Clinical pharmacology of the tyrosine kinase inhibitors imatinib and sunitinib

Erp, P.H. van

Clinical Pharmacology of the Tyrosine Kinase Inhibitors Imatinib and Sunitinib

Nielka van Erp

The research presented in this thesis was performed at the Department of Clinical Pharmacy and Toxicology of Leiden University Medical Center, The Netherlands.

Publication of this thesis was sponsored by:
AZL Onderzoeks- en Ontwikkelingskrediet (OOK) Apotheek
Koninklijke Nederlandse Maatschappij ter Bevordering der Pharmacie (KNMP)

Cover design and layout by: In Zicht Grafisch Ontwerp, Arnhem, www.promotie-inzicht.nl
Printed by: Ipskamp Drukkers, Enschede

ISBN 978-90-9024737-3

Clinical Pharmacology of the Tyrosine Kinase Inhibitors Imatinib and Sunitinib

Proefschrift

ter verkrijging van
de graad Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
ter verdedigen op woensdag 16 december 2009
klokke 13:45 uur

door

Petronella Hubertha van Erp

geboren te Liempde
in 1977

Promotor

Prof. Dr. H.-J. Guchelaar

Copromotor

Dr. A.J. Gelderblom

Overige leden

Prof. Dr. M. Danhof

Prof. Dr. H.M.W. Verheul, *Vrije Universiteit, Amsterdam*

Prof. Dr. J.H. Beijnen, *Universiteit Utrecht*

Dr. A. Sparreboom, *St. Jude Children's Research Hospital, Memphis, USA*

"Nothing in life is to be feared, it is only to be understood"

Marie Curie

Contents

1	General introduction	9
2	Clinical pharmacokinetics of tyrosine kinase inhibitors *Cancer Treatment Reviews 2009 (in press)*	17
Part 1	**Clinical Pharmacology of Imatinib**	**53**
3	Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib *Clinical Cancer Research 2007; 13(24):7394-7400*	55
4	Effect of cigarette smoking on pharmacokinetics, safety and efficacy of imatinib: a study based on data of the Soft Tissue and Bone Sarcoma Group of the EORTC *Clinical Cancer Research 2008; 14(24):8308-8313*	73
5	Is rectal administration of imatinib an alternative route for imatinib? *Cancer Chemotherapy and Pharmacology 2007; 60(4):623-624*	89
Part 2	**Clinical Pharmacology of Sunitinib**	**95**
6	Relationship between CYP3A4 phenotyping and sunitinib exposure in cancer patients	97
7	Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity *Journal of Clinical Oncology 2009 (published online ahead of print, August 10, 2009)*	111
8	Clinically irrelevant effect of grapefruit juice on the steady-state sunitinib exposure *Submitted*	131
9	Mitotane has a strong inducing effect on CYP3A4 activity *Submitted*	149
10	Absorption of cytochrome P450 3A4 inhibiting furanocoumarins from grapefruit juice after oral administration *Submitted*	159
Part 3	**Appendix**	**171**
11	General Discussion and Future Perspectives	173
	Summary	185
	Nederlandse samenvatting	193
	Dankwoord	203
	List of Publications	205
	Curriculum Vitae	207

1 General introduction

General introduction and scope of the thesis

Cancer is the second leading cause of death worldwide, after cardiovascular disease, accounting for 7.9 million deaths; ~ 13% of all deaths in 2007. Additionally the incidence of cancer is increasing. The five most mortal types of cancer are; lung, stomach, liver, colorectal and esophageal cancer. Over 30% of cancer can be prevented by not using tobacco, having a healthy diet, being physically active and by preventing infections that may cause cancer[1]. Once diagnosed, there are several different types of treatment ranging from resection (surgery), to radiation (radiotherapy), to systemic therapy used as adjuvant or palliative therapy. The conventional cytotoxic chemotherapeutic agents have a generic working profile that interact non-specifically with cellular DNA and/ or tubulin resulting in growth arrest of all fast growing cells. With the increased understanding of cancer biology, rational design of targeted drugs has started. Targeted drugs have antitumor activity in selected subgroups of tumors expressing proteins that are specific for the malignant phenotype[2]. The clinical use of targeted therapy started with the development of monoclonal antibodies[3]. Five years later, the first tyrosine kinase inhibitor was approved for cancer treatment. Tyrosine kinase inhibitors are a class of targeted therapy that is designed to compete with adenosine-5'-triphosphate (ATP) for the ATP-binding pocket within the intracellular domain of wild type and/or mutated tyrosine kinase receptor and thereby blocks downstream signaling important for tumor growth. Imatinib is the first rationally designed tyrosine kinase inhibitor approved in 2001 for the treatment of three Philadelphia chromosome positive leukemia subtypes[4]. Since 2001, seven additional tyrosine kinase inhibitors have been approved, all rationally designed to be active against specific tyrosine kinases. These targeted drugs tend to have a better toxicity profile than traditional cytotoxic chemotherapy that interacts non-specifically resulting in more collateral, transient damage in healthy tissues[5]. With the introduction of tyrosine kinase inhibitors a new era of treating tumors has started[6].

All tyrosine kinase inhibitors exhibit rather similar pharmacokinetic characteristics. They are all highly protein bound, have a long half life and a large volume of distribution, they are all primarily metabolized by cytochrome P450 (CYP) 3A, and predominantly excreted with the feces[7-14]. However, several pharmacokinetic aspects of these drugs are also unknown. For example, the absolute bioavailability for most tyrosine kinase inhibitors is unknown as is the clinical relevance of their interactions with (substrates for and/or inhibitors of) drug transporters on intestinal cells, hepatocytes, cancer cells and renal cells. Since these drugs are both substrates and inhibitors of their own metabolic pathways, the metabolism of these drugs at steady-state exposure is complex and unpredictable.

Therefore, the aim of this thesis is to further explore clinical pharmacological aspects of two tyrosine kinase inhibitors; imatinib and sunitinib, to better understand steady-state pharmacokinetics, clinical relevant interactions and genetic determinants that may predispose for specific side effects of these drugs.

Most information of the pharmacokinetic behavior of the tyrosine kinase inhibitors originates from preclinical studies. In addition, clinical studies have revealed important pharmacokinetic data of these drugs. An overview of the current knowledge on absorption, distribution, metabolism, elimination, drug transporter affinity and drug-drug interactions of all approved tyrosine kinase inhibitors as well as their similarities and differences will be presented in **chapter 2**.

Little information is available on the relevance of drug interactions at steady-state pharmacokinetics. According to the drug label of imatinib, CYP3A4 is the most important enzyme responsible for the metabolism. Since many clinically used drugs are known to inhibit or induce CYP3A4, imatinib is prone for drug-drug interactions. In **chapter 3** we will determine the effect of ritonavir, a potent CYP3A4 inhibitor, on the steady-state imatinib exposure (AUC). Multiple CYP enzymes, such as CYP3A4, 3A5, 2D6, 2C9, 2C19, 1A2, 1A1, are capable of metabolizing imatinib in *in vitro* experiments; however there are no data available on the influence of these minor enzymes on imatinib exposure[15]. Since we know that smoking has a pronounced effect on CYP mediated metabolism and hereby on erlotinib exposure a similar effect is hypothesized for imatinib. In **chapter 4**, the effect cigarette smoking on imatinib exposure will be studied[16].

The exact absorption-site of imatinib in the intestines is unknown. Some patients with gastrointestinal stromal tumor (GIST) may not be able to take imatinib orally, due to tumor related gastro-intestinal obstruction. Therefore, in **chapter 5** we will study imatinib pharmacokinetics in a patient after using the rectal route of administration.

Sunitinib, like all tyrosine kinase inhibitors, shows large interpatient variability in drug exposure which might affect the clinical outcome with respect to both toxicity and efficacy. In clinical practice ~ 33% of the patients need a dose interruption or a dose reduction due to drug related toxicities[17-19]. We will explore the use of a noninvasive and harmless phenotypic probe (midazolam) to determine CYP3A4 activity and thereby predict the exposure to sunitinib before starting sunitinib therapy. The results of this study will be described in **chapter 6**. Most interaction studies are performed with a single dose of the drug of interest, whereas the metabolism at steady-state can be distinctively different due to auto-inhibition of the primary metabolic pathway[20]. Some tyrosine kinase inhibitors (imatinib, dasatinib and nilotinib) appear to be both substrates and inhibitors of CYP3A4[12,21,22]. The effect of steady-state sunitinib exposure on CYP3A4 activity is also described in **chapter 6**. Additionally, we will study the association between genetic variants in genes encoding enzymes, transporters and sunitinib targets and sunitinib induced toxicities (**chapter 7**).

Since the absolute bioavailability of sunitinib is unknown, the influence of intestinal CYP3A4 activity on sunitinib exposure is unpredictable. However, in the drug label of sunitinib there is a warning for co-administration of CYP3A4 inhibitors, such as ketoconazole, clarithromycin and indinavir, but also for grapefruit juice which is a potent inhibitor of intestinal CYP3A4.

The suggested effect of grapefruit juice on steady-state sunitinib exposure will be determined (**chapter 8**). A drug-drug interaction in two patients treated with mitotane and sunitinib will be presented in **chapter 9**. In **chapter 10** a possible explanation will be presented for the pronounced effect of grapefruit juice on intestinal but absent effect on hepatic CYP3A4 in healthy volunteers.

Finally the results from these studies will be put into perspective in the general discussion (**chapter 11**).

References

1. WHO. World Health Organization - Cancer. http://www.who.int/cancer/en/ Accessed february 2009.
2. Carden CP, Banerji U, Kaye SB, Workman P, de Bono JS. From darkness to light with biomarkers in early clinical trials of cancer drugs. Clin Pharmacol Ther 2009; 85(2):131-133.
3. Houshmand P, Zlotnik A. Targeting tumor cells. Curr Opin Cell Biol 2003; 15(5):640-644.
4. Druker BJ, Talpaz M, Resta DJ et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001; 344(14):1031-1037.
5. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov 2007; 6(9):734-745.
6. Baker SD, Hu S. Pharmacokinetic considerations for new targeted therapies. Clin Pharmacol Ther 2009; 85(2):208-211.
7. Cohen MH, Williams GA, Sridhara R et al. United States Food and Drug Administration Drug Approval summary: Gefitinib (ZD1839; Iressa) tablets. Clin Cancer Res 2004; 10(4):1212-1218.
8. Cohen MH, Williams G, Johnson JR et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res 2002; 8(5):935-942.
9. Johnson JR, Cohen M, Sridhara R et al. Approval summary for erlotinib for treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of at least one prior chemotherapy regimen. Clin Cancer Res 2005; 11(18):6414-6421.
10. Kane RC, Farrell AT, Saber H et al. Sorafenib for the treatment of advanced renal cell carcinoma. Clin Cancer Res 2006; 12(24):7271-7278.
11. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
12. Brave M, Goodman V, Kaminskas E et al. Sprycel for chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant to or intolerant of imatinib mesylate. Clin Cancer Res 2008; 14(2):352-359.
13. Medina PJ, Goodin S. Lapatinib: a dual inhibitor of human epidermal growth factor receptor tyrosine kinases. Clin Ther 2008; 30(8):1426-1447.
14. Hazarika M, Jiang X, Liu Q et al. Tasigna for chronic and accelerated phase Philadelphia chromosome--positive chronic myelogenous leukemia resistant to or intolerant of imatinib. Clin Cancer Res 2008; 14(17):5325-5331.
15. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005; 44(9):879-894.
16. Hamilton M, Wolf JL, Rusk J et al. Effects of smoking on the pharmacokinetics of erlotinib. Clin Cancer Res 2006; 12(7 Pt 1):2166-2171.
17. Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006; 368(9544):1329-1338.
18. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356(2):115-124.
19. van der Veldt AAM, Boven E, Helgason HH et al. Predictive factors for severe toxicity of sunitinib in unselected patients with advanced renal cell cancer. Br J Cancer 2008; 99(2):259-265.
20. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.
21. O'Brien SG, Meinhardt P, Bond E et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. Br J Cancer 2003; 89(10):1855-1859.
22. Tanaka C, Smith T, Kantarjian H et al. Clinical pharmacokinetics (PK) of AMN107, a novel inhibitor of Bcr-Abl, in healthy subjects and patients with imatinib resistant or intolerant chronic myelogenous leukemia (CML) or replapsed/ refractory Ph+ acute lymphocytic leukemia (Ph+ALL). J Clin Oncol, 2006 ASCO Annual Meeting Proceedings Part I Vol. 24, No. 18S, 3095. 2006.

2

Clinical Pharmacokinetics of Tyrosine Kinase Inhibitors

Nielka P. van Erp, Hans Gelderblom, Henk-Jan Guchelaar

Cancer Treatment Reviews 2009 (in press)

Summary

In the recent years, eight tyrosine kinase inhibitors (TKIs) have been approved for cancer treatment and numerous are under investigation. These drugs are rationally designed to target specific tyrosine kinases that are mutated and/or over-expressed in cancer tissues. Post marketing study commitments have been made upon (accelerated) approval such as additional pharmacokinetic studies in patients with renal- or hepatic impairment, in children, additional interactions studies and studies on the relative or absolute bioavailability. Therefore, much information will emerge on the pharmacokinetic behavior of these drugs after their approval.

In the present manuscript, the pharmacokinetic characteristics; absorption, distribution, metabolism and excretion (ADME), of the available TKIs are reviewed. Results from additional studies on the effect of drug transporters and drug-drug interactions have been incorporated. In general, TKIs reach their maximum plasma levels relatively fast; have an unknown absolute bioavailability, are extensively distributed and highly protein bound. The drugs are primarily metabolized by cytochrome P450 (CYP) 3A4 with other CYP-enzymes playing a secondare role. They are predominantly excreted with the feces and only a minor fraction is eliminated with the urine. All TKIs appear to be transported by the efflux ATP binding cassette transports (ABC) B1 and G2. Additionally these drugs can inhibit some of their own metabolizing enzymes and transporters making steady-state metabolism and drug-drug interactions both complex and unpredictable.

By understanding the pharmacokinetic profile of these drugs and their similarities, factors that influence drug exposure will be better recognized and this knowledge may be used to limit sub- or supra-therapeutic drug exposure.

Introduction

In 1960, a minute chromosome, later known as the Philadelphia chromosome, was discovered in human chronic granulocytic leukemia and a causal relationship was suggested between this abnormal chromosome and the disease[1-3]. Later, a translocation between the long arm of the 22 and the long arm of the 9 chromosome was found and which was associated with an altered heavier human *c-abl* protein with tyrosine kinase activity and assumingly a growth stimulating effect[4-6]. The group of Heisterkamp et al. discovered the linkage between c-abl, positioned at chromosome 9 and the breakpoint cluster region (bcr) on chromosome 22 resulting in the bcr-abl oncogene and corresponding protein supposedly important for the generation and/ or maintenance of the disease[7-10]. Ninety-five percent of all chronic myelogenous leukemia (CML) was suggested to be the result of the altered tyrosine kinase that, under physiological conditions, is under tight control but in fusion is deregulated and expressed constitutively resulting in indefinite proliferation[11]. The involvement of protein tyrosine kinase activity in the development of tumors made them interesting targets for selective chemotherapy and thus for rational drug design. As a result the first series of low molecular weight compounds (tyrphostins) that display specificity for individual tyrosine kinase receptors were synthesized[12].

Also a novel compound (CGP57148, STI571, imatinib) was synthesized that specifically inhibits Bcr-Abl cell proliferation. It competes with ATP for the ATP binding site of the tyrosine kinases. In *in vitro* tests imatinib inhibits Bcr-Abl, c-Abl and platelet-derived growth factor receptor (PDGFR) tyrosine kinase[13, 14]. Only five years after the presentation of the *in vitro* and *animal study* data, the results of the phase I studies were presented[15-17]. Based on the results from three additional phase II studies, the drug that was rationally designed to inhibit the *Bcr-Abl* protein appeared substantially active and received accelerated approval by the FDA on the 5th of May 2001 for the treatment of three Philadelphia chromosome positive leukemia subtypes[18-20]. Additionally imatinib potentially inhibits the kinase activity of the mutated and wild-type c-kit receptor *in vitro* and an effect on malignancies that is completely or partly dependent on c-kit activity was hypothesized and confirmed[21, 22]. The phase I study, presented in 2001, showed imatinib activity in c-kit receptor positive gastrointestinal stromal tumor (GIST)[23]. On the 18th of April 2003 the registration of imatinib was extended to treatment of patients with c-KIT receptor positive unresectable and/or metastatic GISTs and was reassigned to the first line treatment of patients with CML in the chronic phase[24]. With the introduction of imatinib a whole new era of tumor treatment started, with therapy that is rationally designed and given orally on a daily basis. Since the introduction of imatinib seven additional TKIs have been approved (Table I). All TKIs are designed to compete with ATP for the ATP binding pocket of similar or different tyrosine kinases that are mutated and/or over-expressed in specific tumors.

Table I Approved tyrosine kinase inhibitors

Name	Tradename (FDA)	Registration date (FDA)	Research name	Targeted tyrosine kinases
Imatinib	Gleevec	10 May 2001	STI571	Bcr-Abl, PDGFRα, -β, c-KIT
Gefitinib	Iressa	5 May 2003	ZD1839	EGFR
Erlotinib	Tarceva	18 Nov. 2004	OSI774	EGFR
Sorafenib	Nexavar	20 Dec. 2005	BAY 43-9006	C-RAF, B-RAF, c-KIT, FLT3, VEGFR2, -3, PDGFR-β
Sunitinib	Sutent	26 Jan. 2006	SU11248	PDGFRα, -β, VEGFR1, -2, -3, c-KIT, RET, CSF-1R, FLT3
Dasatinib	Sprycel	28 June 2006	BMS354825	Bcr-Abl, SCR-family kinases, PDGFRβ, c-KIT, ephrin (EPH)receptor kinases
Lapatinib	Tykerb	13 March 2007	GW572016	EGFR (HER-1), HER-2
Nilotinib	Tasigna	29 Oct. 2007	AMN107	Bcr-Abl, c-KIT, PDGFRα, -β

Absorption

Imatinib

Imatinib is rapidly absorbed after oral administration with a peak plasma concentration at 2 hrs[25]. For imatinib, the bioavailability is surprisingly well investigated for a drug with no intravenously registered formulation. The exposure after intravenous infusion and after intake of oral capsule or solution was measured to determine the absolute bioavailability[26]. The intravenous formulation was specially made for investigational purposes and the capsule was used at the time the study was performed. The later registered tablet formulation was compared to the capsules to determine the relative bioavailability[27]. The bioavailability of imatinib is ~ 98% which is irrespective of oral formuation (solution, capsule or tablet) or dosage (100mg or 400mg)[26-28]. Imatinib absorption is not influenced by food or concomitant antacid use[29]. Long-term exposure might influence the bioavailability since imatinib inhibits efflux transporters (ABCB1 and ABCG2) and enzymes (CYP3A4 and CYP3A5) present at the intestinal wall, but conflicting data are reported on this matter[30, 31]. The exact gastrointestinal site of absorption is not known yet. In a case of a woman with short bowel syndrome only 20% of the imatinib exposure was measured indicating that absorption takes place over a longer part of the gastrointestinal tract[32]. Another case report describes the absorption from the rectum; the exposure (AUC) was approximately 40% of the orally achieved exposure indicating that absorption of the drug in the rectum takes place[33]. The interpatient variability in imatinib clearance is large ~ 40% and mainly unexplained[34].

Gefitinib

The peak plasma levels of gefitinib occur within 3-7 hrs[35]. The absolute bioavailability is ~ 60% in healthy volunteers and cancer patients[36]. Administration of a granular formulation, a dispersion of the classic tablets or administration by nasogastric tube did not significantly influence the bioavailability[37, 38]. Food has only a moderate and clinical non-significant effect on gefitinib exposure. Data of a study with 50 mg gefitinib showed a 14% decrease in AUC, another study with 250 mg of the drug showed a 37% increase in AUC after co-administration with food; this combined with the large interpatient variability (45-70%) makes the effect of food negligible[35, 36, 39].

Erlotinib

The peak plasma levels of erlotinib occur 4 hrs after dosing[40]. The bioavailability following a 150 mg dose is 100% when applying a noncompartimental approach and ~ 60% using a 2-compartiment nonlinear model[41]. The assumed nonlinearity in the compartmental approach is not confirmed by the data from the phase I dose escalation study[42]. Food increases the bioavailability to almost 100%[40]. Since the effect of food on erlotinib exposure is highly variable, the drug should be taken without food[41]. Erlotinib shows a large interpatient variability (~60%) which is unexplained yet[43].

