammonium acetate in methanol. The gradient applied was the same for both combinations. Peak separation, shape and sensitivity of the assay was poorer for the mobile phase composed of ACN (mobile phase 1) than for the mobile phase containing methanol. After selection of the mobile phase the gradient was further optimized to reduce the run time from 10 to 7 min (Figure 1).

Linearity

The concentration range that needs to be covered for pazopanib is much higher than for the other TKIs. Initially 10 µL was injected onto the chromatographic column for all the analytes. However, for pazopanib nonlinearity of the calibration line was seen during method development. This nonlinearity problem was solved by reducing the injection volume to 1 µL for pazopanib quantification, while keeping the injection volume for the other TKIs at 10 µL. In each analytical run the CS, QC and patient samples are injected two times. The first injection of 10 µL onto the system is for quantification all TKIs except for pazopanib. The second injection of 1 µL is for quantification of pazopanib.

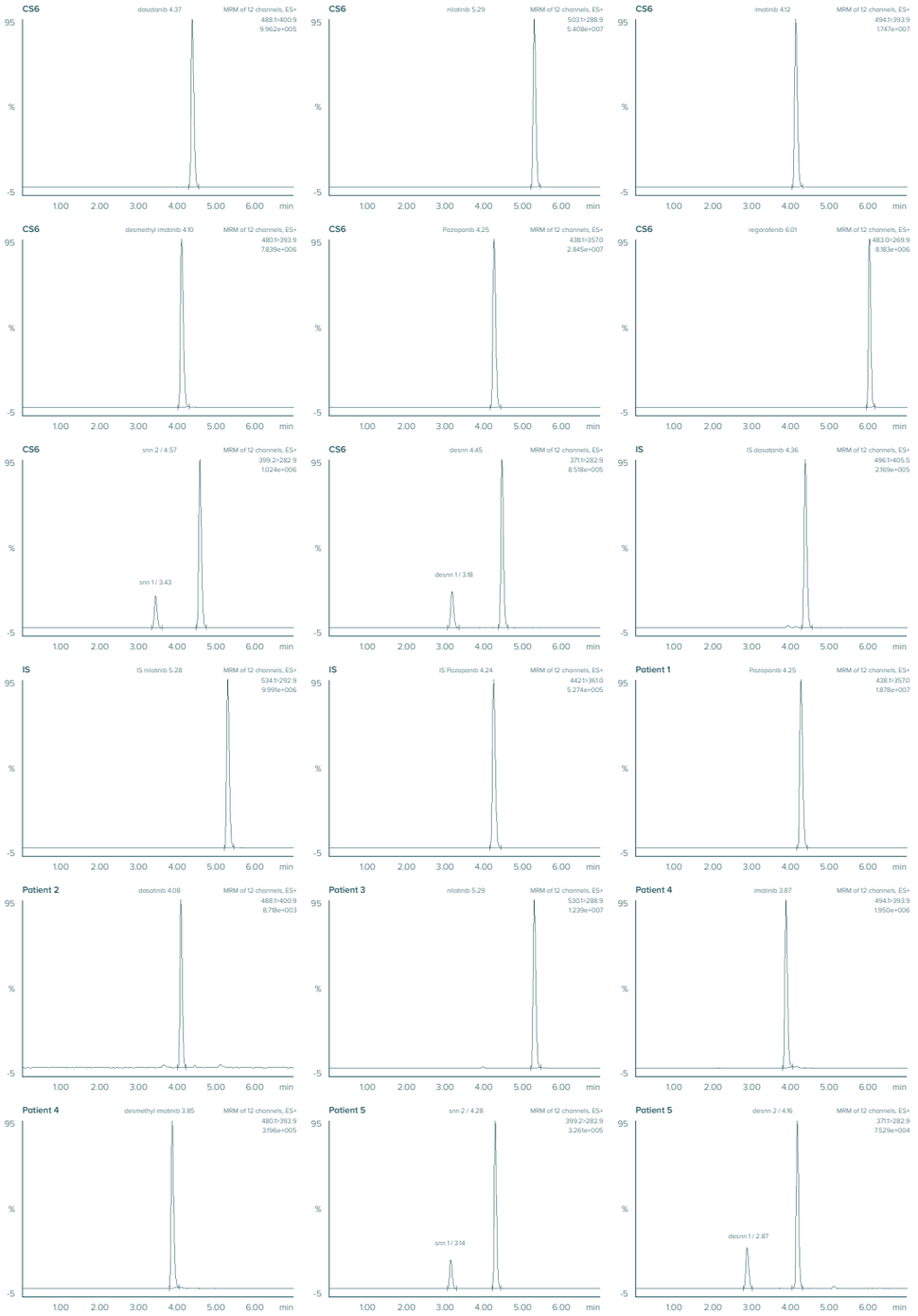


Figure 1 Typical MRM chromatograms of calibration standard 6 (CS 6) of dasatinib, nilotinib, imatinib, desmethyl imatinib, pazopanib, regorafenib, sunitinib isomers (*trans*-isomer = snn1 and *cis*-isomer = snn2), desethyl sunitinib isomers (*trans*-isomer = desnn1 and cis-isomer = desnn2), internal standards (IS) ^{2}H8 dasatinib, ^{13}C3^{15}N nilotinib and^{13}C2H^{3} pazopanib, additionally chromatograms of five patients treated with these tyrosine kinase inhibitors are presented.

Mass spectrometry

Mass spectrometric parameters were optimized by performing direct infusion and flow injection analysis of each analyte. In order to achieve high specificity and sensitivity, the multiple reaction monitoring scan mode was applied to monitor the mass transition to the product ion with the highest abundance in the product ion scan for each analyte. Table 3 shows the selected transitions and the proposed corresponding fragmentation pathways.

Isomerization

As described in the literature, sunitinib and N-desethyl sunitinib showed light induced *trans* to *cis* transformation with an equal MS response [19]. Therefore the sum areas of both isomers of sunitinib and desethyl sunitinib were used for quantification.

Validation

Calibration

The linearity of the assay for all analytes expressed as correlation coefficients (r^2) were at least 0.995. The linear range varied per analyte but was in all cases between the corresponding concentration of the LLOQ and the highest CS as listed in Table 1. At all concentration levels the deviations of the back-calculated concentrations were within ±15% of the nominal concentrations and this is in accordance to FDA guidelines.

Precision and accuracy

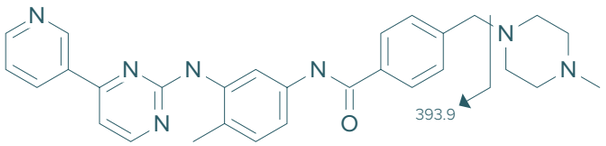
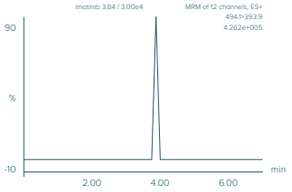
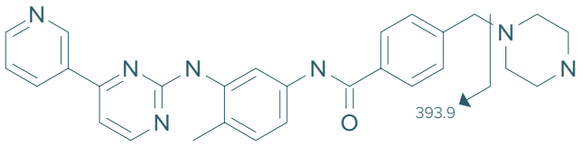
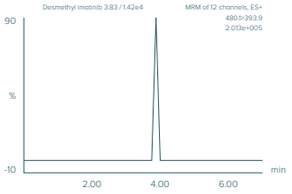
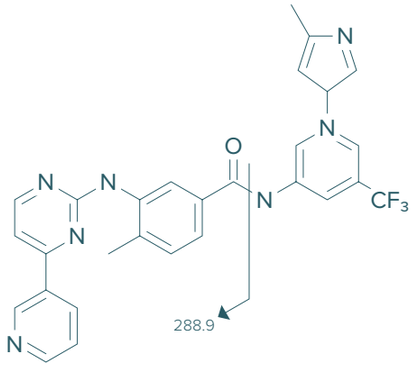
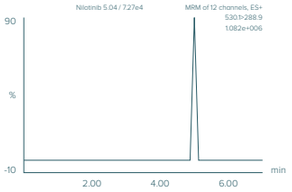
Precision, expressed as CV values and accuracy, expressed as deviations from the nominal concentrations were below 7% and within ±11%, respectively. Therefore, the precision and accuracy were within the acceptance criteria of the FDA guidelines.

Additionally, the signal to noise ratio of the six TKIs and two metabolites at the LLOQ level was > 5. In conclusion, the acceptance criteria for accuracy and precision were met. The assay performance data are summarized in Table 4.

Selectivity

No interference of the different compounds was observed in the test where dilutions of each analyte were injected separately. Only for the

Table 3 Analytes with their selected mass transitions and proposed fragmentation pathways

Compound	Transition	Proposed fragmentation and MRM chromatograms of calibration standard 6
Imatinib	494.1 → 393.9	 
Desmethyl imatinib	480.1 → 393.9	 
Nilotinib	530.1 → 288.9	 

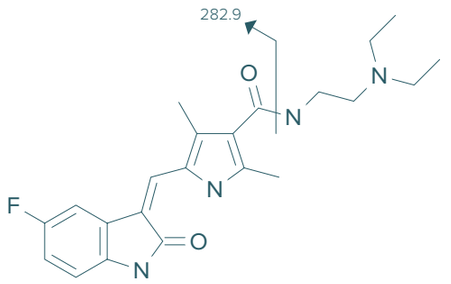
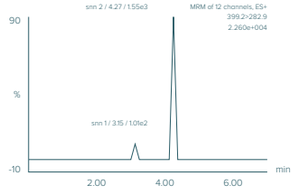
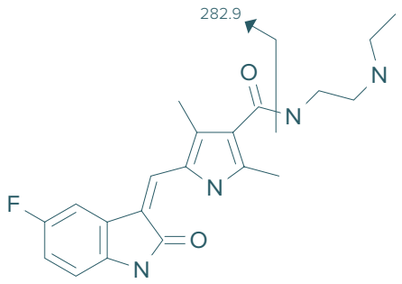
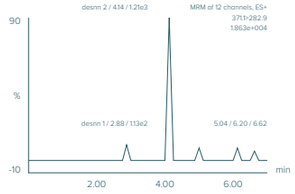
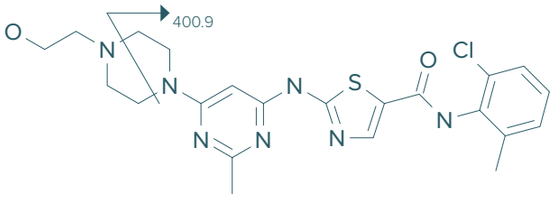
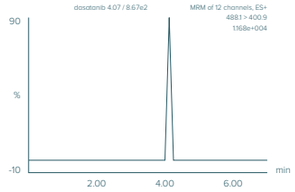
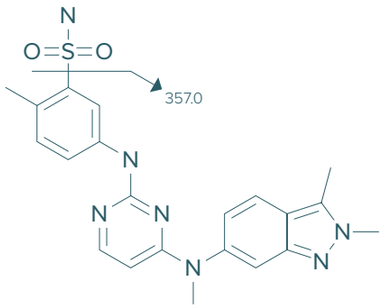
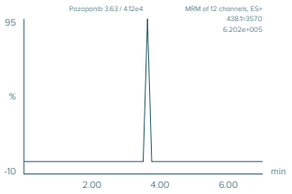
Compound	Transition	Proposed fragmentation and MRM chromatograms of calibration standard 6
Sunitinib	399.2 → 282.9	 
Desethyl sunitinib	371.1 → 282.9	 
Dasatinib	488.1 → 400.9	 

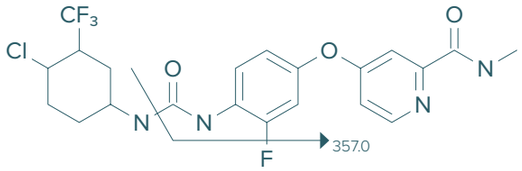
Table 3 [Continued]

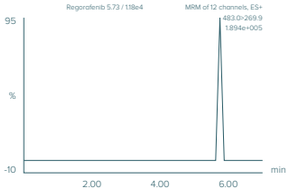
Compound	Transition	Proposed fragmentation and MRM chromatograms of calibration standard 6
Pazopanib	438.1 → 357.0	



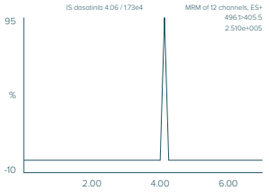


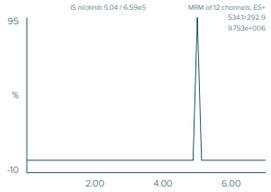
Regorafenib	483.0 → 269.9





IS dasatinib	IS nilotinib	IS pazopanib





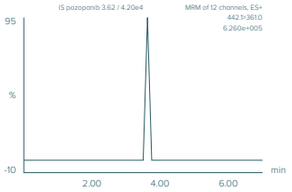


Table 4 Assay performance data of six TKIs and two metabolites from 4 QC samples in six analytical runs

Nominal conc.	Mean within run calculated conc.	Within-run precision (%CV)	Within-run accuracy (% dev.)	Mean between-run calculated conc.	Between-run precision (%CV)	Between-run accuracy (% dev.)
Imatinib (mg/L)						
0.1	-	-	-	0.10	4.0	97
0.3	0.27	3.0	91	0.28	4.2	93
1.5	1.43	3.4	96	1.44	5.7	96
4.0	4.09	1.1	102	3.93	3.8	98
Desmethyl imatinib (mg/L)						
0.1	-	-	-	0.10	3.8	102
0.3	0.31	2.7	102	0.31	3.8	104
1.5	1.57	2.6	105	1.56	6.2	104
4.0	4.20	1.4	105	4.03	4.2	101
Nilotinib (mg/L)						
0.1	-	-	-	0.10	5.4	97
0.3	0.27	1.5	91	0.,28	3.3	94
1.5	1.38	1.0	92	1.43	2.2	95
4.0	3.76	0.6	94	3.75	1.7	94
Suntinib (µg/L)						
2	-	-	-	1.85	11.8	93
5	4.74	4.9	95	5.06	6.6	101
50	47.8	3.5	96	50.,4	3.5	101
180	180.1	1.1	100	180.7	1.1	100
Desethyl sunitinib (µg/L)						
2	-	-	-	1.88	17.6	94
5	4.7	6.6	94	4.95	7.2	99
50	47.5	4.0	95	51.9	7.5	104
180	177.4	1.1	99	186.0	7.7	103
Dasatinib (µg/L)						
5	-	-	-	4.37	17.7	87
15	14.5	5.6	97	14.8	2.8	99
150	146.2	1.9	97	152.,6	3.4	102
400	402.06	1.6	101	400.0	2.1	100
Pazopanib (mg/L)						
1	-	-	-	1.00	4.1	100
3	3.15	2.3	105	3.18	1.5	106
15	15.9	2.4	106	16.5	2.4	110
40	44.3	0.7	111	43.7	1.8	109
Regorafenib (mg/L)						
0.1	-	-	-	0.10	14.6	101
0.3	0.30	5.4	101	0.32	5.1	105
1.5	1.34	2.2	89	1.47	6.8	98
4.0	3.68	1.0	92	3.97	6.3	99

Abbreviations: conc., concentration; dev., deviation; CV, coefficient of variation.

stable isotopes a small amount of the corresponding TKI was measured, the measured signal was < 5% of the LLOQ and therefore considered acceptable. The blank controls (plasma and serum) showed no peaks co-eluting with one of the compounds. The variation (CV%) in the relative area of the low and high controls prepared in the 6 different batches of blank serum were < 10% for all compounds tested. Only the variation of the low control of regorafenib was 15.8% which still meets the < 20% variability allowed for the LLOQ. The low and high controls prepared in six different batches of blank plasma were all within 10% variation except for imatinib with 11.2% and 10.8% variation for the low and high concentration, respectively. The deviation of plasma measurements from serum measurements for all analytes ranged from -15.8% to 4.9%. Based on these results we concluded that the selectivity was sufficient and that the assay was suitable for the detection of the analytes in both serum and plasma.

Recovery

In serum the mean total recovery of imatinib, N-desmethyl imatinib, nilotinib, sunitinib, N-desethyl sunitinib, dasatinib, pazopanib, regorafenib, $^{2}H8$ dasatinib, $^{13}C^{2}H3$ pazopanib and $^{13}C3^{15}N$ nilotinib ranged from 78% to 93% with CV% of 3.4-11.9% (except for regorafenib, LLOQ 23.5%). The variability of the detected regorafenib concentrations might be improved by introducing a structure analog as internal standard. At this point in the method validation we accepted the relative high variability in the recovery of regorafenib.

Carry-over

The response of the blank sample at the retention time of the analytes was < 10% of the corresponding peak area of the LLOQ sample and the response of the internal standard was < 1% of the normal response. Carry-over was therefore considered acceptable.

Stability

Except for regorafenib, all analytes were stable for at least 9 months at -20 °C. Regorafenib was stable for 3 months at -20 °C. At ambient temperature and at 2-8 °C all analytes were stable for 3 months, with the exception of imatinib QC low which was just outside the range of ±15% (18.1%). The effect of six freeze/thaw cycles was tested for the LLOQ of all analytes in serum. The CV's were < 17.7% and the observed concentrations were within 13% of the nominal concentrations. All analytes met the criteria that apply for LLOQ samples and were considered stable during six freeze/thaw cycles. In-(autosampler)vial stability was tested by reinjecting the CS of the calibration line, and the QC low, medium and high samples 24, 48 and 168 hr after the original analysis. The in-vial stability at ambient temperature for all analytes was demonstrated to be at least 168 hr. The

Table 5 Stability data of six TKIs and two metabolites in serum expressed as percentages (%) of the initial concentration

Nominal conc.	Ambient 3 months	2-8 °C 3 months	-20 °C 9 months	Ambient in vial 168 hr
Imatinib (mg/L)				
0.3	81.9	95.2	103.7	100.5
1.5	87.9	93.0	102.5	102.2
4.0	91.0	92.8	98.9	99.7
Desmethyl imatinib (mg/L)				
0.3	87.4	94.8	107.7	100.1
1.5	92.9	91.4	102.7	100.9
4.0	89.0	89.8	100.2	100.6
Nilotinib (mg/L)				
0.3	107.4	105.2	105.9	99.8
1.5	107.3	99.9	102.8	100.0
4.0	105.4	98.5	102.5	100.0
Suntinib (µg/L)				
5	105.6	104.2	98.7	97.9
50	115.2	105.1	99.9	103.7
180	102.1	99.2	102.0	97.1
Desethyl sunitinib (µg/L)				
5	96.3	102.9	98.2	93.0
50	102.1	111.5	104.9	100.5
180	92.2	107.9	103.4	99.0
Dasatinib (µg/L)				
15	96.9	94.3	108.7	105.6
150	102.5	98.9	106.6	104.4
400	97.5	96.4	102.9	104.6
Pazopanib (mg/L)				
3	105.8	105.4	99.7	100.3
15	107.3	100.0	100.6	102.6
40	106.6	98.2	97.8	102.1
Regorafenib (mg/L)				
0.3	99.3	98.7	99.3[a]	99.2
1.5	107.8	101.4	113.3[a]	104.2
4.0	101.4	97.0	107.6[a]	105.1

[a]Regorafenib is stable for 3 months in the freezer.

stability of the individual stock solutions was tested at 12 months and met the criteria. So, stock solutions of all analytes were at least stable for 12 months at -20 °C. The data on the stability of the analytes under different conditions are summarized in Table 5.

Matrix effect

In this study, the potential matrix effect was evaluated by spiking 6 lots of heparin plasma, EDTA plasma and serum at a low (~ the LLOQ concentration) and a high level (~ the highest standard) concentration of each analyte. The variability of the responses were < 15%. Except for sunitinib, SU12662 low level in serum which were slightly higher; 15.5% and 16.0%, respectively and desmethyl imatinib low level in EDTA plasma with a variability of 15.6%. We concluded based on these data that the matrix did not appear to interfere significantly with the integrity of our analytical method.

Clinical application

The described validated assay aimed to support investigator initiated pharmacokinetic studies with imatinib, nilotinib, sunitinib, dasatinib, pazopanib and regorafenib. Additionally, the assay was developed to address unexplained cases of inefficacy or toxicity of TKI therapy in clinical practice. Moreover, the assay can be applied to explore the value of therapeutic drug monitoring for this class of drugs.

To test the assay performance in patient material, we analyzed samples of patients that were treated with imatinib, pazopanib or sunitinib in study protocols and of patients treated with regorafenib in our clinic (Figure 1A-D). Additionally, external commercially controls from Chromsystems® to check the performance of the imatinib, N-desmethyl imatinib, nilotinib and dasatinib quantification were used. These QCs were all within 10% of the declared level (level 1-4).

Conclusion

A sensitive, sophisticated and practical bioanalytical assay for the simultaneous determination of six tyrosine kinase inhibitors (imatinib, sunitinib, nilotinib, dasatinib, regorafenib and pazopanib) and two active metabolites (N-desmethyl imatinib and N-desethyl sunitinib) was developed and validated according to FDA guidelines.

This assay has been applied to support an investigator initiated pharmacokinetic study with pazopanib and sunitinib. Moreover, the assay is being used to explore the possibilities of therapeutic drug monitoring and further understand the pharmacology of this class of drugs [20].

In the literature, multiple bioanalytical assays that quantify TKIs in human matrices have been described. Most of them are developed to quantify a single TKI [19,21,22]. For the clinical practice, it is more efficient to have a bioanalytical method available that can simultaneously determine the most abundantly used TKIs in the clinic. Thus far, nine methods have been published that are developed to simultaneously measure multiple TKIs. Six assays that are developed can simultaneously measure at most six TKIs [23-27], two methods can simultaneously determine eight TKIs [28,29] and one method can simultaneously determine nine TKIs [30].

This present method deviates from previously published assays with regard to the TKIs that are simultaneously measured. Thus far, no multi TKI bioanalytical method has been published that incorporates pazopanib and regorafenib. Pazopanib is possibly not incorporated in other published methods due to the relatively high concentrations that need to be quantified. For pazopanib the serum concentrations are much higher than for the other TKIs. We have overcome the problem of nonlinearity at the highest concentrations by injecting less volume for pazopanib quantification on the analytical column. Regorafenib is potentially not incorporated yet since it was only very recently registered by the FDA for the treatment of metastatic colorectal cancer and is pending registration as third line treatment for GIST. In line with the method of Lankheet et al. [28] we have used protein precipitation, which is for routine measurements in the clinical setting a fast and simple sample pretreatment procedure manageable for any laboratory.

The presented method is robust, easy to perform and currently used for routine patient care in cases where unexpected toxicity, inefficacy and drug interactions are suspected. Additionally, the assay is used to explore the benefit of routine therapeutic drug monitoring for TKIs as well as for investigator initiated studies.

Acknowledgement

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4

Dried blood spot analysis for therapeutic drug monitoring of pazopanib

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ABSTRACT

Background Dried blood spot (DBS) sampling is potentially a more patient-friendly and flexible alternative to venous sampling of pazopanib. This study determines the agreement between pazopanib DBS- and plasma concentrations to facilitate implementation of pazopanib DBS sampling into clinical practice.

Patients and Methods Paired DBS and plasma samples were collected in 12 patients. Pazopanib plasma concentrations were calculated from DBS concentrations using the formula: plasma concentration = $DBS_{concentration}$/(1-haematocrit). Passing-Bablok and Bland-Altman analyses were used to determine the agreement between calculated and measured plasma concentrations. We predefined a clinical acceptance limit of 25% for the Bland-Altman analysis.

Results Passing-Bablok analysis showed a small constant (intercept estimate -8.53(95%-CI; -12.22 to -4.41)) and slightly proportional (slope estimate 1.15 (95%-CI; 1.04-1.24)) bias between calculated and measured concentrations. This bias was clinically non-relevant as shown by Bland-Altman analysis; the mean ratio of calculated to measured concentrations was 0.94 (95%-CI; 0.65-1.23). The clinical acceptance limits were well within these 95% limits of agreement. More specifically, 92.6% of the data points were within the predefined acceptance limits.

Conclusion Pazopanib plasma concentrations can be accurately calculated from DBS concentrations. Although validation of DBS cards prepared by patients themselves is required, these results show that DBS sampling can be used to monitor pazopanib therapy in clinical practice.

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Introduction

Pazopanib hydrochloride (Votrient®) is an oral multi-targeted tyrosine kinase inhibitor (TKI) used for the treatment of metastatic renal cell carcinoma (mRCC) and metastatic soft tissue sarcoma (STS) [1,2]. For patients with mRCC, a correlation between pazopanib exposure and treatment outcome has been demonstrated [3]. Suttle et al. showed that a higher treatment response was seen for patients with a C_{trough} level > 20.5 mg/L and a higher incidence of toxicity was seen in patients with a C_{trough} level > 36 mg/L. This implies that the optimal therapeutic window for pazopanib in patients with mRCC lies between a C_{trough} level of 20.5 - 36 mg/L. There is a large variability in pazopanib exposure between patients (40-60 CV% in AUC) leading to the risk of sub- or supra-therapeutic C_{trough} levels and therefore to decreased therapeutic effects or more toxicity [3,4].

Therapeutic drug monitoring (TDM) can be useful to reach concentrations within the therapeutic window in order to optimize the efficacy and minimize the toxicity of therapy. The evidence for usefulness of TDM of pazopanib is accumulating and monitoring of C_{trough} levels is currently indicated in case of extreme or unexpected toxicity, a lack of clinical benefit, suspected drug interactions or suspected non-adherence to therapy [4-6]. In the first phase I trial, pazopanib exposure increased as the dose increased, although a plateau at 800 mg once daily was seen [7]. Theoretically, this might introduce problems when a patient has an exposure below the threshold for efficacy and needs an increased dose. However, the finding of a plateau is based on a limited number of patients and therefore not conclusive yet. In addition, results from two pazopanib TDM study suggest that exposure does increase with doses above 800 mg [4,8].

At present, pazopanib concentrations are monitored in plasma collected by venous sampling [9]. However, sampling by venipuncture has several disadvantages including its invasive character, the requirement for patients to travel to the clinic and the need for trained personnel. Compared to venous sampling, dried blood spot (DBS) sampling is a convenient, simple, flexible and more patient friendly alternative to collect blood in an at home setting. With clear instructions and after adequate training, patients should be able to self-collect DBS samples. The added value and feasibility of DBS collection for TDM has been shown effective for several drugs including anti-epileptics, immunosuppressants and antiretroviral drugs [10-12].

Here, we describe the results of a study investigating the feasibility of DBS for TDM of pazopanib. The objective of this study is to determine the agreement between pazopanib DBS- and plasma concentrations in order to facilitate the future implementation of pazopanib DBS sampling into clinical practice.

Methods

Patients

The collection and analysis of DBS and plasma samples was part of a larger phase I study that investigated the feasibility of TDM for dose individualization of pazopanib [4]. Included patients were ≥ 18 years with progressive disease from an advanced solid tumor, a WHO performance status ≤ 2 that had no standard treatment options available. All patients had adequate haematologic, renal and liver function reserves. The study was approved by the institutional ethics committee (Leiden University Medical Center, The Netherlands) and all patients gave written informed consent before entering the study. Between July 2012 and June 2013, 13 patients were included of whom 12 also participated in the DBS part of the study

Sampling

At day 14 of standard 800 mg pazopanib therapy, patients were admitted to the hospital for pharmacokinetic sampling. EDTA-blood samples were collected by venepuncture pre-dose and 1, 2, 3, 4, 6, 8, 10 and 24 hours after pazopanib intake. From these EDTA blood samples, 15 µL blood was collected into an EDTA capillary tube and spotted onto a pre-marked circle on a Whatman FTA® DBS card. This procedure was repeated 2 times to fill the 3 pre-marked circles on the card. After spotting the DBS cards, venous blood samples were centrifuged at 3,000 rpm for 5 minutes; the supernatant plasma was stored at -20°C until the day of analysis.