Sorafenib

The peak plasma levels of sorafenib occurs ~3 hrs after dosing[44]. The absolute bioavailability is unknown. The relative bioavailability of tablets compared to oral solution is 38-49%[45]. Conflicting data are published on the effect of food on the pharmacokinetics of sorafenib. In the phase I studies no major effect of food was observed[46]. However, the FDA approval reports a reduction of the bioavailability of 29% when taken with food and advices to take sorafenib without food [47]. Sorafenib pharmacokinetics show a large interpatient variability[44]. The large interpatient variability is supposed to be the result of slow dissolution in the gastrointestinal tract and enterohepatic circulation[46]. The drug shows a less than proportional increase in exposure with dose escalation. The underlying reason for this nonlinearity is not known yet[46].

Sunitinib

The maximum plasma concentration of sunitinib is achieved within 6-12 hrs and the absolute bioavailability is unknown. The drug may be taken with or without food since food only has

a marginal effect on the exposure[48]. The interpatient variability is large ~40%[49]. A recent case report describes a significant decrease in sunitinib exposure (AUC) in an obese patient, which might indicate that body mass index has a pronounced effect on drug exposure and might thereby explain partly the large interpatient variability[50].

Dasatinib

The maximum plasma concentration of dasatinib is achieved within 3-5 hrs and the bioavailability in humans is unknown. A 14% AUC increase may occur in patients taking the drug with a high-fat meal, however, this effect is not supposed to be clinically significant[51]. The interpatient and inter-occasion variability is large and ranges from 32-118%. A substantial proportion of the inter-occasion variability is supposed be explained by the bioavailability[52]. The origin of the interpatient variability has not been elucidated yet.

Lapatinib

The maximum plasma concentration of lapatinib is achieved within 3-4 hrs[53]. The absolute bioavailability has not been studied. However, the bioavailability of the drug must be low since food has such an extraordinary effect on the bioavailability. The largest effect is seen with a high-fat meal, which increased the exposure of lapatinib by 325% while a low-fat meal increased the exposure by 167%[54]. Possible explanations for this pronounced effect are: 1] A delayed gastric emptying induced by food allows more time for the tablets to dissolve and/or 2] Food increases the formation of micelles by bile salts of hydrophobic substances such as lapatinib which might be of great influence on the bioavailability. Food does not influence the half life which suggests that the increased exposure is mainly caused presystemically[54]. The interpatient variability is large (68%) and not significantly reduced by the co-administration of food (52%)[54].

Nilotinib

The maximum plasma concentration of nilotinib is reached 3 hrs after oral administration[55]. The absolute bioavailability is unknown but again cannot be high since the systemic exposure is increased by 82% when the drug is given with a high fat meal compared to fasted state[56]. The interpatient variability in exposure is 32-64% and unexplained yet[57]. In the phase I dose escalation study a saturation of serum levels was observed with doses ranging from 400 – 1200mg daily. A possible explanation might be that the uptake of nilotinib is saturated at doses exceeding 400mg since a modified dose schedule to a twice-daily regimen results in an increased exposure[55].

Absorption: In summary most TKIs reach the maximum plasma concentration relatively fast (3-6 hrs) with sunitinib as the only exception (6-12 hrs) (Table II). The absolute bioavailability

Table II Pharmacokinetic parameters of the individual tyrosine kinase inhibitors

Name	F (%)	Protein binding (%)	t_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ug*hr/mL)	Vd/F (L)	Cl/F (L/hr)	C_{trough} (ng/mL)	Ref.
Imatinib	98	~95	2 - 4	18	40.1	295	11.8	1215.8	15, 26, 34
Gefitinib	60	~91	3 - 7	48	5.6	1400	35.7	60	36, 39, 153
Erlotinib	60 - 100	~93	4	36.2	26.5	232	5.3	1168	40, 41
Sorafenib	unknown	~99.5	3	25 - 48	143.4	unknown	unknown	unknown	46, 47
Sunitinib	unknown	~95	6 - 12	40 - 60	1.11	2230	34-62	44	49, 93
Dasatinib	unknown	~96	0.5 - 6	3 - 5	unknown	2505	unknown	unknown	51
Lapatinib	unknown	>99	3 - 4	24	14.3 - 36.2	>2200	unknown	300	53, 79, 154, 155
Nilotinib	unknown	~98	3	17	36.0	579	29.1	900.2	57, 156

Abbreviations: F, absolute bioavailability; T_{max}, time to peak concentration; $t_{1/2}$, elimination half-life; AUC, area under the concentration-time curve; Vd/F, apparent volume of distribution; CL/F, apparent oral clearance; C_{trough}, trough concentration

is only known for the three earliest registered TKIs (imatinib, gefitinib and erlotinib). It is remarkable that the bioavailability is not mandatory for registration since this information is used in the clinical practice to treat patients with altered gastrointestinal anatomy/physiology. TKIs are generally well soluble in acidic environment and the solubility rapidly declines above pH 4-6. A pronounced effect of food was expected for all TKIs since food can rapidly buffer gastric acid and thereby negatively influence the drug's solubility. However, food has an effect on only a few TKIs and even then in the opposite direction, indicating that other possible factors such as micelle formation or a hydrophobic vehicle (fat) are more important for the absorption of TKIs than the drug's solubility is.

The bioavailability of lapatinib and nilotinib was pronouncedly increased by food, the bioavailability of erlotinib was marginally increased, the bioavailability of gefitinib, sorafenib and dasatinib is not clinically significant increased by food and food has no effect on the bioavailability of imatinib and sunitinib. Only sorafenib and

nilotinib showed a less than proportional increase in exposure with dose escalation which could be result of multiple mechanisms e.g. saturation at the absorption site, solubility aspects and transporter interactions. This non-proportionality distinguishes them from the other TKIs and might be addressed in future research. Also the large and unexplained inter-patient variability of all TKIs warrants further research..

Distribution

Imatinib

Imatinib is extensively distributed into tissues and highly protein bound, predominantly to albumin and α1-glycoprotein (AGP), which is translated into a large volume of distribution of 435 L and a long half life of 18 hrs[26, 58-60]. Changes in the unbound drug fraction had a large effect on the intracellular drug concentration in *in vitro* experiments[61]. The role of AGP on the pharmacokinetics is underscored in *in vivo* studies, and a possible relation was suggested between imatinib-free plasma levels and the treatment efficacy[62-65]. Imatinib only penetrates in the cerebrospinal fluid (CSF) to a limited extent; ~100-fold lower levels were measured in the central nervous system (CNS) compared to plasma[61, 66-70]. This limited penetration in the CNS was confirmed in a non-human primate model. The drug appears to concentrate in the sinuses and tissues surrounding the brain[58, 71]. ABCB1 and to a lesser extent ABCG2 are suggested to strongly regulate the uptake in the CNS and malignant cells. Inhibition of ABCB1 in *in vitro* and *animal* studies resulted in a 2-10 fold increase in CNS penetration[66, 67, 69, 70]. However, the clinical relevance of the efflux transporters has to be investigated in humans. In *in vivo* and *in vitro* studies a 5-8 cell/plasma ratio was observed which indicates that imatinib is actively transported into the leukemia cells and a possible role for the organic cation transporter (OCT) 1 is hypothesized[61, 62].

Gefitinib

Gefitinib is extensively distributed into the tissues and highly protein bound (to albumin and AGP) which results in a large volume of distribution of 1400L and a long half life of 48hrs[72, 73]. The blood to plasma ratio of 0.76 suggests that the drug mainly binds to plasma proteins, with a preference for AGP, and to a lesser degree to blood cells[72]. The penetration in the CNS is poor, probably as a result of ABCB1 mediated efflux at the blood-brain barrier[73]. The drug preferably distributes into highly perfused tissues (lung, liver, kidney and gastrointestinal tract) including tumor tissues[73]. In mice bearing human tumor xenografts the tumor cell/plasma ratio was 11-fold as was the skin/plasma ratio which points into the direction of active transport into specific tissues[74].

Erlotinib

Erlotinib and gefitinib have a common chemical backbone structure and are distributed very similarly in the human body. Erlotinib is also extensively protein bound, predominantly to albumin and AGP, has a long half life of 36.2 hrs and an accompanying large volume of distribution of 232 L[40]. AGP concentration and steady state exposure (AUC) are tightly linked[43]. AGP together with total bilirubin and smoking status were the most important factors affecting the drug clearance[75]. The penetration of erlotinib in the CNS is poor, with CNS levels that represent ~7% of the plasma exposure[76].

Sorafenib

The volume of distribution of sorafenib is not reported. However, since the drug is highly protein bound (~99.5%) and has a long half life of 25-48hrs, a large volume of distribution is expected[47].

Sunitinib

Sunitinib has a large volume of distribution of 2230 L and is highly (95%) protein bound. The half life of the drug is 40-60 hrs[49].

Dasatinib

Dasatinib is extensively distributed in the extravascular space and is highly protein bound (~94%) which results in a large volume of distribution of 2505 L and a half life of 3-5 hrs[77]. The distribution between plasma and blood cells was equal in *in vitro* experiments[77]. The brain penetration is poor. In three patients the CSF: plasma ratios ranged from 0.05-0.28. However, dasatinib appears to be more potent against CNS tumors than imatinib which might be the result of a much greater potency (325-fold) along with the low amount of proteins in the CNS resulting in a relatively large fraction of unbound drug[78].

Lapatinib

The volume of distribution of the terminal phase of lapatinib is >2200 L and the half life is 24 hrs. The drug is highly protein bound (> 99%) to albumin and AGP[79]. *Rat* and *mouse* studies demonstrated a very limited penetration of the drug in the CNS which was increased with 40-fold in ABCB1/ ABCG2 knockout mice though single transporter knockout mice have only limited effect on the CNS penetration[80,81]. The translation of the results of these animal studies to human remains difficult and therefore additional studies in humans are warranted.

Nilotinib

The volume of distribution of nilotinib is not reported. Although the high level of protein binding (98%) and the long half life (~17 hrs) suggest that the volume of distribution is presumably large.

Distribution: In summary TKIs are extensively distributed into tissues and are highly protein bound, resulting in a large volume of distribution and a long terminal half life (Table II). The volume of distribution, the affinity for specific plasma proteins and the CNS penetration is not reported for all TKIs yet. However, since the TKIs share multiple pharmacokinetic characteristics, parallels might be drawn between the TKIs. Especially, the influence of AGP on the pharmacokinetics and efficacy of TKIs might be interesting, since TKIs are preferably bound to this plasma protein and AGP is often elevated in cancer patients and could therefore interfere with an effective treatment.

Metabolism

Imatinib

Imatinib is primarily metabolized through CYP3A4 and CYP3A5 with CYP2D6, CYP2C9, CYP2C19 and CYP1A2 playing a minor role[28, 82-84] . A recent study identified two extrahepatic enzymes (CYP1A1 and CYP1B1) and the flavin-containing monooxygenase 3 (FMO3) enzyme as being capable of extensively metabolizing the drug[83]. Additionally, imatinib can inhibit CYP3A4 and CYP2D6 metabolism[34, 84, 85]. Patients carrying a polymorphism in CYP2D6 (*4 allele) show a reduced apparent clearance indicating that CYP2D6 appears to be important *in vivo* in the metabolism of imatinib[86]. The clinical relevance of these enzymes at steady-state pharmacokinetics, under auto inhibition of metabolic pathways, is mainly unsolved and needs to be addressed in additional studies. The main metabolite is CGP74588 which represents approximately 10% of the imatinib AUC and has similar potency *in vitro*[25].

Gefitinib

In vitro studies indicate that gefitinib is metabolized by CYP3A4, CYP3A5, CYP2D6 and by the extrahepatic enzyme CYP1A1[39, 87]. The drug inhibits CYP2C19 and CYP2D6 although the clinical relevance is questioned[88]. The main metabolite is the O-desmethyl derivate (M523595) which is present at concentrations similar to gefitinib and is formed through CYP2D6 metabolism[87, 89]. M523595 and gefitinib have similar potency against epithelial growth factor receptor (EGFR) tyrosine kinase activity in isolated enzyme assays. However, the metabolite has lower activity in a cell based assay due to the poor penetration into the cell and is therefore unlikely to contribute significantly to the therapeutic activity[74]. In CYP2D6 poor metabolizers a higher exposure to gefitinib was observed compared to the extensive metabolizers. Additionally, M523595 was undetectable in poor metabolizers. CYP3A4 activity and CYP3A5 polymorphisms did not explain the large interindividual variability[89]. *In vitro* studies claim that CYP3A4 is the most prominent enzyme in gefitinib metabolism though conflicting data are presented[73, 87, 88]. However *in vivo* data suggests that besides CYP3A4 also CYP2D6 activity has a significant influence on the exposure[89, 90].

Erlotinib

The overall metabolism of erlotinib, and formation of O-desmethyl-erlotinib (OSI-420), is predominantly through CYP3A4 and CYP3A5 and to a lesser extent by CYP1A2 and the extrahepatic isoform CYP1A1 and CYP1B1, with only a minor role for CYP2D6 and CYP2C8[40, 75, 87, 91]. However, induction of the enzymes CYP1A2 and CYP1A1 has a pronounced effect on the drug exposure, indicating that both enzymes might have a more prominent role in the *in vivo* erlotinib metabolism as suggested by the *in vitro* results[91]. Erlotinib is a moderate pregnane X receptor (PXR) inducer and strongly induces CYP3A4 mRNA levels, although the formation of 1-hydroxymidazolam is decreased in *in vitro* experiment showing the potency of erlotinib to inhibit CYP3A4 metabolism[92]. Conflicting data are published on the effect the drug has on CYP3A4 metabolism[87].

Sorafenib

Oxidative metabolism of sorafenib is mediated by CYP3A4, additionally the drug is glucuronidated by UDP glucuronosyltransferase (UGT) 1A9[47]. Around 50% is eliminated in the unchanged form which is either the result of poor metabolism capacity or the result of a low fraction of the drug that is absorbed from the intestines.

Sunitinib

Sunitinib is primarily metabolized by CYP3A4 to produce its primary active metabolite SU12662 which is further metabolized by CYP3A4 into inactive metabolites[93]. Data on additional enzymes involved in the metabolism are lacking.

Dasatinib

Dasatinib is extensively metabolized and thus relatively small amount of unchanged drug is excreted[94]. Dasatinib is primarily metabolized by CYP3A4 to produce its pharmacologically active metabolites; M4, M5, M6, M20 and M24 that represent around 5% of the parent compound AUC. Flavin-containing mono-oxygenase 3 (FMO-3) and UGT are also involved in the formation drug metabolites[51]. *In vitro* data demonstrate that multiple CYP isoforms (e.g. CYP1A1, 1B1 and 3A5) are capable of metabolizing dasatinib, however the relevance of these other CYP-enzymes *in vivo* requires further investigation[95].

Lapatinib

In vitro studies indicate that lapatinib is primarily metabolized to oxidation products by CYP3A4, 3A5, 2C19 and 2C8[79]. The major enzyme is CYP3A4 which accounts for approximately 70% of the metabolism. One metabolite GW690006 remains active against EGFR however it has lost activity against HER2, whereas other metabolites appear to be inactive[79]. Lapatinib is an inhibitor of CYP3A4 and CYP2C8 and may therefore interact with substrates of these

enzymes; additional studies to investigate this effect are ongoing[79].

Nilotinib

Nilotinib is mainly metabolized through CYP3A4. *In vitro* data demonstrate that the drug is a competitive inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2D6 and UGT1A1. Additional *in vitro* data show that nilotinib may induce CYP2B6, CYP2C8 and CYP2C9[57]. *In vivo* data have been presented on the clinical relevance of CYP3A4 inhibition in an interaction study with midazolam and for UGT1A1 in a genetic polymorphism study describing an increased risk of nilotinib induced hyperbilirubinemia for the UGT1A1 *28 genotype[56,96].

Metabolism: In summary all TKIs are metabolized in a very similar way (Table III, Figure I). All TKIs are primarily metabolized by CYP3A4, with other CYP-enzymes and for some TKIs (sorafenib, dasatinib) UGT playing only a minor role. The enzymes that show affinity are mostly identified in *in vitro* experiments, whereas the clinical effects of the major enzymes is typically investigated in *in vivo* interaction studies in healthy volunteers. The clinical relevance of the involvement of minor enzymes is largely unsolved at the time of registration and needs to be addressed in additional studies after registration. Several TKIs (imatinib, gefitinib, lapatinib and nilotinib) are inhibitors of enzymes by which they are primarily metabolized themselves, this could alter their metabolism substantially upon multidose use at steady-state. There is little insight in the steady-state metabolism at this point, which is surprising since these drugs are used on a daily basis. Some TKIs (erlotinib, sorafenib, sunitinib and dasatinib) are thought to have no effect on CYP-enzyme activity which might be the result of a lack of data rather than an absent effect. Additional research to investigate the effect of these drugs on CYP-enzyme activity is needed.

Excretion

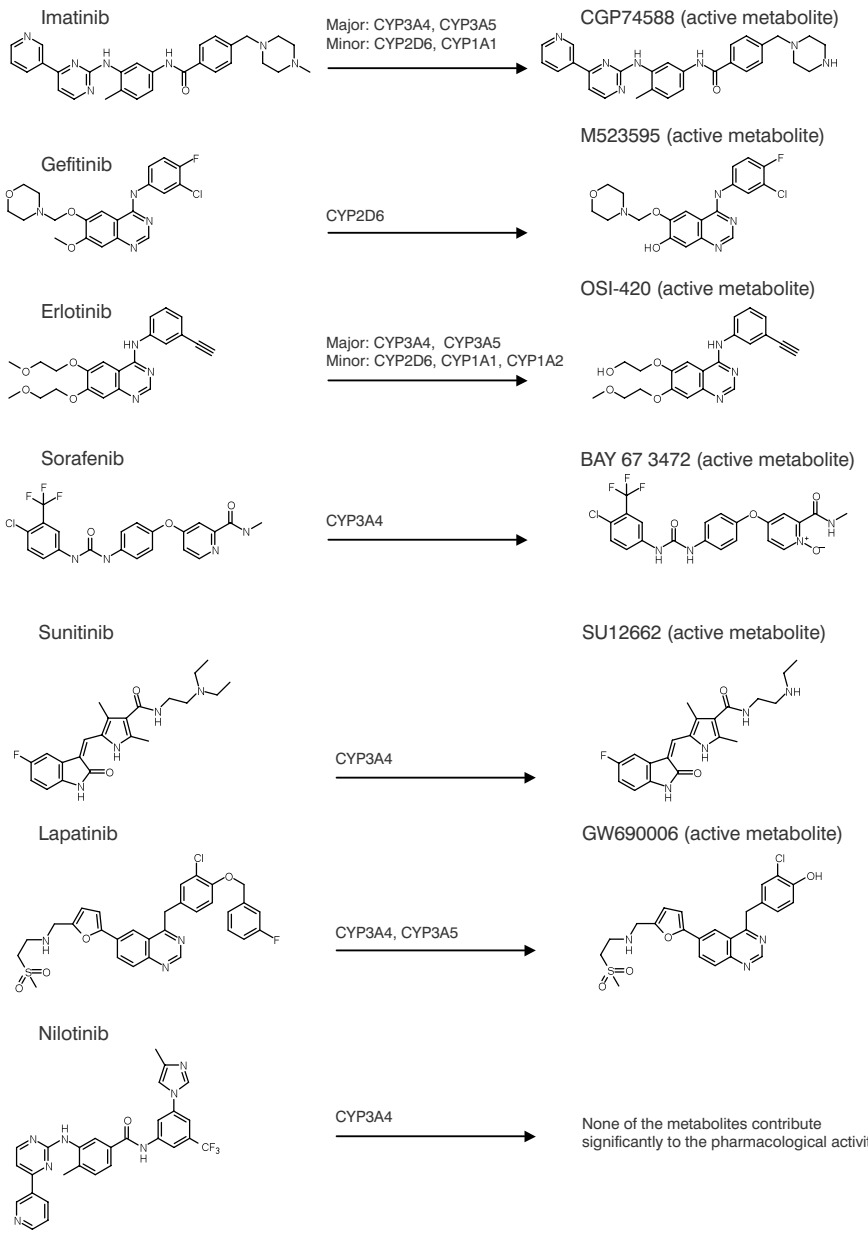
Imatinib

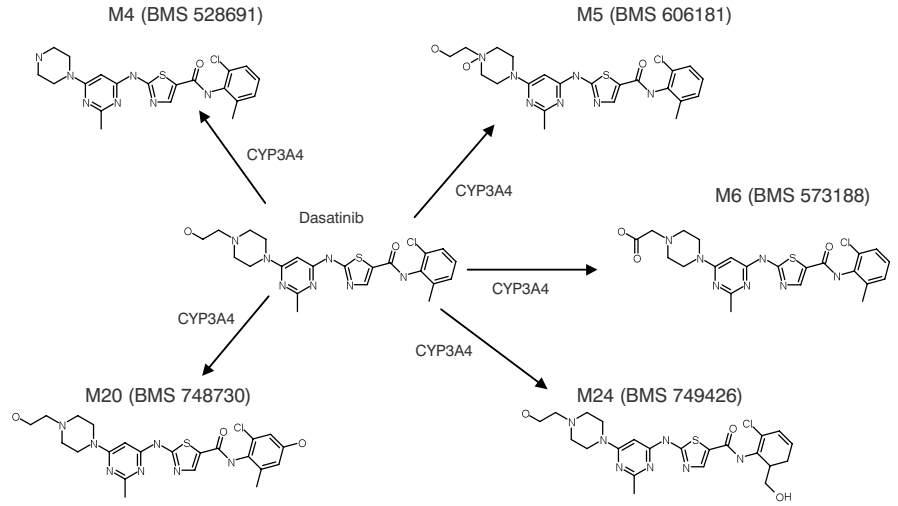
Imatinib is mainly eliminated trough the liver. The kidneys only excrete a minimal amount of the drug and its metabolites. At this point there is still a discussion ongoing whether the apparent clearance increases, decreases or remains the same at steady-state[97-100]. However, a decrease in clearance seems more plausible since imatinib is capable of inhibiting its own metabolic pathways. Of a single dose imatinib in healthy volunteers 81% of the dose was recovered in urine (13.2%; 5% as unchanged imatinib) and feces (67.8%; 23% as unchanged imatinib) in 7 days[25]. This suggests that the drug clearance will more likely be affected by hepatic impairment than by renal dysfunction[61]. Surprisingly, two independent groups found that renal impairment has a pronounced effect on imatinib pharmacokinetics[62,101]. In contrary

Table III enzymes and transporters involved in the pharmacokinetics

Name	Enzymes Phase I - oxidation	Enzymes Phase II - conjugation	Efflux transporters	ref
Imatinib	Major: CYP3A4 and CYP3A5 Minor: CYP1A2, CYP2D6, CYP2C9, CYP2C19, CYP1A1, CYP1B1	Minor: FMO3	ABCB1, ABCG2, ABCC4 Suggested: OCT1, OATP1B3 and OCTN2	28, 117, 120, 122
Gefitinib	Major: CYP3A4, CYP3A5, CYP2D6 & CYP1A1		ABCG2 Suggested: ABCB1	39, 123
Erlotinib	Major: CYP3A4 and CYP3A5, Minor CYP1A1, CYP1A2, CYP2C8, CYP2D6		ABCB1, ABCG2	40, 87, 127
Sorafenib	CYP3A4	UGT1A9		47
Sunitinib	CYP3A4		ABCB1, ABCG2	49, 130
Dasatinib	Major: CYP3A4	Minor FMO-3, UGT	ABCB1, ABCG2 Suggested: OCT1	51, 77, 132
Lapatinib	Major: CYP3A4, CYP3A5 Minor CYP2C19, CYP2C8		ABCB1, ABCG2	79, 80
Nilotinib	CYP3A4		ABCB1, ABCG2	57, 134

Figure I Tyrosine kinase inhibitors with their active metabolites





The tyrosine kinase inhibitors with only their active metabolites are demonstrated. The enzymes involved according to literature are presented, possible other enzymes involved in the formation of the metabolite are absent.

a case study in an end-stage renal function patient claims no effect on the pharmacokinetics, however the clearance in this patient was significantly reduced compared to patients with normal renal function[102]. Two possible explanations for this apparent discrepancy were put forward: a correlation between renal failure and AGP levels and an effect of elevated levels of uremic toxins in renal failure on the organic anion transporting polypeptide (OATP) 1B3 and hereby influencing the hepatic elimination[101, 103, 104]. Moreover, there was no effect observed of mild and moderate liver dysfunction on the pharmacokinetics of imatinib and CGP74588 in three independent studies[105-107]. Severe liver dysfunction resulted in elevated drug exposure levels[106]. Renal and hepatic impairment is no reason for abstaining patients from imatinib treatment though patients with moderate renal failure should start at a 50% decreased dose and patients with severe liver dysfunction are advised to start with a 25% dose reduction[24].

Gefitinib

About 90% of gefitinib is recovered in feces (86%) and urine (0.5%) over 10 days indicating that renal excretion is not a major route of elimination[35]. Surprisingly, in patients with moderate and severe elevated liver tests the pharmacokinetics was not altered. No data are available on the influence of renal impairment on the pharmacokinetics[39].

Erlotinib

Following a 100 mg oral dose of erlotinib, 91% of the dose was recovered over 11 days: 83% in feces and 8% in urine of which 1% and 0.3% as parent drug respectively[108]. No data are available regarding the influence of hepatic dysfunction and/or hepatic metastases and renal dysfunction on the drug pharmacokinetics[40].

Sorafenib

Sorafenib is eliminated primarily through the liver. Of a 100mg dose 77% is excreted with the feces and 19% is excreted as glucuronidated metabolites in the urine[47]. Approximately 50% of an oral dose is recovered as unchanged drug in the feces, due to either inefficient metabolism or lack of absorption[47]. Mild to moderate hepatic impairment does not significantly alter the exposure. Sorafenib pharmacokinetics has only recently been studied in patients with severe hepatic and renal impairment[109]. After a single dose of 400mg no significant alterations were observed in drug and metabolite AUC regardless of the severity of renal or hepatic impairment. However, only patients with normal or mild hepatic and renal dysfunction tolerated (without experiencing dose limiting toxicities) a dose of 400mg twice daily at steady state. Patients with moderate renal en hepatic dysfunction needed a dose reduction of 50%, while patients with severe hepatic impairment did not tolerate sorafenib. Patients with very severe hepatic and renal dysfunction only tolerated 200mg once daily, no explanation for the discrepancy between the tolerance in severe and very severe hepatic impairment is provided[109]. This recent study provides valuable information since sorafenib is used for the treatment of patients with hepatocellular carcinoma that is often accompanied by severe hepatic impairment.

Sunitinib

Sunitinib is primarily eliminated with the feces (61%), with renal elimination accounting for only 16% of the administered dose. There are no studies on the pharmacokinetics in patients with serious hepatic or renal insufficiency. However, in pharmacokinetic studies where also the creatinine clearance was assessed, there appeared to be no pharmacokinetic alterations in volunteers with a wide range of creatinine clearances[110]. Additionally in a case report describing two hemodialyzed patients on sunitinib therapy the plasma concentration of the drug and its major metabolite at steady-state were comparable to patients with normal renal function[111].

Dasatinib

Dasatinib is mainly excreted via feces, 85% of which 19% as intact drug. Urine excretion is around 4% of which <1% as unchanged dasatinib[51, 112]. No data are available on the effect of hepatic and renal impairment on dasatinib pharmacokinetics[51].

Lapatinib

Lapitinib is primarily eliminated hepatically, with 27% of the oral dose recovered in the feces and <2% recovered in the urine[79]. It is suggested that a large part of the oral dose remains in the intestines and is not absorbed which may contribute to the most prevalent dose limiting toxicity diarrhea. Indeed, diarrhea showed no relation to serum levels of lapatinib[113]. In patients with severe hepatic impairment the AUC of lapatinib was increased by > 60% and the half life was ~3-fold increased compared to patients with normal hepatic function[79]. No data are available on the influence of severe renal impairment.

Nilotinib

Nilotinib recovery was assessed over 7 days after a single dose and showed 4.4% of the drug being recovered in urine and 93.5% in feces (69% unchanged nilotinib). A large amount (31%) of unchanged nilotinib excreted via the feces was suggested to be the result of unabsorbed drug[114]. Nilotinib pharmacokinetics has not been studied in patients with hepatic or renal impairment, however the drug label warns for the possible risk of giving nilotinib to patients with hepatic impairment[57].

Excretion: In summary all TKIs are predominantly excreted via the feces and only a minor fraction is eliminated with the urine. The fraction of unchanged drug in the feces can vary widely among the TKIs. Large fraction of unchanged drug in the feces can either be the result of a relatively large fraction that is not absorbed and directly eliminated or by a low efficient metabolism. Without data on the absolute bioavailability or the time frame of the fecal elimination it is difficult to distinguish between both mechanisms. Data on the effects of mild, moderate or severe renal and hepatic impairment on the pharmacokinetics of TKIs are mainly absent. For the few TKIs where the effect is studied some unexpected results are observed. Mild to moderate hepatic impairment did not affect the pharmacokinetics of imatinib and gefitinib whereas severe hepatic impairment did affect the pharmacokinetics of imatinib and lapatinib and did not affect the pharmacokinetics of gefitinib. Surprisingly, mild to moderate renal impairment did affect the pharmacokinetics of imatinib pharmacokinetics. Since the patients treated with these drugs are at risk to develop renal or hepatic impairment at any stage of their disease it is necessary that more data become available on the possible influence of these impairments on the pharmacokinetics of these drugs.

Drug transporters

The ABCB1 (P-glycoprotein; P-gp), ABCC1 (multidrug resistance-associated protein; MRP1) and ABCG2 (breast cancer resistance protein; MXR) are efflux transporters and are now

recognized to have an important role in the absorption, distribution, excretion and toxicity of xenobiotics[115]. Also the solute carrier family (SLC) transporters, which are influx transporters, are receiving more attention although their effect on drug kinetics is less well established at this point[116]. Members of the SLC family are the solute carrier OATP, solute carrier peptide transporter family (PepT1), and organic zwitterion/cation transporters (OCTNs)[116]. Also for the disposition of TKIs efflux and influx transporters are gaining interest.

Imatinib

The high bioavailability of imatinib, a substrate for multiple CYP enzymes (especially CYP3A4 and CYP3A5), and also for ABCB1, ABCG2 with ambiguous affinity for SLC transporters, is remarkable and can only be explained by a low hepatic extraction and low efficient transport of imatinib by the efflux transporters[115, 117-119]. Although conflicting results have been published, imatinib is most likely a substrate and an inhibitor of ABCB1 and ABCG2[120]. The *ABCG2 421C/A* polymorphism is associated with a reduced clearance in humans[65]. A recent study in 90 CML patients showed a pronounced effect of *ABCB1 1236C/T* and *2677G/T* polymorphisms on trough drug levels and an corresponding clinical effect (major molecular response)[121]. However, additional studies are necessary to conclusively determine the role of ABC-transporters on imatinib pharmacokinetics and efficacy. There appears to be a modest role for the organic cation transporter 1 (OCT1) as observed in *in vitro* experiments. OAT1, OAT3 and OCT2 do not transport imatinib *in vitro* which is consistent with their presence on the kidneys and the relative low renal clearance[117, 122]. OATP1B3 and OCTN2 appeared to have affinity for the drug, however the in vivo relevance is not yet studied [Oostendorp RL The role of Organic Cation Transporter 1 and 2 in the in vivo pharmacokinetics of imatinib Submitted]. Since the precise role of the transporters on imatinib disposition and the effect of transporter inhibition by the drug is not completely understood, no additional warnings have been added to the drug label. However, alertness is necessary for possible drug interactions on drug transporter level. Moreover, the highly polymorphic transporters might explain at least in part the large interpatient variability.

Gefitinib

Gefitinib also interacts with ABCG2 and to a lesser extend with ABCB1[123]. In *in vitro* experiments the drug appeared to reverse ABCG2 mediated resistance by inhibiting ABCG2 at relatively high drug concentrations[123-125]. It is a substrate of ABCG2 in *in vitro* experiments at clinically relevant drug concentration. Additionally patients carrying the *ABCG2 421C/A* polymorphism have higher gefitinib exposure and more diarrhea compared to those carrying the wild-type *ABCG2* genotype[125, 126]. No association was found between the *ABCB1 3435 C/T* genotype and gefitinib pharmacokinetics[125].

Erlotinib

In *in vitro* experiments erlotinib was shown to be a substrate for ABCB1 and ABCG2 but not for ABCC2. In mice studies the absence of ABCB1 and ABCG2 significantly affected the oral bioavailability[127]. Erlotinib also inhibits the ABCB1 and ABCG2 drug efflux function[128]. In a recent study in humans the *ABCG2 -15622C/T* and *1143C/T* polymorphisms, resulting in a reduced expression of the transporter, were associated with increased AUC and Cmax[129].

Sorafenib

The role of transporters on the disposition of sorafenib is yet unknown.

Sunitinib

Recently, an *in vitro* study demonstrated that sunitinib is a high affinity inhibitor of ABCG2 and inhibits ABCB1, albeit more weakly. Moreover, the drug is also a substrate of both transporters[130]. The bioavailability might therefore be affected by polymorphisms in the genes encoding for these transporters but this needs to be addressed in clinical studies[130].

Dasatinib

In vitro data demonstrated that dasatinib is a substrate of ABCB1 and ABCG2 but not a potent inhibitor of these transporters[77, 131, 132]. Additional *in vitro* studies suggested that the drug is also a substrate for hOCT1 however the uptake is much less hOCT1 dependent compared to imatinib. Inhibitors of hOCT1 did not interfere with the uptake of dasatanib and it is hypothesized that the uptake *in vivo* is more likely driven by diffusion than by active transport[131, 132].

Lapatinib

Results from *in vitro* studies indicated that lapatinib is a substrate and an inhibitor of the efflux transporters ABCB1, ABCG2 and solely an inhibitor of OATP1B1[80]. It has the potency to reverse the ABCB1 and ABCG2 driven resistance on multi drug resistant cells *in vitro*[133]. In addition, lapatinib did not inhibit nor was a substrate of OAT, OCT and uric acid transporter (URAT) transporters which is in line with the marginal renal clearance of the drug[80]. Further studies in humans are warranted to further clarify the role of transporters on the efficacy, disposition, toxicity and drug interactions[80].

Nilotinib

Nilotinib appears to be a substrate and an inhibitor of ABCB1 and ABCG2, however the clinical relevance of these *in vitro* assessments need to be addressed[57, 134].

Drug transporters: In summary all TKIs are substrates and inhibitors of ABCB1 and ABCG2, except for dasatinib which appears to be no inhibitor of these transporters and for sorafenib of which no data are available. Additionally, imatinib and dasatinib might interact with OCT1. No data are available on the affinity of the other TKIs for the SLC transporter family. Multiple *in vitro* studies have been published on the effect TKIs have on drug transporters and visa versa. At this point the clinical significance of polymorphic transporters and interactions between drugs on transporters are mainly undefined.

Drug-drug interactions

Imatinib

The drug label of imatinib warns for co-administration of potent CYP3A4 inhibitors and for co-administration of substrates of CYP3A4 with a narrow therapeutic window. This warning makes the clinical practice difficult since a large group of drugs is either a substrate or an inhibitor of CYP3A4. The inhibitory effect of the drug on CYP3A4 was investigated by an interaction study with simvastatin. Simvastatin clearance was reduced by 70% indicating a clinically relevant strong CYP3A4 inhibitory effect[85]. Contrary results are presented in interaction studies with CYP3A4 inhibitors after a single dose (ketoconazole) and at steady-state (ritonavir)[84, 135]. CYP3A4 inducers (rifampicin, St. John's wort, phenytoin and enzyme inducing anti-epileptic drugs (EIAED's)) very constantly show a decrease in imatinib exposure[15, 100, 136-139]. Administration of the drug together with metoprolol, a CYP2D6 substrate, resulted in an increase in metoprolol exposure of 23% with moderate differences between the intermediate and extensive metabolizers[140]. Smoking does not alter imatinib exposure, indicating no major contribution of CYP1A2 in the metabolism of the drug[141]. An interaction between imatinib and warfarin is hypothesized since both increases and decreases in INR have been reported after starting therapy. Warfarin is a substrate of CYP2C9 and CYP3A4 and both enzymes are involved in the metabolism of imatinib[28]. Interactions through the other enzymes are hypothesized and warned for in the drug label but not yet investigated. Also the effect of drug transporter inhibitors (e.g. pantoprazol, cyclosporine) on the disposition of the drug in humans is not evolved yet and neither is the influence of imatinib on drugs that are transported by ABCB1 (e.g. digoxine) or ABCG2 (e.g. nitrofurantoin). The cellular uptake of nilotinib is enhanced by the co-administration of imatinib due to ABCB1 and possibly ABCG2 inhibition in *in vitro* studies[142]. The drug label does not include warnings with regard to risks related to drug transporter interactions though alertness is on its place.

Gefitinib

Inhibitors and inducers of CYP3A4 interfere with gefitinib exposure. Itraconazole elevated the exposure (AUC) with 78%. Concomitant administration of rifampicin reduced the AUC with 83%[88]. In *in vitro* experiments gefitinib stimulates midazolam metabolism through CYP3A4. An explanation for this observation is not provided yet[87]. High doses of ranitidine, inducing a gastric pH > 5.0, resulted in a decreased gefitinib AUC[143]. Gefitinib co-administration resulted in a 35% increase in metoprolol exposure indicating that the drug is a CYP2D6 inhibitor at therapeutic levels[88]. In a case report the possible interaction between herbal medicines (e.g. ginseng) and gefitinib is suggested since interruption of the herbal medicine treatment turned the patient from a non-responder into a responder. Unfortunately, in this case the gefitinib plasma levels were not measured[144]. Surprisingly, sorafenib reduced the AUC of gefitinib by 38%, where gefitinib has no effect on sorafenib exposure[145]. There is no explanation for this observation.

Erlotinib

Inhibitors and inducers of CYP3A4 interfere with erlotinib exposure. Co-administration of rifampicin results in a 67% decreased drug exposure (AUC)[146]. Ketoconazole increases the drug exposure ($AUC_{0-\infty}$) and C_{max} with 86% and 102% respectively[147].

Co-administration of BAS 100, a substance in grapefruit juice, resulted in a 2.1 fold increase in the AUC of erlotinib in mice, most likely due to an increased uptake by inhibiting CYP3A4 or ABCB1[148]. Smoking results in a decreased erlotinib AUC (35.9%) possibly by inducing CYP1A1 and CYP1A2 metabolism[91]. The maximum tolerated dose in smokers was 300 mg compared to 150 mg in non-smokers. Additionally the steady-state trough levels and incidence of rash and diarrhea in smokers at 300mg were similar as the data for non smokers receiving 150mg erlotinib[149].

Sorafenib

Since sorafenib is metabolized by CYP3A4, an interaction with CYP3A4 inhibitors was expected. In a drug interaction study with ketoconazole and sorafenib, ketoconazole did not alter the exposure. However it did decrease the plasma concentration of sorafenib-N-oxide which is formed through CYP3A4. This finding is consistent with an earlier mass-balance study showing that 15% of the administered dose was eliminated by glucuronidation where only 5% was eliminated as oxidative metabolites[150]. Co-administration of sorafenib with gefitinib causes an decrease in gefitinib exposure of 38%. The interaction can not be mediated through CYP3A4 inhibition since sorafenib does not influence the exposure of midazolam which is suggested to be solely metabolized through CYP3A4[145]. *In vitro* data demonstrated that sorafenib is a competitive inhibitor of CYP2C19, CYP2D6 and CYP3A4 although the inhibitory potency does not appear in clinical studies where the drug was given concomitantly with midazolam (CYP3A4 substrate), dextromethorphan (CYP2D6 substrate) or omeprazol (CYP2C19 substrate)[45, 47]. Sorafenib is also a competitive inhibitor of CYP2B6 and CYP2C8 though the clinical relevance of this inhibition is not studied yet[151]. The hypothetical effect

on CYP2C9 was indirectly measured by the effect on warfarin therapy. The INR in sorafenib treated patients was similar in placebo treated patients. The effect of CYP3A4 inducers (e.g. rifampicin) is not studied, however a warning is included in the drug label of sorafenib[47]. The drug does interfere with the pharmacokinetics of concomitantly administered antineoplastic agents doxorubicin and irinotecan. The exposure of doxorubicin was increased by 21%. The increase in SN-38 exposure was 67-120% most likely through competition or inhibition of UGT1A1 and additionally the irinotecan exposure increased with 26-41%[47].

Sunitinib

Since sunitinib is primarily metabolized through CYP3A4, the influence of ketoconazole and rifampicin was investigated in healthy volunteers. Co-administration of ketoconazole increased the cumulative exposure of sunitinib and SU12662 with ~ 50%. Rifampicin coadministration resulted in a 50% decrease in combined systemic exposure[110]. It is suggested that sunitinib has no influence on other co-administered drugs.

Dasatinib

The exposure of dasatinib is increased five fold on the co-ingestion of ketoconazole in healthy volunteers. Rifampicin decreased the exposure by 82%. *In vitro* data demonstrated that the drug does not induce human CYP-enzymes. It however does appear to be a time dependent CYP3A4 inhibitor. As a result, the co-ingestion of dasatinib with simvastatin (a CYP3A4 substrate) resulted in a 20% increased exposure to simvastatin. The solubility of dasatinib appears to be pH dependent. Famotidine reduced the exposure by 61%, the co-administration of agents that provide prolonged gastric acid suppression is therefore not recommended[51].

Lapatinib

Lapatinib is a substrate and an inhibitor of CYP3A4 and an inhibitor of CYP2C8. A single dose of a CYP3A4 inhibitor (ketoconazole) increases the exposure by 3.6-fold. In contrast carbamazepine, a CYP3A4 inducer, decreases the exposure by ~75%[79]. An extrapolation is made to other CYP3A4 inhibitors and inducers although no clinical data are available on these interactions. Dose adjustment advices are given in the package insert when combining lapatinib with an inducer or an inhibitor. These advices are not tested in clinical setting and/or on steady-state lapatinib exposure.