In addition to the DBS sampling cards prepared with venous blood, DBS sampling cards prepared by finger prick were collected pre-dose, and 3 and 8 hours after pazopanib intake. After disinfection of the skin with alcohol 70%, a lancet puncture was performed. The first drop of blood was discarded, thereafter 15 µL blood from the finger was collected using the above described capillary tube and spotted onto the DBS card. This procedure was repeated 2 times to fill the 3 pre-marked circles on the card

After drying for at least 2 hours, DBS cards were stored at room temperature in a closed plastic bag containing 2 sachets of desiccant. Thereafter, finger prick DBS cards (n = 3), venous DBS cards (n = 9) and plasma samples (n = 9) were all sent to GlaxoSmithKline, USA for further bio-analytical analysis.

Analysis

For the analysis of DBS pazopanib concentrations, a 4 mm diameter disc was punched out from the 15 µL dried blood spot. Per subject only 1 blood spot out of 3 was analyzed. Pazopanib was extracted from this disc with the use of 50 µL formic acid and 400 µL methanol containing an isotopic labelled internal standard, [$^{2}H_{3}$ ^{13}C]-pazopanib. After through mixing and centrifugation, 200 µL of the extract was taken into an auto-sample

tube where it was diluted with 200 µL of water before injection onto a HPLC-MS/MS system for analysis. This validated method was linear within the concentration range of 0.1-50 µg/mL pazopanib. The within- and between-run imprecisions were ≤ 11.4% and ≤ 6.2% respectively and the accuracy of this method was between -10.5% and 5.5%. Samples were stable on the DBS card for at least 75 days at ambient temperature. There was no influence of haematocrit levels (0.2 to 0.65) on the performance of this assay.

For analysis of pazopanib concentrations in plasma, 20 µL of plasma was extracted by adding 500 µL of acetonitrile/10 mM ammonium acetate (80/20 v/v) containing 100 ng/mL of [$^{2}H_{3}$ ^{13}C]-pazopanib as the internal standard. This was followed by vortex mixing and centrifugation at approximately 6200 g for 20 minutes. The supernatant was transferred into clean tubes and injected onto a HPLC-MS/MS system for analysis. This validated method was linear within the concentration range of 0.1 - 50 µg/mL pazopanib. The within- and between-run imprecisions were ≤ 14.7% and ≤ 2.9% respectively and the accuracy of this method was between -4.3% and 5.5%. Samples were stable in plasma for at least 530 days at -20 °C and 24 hours at ambient temperature.

Calculation of plasma concentration

Pazopanib plasma concentrations were calculated from DBS concentrations using the previously described formula: plasma concentration = $DBS_{concentration}$ /(1-haematocrit) [13]. The blood:plasma ratio of pazopanib ranges from 0.59 to 0.93 which suggests only a minimal association of pazopanib with blood cells [14]. In addition, only the unbound fraction of a drug can partition into blood cells [15]. Since pazopanib has a high protein binding of > 99.9%, the unbound fraction will be negligible [16]. Therefore, the fraction of pazopanib bound to red blood cells (haematocrit) was ignored in the above described formula. Plasma concentrations were calculated using both patient specific measured haematocrit values and fixed haematocrit values of 0.40 and 0.45 for males and females, respectively. A paired Student's t-test was used to test for a difference in calculated plasma concentrations using measured and fixed haematocrit values.

Statistics

Passing-Bablok regression and Bland-Altman analysis were used to determine the agreement between the two sampling methods [17,18]. Passing-Bablok regression analysis tests for a constant bias and proportional bias between two methods. If the 95%-CI for the intercept of the regression line includes 0, no constant bias is observed. If the 95%-CI for the slope of regression line includes 1, there is no proportional bias between the two tested methods. We used Bland-Altman analysis to define the

clinical relevance of any found bias. As suggested by Bland and Altman, a clinical and practical acceptance limit for the found ratio was determined [18]. A 25% range around the found ratio of the two methods was determined to be clinically and practically relevant since pazopanib can only be dose adjusted in steps of 25% of the total dose (200 mg tablets are the lowest dose available). Hence, the difference should be > 25% to result in a possibility to adjust the dose.

Analysis was performed with Microsoft office Excel (Microsoft Inc, Redmond, WA) and add-in Analyse-it statistics software (Analyse-it Software, Ltd, Leeds, UK).

Results

Patients

Between July 2012 and June 2013, 12 patients were enrolled in this DBS study. Characteristics of the patients included are summarized in Table 1.

Agreement between DBS concentrations prepared by finger prick and with venous blood

Concentrations measured at the same time points in DBS samples prepared by finger prick and prepared with venous blood were in good agreement with each other (Figure 1A). Passing-Bablok regression showed that that there was no constant (intercept estimate -0.71 (95%-CI; -3.41 to 2.23)) or proportional bias (slope estimate 1.05 (95%-CI; 0.93 to 1.17)) between the two sampling methods for the preparation of DBS cards. In this study, we collected 3 DBS cards prepared by finger prick per patient compared to 9 DBS cards prepared with venous blood. Since both methods for DBS card preparation were in agreement with each other and values therefore interchangeable, we used the (more extensive) data from the venous DBS cards for all further described analysis.

DBS vs. plasma concentrations

Pazopanib DBS concentrations (uncorrected concentrations in blood) were on average 48.0% (SD 8.5%) lower than measured plasma concentrations (Figure 1B). Passing-Bablok regression analysis showed that there was a constant (intercept estimate -4.68 (95%-CI; -6.48 to -2.47)) and proportional bias (slope estimate 0.63 (95%-CI; 0.57 to 0.68)).

Calculated vs. measured plasma concentrations – Passing-Bablok analysis

Calculated plasma concentrations using patient specific haematocrit values were on average 94.0% of measured plasma concentrations. Variability was relatively large (SD 14.7%, range 61.6% - 134.9%). Using a fixed haematocrit value, calculated plasma concentrations were on

Table 1 Patient baseline characteristics

Characteristic	
N	12
Age (years)	48 (23 - 68)
Sex (n)	
Male	11 (92%)
Female	1 (8%)
Length (cm)	183 (168 - 186)
Weight (kg)	90 (71 - 101)
ECOG PS (n)	
0	3 (25%)
1	9 (75%)
Hematology	
Haematocrit	0.45 (0.40 - 0.49)
ANC (× 10^9/L)	4.12 (2.49 - 6.42)
Platelets (× 10^9/L)	261 (150 - 394)
Hemoglobin (mmol/L)	9.05 (6.9 - 9.9)
Chemistry	
AST (U/L)	23 (17 - 33)
ALT (U/L)	21 (12 - 33)
Creatinine (mg/dL)	0.85 (0.71 - 0.97)
Total bilirubin (mg/dL)	0.52 (0.35 - 0.70)
Blood pressure (mmHg)	
Systolic	129 (113 - 142)
Diastolic	79 (60 - 93)
Tumor type (n)	
Chordoma	4
Sarcoma	3
Pancreatic cancer	1
Schwannoma	2
Ganglioneuroma	1
Granulair cell tumor	1

Data are presented as median (range) unless stated otherwise. Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; ECOG PS, Eastern Cooperative Oncology Group performance score; WBC, white blood count.

average 95.0% of measured concentrations with comparable variability (SD 15.5%, range 65.0 - 144.0%). No significant differences between both approaches (measured and fixed haematocrit values) was observed (95%-CI of difference in calculated plasma concentrations; -0.19 to 0.57, $P = 0.315$).

Passing-Bablok analysis showed a small constant bias (intercept estimate -8.53 (95%-CI; -12.22 to -4.41)) and slightly proportional bias (slope estimate 1.15 (95%-CI; 1.04 to 1.24)) between calculated and measured plasma concentration when patient specific haematocrit values were used (Figure 2). Similar results were found when a fixed haematocrit value was used; intercept estimate -9.67 (95%-CI; -13.28 to -5.51) and slope estimate 1.17 (95%-CI; 1.07 to 1.26).

Figure 1 Agreement between A) DBS concentrations obtained by finger prick and DBS concentrations obtained by venous blood. B) DBS concentrations and measured plasma concentrations

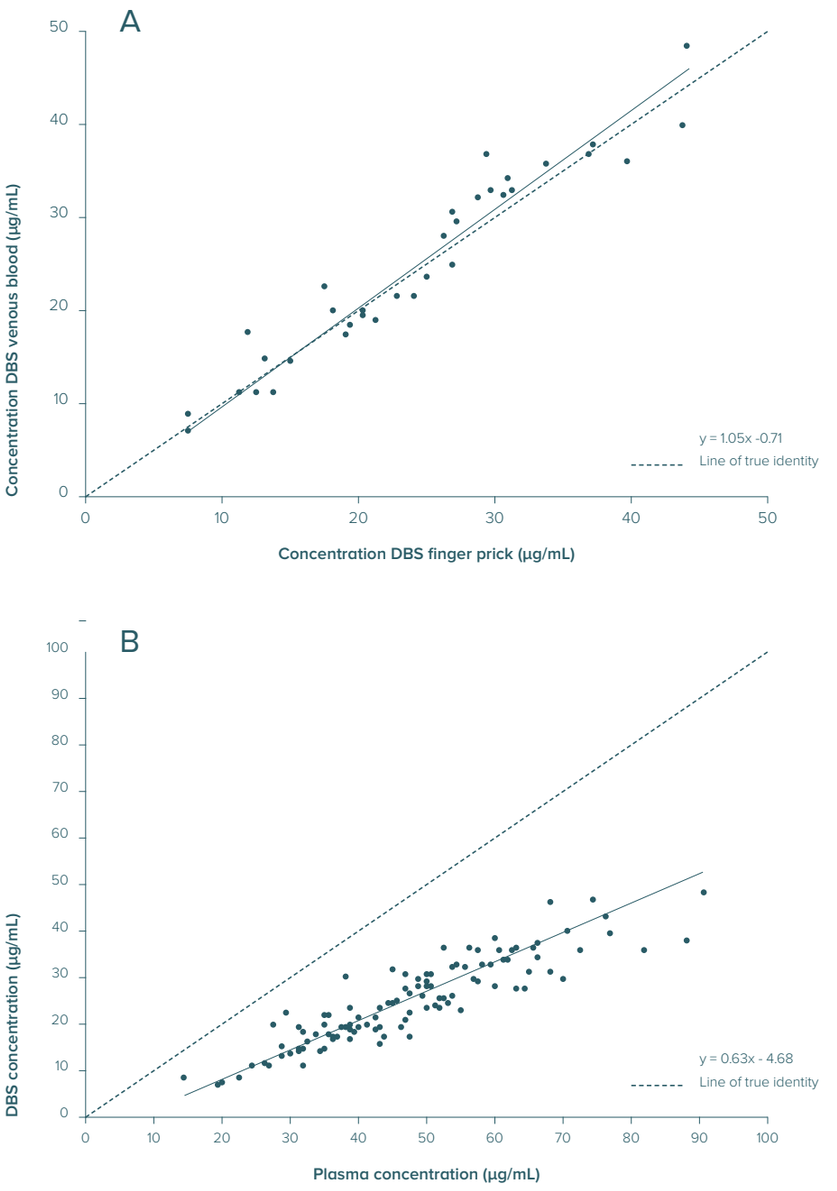
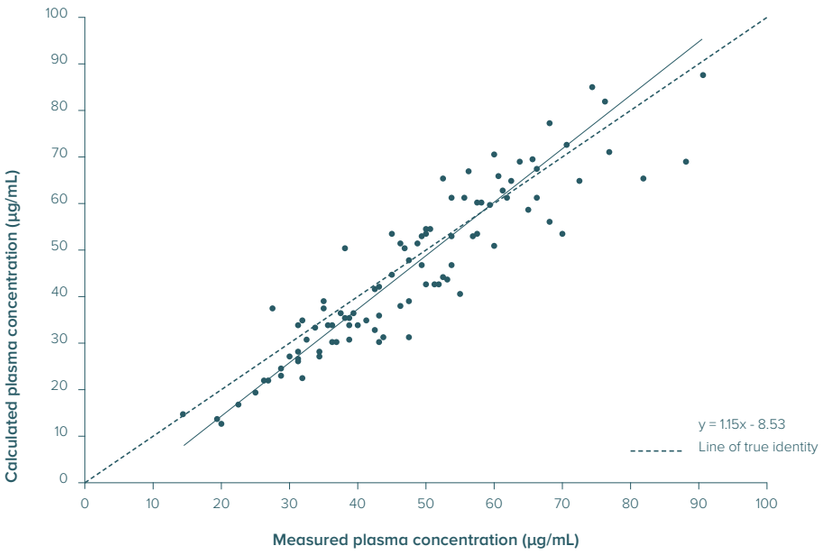


Figure 2 Agreement between measured and calculated plasma concentrations using patient specific haematocrit.

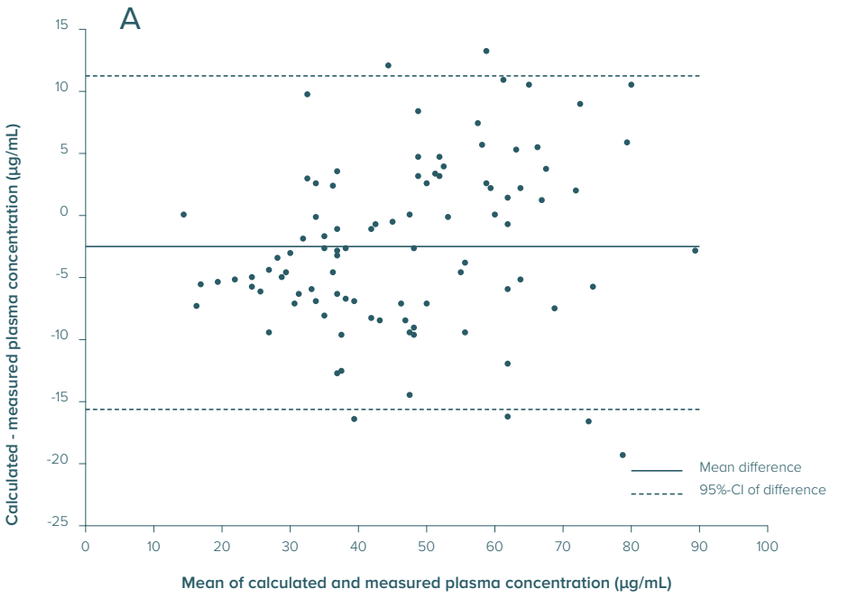


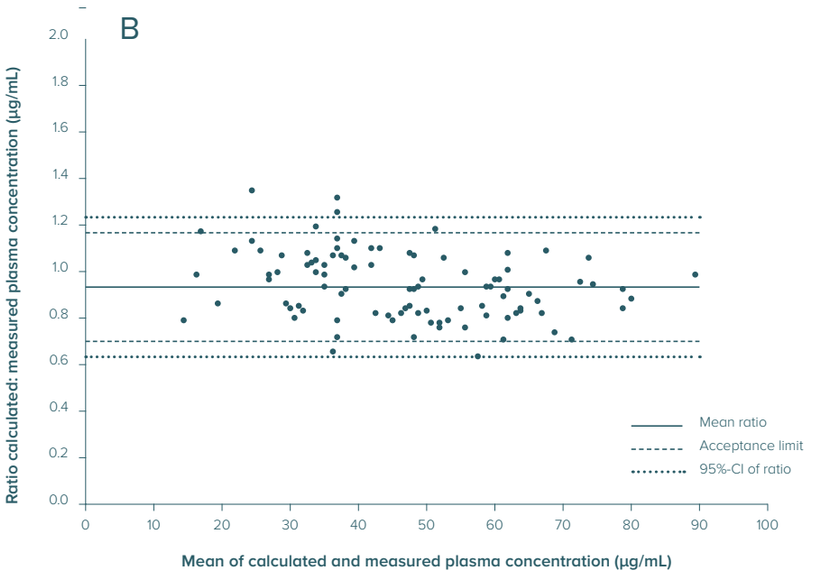
Calculated vs. measured plasma concentrations – Bland-Altman analysis

The difference in pazopanib concentrations between calculated and measured plasma concentrations using patient specific haematocrit values ranged from -19.2 to 13.2 µg/mL with a mean difference of -2.4 µg/mL (SD 6.8 µg/mL, Figure 3A). The mean ratio of calculated to measured plasma concentrations was 0.94 with the 95% limits of agreement of this ratio being 0.65 to 1.23 (Figure 3B). The clinical acceptance limits which were set at 25% around the found mean ratio, fell well within the 95% limits of agreement (0.71 to 1.18). More specifically, 92.6% (88 out of 95) of the data points were within the clinical acceptance limits.

Similar results were found when fixed haematocrit values were used. The difference ranged from -18.7 to 16.7 with a mean difference of -2.0 µg/mL (SD 7.1 µg/mL). The mean ratio of calculated to measured plasma concentrations was 0.95 with the 95% limits of agreement of this ratio being 0.65 to 1.25. The clinical acceptance limits (0.71 to 1.19) also fell well within the agreement limits. Using fixed haematocrit values, 12.6% (13 out of 103) of the data points exceeded the clinical acceptance limits.

Figure 3 Bland-Altman plot of A) difference between calculated and measured against mean plasma concentrations using patient specific haematocrit. B) ratio of calculated and measured against mean plasma concentrations using patient specific haematocrit.





Clinical relevance

A pazopanib trough concentration of 20.5 µg/mL is suggested as the threshold for efficacy in mRCC patients [3]. In Table 2, decision making based on measured and calculated plasma concentrations are compared. In 1 case (9.1%), there would have been a difference in decision making based on exposure. The measured plasma C_{trough} was 24.7 µg/mL compared to a calculated C_{trough} of 19.5 µg/mL from DBS. In all other cases, clinical decision making would have been the same based on either the measured or calculated plasma concentration. The same results were found when a fixed haematocrit value was used.

Table 2 Clinical decision making based on calculated and measured C_{trough} levels

	Calculated C_{trough} level < 20.5 µg/mL	Calculated C_{trough} level ≥ 20.5 µg/mL
measured C_{trough} level < 20.5 µg/mL	2	0
measured C_{trough} level ≥ 20.5 µg/mL	**1**	8

In **bold** a difference in clinical decision making based on calculated plasma C_{trough} levels using patient specific haematocrit and measured plasma C_{trough} levels

Discussion

The present study shows that pazopanib plasma concentrations calculated with the use of DBS, are in good agreement with actually measured pazopanib plasma concentrations. This implicates that DBS sampling can be used as an alternative sampling strategy for the determination of plasma concentrations to monitor pazopanib therapy.

A small constant, and slightly proportional bias was shown between calculated and measured pazopanib plasma concentrations. However, these biases were clinically not relevant as the vast majority of data points were within the predefined clinical acceptance limits. In addition, the difference between calculated and measured plasma concentrations would have resulted in different clinical decision making in only one out of 11 cases. It should be noted that in this case the difference between calculated and measured concentrations was small and concentrations were close the defined target of 20.5 µg/mL. Overall, these results show that DBS sampling can be used as an alternative – more patient friendly – sampling strategy to monitor pazopanib therapy in clinical practice.

Previously, Kralj et al investigated DBS sampling and analysis for the TKIs imatinib, nilotinib and dasatinib [19]. They used the same formula and also found good agreement between calculated and measured plasma concentrations. In the current study, we used both patient specific as well as fixed haematocrit values for the estimation of pazopanib plasma concentrations. The percentage of data points within the clinical acceptance limits when fixed haematocrit values were used, was slightly lower in comparison to when patient specific haematocrit values were used. However, no significant difference between calculated plasma concentrations using patient specific or fixed haematocrit levels could be shown. In addition, there was no difference in clinical decision making based on C_{trough} levels when patient specific or fixed haematocrit values were used. This indicates that fixed haematocrit values can be interchangeably used instead of measured haematocrit values for the calculation of pazopanib plasma concentrations when patient haematocrit levels are within the normal range.

The binding of pazopanib to red blood cells is thought to be limited and we did not take this into account for the calculation of pazopanib plasma concentrations. This may potentially cause bias in the calculation of plasma concentrations from DBS concentrations. However, calculated plasma concentrations from DBS were on average 6% lower than the measured concentrations. This demonstrates that the possibility of pazopanib partitioning into red blood cells is minimal since the calculated concentration would then have been higher otherwise. In addition, plasma concentrations could be readily predicted from DBS concentrations which also indicates that the uptake of pazopanib into red blood cells is small. This is also in agreement with the fact that pazopanib has a high plasma protein binding (> 99.9%) and the assumption that only the free unbound amount of a drug can participate into red blood cells.

In this study, DBS cards contained relatively high concentrations of pazopanib since samples were taken as part of rich PK-curves shortly after pazopanib intake to calculate pazopanib AUCs. As a consequence, there is only a limited number of DBS samples within the lower concentration range. Although splitting the Passing-Bablok regression into C_{trough} DBS samples and all other samples or into different concentration ranges did not change the results, it can be doubted whether the small proportional bias is caused by the fact that there are not enough samples in the lower concentration range or whether there is truly a proportional bias. In addition, this limits the amount of data on which agreement is based within the lower concentration range. Both bio-analytical assays for the determination of pazopanib in DBS and plasma were validated according to international guidelines, excluding an analytical cause.

DBS cards were prepared by the research nurse with the use of a 15 µl capillary. It can be argued that sampling by the research nurse

with a capillary does not truly reflect an at home sampling setting where patients spot themselves, which is a limitation of this study. On the other hand, previous studies with antiretroviral and immunosuppressive drugs have shown that 87.5 to 98% of the DBS samples obtained by patients were suitable for analysis [20,21]. Although these cards were prepared by blood drop and not capillary, it suggests that preparation of a DBS card by patients after a clear instruction is highly feasible. The perfect agreement between DBS cards prepared by finger prick and those prepared with whole blood shows that there is no engorgement of blood when a DBS is prepared by finger prick.

Conclusion

This study shows a good agreement between pazopanib levels measured in plasma and concentrations calculated from the corresponding DBS card. Although validation of clinical utility with DBS cards prepared by patients themselves is necessary, the results from this study show the feasibility of the use of DBS cards. With the ease and convenience of sample collection, DBS could be very useful for TDM of patients treated with pazopanib and potentially other TKIs in the future.

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5

Therapeutic drug monitoring to individualize the dosing of pazopanib: a pharmacokinetic feasibility study

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ABSTRACT

Background Patients treated with the standard dose of pazopanib show a large inter-patient variability in drug exposure defined as the area under the plasma concentration time curve ($AUC_{0\text{-}24}$). The primary objective of this study was to evaluate the feasibility of pharmacokinetics (PK)-guided individualized dosing to reduce the inter-patient variability in pazopanib exposure.

Patients and Methods Thirteen patients were treated with pazopanib for 3 consecutive periods of 2 weeks. During the first period, all patients received 800 mg of pazopanib once daily to reach steady-state exposure. During the second period, patients either received a PK-guided individualized pazopanib dose or the registered fixed 800 mg dose. During the third period, these 2 dosing regimens were switched.

Results The inter-patient variability in pazopanib $AUC_{0\text{-}24}$ during fixed dosing (27.3 CV%) was not significantly different when compared with the variability in $AUC_{0\text{-}24}$ during PK-guided dosing (24.8 CV%). The percentage of patients within the target window during PK-guided dosing (53.9%) was not significantly different from the percentage during fixed dosing (46.2%). Both C_{trough} and C_{24} were significantly ($P < 0.001$) correlated to pazopanib $AUC_{0\text{-}24}$ ($r^2 = 0.596$ and $r^2 = 0.940$, respectively). Pazopanib $AUC_{0\text{-}24}$ decreased 17% over time.

Conclusion PK-guided dosing did not reduce the inter-patient variability in pazopanib exposure. In this study, the intra-patient variability in pazopanib exposure was relatively large compared with inter-patient variability. This makes it challenging to achieve a target exposure within a predefined window. The causes of intra-patient variability must first be better understood and controlled, before PK-guided dosing can reduce the inter-patient variability.

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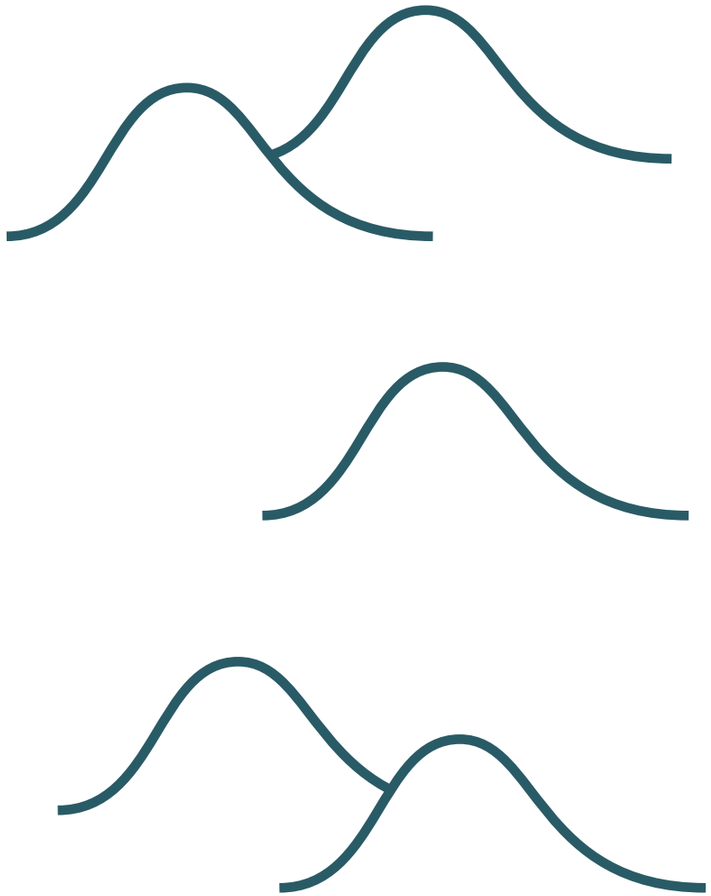


Introduction

Pazopanib hydrochloride (Votrient, GlaxoSmithKline; GW786034) is an oral multi-targeted tyrosine kinase inhibitor (TKI) with activity against vascular endothelial growth factor receptors 1-3, platelet-derived growth factor receptors α and β, and c-KIT [1-3]. Pazopanib is approved for the treatment of metastatic renal cell carcinoma (mRCC) and metastatic non-adipocytic soft tissue sarcoma [1-3].

Similar to other TKIs, pazopanib shows a large inter-patient variability in drug exposure of 40-70 coefficient of variation (CV%) [4-6]. Despite this large inter-patient variability in pharmacokinetics (PK), pazopanib is approved at a fixed oral dose of 800 mg once daily. In addition, a correlation between pazopanib exposure and efficacy and toxicity in mRCC has been demonstrated [7,8]. Subsequently, the reported variability in PK can potentially result for some patients in subtherapeutic exposure levels leading to decreased therapeutic effects. For other patients, the reported variability could result in supratherapeutic drug exposure levels with an increased incidence of adverse events. If this variability in PK can be controlled, the individual benefit – risk ratio for patients treated with pazopanib could be optimized.

The use of therapeutic drug monitoring (TDM) could potentially lower the inter-patient variability in pazopanib area under the plasma concentration time curve (AUC). TDM is the measurement of plasma drug concentrations to individualize the dosage to achieve a target plasma concentration. This individualized dose will ultimately result in an optimal exposure to a predefined target drug level with maximal therapeutic effects and minimal toxicity. TDM has already shown to be of value for the dose individualization of different drugs including antibiotics, antiretroviral drugs, immunosuppressive agents, and anti-epileptics [9-12]. However, for the new orally administered targeted anticancer agents used in oncology, it has not yet been demonstrated whether the use of TDM is feasible or whether it will result in exposures within a predefined target window. This must be demonstrated first, before PK-guided dosing of pazopanib can be recommended.

Clinically, the main prerequisites for TDM are a proven drug exposure-response relationship, a large inter-patient variability in PK, and a well-defined narrow therapeutic window [13,14]. For pazopanib, a drug exposure-response relationship seems to be present, and the reported inter-patient variability is large. This makes it seem likely that TDM is suitable for the individualization of pazopanib therapy. TDM of pazopanib could thereby ultimately result in more efficacy and less toxicity of therapy.

We conducted a prospective study to evaluate the feasibility of PK-guided individualized dosing of pazopanib in patients with cancer. The primary objective of this study was to assess whether individualized PK-guided dosing could reduce the inter-patient variability in pazopanib exposure and whether a predefined target exposure could be achieved. This study was also used to determine the correlation between pazopanib C_{trough} levels (plasma concentration just before pazopanib intake), C_{24} (plasma concentration 24 hours after pazopanib intake), and pazopanib exposure. These analyses may justify the use of trough level measurement for monitoring and guiding pazopanib therapy in clinical practice. Further, we explored whether there is a change in pazopanib exposure over time, which has been shown for other TKIs [15,16].

Patients and Methods

Patients

Patients eligible for this study were 18 years or older with progressive disease from an advanced solid tumor with a World Health Organization performance status ≤ 2 and for whom no standard treatment options were available. All patients had adequate hematologic, renal, and liver function reserves as defined by a hemoglobin ≥ 5.6 mmol/L, absolute neutrophil count ≥ 1.5 × 10^9/L, platelets ≥ 100 ×10^9/L, creatinine clearance ≥ 50 mL/min, total bilirubin ≤ 1.5 × the upper limit of institutional normal value, alanine aminotransferase, and aspartate aminotransferase ≤ 2.5 × upper limit of the institutional normal value.

Cytotoxic chemotherapy or radiation therapy for a period of 4 weeks before entering the study was not allowed. Further, patients receiving concurrent study treatment, patients with clinical evidence of central nervous system metastases or with poorly controlled hypertension (defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg) were not eligible for study entry. Patients were not allowed to use substances known or likely to interfere with the PK of pazopanib, which included CYP3A4 inhibitors (eg, ritonavir, clarithromycin) or inducers (eg, phenytoin, rifampicin) within 14 days or 5 half-lives of the substance (whichever was longer) before study entry. This study was approved by the Institutional Ethics Committee (Leiden University Medical Center, The Netherlands), and all patients gave written informed consent before entering the study.

Study design and treatment

This study was a multicenter, open-label, 3-period, randomized, 2-sequence, crossover pharmacokinetic study. Pazopanib (Votrient) was supplied as 200 mg tablets for oral administration 1 hour before or 2 hours after food intake. The study was performed over a timeframe of 6 weeks. Within these 6 weeks, the patients received pazopanib at the registered dose of 800 mg once daily for 4 weeks and a PK-guided individualized dose for 2 weeks. This PK-guided individualized dose was based on

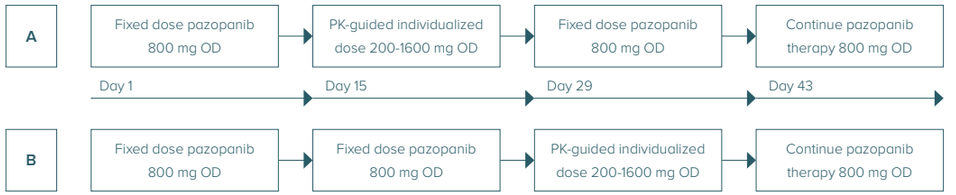
the deviation from a predefined target and measured exposure. Because a therapeutic window with an optimal balance between exposure (AUC) and efficacy on the one hand and toxicity on the other hand was lacking for pazopanib, a safe and effective target exposure had to be established first. For this, we used the results of 2 previous phase I studies in which the median steady-state AUCs for 800 mg of pazopanib were determined with non-compartmental methods. With the results from these trials, we defined a target exposure of 805 mg·hr/L (range 715 - 920 mg·hr/L) [4,17].

At study entry, patients were allocated to treatment group A or B (Figure 1). All patients started with the fixed pazopanib dose of 800 mg once daily for a period of 2 weeks to reach steady-state PK. After 2 weeks, pazopanib AUCs were assessed. Thereafter, patients allocated to treatment group A switched over to a PK-guided individualized dose (based on measured pazopanib exposure on day 14), and patients in treatment group B continued with the fixed 800 mg dose. After a further 2 weeks of pazopanib therapy and having reached a new steady state, the AUCs of pazopanib were assessed on day 28. Patients in treatment group A returned to the fixed 800 mg pazopanib, and patients in treatment group B switched over to the PK-guided individualized dose (based on pazopanib exposure on day 28). After another 2 weeks of pazopanib therapy and a third PK assessment on day 42, all patients returned to the standard dose of 800 mg of pazopanib once daily. This crossover design was chosen to test for changes in pazopanib exposure over time.

Patients were instructed to take pazopanib at the same time and under the same conditions (1 hour before or 2 hours after breakfast) every day. The exact time of pazopanib intake was recorded for the 3 days preceding PK assessment.

On days 14, 28, and 42, adverse events were monitored using the Common Terminology Criteria for Adverse Event version 4.0. Radiological response was determined by computed tomography scan using Response Evaluation Criteria in Solid Tumors version 1.1, at 7 weeks after the start of treatment with pazopanib and reassessed thereafter at 8- to 12-week intervals. Patients were withdrawn from the study if the disease progressed

Figure 1 Study design



Abbreviations: OD, once daily.

or if toxicity was unacceptable. This trial was registered at www.trialregister.nl under the ID: NTR3967.

Pazopanib pharmacokinetics

For pazopanib PK assessment, EDTA-blood samples were collected on 3 days (ie, days 14, 28, and 42 after the start of treatment) at pre-dose (C_{trough}) and 1, 2, 3, 4, 6, 8, 10, and 24 (C_{24}) hours after pazopanib intake. Samples were centrifuged at 1000 relative centrifugal force (RCF) for 5 minutes at room temperature; plasma was split into 2 aliquots and stored at -20 °C until analysis. Pazopanib plasma concentrations were measured within 3 days of the last sample being collected. Pazopanib plasma concentrations were determined using a validated ultraperformance liquid chromatography – tandem mass spectrometric method [18]. The calibration line of this method was linear over the range from 1.0 to 50.0 mg/L of pazopanib. The within- and between-day imprecisions were 2.4% and 4.1%, respectively, and the within- and between-day inaccuracies were 11% and 9%, respectively. Pazopanib exposures were calculated using a non-compartmental trapezoidal approach (Phoenix WinNonlin v6.3).

Sample size calculation

In this study design, patients served as their own control. We hypothesized that the inter-patient variability (SD) in pazopanib exposure could be reduced by 50%, in other words that the variance ratio (SD^2 of fixed dosing relative to SD^2 of PK-guided dosing) would equal 4 by introducing individualized PK-guided dosing. Because the intra-patient variability was unknown, we assumed that the correlation between the AUC measurements within patients equals 0.5, or in other words, that the intra-patient variability in AUC was 50% of the inter-patient variability as has been shown for other TKIs. We used simulation (sampling 100,000 times) in SPSS (version 20.0) to calculate the power of the appropriate F test taking into account this assumed correlation of 0.5 between the 2 variances [19]. A sample size of 13 resulted in a power of at least 80% to reject the null hypothesis of equal variances.

Statistical analysis

The inter-patient variability in $AUC_{0\text{-}24}$ was evaluated by determining the sample variances from the fixed and individualized PK-guided dosing regimen (σ^2). To calculate the limits of a 95% confidence interval (CI) for the population variance ratio, we used likelihood-ratio tests in linear mixed models (profile likelihood-like analysis, SAS, version 9.2). To calculate the intra-patient variability in exposure, the 2 $AUCs_{0\text{-}24}$ during fixed 800 mg dosing (PK day 14 and PK day 28 or 42) were used. When there was no dose adjustment during PK-guided dosing and patients were dosed with

800 mg, this third AUC_{0-24} was also included to calculate the individual intra-patient variability. The mean biases from the target AUC_{0-24} (ie, individual AUC_{0-24} values minus the target AUC_{0-24}) during fixed and PK-guided dosing were compared using a paired sample t-test.

The relationship between pazopanib C_{trough} and C_{24} and AUC_{0-24} was examined by Pearson correlation analysis.

To test for changes in pazopanib AUC_{0-24} over time, we used the statistical method described for the assessment of bioequivalence because these studies also determine possible differences in exposure [20]. We calculated the 90% CI of the geometric mean ratios AUC_{fixed} on day 28 (treatment arm B) or 42 (treatment arm A): AUC_{fixed} on day 14. The CI of this ratio should be between 0.80 and 1.25 to conclude the absence of a decrease or increase of pazopanib exposure over time. Statistical calculations were performed with SPSS, version 20.0 and SAS version 9.2.

Table 1 Patient baseline characteristics

Characteristic	Treatment Arm A	Treatment Arm B	Total
N	7	6	13
Age (years)	58 (23 - 68)	45 (30 - 60)	48 (23 - 68)
Sex (n)			
Male	6 (86%)	6 (100%)	12 (92%)
Female	1 (14%)	0 (0%)	1 (8%)
Length (cm)	182 (168 - 183)	182 (170 - 184)	182 (168 - 184)
Weight (kg)	84 (62 - 101)	90 (77 - 98)	89 (62 - 101)
ECOG PS (n)			
0	1 (14%)	2 (33%)	3 (23%)
1	5 (71%)	4 (67%)	9 (69%)
2	1 (14%)	0 (0)	1 (8%)
Hematology			
ANC ($\times 10^9$/L)	4.9 (3.1 - 6.0)	4.4 (2.5 - 6.4)	4.4 (2.5 - 6.4)
Platelets ($\times 10^9$/L)	278 (158 - 651)	261 (150 - 394)	271 (150 - 651)
Hemoglobin (mmol/L)	9.2 (6.8 - 9.9)	8.8 (7.8 - 9.9)	9.0 (6.8 - 9.9)
Chemistry			
AST (U/L)	22 (17 - 33)	25 (17 - 32)	22 (17 - 33)
ALT (U/L)	17 (12 - 26)	23 (12 - 33)	20 (12 - 33)
Creatinine (µmol/L)	70 (63 - 88)	77 (71 - 86)	73 (64 - 88)
Total bilirubin (µmol/L)	7 (5 - 11)	10 (7 - 12)	10 (5 - 12)
Blood pressure (mmHg)			
Systolic	129 (112 - 142)	129 (113 - 138)	129 (112 - 142)
Diastolic	77 (60 - 93)	80 (70 - 88)	79 (60 - 93)
Tumor type (n)			
Chordoma	3	1	4
Sarcoma	2	1	3
mRCC	1	0	1
Pancreas carcinoma	1	0	1
Schwannoma	0	2	2
Ganglioneuroma	0	1	1
Granulair cell myoblastoma	0	1	1

Data are presented as median (range) unless stated otherwise. Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; ECOG PS, Eastern Cooperative Oncology Group performance score; mRCC, metastatic Renal Cell Carcinoma; WBC, white blood count.

Results

Patient characteristics

Between July 2012 and June 2013, 14 patients were enrolled in the study. One patient withdrew informed consent before starting pazopanib therapy. Seven patients were assigned to group A, and 6 patients were assigned to group B. Baseline patient characteristics are summarized in Table 1.

Table 2 Summary of pazopanib PK parameters during individualized and fixed dosing

Parameter	Fixed dose D14	PK-guided dose D28/42	Fixed dose D28/42
AUC_{0-24} (mg·hr/L)			
Mean	1087	838	881
SD	349	207	241
CV%	32.1	24.8	27.3
ln AUC_{0-24} (mg·hr/L)			
Mean	6.94	6.70	6.75
SD	0.33	0.29	0.26
C_{trough} (mg/L)			
Mean	32.3	25.9	29.0
SD	11.7	9.6	9.2
CV%	36.2	36.9	31.7
ln C_{trough} (mg/L)			
Mean	3.41	3.18	3.30
SD	0.39	0.41	0.41

Abbreviations: D14/D28/D42, treatment day 14, 28 and 42, respectively; ln AUC_{0-24}, natural log-transformed AUC_{0-24}; ln C_{trough}, natural log-transformed C_{trough}.

Pharmacokinetics

Pazopanib pharmacokinetic data were obtained from all 13 patients on days 14, 28, and 42 of treatment. Table 2 summarizes the pharmacokinetic parameters of pazopanib during the fixed and individualized PK-guided dosing regimens.

The mean exposures (AUC_{0-24}) were 881 mg·hr/L (range, 600 - 1296 mg·hr/L) and 838 (range, 361 - 1191 mg·hr/L) during, respectively, fixed dosing and PK-guided dosing with accompanying SDs of 241 and 207 mg·hr/L, respectively. The corresponding inter-patient variability (CV%) in AUC_{0-24} was 27.3% for fixed dosing and 24.8% for PK-guided dosing. The inter-patient variability in AUC_{0-24} was not significantly reduced with the introduction of PK-guided dosing; the ratio of variance (σ^2)

Figure 2 AUC_{0-24} during individualized and fixed pazopanib dosing

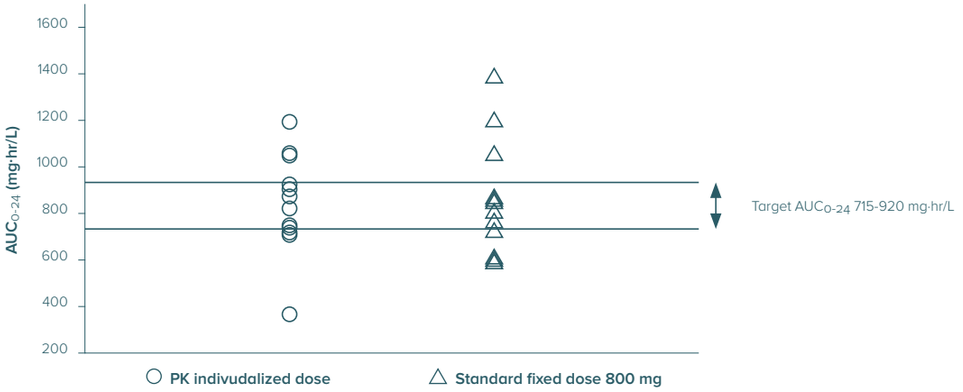
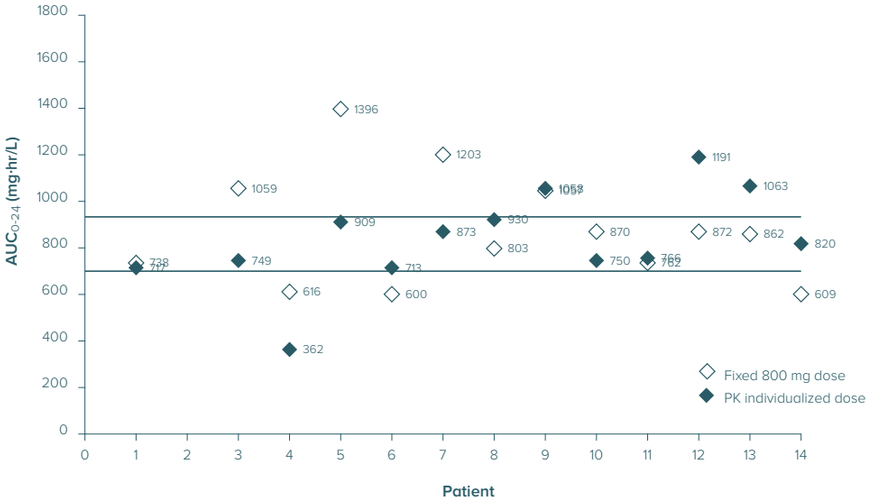


Figure 3 AUC_{0-24} per subject



during fixed dosing relative to PK-guided dosing was 1.35 (95%-CI: 0.48 - 3.9) (Figure 2). Individual intra-patient variability was calculated based on 3 AUCs for 6 patients and based on 2 AUCs for 7 patients; the mean intra-patient variability in AUC_{0-24} was 24.7 CV% (range, 8.3 - 48.7 CV%).

During PK-guided dosing, patients received daily doses ranging from 400 to 1200 mg of pazopanib. Seven of 13 patients received an adjusted dose during PK-guided dosing; 6 patients received a lowered

dose, and 1 patient an increased pazopanib dose. Dose reduction resulted in a dose-proportional decrease in pazopanib AUC_{0-24} in 4 of 6 patients. The only patient with an increased dose showed a dose-proportional increase in AUC_{0-24}. The percentage of patients with an AUC_{0-24} within the target window was not significantly different during PK-guided dosing when compared with that in fixed dosing (7 versus 6 out of 13 patients, 53.9% and 46.2%, respectively) (Figure 3). The biases from the target AUC in the fixed dosing arm and in the individualized arm were 75.9 mg·hr/L (95%-CI: 254.9 - 206.7) and 33.1 mg·hr/L (95%-CI: 297.7 - 145.8), respectively ($P = 0.538$).

Correlation between C_{trough}, C_{24} and AUC_{0-24}

Both C_{trough} and C_{24} were significantly associated with pazopanib AUC_{0-24} as shown in Figures 4A and 4B ($r^2 = 0.596$, $P < 0.001$ for C_{trough} and $r^2 = 0.940$, $P < 0.001$ for C_{24}, respectively). C_{trough} levels were taken after an uncontrolled pazopanib intake at home the day before hospital admission for PK sampling. The C_{trough} levels that were taken earlier or later than 24 hours after the previous dose tended to be, respectively, higher and lower than the controlled intake C_{24} levels (which is the same as a C_{trough} level taken exactly 24 hours after in-hospital pazopanib administration) (Figure. 4C).

Figure 4 Correlation between (A) pazopanib C_{trough} and AUC_{0-24}; (B) pazopanib C_{24}, and AUC_{0-24}; (C) time after prior dose at C_{trough} sampling and difference between C_{trough} and C_{24}

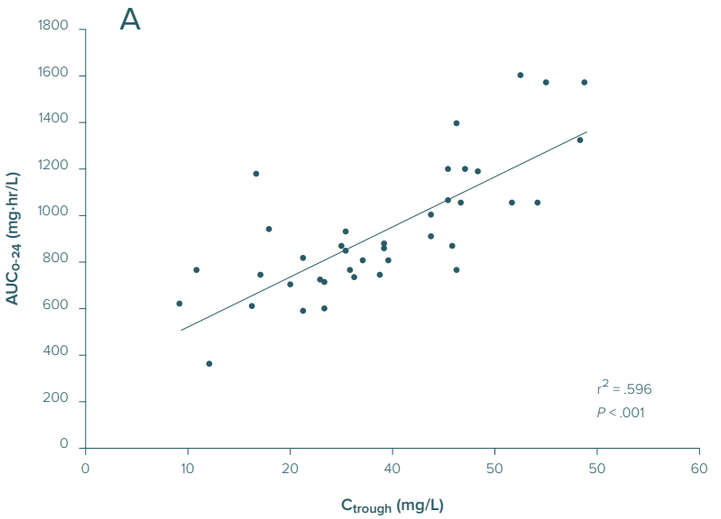
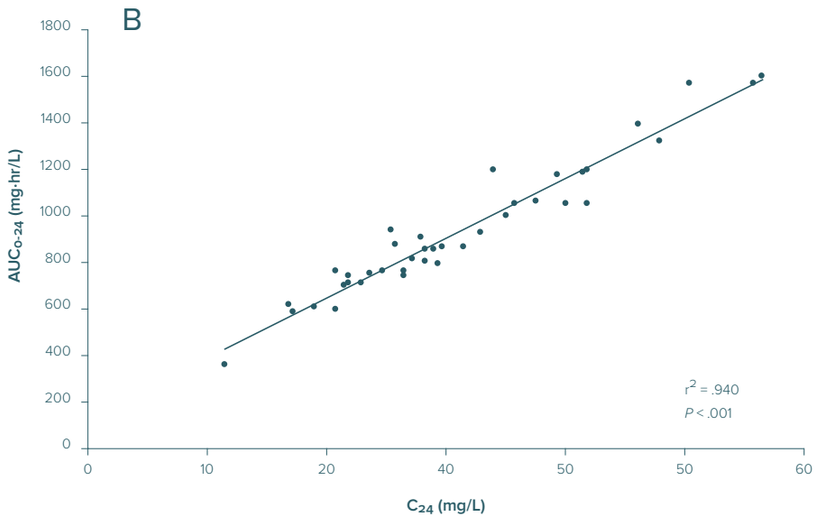


Figure 4 [Continued]



Change in pazopanib exposure over time

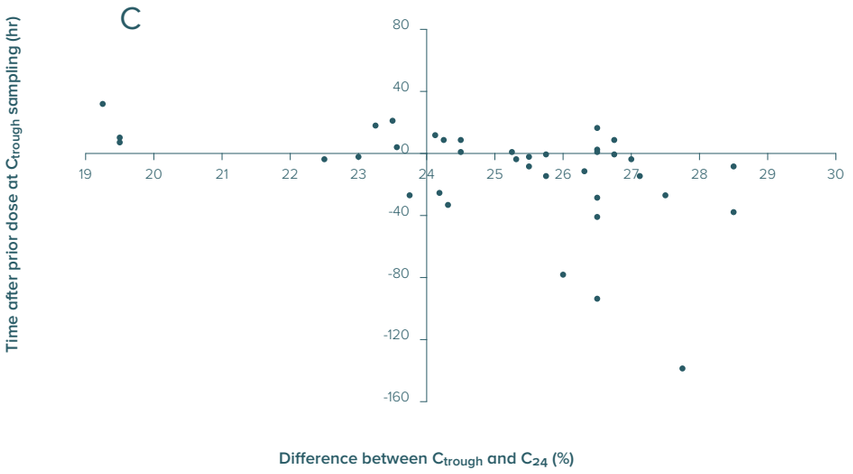
The ratio of the fixed dose pazopanib $AUC_{0\text{-}24}$ (day 28 or 42) versus fixed dose pazopanib $AUC_{0\text{-}24}$ (day 14) was 0.83 (90%-CI: 0.69-0.99) indicating a significant decrease in pazopanib $AUC_{0\text{-}24}$ of 17% over time.

Discussion

The reported large inter-patient variability in pazopanib PK may result in subtherapeutic or supratherapeutic exposure, which could potentially lead to either decreased efficacy or increased toxicity. In this study, we assessed whether individualized PK-guided dosing could reduce the inter-patient variability. The primary aim was to decrease the inter-patient variability in pazopanib $AUC_{0\text{-}24h}$ by 50%. This aim was not achieved; the results of our study indicate that PK-guided dosing to reach a pazopanib exposure within a predefined target window is not yet feasible.

Because the intra-patient variability in exposure has not been described before, we hypothesized this to be approximately 50% of the inter-patient variability. This is comparable with the results seen for other TKIs [21-23]. However, in this study, we found that the intra-patient variability was actually within the same range (24.7%) as that of the inter-patient variability (27.3%). Moreover, the inter-patient variability in exposure was much lower than that reported in the literature. The relatively large intra-patient variability described for the first time here is the main reason why

Figure 4 [Continued]



this study could not show the feasibility of TDM for the dose individualization of pazopanib therapy.

According to the Biopharmaceutics Classification System, pazopanib is a class II active substance and characterized by poor water solubility and low oral bioavailability of 14% to 39% [24]. These physicochemical properties, when combined with oral administration, cause variability in the absorption of pazopanib within and between individuals as shown in this study. However, other factors influencing the exposure cannot be completely ruled out.

Administration of pazopanib with both low and high fat meals has been shown to increase the $AUC_{0\text{-}24}$ by approximately 2-fold compared with that in a fasted condition [17]. Although in this study the intake of pazopanib has been standardized to the advised administration of 1 hour before or 2 hours after the intake of food, the intra-patient variability is relatively large. Possibly, this time interval of no food intake around pazopanib administration is insufficient to prevent an effect of food on pazopanib absorption. In addition, we did not standardize diet composition, and this could also have influenced the results. A possible option to reduce the intra-patient variability could be to increase the interval between food consumption and pazopanib intake. An alternative approach could be the administration of pazopanib at a lower dose in combination with a standardized meal to regulate the factors that influence its absorption. An additional benefit of this approach would be decreased costs of thera-

py with pazopanib [25]. The lower inter-patient variability found in this study, when compared with what is reported in the literature, is possibly the result of the inclusion and exclusion criteria used, controlled drug adherence, and the standardized intake of pazopanib [4].

Of the 13 patients included, 7 received an adjusted dose during PK-guided dosing. Dose reduction resulted in a dose-proportional decrease in AUC_{0-24} in 4 of 6 patients. Previous research has suggested that the steady-state exposure to pazopanib seems to plateau at 800 mg. However, in this study, the increased dose from 800 to 1200 mg in 1 patient did result in a dose-proportional increase in AUC_{0-24} [4].

The pazopanib plasma concentration at 24 hours after pazopanib intake (C_{24}) was much better correlated with the AUC_{0-24} than C_{trough} levels. Besides the intra-patient variability, 2 other reasons could possibly explain this finding; First, C_{trough} levels were taken around 24 hours (19.5 - 28.5 hours) after pazopanib intake, whereas C_{24} levels were taken exactly 24 hours after pazopanib intake. When C_{trough} levels were drawn > 24 hours after pazopanib intake, C_{trough} was lower than C24 and vice versa supporting our hypothesis. Second, C_{trough} levels reflected an at home - uncontrolled - pazopanib administration, whereas C_{24} levels were drawn after in hospital - controlled - administration of pazopanib. However, these findings are the reality of clinical practice and should be kept in mind when interpreting pazopanib C_{trough} levels in the clinic. A possible option to address this issue may be dry blood spot sampling. Patients could then take samples at home at exactly 24 hours after their last pazopanib intake. However, the feasibility and accuracy of this at home approach needs prospective validation.

A decrease of 17% in pazopanib exposure over time was observed. Similar decreases have been shown for imatinib and sorafenib [15,16]. Changes in the activity or expression of drug transporters or upregulation of liver enzymatic function might explain our observation. However, due to the small number of patients, this finding should be regarded as hypothesis generating and needs to be confirmed in a larger group of patients.

Although this study could not show the feasibility of TDM to reach a target exposure, measuring of pazopanib plasma concentrations may still be of clinical importance. A plasma concentration of 20.5 mg/L is retrospectively defined as the threshold for improved efficacy of pazopanib therapy in patients with mRCC [8]. In this study, 20% of the patients had C_{trough} levels below this threshold (data not shown). However, the incidence of different pazopanib-induced toxicities has also shown to be concentration dependent; there was a ≥ 2-fold increase in the incidence of hypertension, diarrhea, hair color change, alanine aminotransferase increase, and stomatitis when C_{trough} increased from 12.6 to 46 mg/L [7].

Although the problem of intra-patient variability remains to be solved, threshold-driven dosing might be beneficial and safe because target levels are much cruder. The concentration window between efficacy and toxicity seems to be much larger (> 2-fold (20.5 - 46 mg/L)) than the target window used in this study (~1.25-fold (715 - 920 mg·hr/L)). Therefore, it seems justified to target a C_{trough} level > 20.5 mg/L in clinical practice to prevent under dosing and unjustified discontinuation of treatment. Additionally, in patients that experience pazopanib-induced toxicity, measurement of pazopanib concentrations could help to determine whether the dose can be reduced or an alternative therapy should be initiated.

Conclusion

In this study, the feasibility of PK-guided dosing to reduce the inter-patient variability in pazopanib exposure could not be shown due to the relatively large intra-patient variability. The causes of the intra-patient variability must first be better understood and controlled, before PK-guided dosing will result in less inter-patient variability. Further research is needed to confirm whether there is a decrease in pazopanib exposure over time. Measuring of pazopanib plasma concentrations may still be of clinical benefit, especially to target a threshold pazopanib exposure with increased efficacy and limited risk to toxicity. For the interpretation of these plasma concentrations in the clinic, samples for C_{trough} levels are preferably taken exactly 24 hours after pazopanib intake.

Acknowledgment

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6

Midazolam as a phenotyping probe to predict sunitinib exposure in patients with cancer

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ABSTRACT

Background Patients treated with sunitinib show substantial inter-patient variability in drug exposure (~30-40%), which is largely unexplained. Since sunitinib is metabolized by cytochrome P450 (CYP) 3A4, variability in the activity of this enzyme may explain a considerable proportion of this inter-patient variability. Midazolam is widely used as a phenotyping probe to assess CYP3A4 activity. The objective of this study was to prospectively evaluate the relationship between midazolam and sunitinib exposure. Additionally, the correlation between sunitinib C_{trough} levels and exposure and the influence of sunitinib on midazolam exposure was determined.