In the combination of lapatinib with folinic acid, 5-fluorouracil and irinotecan the AUC of SN-38, the active metabolite of irinotecan, was increased by 41%. There are multiple suggested explanations for this interaction. Lapatinib showed inhibition of CYP3A4, OATP1B1, ABCB1 and ABCG2 *in vitro* which are enzymes and transporters important in the metabolism and disposition of SN-38. However, further investigation is needed to determine whether

one or all mechanisms are responsible for this *in vivo* interaction[152]. In the combination of lapatinib with paclitaxel the exposure of lapatinib as well as paclitaxel was increased with 21% and 23% respectively[79]. The mechanism behind the described interactions is yet unknown. Since in vitro data suggest that lapatinib is an inhibitor of ABCB1, alertness may be warranted when the drug is co-administered with ABCB1 substrates[79]. However, no clinical studies are available to confirm this interaction.

Nilotinib

Nilotinib is mainly metabolized through CYP3A4. The concomitant administration of ketoconazole with nilotinib produces a 3-fold increase in systemic exposure. Nilotinib itself appears to be a weak inhibitor of CYP3A4[56]. The co-administration of midazolam with the drug in healthy volunteers resulted in a 30% increase in the systemic exposure of midazolam[56].

Drug-drug interactions: In summary most clinical interaction studies investigate interactions in healthy volunteers with a single dose of the TKI given together with a CYP3A4 inhibitor (e.g. ketoconazole, itraconazole) or a single dose of the TKI given after a few days of CYP3A4 inducing therapy (e.g. rifampicin, carbamazepine) (Table IV, V). This study design might not represent the metabolism at steady-state pharmacokinetics, since most TKIs are capable of inhibiting at least partly the enzymes by which they are metabolized. Auto-inhibition could result in the shunting of the metabolism through less prominent metabolic pathways at steady-state. Therefore, interaction studies performed at steady-state pharmacokinetics are more informative and representative for the clinical relevance of the investigated enzymes. Fortunately, the FDA is becoming stricter and demands additional research at steady-state for the newer TKIs (lapatinib, nilotinib) that have potency to inhibit enzymes and transporters.

Table IV Drugs that interact with tyrosine kinase exposure

Name	Inducing drug	Inhibitory drug	Effect observed	ref
Imatinib	Phenytoin		Decreased imatinib AUC	15
	Rifampicin		Decreased imatinib C_{max} (54%) and $AUC_{24\&\infty}$ (68%; 74%)	136
	St. John's Wort		Increased Cl/F (44%) of imatinib	137
	Enzyme-inducing antiepileptic drug (carbamazepine, oxcarbazepine, phenytoin, phenobarbital or primidone)		Decreased C_{max}, AUC, Tmax and $t_{1/2}$ Increased Cl/F	100
		Ketoconazole	Decreased clearance (28.6%) and increased Cmax (26%) and $AUC_{24\&\infty}$ (40%; 40%)	135
		Cyclosporin	Increased exposure (2-10%) of imatinib	28
		Elacridar*	Brain penetration increases, increased systemic exposure	67, 157
		Pantoprazol*	Brain penetration increases, increased systemic exposure	157
		Valspodar*	Brain penetration increases	67
		Zosuquidar*	Brain penetration increases	67
Gefitinib	Rifampicin		Decreased gefitinib AUC (83%)	88
		Itraconazol	Increased gefitinib AUC (78%)	88
		Sorafenib	Increased gefitinib AUC (38%)	145
	High dose ranitidine		Decreased gefitinib exposure	143
Erlotinib	Rifampicin		Decreased erlotinib AUC (67%)	146
	Smoking		Decreased erlotinib AUC, C_{max} and C_{trough}	91
	Phenytoin		Highest Cl/F and lowest AUC observed in patient treated with phenytoin	43
		Ketoconazole	Increased erlotinib AUC (86%) and Cmax (102%)	147
		BAS 100*	2.1-fold increase in erlotinib AUC	148
Sorafenib	-			
Sunitinib	Rifampicin		Decreased combined AUC (sunitinib + metabolite) (46.5%)	49
		Ketoconazole	Increased combined AUC (sunitinib + metabolite) (51%)	49
Dasatinib	Rifampicin		Decreased dasatinib AUC (82%)	51
		Ketoconazole	Increased dasatinib AUC (5-fold)	51
	Famotidine		Decreased dasatinib exposure (61%)	51
Lapatinib	Carbamazepine		Decreased lapatinib AUC (75%)	79
		Ketoconazole	Increased lapatinib AUC (3.6-fold)	79
		Paclitaxel	Increased lapatinib AUC (21%)	79
Nilotinib	Rifampicin		Decreased nilotinib AUC (80%)	57
		Ketoconazole	Increased niltoinib AUC (3-fold)	56

Abbreviations: C_{max}, peak concentration; AUC, area under the concentration-time curve; CL/F, apparent oral clearance; T_{max}, time to peak concentration; $t_{1/2}$, elimination half-life; C_{trough}, trough concentration

* Results from animal studies

Table V Effect of tyrosine kinases on the exposure of co-administered drug

Name	Drug	Effect observed	ref
Imatinib	Simvastatin	Decreased Cl/F (70%) of simvastatin	85
	Cyclosporin	Increased C_{max} and AUC (20-23%) of cyclosporin	28
	Warfarin	Increased and decreased international normalized ratio (INR)	158
	Nilotinib	Increased intracellular uptake in *in vitro* study of nilotinib under influence of imatinib	142
	Metoprolol	Increased AUC (17% IM; 24% EM)	140
	Methotrexate	Decreased elimination	159
Gefitinib	Metoprolol	Increased AUC (35%)	88
	Warfarin	Elevations and/or bleeding events have been reported	143
	SN-38 *in vitro*	Increased uptake in initially resistant cell lines	124
	Topotecan *in vitro*	Increased uptake in initially resistant cell lines	124, 160
	Mitoxantrone *in vitro*	Increased uptake in initially resistant cell lines	124, 161
	Paclitaxel *in vitro*	Increased uptake in initially resistant cell lines	160
	Doxorubicin *in vitro*	Increased uptake in initially resistant cell lines	160
Erlotinib	Warfarin	Elevations and/or bleeding events have been reported	162
Sorafenib	Doxorubicin	Increases doxorubicin AUC (21%)	47
	Irinotecan & SN-38	Increases SN-38 AUC (67-120%) and irinotecan AUC (26-41%)	47
	Gefitinib	Increased gefitinib AUC (38%)	145
Sunitinib	-		
Dasatinib	Simvastatin	Increased simvastatin exposure (20%)	51
Lapatinib	Paclitaxel	Increased paclitaxel exposure (23%)	79
	SN-38	Increased SN-38 exposure (40%)	152
Nilotinib	Midazolam	Increased midazolam exposure (30%)	56

Abbreviations: C_{max}, peak concentration; AUC, area under the concentration-time curve; CL/F, apparent oral clearance

Table VI Effect of renal and hepatic impairment on the pharmacokinetics of tyrosine kinase inhibitors

Name	Hepatic impairment			Renal impairment			ref
	Mild	Moderate	Severe/ Very severe	Mild	Moderate	Severe/ Very severe	
Imatinib	No effect	No effect	Effect: Increased imatinib & CGP74588 exposure Advice: start with 300 mg/day	Effect: increased imatinib exposure Advice: no dose adjustment	Effect: increased imatinib exposure Advice: start with 200 mg/day	Unknown	101, 106
Gefitinib	No effect	No effect	No effect	Unknown	Unknown	Unknown	39
Erlotinib	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	40
Sorafenib	No effect	Effect: DLT observed at reduced dose Advice: start with 200mg bid	Effect: DLT observed at reduced dose. No dose advice possible due to conflicting data	No effect	Effect: DLT observed at reduced dose Advice: start with 200mg bid	Effect: DLT observed at reduced dose Advice: start with 200mg daily	109
Sunitinib	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown Effect: case report described no PK effect in hemodialyzed patients	110, 111
Dasatinib	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	51
Lapatinib	Unknown	No effect	Effect: 60% increase in lapatinib exposure Advice: start with 750mg/ day	Unknown	Unknown	Unknown	79
Nilotinib	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	57

Conclusions

TKIs are a relatively new and fast growing group of anticancer drugs developed as oral formulations and administered on a daily basis. In general, these TKIs are substrates of several drug transporters and metabolizing enzymes. Some of them are also capable to inhibit drug transporters and enzymes making their disposition and metabolism at steady-state pharmacokinetics rather complex and unpredictable. Most of the available pharmacokinetic information is based on information obtained from *in vitro* experiments, animal studies, drug-drug interaction studies and mass balance studies in healthy volunteers with a single dose of the aimed TKI. However, it is difficult to translate the results of these studies to the clinical oncology practice where these drugs are administered on a daily basis with possible auto-inhibiting mechanisms significantly altering the pharmacokinetics outcomes as well as the relevance of claimed drug interactions. Most information is available for the TKIs that are used for the longest time in clinical practice. A question that arises is whether the knowledge obtained for one TKI should not be used for the rational design of studies with the other TKIs and whether translations between these drugs are possible when confronted with unexpected low or high drug exposure.

In this review the current knowledge on the pharmacokinetic aspects; ADME, drug transporters and drug-drug interactions of the individual TKIs are described. Similarities and differences between the TKIs are summarized. It appears that several pharmacokinetic aspects are unfortunately not investigated yet for these drugs. While awaiting the results the only way to anticipate on clinical features and drug interaction potential in the clinical practice is by translating the knowledge obtained from the other TKIs as described in this review.

References

1. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960; 132:1497.
2. Rudkin CT, Hungerford DA, Nowell PC. DNA contents of chromosome PH1 and chromosome 21 in human chronic granulocytic leukemia. Science 1964; 144:1229-1231.
3. A proposed standard system of nomenclature of human mitotic chromosomes. Lancet 1960; 1(7133):1063-1065.
4. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243(5405):290-293.
5. Mayall BH, Carrano AV, Moore DH, Rowley JD. Quantification by DNA-based cytophotometry of the 9q+/22q-chromosomal translocation associated with chronic myelogenous leukemia. Cancer Res 1977; 37(10):3590-3593.
6. Konopka JB, Watanabe SM, Witte ON. An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell 1984; 37(3):1035-1042.
7. Heisterkamp N, Stam K, Groffen J, De KA, Grosveld G. Structural organization of the bcr gene and its role in the Ph' translocation. Nature 1985; 315(6022):758-761.
8. Stam K, Heisterkamp N, Grosveld G et al. Evidence of a new chimeric bcr/c-abl mRNA in patients with chronic myelocytic leukemia and the Philadelphia chromosome. N Engl J Med 1985; 313(23):1429-1433.
9. Grosveld G, Hermans A, De KA, Bootsma D, Heisterkamp N, Groffen J. The role of the Philadelphia translocation in chronic myelocytic leukemia. Ann N Y Acad Sci 1987; 511:262-269.
10. Naldini L, Stacchini A, Cirillo DM, Aglietta M, Gavosto F, Comoglio PM. Phosphotyrosine antibodies identify the p210c-abl tyrosine kinase and proteins phosphorylated on tyrosine in human chronic myelogenous leukemia cells. Mol Cell Biol 1986; 6(5):1803-1811.
11. Comoglio PM, Di Renzo MF, Gaudino G. Protein tyrosine kinases associated with human malignancies. Ann N Y Acad Sci 1987; 511:256-261.
12. Yaish P, Gazit A, Gilon C, Levitzki A. Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. Science 1988; 242(4880):933-935.
13. Buchdunger E, Zimmermann J, Mett H et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 1996; 56(1):100-104.
14. Druker BJ, Tamura S, Buchdunger E et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 1996; 2(5):561-566.
15. Peng B, Hayes M, Resta D et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. J Clin Oncol 2004; 22(5):935-942.
16. Druker BJ, Talpaz M, Resta DJ et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001; 344(14):1031-1037.
17. Druker BJ, Sawyers CL, Kantarjian H et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001; 344(14):1038-1042.
18. Sawyers CL, Hochhaus A, Feldman E et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 2002; 99(10):3530-3539.
19. Kantarjian H, Sawyers C, Hochhaus A et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 2002; 346(9):645-652.
20. Talpaz M, Silver RT, Druker BJ et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 2002; 99(6):1928-1937.
21. Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA, Zigler AJ. Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood 2000; 96(3):925-932.
22. Tuveson DA, Willis NA, Jacks T et al. STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene 2001; 20(36):5054-5058.
23. van Oosterom AT, Judson I, Verweij J et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 2001; 358(9291):1421-1423.
24. FDA. Drug label Gleevec approved on 05/20/2003. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ Accessed on 01/19/2009.
25. Gschwind HP, Pfaar U, Waldmeier F et al. Metabolism and disposition of imatinib mesylate in healthy volunteers. Drug Metab Dispos 2005; 33(10):1503-1512.
26. Peng B, Dutreix C, Mehring G et al. Absolute bioavailability of imatinib (Glivec) orally versus intravenous infusion. J Clin Pharmacol 2004; 44(2):158-162.
27. Nikolova Z, Peng B, Hubert M et al. Bioequivalence, safety, and tolerability of imatinib tablets compared with capsules. Cancer Chemother Pharmacol 2004; 53(5):433-438.
28. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005;

44(9):879-894.
29. Sparano BA, Egorin MJ, Parise RA et al. Effect of antacid on imatinib absorption. Cancer Chemother Pharmacol 2009; 63(3):525-528.
30. Gurney H, Wong M, Balleine RL et al. Imatinib disposition and ABCB1 (MDR1, P-glycoprotein) genotype. Clin Pharmacol Ther 2007; 82(1):33-40.
31. Burger H, Nooter K. Pharmacokinetic resistance to imatinib mesylate: role of the ABC drug pumps ABCG2 (BCRP) and ABCB1 (MDR1) in the oral bioavailability of imatinib. Cell Cycle 2004; 3(12):1502-1505.
32. Beumer JH, Natale JJ, Lagattuta TF, Raptis A, Egorin MJ. Disposition of imatinib and its metabolite CGP74588 in a patient with chronic myelogenous leukemia and short-bowel syndrome. Pharmacotherapy 2006; 26(7):903-907.
33. van Erp NP, Oostendorp RL, Guchelaar HJ, Schellens JH, Gelderblom H. Is rectal administration an alternative route for imatinib? Cancer Chemother Pharmacol 2007; 60(4):623-624.
34. Cohen MH, Williams G, Johnson JR et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res 2002; 8(5):935-942.
35. Swaisland H, Laight A, Stafford L et al. Pharmacokinetics and tolerability of the orally active selective epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 in healthy volunteers. Clin Pharmacokinet 2001; 40(4):297-306.
36. Swaisland HC, Smith RP, Laight A et al. Single-dose clinical pharmacokinetic studies of gefitinib. Clin Pharmacokinet 2005; 44(11):1165-1177.
37. Cantarini MV, McFarquhar T, Smith RP, Bailey C, Marshall AL. Relative bioavailability and safety profile of gefitinib administered as a tablet or as a dispersion preparation via drink or nasogastric tube: results of a randomized, open-label, three-period crossover study in healthy volunteers. Clin Ther 2004; 26(10):1630-1636.
38. Cantarini MV, Bailey CJ, Collins B, Smith RP. The relative bioavailability of gefitinib administered by granular formulation. Cancer Chemother Pharmacol 2008; 62(2):203-208.
39. Cohen MH, Williams GA, Sridhara R et al. United States Food and Drug Administration Drug Approval summary: Gefitinib (ZD1839; Iressa) tablets. Clin Cancer Res 2004; 10(4):1212-1218.
40. Johnson JR, Cohen M, Sridhara R et al. Approval summary for erlotinib for treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of at least one prior chemotherapy regimen. Clin Cancer Res 2005; 11(18):6414-6421.
41. Frohna P, Lu J, Eppler S et al. Evaluation of the absolute oral bioavailability and bioequivalence of erlotinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in a randomized, crossover study in healthy subjects. J Clin Pharmacol 2006; 46(3):282-290.
42. Hidalgo M, Siu LL, Nemunaitis J et al. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 2001; 19(13):3267-3279.
43. Tan AR, Yang X, Hewitt SM et al. Evaluation of biologic end points and pharmacokinetics in patients with metastatic breast cancer after treatment with erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor. J Clin Oncol 2004; 22(15):3080-3090.
44. Moore M, Hirte HW, Siu L et al. Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. Ann Oncol 2005; 16(10):1688-1694.
45. FDA. Drug label Nexavar approved 12/20/2005. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ Accessed on 02/03/2009.
46. Strumberg D, Richly H, Hilger RA et al. Phase I clinical and pharmacokinetic study of the Novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. J Clin Oncol 2005; 23(5):965-972.
47. Kane RC, Farrell AT, Saber H et al. Sorafenib for the treatment of advanced renal cell carcinoma. Clin Cancer Res 2006; 12(24):7271-7278.
48. Bello CL, Sherman L, Zhou J et al. Effect of food on the pharmacokinetics of sunitinib malate (SU11248), a multi-targeted receptor tyrosine kinase inhibitor: results from a phase I study in healthy subjects. Anticancer Drugs 2006; 17(3):353-358.
49. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
50. Desar IM, Burger DM, Van Hoesel QG, Beijnen JH, Van Herpen CM, Van der Graaf WT. Pharmacokinetics of sunitinib in an obese patient with a GIST. Ann Oncol 2009; 20(3):599-600.
51. Brave M, Goodman V, Kaminskas E et al. Sprycel for chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant to or intolerant of imatinib mesylate. Clin Cancer Res 2008; 14(2):352-359.
52. Dai G, Pfister M, Blackwood-Chirchir A, Roy A. Importance of characterizing determinants of variability in exposure: application to dasatinib in subjects with chronic myeloid leukemia. J Clin Pharmacol 2008; 48(11):1254-1269.
53. Burris HA, III, Hurwitz HI, Dees EC et al. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. J Clin Oncol 2005; 23(23):5305-5313.
54. Koch KM, Reddy NJ, Cohen RB et al. Effects of Food on the Relative Bioavailability of Lapatinib in Cancer Patients. J Clin Oncol 2009; 27(8):1191-1196.
55. Kantarjian H, Giles F, Wunderle L et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 2006; 354(24):2542-2551.
56. Tanaka C, Smith T, Kantarjian H et al. Clinical pharmacokinetics (PK) of AMN107, a novel inhibitor of Bcr-Abl, in healthy subjects and patients with imatinib resistant or intolerant chronic myelogenous leukemia (CML) or replapsed/refractory Ph+ acute lymphocytic leukemia (Ph+ALL). J.Clin.Oncol., 2006 ASCO Annual Meeting Proceedings Part I Vol. 24, No. 18S, 3095.
57. Hazarika M, Jiang X, Liu Q et al. Tasigna for chronic and accelerated phase Philadelphia chromosome--positive chronic myelogenous leukemia resistant to or intolerant of imatinib. Clin Cancer Res 2008; 14(17):5325-5331.
58. Kil KE, Ding YS, Lin KS et al. Synthesis and positron emission tomography studies of carbon-11-labeled imatinib (Gleevec). Nucl Med Biol 2007; 34(2):153-163.
59. Gambacorti-Passerini C, Barni R, le CP et al. Role of alpha1 acid glycoprotein in the in vivo resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. J Natl Cancer Inst 2000; 92(20):1641-1650.
60. Gambacorti-Passerini C, Zucchetti M, Russo D et al. Alpha1 acid glycoprotein binds to imatinib (STI571) and substantially alters its pharmacokinetics in chronic myeloid leukemia patients. Clin Cancer Res 2003; 9(2):625-632.
61. le Coutre P, Kreuzer KA, Pursche S et al. Pharmacokinetics and cellular uptake of imatinib and its main metabolite CGP74588. Cancer Chemother Pharmacol 2004; 53(4):313-323.
62. Widmer N, Decosterd LA, Csajka C et al. Population pharmacokinetics of imatinib and the role of alpha-acid glycoprotein. Br J Clin Pharmacol 2006; 62(1):97-112.
63. Widmer N, Decosterd LA, Leyvraz S et al. Relationship of imatinib-free plasma levels and target genotype with efficacy and tolerability. Br J Cancer 2008; 98(10):1633-1640.
64. Delbaldo C, Chatelut E, Re M et al. Pharmacokinetic-pharmacodynamic relationships of imatinib and its main metabolite in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 2006; 12(20 Pt 1):6073-6078.
65. Petain A, Kattygnarath D, Azard J et al. Population pharmacokinetics and pharmacogenetics of imatinib in children and adults. Clin Cancer Res 2008; 14(21):7102-7109.
66. Dai H, Marbach P, Lemaire M, Hayes M, Elmquist WF. Distribution of STI-571 to the brain is limited by P-glycoprotein-mediated efflux. J Pharmacol Exp Ther 2003; 304(3):1085-1092.
67. Bihorel S, Camenisch G, Lemaire M, Scherrmann JM. Modulation of the brain distribution of imatinib and its metabolites in mice by valspodar, zosuquidar and elacridar. Pharm Res 2007; 24(9):1720-1728.
68. Wolff NC, Richardson JA, Egorin M, Ilaria RL, Jr. The CNS is a sanctuary for leukemic cells in mice receiving imatinib mesylate for Bcr/Abl-induced leukemia. Blood 2003; 101(12):5010-5013.
69. Breedveld P, Pluim D, Cipriani G et al. The effect of Bcrp1 (Abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. Cancer Res 2005; 65(7):2577-2582.
70. Bihorel S, Camenisch G, Lemaire M, Scherrmann JM. Influence of breast cancer resistance protein (Abcg2) and p-glycoprotein (Abcb1a) on the transport of imatinib mesylate (Gleevec) across the mouse blood-brain barrier. J Neurochem 2007; 102(6):1749-1757.
71. Neville K, Parise RA, Thompson P et al. Plasma and cerebrospinal fluid pharmacokinetics of imatinib after administration to nonhuman primates. Clin Cancer Res 2004; 10(7):2525-2529.
72. Li J, Brahmer J, Messersmith W, Hidalgo M, Baker SD. Binding of gefitinib, an inhibitor of epidermal growth factor receptor-tyrosine kinase, to plasma proteins and blood cells: in vitro and in cancer patients. Invest New Drugs 2006; 24(4):291-297.
73. McKillop D, Hutchison M, Partridge EA et al. Metabolic disposition of gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor, in rat, dog and man. Xenobiotica 2004; 34(10):917-934.
74. McKillop D, Partridge EA, Kemp JV et al. Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor. Mol Cancer Ther 2005; 4(4):641-649.
75. Lu JF, Eppler SM, Wolf J et al. Clinical pharmacokinetics of erlotinib in patients with solid tumors and exposure-safety relationship in patients with non-small cell lung cancer. Clin Pharmacol Ther 2006; 80(2):136-145.
76. Broniscer A, Panetta JC, O'Shaughnessy M et al. Plasma and cerebrospinal fluid pharmacokinetics of erlotinib and its active metabolite OSI-420. Clin Cancer Res 2007; 13(5):1511-1515.
77. Kamath AV, Wang J, Lee FY, Marathe PH. Preclinical pharmacokinetics and in vitro metabolism of dasatinib (BMS-354825): a potent oral multi-targeted kinase inhibitor against SRC and BCR-ABL. Cancer Chemother Pharmacol 2008; 61(3):365-376.