Patients and Methods Thirteen patients treated with sunitinib in a '4 weeks on - 2 weeks off' regimen received twice 7.5 mg midazolam; once with and once without sunitinib. Steady-state sunitinib, its active metabolite SU12662 and midazolam exposures were determined.

Results A significant correlation between midazolam exposure (AUC_{0-7}) and steady-state sunitinib and sunitinib + SU12662 exposure (AUC_{0-24}) was found ($P = 0.006$ and $P = 0.0018$, respectively); midazolam exposure explained 51% and 41% of the inter-patient variability in sunitinib and sunitinib + SU12622 exposure. Furthermore, C_{trough} was highly correlated ($r^2 = 0.94$) with sunitinib AUC_{0-24}. Sunitinib decreased midazolam exposure with 24% ($P = 0.034$).

Conclusion Midazolam exposure is highly correlated with sunitinib exposure and explains a large proportion of the observed inter-patient variability in sunitinib pharmacokinetics. Consequently, midazolam could be used to identify patients that are at risk of under- or overtreatment, respectively, at the start of sunitinib therapy. Moreover, sunitinib and sunitinib + SU12662 C_{trough} levels are highly correlated with drug exposure and can thus be used in clinical practice to individualize sunitinib therapy. The decrease in midazolam exposure by sunitinib needs further investigation.

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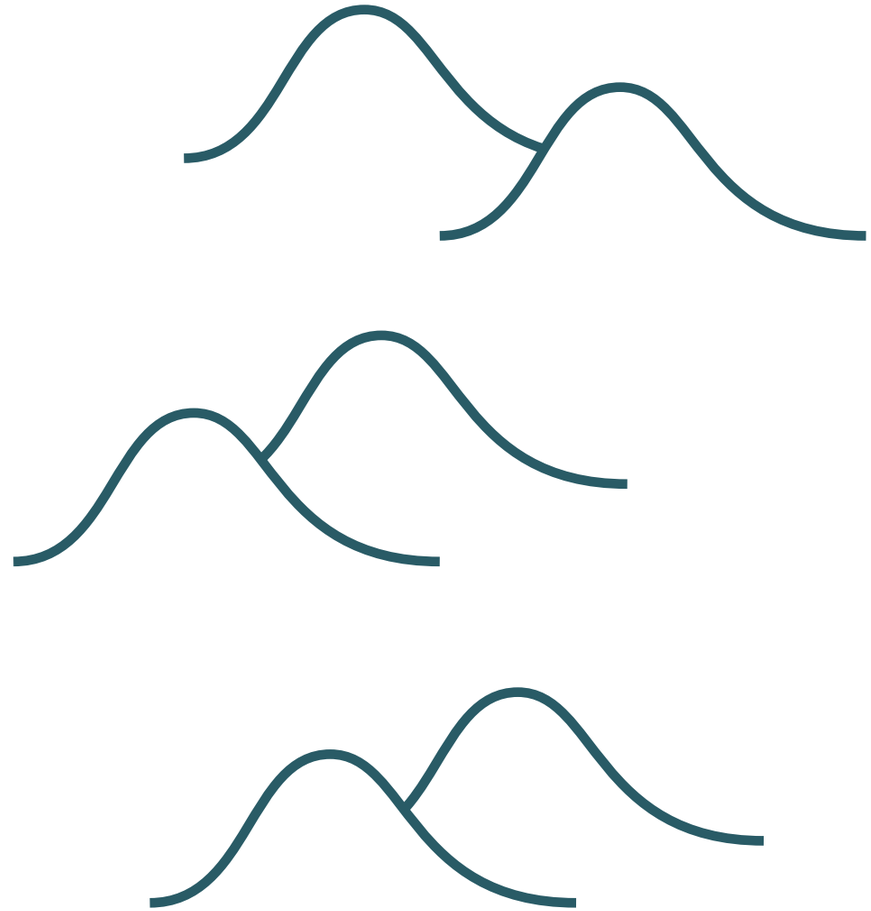


Introduction

Sunitinib malate (Sutent®; SU11248) is an oral multi-targeted tyrosine kinase inhibitor with both antitumor and antiangiogenic activity. It is approved for the treatment of metastatic renal cell carcinoma (mRCC), metastatic gastrointestinal stromal tumors (GIST) and advanced pancreatic neuroendocrine tumors [1-4]. Sunitinib is primarily metabolized by cytochrome P450 3A4 to its equally active *N*-desethyl metabolite SU12662, which is further metabolized to inactive moieties by CYP3A4 as well [5]. Steady-state concentrations of sunitinib and SU12662 are approximately reached after 14 days of daily dosing [6].

Patients treated with sunitinib show substantial inter-patient variability in drug exposure (~30-40%), which is largely unexplained [3,7,8]. Previously, a relationship between systemic sunitinib exposure, efficacy and adverse events has been demonstrated; Houk et al. showed that patients with mRCC, GIST or solid tumors and a sunitinib AUC_{ss} > 800, 600 and 700 µg·hr/L, respectively, had longer time to progression (TTP) and better overall survival (OS) [9]. This study also showed that there was a positive relationship between exposure and fatigue, decreased absolute neutrophil count and change in diastolic blood pressure. Consequently, the inter-patient variability in the pharmacokinetics of sunitinib can result in either sub- or supratherapeutic levels leading to inefficacy or toxicity, respectively. Since sunitinib is predominantly metabolized by CYP3A4, differences in the activity of this enzyme may explain a considerable proportion of the unexplained observed inter-patient variability in pharmacokinetics.

Midazolam is widely used as a noninvasive phenotyping probe to assess CYP3A4 activity. By using this probe, patients potentially at risk of under- or overtreatment, respectively, at the standard dosage regimen of sunitinib can be identified before the start of therapy. Hence, midazolam exposure can be used as a surrogate parameter for sunitinib exposure and could possibly prevent inefficacy or toxicity of sunitinib therapy.

The predictive value of different phenotyping probes for the exposure of various cytotoxic drugs including vinorelbine, docetaxel, irinotecan and tamoxifen, as well as the tyrosine kinase inhibitors gefitinib and imatinib has been investigated before [10-20]. Since, CYP3A4 plays an important role in the metabolism of nearly all tyrosine kinase inhibitors (TKI) and these TKIs also show a large inter-patient variability in pharmacokinetics, phenotyping with midazolam may also be useful for these drugs [21].

This study prospectively evaluated the relationship between midazolam and sunitinib exposure in patients with cancer in order to assess the feasibility of midazolam as a phenotyping probe for sunitinib pharmacokinetics. Total drug exposure is usually determined by collecting multiple samples over the dosing interval. However, for daily clinical practice, less intensive sampling would be a more feasible approach. Therefore, this study also determined the correlation between C_{trough} levels and total sunitinib exposure. This potentially justifies the use of only C_{trough} levels for monitoring and guiding sunitinib therapy in clinical practice. Finally, this study evaluated the effect of sunitinib on the pharmacokinetics of midazolam in patients with cancer.

Patients and Methods

Patients

Patients that used sunitinib for the palliative treatment of various tumors were eligible for study entry. Patients were ≥ 18 years old, had a WHO performance status ≤ 2 and a life expectancy of at least 12 weeks. Cytotoxic chemotherapy or radiation therapy within 4 weeks before study entry and concurrent use of substances known or likely to interfere with CYP3A4 activity within 14 days before study entry were not allowed. All patients had adequate clinical functional reserves as defined by hemoglobin ≥ 6.0 mmol/L, WBC ≥ 3.0×10^9/L, ANC ≥ 1.5×10^9/L, platelets ≥ 100×10^9/L, creatinine clearance ≥ 60 mL/min, bilirubin ≤ 1.75 × the upper limit of institutional normal value. The study was approved by the institutional ethics committee (Leiden University Medical Center, the Netherlands) and all patients gave written informed consent before entering the study.

Study design

All patients were treated in a '4 weeks on - 2 weeks off' dosing schedule, with commercially available sunitinib malate hard capsules (Pfizer, Kent, United Kingdom) at an once daily oral dose of 37.5 or 50 mg. The study was performed during one sunitinib treatment cycle of 6 weeks. Patients were admitted to the hospital for pharmacokinetic (PK) sampling on two separate days. The first PK day was at steady-state sunitinib pharmacokinetics (between days 14-20). The second PK day was on day 42, the final day of the 2 weeks 'off period' after the wash out of sunitinib. On both PK days, patients were given one midazolam 7.5 mg tablet (Roche, Woerden, The Netherlands) which was used either with (first PK day) or without sunitinib (second PK day).

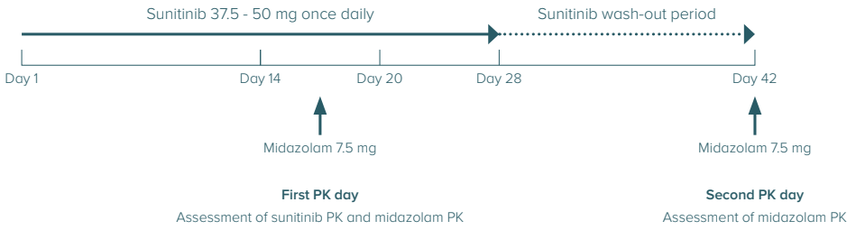
To determine the correlation between midazolam and sunitinib exposure, the $AUC_{0\text{-}7}$ of midazolam without the concomitant use of sunitinib (second PK day) was related to the $AUC_{0\text{-}24}$ and C_{trough} (t = 0) of both sunitinib and sunitinib + SU12662 at steady-state pharmacokinetics (first PK day). Additionally, the steady-state sunitinib data were used to assess the relationship between sunitinib and sunitinib + SU12662 C_{trough} levels (t = 0) and exposure ($AUC_{0\text{-}24}$). To determine the influence of sunitinib on CYP3A4 activity, midazolam exposures ($AUC_{0\text{-}7}$) with

(first PK day) and without (second PK day) the concomitant use of sunitinib were compared (Study Design; Figure 1).

Sunitinib pharmacokinetic analysis

To assess steady-state sunitinib pharmacokinetics, blood samples were collected after 14-20 days of sunitinib therapy (first PK day). The samples were collected into heparin containing tubes at 0, 10, 20, 40 min and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hr after the sunitinib dose. Blood samples were centrifuged at 3,000 rpm for 10 min; plasma was divided into two aliquots and stored at -80 °C until the day of analysis. Sunitinib and SU12662 plasma concentrations were determined using a validated Ultra Performance Liquid Chromatography-tandem Mass Spectrometric (UPLC-MS/MS) assay [22].

Figure 1 Study design



Midazolam pharmacokinetic analysis

Blood samples to assess midazolam pharmacokinetics were collected on the first and second PK day after a single oral dose of midazolam. The samples were collected into heparin containing tubes at the following time points: 0, 10, 20, 40 min and 1, 2, 3, 4, 5, 7 hr after the midazolam dose. Blood samples were centrifuged at 3,000 rpm for 10 min and plasma was stored at -80 °C until the day of analysis. Midazolam and its metabolite α-hydroxy midazolam were measured using a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay. Briefly, 200 µL plasma was extracted by adding 500 µL of acetonitrile containing midazolam D4 (4 µg/L) as the internal standard, followed by vortex mixing and centrifugation at 13,000 rpm for 5 min at ambient temperature. The supernatant was collected and 10 µL was separated on an Atlantis T3 C18 analytical column (50 × 2.1 mm i.d. particle size 3 µm) with a linear gradient. Mobile phase A consisted of 0.1 % v/v formic acid in water and mobile phase B consisted of 0.1 % v/v formic acid in acetonitril. Midazolam was eluted with the following gradient [time scale (min-min): flow rate (mL/min): mobile phase A (%)/mobile phase B (%)] 0-0.5:0.3: 85/15; 0.5-1.5:0.3: 85/15 → 10/90; 1.5-5.8:0.3:10/90; 5.8-6.2:0.5:10/90 → 85/15; 6.2-9.5:0.5:85/15. The effluent was monitored with a Micromass Quattro LC triple-quadrupole mass spectrometric detector (Waters, Milford, MA) using the electrospray positive ionization mode. The retention times for midazolam and α-hydroxy midazolam were 4.82 and 4.83 min, respectively; MRM transitions for midazolam 326.0 > 291.0 and for α-hydroxy midazolam 342.0 > 323.9. The calibration line of midazolam (Bufa, IJsselstein, Netherlands) was linear over the range from 1 to 100 µg/L. The within day and between day imprecision and inaccuracy were less than 5 % within this concentration range.

Pharmacokinetic analysis

Sunitinib and midazolam plasma concentrations were analyzed using a non-compartmental trapezoidal approach (Phoenix® Winnonlin® v6.3). For sunitinib and SU12662 the following pharmacokinetic parameters were assessed: AUC over the dosing interval (0-24); C_{trough} = trough plasma concentration; T_{max} = time to reach peak plasma concentration and C_{max} = peak plasma concentration. Since sunitinib and SU12662 pharmacokinetics are known to be dose-proportional over at least the dose range of 25-100 mg, the AUC_{0-24}, C_{trough} and C_{max} were dose normalized to a sunitinib dose of 50 mg in order to account for sunitinib dose differences (37.5 and 50 mg) between patients [6]. For midazolam and its metabolite

Table 1 Patient characteristics

Characteristic	Value
Number of patients	15
Sex	
Male (n)	12
Female (n)	3
Age (years)	61 (41 - 78)
Creatinine (µmol/L)	76 (56 - 122)
Total bilirubine (µmol/L)	9 (6 - 27)
ALT (µmol/L)	24 (9 - 68)
AST(µmol/L)	38 (23 - 203)
Hb (mmol/L)	8.8 (7.0 - 10.3)
WBC (x109)	5.0 (3.2 - 38.2)
Thrombocytes (x109/L)	158 (82 - 318)
Neutrophils (%)	53.3 (31.3 - 96.6)
WHO performance score	
0 (n)	3
1 (n)	12

Data are presented as median values with lower and upper limit. Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Hb, hemoglobin; WBC, white blood count.

α-hydroxy midazolam the following pharmacokinetic parameters were assessed: AUC over the sampling period (0-7), T_{max} and C_{max}.

Statistical analysis

The relationship between midazolam exposure and both sunitinib and sunitinib + SU12662 exposure, as well as the relation between both sunitinib and sunitinib + SU12662 C_{trough} and AUC_{0-24} were examined by correlation analysis. The Pearson square correlation coefficient (r^2) was used to assess the percentage of variability in sunitinib exposure that could be explained by midazolam exposure. To determine the influence of sunitinib on midazolam exposure, midazolam AUC_{0-7} on the first and second PK day were compared using a two-tailed paired student's t-test. *P* values < 0.05 were considered statistically significant. Statistical calculations were performed using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Patients and treatment

Fifteen patients using sunitinib were included in this pharmacokinetic study. Of these patients, 12 received 50 mg and 3 received 37.5 mg sunitinib once daily. Patient characteristics are summarized in Table 1. No unexpected side effects were observed during sunitinib treatment or on the day of midazolam co-administration. Two patients used or recently stopped using mitotane therapy during the study. At the time of inclusion it was unknown that mitotane is a very potent inducer of CYP3A4 and that the combination of both drugs would thus result in markedly decreased sunitinib and midazolam concentrations. The observed interaction between sunitinib and mitotane in this present study is reported separately [23] and thereafter also described elsewhere [24-26].

Table 2 Summary of sunitinib and midazolam pharmacokinetic parameters

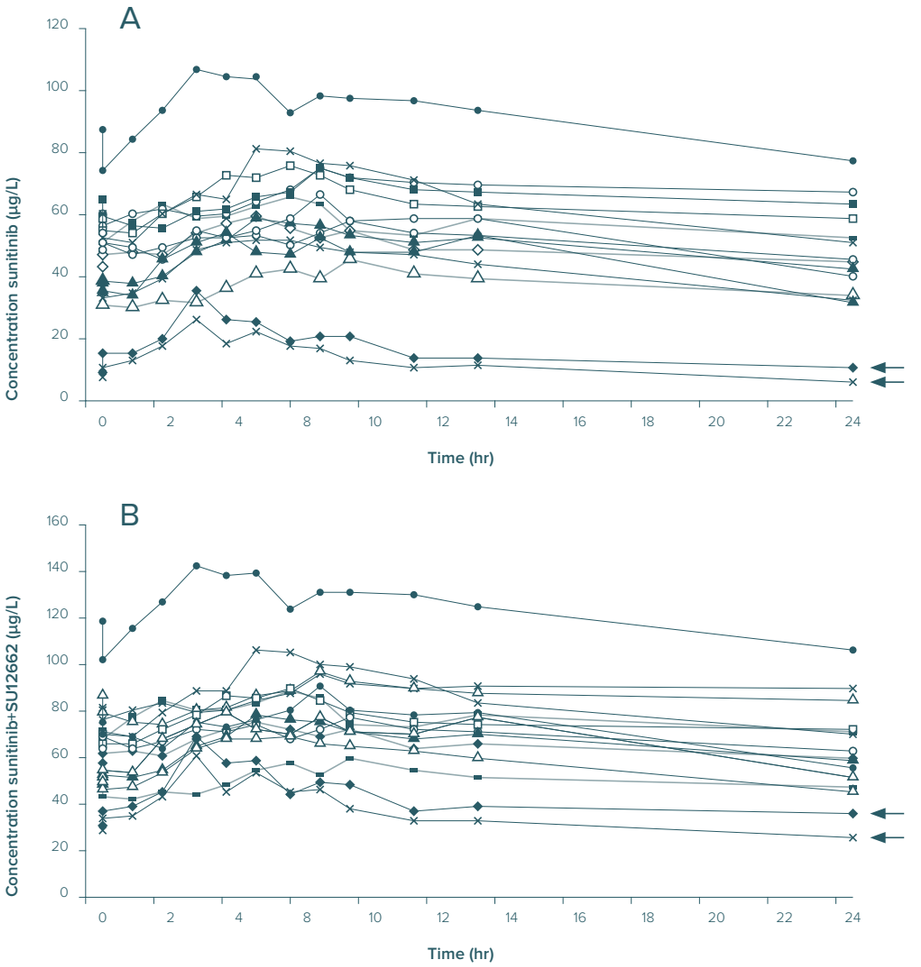
	Sunitinib (n = 13)	Sunitinib + SU12662 (n = 13)		Midazolam[a] (n = 13)	α-OH-midazolam[a] (n = 13)
AUC_{0-24} (μg·hr/L)	1442 (329)	1929 (423)	AUC_{0-7}[a] (μg·hr/L)	152 (54)	40 (13)
C_{trough} (μg/L)	55.2 (14.6)	74.4 (19.2)	C_{trough} (μg/L)	N/A	N/A
T_{max} (hr)	6.0 (1.5)	6.0 (1.5)	T_{max} (hr)	1.0 (1.0)	1.0 (0.9)
C_{max} (μg/L)	71.6 (15.4)	94.1 (19.7)	C_{max} (μg/L)	69.4 (28.9)	20.2 (10.8)

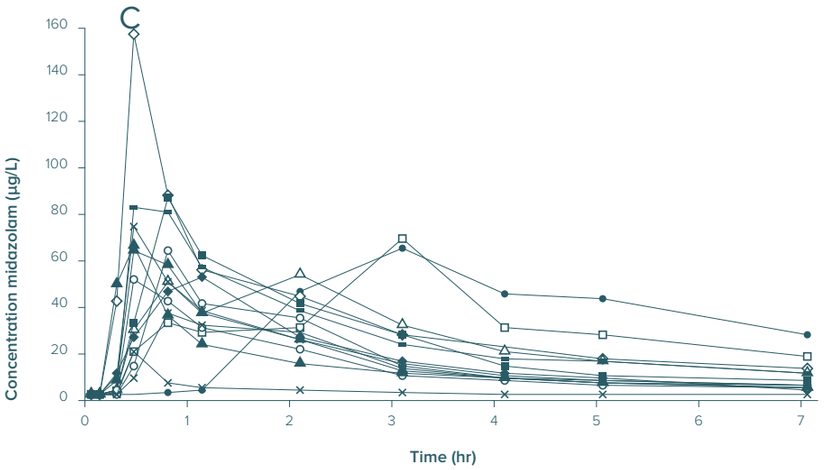
Data are presented as mean values (standard deviation). Parameters were dose normalized to sunitinib 50 mg.
Abbreviations: AUC, area under the concentration time curve; C_{max}, peak plasma concentration and T_{max}, time to reach peak plasma concentration.
[a]Midazolam data from PK day 2 were used; midazolam was then given without the co-administration of sunitinib.

Sunitinib and midazolam pharmacokinetics

Individual sunitinib, sunitinib + SU12662 and midazolam concentration versus time profiles are shown in Figure 2. A wide overlap is seen in the curves of patients that received 37.5 mg sunitinib and those that received 50 mg. The two patients that (recently) used mitotane are represented by the two lowest curves (indicated with an arrow). Since one of the

Figure 2 Individual observed concentration versus time profiles for A) sunitinib B) sunitinib +SU12662 C) midazolam. The *gray lines* represent the patients that received 37.5 mg sunitinib, and the *black lines* patients that received 50 mg sunitinib. The *arrows* indicate the two patients that used or recently stopped using mitotane





exclusion criteria was the use of CYP3A4 inducers, these two patients were excluded from further analysis after discovering that mitotane is a potent CYP3A4 inducer. Summaries of the pharmacokinetics of sunitinib and midazolam are presented in Table 2. The mean dose-normalized AUC_{0-24} (standard deviation) for sunitinib and sunitinib + SU12662 were 1442 µg·hr/L (330 µg·hr/L) and 1929 µg·hr/L (423 µg·hr/L), respectively. C_{trough} levels were 55.2 µg/L (14.6 µg/L) and 74.4 µg/L (19.2 µg/L) for, respectively, sunitinib and sunitinib + SU12662. The two patients that used mitotane had a sunitinib + SU12662 AUC_{0-24} of 1014 and 855 µg·hr/L. Their C_{trough} sunitinib + SU12662 levels were, respectively, 36.7 and 31.6 µg/L.

Correlation between sunitinib and midazolam pharmacokinetic parameters

Both sunitinib C_{trough} and sunitinib exposure (AUC_{0-24}), as well as sunitinib + SU12662 C_{trough} and sunitinib + SU12662 AUC_{0-24} were highly correlated ($r^2 = 0.94$, $P < 0.001$ and $r^2 = 0.93$, $P < 0.001$, respectively) as shown in Figure 3.

Significant correlations were observed between midazolam and sunitinib pharmacokinetics: midazolam exposure (AUC_{0-7}) could explain 51% of the variability in sunitinib AUC_{0-24} ($P = 0.006$), 47% of the variability in sunitinib C_{trough} ($P = 0.010$), 41% of the variability in sunitinib + SU12622 AUC_{0-24} ($P = 0.0018$) and 39% of the variability in sunitinib + SU12622 C_{trough} ($P = 0.023$) (Figure 4).

Influence of sunitinib on midazolam exposure

The mean midazolam exposures (AUC_{0-7}) without and with concomitant sunitinib use were 151.8 µg·hr/L and 115.3 µg·hr/L respectively. This indi-

Figure 3 Correlations between sunitinib pharmacokinetics for A) sunitinib C_{trough} levels and AUC_{0-24} B) sunitinib + SU12662 C_{trough} levels and AUC_{0-24} and C) sunitinib AUC_{0-24} and sunitinib + SU12662 AUC_{0-24}

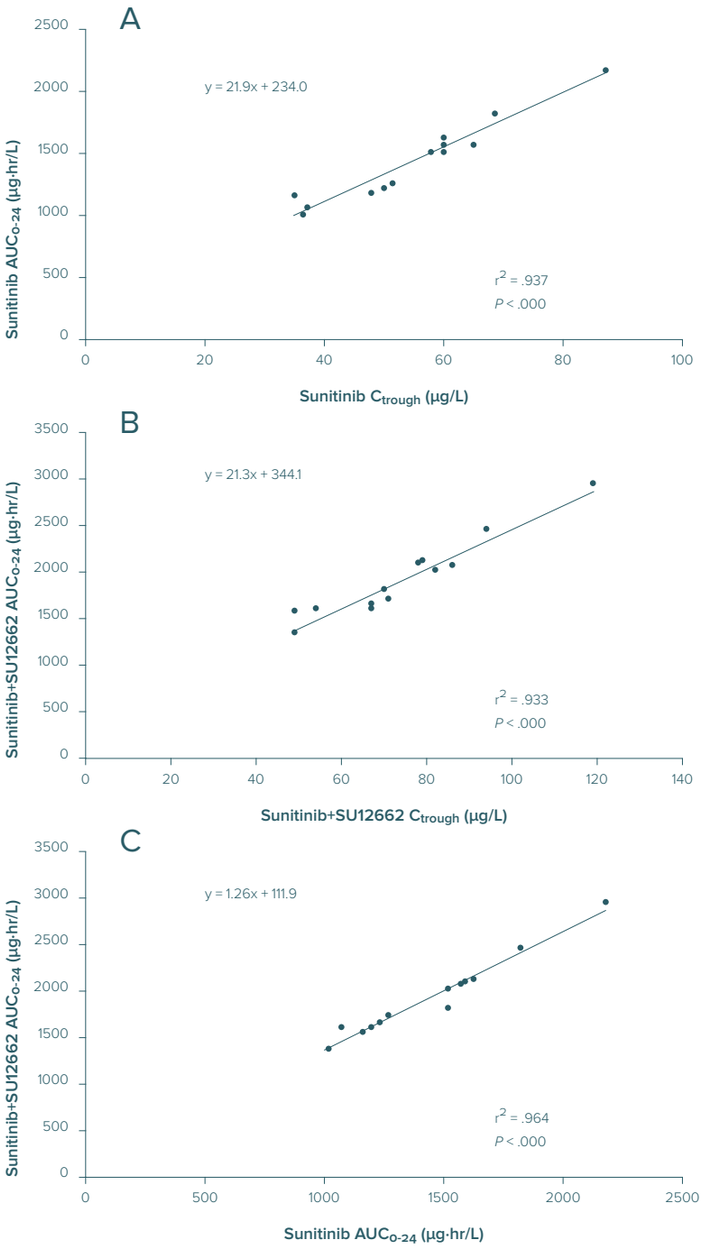
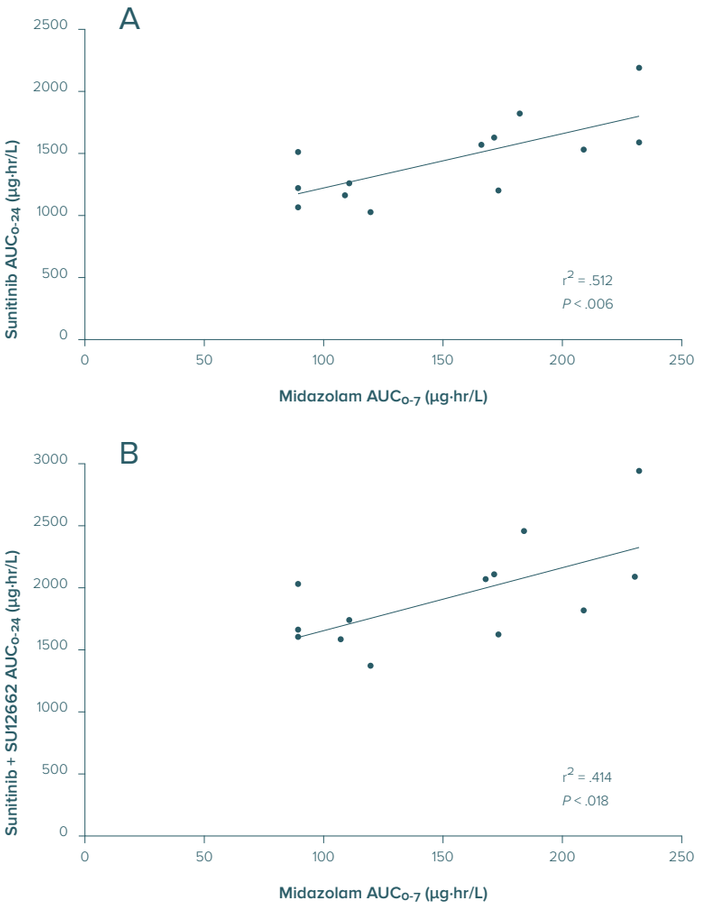


Figure 4 Correlations between midazolam and sunitinib pharmacokinetics A) midazolam AUC_{0-7} and sunitinib AUC_{0-24} B) midazolam AUC_{0-7} and sunitinib + SU12662 AUC_{0-24}



cates a decrease of 24% in midazolam exposure due to the co-administration of sunitinib ($P = 0.034$).

Discussion

This study shows that midazolam exposure is highly correlated with sunitinib exposure and explains a large proportion of the observed inter-patient variability in sunitinib pharmacokinetics. In addition, sunitinib and sunitinib + SU12662 C_{trough} levels were highly correlated with, respectively, sunitinib and sunitinib + SU12662 AUC_{0-24}. Finally, this study indicates that sunitinib significantly affects midazolam exposure in vivo.

This study not only demonstrates the excellent correlation of midazolam exposure with sunitinib pharmacokinetics, but also that up to 51% of the previously unexplained inter-patient variability in sunitinib pharmacokinetics can actually be elucidated by differences in midazolam exposure. By using midazolam as a phenotypic probe, patients at risk for either under- or overtreatment with sunitinib at the standard dosage regimen can be identified before the start of therapy. In fact, the two patients that used mitotane had very low sunitinib exposures with almost unmeasurable midazolam exposure. Since mitotane turned out to be a very potent CYP3A4 inducer which was one of the a priori exclusion criteria of this study, these two patients were excluded from statistical analyses. However, this interaction was unknown at the time of inclusion and actually became apparent by phenotyping these two patients, which shows us the clinical value of phenotyping [23]. By starting sunitinib therapy at an individualized dose, inefficacy due to under treatment as well as dose modifications (~50%) and discontinuations (~19%) due to toxicity can be prevented [1]. Moreover, for patients whose correct starting dose is debatable (e.g., due to comorbidities), midazolam could be useful to establish a safe and effective individualized sunitinib dose. However, before midazolam phenotyping can be used in clinical practice, the suitability of an individualized dosing strategy for sunitinib based on midazolam exposure would require prospective validation in a clinical trial.

Another approach to individualize sunitinib therapy could be therapeutic drug monitoring (TDM). Whereas TDM can be used to identify those patients that are over-/under dosed while on sunitinib therapy, phenotyping could be used to identify these patients beforehand.

Earlier identified covariates including tumor type, race, gender, body weight and elevated ECOG score could only explain 2-17 % of the observed changes in AUC and/or C_{max} [7,27]. Additionally, one study correlated pharmacogenetics with sunitinib exposure and treatment outcome; a significant higher exposure and more toxicity were found in patients harboring a polymorphism in the efflux transporter gene ABCG2 [28,29]. Phenotyping with midazolam might explain such a large percentage of the variability in sunitinib pharmacokinetics because it represents the influence of both genetic differences as well as environmental covariates (comorbidities, medication, life style, etc.) that might affect sunitinib exposure [7].

Although midazolam is officially recommended by the FDA for CYP3A4 activity phenotyping, variability in the oral exposure to midazolam can also be caused by co-influencing factors that affect both sunitinib and midazolam exposure (e.g., body composition, age, gender). Therefore, the percentage of sunitinib variability that can be truly explained by

CYP3A4-activity will be somewhat lower than the 51 % found in this present study.

The time frame of 0-7 hours over which midazolam samples were collected is based on the pharmacokinetic characteristics of midazolam. This frame represents 2-3 times the half-life of midazolam and therefore adequately describes the exposure to midazolam. Accordingly, the difference between the calculated midazolam AUC_{0-7} and extrapolated AUC_{inf} is < 15 % (not shown). Therefore, a longer midazolam sampling schedule would not have substantially improved the correlation between midazolam and sunitinib exposure. Midazolam is, besides CYP3A4, also metabolized by CYP3A5. Since sunitinib is only metabolized by CYP3A4, this difference in metabolism could have influenced the primary outcomes of this study [6, 8]. However, CYP3A5 is only active in 20% of the Caucasian population and in Dutch Caucasians even less [30]. Since all included patients were Dutch Caucasians, we do not expect that the correlation between midazolam and sunitinib exposure is considerably affected by the influence of CYP3A5 activity on midazolam metabolism.

According to the secondary objectives, this study shows the excellent correlation between sunitinib C_{trough} and sunitinib AUC_{0-24}, as well as sunitinib + SU12662 C_{trough} levels and sunitinib + SU12662 exposure. Taking into account the long half-life of sunitinib, it was expected that steady-state C_{trough} levels would well represent total drug exposure. However, the exact correlation between both parameters has, to our knowledge, not been reported before. The results of this present study legitimate the use of only C_{trough} levels to individualize sunitinib therapy in clinical practice. Additionally, the results of this study could be used to translate the threshold sunitinib AUCs associated with a beneficial clinical response into corresponding sunitinib C_{trough} levels [9]. These sunitinib AUCs would correspond with sunitinib + SU12662 AUC_{ss} greater than 1120, 868 and 994 µg·h/L and C_{trough} levels of sunitinib + SU12662 greater than 36.4, 24.6 and 30.5 µg/L for patients with mRCC, GIST and solid tumors, respectively, to achieve longer TTP and OS. Preclinical in vivo research identified 50 - 100 µg/L as the minimum concentration of sunitinib + SU12662 required to show anti-tumor activity which is in the same range [31].

In the current study, we found that treatment with sunitinib resulted in a 24% decrease in midazolam exposure. The mechanism by which this interaction occurs is not entirely clear. The prescribing information states that sunitinib does not induce CYP3A4 [6]. This is consistent with previous studies indicating that sunitinib does not influence the metabolism of the CYP3A4 substrates paclitaxel, docetaxel, or irinotecan [32-35]. A possible mechanistic explanation for the change in midazolam pharmacokinetics observed in this present study is heterotrophic cooperativity, whereby reversible binding of sunitinib causes a three-dimensional change in enzyme structure that ultimately affects a distant active site for some,

but not all substrates. This mechanism was previously proposed in an in vitro study where sunitinib was found to enhance CYP3A5-mediated hydroxylation of midazolam [36]. Since sunitinib and midazolam were administered simultaneously, a decreased uptake of midazolam due to sunitinib cannot be ruled out. Additional investigation is warranted to confirm the exact mechanism of the identified interaction observed in our current study.

In conclusion, this study demonstrates that midazolam exposure is highly correlated with sunitinib exposure and explains a large proportion of the observed inter-patient variability in sunitinib pharmacokinetics. Midazolam could be useful in clinical practice to identify those patients that are at risk of under- or overtreatment, respectively, at start of the standard sunitinib dosage regimen. Moreover, using C_{trough} levels as a surrogate parameter of total sunitinib exposure is a good and feasible approach for monitoring and guiding sunitinib therapy in clinical practice. Finally, the exposure to midazolam is decreased by the co-administration of sunitinib therapy, but this finding needs further investigation.

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7

Effect of gastrointestinal resection on sunitinib exposure in patients with GIST

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ABSTRACT

Background GIST patients often undergo gastrointestinal (GI) surgery. Previous studies have shown that imatinib and nilotinib exposures were decreased in GIST patients with prior major gastrectomy. We investigated whether major gastrectomy influences the exposure to sunitinib and its active metabolite SU12662.

Patients and Methods Pharmacokinetic data from 305 GIST patients included in 4 phase I-III trials were analyzed. Patients were subdivided into 6 groups according to their prior GI surgery. Apparent clearance (CI/F) and dose-corrected steady-state plasma exposures (AUC_{0-24}) of sunitinib and SU12662 were estimated using a population PK approach. ANCOVA was performed to test for differences in AUC_{0-24} and CI/F between each surgery subgroup and controls.

Results Major gastrectomy did not influence sunitinib or SU12662 exposure. The geometric mean of sunitinib and SU12662 AUC_{0-24} was decreased by 21% and 28% in patients with both gastrectomy and small bowel resection (n = 8) compared to controls (n = 63) for sunitinib (931 ng·hr/mL (95%-CI; 676 - 1283) versus 1177 ng·hr/mL (95%-CI; 1097 - 1263); $P < 0.05$) and SU12662 (354 ng·hr/mL (95%-CI; 174 - 720) versus 492 ng·hr/mL (95%-CI; 435 - 555); $P < 0.05$). No significant differences in exposure were observed in each of the other subgroups versus controls.

Conclusion In contrast to previous results for imatinib and nilotinib, gastrectomy alone does not influence sunitinib or SU12662 exposure. This should be taken into account for the treatment of gastrectomized GIST patients with TKIs. In patients who had undergone both gastrectomy and small bowel resection, sunitinib and SU12662 exposures are significantly, although clinically not relevantly, decreased.

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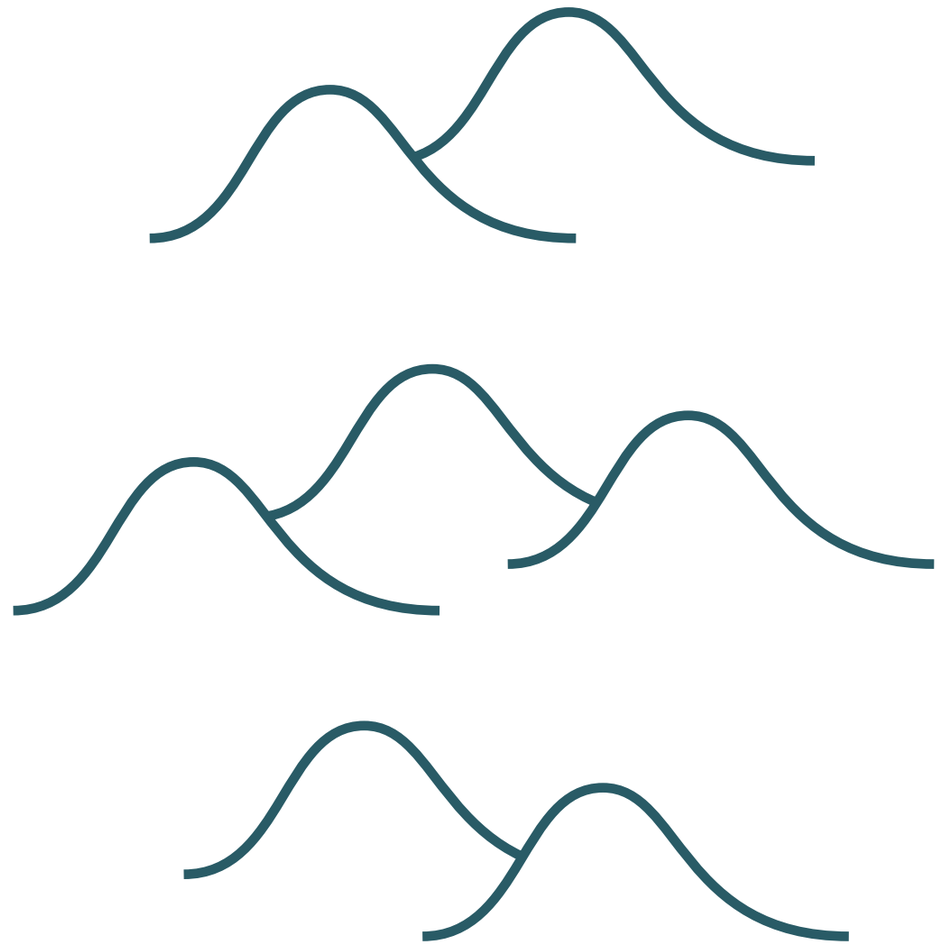


Introduction

Gastrointestinal stromal tumors (GISTs) are the most common sarcoma of the gastrointestinal tract and highly resistant to conventional chemotherapy [1]. In 2001, imatinib was registered as first-line therapy for patients with primary unresectable and/or metastatic GIST [2,3]. Thereafter in 2006, sunitinib was approved as second-line treatment for patients intolerant or refractory to imatinib therapy [4]. Recently, regorafenib was approved by the FDA as third-line therapy for GIST after failure of imatinib and sunitinib [5]. With the introduction of imatinib, sunitinib and regorafenib, survival of patients with metastatic GIST has substantially improved [4-6].

Imatinib C_{trough} levels and total sunitinib exposure have been reported to correlate with treatment benefit in patients with GIST [7,8]. However, GIST patients often have an altered GI tract due to either resection of the primary tumor or subsequent surgery for recurrence and/or metastasis. Whether these alterations influence drug absorption and thus exposure and clinical outcome of treatment, depends on the physicochemical properties of the oral tyrosine kinase inhibitor given (Table 1).

A cross-sectional study in GIST patients treated with imatinib revealed that C_{trough} levels were significantly lower in patients that previously had a major gastrectomy compared to patients without gastric surgery [9]. Comparable results, relating decreased plasma exposures with prior major gastrectomy, were seen for GIST patients treated with nilotinib [10]. Since the solubility of imatinib and nilotinib rapidly declines above pH 5.5 and 4.5 respectively, it is suggested that in gastrectomized patients a decreased acid secretion may contribute to a decreased solubility and thereby decreased absorption of both TKIs [9-12]. Each segment of the gastrointestinal tract has its own characteristic pH level; acidity declines over the GI tract from the stomach (pH 1-3) to the small intestine (pH 5-7) and the colon (pH 7-8) [13,14]. For imatinib and nilotinib solubility and absorption therefore rapidly decreases after the stomach [15]. This is further supported by the relative short time to reach maximum plasma concentration (T_{max}) for these drugs; 2-4 hours for imatinib and 3 hours for nilotinib [11,12]. Hence, due to the physicochemical properties of imatinib and nilotinib, the stomach is essential for dissolution and absorption of these TKIs.

Table 1 Physicochemical properties of imatinib, nilotinib and sunitinib

Drug	MW (g/mol)	pKa	Solubility	BCS class	Ref
Imatinib	493.60	7.7	Freely soluble (100-1000 mg/mL) up to pH 5.5. Solubility declines at higher pH; lowest solubility is 1 mg/mL.	II	[11]
Nilotinib	565.98	2.1 and 5.4	Slightly soluble (1-10 mg/mL) at pH 1.0, very slightly soluble (0.1-1 mg/mL) in water, at pH 3.0. Practically insoluble (< 0.1 mg/mL) in buffer solutions of pH ≥ 4.5	IV	[12]
Sunitinib	398.47	9.0	25 mg/mL at pH 1.2-6.8. Solubility reduces at pH ≥ 6.8	IV	[16]

Abbreviation: BCS, Biopharmaceutics Classification System; MW, molecular weight.

For sunitinib however, solubility does not decline until pH 6.8 [16]. This makes in theory the involvement of the stomach less critical for dissolution and absorption of sunitinib. This is further supported by the relative broad surface over which sunitinib is absorbed from the GI tract, reflected by a long time to reach maximum plasma concentration of sunitinib, e.g. 6-12 hours [16]. We postulated that major gastrectomy would most likely not affect the exposure to sunitinib and its active metabolite SU12662.

To confirm this hypothesis, we retrospectively investigated the effect of GI resections on sunitinib and SU12662 exposures in patients with GIST across 4 different phase I-III clinical trials. Our primary objective was to investigate the effect of major gastrectomy; secondary objectives were to determine the effect of other GI resections on sunitinib exposure.

Patients and Methods

Patient selection

A total of 635 patients were treated with sunitinib in 4 different phase I/II, II, or III clinical trials that investigated the safety, efficacy, and/or pharmacokinetics of sunitinib in patients with GIST [17-20]. Of these 635 patients, a total of 364 patients had pharmacokinetic (PK) samples available which were included in population pharmacokinetic analysis. Out of these 364 patients (for sunitinib total number of samples = 3394 and for SU12662 total number of samples = 3410), a total of 305 patients had comprehensive GI resection data available and were therefore eligible for the present analysis.

Inclusion criteria in these trials were: a histopathologically confirmed diagnosis of metastatic or unresectable GIST with progression on or toxicity of previous imatinib therapy; age > 18 years or between 20 to 75 years; adequate hematologic, renal, liver and cardiac function; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; willingness and ability to comply with scheduled visits, treatment plans, laboratory test, and other study procedures.

Exclusion criteria in these trials were: current treatment in another clinical trial or ≤ 4 weeks prior to starting sunitinib, ≤ 2 weeks for imatinib therapy; non recovery from acute toxic effect of previous chemotherapy or imatinib; a history of known brain metastases; any serious co-morbidity; and pregnancy or breastfeeding.

All studies were done in accordance with Good Clinical Practice and under the ethical principles established by the Declaration of Helsinki.

Each protocol was reviewed and approved by the Institutional Review Board and informed consent was obtained from each patient. The sub-analysis on the existing dataset of Pfizer was requested by the non-Pfizer affiliated authors of this manuscript and was reviewed and granted by Pfizer.

Sunitinib pharmacokinetic data collection and statistical analysis

Patients were treated with sunitinib in doses ranging from 25 mg to 75 mg once daily on 4/2 (4 weeks on treatment followed by 2 weeks off treatment), 2/1 (2 weeks on treatment followed by 1 week off treatment), 2/2 (2 weeks on treatment followed by 2 weeks off treatment) or CDD (continuous daily dosing) schedules. Blood samples for pharmacokinetic assessment of sunitinib and its active metabolite SU12662 were collected pre-dose or post-dose on different days with details provided in Table 2. Blood samples were collected in EDTA tubes and shortly after collection centrifuged at 4 °C for 10 minutes at 3500 rpm. Plasma samples were separated and stored at -20 °C or lower until shipped. Shipment of samples was on dry ice to Bioanalytical Systems Inc (West Lafayette, IN) where they were stored at -20 °C or lower until assayed within the established stability window. For quantification a validated, sensitive and specific isocratic liquid chromatographic tandem mass spectrometric (LC-MS/MS) method in positive ionization mode was used [21].

Table 2 Summary of characteristics of studies used for analyses

Study number	Study design	n[a]	Dosing schedule: dose	Day(s) of PK sampling	Time point(s) of sampling	Ref
RTKC-0511-013	Phase II	74	2/1: 50 mg 2/2: 25, 50, 75 mg 4/2: 50 mg	Days 1, 14, 28 (only 4/2) of Cycles 1, 2, and 3 (optional) Day 1 of Cycles 4, 5 (optional), and 6	Pre-dose On 1st day of Cycle 1 and on last day of Cycles 1 and 2: 0, 1, 4, 6, 8, 10, 12, 24, and 48 hr post-dose (10 and 12 hr optional)	[19]
A6181004	Phase III	179	4/2: 50 mg	Days 1, 14, and 28 of Cycle 1; Days 1 and 28 of Cycles 2 and beyond	Pre-dose	[20]
A6181045	Phase I/II	33	4/2: 25, 50, 75 mg	Phase I: Days 1, 2, 7, 14, 21, and 28 of Cycle 1 Phase II: Days 1, 14, and 28 of Cycles 1-4	Pre-dose On Days 1 and 28 of Cycle 1 (Phase I Only): 0, 1, 2, 4, 6, 8, 10, 24 (Only Day 28) and 48 (Only Day 28) hr post-dose	[18]
A6181047	Phase II	19	CDD: 37.5 mg	Day 1 of each cycle	Pre-dose	[17]

Abbreviations: 2/1, 2 weeks on treatment followed by 1 week off treatment; 2/2/, 2 weeks on treatment followed by 2 weeks off treatment; 4/2, 4 weeks on treatment followed by 1 week off treatment; CDD, continuous daily dosing; PK, pharmacokinetics.

[a] Number of subjects from each study contributing to the ANCOVA

All quantifiable plasma samples were included to develop population PK models for sunitinib and SU12662 using Nonlinear Mixed Effect Modeling software (NONMEM; version 7.1.2), following exclusion of plasma samples with inadequate dosing records and those identified to be extreme outliers (eg, 6 < Conditional Weighted Residual (CWRES) < −6). Sunitinib data were best described by a two-compartment model with first-order order absorption with a lag time and first-order elimination. Similarly, SU12662 data were best described by a two-compartment model with first-order formation without lag time and first-order elimination.

Patients were subdivided into 6 subgroups according to their previous GI surgery: 1) Major gastrectomy (defined as total or subtotal gastrectomy), 2) Partial gastrectomy, 3) Small bowel resection, 4) Both gastrectomy (either partial or (sub)total) and small bowel resection, 5) Colon resection, and 6) Controls with no prior surgery. Patients with uncertain or unclear defined GI resections (n = 59) were excluded from analysis.

Following population PK analyses, the individual post-hoc estimates for CI/F of sunitinib and SU12662 were used to calculate steady-state total plasma exposures ($AUC_{0\text{-}24}$) of sunitinib and SU12662 at 50 mg of sunitinib for each individual patient, by dividing the dose (i.e., 50 mg) by individual patient post-hoc CI/F estimate. Thereafter, an analysis of covariance (ANCOVA) on log transformed data was performed to test for significant differences in $AUC_{0\text{-}24}$ and CI/F of both sunitinib and SU12662 between each surgical subgroup and control. Covariates previously identified by Houk et al. which were initially included in the ANCOVA model were sex and race for sunitinib CI/F, and sex, race, body weight and ECOG performance status for SU12662 CI/F [22]. Within the ANCOVA models, Multiple Comparisons with Control (i.e., MCC) using Dunnett's test were performed and significant increases in CI/F and decreases in $AUC_{0\text{-}24}$ were identified. For sunitinib and SU12662 CI/F the difference was considered statistically significant ($P \leq 0.05$), if the 95% lower bound for the difference from controls on the log scale did not include zero. Conversely, for sunitinib and SU12662 $AUC_{0\text{-}24}$, if the 95% upper bound for the difference from the control, on the log scale, did not include zero, the difference was considered statistically significant ($P \leq 0.05$). Subsequently, previously identified covariates which were not statistically significant ($P > 0.05$) within the ANCOVA model were later removed from the ANCOVA models for sunitinib and SU12662. The number of observation for each individual was added as an additional covariate to the ANCOVA models to make sure it did not affect the final ANCOVA models overall results and conclusions. All statistical analyses were performed using S-Plus Version 8.0 (TIBCO Software Inc., Palo Alto, USA). The population pharmacokinetic and statistical analysis on the existing dataset was done by Pfizer Inc. Independent

reviewers, blinded to the PK and patient data and not related to Pfizer, subdivided the included patients into 6 groups according to their previous GI surgery.

Results

Patient characteristics

A total of 305 patients had both population PK parameter estimates and comprehensive GI resection data available and were therefore included in the descriptive statistics presented as well as in the analysis of covariance (ANCOVA) models for sunitinib and SU12662. Of these patients, 45 underwent major gastrectomy (subgroup 1), 58 partial gastrectomy (subgroup 2), 118 small bowel resection (subgroup 3), 8 both gastrectomy and small bowel resection (subgroup 4) and 13 patients a colon resection (subgroup 5). Sixty-three patients served as controls and did not have any prior surgery (subgroup 6). Baseline characteristics including sex, age, bodyweight, ethnicity and ECOG performing status are shown in Table 3 per subgroup.

Effect of prior gastrointestinal surgery on sunitinib pharmacokinetics

Sunitinib and SU12662 apparent clearance (CI/F) was not increased in patients that previously had a major gastrectomy. Consequently, the geometric mean of sunitinib and SU12662 $AUC_{0\text{-}24}$ were not decreased in patients with a major gastrectomy, compared to patients in the control subgroup for sunitinib (1171 ng·hr/mL versus 1177 ng·hr/mL; $P > 0.05$) and SU12662 (520 ng·hr/mL versus 492 ng·hr/mL $P > 0.05$) (Table 4 and Figure 1).

A significant increase in apparent clearance (CI/F) of sunitinib and SU12662 was seen in patients that had undergone both gastrectomy and small bowel resection relative to the controls. The geometric mean of CI/F for sunitinib and SU12662 was increased by 26% and 39% in subgroup 4, patients with both gastrectomy and small bowel resection, compared to those in the control subgroup for sunitinib (53.7 L/hr versus 42.5 L/hr; $P \leq 0.05$) and for SU12662 (29.7 L/hr versus 21.4 L/hr; $P \leq 0.05$), respectively. No statistically significant ($P > 0.05$) increases in apparent clearance for each of the other subgroups from controls were observed (Table 4 and Figure 1).

Consequently, a decreased total plasma exposure ($AUC_{0\text{-}24}$) to sunitinib and SU12662 was seen in patients that had undergone both gastrectomy and small bowel resection. The geometric mean of total plasma exposure ($AUC_{0\text{-}24}$) to sunitinib and SU12662 was 21% and 28% lower in subgroup 4, patients that underwent both gastrectomy and small bowel resection, compared to those in the control subgroup sunitinib (931 ng·hr/mL versus 1177 ng·hr/mL; $P \leq 0.05$) and for SU12662 (354 ng·hr/mL versus 492

Table 3 Patient characteristics for each past GI surgery subgroup

	Major gastrectomy (n = 45)	Partial gastrectomy (n = 58)	Small bowel resection (n = 118)	Small bowel resection (n = 118)	Combined gastrectomy and small bowel resection (n = 8)	Colon resection (n = 13)	Controls (n = 63)
Sex, n (%)							
Male	30 (66.7%)	35 (60.3%)	75 (63.6%)	6 (75%)	9 (69.2%)	40 (63.5%)
Female	15 (33.3%)	23 (39.7%)	43 (36.4%)	2 (25%)	4 (30.8%)	23 (36.5%)
Age (years)*	56 (36 - 77)	57 (28 - 79)	53 (23 - 81)	49 (45 - 54)	68 (50 - 84)	58 (36 - 84)
Bodyweight (kg)*	65 (40 - 100)	70 (39 - 121)	71 (40 - 140)	64 (45 - 139)	80 (56 - 114)	74 (44 -137)
Race, n (%)							
Non-Asian	37 (82.2%)	52 (89.7%)	94 (79.7%)	7 (87.5%)	12 (92.3%)	59 (93.7%)
Asian	8 (17.8%)	6 (10.3%)	24 (20.3%)	1 (12.5%)	1 (7.7%)	4 (6.3%)
ECOG performing status, n (%)							
≤ 1	42 (93.3%)	58 (100%)	116 (98.3%)	8 (100%)	13 (100%)	62 (98.4%)
≥ 2	3 (6.7%)	0 (0%)	2 (1.7%)	0 (0%)	0 (0%)	1 (1.6%)

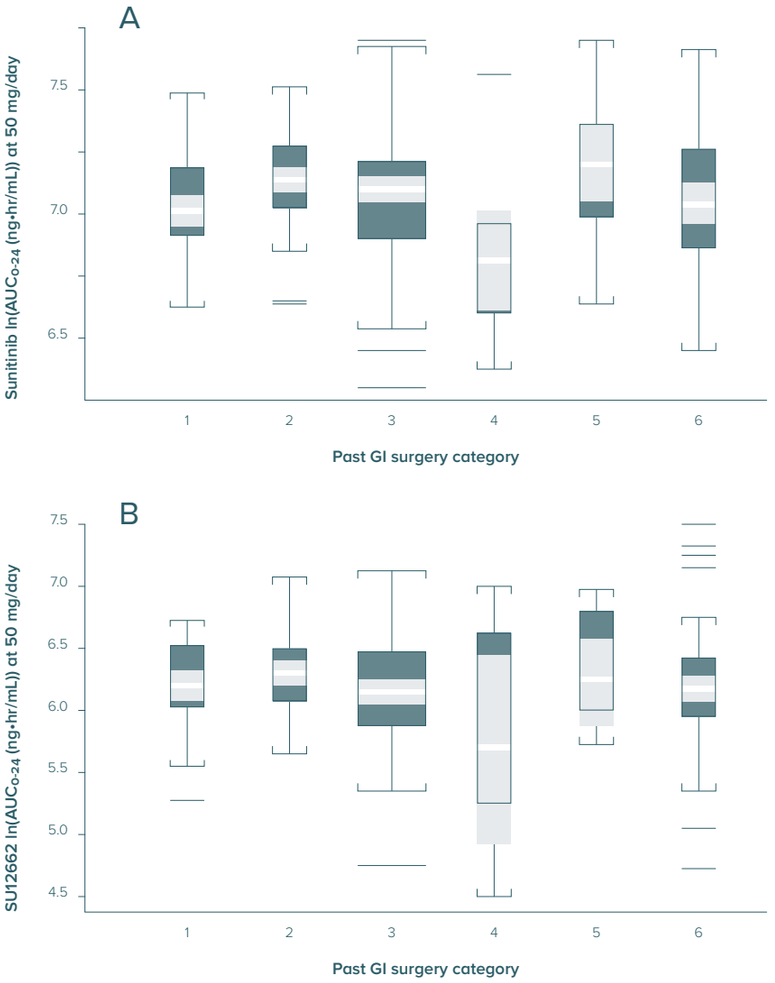
* Data are presented as median values with lower and upper limit

Table 4 Sunitinib and SU12662 CI/F and $AUC_{0\text{-}24}$ estimates for each past GI surgery subgroup

Parameter	Past GI Surgery Subgroup					
	Major gastrectomy (n = 45)	Partial gastrectomy (n = 58)	Small bowel resection (n = 118)	Combined gastrectomy and small bowel resection (n = 8)	Colon resection (n = 13)	Controls (n = 63)
Number of PK samples per subject, median (range)	7 (1 - 32)	9 (1 - 38)	7 (1 - 35)	10 (2 - 35)	13 (2 - 30)	7 (1 - 37)
Sunitinib						
$AUC_{0\text{-}24}$ (ng·hr/mL)	1171 (1099 - 1248)	1294 (1228 - 1365)	1194 (1141 - 1250)	931 (676 - 1283)*	1325 (1109 - 1583)	1177 (1097 - 1263)
CI/F (L/hr)	42.7 (40.1 - 45.5)	38.6 (36.6 - 40.7)	41.9 (40.0 - 43.8)	53.7 (39.0 - 74.0)*	37.7 (31.6 - 45.1)	42.5 (39.6 - 45.6)
SU12662						
$AUC_{0\text{-}24}$ (ng·hr/mL)	520 (474 - 571)	567 (522 - 617)	492 (458 - 529)	354 (174 - 720)*	597 (457 - 779)	492 (435 - 555)
CI/F (L/hr)	20.2 (18.4 - 22.1)	18.5 (17.0 - 20.1)	21.4 (19.9 - 23.0)	29.7 (14.6 - 60.4)*	17.6 (13.5 - 23.0)	21.4 (18.9 - 24.1)

Abbreviations: $AUC_{0\text{-}24}$, Area Under the Concentration-time curve from time zero to 24 hours post-dose at steady state; CI/F, apparent clearance; PK, pharmacokinetic. Data are presented as geometric mean (95% CI) unless stated otherwise. * Significantly different compared to controls (p<0.05).