78. Porkka K, Koskenvesa P, Lundan T et al. Dasatinib crosses the blood-brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. Blood 2008; 112(4):1005-1012.
79. Medina PJ, Goodin S. Lapatinib: a dual inhibitor of human epidermal growth factor receptor tyrosine kinases. Clin Ther 2008; 30(8):1426-1447.
80. Polli JW, Humphreys JE, Harmon KA et al. The role of efflux and uptake transporters in [N-{3-chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethy l]amino}methyl)-2-furyl]-4-quinazolinamine (GW572016, lapatinib) disposition and drug interactions. Drug Metab Dispos 2008; 36(4):695-701.
81. Polli JW, Olson KL, Chism JP et al. An unexpected synergist role of P-glycoprotein and breast cancer resistance protein on the central nervous system penetration of the tyrosine kinase inhibitor lapatinib (N-{3-chloro-4-[(3-fluorobenzyl)oxy] phenyl}-6-[5-({[2-(methylsulfonyl)ethy l]amino} methyl)-2-furyl]-4-quinazolinamine; GW572016). Drug Metab Dispos 2009; 37(2):439-442.
82. Rochat B. Role of cytochrome P450 activity in the fate of anticancer agents and in drug resistance: focus on tamoxifen, paclitaxel and imatinib metabolism. Clin Pharmacokinet 2005; 44(4):349-366.
83. Rochat B, Zoete V, Grosdidier A, von GS, Marull M, Michielin O. In vitro biotransformation of imatinib by the tumor expressed CYP1A1 and CYP1B1. Biopharm Drug Dispos 2008; 29(2):103-118.
84. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.
85. O'Brien SG, Meinhardt P, Bond E et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. Br J Cancer 2003; 89(10):1855-1859.
86. Gardner ER, Burger H, van Schaik RH et al. Association of enzyme and transporter genotypes with the pharmacokinetics of imatinib. Clin Pharmacol Ther 2006; 80(2):192-201.
87. Li J, Zhao M, He P, Hidalgo M, Baker SD. Differential metabolism of gefitinib and erlotinib by human cytochrome P450 enzymes. Clin Cancer Res 2007; 13(12):3731-3737.
88. Swaisland HC, Ranson M, Smith RP et al. Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. Clin Pharmacokinet 2005; 44(10):1067-1081.
89. Swaisland HC, Cantarini MV, Fuhr R, Holt A. Exploring the relationship between expression of cytochrome P450 enzymes and gefitinib pharmacokinetics. Clin Pharmacokinet 2006; 45(6):633-644.
90. Li J, Karlsson MO, Brahmer J et al. CYP3A phenotyping approach to predict systemic exposure to EGFR tyrosine kinase inhibitors. J Natl Cancer Inst 2006; 98(23):1714-1723.
91. Hamilton M, Wolf JL, Rusk J et al. Effects of smoking on the pharmacokinetics of erlotinib. Clin Cancer Res 2006; 12(7 Pt 1):2166-2171.
92. Harmsen S, Meijerman I, Beijnen JH, Schellens JH. Nuclear receptor mediated induction of cytochrome P450 3A4 by anticancer drugs: a key role for the pregnane X receptor. Cancer Chemother Pharmacol 2008; 64(1): 35-43.
93. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006; 24(1):25-35.
94. Christopher LJ, Cui D, Li W et al. Biotransformation of [14C]dasatinib: in vitro studies in rat, monkey, and human and disposition after administration to rats and monkeys. Drug Metab Dispos 2008; 36(7):1341-1356.
95. Wang L, Christopher LJ, Cui D et al. Identification of the human enzymes involved in the oxidative metabolism of dasatinib: an effective approach for determining metabolite formation kinetics. Drug Metab Dispos 2008; 36(9):1828-1839.
96. Singer JB, Shou Y, Giles F et al. UGT1A1 promoter polymorphism increases risk of nilotinib-induced hyperbilirubinemia. Leukemia 2007; 21(11):2311-2315.
97. Schmidli H, Peng B, Riviere GJ et al. Population pharmacokinetics of imatinib mesylate in patients with chronic-phase chronic myeloid leukaemia: results of a phase III study. Br J Clin Pharmacol 2005; 60(1):35-44.
98. Judson I, Ma P, Peng B et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005; 55(4):379-386.
99. Menon-Andersen D, Mondick JT, Jayaraman B et al. Population pharmacokinetics of imatinib mesylate and its metabolite in children and young adults. Cancer Chemother Pharmacol 2009; 63(2):229-238.
100. Reardon DA, Egorin MJ, Quinn JA et al. Phase II study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. J Clin Oncol 2005; 23(36):9359-9368.
101. Gibbons J, Egorin MJ, Ramanathan RK et al. Phase I and pharmacokinetic study of imatinib mesylate in patients with advanced malignancies and varying degrees of renal dysfunction: a study by the National Cancer Institute Organ Dysfunction Working Group. J Clin Oncol 2008; 26(4):570-576.
102. Pappas P, Karavasilis V, Briasoulis E, Pavlidis N, Marselos M. Pharmacokinetics of imatinib mesylate in end stage renal disease. A case study. Cancer Chemother Pharmacol 2005; 56(4):358-360.
103. Judson IR. Imatinib for patients with liver or kidney dysfunction: no need to modify the dose. J Clin Oncol 2008; 26(4):521-522.
104. Franke RM, Sparreboom A. Inhibition of imatinib transport by uremic toxins during renal failure. J Clin Oncol 2008; 26(25):4226-4227.
105. Eckel F, von DS, Mayr M et al. Pharmacokinetic and clinical phase II trial of imatinib in patients with impaired liver function and advanced hepatocellular carcinoma. Oncology 2005; 69(5):363-371.
106. Ramanathan RK, Egorin MJ, Takimoto CH et al. Phase I and pharmacokinetic study of imatinib mesylate in patients with advanced malignancies and varying degrees of liver dysfunction: a study by the National Cancer Institute Organ Dysfunction Working Group. J Clin Oncol 2008; 26(4):563-569.
107. Treiber G, Wex T, Schleyer E, Troeger U, Hosius C, Malfertheiner P. Imatinib for hepatocellular cancer--focus on pharmacokinetic/pharmacodynamic modelling and liver function. Cancer Lett 2008; 260(1-2):146-154.
108. Ling J, Johnson KA, Miao Z et al. Metabolism and excretion of erlotinib, a small molecule inhibitor of epidermal growth factor receptor tyrosine kinase, in healthy male volunteers. Drug Metab Dispos 2006; 34(3):420-426.
109. Miller AA, Murry DJ, Owzar K et al. Phase I and Pharmacokinetic Study of Sorafenib in Patients With Hepatic or Renal Dysfunction: CALGB 60301. J Clin Oncol 2009; 27(11):1800-1805.
110. Adams VR, Leggas M. Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Ther 2007; 29(7):1338-1353.
111. Izzedine H, Etienne-Grimaldi MC, Renee N, Vignot S, Milano G. Pharmacokinetics of sunitinib in hemodialysis. Ann Oncol 2009; 20(1):190-192.
112. Christopher LJ, Cui D, Wu C et al. Metabolism and disposition of dasatinib after oral administration to humans. Drug Metab Dispos 2008; 36(7):1357-1364.
113. Ratain MJ, Cohen EE. The value meal: how to save $1,700 per month or more on lapatinib. J Clin Oncol 2007; 25(23):3397-3398.
114. Kagan M, Tran P, Fischer V et al. Safety, Pharmacokinetics (PK), Metabolism, and Mass Balance of [^{14}C]-AMN107, a Novel Aminopyrimidine Inhibitor of Bcr-Abl Tyrosine Kinase, in Healthy Subjects. Blood ASH Annual Meeting Abstracts 2005 106 abstract 4887.
115. Lepper ER, Nooter K, Verweij J, Acharya MR, Figg WD, Sparreboom A. Mechanisms of resistance to anticancer drugs: the role of the polymorphic ABC transporters ABCB1 and ABCG2. Pharmacogenomics 2005; 6(2):115-138.
116. Oostendorp RL, Beijnen JH, Schellens JH. The biological and clinical role of drug transporters at the intestinal barrier. Cancer Treat Rev 2008; 35(2):137-147.
117. Hu S, Franke RM, Filipski KK et al. Interaction of imatinib with human organic ion carriers. Clin Cancer Res 2008; 14(10):3141-3148.
118. White DL, Saunders VA, Dang P et al. OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. Blood 2006; 108(2):697-704.
119. Wang L, Giannoudis A, Lane S, Williamson P, Pirmohamed M, Clark RE. Expression of the uptake drug transporter hOCT1 is an important clinical determinant of the response to imatinib in chronic myeloid leukemia. Clin Pharmacol Ther 2008; 83(2):258-264.
120. Shukla S, Sauna ZE, Ambudkar SV. Evidence for the interaction of imatinib at the transport-substrate site(s) of the multidrug-resistance-linked ABC drug transporters ABCB1 (P-glycoprotein) and ABCG2. Leukemia 2008; 22(2):445-447.
121. Dulucq S, Bouchet S, Turcq B et al. Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood 2008; 112(5):2024-2027.
122. Thomas J, Wang L, Clark RE, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. Blood 2004; 104(12):3739-3745.
123. Ozvegy-Laczka C, Hegedus T, Varady G et al. High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. Mol Pharmacol 2004; 65(6):1485-1495.
124. Nakamura Y, Oka M, Soda H et al. Gefitinib ("Iressa", ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, reverses breast cancer resistance protein/ABCG2-mediated drug resistance. Cancer Res 2005; 65(4):1541-1546.
125. Li J, Cusatis G, Brahmer J et al. Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. Cancer Biol Ther 2007; 6(3):432-438.
126. Cusatis G, Sparreboom A. Pharmacogenomic importance of ABCG2. Pharmacogenomics 2008; 9(8):1005-1009.
127. Marchetti S, de Vries NA, Buckle T et al. Effect of the ATP-binding cassette drug transporters ABCB1, ABCG2, and ABCC2 on erlotinib hydrochloride (Tarceva) disposition in in vitro and in vivo pharmacokinetic studies employing Bcrp1-/-/Mdr1a/1b-/- (triple-knockout) and wild-type mice. Mol Cancer Ther 2008; 7(8):2280-2287.
128. Shi Z, Peng XX, Kim IW et al. Erlotinib (Tarceva, OSI-774) antagonizes ATP-binding cassette subfamily B member 1 and ATP-binding cassette subfamily G member 2-mediated drug resistance.

Cancer Res 2007; 67(22):11012-11020.
129. Rudin CM, Liu W, Desai A et al. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. J Clin Oncol 2008; 26(7):1119-1127.
130. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. Drug Metab Dispos 2009; 37(2):359-365.
131. Giannoudis A, Davies A, Lucas CM, Harris RJ, Pirmohamed M, Clark RE. Effective dasatinib uptake may occur without human organic cation transporter 1 (hOCT1): implications for the treatment of imatinib-resistant chronic myeloid leukemia. Blood 2008; 112(8):3348-3354.
132. Hiwase DK, Saunders V, Hewett D et al. Dasatinib cellular uptake and efflux in chronic myeloid leukemia cells: therapeutic implications. Clin Cancer Res 2008; 14(12):3881-3888.
133. Dai CL, Tiwari AK, Wu CP et al. Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2. Cancer Res 2008; 68(19):7905-7914.
134. Brendel C, Scharenberg C, Dohse M et al. Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. Leukemia 2007; 21(6):1267-1275.
135. Dutreix C, Peng B, Mehring G et al. Pharmacokinetic interaction between ketoconazole and imatinib mesylate (Glivec) in healthy subjects. Cancer Chemother Pharmacol 2004; 54(4):290-294.
136. Bolton AE, Peng B, Hubert M et al. Effect of rifampicin on the pharmacokinetics of imatinib mesylate (Gleevec, STI571) in healthy subjects. Cancer Chemother Pharmacol 2004; 53(2):102-106.
137. Frye RF, Fitzgerald SM, Lagattuta TF, Hruska MW, Egorin MJ. Effect of St John's wort on imatinib mesylate pharmacokinetics. Clin Pharmacol Ther 2004; 76(4):323-329.
138. Wen PY, Yung WK, Lamborn KR et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99-08. Clin Cancer Res 2006; 12(16):4899-4907.
139. Pursche S, Schleyer E, von BM et al. Influence of enzyme-inducing antiepileptic drugs on trough level of imatinib in glioblastoma patients. Curr Clin Pharmacol 2008; 3(3):198-203.
140. Wang Y, Zhou L, Dutreix C et al. Effects of imatinib (Glivec) on the pharmacokinetics of metoprolol, a CYP2D6 substrate, in Chinese patients with chronic myelogenous leukaemia. Br J Clin Pharmacol 2008; 65(6):885-892.
141. van Erp NP, Gelderblom H, Van GM et al. Effect of cigarette smoking on imatinib in patients in the soft tissue and bone sarcoma group of the EORTC. Clin Cancer Res 2008; 14(24):8308-8313.
142. White DL, Saunders VA, Quinn SR, Manley PW, Hughes TP. Imatinib increases the intracellular concentration of nilotinib, which may explain the observed synergy between these drugs. Blood 2007; 109(8):3609-3610.
143. AstraZenica. Drug Label Gefitinib approved 05/05/2003. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ Accessed 03/02/2009.\
144. Hwang SW, Han HS, Lim KY, Han JY. Drug interaction between complementary herbal medicines and gefitinib. J Thorac Oncol 2008; 3(8):942-943.
145. Adjei AA, Molina JR, Mandrekar SJ et al. Phase I trial of sorafenib in combination with gefitinib in patients with refractory or recurrent non-small cell lung cancer. Clin Cancer Res 2007; 13(9):2684-2691.
146. Abbas R, Fettner S, Riek M et al. A drug-drug interaction study to evaluate the effect of rifampicin on the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib in healthy subjects [abstract 548]. Proc Am Soc Clin Oncol 22, 137. 2003.
147. Rakhit A, Pantze MP, Fettner S et al. The effects of CYP3A4 inhibition on erlotinib pharmacokinetics: computer-based simulation (SimCYP) predicts in vivo metabolic inhibition. Eur J Clin Pharmacol 2008; 64(1):31-41.
148. Smith NF, Baker SD, Gonzalez FJ, Harris JW, Figg WD, Sparreboom A. Modulation of erlotinib pharmacokinetics in mice by a novel cytochrome P450 3A4 inhibitor, BAS 100. Br J Cancer 2008; 98(10):1630-1632.
149. Hughes AN, O'Brien ME, Petty WJ et al. Overcoming CYP1A1/1A2 Mediated Induction of Metabolism by Escalating Erlotinib Dose in Current Smokers. J Clin Oncol 2009; 27(8):1220-1226.
150. Lathia C, Lettieri J, Cihon F, Gallentine M, Radtke M, Sundaresan P. Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. Cancer Chemother Pharmacol 2006; 57(5):685-692.
151. Hutson TE, Figlin RA, Kuhn JG, Motzer RJ. Targeted therapies for metastatic renal cell carcinoma: an overview of toxicity and dosing strategies. Oncologist 2008; 13(10):1084-1096.
152. Midgley RS, Kerr DJ, Flaherty KT et al. A phase I and pharmacokinetic study of lapatinib in combination with infusional 5-fluorouracil, leucovorin and irinotecan. Ann Oncol 2007; 18(12):2025-2029.
153. Ranson M, Hammond LA, Ferry D et al. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. J Clin Oncol 2002; 20(9):2240-2250.
154. Chu QS, Schwartz G, de BJ et al. Phase I and pharmacokinetic study of lapatinib in combination with capecitabine in patients with advanced solid malignancies. J Clin Oncol 2007; 25(24):3753-3758.
155. LoRusso PM, Jones SF, Koch KM et al. Phase I and pharmacokinetic study of lapatinib and docetaxel in patients with advanced cancer. J Clin Oncol 2008; 26(18):3051-3056.
156. Novartis. Scientific Discussion Tasigna Approved on 11/19/2007. http://www.emea.europa.eu/ Accessed on 03/06/2009.
157. Oostendorp RL, Buckle T, Beijnen JH, van TO, Schellens JH. The effect of P-gp (Mdr1a/1b), BCRP (Bcrp1) and P-gp/BCRP inhibitors on the in vivo absorption, distribution, metabolism and excretion of imatinib. Invest New Drugs 2009; 27(1):31-40.
158. Deininger MW, O'Brien SG, Ford JM, Druker BJ. Practical management of patients with chronic myeloid leukemia receiving imatinib. J Clin Oncol 2003; 21(8):1637-1647.
159. Van Hest RM, Schnog JB, Van't Veer MB, Cornelissen JJ. Extremely slow methotrexate elimination in a patient with t(9;22) positive acute lymphoblastic leukemia treated with imatinib. Am J Hematol 2008; 83(9):757-758.
160. Yang CH, Huang CJ, Yang CS et al. Gefitinib reverses chemotherapy resistance in gefitinib-insensitive multidrug resistant cancer cells expressing ATP-binding cassette family protein. Cancer Res 2005; 65(15):6943-6949.
161. Stewart CF, Leggas M, Schuetz JD et al. Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. Cancer Res 2004; 64(20):7491-7499.
162. OSI Pharms. Drug Label Erlotinib approved 11/18/2004. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ Accessed 03/02/2009.

Part 1 | Clinical Pharmacology of Imatinib

3

Influence of CYP3A4 Inhibition on the Steady-State Pharmacokinetics of Imatinib

Nielka P. van Erp, Hans Gelderblom, Mats O. Karlsson, Jing Li, Ming Zhao, Jan Ouwerkerk, Johan W. Nortier, Henk-Jan Guchelaar, Sharyn D Baker, and Alex Sparreboom

Clinical Cancer Research 2007, 13(24):7394-7400

Abstract

Purpose: To evaluate the effects of ritonavir, a potent inhibitor of CYP3A4, on the steady-state pharmacokinetics of imatinib.

Experimental Design: Imatinib pharmacokinetics were evaluated in cancer patients receiving the drug for at least 2 months, after which ritonavir (600 mg) was administered daily for 3 days. Samples were obtained on the day before ritonavir (day 1) and on the third day (day 4). The *in vitro* metabolism of imatinib with or without ritonavir and the effect of imatinib on 1-OH-midazolam formation rate, a probe for CYP3A4 activity, were evaluated with human CYP3A4 and pooled liver microsomes.