Figure 1 Exposures in patients with different GI resections. A) Sunitinib exposure. B) SU12662 exposure. Major gastrectomy; 2 = Partial gastrectomy; 3 = Small bowel resection; 4 = Combined gastrectomy and small bowel resection; 5 = Colon resection; 6 = Controls with no prior surgery



ng·hr/mL; $P \leq 0.05$), respectively. No statistically significant ($P > 0.05$) decreases in total plasma exposures for each of the other subgroups compared to controls were observed (Table 4 and Figure 1).

Discussion

This study shows that major gastrectomy did not affect sunitinib or SU12662 plasma exposures in patients with GIST. This is in contrast to prior data regarding the impact of gastrectomy on both imatinib and nilotinib exposure [9,10]. Sunitinib and SU12662 exposures were however significantly decreased in patients who had previously undergone both gastrectomy and small bowel resection, although this observation was in a small subgroup of patients. All other types of GI resections studied, showed no impact on sunitinib or SU12662 pharmacokinetics.

The results from this study support our hypothesis that the influence of GI resections on TKI exposure depends on two variables: the specific physicochemical properties of the TKI given and the part of the GI tract that has undergone resection. So although most TKIs exhibit pH-dependent solubility, small differences in their physicochemical properties (e.g. declined solubility in pH conditions higher than pH 5.5 for imatinib versus 6.8 for sunitinib) may cause great differences in the impact of gastrectomy on their GI solubility and absorption. In addition, the absorption characteristics of a drug under normal conditions [i.e. whether it is absorbed throughout the GI tract (e.g. sunitinib) or whether it is mainly absorbed through the stomach and the upper part of the small intestine (e.g. imatinib)] may affect the extent to which site specific GI resections can decrease the GI availability (F_{gut}) and subsequently the bioavailability ($F = F_{gut} \cdot F_{hepatic}$) of a drug. The finding that imatinib exposure is not affected by the co-administration of the proton pump inhibitor omeprazole somewhat contradicts our hypothesis considering reduced solubility [23]. However, 40 mg omeprazole only increases the gastric pH to 4.6 which is still an adequate level for imatinib to freely dissolve [24]. Major gastrectomy might result in a further rise in pH equally to that of the small intestines and this therefore could interfere with imatinib dissolution.

Currently, the approved and accepted first line treatment for GIST is imatinib [11]. The stomach is the most common primary site for GIST (~60%), and a proportion of these patients will therefore undergo major gastrectomy procedures prior to systemic imatinib therapy for metastasis [25]. Imatinib C_{trough} levels in ~80% of the gastrectomized patients were reported to be below 1100 ng/mL which has been correlated to shorter progression free survival (PFS) [7,9]. In addition, increasing the imatinib dose might not result in an increased exposure due to the limited solubility of imatinib in a patient with limited gastric physiology. By measuring plasma concentrations in patients with prior major gastrectomy, a decreased exposure to imatinib could be identified early in treatment, prior to development of clinical drug failure. Sunitinib is currently approved and accepted as the second line treatment for GIST patients and also for sunitinib a relationship between systemic exposure and efficacy has been demonstrated before [8,16]. The results from this present study

show that sunitinib exposure is, in contrast to the results for imatinib, not decreased in gastrectomized patients. These findings should be taken into account for the treatment of gastrectomized GIST patients with TKIs. Hypothetically, gastrectomized patients have less and/or shorter treatment benefit from first-line imatinib therapy due to decreased imatinib plasma levels. Yet, these patients theoretically have a high chance of treatment benefit from second line sunitinib therapy. However, further prospective research to investigate this hypothesis and whether there is a difference in clinical outcome between gastrectomized patients treated with imatinib or sunitinib is needed.

The results from this present analysis also show that patients who had undergone both gastrectomy and small bowel resection did have statistically significantly ($P \leq 0.05$) lower sunitinib and SU12662 exposures, which is an extension of prior data showing such effects of combined surgery on plasma exposure of both imatinib and nilotinib [9,10]. An effect of both gastrectomy and small bowel resection on the exposure to all three studied TKIs and other drugs is not surprising, since resections of large portions of the GI tract will significantly reduce the absorption surface available. Houk et al. showed that patients with GIST and a sunitinib $AUC_{0\text{-}24} > 600$ ng·hr/mL had longer time to progression (TTP) and overall survival (OS) [8]. The patients with a combined gastrectomy and small bowel resection in our study had an average sunitinib exposure of 931 ng·hr/mL and none of the patients in this subgroup had a sunitinib exposure < 600 ng·hr/mL. So although patients with both a gastrectomy and small bowel resection in this study had a statistically significant ($P \leq 0.05$) decrease in sunitinib and SU12662 exposure, this decrease does not appear to be clinically relevant.

Hypothetically, the extent of small bowel resection will be critical for the remaining absorption surface and whether and to what extent sunitinib exposure is affected or not. Unfortunately, the length of resected intestine was not registered in the database used for this retrospective study, which limits the ability to analyze this variable. Measuring plasma concentrations of sunitinib could be suggested in patients that underwent an unknown or very large resection of the GI tract to identify those patients that do have a clinically relevant decreased sunitinib exposure. In clinics where the measurement of sunitinib plasma concentration is not feasible, an alternative and practical approach could be to gradually increase the dose based on the individual patient safety and tolerability. The relatively small number of patients who underwent a combined gastrectomy and small bowel resection (n = 8) can be considered as a limitation of this present study. Therefore, the results in this subgroup of patients should be interpreted with caution and need to be verified in a larger group of patients with extended GI resections.

It is generally assumed that for most weakly basic drugs, the dissolution process is often the rate-limiting step for absorption of these drugs from the GI tract. However, besides pH and physicochemical properties, there are other variables within the GI tract that determine the rate and extent of dissolution including the fluid volume available for dissolution that is added in the stomach, gastric motility and the maximum dose strength. Also the maximum dose strength is rather different between imatinib, nilotinib and sunitinib. Imatinib and nilotinib are dosed at 400-800 mg a day compared to sunitinib which is dosed at 25-50 mg a day. This could be an additional explanation why sunitinib exposure is not influenced by gastrectomy whereas imatinib and nilotinib exposure are. Apparently, pH and dosage rather than fluid volume and gastric motility is of influence on the absorption of TKIs. An alternative pre-clinical explanation for the found differences is the removal due to gastrectomy of transporters that facilitate the gastric absorption of TKIs, whereby imatinib might depend more on this transporter for absorption than sunitinib does [26,27].

Conclusion

In conclusion, major gastrectomy alone does not influence exposure to sunitinib or its active metabolite SU12662, which is contrary to previous results for imatinib and nilotinib. This should be taken into account for the treatment of gastrectomized GIST patients with TKIs. Patients with a combined gastrectomy and small bowel resection had a statistically significantly, though clinically not relevant, decreased plasma exposure to sunitinib and SU12662 which in theory might depend on the length of intestine resected.

Acknowledgement

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8

Everolimus pharmacokinetics and its exposure-toxicity relationship in patients with cancer

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ABSTRACT

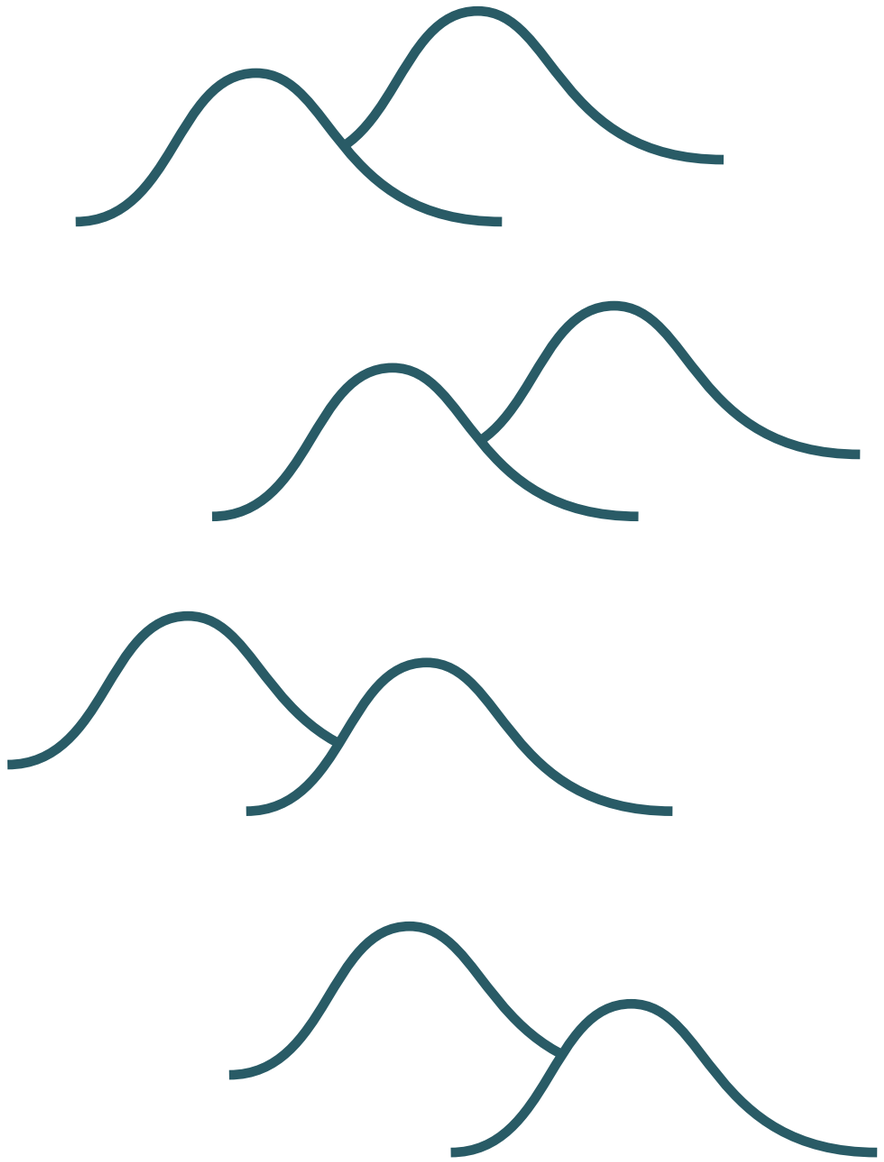
Background Everolimus is a mTOR inhibitor used for the treatment of different solid malignancies. Many patients treated with the registered fixed 10 mg dose once daily are in need of dose interruptions, reductions or treatment discontinuation due to severe adverse events. This study determined the correlation between systemic everolimus exposure and toxicity. Additionally, the effect of different covariates on everolimus pharmacokinetics (PK) was explored.

Patients and Methods Forty-two patients with advanced thyroid carcinoma were treated with 10 mg everolimus once daily. Serial pharmacokinetic sampling was performed on day 1 and 15 of treatment. Subsequently, a population PK model was developed using NONMEM to estimate individual PK values which were used for the assessment of an exposure-toxicity relationship. In addition, the population PK model was used to investigate the influence of patient characteristics and genetic polymorphisms in genes coding for ABCB1, CYP3A, CYP2C8 and PXR on everolimus PK.

Results Forty patients were evaluable for PK analysis. Patients who required a dose reduction (n = 18) due to toxicity at any time during treatment had significant higher everolimus exposures (mean $AUC_{0\text{-}24}$ (SD) 600 (274) vs. 395 (129) µg·hr/L, $P = 0.008$) than patients without a dose reduction (n = 22). A significant association between everolimus exposure and stomatitis was found in the four-level order logistic regression analysis ($P = 0.047$). The presence of at least one TTT haplotype in the *ABCB1* gene was associated with a 21% decrease in everolimus exposure.

Conclusion The current study showed that dose reductions and everolimus induced stomatitis were strongly associated with systemic everolimus drug exposure in patients with cancer.

* Contributed equally Submitted



Introduction

Everolimus is an orally administered rapamycin derivative inhibiting the mammalian target of rapamycin (mTOR) [1]. This is a key signaling molecule in the phosphatidylinositol 3-kinase (PI3K)/Akt pathway which is involved in the regulation of growth, proliferation, metabolism, survival and angiogenesis of cells and often dysregulated in cancer [1]. Currently, everolimus is registered for the treatment of advanced hormone receptor positive (HR^+), human epidermal growth factor-2 negative ($HER2^-$) breast cancer in postmenopausal women in combination with exemestane, for metastatic renal cell carcinoma (mRCC), for irresectable or metastatic pancreatic neuroendocrine tumors (pNET) and subependymal giant cell astrocytoma (SEGA) [2-4].

Despite its proven efficacy, everolimus is also associated with a number of serious side effects. Most common toxicities associated with everolimus therapy include stomatitis, rash, fatigue, diarrhea, infections, nausea, loss of appetite, hematologic toxicities, dyspnea, noninfectious pneumonitis and metabolic abnormalities such as hypercholesterolemia and -glycaemia [5]. While it is reported that the majority of these adverse events are manageable and of mild to moderate severity, many patients are in need of dose interruptions, reductions or treatment discontinuation due to toxicity [6]. Indeed, in the pivotal breast cancer, mRCC and pNET phase III trials, 10 to 35% of the patients discontinued everolimus treatment due to adverse events [2-4]. In addition, ~62% of the patients needed dose interruptions or reductions compared to 12-29% in the placebo arms [2, 4].

The large number of dose reductions and treatment discontinuation make toxicity currently one of the main challenges in the optimal use of everolimus for the treatment of cancer. In oncology, everolimus is registered as a fixed oral dose of 10 mg once daily. However, in transplantation medicine therapeutic drug monitoring (TDM) with individualized dosing is routinely applied due to everolimus' narrow therapeutic window and high inter-patient variability in pharmacokinetics (PK) [7]. In transplantation medicine, everolimus is used as an immunosuppressant to prevent rejections. Dose individualization is not only applied to prevent toxicity, but also to optimize treatment efficacy. In oncology, the same high inter-patient variability in PK is seen (AUC; 45 CV%, C_{trough}; 60 CV%) [8]. This substantial variability, in combination with the fixed 10 mg dosing, results in large differences in everolimus exposure between patients. This could result in either supra-therapeutic drug exposure with an increased incidence of toxicity, but also in sub-therapeutic drug exposure leading to decreased anticancer effects.

The primary objective of this study was to assess the correlation between everolimus exposure and toxicity in patients with advanced thyroid cancer. Additionally, we explored the influence of different covariates on everolimus PK, including genetic polymorphisms in genes encoding enzymes involved in the absorption and metabolism of everolimus.

Patients and Methods

Patients

Forty-two patients were enrolled in this phase II study investigating the efficacy and pharmacokinetics of everolimus for the treatment of progressive or recurrent, unresectable or metastatic thyroid cancer. The efficacy data of this study will be reported separately. Participating medical centers were the Leiden University Medical Center and the University Medical Center Groningen. Patients were treated continuously with everolimus at an once daily oral dose of 10 mg until tumor progression, unacceptable toxicity, death or discontinuation from the study for other reasons. Toxicities were assessed at baseline, day 1, 14 and 28 of therapy and monthly thereafter according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTC-AE) version 4.0. Dose adjustments were permitted for adverse events suspected to be related to everolimus. The first dose reduction was to 5 mg once daily. If another dose reduction was needed, everolimus was dosed as 5 mg every other day. The study was approved by the institutional ethics committees (Leiden University Medical Center and University Medical Center Groningen, The Netherlands) and all patients gave written informed consent before entering the study.

Pharmacokinetic sample collection and analysis

For everolimus PK assessment, whole blood samples were obtained at day 1 and 15 of therapy. Samples were collected into EDTA-tubes at pre-dose and 1, 2, and 3 hours after everolimus intake (sparse schedule). More extensive PK sampling at 4, 5, 6, 7 and 8 hours after everolimus intake was optional for patients (extensive schedule). Samples were stored at -20 °C until the day of analysis.

Everolimus concentrations in whole blood were measured using a validated Ultra Performance Liquid Chromatography - Tandem Mass Spectrometric (UPLC-MS/MS) assay. Validation of the assay was performed according to the EMA guidelines of bioanalytical method development [9]. The calibration line was linear over the range from 2 to 160 µg/L and the lower limit of quantification (LLOQ) was 0.6 µg/L. Assay performance was in agreement with guidelines for bioanalytical method development and validation.

Pharmacogenetic analysis

Single Nucleotide Polymorphisms and haplotype selection

Everolimus is metabolized by the cytochrome P450 (CYP) enzymes CYP3A4, CYP3A5 and CYP2C8 and is also a substrate for the efflux pump P-glycoprotein (P-gp) encoded by the *ABCB1* gene [10]. The nuclear pregnane X receptor (PXR; NR1I2) regulates the expression of CYP3A4 and could therefore also influence everolimus PK [11]. Eleven single nucleotide polymorphisms (SNPs) in these genes were selected based upon a candidate gene approach (Supplementary Data S1). For the *ABCB1* and *CYP2C8* gene, selected SNPs were used for haplotype analysis performed in gPLINK (Supplementary Data S2). Haplotypes were set at a certainty greater than 0.97. For the *ABCB1* and *CYP2C8* gene, only haplotypes and no individual SNPs were tested.

Genotyping assays

Germline DNA was isolated from 400 µl EDTA-blood using MagNa Pure Compact (Roche, Almere, the Netherlands). DNA concentrations were thereafter measured using Nanodrop (Isogen, De Meern, The Netherlands). Genotyping was performed using pre-designed genotyping assays (Supplementary Data S1, online). Samples were analyzed on a Viia7 real-time PCR system according to the manufacturers' instruction (Life Technologies, Bleiswijk, The Netherlands). Call rates of all assays were >98%. As a quality control, at least 5% of the samples were genotyped in duplicate. No inconsistencies were observed. Minor allele frequencies (MAF) of all 11 SNPs were calculated and compared with reported MAF for European Populations (HAPMAP). No significant deviations were observed and derived allele frequencies were all in Hardy-Weinberg equilibrium ($P \geq 0.05$) (Supplementary Data S1).

Pharmacokinetic modelling

Base model

Thirty patients completed the extensive PK sampling and ten patients the sparse PK sampling schedule. After PK sampling, nonlinear mixed-effects modeling (NONMEM) was used to describe the population pharmacokinetics of everolimus. Subsequently, the developed population PK model was used to estimate individual everolimus exposure. NONMEM version 7.2 (Icon Development Solutions, Ellicott City, MD, USA) was used with Piranã (version 2.9.0) as the modelling environment. Statistical software package R (version 2.15.1) was used for handling of data and plots generation. We also used NONMEM to explore the influence of different covariates on everolimus PK.

A first-order conditional estimation method with interaction (FOCE-I) was used to fit models throughout the building process. One- and two-compartment models with first-order elimination were explored. It was also assessed whether there was a change in clearance from day 1 to day 15 of treatment. Model selection was based on goodness of fit and statistical significance. An adjusted model was chosen over the original model if the drop in the objective function value (OFV) was > 3.84 ($P < 0.05$ with one degree of freedom (df), assuming χ^2-distribution).

Since the bioavailability (F) of everolimus is unknown, F was fixed at 1 and PK parameter estimates reported are proportional to F except K_a. In addition, both clearance (CI/F) and the volume of distribution (Vd/F) were allometrically scaled [12].

Covariate analysis

After the base model was determined, covariates were tested to explore the influence of bilirubin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine, body surface area (BSA) and haematocrit on CI/F. Individual effect sizes were estimated with the formula $CL/F_{typical\ value} = \theta_1 * (COV/COV_{median})^{\theta 2}$, whereby θ_1 is the population estimate for CI/F, COV the tested covariate and θ_2 the covariant effect size estimate.

The influence of SNPs and haplotypes were all tested as a covariate on CI/F, except for *ABCB1* haplotypes which were tested for an effect on F as this is physiologically more plausible. Effect sizes were estimated with the formula $CL/F_{typical\ value}$ (or F) $= \theta_1 * \theta_2^{\wedge pg1} * \theta_3^{\wedge pg2}$, whereby θ_1 is the population CI/F or F estimate in wild-type patients, θ_2 the covariate effect size of the heterozygote mutation status and θ_3 the effect size of the homozygote mutation status. The heterozygote (pg1) and homozygote (pg2) mutation status was scored as 1 if present or 0 if not present. If the genotype frequency was < 0.1, homozygote mutant and heterozygote mutant genotypes were combined (Supplementary Data S1 and S2)

All covariates were first tested for statistical significance with univariate forward inclusion into the base model (drop in OFV > 3.84, df = 1, $P < 0.05$). After inclusion of significant covariates in the intermediate model, a stepwise backward elimination procedure was performed. Covariates were remained in the final model if the threshold for statistical significance of backward elimination was reached (increase in OFV > 6.64, df = 1, $P < 0.01$).

Evaluation of model fit

Next to goodness of fit plots, a visual predictive check (VPC) was used to assess the performance of the final model by comparing the 10th and 90th percentiles of the simulated concentrations with those of the observed concentrations. In addition, a bootstrap analysis was performed to evaluate the precision of parameter estimation. Shrinkage in inter-individual variability and residual errors were automatically calculated by NONMEM.

Assessment of systemic exposure toxicity relationship

Selection of toxicities

In this study, all experienced toxicities were scored according to CTC-AE version 4.0. However, due to the number of patients included, only a limited number of toxicities were selected to be tested for an association with everolimus exposure in order to prevent false positive findings.

We choose dose reductions as the first outcome of toxicity as this is the sum of all different toxicities experienced by patients and these are also the toxicities that lead to clinical action by the treating physician. In addition, we selected stomatitis and pneumonitis as toxicity outcomes. The rationale for selection of these toxicities was based on their prevalence and the fact that these toxicities are 1) objectively measurable, 2) clinically relevant and 3) untreatable and therefore leading to dose reductions or discontinuation of therapy. Toxicities were scored as the highest grade experienced until dose reduction and if no reduction occurred until the end of study.

Table 1 Patient baseline characteristics

Characteristic	
N	40
Age (years)	63 (40 - 80)
Gender (n)	
Male	21 (52.5%)
Female	19 (47.5%)
Length (cm)	173 (154 - 189)
Weight (kg)	75 (45 - 105)
Hematology	
WBC (× 10^9/L)	7.1 (3.6 - 25)
ANC (× 10^9/L)	4.8 (2.7 - 13.0)
Platelets (× 10^9/L)	254 (147 - 995)
Hemoglobin (mmol/L)	7.5 (5.3 - 10.7)
Haematocrit	0.39 (0.29 - 0.50)
Chemistry	
AST (U/L)	22 (12 - 61)
ALT (U/L)	22 (7 - 19)
Creatinine (µmol/L)	66 (42 - 205)
Total bilirubin (µmol/L)	9 (4 - 16)
Tumor type (n)	
Differentiated	26 (65%)
Undifferentiated	7 (17.5%)
Medullary	7 (17.5%)

Data are presented as median (range) unless stated otherwise. Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; WBC, white blood count

Statistical analysis

The difference in day 15 steady-state everolimus exposure (AUC_{0-24}) between patients with and without dose reductions was tested with an unpaired t-test. The relationships between day 15 everolimus exposure and stomatitis and pneumonitis were evaluated using a four-level ordered logistic regression in SPSS version 20.0 (IBM).

Results

Patient characteristics

Forty-two adult patients with thyroid carcinoma, 22 men and 20 women were included in the phase II trial that investigated everolimus for the treatment of thyroid cancer. Of these patients, 28 (66.7%) had differentiated, 7 (16.7%) had undifferentiated (anaplastic) and 7 (16.7%) had medullary advanced thyroid carcinoma. Two patients were excluded for PK analysis; in one patient no PK samples were collected, and in the other patient no measurable everolimus levels could be detected. Patient baseline characteristics are shown in Table 1.

Pharmacokinetics

A total of 669 samples from 40 patients were used to build the population PK model. The pharmacokinetic data for everolimus were best described by a two-compartmental model with first order absorption and first order elimination from the central compartment (Supplementary Data S3). No difference in clearance over time between day 1 and day 15 of treatment was found.

Forward inclusion of BSA, creatinine, ASAT, ALAT, bilirubin and haematocrit did not improve the PK model and no association between these covariates and clearance was found. With forward inclusion of the *ABCB1* TTT and CCG haplotype the base model significantly improved (ΔOFV = -7.2 and -6.4 respectively, $P < 0.05$). The other SNPs and the *CYP2C8* haplotype did not improve the model. With multivariate backward elimination, only the presence of at least one *ABCB1* TTT haplotype remained significant (ΔOFV = 9.6, $P < 0.01$). A 21% decrease in F was observed in the presence of at least one *ABCB1* TTT haplotype. Inclusion of this covariate in the final PK model, reduced the inter-patient variability in CI/F from 38.1 to 35.1 CV%. Parameter estimates of the base and final model are shown in Table 2.

Evaluation of the final model was, next to inspection of the goodness of fit plots, done with VPC and a bootstrap procedure. Results of the VPC show that predicted and observed concentration intervals are almost identical, indicating accuracy and good predictive performance of the final model (Figure 1).

There is a small tendency for a difference between predicted and observed concentrations in the absorption part of the curve due to limited

Figure 1 Visual predictive check (VPC) of final everolimus PK model

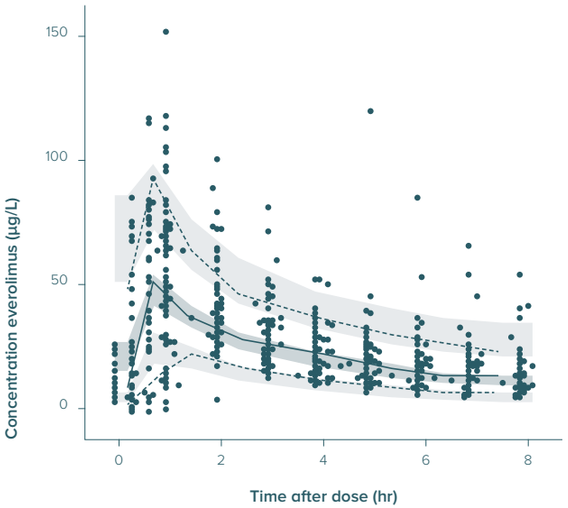


Table 2 Summary of model parameter estimates

Parameter	Base model			Final model			1000 Bootstrap runs	
	Estimate	RSE (%)	Shrinkage (%)	Estimate	RSE (%)	Shrinkage (%)	Median value	95%-CI
Cl/F (L/hr)	20.3	7.0		17.4	8.4		18.0	15.5 - 20.8
F	1	-		1	-		1	-
V_1/F (L)	29.1	18.5		25.2	17.8		25.7	18.1 - 40.4
k_a (hr^{-1})	0.643	5.3		0.647	6.2		0.653	0.583 - 0.740
Q (L/hr)	60	4.7		51.1	7.3		52.1	45.5 - 59.1
V_2 (L)	475	5.4		400	-		400	-
θ_{TTT} on F	NA	NA		0.792	6.5%		0.81	0.71 - 0.90
Inter-individual variablity								
Cl/F (CV%)	38.1%	34.4	10	35.1%	30.5	11	35.0%	22.1 - 49.1%
V1/F (CV%)	87.3%	35.7	27	86.4%	35.3	27	90.5%	53.7 - 138.9%
Inter-occasion variability								
F (CV%)	20.7%	37.7	9	19.2%	38.1	12	19.4%	12.9 - 30.5%
Residual variability								
σ (proportional error)	27.2%	20.7	7	27.3%	20.8	7	27.9%	22.6 - 32.9%

number of samples during this phase. Since we mainly used the model to estimate individual values for CL/F, the modest under-prediction of the absorption did not affect our analysis. The successful bootstrap procedure with 1000 runs is shown in Table 2. The median values for PK parameters found were within 10% of those estimated with the final model indicating that the model is precise and reliable in its parameter estimation.

Exposure-toxicity relationship

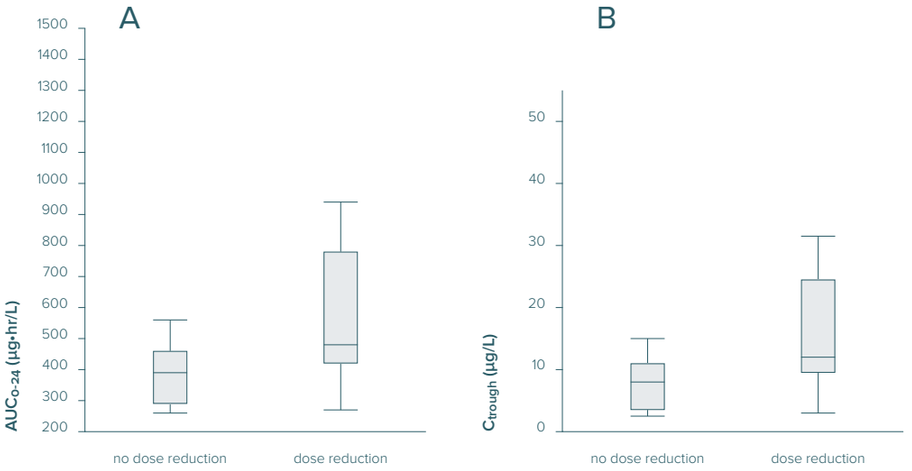
The relationships between everolimus exposure and dose reductions as well as stomatitis and pneumonitis were examined. In total, 45% of the patients had their everolimus 10 mg dose reduced to a lower dose due to toxicity (Table 3). Toxicities leading to dose reduction included stomatitis, pneumonitis, fatigue, loss of appetite, diarrhea, liver and kidney toxicity and oedema. Considering stomatitis, 42.5% of the patients experienced any grade stomatitis and 7.5% experienced grade 3 stomatitis. In addition, 10% had a non-infectious pneumonitis.

Figure 2 shows boxplots of everolimus exposures in patients with and without dose reduction. Mean $AUC_{0\text{-}24}$(SD) were 600(274) and 395(129) µg·hr/L for patients with and without dose reductions respectively. The ex-

Table 3 Dose reductions and toxicity incidence

Dose reductions	
No	22 (55%)
Yes	18 (45%)
Stomatitis	
None	23 (57.5%)
Grade 1	12 (30%)
Grade 2	2 (5%)
Grade 3	3 (7.5%)
Pneumonitis	
None	36 (90%)
Grade 1	2 (5%)
Grade 2	1 (2.5%)
Grade 3	1 (2.5%)
Reason for reduction	
Stomatitis	4 (22.2%)
Pneumonitis	4 (22.2%)
Fatigue	5 (27.8%)
Loss of appetite	1 (5.6%)
Diarrhea	1 (5.6%)
Liver toxicity	1 (5.6%)
Kidney toxicity	1 (5.6%)
Edema	1 (5.6%)

Figure 2 Boxplot of everolimus exposure in patient with and without dose reduction



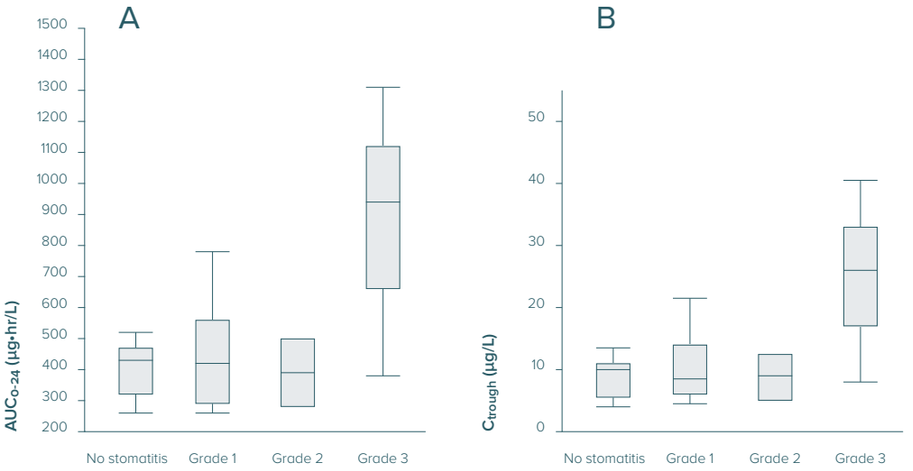
posure to everolimus was significantly different between the two groups (mean difference 204 µg·hr/L (95%-CI; -340 to -69 µg·hr/L, $P = 0.008$). Figure 3 shows boxplots of AUCs in patients experiencing different grades of stomatitis. A positive association between everolimus exposure and stomatitis was identified ($P = 0.047$). The odd ratio for stomatitis was 1.16 (95%-CI; 1.06 to 1.26) for every 50 µg·hr/L increase in $AUC_{0\text{-}24}$. Patients with grade 3 stomatitis had an everolimus exposure that was two times that of patients with ≤ 2 stomatitis (896 vs. 456 µg·hr/L, $P > 0.05$). No association of everolimus exposure with pneumonitis was found.

Discussion

This study was primarily performed to assess the correlation between everolimus exposure and toxicity. Results show that patients who had their everolimus dose reduced due to toxicity, had significantly higher drug exposures than patients without the need for dose reductions. Moreover, everolimus exposure was associated with the probability for stomatitis and patients with grade 3 stomatitis had an everolimus exposure two times that of patients with ≤ 2 stomatitis. Additionally, we found that the presence of at least one TTT allele in the *ABCB1* gene was associated with lower everolimus exposure due to decreased absorption.

A clear relationship between everolimus exposure and toxicity was established in the present analysis. These findings are in line with results from other studies in patients with cancer treated with everolimus.

Figure 3 Boxplot of CTC-AE v 4.0 severity of stomatitis versus everolimus exposure. Abbreviation: CTC-AE, Common Terminology Criteria for Adverse Events.



Previously, it has been shown that a 2-fold increase in everolimus exposure increased the risk of ≥ grade 3 pulmonary events, ≥ grade 3 stomatitis and ≥ grade 3 metabolic events with 1.9, 1.5 and 1.3-fold respectively in patients with advanced solid tumors [13]. The present analysis could not confirm the earlier identified association of everolimus exposure with pneumonitis, but this may be due to the limited number of patients with pneumonitis in our study cohort.

The present study underscores the high inter-patient variability in everolimus PK which is in line with previous observations [8]. This is also analogue to the variability in PK seen for other oral targeted therapies for the treatment of cancer such as tyrosine kinase inhibitors (TKIs). For TKIs the evidence for relationships between systemic drug exposure and efficacy or toxicity endpoints is growing [14, 15]. The currently available data suggest that an individualized dosing approach seems justified in certain circumstances and different studies support the feasibility of an individualized dosing approach for TKIs [16, 17].

In the exploration of covariates of influence on everolimus PK, the presence of at least one TTT haplotype was responsible for a decrease in everolimus exposure due to decreased absorption. Previously, the TTT haplotype has been demonstrated to be associated with enhanced function of the P-glycoprotein transporter and indeed reduced exposure or efficacy of treatment [18-20]. However, decreased function of the transporter and thus increased exposure have also been reported, as well as studies that could not show an effect [7, 21, 22]. The association we found should be

regarded as preliminary and needs further validation. If this association is confirmed, it might be argued whether a decrease in exposure of 21% can be considered as clinically relevant when taking into account the inter-patient variability in everolimus PK.

To the best of our knowledge, we are the first to describe the population pharmacokinetics of everolimus 10 mg once daily in patient with cancer. Previously, population PK models have been described, but only within the field of transplantation medicine where everolimus is used in a much lower dose. Taking this and differences in modeling into account, pharmacokinetic parameter estimates were in agreement with those previously found [7].

Everolimus exposures were assessed at day 1 and 15 of therapy and not necessarily at the time when adverse events occurred. This may be considered as a limitation and future studies should preferably measure everolimus exposure at the time that toxicity occurs. However, the variability in everolimus PK within a patient (intra-patient) is reported to be much smaller than the variability between patients [23, 24]. In addition, we observed a constant clearance of everolimus over time. While treated at the same dose (10 mg once daily), this restricts the probability for large differences between the exposures that we have measured and the actual exposures that would have been measured at the moment that toxicity occurred. In addition, the study that previously described a correlation between everolimus exposure and toxicity, found similar results with the use of C_{trough} at the time of toxicity or when C_{trough} averaged over a given time period was used [13].

The present results both underscore the correlation between everolimus exposure and toxicity as well as the high inter-patient variability in everolimus PK. These observations should be taken into account in the use of everolimus for the treatment of solid tumors. Preventing high drug exposures by dose individualization may have the potential to reduce the side effects of everolimus therapy while remaining its efficacy. However, prospective validation within oncology patients in necessary. Moreover, it has been shown that high early everolimus exposure (C_{trough} >14.1 µg/L) is associated with longer progression free survival (PFS) and overall survival (OS) (13.3 and 26.2 months vs. 3.9 and 9.9 months for PFS and OS respectively) in patients with mRCC [25]. Hence, an individualized dosing approach may also be of value for some patients with treatment inefficacy due to subtherapeutic exposures. On the other hand, in this present analysis there were also patients in need of dose reductions in whom the exposure to everolimus was not elevated. This finding suggest that a subpopulation may not benefit from dose individualization but maybe more from treatment switch if available. In summary, future studies are required to define the therapeutic window of everolimus for the treatment of different malignancies and these studies should aim to optimize

both treatment toxicity as well as efficacy outcomes possibly by using everolimus in a more individualized way.

Conclusion

In conclusion, this study shows a clear association between everolimus exposure and toxicity in patients with cancer using a newly developed population PK model. Our findings confirm observations from another study in patients with cancer and show us that everolimus is a good candidate for individualized dosing in patients with cancer.

Acknowledgement

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Supplementary data 1 Selected polymorphisms in genes involved in the absorption and metabolism of everolimus

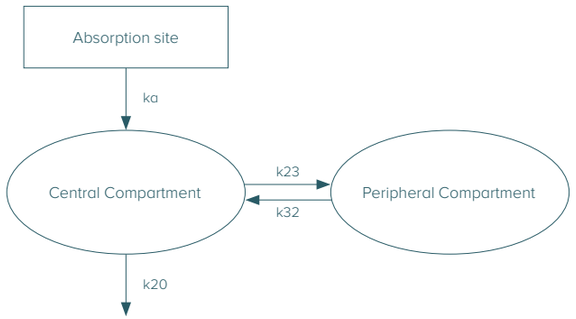
Gene	rs number	Polymorphism	Genotype	Frequency N (%)		Observed Minor Allele Frequency (%)	HWE (p-value)	Assay ID	Covariate testing
ABCB1	rs1128503	1236C>T	CC (wt)	12	(29.2)	T = 40.2%	0.09	C___7586662_10	in haploblock
			TC	25	(61.0)				
			TT	4	(9.8)				
ABCB1	rs2032582	2677G>T/A	GG (wt)	12	(30.0)	T = 41.3%	0.24	C_11711720C_30	in haploblock
			GT	23	(57.5)				
			TT	5	(12.5)				
ABCB1	rs1045642	3435T>C	TT (wt)	10	(24.4)	C = 46.3%	0.26	C___7586657_20	in haploblock
			TC	24	(58.5)				
			CC	7	(17.1)				
NR1I2	rs2276707	8055C>T	CC (wt)	30	(73.2)	T = 17.1%	0.05	C__15882324_10	CC vs. CT+TT
			CT	8	(19.5)				
			TT	3	(7.3)				
NR1I2	rs6785049	7635A>G	AA (wt)	15	(36.6)	G = 40.2%	0.82	C__29280426_10	AA vs. AG vs. GG
			AG	19	(46.3)				
			GG	7	(17.1)				
CYP3A5	rs776746	6986A>G	GG/*3*3(wt)	32	(78.0)	A = 13.4%	0.09	C__26201809_30	GG vs. AG+AA
			AG/*1*3	7	(17.1)				
			AA/*1*1	2	(4.9)				
CYP3A4	rs2246709	16090A>G	AA (wt)	19	(47.5)	G = 33.8%	0.31	C___1845287_10	AA vs. AG vs. GG
			AG	15	(37.5)				
			GG	6	(15.0)				
CYP2C8	rs7909236	-271G>T	GG (wt)	21	(51.2)	T = 25.6%	0.17	custom designed *	GG vs. GT+TT
			GT	19	(46.4)				
			TT	1	(2.4)				
CYP2C8	rs10509681	47603213T>C	TT (wt)	36	(87.8)	C = 6.1%	0.68	C__25625782_20	in haploblock
			CT	5	(12.2)				
CYP2C8	rs11572080	47631494C>T	CC(wt)	36	(87.8)	T = 6.1%	0.68	C__25625794_10	in haploblock
			TC	5	(12.2)				
CYP3A4	rs35599367	522-191C>T	CC (wt)	39	(95.1)	T = 4.9%	0.87	C_59013445_10	not tested due to too low frequency
			CT	2	(4.9)				

*custom designed assay: PCR primers, forward: 5'-GTATTGGATTGGAGCCCAGGTATTT-3', reverse: 5'-TGTTTCTCCATCATCACAGCACAT-3'; probes, VIC: AAGTCCCTGGTTGTTCCA, FAM: TCCCTGGTTTTTCCA

Supplementary data 2 Selected haploblocks in genes involved in the absorption and metabolism of everolimus

Gene	rs number	Polymorphism	Genotype	Frequency N (%)		Observed Minor Allele Frequency (%)	Covariate testing
ABCB1	rs1128503	1236C>T	other-other	23	(56.1)	TTT= 26.8%	other-other vs. TTT-other + TTT-TTT
haploblock	rs2032582	2677G>T/A	TTT-other	14	(34.1)		
	rs1045642	3435T>C	TTT-TTT	4	(9.8)		
			other-other	30	(73.2)	CTG = 14.6%	other-other vs. CTG-other + CTG-CTG
			CTG-other	10	(24.4)		
			CTG-CTG	1	2.4)		
			other-other	10	(24.4)	CCG = 43.9	other-other vs. CCG-other vs. CCG-CCG
			CCG-other	26	(63.4)		
			CCG-CCG	5	(12.2)		
CYP2C8	rs10509681	47603213T>C	CT-CT	36	(87.8)	TC = 6.1%	CT-CT vs. TC-CT
haploblock	rs11572080	47631494C>T	TC-CT	5	(12.2)		

Supplementary data 3 Schematic presentation of PK model



9
General discussion & future perspectives

Introduction

More and more progress is being made in the unraveling of cancer pathophysiology. With this increased understanding, a whole new era of rationally designed oral targeted therapies has been developed over the last one and a half decade. Both tyrosine kinase inhibitors (TKIs) and mammalian target of rapamycin (mTOR) inhibitors block the growth of cancer by interfering with specific target molecules involved in the growth, activation and differentiation of cancer cells. Therefore, they act more specific when compared to conventional therapies.

It is important to continue the development of innovative targeted therapies for the treatment of cancer. However, we should also try to optimize the treatment options that are currently available. Especially, since not all patients have the same beneficial treatment outcomes in term of efficacy when given the same therapy. Moreover, it was expected that (oral) targeted therapies would be less toxic than conventional chemotherapy due to their selective mode of action. However, still a significant number of patients experience, sometimes severe, adverse events leading to dose interruption and -reductions and even non-compliance or treatment discontinuation.

For many TKIs as well as everolimus, correlations between drug exposure and treatment outcome have been described, and the evidence for such relationships is gradually growing. This, in combination with their fixed dosing and reported high inter-patient variability in pharmacokinetics, has raised the hypothesis that dose optimization of these drugs may lead to better treatment outcomes both in terms of more efficacy and less toxicity.

The aim of this thesis was to investigate and develop dose optimization strategies of targeted therapies used in oncology, in particular for the TKIs pazopanib and sunitinib and the mTOR inhibitor everolimus.

Measurement of drug exposure

One of the goals of this thesis was to develop strategies to easily measure drug exposure. Both for the purposes of clinical research and for clinical practice, accurate and specific bioanalytical methods are necessary in order to retrieve reliable and comparable results. In the literature, different assays including liquid chromatography-tandem mass spectrometric (LC-MS/MS) and high pressure liquid chromatography -ultraviolet (HPLC-UV) assays have been described for the single quantification of most TKIs as well as everolimus [1-14]. However, for clinical practice it is more efficient to have a bioanalytical method that can quantify various TKIs within one run. We have successfully developed a sensitive LC-MS/MS method for the simultaneous determination of six TKIs (pazopanib, sunitinib, imatinib, nilotinib, dasatinib and regorafenib) and two active metabolites (N-desmethyl imatinib and N-desethyl sunitinib) in human serum or plasma as described in **chapter 3**. This multi-TKI bioanalytical assay was successfully validated according to FDA guidelines. In comparison with the existing assays that determine multiple TKIs, we were the first to incorporate pazopanib and regorafenib [15-23]. This assay has been used for the clinical pharmacokinetic studies with pazopanib and sunitinib that are described in **chapter 5** and **chapter 6** of this thesis, respectively. In addition, this method is used in routine patient care to monitor and individualize the treatment with certain TKIs.

Monitoring of drug levels in serum or plasma makes sampling by venapuncture necessary. Sampling by dried blood spot (DBS) may be a more patient friendly alternative that can be performed at home. In **chapter 4** we studied the feasibility of DBS sampling to monitor pazopanib therapy. Thus far, the measurements of imatinib, nilotinib, dasatinib and dabrafenib in DBS have been described [24, 25]. However, focus of these studies was on assay development and validation, whereas our study focused on the next step towards clinical application; the agreement between pazopanib levels measured in plasma and calculated plasma concentrations from the corresponding DBS card. Results showed that these concentrations were in good agreement with each other and thus show the feasibility of measuring pazopanib concentrations on DBS cards in a clinical setting. Since DBS cards were prepared by the research nurse, validation of DBS cards prepared by patients remains necessary. However, we do not expected major problems as previously DBS samples prepared by patients have shown to be suitable for analysis [26, 27]. With the ease and convenience of sample

collection, DBS could be very useful to measure drug exposure in patients that are treated with pazopanib in an at home setting. If monitoring and dose optimization of oral targeted therapies is becoming more widespread, DBS will potentially also be of use for the measurement of other oral anticancer drugs that are mainly used in an at home setting.

Dose Optimization Strategies

A second goal of this thesis was to investigate the feasibility of dose optimization strategies for oral targeted therapies. The use of therapeutic drug monitoring (TDM) is one of such strategies. In **chapter 5** we investigated the feasibility of TDM to reduce the inter-patient variability in pazopanib exposure. The previously unreported high intra-patient variability in PK was the main reason why this study could not show the feasibility of TDM to reduce the inter-patient variability in exposure. Pazopanib intake was standardized to the advised use of pazopanib; 1 hour before or 2 hours after the intake of food as stated in the drug label. However, in the food interaction study that showed a 2-fold increase in exposure when pazopanib was administered with food, fasted was defined as no food intake for at least 8 hours [29]. Hence, the time interval of no food in our study was possibly insufficient to prevent an effect of food on pazopanib absorption. In addition, we did not standardize diet composition. A main lesson learned from this study is that, when there is an interaction of a particular drug with food (which is present for several TKIs), one should always keep in mind the interval of no food consumption and also try to standardize the composition of meals taken by patients. This is support by the preliminary results from two ongoing studies (NCT02138526 and NCT01995981) in which a much smaller intra-patient variability is found, most likely as a consequence of standardization of pazopanib intake in relation to food.

Recently, the final data that show an exposure-response relationship for pazopanib are reported [30]. These data indicate that the optimal window for pazopanib exposure is a C_{trough} level between 20.5 to 36 mg/L. Compared to the target window that we used in our study, this concentration window is much wider. Possibly, we might have been too stringent in our study. This is supported by the (preliminary) results from a study that also investigated the feasibility of PK-guided dosing of pazopanib [31]. This study was designed to reach a target $C_{trough} > 20$ mg/L. Dose modifications were based on measured C_{trough} levels as well as the grade of toxicity experienced. Of the patients with a $C_{trough} < 20$ mg/L that experienced no grade ≥ 3 toxicity, 40% achieved an exposure above the target at week 8 of treatment after dose modification. During study follow up this percentage was further increased to 70%. These results suggest that individualized dosing of pazopanib with the aim to reach a target C_{trough} level is feasible and leads to additional patients reaching the target exposure. Yet, prospective studies that show an effect on clinical outcome in terms of overall survival (OS) and progression free survival (PFS) are needed.

Next to TDM, another approach for dose optimization is the use of a non-invasive phenotyping probe in order to predict drug exposure before the start of therapy. In **Chapter 6** the feasibility of midazolam as a phenotyping probe for CYP3A4 activity to predict sunitinib exposure is described. The results of this study show that midazolam exposure was highly correlated with sunitinib exposure. This suggests that midazolam could be useful in clinical practice to identify those patients that are at risk for under- respectively overtreatment at the standard sunitinib dosage regimen. However, the suitability of an individualized dosing strategy for sunitinib based on phenotyping by midazolam would require prospective validation.

Explaining Inter-Patient Variability

The third goal of this thesis was to gain more knowledge of the underlying causes of the inter-patient variability in dug exposure of oral targeted therapies. The results of our phenotyping study suggest that half of the observed inter-patient variability in sunitinib PK can be explained by differences in CYP3A4 activity which is much more than earlier identified covariates [32, 33]. Phenotyping with midazolam possibly explains such a large percentage of the inter-patient variability in sunitinib pharmacokinetics because it represents the influence of both genetic differences as well as environmental covariates.

Patients with GIST often have an altered anatomy of the gastrointestinal tract due to either resection of the primary tumor or subsequent surgery for recurrence and/or metastasis. Previous research has shown that imatinib and nilotinib C_{trough} levels were significantly lower in patients that previously had a major gastrectomy compared to patients without gastric surgery [34]. The suggested cause for this decreased exposure is the lack of gastric acid secretion in combination with poor solubility of imatinib and nilotinib at a pH above 5.5 and 4.5, respectively. Due to small differences in physicochemical properties, we hypothesized that a major gastrectomy would not have an influence on sunitinib exposure.

Indeed, as described in **chapter 7,** major gastrectomy alone did not influence the exposure to sunitinib or its active metabolite SU12662. We also found that patients with a combined gastrectomy and small bowel resection did have a statistically significantly decreased plasma exposure to sunitinib and its active metabolite. However, this observation was considered as clinically non relevant since exposures were still above

the threshold previously associated with sunitinib efficacy. Due to the retrospective character of our analysis, the length of intestine resected was unfortunately unknown. Theoretically, the influence of a combined gastrectomy and small bowel resection depends on the length of intestine resected and monitoring of sunitinib plasma concentrations is indicated in such situations.

The lack of an effect of major gastrectomy on sunitinib exposure, is in contrast to the results found for imatinib. This should be taken into account when treating gastrectomized GIST patients with TKIs. Hypothetically, gastrectomized patients have less and/or shorter treatment benefit from first-line imatinib therapy, when administered as a fixed dose, due to decreased imatinib plasma levels. Yet, these patients might have a high chance of benefit from second line sunitinib therapy. Another approach could be the administration of imatinib with an acidic containing beverage analogue to administration of, for example, itraconazol with coca cola when a proton pump inhibitor is used to increase exposure. This hypothesis is currently investigated in an ongoing study in the LUMC and Radboud UMC (NCT02185937). For now, depending on the type of resection of the GI tract, measuring exposure levels to imatinib, sunitinib and presumably also regorafenib could be helpful to decide whether there is sufficient exposure to these drugs.

In **chapter 8** we assessed the correlation between everolimus exposure and toxicity. Results show that patients who had their everolimus dose reduced due to toxicity, had significantly higher drug exposures than patients without reductions. Moreover, everolimus exposure was associated with the probability for stomatitis. Results were in line with findings from another study in patients with cancer [35]. The results of our study underscore the high inter-patient variability in everolimus PK as well as its correlation with toxicity. We should take this inter-patient variability, in combination with the growing evidence for a correlation between exposure and treatment efficacy and toxicity in the field of oncology, into account in the use of everolimus for the treatment of solid tumors. Future studies should first aim to clearly identify the optimum therapeutic window of everolimus exposure for different cancers. As TDM of everolimus is already the standard of care within transplantation medicine, dose individualization of everolimus in the field oncology is maybe not that far away.

Future Perspectives

Multiple opinion articles and reviews about the dose optimization of oral targeted therapies have been published in the last few years [36-48]. Next to the exposure-response relationship described in **chapter 2** which were published until February 2014, several new relationships have been shown [30, 49-52]. Furthermore, a few studies that investigated the feasibility of dose optimization to either reach a target exposure or reduce the inter-patient variability in PK have been conducted since then [31, 53-55]. Moreover, a recent study showed that about half of the plasma concentrations for imatinib, sunitinib and erlotinib in the outpatient population appear to be below their supposed target level [56]. However, thus far only one randomized controlled study that prospectively investigated the effect of imatinib TDM on clinical outcome has been published [57].