Results: In 11 evaluable patients, the geometric mean (95% confidence interval) area under the curve of imatinib on days 1 and 4 were 42.6 (33.0-54.9) μg·h/mL and 41.2 (32.1-53.1) μg·h/mL, respectively (P = 0.65). A population analysis performed in NONMEM with a time-dependent covariate confirmed that ritonavir did not influence the clearance or bioavailability of imatinib. *In vitro*, imatinib was metabolized to the active metabolite CGP74588 by CYP3A4 and CYP3A5 and, to a lesser extent, by CYP2D6. Ritonavir (1 μmol/L) completely inhibited CYP3A4-mediated metabolism of imatinib to CGP74588, but inhibited metabolism in microsomes by only 50%. Imatinib significantly inhibited CYP3A4 activity *in vitro*.

Conclusion: At steady-state, imatinib is insensitive to potent CYP3A4 inhibition and relies on alternate elimination pathways. For agents with complex elimination pathways that involve autoinhibition, interaction studies that are done after a single dose may not be applicable when drugs are administered chronically.

Introduction

The first rationally designed inhibitor of a signal transduction pathway, imatinib, is a competitive inhibitor of Bcr-Abl, platelet-derived growth factor receptors (α and β), and c-KIT receptor tyrosine kinases[1-4]. It was first approved for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia and, shortly thereafter, for c-KIT positive metastatic and unresectable gastrointestinal stromal tumor[5, 6].

The pharmacokinetic properties of imatinib have been investigated in healthy volunteers and in patients with chronic myelogenous leukemia, gastrointestinal stromal tumor, and other tumors[7, 8]. Imatinib is well absorbed after oral administration with a bioavailability exceeding 90%[9]. It is extensively metabolized, with up to 80% of the administered dose being recovered in feces, predominantly as metabolites[10]. Imatinib is metabolized *in vitro* principally by cytochrome *P*450 (CYP) 3A4 and CYP3A5 with CYP1A2, CYP2C9, CYP2C19, and CYP2D6 playing a minor role[8]. The main circulating metabolite of imatinib is an N-desmethyl derivative, CGP74588, which has *in vitro* activity similar to that of imatinib, and the systemic exposure represents approximately 10% to15% of that for imatinib[10]. The pharmacokinetic profile of a single dose of imatinib is sensitive to CYP3A4 modulation, with a 74% and 30% reduction in imatinib area under the curve (AUC) observed with coadministration of the CYP3A4 inducers rifampin[11] or St. John's wort[12], respectively, and a 40% increase in imatinib AUC observed with coadministration of the CYP3A4 inhibitor ketoconazole[8]. Interestingly, imatinib itself is known to decreased the clearance of simvastatin, a CYP3A4 substrate, by 70% in patients with chronic myelogenous leukemia[13].

Because imatinib is a substrate for CYP3A4, there is great potential for drug interactions with co-administered drugs, food, and herbal and nutritional supplements potentially leading to subtherapeutic exposure or concentrations associated with greater than acceptable toxicity for imatinib[14, 15]. The prescribing information for imatinib mesylate indicates a need for caution when imatinib is administered with inhibitors and inducers of CYP3A4, based on drug interaction studies involving single-dose administration of imatinib[i]. However, it is unknown if similar drug interactions occur when imatinib concentrations are at steady-state. The purpose of this study was to evaluate the effect of acute administration of ritonavir, a potent inhibitor of CYP3A4, on the steady-state pharmacokinetics of imatinib.

i Novartis Pharma Stein AG, Gleevec (imatinib mesylate): prescribing information [accessed 2007 July 31]. Available from: http://www.gleevec.com/info/page/prescribing.info

Methods

Patients. Eligibility for study entry included a histologically or cytologically confirmed diagnosis of gastrointestinal stromal tumor. Patients had to be on single-agent imatinib treatment for at least 2 months and receive a daily dose of at least 400 mg. Patients were ≥ 18 years old, HIV negative, and had a WHO performance status ≤ 2. Patients were not allowed to have been in surgery within four weeks before entering the study protocol nor experience gastrointestinal toxicity on imatinib treatment during the last 2 weeks before enrollment. Concurrent use of substances known or likely to interfere with the pharmacokinetics of imatinib was not allowed. All patients had adequate clinical functional reserves as defined by absolute neutrophil count > 1.5×10^9/L, platelets > 100×10^9/L, creatinine clearance > 65 mL/min, and bilirubin < 1.75 × the upper limit of institutional normal. The study was approved by the institutional ethics committee (Leiden University Medical Center, Leiden, The Netherlands), and all patients gave written inform consent before entering the study.

Study design. The study was designed to evaluate the effect of ritonavir on imatinib pharmacokinetics at steady-state. All patients were treated daily, for at least 2 months, with commercially available imatinib mesylate film-coated tablets (Novartis International AG) at an oral dose ranging between 400 and 800 mg. The study was done over 5 consecutive days. Ritonavir at a dose of 600 mg (6 capsules of 100 mg; Abbott Laboratories) was coadministered on days 2, 3 and 4 of the study, ~ 30 min before the planned administration of imatinib. The selected dose and schedule of ritonavir are associated with significant inhibition of CYP3A4[16, 17]. On days 2 to 4 during coadministration of ritonavir, the dose of imatinib was reduced by 50% for safety reasons. On the 5th day of the study, patients returned to receiving the imatinib dose they were taking before entering the study.

Pharmacokinetic sampling and analytical assays. Blood samples were collected on the 1st and 4th day of the study for assessment of imatinib and ritonavir pharmacokinetics. Blood was collected into heparin-containing tubes at the following time points: before treatment and after imatinib administration at 1, 2, 3, 4, 5, 6, 8, 10 and 24 hours. Blood samples were centrifuged at 3,000 x *g* for 10 min and plasma was divided into two tubes, one each for imatinib and ritonavir pharmacokinetics, and stored at –20˚C until the day of analysis.
Imatinib and CGP74588 concentrations in plasma were measured using a validated method based on reversed-phase high-performance liquid chromatography with UV detection at a wavelength of 270 nm using a Water 2690 Alliance Separation Module and 2487 UV/Vis Dual Wavelength Detector (Waters Corp.). Each analytical run included a calibration curve of imatinib spiked in plasma over the concentration range of 0.2 to 10 μg/mL and quality control samples analyzed in duplicate at three different concentrations of 0.6, 4.0, and 8.0 μg/mL. Analytes were extracted from plasma by protein precipitation with 10% perchloric acid. Separation was achieved on a column (4 × 125 mm internal diameter) packed with 5-μm particle size Nucleosil C_{18}. The mobile phase consisted of a mixture of acetonitrile-water (20:80, v/v) containing 0.05% trifluoroacetic acid and was delivered isocratically at a flow rate of 1 mL/min. Imatinib eluted at 5.5 ± 0.2 minutes, and CGP74588 eluted at 4.4 ± 0.1 minutes. The metabolite CGP74588 was quantitated indirectly using the imatinib calibration curve at a wavelength of 270 nm. A small amount of CGP74588 was available to confirm the retention time and to ensure the peak areas of imatinib and CGP74588 were similar (within 90-110% of each other) at a concentration of 10 μmol/L. Over 4 days of validation, the within- and between-day precision was always < 10%.
Ritonavir plasma concentrations were measured using a validated method based on high-performance liquid chromatography with UV detection as described previously, with minor modifications[18].

Non-compartmental analysis. Individual plasma concentrations of imatinib, CGP74588, and ritonavir were analyzed by noncompartmental methods using WinNonlin version 5.0 (Pharsight, Inc., CA, USA). Pharmacokinetic variables assessed included peak concentration (C_{max}), AUC during the dosing interval (0-24 h), and apparent oral clearance (CL/F), calculated as dose/AUC. To account for the 50% reduction in imatinib dose between days 1 and 4, C_{max} and AUC for imatinib and CGP74588 on both days 1 and 4 were normalized to an imatinib dose of 400 mg. The relative extent of conversion of imatinib to CPG74588 was calculated as the AUC ratio of CPG74588 to imatinib and expressed as a percentage.

Modeling conditions. To more accurately account for variations in drug doses and to apply a more formal method for estimating CL/F values, additional analyses were done with the first-order conditional estimation method in the NONMEM program, version VI (Icon Development Solutions). Exponential variable distributions were used with exploration of off-diagonal elements (covariances). An additive residual error model following log transformation of imatinib concentrations was used. Identification of a structural model was initially done using the imatinib data obtained on day 1. The same model was then applied to all imatinib data (days 1 and 4), and the variables were reestimated. Next, a dichotomous covariate was introduced (RITA), which was given the value zero before the first ritonavir dose and the value one thereafter. The basic model using all data was compared to a model where CL and/or bioavailability (F) were allowed to change with the value of RITA. A change in CL would affect CL/F only, whereas a change in F would affect both CL/F and the apparent volume of distribution (V/F). Based on anticipated changes in imatinib pharmacokinetic variables, the effect of RITA was constrained to potentially decrease CL and/or an increase F. The basic model was then refined by introducing interoccasion variability, where each

dosing interval was treated as a new occasion. This model was further refined by allowing the morning trough samples to have a different (higher) residual variability magnitude compared with other samples. As an alternative to the dichotomous RITA covariate, the predicted ritonavir concentration was evaluated. This was based on individual predictions from a linear one-compartment model with first-order absorption and an absorption lag time (not shown). It was hypothesized that ritonavir potentially could increase F and/or decrease CL, this time via maximum-effect models. Identification of the best structural model and subsequent improvement of the model was based on differences in the objective function value (ΔOFV) from the NONMEM output and on interpretation of diagnostic plots using the Xpose program, version 4 (Uppsala University, Uppsala, Sweden).

In vitro *metabolism studies.* The *in vitro* metabolism of imatinib and CGP74588 (50 μmol/L each) was determined using human CYP3A4, CYP3A5, CYP1A2, CYP1A1, CYP2D6, CYP2C9 and CYP2C19 Supersomes (10 – 160 pmol/mL) and pooled human liver microsomes (1.6 mg/mL; Gentest, BD Biosciences). The effect of 30 min coincubation of ritonavir (0.1 – 20 μmol/L) with imatinib on the formation of CGP74588 from imatinib (50 μmol/L), as well as the effect of imatinib (1 – 20 μmol/L) on the formation rate of 1-OH-midazolam from midazolam (10 μmol/L) was assessed in CYP3A4 Supersomes and pooled human liver microsomes. Reaction mixtures were made in duplicate and consisted of 1.3 mmol/L NADP+, 3.3 mmol/L glucose-6-phosphate, 0.4 units/mL glucose-6-phosphate dehydrogenase, 3.3 mmol/L magnesium chloride, 50 μmol/L sodium citrate, and 100 mmol/L potassium phosphate buffer in a total volume of 0.2 mL (pH 7.4). Reaction mixtures were incubated for 30 min at 37˚C and terminated by adding 100 μL of acetonitrile and centrifugation at 14,000 rpm at 4°C for 10 min. The supernatant was analyzed for imatinib, CGP74588 and any other unknown metabolites absorbing at 270 nm as described above with minor modifications. Using this modified assay, imatinib and CGP74588 eluted at approximately 12.0 and 9.5 minutes, respectively. Like CGP74588, concentrations of unknown metabolites were quantitated by interpolation on the imatinib calibration curve. Column effluents containing suspected unknown metabolites were subjected to high-performance liquid chromatography analysis with tandem mass spectrometric detection in the scan mode using a Micromass Quattro LC triple-quadrupole mass spectrometer (Waters) to obtain initial structural information. Midazolam and 1-OH-midazolam were analyzed in supernatant using a previously described method based on high-performance liquid chromatography with tandem mass spectrometric detection[19].

Statistical considerations. Data are presented as a geometric mean along with 95% confidence intervals, unless stated otherwise. Statistical analysis was based on a two-tailed paired t-test of logarithmically transformed data, and *P* values of < 0.05 were considered to be statically significant. Calculations were done using the software package Number Cruncher Statistical Systems, version 2005 (NCSS, J. Hintze).

Results

Patients. Twelve patients with a diagnosis of gastrointestinal stromal tumor were enrolled on the study, and 11 were evaluable for pharmacokinetic analysis (Table 1). One patient did not take the ritonavir dose on days 2, 3 and 4 and was excluded from analysis. No severe or unexpected side effects were observed during the concurrent administration of imatinib and ritonavir for 3 days.

Table 1 Patient characteristics

Characteristic	Value
Number of patients	11
Sex (female / male)	6 / 5
Age, years [a]	62 (51 – 79)
Baseline renal and liver function parameters [a]	
Creatinine, μM	86 (70 – 95)
Total bilirubin, μM	7 (6 – 24)
ALT, units/L	20 (9 – 27)
AST, units/L	32 (22 – 45)
Gamma-glutamyltransferase, units/L	16 (9 – 38)
Alkaline phosphatase, units/L	85 (61 – 112)

[a] Values are median with range in parenthesis

Imatinib pharmacokinetics. Ritonavir did not significantly alter the steady-state exposure to imatinib with dose-normalized geometric mean AUC values (95% confidence interval) on days 1 and 4 of 42.6 (33.0 – 54.9) μg·h/mL and 41.2 (32.1 – 53.1) μg·h/mL, respectively (*P* = 0.65; Table 2; Fig. 1). Imatinib dose-normalized C_{max} and CL/F values were also unchanged after 3 days of ritonavir administration. However, ritonavir administration resulted in a > 40% increase in plasma exposure to CGP74588, with mean values for CGP74588 to imatinib AUC ratio on days 1 and 4 of 16.8% (14.6 – 19.4%) and 24.0% (19.9 – 29.0%), respectively (*P* < 0.0001; Table 2; Fig. 1).

Population analysis. A one-compartment model with linear elimination and first-order absorption adequately described the imatinib concentration-time data, and a two-compart-

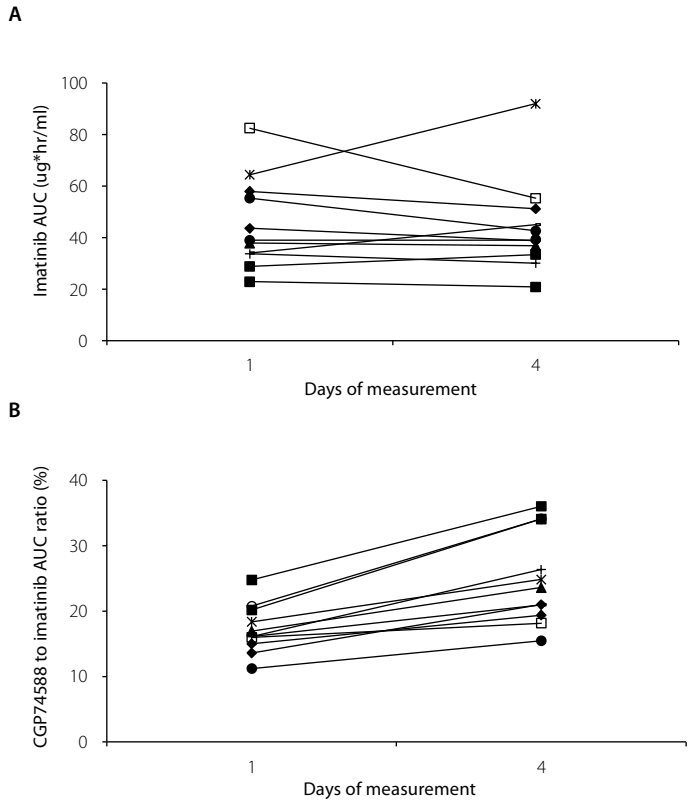
ment model did not significantly improve the fit (ΔOFV = 7.3; $P > 0.05$). For the one-compartment model, CL/F (percent interindividual variability) was estimated to 9.18 ± 0.95 L/h (33%), the apparent volume of distribution to 225 ± 31 L (38%), and absorption rate constant to 1.64 ± 0.39 h^{-1} (38%). The estimate of CL/F obtained when applying the same model to all imatinib data was 9.99 ± 1.05 L/h. Introduction of the dichotomous covariate RITA did not offer an improvement of the model compared with the basic model (ΔOFV = 0.0; $P > 0.05$). Introducing interoccasion variability into the basic model resulted in a significant decrease in ΔOFV of 121.1 ($P < 0.001$) and provided an estimate for CL/F of 9.40 ± 1.16 L/h. This model was further refined by allowing the morning trough samples to have a higher residual variability magnitude compared to other samples (ΔOFV = 34.7; $P < 0.001$), with an error magnitude of 42.3% for morning trough samples and 10.2% for all other samples. To this refined model, the influence of RITA on CL and/or F was again tested, but the improvement in the model fit was negligible (ΔOFV = 0.3; $P > 0.05$). Similarly, a model that incorporated a predicted ritonavir concentration offered no improvement in the description of imatinib data (ΔOFV = 0.0; $P > 0.05$).

Table 2 Pharmacokinetic parameters obtained with non-compartmental analysis

Parameter [a,b]	Day 1 (imatinib alone)	Day 4 (imatinib with ritonavir)	Day 4 / Day 1 ratio	*P*
Imatinib				
C_{max}, µg/mL	2.88 (2.27 – 3.65)	2.50 (1.93 – 3.24)	0.869 (0.744 – 1.02)	.072
AUC, µg·h/mL	42.6 (33.0 – 54.9)	41.2 (32.1 – 53.1)	0.969 (0.835 – 1.125)	.65
CL/F, L/h	9.40 (7.29 – 12.1)	9.69 (7.53 – 12.5)	1.032 (0.889 – 1.198)	.65
CGP74588				
C_{max}, µg/mL	0.467 (0.356 – 0.612)	0.521 (0.411 – 0.661)	1.050 (0.893 – 1.235)	.52
AUC, µg·h/mL	7.16 (5.74 – 8.93)	9.92 (7.85 – 12.5)	1.385 (1.159 – 1.656)	.0023
CGP74588/imatinib AUC ratio, %	16.8 (14.6 – 19.4)	24.0 (19.9 – 29.0)	1.429 (1.317 – 1.551)	<.0001

[a] Values are geometric mean with 95% confidence interval in parenthesis; P-values were obtained from a paired t-test. [b] Cmax and AUC values are normalized to an imatinib dose of 400 mg.
Abbreviations: Cmax, peak concentration; AUC, area under the concentration-time curve; CL/F, apparent oral clearance.

Figure 1

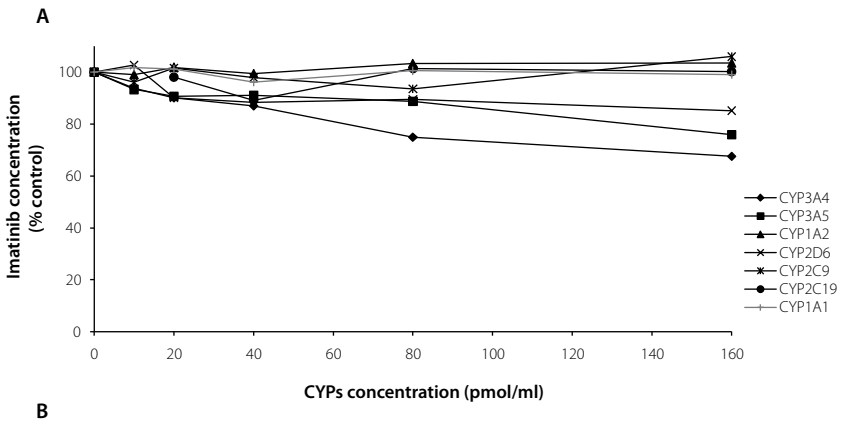


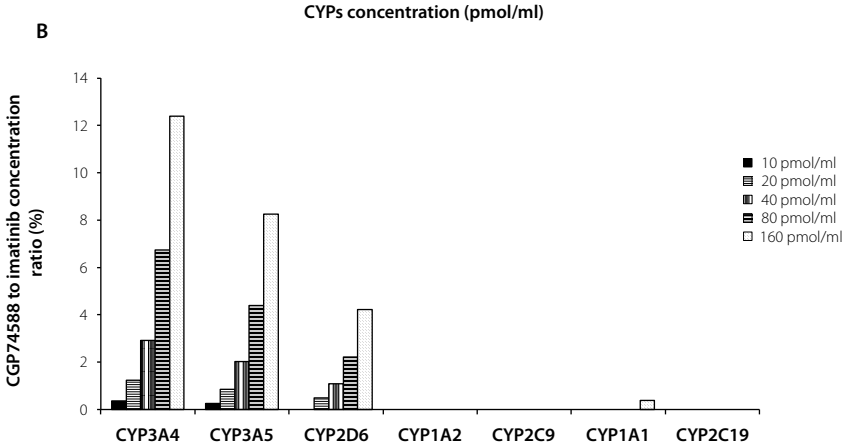
Imatinib dose normalized area under the concentration-time curve (AUC) (**A**) and CGP74588 to imatinib AUC ratio (**B**) before (day 1) and after (day 4) ritonavir co-administration in 11 patients.