In this study, event free was defined as remaining without treatment failure, disease progression, occurrence of moderate clinical or severe laboratory adverse events or treatment discontinuation. In contrast to what was expected, this study could not demonstrate a benefit of TDM in terms of the percentage of patients that were event free. However, failure of this trial can be fully explained by the fact that adherence to dosage recommendations by prescribers was only 50% in the TDM arm. Of the patients that *did* receive the recommended TDM guided dose, 71% remained event free compared to 23% of the patients who did not or only partially received this recommended dose (absolute risk reduction 48%, $P = 0.033$). Therefore, I would like to oppose that this study actually does show us the beneficial effect of TDM to improve treatment outcome. Nevertheless, this study also highlights the challenges to prospectively investigate the benefit of TDM of targeted therapies on treatment outcome. Despite oral and written communication of dose recommendations, dose adjustments were not adhered to by the treating physician. Moreover, this study could only include 56 of the supposed 300 patients within the planned timeframe. Possibly, at present TDM does not belong as much to the culture of oncological patient care and is actually rarely used [37]. Hence, education about dose optimization within the field of oncology will become very important in the nearby future. This should lead to adequate patient recruitment and adherence to dose recommendations. Another challenge is the reimbursement by health insurances of the costs of administration of higher than registered doses of TKIs.

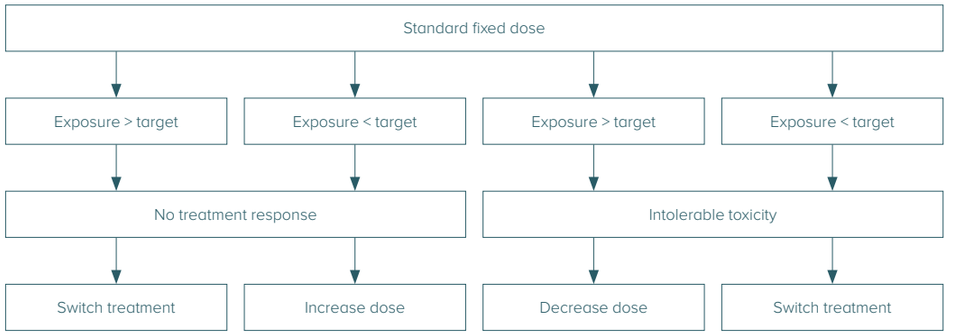
The main arguments for withholding dose optimization of oral targeted therapies from clinical practice are the lack of 1) studies that prospectively determine the relation between, or thresholds for, systemic drug exposure and treatment outcome and 2) studies that prospectively assess the influence of dose optimization on primary treatment outcome parameters both in terms of efficacy as well as toxicity. However, as long as the assessment of exposure-response relationships will not become a requirement by the regulatory agencies and the assessment of pharmacokinetics are not involved in phase III trials in which the efficacy of a new treatment is assessed, the lack of prospectively assessed correlations will remain.

As suggested by others, regulatory incentives for drug developers and healthcare providers maybe need to be put in place in order to generate forces that reward the exploration of exposure-response relationships and also dose optimization approaches [47]. At first it may seem that there are no apparent benefits for pharmaceutical companies to investigate dose optimization. However, actually it can be argued whether the failure of some clinical studies due to a lack of efficacy is possibly also a result of fixed dosing leading to under exposure of oral targeted therapies in a significant number of patients [42]. In addition, fixed dosing can lead to over exposure resulting in therapies that are effective but also extremely toxic in a significant number patients, such as everolimus and regorafenib.

Meanwhile, for oral targeted therapies that are already registered, retrospective data are the best we have and it can be discussed whether this is perhaps also good enough. In addition, it can be argued whether it is ethical to not measure since this means that we ignore the data on exposure-response relationships that we currently have. In my opinion and as also proposed by others, we should therefor just 'quit guessing and start measuring' [38]. Only by starting to measure, we can build comprehensive databases that can be used for further investigation of exposure-response relationships. Oral targeted therapies have different indications for sometimes small patient populations and exposure-response relationships should be defined separately per drug for each tumor type. Therefore, I believe that collaboration between research groups is of utmost importance. An example of such collaborations is the Dutch GIST consortium. Another novel example of such collaboration is the Dutch Pharmacology Oncology Group which aims at collaboration on dose optimization studies within oncology (www.dpog.nl).

As said, future research should focus on the added value of routine dose optimization strategies (such as TDM) of oral targeted therapies on clinical outcome to make dose optimization an evidence based approach. In my opinion, the thresholds defined by retrospective analysis could be used as target exposure for these dose optimization studies. With such an approach it can be tested at the same time within one study whether retrospectively defined correlations hold when prospectively investigated. Meanwhile, I suggest that the measurement of drug exposure is indicated in clinical practice in case of extreme or unexpected toxicity, a lack of expected clinical benefit, suspected PK drug-drug interactions, in patients with an altered anatomy of the GI tract or in case of suspected therapy nonadherence, to support clinical decision making for at least imatinib, sunitinib and pazopanib. Actually, the ESMO guideline for the diagnosis, treatment and follow-up of GIST recognizes the potential of measuring imatinib concentrations in these situations [58]. Recently, also the Dutch

Figure 1 Supposed dosing algorithm for future treatment with oral targeted therapies.
All patients start with the registered fixed dose of an oral targeted therapy. When steady-state is reached, exposures are measured. Based on exposure and clinical outcomes, the dose should be increased, decreased or a treatment switch should be made.



Association of Hospital Pharmacists published a document regarding TDM of imatinib [59]. This shows us that the dose optimization of oral targeted therapies is starting to become part of clinical practice. For targeted therapies without clearly defined thresholds, the average exposures identified in phase I or II trials on the registered dose could be used as second best alternative in specific circumstances.

Obviously, drug exposure is not the sole determinant of clinical outcome in patients with cancer and other factors such as patient- or tumor specific characteristics also contribute to the efficacy of oral targeted therapies. For different reasons, such as unnecessary toxicity, treatment delay, compliance, *de novo* inefficacy but also costs, it is crucial to identify those patients who are most likely to respond to oral targeted therapies. However, after selecting the most effective drug for a specific tumor type, dose individualization could further help to optimize the individual benefit-risk ratio, with the highest possible efficacy and the lowest possible toxicity of therapy.

In the future 'ideal, evidence based, dose optimized world', when we have more results from prospective randomized trials on dose optimization, dose adjustments and treatment switch are made according to the algorithm depicted in Figure 1. I expect that this strategy will lead to better treatment outcomes. Especially, since normally the dose of an oral targeted therapy is not increased when a patient does not show any toxic effects. However, this absence of toxicity is potentially also a sign of under exposure that could lead to treatment failure. On the other hand, in case

of toxicity the dose if often pragmatically lowered. However, for patients that already have exposures below the threshold associated with efficacy, a treatment switch to another therapy might be a better option compared to reducing the dose. I believe that in the 'ideal, evidence based, dose optimized world' drug exposure should be measured in all patients treated with oral targeted therapies. As said, this a future world and obviously trials that asses the feasibility of dose optimization on clinical outcome are warranted.

In 1892 Sir William Osler, a Canadian physician and one of the four founding professors of the Johns Hopkins Hospital, said: 'If it were not for the great variability among individuals, medicine might as well be a science, not an art'. He referred to the decisions that doctors make when prescribing medication as an art since objective data considering the benefits and harms on an individual patient level were lacking at that time. However, about one century later we should maybe reconsider these words as we are actually getting closer to a more evidence based approach for individualized treatments. This eventually should not only lead to the right drug for the right patient, but also to the administration of this drug in the right dose in order to achieve the right drug exposure level within each individual patient.

Conclusion

Future research should focus at showing the added value of dose optimization of oral targeted therapies on clinical outcome. The thresholds defined by either retrospective or future prospective analysis could be used as target exposure for these dose optimization studies Education about dose optimization within the field of oncology will become important in the nearby future both for the recruitment of trials as well as adherence to recommendations considering dosing. Meanwhile, the measurement of drug exposure seems justified in situations of extreme or unexpected toxicity, a lack of expected clinical benefit, suspected PK drug-drug interactions, in patients with major resections of the GI tract or in case of suspected therapy nonadherence, to support clinical decision making.

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Summary/Samenvatting

Both tyrosine kinase inhibitors (TKI) and mammalian target of rapamycin (mTOR) inhibitors are oral targeted therapies that are used for the treatment of a variety of malignancies. Due to the growing evidence for drug exposure-response relationships, in combination with their high inter-patient variability in pharmacokinetics (PK) and a fixed dosing regimen, it is hypothesized that dose individualization of oral targeted therapies may lead to better treatment outcomes both in terms of efficacy as well as toxicity. This thesis describes the results of different studies that investigated dose optimization strategies of oral targeted therapies used in oncology, with a focus on the TKIs pazopanib and sunitinib and the mTOR inhibitor everolimus.

In **chapter 2** an overview of the current knowledge and evidence for individualized dosing of TKIs used for the treatment of solid tumors is given. Despite limitations such as retrospective analysis, the monitoring of C_{trough} levels of imatinib, sunitinib, and pazopanib seems indicated in certain circumstances such as extreme or unexpected toxicity, a lack of clinical benefit, suspected PK drug-drug interactions or suspected therapy nonadherence.

In **chapter 3** and **4** different strategies to measure drug exposure of oral targeted therapies are investigated. In **chapter 3** the successful development and validation of a LC-MS/MS method for the simultaneous determination of six TKIs and two active metabolites in human serum or plasma is described. In comparison with the existing assays that simultaneously determine multiple TKIs, we were the first to incorporate pazopanib and regorafenib in the assay. This method has been used for the clinical studies described in **chapter 5** and **6** of this thesis and is currently also used for routine patient care.

In **chapter 4** we studied the feasibility of dried blood spot (DBS) sampling as an alternative for venous sampling to monitor pazopanib therapy in clinical practice. A good agreement between pazopanib levels measured in plasma and concentrations calculated from the corresponding DBS card was demonstrated. Although validation of DBS cards prepared by patients remains necessary, the results show that DBS could be very useful to measure drug exposure in patients that are treated with pazopanib.

In **chapters 5, 6, 7,** and **8** the causes and extent of inter-patient variability in drug exposure as well as the feasibility of different dose individualization strategies for oral targeted therapies are described. In **chapter 5** we investigated the possibility of therapeutic drug monitoring (TDM) to reduce the inter-patient variability in pazopanib exposure. In this study, the previously unknown intra-patient variability in pazopanib exposure was relatively large compared to inter-patient variability. This made it challenging to achieve a target exposure within a predefined window and this study could not show that individualized dosing results in a reduction of the inter-patient variability in pazopanib exposure. The most plausible explanation for the large intra-patient variability in PK, is the major effect of food on pazopanib absorption which should be tightly controlled. Interestingly, this study also suggested a decrease in pazopanib exposure over the studied period of 6 weeks.

In **chapter 6** the results of a phenotyping study for the dose individualization of sunitinib are described. It was hypothesized that with the use of midazolam as a phenotyping probe for CYP3A4 activity, patients that are potentially at risk for under- or overtreatment at the standard sunitinib dosage regimen could be identified before the start of therapy. The results of this study showed that midazolam exposure was highly correlated with sunitinib exposure and could explain up to 51% of the inter-patient variability in sunitinib exposure. This suggests that midazolam could be useful in clinical practice to dose individualize sunitinib therapy. However, prospective validation in a clinical study remains required.

In **chapter 7** the effect of a major gastrectomy on sunitinib exposure in patients with GIST was investigated. Our retrospective analysis across 4 phase I-III trials showed that major gastrectomy alone did not influence the exposure to sunitinib or its active metabolite. However, it was shown that patients with a combined gastrectomy and small bowel resection did have a statistically significantly decreased plasma exposure to sunitinib and its active metabolite. This change was considered clinically not relevant as exposures were still above the threshold associated with sunitinib efficacy. The lack of an effect of major gastrectomy on sunitinib exposure,

is in contrast to the results found for imatinib and should be taken into account when treating gastrectomized GIST patients with TKIs.

In **chapter 8** the correlation between everolimus exposure and toxicity was assessed in patients with thyroid cancer. It was shown that patients who had their everolimus dose reduced due to toxicity, had significantly higher drug exposure levels than patients without dose reductions. Moreover, everolimus exposure was found associated with the risk to develop stomatitis. These results suggest that the high inter-patient variability in everolimus exposure, in combination with the growing evidence for a correlation between exposure and treatment outcome should be taken into account in the treatment with everolimus of patients with cancer.

In **chapter 9** the results from the studies performed are discussed and future perspectives are described. Future research should focus on showing the added value of dose optimization of oral targeted therapies on clinical outcome. Also education about dose optimization within the field of oncology will become important in the nearby future. Meanwhile, the measurement of drug exposure seems justified in situations of extreme or unexpected toxicity, a lack of expected clinical benefit, suspected PK drug-drug interactions, in patients with major resections of the GI tract or in case of suspected therapy nonadherence, to support clinical decision making.

>

Kanker behoort wereldwijd tot een van de meest voorkomende doodsoorzaken. In Nederland kregen in 2012 ongeveer 100.000 mensen de diagnose kanker te horen. Daarnaast overleden in datzelfde jaar 42.000 mensen aan de gevolgen van kanker. Na diagnose zijn er vaak verschillende behandelingen en combinaties mogelijk voor patiënten waaronder chirurgie, radiotherapie en systemische behandeling met geneesmiddelen.

Doelgerichte Orale Antikanker Geneesmiddelen

Met de toegenomen kennis over de onderliggende ziekteprocessen van kanker, is het aantal beschikbare systemische behandelingen in de afgelopen 20 jaar enorm toegenomen. Naast de behandeling met conventionele niet-specifieke chemotherapie die aangrijpt op alle snel delende cellen, is er nu ook behandeling mogelijk met meer specifieke middelen zoals doelgerichte orale geneesmiddelen (*'oral targeted therapies'*) en monoklonale antilichamen en sinds recent ook immunotherapie. Doelgerichte orale antikanker geneesmiddelen grijpen aan op eiwitten die specifiek betrokken zijn bij de activering en coördinatie van processen van belang voor de groei en overleving van cellen. De activiteit van deze eiwitten

is normaalgesproken nauwkeurig gereguleerd. Bij verschillende soorten kanker is deze regulatie echter weggevallen en kan er dus ongecontroleerde celgroei plaatvinden. Voorbeelden van dit soort coördinerende eiwitten zijn tyrosine kinasen en de mammalian target of rapamycin (mTOR).

De ontdekking van de rol van tyrosine kinasen bij kanker heeft geleid tot de ontwikkeling van verschillende tyrosine kinase remmers (*'tyrosine kinase inhibitors', TKIs*). Sinds de registratie van imatinib in 2001, zijn er nog 22 andere TKIs geregistreerd voor de behandeling van kanker. Tevens zijn er ook remmers van het eiwit mTOR beschikbaar gekomen zoals bijvoorbeeld everolimus en temsirolimus.

Dosis Optimalisatie

Hoewel de ontwikkeling van nieuwe geneesmiddelen voor de behandeling van kanker moet worden voortgezet, is het ook van belang het effect van de huidige beschikbare geneesmiddelen te optimaliseren. Niet iedere patiënt behaalt namelijk dezelfde mate van effectiviteit met de zelfde therapie. Daarnaast ervaren veel patiënten, soms ernstige, bijwerkingen welke er voor zorgen dat de behandeling moet worden onderbroken, de dosis verlaagd of de therapie soms zelfs in zijn geheel moet worden gestopt. Dit ondanks de verwachting dat orale doelgerichte therapieën vanwege hun specifieke karakter minder bijwerkingen zouden veroorzaken.

Voor verschillende TKIs en ook everolimus zijn er relaties aangetoond tussen de blootstelling of concentratie (farmacokinetiek) van het geneesmiddel in het bloed en de effectiviteit en bijwerkingen van het geneesmiddel. Dit betekent dat wanneer de blootstelling te laag is, er een kans is dat de therapie daardoor niet effectief is. Wanneer de blootstelling te hoog is kan dit juist leiden tot (onnodige) bijwerkingen. Daarnaast is er veel variatie in de mate van blootstelling tussen patiënten. Ondanks deze verschillen in blootstelling tussen patiënten en de relaties tussen blootstelling en effect, krijgen alle patiënten standaard eenzelfde dosis bij de behandeling met orale doelgerichte therapie. De combinatie van deze eigenschappen vormt de basis voor het idee dat dosis optimalisatie mogelijk kan zorgen voor meer effectiviteit en minder bijwerkingen van deze therapieën.

Doel van het onderzoek

Het is belangrijk om de kennis over de onderliggende oorzaken van deze variatie in blootstelling tussen patiënten te vergroten. Ook is het belangrijk om methoden te ontwikkelen waarmee we gemakkelijk de geneesmiddelblootstelling in patiënten kunnen bepalen. Ten slotte is er meer onderzoek nodig naar de geschiktheid van dosis individualisatie van orale doelgerichte antikanker geneesmiddelen met als streven om de verschillen in blootstelling tussen patiënten te verkleinen.

Het doel van het onderzoek beschreven in dit proefschrift is om verschillende dosisoptimalisatie strategieën te ontwikkelen en te onderzoeken voor orale doelgerichte geneesmiddelen die binnen de oncologie worden gebruikt. De focus lag hierbij op de TKIs pazopanib en sunitinib en de mTOR-remmer everolimus.

In **hoofdstuk 2** van dit proefschrift is een overzicht gegeven van de eerdere onderzoeken die zijn gedaan naar dosis optimalisatie van TKIs die worden gebruikt voor de behandeling van solide tumoren. Hoewel deze onderzoeken een aantal beperkingen kennen, lijkt het meten en monitoren van de blootstelling aan het geneesmiddel in het bloed op dit moment voldoende onderbouwd voor de TKIs imatinib, sunitinib en pazopanib. Vooral in situaties zoals extreme of onverwachte bijwerkingen, het uitblijven van een klinisch effect, verdenking van een interactie met andere geneesmiddelen en bij verdenking van therapieontrouw lijkt het meten van bloedspiegels aangewezen.

In **hoofdstuk 3** en **4** worden verschillende strategieën onderzocht om de blootstelling in het lichaam aan TKIs te meten. In **hoofdstuk 3** wordt de ontwikkeling en validatie van een analytische bepalingsmethode voor het meten van de concentratie geneesmiddel in serum of plasma van zes verschillende TKIs en twee actieve metabolieten beschreven. Het gaat hierbij om een *liquid chromatography - tandem mass spectrometry* (LC-MS/MS) bioanalytische bepalingsmethode. In vergelijking met bestaande bepalingsmethoden die ook tegelijkertijd verschillende TKIs kunnen bepalen, hebben wij als eerste pazopanib en regorafenib aan de methode toegevoegd. Deze methode wordt gebruikt voor de onderzoeken die zijn beschreven in **hoofdstuk 5** en **6** van dit proefschrift en wordt tevens gebruikt in de dagelijkse patiëntenzorg.

Voor het bepalen van geneesmiddelconcentraties in serum of plasma is het afnemen van een bloedmonster noodzakelijk. Dit is echter niet zo patiëntvriendelijk, o.a. omdat bloedprikken soms als belastend wordt ervaren en patiënten hiervoor naar het ziekenhuis dan wel naar een dokterspost moeten komen. In **hoofdstuk 4** is er gekeken naar de geschiktheid van de droge bloedspot methode (*'dried blood spot'*, DBS) als meer patiëntvriendelijk alternatief voor het monitoren van de behandeling met pazopanib. Dit is een methode waarbij patiënten na een vingerprik een druppel bloed opvangen op een speciaal DBS kaartje. In het beschreven onderzoek werd een goede overeenkomst tussen pazopanib concentraties gemeten in plasma en de concentraties zoals berekend met behulp van de DBS aangetoond. Ondanks dat controle van de bruikbaarheid van deze methode met DBS kaarten die door patiënten zelf zijn gemaakt nog nodig is, laten onze resultaten zien dat DBS zeer waardevol

kan zijn voor het meten van de geneesmiddel blootstelling in patiënten die met pazopanib worden behandeld.

In **hoofdstuk 5, 6, 7,** en **8** worden de oorzaken en mate van variabiliteit in geneesmiddel blootstelling tussen patiënten, als ook de geschiktheid van verschillende dosis individualisatie strategieën voor orale doelgerichte antikanker geneesmiddelen beschreven.

In **hoofdstuk 5** is gekeken naar het toepassen van *therapeutic drug monitoring* (TDM) voor het verminderen van variabiliteit in pazopanib blootstelling tussen patiënten. TDM is een vorm van dosis optimalisatie waarbij de dosering van een geneesmiddel wordt aangepast op basis van gemeten geneesmiddelconcentraties. Op deze manier wordt geprobeerd een streefblootstelling (therapeutisch venster) te bereiken met zoveel mogelijk effectiviteit en zo min mogelijk toxiciteit van de therapie. Voor geneesmiddel blootstelling geldt dat deze niet alleen varieert tussen patiënten, maar ook binnen één patiënt. Dit wordt intra-patiënt variatie genoemd en ontstaat bijvoorbeeld doordat de opname van het geneesmiddel per dag beïnvloed kan worden door verschillende factoren zoals voedsel. Omdat de intra-patiënt variatie van pazopanib blootstelling onbekend was, hadden we ingeschat dat deze ongeveer de helft van de variatie tussen patiënten zou zijn, zoals ook voor andere TKIs is aangetoond. Echter, de eerder onbekende intra-patiënt variatie van pazopanib bleek achteraf relatief groot te zijn in ons onderzoek en ongeveer gelijk aan de variatie tussen patiënten. Deze grote variatie binnen een patiënt is de voornaamste reden waarom het in deze studie niet gelukt is de variatie in blootstelling tussen patiënten te reduceren. De lage biologische beschikbaarheid van pazopanib in combinatie met de invloed van voedsel op de opname van pazopanib worden als de voornaamste redenen beschouwd voor de gevonden resultaten. In dit onderzoek leek tevens een afname in pazopanib blootstelling in de tijd op te treden zoals ook voor enkele andere TKIs is aangetoond.

In **hoofdstuk 6** zijn de resultaten weergegeven van een fenotypering studie naar de dosis individualisatie van sunitinib. Hierbij is midazolam blootstelling in het bloed gebruikt als maat voor de CYP3A4 activiteit. CYP3A4 is een enzym in voornamelijk de darm en lever dat betrokken is bij het omzetten en afbreken van sunitinib in andere stoffen (metabolisme). De veronderstelling is hierbij dat iemand met veel CYP3A4 activiteit, midazolam (en dus ook sunitinib) sneller zal omzetten en dus mogelijk een hogere dosering sunitinib nodig heeft. Omgekeerd geldt dat personen met weinig CYP3A4 activiteit mogelijk een lagere dosering nodig hebben. Op deze manier kunnen voor de start van de behandeling patiënten worden geïdentificeerd die het risico lopen op onder- dan wel overbehandeling met sunitinib. Onze studie toonde aan dat de midazolam blootstelling als maat voor CYP3A4 activiteit sterk gecorreleerd was aan

sunitinib blootstelling en tot 51% van de variatie tussen patiënten in sunitinib blootstelling kon verklaren. Dit suggereert dat midazolam zinvol kan zijn voor dosis optimalisatie van sunitinib, hoewel prospectieve validatie nodig blijft.

In **hoofdstuk 7** is het effect van chirurgische verwijdering van een groot gedeelte van de maag (gastrectomie) op sunitinib blootstelling onderzocht. Imatinib, sunitinib en regorafenib zijn de TKIs die op dit moment geregistreerd zijn voor de 1e, 2e en 3e lijns behandeling van patiënten met gastro-intestinale stroma tumoren (GIST). Bij een aanzienlijk deel van de patiënten wordt (een groot gedeelte van) de maag (maagresectie) of de darmen of beiden verwijderd om zodoende de tumor te verwijderen. Na orale inname moeten TKIs eerst oplossen in het maagdarmkanaal voordat ze via de darmwand in het bloed kunnen worden opgenomen. Eerder onderzoek heeft aangetoond dat patiënten met een maagresectie, een verminderde blootstelling aan imatinib laten zien. De oorzaak hiervoor wordt gezocht in het feit dat imatinib een zure omgeving nodig heeft (de maag) om op te lossen en deze door een maagresectie ontbreekt. Voor sunitinib geldt dat deze zure omgeving iets minder van belang is voor oplossen en opname in het bloed. Dit komt door verschillen in de fysische chemische eigenschappen van sunitinib ten opzichte van imatinib. Dit is de reden dat wij geen verminderde blootstelling verwachtten in patiënten die een maagresectie hebben gehad en worden behandeld met sunitinib. Dit is retrospectief onderzocht in een grote groep patiënten die deelnamen aan verschillende fase I-III onderzoeken. De resultaten lieten zien dat patiënten die een maagresectie hadden ondergaan geen verminderde sunitinib blootstelling hadden ten opzichte van de controle groep zonder die ingreep. De resultaten lieten ook zien dat patiënten bij wie de maag samen met een gedeelte van de dunne darm was verwijderd, wel een iets verminderde blootstelling hadden. Klinisch wordt dit echter niet relevant geacht omdat de blootstelling nog steeds boven de afkapwaarde was die is gerelateerd aan effectiviteit. Het feit dat de sunitinib blootstelling niet, en imatinib blootstelling wel verminderd is in patiënten die een maagresectie hebben gehad moet in overweging worden genomen voor de behandeling van de patiënten met dit type kanker met TKIs.

In **hoofdstuk 8** is gekeken naar de correlatie tussen blootstelling van de mTOR inhibitor everolimus en toxiciteit in patiënten met schilklierkanker. De resultaten lieten zien dat patiënten bij wie de dosis everolimus verlaagd moest worden vanwege bijwerkingen aan aanzienlijk hogere blootstelling hadden dan patiënten die geen dosisreductie nodig hadden. Daarnaast was de blootstelling aan everolimus geassocieerd met de kans op stomatitis. Dit is een pijnlijke ontsteking van het mondslijmvlies welke in sommige gevallen heel ernstig kan zijn en kan leiden tot infecties, maar bijvoorbeeld ook gewichtsverlies door verminderde inname van voedsel. Stomatitis is een frequent voorkomende reden de dosering te verlagen of de behandeling te staken. De resultaten van het beschreven onderzoek suggereren dat de grote variatie in everolimus blootstelling tussen patiënten in combinatie met het groeiende bewijs voor relaties tussen everolimus blootstelling en behandeluitkomst in overweging moeten worden genomen.

In **hoofdstuk 9** van dit proefschrift worden de uitgevoerde onderzoeken bediscussieerd en in een toekomstig perspectief geplaatst. Toekomstig onderzoek moet zich richten op het aantonen van de toegevoegde waarde van dosis optimalisatie van orale doelgerichte geneesmiddelen op klinische uitkomsten. Educatie over dosis optimalisatie aan medisch oncologen zal daarbij een belangrijke plaats moeten krijgen. Totdat resultaten van genoemde onderzoeken beschikbaar komen, lijkt het gerechtvaardigd om blootstelling in patiënten te meten in situaties van extreme of onverwachte toxiciteit, het uitblijven van een klinisch effect, verdenking van interactie met andere geneesmiddelen, in patiënten die een grote resectie van het maagdarmkanaal hebben gehad of bij verdenking van therapieontrouw ter ondersteuning van het maken van klinische keuzes.

Curriculum vitae

Djoeke de Wit was born in Alkmaar, the Netherlands on January 8th of 1986. After she finished her secondary school in 2004 at the Murmellius Gymnasium in Alkmaar, she started with the study Pharmacy at the University of Utrecht. In 2011, Djoeke finished her Master's degree in Pharmacy and started working as a project pharmacist at the hospital pharmacy of the Westfriesgasthuis in Hoorn. In August 2012, she made a switch to the department of Clinical Pharmacy and Toxicology of the Leiden University Medical Center. Here, she started with her PhD on dose optimization of oral targeted therapies in oncology. This project was a collaboration between the Clinical Pharmacy and Toxicology (supervisor prof. dr. H-J. Guchelaar) and Medical Oncology (supervisor prof. dr. A.J. Gelderblom) of the Leiden University Medical Center. In January 2014, she also started with her residency in the Leiden University Medical Center to become a hospital pharmacist. After completion of her PhD thesis, Djoeke will continue with her residency which will be finished in December 2017.

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