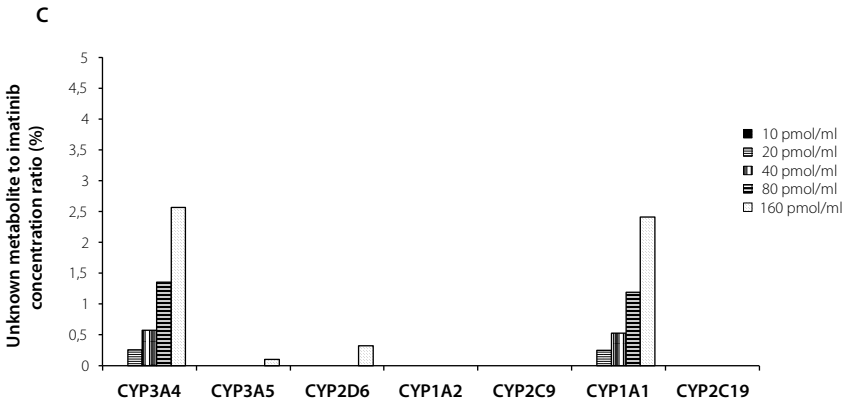
Ritonavir pharmacokinetics. On day 4, the mean C_{max} and AUC values of ritonavir were 14.7 ± 6.3 µg/mL and 85.3 ± 23 µg·h/mL, respectively, which are similar to those previously published for ritonavir in drug interaction studies to inhibit CYP3A4 when it was administered to enhance the oral absorption of antiretroviral agents[20, 21].

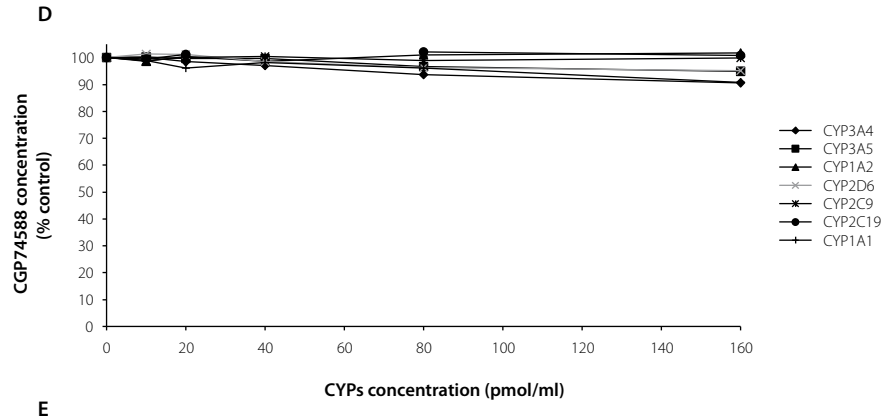
In vitro *metabolism studies.* Imatinib was metabolized to CGP74588 by CYP3A4 and CYP3A5, and to a lesser extent by CYP2D6, with CYP1A1 having minor involvement (Fig. 2A and B). Imatinib was also converted to a metabolite with a retention time of 5.5 min by CYP3A4 and CYP1A1, with a minor fraction formed by CYP3A5 and CYP2D6 (Fig. 2C). The UV

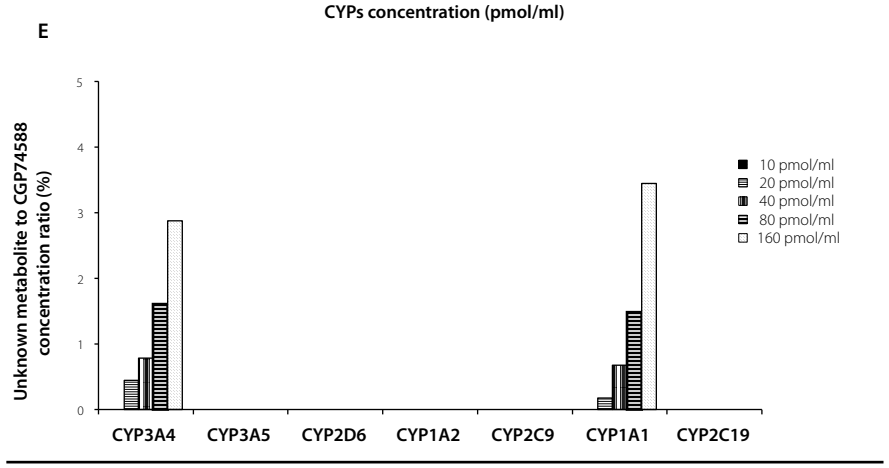
Figure 2









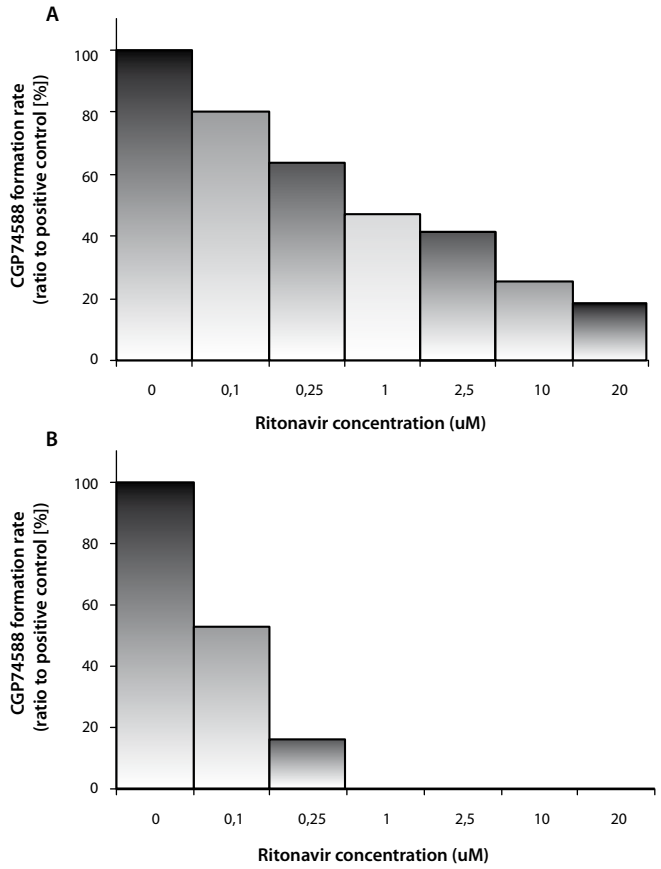


In vitro metabolism of imatinib (**A-C**) and CGP74588 (**D-E**) by different cytochrome P450 (CYP) isoforms. Experimental details are provided in the Methods section.

spectrum of this unknown compound indicated the presence of at least two metabolites. Analysis by mass spectrometry suggested that one of the metabolites formed from imatinib is AFN911, whereas the other could be CGP72383 or CGP71422 or a combination of both, as described previously[10]. No biotransformation of imatinib was observed by CYP1A2, CYP2C9 and CYP2C19 (Fig. 2A-C). CGP74588 was less sensitive to CYP-mediated metabolism, but an unknown metabolite at a retention time of 4.3 min was observed in the presence of CYP3A4 and CYP1A1 (Fig. 2D). The UV spectrum of this unknown metabolite also indicated the presence of two or more compounds, and mass-spectral analysis suggested that these metabolites are structurally similar to the metabolites formed from imatinib without the N-methyl group.

Ritonavir concentrations of 1 µmol/L and higher completely inhibited the metabolism of imatinib to CGP74588 by CYP3A4 (Fig. 3A). However, in pooled human liver microsomes only 50% inhibition of imatinib metabolism by ritonavir was noted at 1 µmol/L, and ~ 80% inhibition at the highest ritonavir concentration tested (Fig. 3B). This suggests the involvement of other CYPs in imatinib metabolism when CYP3A4 function is inhibited. Finally, imatinib concentrations of 1 µmol/L and higher inhibited the metabolism of midazolam to 1-OH-midazolam both in a CYP3A4-expressed system as well as in human liver microsomes (Table 3).

Figure 3



Influence of ritonavir on the *in vitro* metabolism of imatinib by human liver microsomes (**A**) and cytochrome P-450 (CYP) 3A4 (**B**). Experimental details are provided in the Methods section.

Table 3 Effect of imatinib on 1-OH-midazolam formation rate in human liver microsomes and by CYP3A4[a]

Imatinib Concentration (µM)	1-OH-midazolam formation rate (% control)[b]
CYP3A4	
1 µM	92.3
5 µM	86.4
20 µM	57.1
Liver microsomes	
1 µM	64.7
5 µM	60.9
20 µM	56.8

[a] Midazolam concentration used was 10 µM. Experimental details are provided in the Methods section.
[b] The control is 1-OH-midazolam formation rate in the absence of imatinib.

Discussion

This study shows that acute inhibition of CYP3A4 by the potent enzyme inhibitor ritonavir does not result in a substantial pharmacokinetic interaction with imatinib at steady state. These data not only emphasize the need to consider appropriate trial designs to evaluate the plausibility of pharmacokinetic interactions in the development of anticancer drugs that require daily chronic dosing but also have direct clinical relevance for chemotherapeutic treatment with imatinib.

It was previously established that the most prominent pathway of imatinib elimination consists of CYP3A4-mediated metabolism leading to the formation of CGP74588 and several other metabolites[8]. This suggested that imatinib was potentially subject to a host of enzyme-mediated drug interactions with commonly prescribed medications[14]. Indeed, the prototypical CYP3A4 inhibitor ketoconazole has been shown to inhibit the CL/F of imatinib by 40% in healthy volunteers after single-dose imatinib administration[8]. This led to the concern that some degree of interaction is to be expected with simultaneous administration of other potent CYP3A4 inhibitors with imatinib and that concurrent administration should be avoided or that dose adjustments for imatinib should be considered[i].

In consideration of prior knowledge[8], the current observation that acute inhibition of CYP3A4-mediated metabolism by ritonavir does not lead to substantially altered imatinib steady-state exposure was somewhat unexpected. In particular, ritonavir is generally considered to have similar CYP3A4-inhibitory potency as compared with ketoconazole[22], and hence, it is unlikely

that the degree of interaction between imatinib at steady-state and inhibitors of CYP3A4 other than ritonavir would be more substantial than that observed in the current study.

To reconcile the apparent inconsistencies with reported studies on the drug interaction potential of CYP3A4 inhibitors given in combination with imatinib, several additional *in vitro* experiments were done. We found that ritonavir completely inhibited the metabolism of imatinib in CYP3A4 expression system but had only a limited effect on imatinib biotransformation in human liver microsomes. This suggests that the lack of pharmacokinetic interference with ritonavir might be the result of inhibition of only one of multiple enzymes involved in the hepatic metabolism of imatinib, which results in shunting of parent drug to alternative elimination pathways. The present study also showed that imatinib itself is a potent inhibitor of CYP3A4 *in vitro*, and it is plausible that, at steady state, continuous administration of imatinib causes auto-inhibition of the primary metabolic pathway (CYP3A4) and that the presence of another modulator of this route does not result in additional changes in systemic exposure to imatinib.

It should be pointed out that the effect of ritonavir on CYP3A4 activity may be time dependent. Although acute exposure to ritonavir inhibits CYP3A4, extended daily administration of ritonavir may induce CYP3A4. For example, exposure to ritonavir for 7 days or more increased the clearance of the CYP3A4 substrate drugs methadone, alprazolam, mefloquine, dapsone, and cortisol[20, 23-25]. Several recent trials have evaluated the effects of acute and extended exposure to ritonavir on CYP3A activity in the same individuals. In contrast to previous studies, 200 mg ritonavir given twice daily for three doses (acute exposure) and for 10 days (extended exposure) increased the AUC of triazolam, a CYP3A4 probe drug, by 50-fold and 20-fold, respectively[16]. Likewise, acute and extended exposure to 200 mg ritonavir twice daily increased exposure to midazolam, a CYP3A4 substrate probe, by up to 50-fold[17]. If induction does occur, it is likely that ritonavir exposure for more than 10 days is required for this phenomenon. Therefore, the present observations may not be extrapolated to the situation where imatinib is coadministered with ritonavir for an extended period of time.

Interestingly, ritonavir may also affect CYP2C9 and CYP2C19 activity[20], but this may not be of concern clinically, because CYP2C9 and CYP2C19 seem to play only a minor role in imatinib metabolism (Fig. 2). Compared with imatinib, the *in vitro* experiments indicated that the catalytic activity and the relative affinity of CGP74588 for CYP3A4 were substantially weaker. However, subsequent elimination of CGP74588 seem to be highly dependent on the activity of CYP3A4, and therefore, this metabolite is likely to be more sensitive to an acute interaction with ritonavir. This hypothesis is consistent with the current observation that the systemic exposure to CGP74588 was increased by ritonavir (Table 2).

It is noteworthy that imatinib is also both a substrate and an inhibitor for the ATP-binding cassette transporters ABCB1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein)[26-30]. These transporters influence the oral bioavailability of various substrate drugs by decreasing the amount of drug absorbed after oral intake due to their localization on the apical surface of intestinal epithelial cells[31, 32]. Furthermore, these efflux transporters may alter systemic drug elimination, as they are expressed in proximal renal tubular cells and on the biliary surface of hepatocytes[32]. Indeed, inhibitors of ABCB1 and ABCG2 function, such as elacridar and pantoprazol, have been shown to significantly reduce the systemic clearance of imatinib in mice[33]. Because ritonavir is a known inhibitor of both ABCB1 and ABCG2[34, 35], the current data suggest that modulation of the activity of these transporters in humans would not result in substantially altered exposure to imatinib under steady-state conditions.

In conclusion, this study suggests that acute inhibition of CYP3A4 by ritonavir does not result in increased steady-state plasma concentrations of imatinib. The current findings suggest that the warning in the prescribing information for imatinib related to the concomitant use of substrates or inhibitors of CYP3A4 should be reconsidered. Furthermore, the design of drug interaction studies with novel agents that require continuous administration should consider additional evaluation at steady-state.

Acknowledgments

We thank Carol Hartke, Ping He, Alex Mnatsakanyan, Yelena Zabelina (Baltimore, MD), Marco Tiller, Jacqueline Koning, Ed de Haas, Judith Boogaerts and Trees Hessing (Leiden, The Netherlands) for expert analytical and technical assistance, and the nurses of the oncology ward for their assistance with sample collection. We also thank the Dutch Cancer Society for funding travel and accommodations for NPvE during her sabbatical at Johns Hopkins University (Baltimore, MD, USA). This study was supported, in part, by NIH grant P50 AT00437 (SDB).

References

1. Buchdunger E, Zimmermann J, Mett H et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 1996; 56(1):100-104.
2. Lydon NB, Druker BJ. Lessons learned from the development of imatinib. Leuk Res 2004; 28 Suppl 1:S29-S38.
3. van Oosterom AT, Judson I, Verweij J et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 2001; 358(9291):1421-1423.
4. Verweij J, Judson I, Van Oosterom A. STI571: a magic bullet? Eur J Cancer 2001; 37(15):1816-1819.
5. Cohen MH, Williams G, Johnson JR et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res 2002; 8(5):935-942.
6. Dagher R, Cohen M, Williams G et al. Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clin Cancer Res 2002; 8(10):3034-3038.
7. Judson I, Ma P, Peng B et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005; 55(4):379-386.
8. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005; 44(9):879-894.
9. Peng B, Dutreix C, Mehring G et al. Absolute bioavailability of imatinib (Glivec) orally versus intravenous infusion. J Clin Pharmacol 2004; 44(2):158-162.
10. Gschwind HP, Pfaar U, Waldmeier F et al. Metabolism and disposition of imatinib mesylate in healthy volunteers. Drug Metab Dispos 2005; 33(10):1503-1512.
11. Bolton AE, Peng B, Hubert M et al. Effect of rifampicin on the pharmacokinetics of imatinib mesylate (Gleevec, STI571) in healthy subjects. Cancer Chemother Pharmacol 2004; 53(2):102-106.
12. Frye RF, Fitzgerald SM, Lagattuta TF, Hruska MW, Egorin MJ. Effect of St John's wort on imatinib mesylate pharmacokinetics. Clin Pharmacol Ther 2004; 76(4):323-329.
13. O'Brien SG, Meinhardt P, Bond E et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. Br J Cancer 2003; 89(10):1855-1859.
14. Beijnen JH, Schellens JH. Drug interactions in oncology. Lancet Oncol 2004; 5(8):489-496.
15. Scripture CD, Sparreboom A, Figg WD. Modulation of cytochrome P450 activity: implications for cancer therapy. Lancet Oncol 2005; 6(10):780-789.
16. Culm-Merdek KE, von Moltke LL, Gan L et al. Effect of extended exposure to grapefruit juice on cytochrome P450 3A activity in humans: comparison with ritonavir. Clin Pharmacol Ther 2006; 79(3):243-254.
17. Fellay J, Marzolini C, Decosterd L et al. Variations of CYP3A activity induced by antiretroviral treatment in HIV-1 infected patients. Eur J Clin Pharmacol 2005; 60(12):865-873.
18. Hugen PW, Verweij-van Wissen CP, Burger DM, Wuis EW, Koopmans PP, Hekster YA. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1999; 727(1-2):139-149.
19. Li J, Karlsson MO, Brahmer J et al. CYP3A phenotyping approach to predict systemic exposure to EGFR tyrosine kinase inhibitors. J Natl Cancer Inst 2006; 98(23):1714-1723.
20. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 1998; 35(4):275-291.
21. King JR, Wynn H, Brundage R, Acosta EP. Pharmacokinetic enhancement of protease inhibitor therapy. Clin Pharmacokinet 2004; 43(5):291-310.
22. von Moltke LL, Greenblatt DJ, Grassi JM et al. Protease inhibitors as inhibitors of human cytochromes P450: high risk associated with ritonavir. J Clin Pharmacol 1998; 38(2):106-111.
23. Gass RJ, Gal J, Fogle PW, tmar-Hanna D, Gerber JG. Neither dapsone hydroxylation nor cortisol 6beta-hydroxylation detects the inhibition of CYP3A4 by HIV-1 protease inhibitors. Eur J Clin Pharmacol 1998; 54(9-10):741-747.
24. Greenblatt DJ, von Moltke LL, Harmatz JS et al. Alprazolam-ritonavir interaction: implications for product labeling. Clin Pharmacol Ther 2000; 67(4):335-341.
25. Khaliq Y, Gallicano K, Tisdale C, Carignan G, Cooper C, McCarthy A. Pharmacokinetic interaction between mefloquine and ritonavir in healthy volunteers. Br J Clin Pharmacol 2001; 51(6):591-600.
26. Burger H, van Tol H, Boersma AW et al. Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. Blood 2004; 104(9):2940-2942.
27. Houghton PJ, Germain GS, Harwood FC et al. Imatinib mesylate is a potent inhibitor of the ABCG2 (BCRP) transporter and reverses resistance to topotecan and SN-38 in vitro. Cancer Res 2004; 64(7):2333-2337.
28. Jordanides NE, Jorgensen HG, Holyoake TL, Mountford JC. Functional ABCG2 is overexpressed on primary CML CD34+ cells and is inhibited by imatinib mesylate. Blood 2006; 108(4):1370-1373.
29. Nakanishi T, Shiozawa K, Hassel BA, Ross DD. Complex interaction of BCRP/ABCG2 and imatinib in BCR-ABL-expressing cells: BCRP-mediated resistance to imatinib is attenuated by imatinib-induced reduction of BCRP expression. Blood 2006; 108(2):678-684.
30. Thomas J, Wang L, Clark RE, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. Blood 2004; 104(12):3739-3745.
31. Kuppens IE, Breedveld P, Beijnen JH, Schellens JH. Modulation of oral drug bioavailability: from preclinical mechanism to therapeutic application. Cancer Invest 2005; 23(5):443-464.
32. Lepper ER, Nooter K, Verweij J, Acharya MR, Figg WD, Sparreboom A. Mechanisms of resistance to anticancer drugs: the role of the polymorphic ABC transporters ABCB1 and ABCG2. Pharmacogenomics 2005; 6(2):115-138.
33. Breedveld P, Pluim D, Cipriani G et al. The effect of Bcrp1 (Abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. Cancer Res 2005; 65(7):2577-2582.
34. Profit L, Eagling VA, Back DJ. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. AIDS 1999; 13(13):1623-1627.
35. Gupta A, Zhang Y, Unadkat JD, Mao Q. HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). J Pharmacol Exp Ther 2004; 310(1):334-341.

4

Effect of cigarette smoking on pharmacokinetics, safety and efficacy of imatinib: a study based on data of the Soft Tissue and Bone Sarcoma Group of the EORTC

Nielka P. van Erp, Hans Gelderblom, Martine Van Glabbeke, Allan Van Oosterom, Jaap Verweij, Henk-Jan Guchelaar, Maria Debiec-Rychter, Bin Peng, Jean-Yves Blay and Ian Judson

Clinical Cancer Research 2008, 14(24): 8308-8313

Abstract

Purpose: Smoking is a potent inducer of cytochrome *P*450 (CYP) 1A2 and may affect the pharmacokinetics of CYP1A2 metabolized drugs. The effect of smoking on the pharmacokinetics of imatinib, which is metabolized by CYP3A4 and partly by CYP1A2, is unknown. We studied the effect of smoking on imatinib pharmacokinetics, safety, and efficacy.

Experimental Design: Imatinib pharmacokinetics, safety, and efficacy were analyzed in 45 patients with gastrointestinal stromal tumors (GIST) or soft-tissue sarcoma included in two European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group trials, including 15 smokers and 30 nonsmokers. Apparent oral clearance, distribution volume, elimination half-life, and dose-standardized area under the concentration curve (AUC) were assessed in 34 patients using nonlinear mixed-effect modeling.

Results: Mean ± SD pharmacokinetic variables in smokers (n = 9) versus nonsmokers (n = 25) groups were 9.6 ± 5.5 versus 9.2 ± 4.6L/h (apparent oral clearance), 216.5 ± 114.3 versus 207.0 ± 116.9 L (distribution volume), 16.1 ± 6.0 versus 16.5 ± 6.0 h (elimination half-life), and 133.6 ± 71.0 versus 142.3 ± 84.0 ng·h/mL·mg area under the concentration curve; $P > 0.05$. Smokers experienced more grade 2/3 anemia ($P = 0.010$) and fatigue ($P = 0.011$) and those with GIST had a significantly shorter overall survival ($P = 0.037$) and time to progression ($P = 0.052$).

Conclusions: This retrospective study suggests that the pharmacokinetics of imatinib is not affected by smoking. However, smokers have an increased risk of anemia and fatigue. Smokers with GIST have a shorter overall survival and time to progression.

Introduction

Tobacco smoking is a major problem for public health[i]. Despite all the attention paid to the negative effects of smoking cigarettes by the medical profession and media, its prevalence remains high[ii]. Between 2002 and 2005, ~ 34% and 23% of men and women, respectively, smoked in the European Union[iii]. The smoking prevalence in the United States is similar, with estimates of 24% to 32% of men and 18% to 21% of women smoking[1]. Among the various biological effects, tobacco smoke induces several drug-metabolizing enzymes. One of the constituents in tobacco smoke known to be involved in the induction of cytochrome P450 (CYP) 1A1, 1A2, 2E1 and UDP-glucuronosyltransferases are the polycyclic aromatic hydrocarbons, a product of incomplete combustion of organic matter[2]. CYP1A1 and CYP1A2 are involved in the metabolism of a variety of drugs. By inducing these CYPs, smoking can interfere with the pharmacokinetics of many drugs. The most extensively described pharmacokinetic interaction between smoking and drug clearance is that of clozapine, which is primarily metabolized by CYP1A2[3-5]. However, in addition, the metabolism of drugs that are not predominantly metabolized by CYP1A2 can be influenced by smoking. For example, erlotinib is principally metabolized by CYP3A4 and to a minor extent, by CYP1A2. Smoking has been shown to increase the clearance of erlotinib by 23.5% and may therefore reduce the efficacy of the drug in patients with non-small-cell lung cancer[6]. Likewise, imatinib, also a receptor tyrosine kinase inhibitor, is principally metabolized by CYP3A4 and CYP3A5 with CYP1A1, CYP1A2, CYP2C9, CYP2C19, and CYP2D6 playing a secondary role[7, 8]. However, the role of CYP3A4 in imatinib metabolism is under discussion since acute inhibition of CYP3A4, by the potent CYP3A4 inhibitor ritonavir, did not result in a substantial change in the PK of imatinib at steady-state exposure levels [8]. This might be a result of the activity of other CYP enzymes, which while playing only a secondary role in *in vitro* experiments become the principal catabolic enzymes when the main metabolic route is blocked. Induction of an enzyme that only plays a secondary role in the metabolism of imatininb might likewise result in a shift in importance of the individual CYPs.

Therefore, we aimed to explore the effect of smoking on the pharmacokinetics of imatinib as a primary endpoint and the effect of smoking on adverse events and treatment outcome as secondary endpoints.

i European Commission. Tobacco and Health in the European Union. Visited Sept 2007 http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/tobacco_fr_en.pdf

ii Lopez AD, Collishaw N.E., Piha T.A. Tobacco Control Country Profiles. Visited Sept 2007 http://www.who.int/tobacco/statistics/country_profiles/en/Introduction.pdf

iii World Health Organization. Tobacco control database; Adults. Visited Sept 2007 http://data.euro.who.int/tobacco/

Materials and methods

Patients and treatment

A total of 91 patients were included in 2 European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group phase I and II trials of imatinib in patients with gastrointestinal stromal tumors (GIST) and other soft-tissue sarcomas[9-11]. Smoking data were only available for 45 patients; pharmacokinetic data were available for 34 of these patients. The patients from three centers (Leuven, Belgium; London, United Kingdom; and Rotterdam, The Netherlands) were included in this retrospective analysis[9-12]. Eligible patients had histologically proven soft-tissue sarcomas, and those with GISTs were required to be KIT positive by CD117 expression on immunohistochemical staining. Patients had to have a measurable lesion with evidence of progression of < 6 weeks before treatment. Previous chemotherapy was allowed, but had to be discontinued for at least 4 weeks. Additional s elected eligibility criteria for inclusion were WHO performance status of ≤ 2; adequate haematological, renal, and hepatic function; no other severe illness; and no concomitant use of coumarin, other investigational drugs, or systemic corticosteroid therapy; before patient registration, written informed consent was given according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use/European Union-Good Clinical Practice and national-local regulations.

Patients were treated with imatinib 400 mg once daily (7 patients), 300 mg twice daily (7 patients), 400 mg twice daily (25 patients), or 500 mg twice daily (6 patients). All toxicities were graded using the National Cancer Institute Common Toxicity Criteria version 2.0.

Smoking status

Patients were categorized as smokers or non-smokers based on information collected and recorded before study entry. If the smoking behavior was not clearly described in the medical record, the patients were excluded from analyses. In the *de novo* analysis, we divided the patients in two groups: ever versus never smokers. The rationale for this is that it is unclear for how long after cessation smoking could influence the toxicity profiles and overall survival as well as other clinical endpoints such as time to progression. In contrast, with regard to alteration of pharmacokinetic variables by smoking, the *a priori* hypothesis is that smoking induces CYP1A2. In that case, it is clear that the influence of smoking will last for a maximum of 9 days, because the half life of CYP1A2 is estimated to be ~ 38.6 h after smoking cessation[13]. In the sensitivity analysis, we have studied the possible effect of the two different ways of categorization and repeated the analysis with the criterion of current smokers. In fact, two patients who were classified as ever smokers stopped smoking >1 year before imatinib therapy started. This analysis showed that the two different ways of categorization did not alter the outcome of the statistics (the association remained not significant).

Pharmacokinetic Analysis

Blood samples were taken for pharmacokinetic analysis as described in the phase I and II studies. On day 1 blood samples were taken pre-dose, and 1, 2, 3, 4, 8, 12, 14 and 24 h after dosing for patients receiving one daily dose. For patients being dosed twice daily, the 12 h sample was taken after the first dose, just before the second dose, and the 14 h sample 2 h after the second dose. In both groups of patients, the 24 h sample was before the second and the third dose of imatinib respectively[9, 11].

The plasma imatinib concentrations were determined in the Novartis USA bioanalytical laboratory using a validated liquid chromatography-tandem mass spectrometry assay[14].

The pharmacokinetic variables were estimated with a one-compartment model with zero-order absorption and first-order elimination. The model was developed with nonlinear mixed-effect modeling in the study of Judson et al. The following pharmacokinetic variables were estimated: volume of distribution (V_d), apparent oral clearance (Cl/F), elimination half life ($t_{1/2}$), and absolute and dose-standardized area under the concentration time curve (AUC). Details on the model can be found in the original article[12].

Statistics

The estimated pharmacokinetics variables were compared between the smoker and non-smoker populations using the Student's *t*-test. The maximum grades of observed toxicities were compared between those populations using a Cochrane-Armitage χ^2 test for trend; if this test was significant, the probability of undergoing a grade ≥ 2 toxicity was analyzed in a logistic model including the initial imatinib daily dose and the smoking status; if this test was significant, the probability of undergoing a grade ≥ 2 toxicity was analyzed in a logistic model stratified by imatinib daily dose. The time to progression and overall survival were compared between smokers and non-smokers using the log-rank test. All statistical tests were done two-sided with rejection of the null hypothesis at $P < 0.05$. All statistical analyses were performed using SAS, version 9.1 for Windows (SAS Institute). The sample size was based on the available data. A retrospective power computation shows that the study had a 69% power to detect a 50% elevation of the Cl/F calculated with a two-sided *t*-test and a 62% power to detect a 50% decrease of the dose-standardized AUC.

Results

Smoking data were available for 45 patients and pharmacokinetic data for 34 of these. Therefore, correlation of the smoking status with pharmacokinetics, the primary endpoint, is based on the analysis of 34 cases, whereas the correlation of the smoking status with treatment outcome, the secondary endpoint, is based on 45 patients. In the group of

45 patients with smoking data 15 patients were categorized as smokers and 30 were categorized as non-smokers. The patient characteristics and the distribution of the smokers and the non-smokers over the different treatment arms are listed in Tables 1 and 2.

Table 1 Patient characteristics

	Smokers (n=15)	Non-smokers (n=30)	Total (n=45)
Sex			
Male	10	18	28
Female	5	12	17
Age group			
<40	3	5	8
40-50	2	9	11
50-60	7	9	16
60-70	3	7	10
Weight			
Median (range)	77.0 (46.2 - 104.7)	70.3 (30.6 – 102.2)	70.4 (30.6 – 104.7)
Prior chemotherapy			
No	6	15	21
Yes	9	15	24
GIST			
No	2	9	11
Yes	13	21	34
Age			
Median (range)	55.1 (35.9 – 67.7)	50.7 (21.0 – 69.9)	51.3 (21.0 – 69.9)
Time since diagnosis			
Median (range)	430 (9.0 – 5694.0)	476 (28.0 – 1933.0)	430 (9.0 – 5694.0)
Haemoglobin			
Median (range)	7.8 (5.7 – 10.4)	8.0 (5.7 – 9.8)	7.9 (5.7 – 10.4)
White Blood cell Counts			
Median (range)	7.4 (4.5 – 11.3)	6.1 (4.0 – 17.1)	6.4 (4.0 – 17.1)
Creatinine clearance			
Median (range)	79.3 (26.4 – 140.8)	82.2 (41.2 – 146.7)	81.7 (26.4 – 146.7)

Smoking and imatinib pharmacokinetics

The imatinib exposure in smokers versus non smokers was not significantly different; the mean ± SD dose-standardized AUC was 133.6 ± 71.0 ng·h/mL·mg in smokers versus 142.3 ± 84.0 ng·h/mL·mg in non-smokers ($P = 0.78$); the mean Cl/F was 9.6 ± 5.5 L/hr in smokers versus 9.2 ± 4.6 L/hr in non-smokers ($P = 0.84$); the volume of distribution (V_d) was 216.5 ± 114.3 L in smokers versus 207.0 ± 116.9 L in non-smokers ($P = 0.84$) and the half life ($t_{1/2}$) was 16.1 ± 6 h in smokers versus 16.5 ± 6 h in non-smokers ($P = 0.87$; Table 3).

Table 2 Distribution of smokers and non-smokers in the different treatment groups

	Smokers	Non-smokers
400 mg od	1	6
	6.7%	20%
300 mg bid	3	4
	20%	13.3%
400 mg bid	7	18
	47.7%	60%
500 mg bid	4	2
	26.7%	6.7%
Total	15	30

Smoking and imatinib toxicity

The maximum grade of the principal toxicities observed in the study has been tabulated for non-smokers and smokers (Table 4). Grade 2/3 fatigue and anemia were more frequently observed in smokers ($P = 0.0493$ and $P = 0.0258$, respectively). The probability of grade 2/3 fatigue and anemia remained higher in smokers after adjustment for the imatinib dose (logistic model adjusted by dose, P=0.011 and 0.010, respectively).

Smoking and time to progression and overall survival

In the entire population ($n = 45$), non-smoking patients showed a favorable but non significant difference in the overall survival analysis ($P = 0.12$) but not in the time to progression ($P = 0.36$). However, in GIST patients, nonsmokers showed a favorable time to progression ($P = 0.052$) and overall survival ($P = 0.037$; Figs. 1 and 2)

Table 4 Effect of smoking on imatinib induced toxicities

Variables		Smokers (n = 15)	Non-smokers (n = 30)	*P* value
Edema	Grade 0	1	3	0.1106
	Grade 1	5	19	
	Grade 2	9	7	
	Grade 3	0	1	
Fatigue	Grade 0	1	6	0.0493
	Grade 1	2	13	
	Grade 2	11	8	
	Grade 3	1	3	
Dyspnea	Grade 0	9	24	0.2251
	Grade 1	1	0	
	Grade 2	4	5	
	Grade 3	1	1	
Rash	Grade 0	3	3	0.0628
	Grade 1	10	16	
	Grade 2	2	7	
	Grade 3	0	4	
Infection	Grade 0	10	16	0.4071
	Grade 1	2	6	
	Grade 2	3	7	
	Grade 3	0	1	
Leukopenia	Grade 0	5	7	0.4125
	Grade 1	5	9	
	Grade 2	3	9	
	Grade 3	2	5	
Neutropenia	Grade 0	7	9	0.3581
	Grade 1	5	6	
	Grade 2	0	9	
	Grade 3	1	5	
	Grade 4	2	1	
Thrombocytopenia	Grade 0	12	19	0.0346
	Grade 1	1	1	
	Grade 2	0	0	
	Grade 3	1	0	
	Grade4	1	0	
Anemia	Grade 0	1	3	0.0258
	Grade 1	2	15	
	Grade 2	7	6	
	Grade 3	5	4	

Figure 1 Time to progression in smokers versus non-smokers in GIST patients

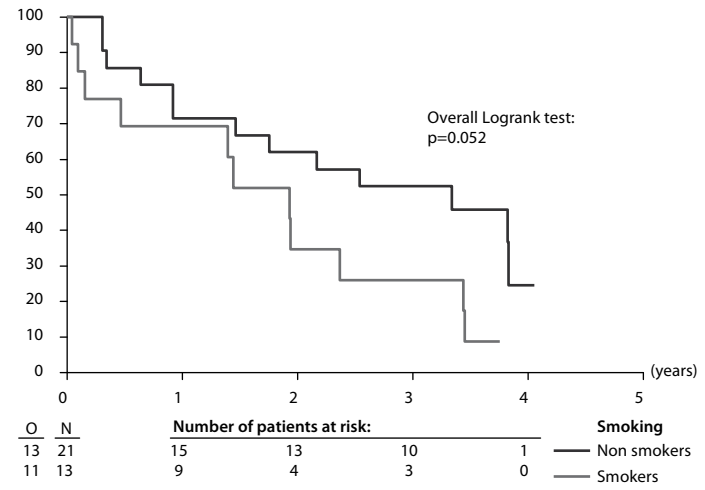
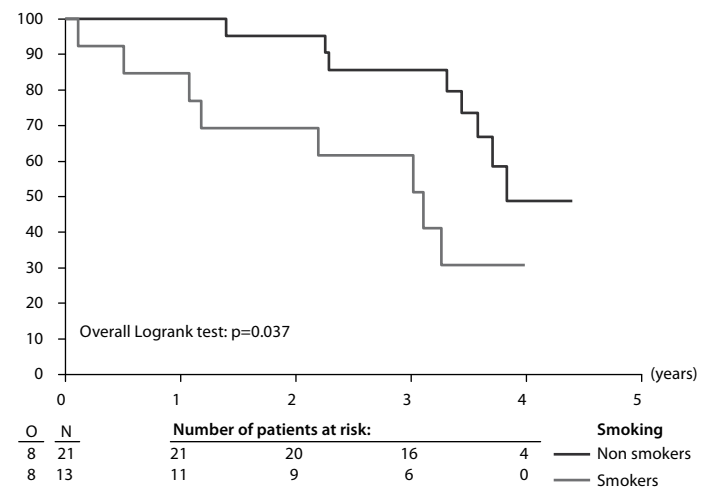


Figure 2 Overall survival in smokers versus non-smokers in GIST patients



Discussion

This study suggests that smoking does not affect the pharmacokinetics of imatinib. However, smoking did increase the risk of some toxicities such as grade ≥ 2 anemia and fatigue. Interestingly, in patients treated with imatinib for GIST, nonsmokers showed a favorable outcome with respect to both time to progression and overall survival.

To date, all interaction studies with imatinib have focused on its primary metabolizing enzyme, CYP3A4 (e.g., the description of the interactions with ketoconazole, rifampicin and St. John's wort)[15-17]. There is one study describing the relationship between genotypes encoding for CYP450 enzymes and ATP-binding cassette transporters thought to play a role in imatinib metabolism and transport, indicating that they do not appear to influence the pharmacokinetics of imatinib significantly in humans. However, the study did show that the Cl/F of orally administered imatinib was slightly reduced in carriers of at least one CYP2D6*4 allele compared with individuals carrying two wild-type CYP2D6 alleles [18]. This might indicate that CYP2D6 plays a more important role in imatinib metabolism *in vivo* than that observed *in vitro*. Imatinib can inhibit certain CYP450 enzymes; hence, although a substrate for CYP3A4 and CYP3A5, it may also inhibit their action, directing metabolism towards other enzymes for which it is a less preferred substrate, such as CYP2D6 and CYP1A2. This emphasizes the importance of exploring the influence of enzymes *in vivo* that appear to only play a minor role in *in vitro* experiments.

In the current retrospective study, smoking habits were retrieved from the medical record and were originally recorded following direct questions about smoking habits before entering the study. We could not validate the smoking status of the patients by measuring the plasma cotinine levels because the plasma samples were not available anymore in this retrospective study. Because ~10% of the patients report not to smoke while smoking, there is a chance of potentially misclassified patients[19]. However this would dilute the outcome of our study because their toxicity profile would be less favorable than the correctly classified patients and they also would negatively influence the time to progression and overall survival. Therefore, 10% misclassification would not influence the conclusion of our study. The specified smoking attitudes of the patients were not noted (e.g., how many tobacco-containing units were consumed what type of tobacco product was used). Therefore, we defined smoking regardless of the quantity of cigarettes smoked per day. We excluded all patients from the analysis for whom the smoking status was not clearly noted, either positively or negatively, in the patients' medical records.

This study is marginally powered (69%) to detect a 50% elevation of the Cl/F of imatinib. Obviously, it could be suggested that a 50% decrease in exposure to a drug is a large effect. However, there are several interactions with cigarette smoking described, which resulted in a >50% decline in exposure (e.g., smoking interactions with theophylline, caffeine, clozapine, olanzapine, flecainide and propranolol)[2, 4, 20-23]. Because our data do not show any effect of smoking on the pharmacokinetics of imatinib, we decided not to expand the study with additional patients.

The absence of an interaction between cigarette smoke and imatinib will most likely be explained by at best a minor role of CYP1A1 and CYP1A2 in imatinib pharmacokinetics *in vivo*. The pharmacokinetic data analyzed in this study were obtained after the first dose of imatinib. Because imatinib is a potent inhibitor of CYP3A4, one may hypothesize that at steady-state pharmacokinetics, imatinib inhibits its own primary metabolizing CYP3A4 pathway and its metabolism is shunted to CYP1A1 and CYP1A2[24]. We can only conclude from our data that metabolism through CYP1A1 and CYP1A2 is not important immediately after starting imatinib therapy, but we cannot exclude an effect of smoking at steady-state pharmacokinetics. Also, other factors that are known to influence the apparent clearance of imatinib should be considered (α1-acid glycoprotein , albumin, body weight, hemoglobin and WBC counts)[25-27]. Elevated α1-acid glycoprotein levels are often seen in cancer patients and with increasing age[12, 28]. However, smoking does not significantly influence the α1-acid glycoprotein levels[29]. In our study, age, body weight, albumin, hemoglobin and WBC counts seems to be equally distributed between the two groups. α1-Acid glycoprotein was not measured, but we have no reason to believe that these factors are unequally distributed between the smoking and non-smoking groups. We studied the most prevalent imatinib toxicities: edema, fatigue, nausea, skin rash, anemia, infection, leucopenia, neutropenia and thrombocytopenia. Except for neutropenia, all toxicities have been shown to be highly dose dependent[30]. In our study, smokers received a higher mean dose of imatinib compared with nonsmokers, which could explain the higher incidence of toxicities in the former group. However, on adjustment for the imatinib dose using multivariate analysis, the increased risk of toxicity in smokers remained significant. Therefore, it is more plausible that the relation of smoking with toxicity is causal[31].

Interestingly, in patients treated for GIST with imatinib, smokers had a significant shorter time to progression and overall survival, which is obviously not explained by differences in imatinib exposure. A possible explanation may be that smokers harbor unfavorable somatic mutations that make the tumor less sensitive to the tyrosine kinase inhibitor and hence are related to a worse outcome. However, in 19 nonsmokers and 11 smokers, we detected a limited set of somatic mutations in *cKIT* exons 11, 9, 13 and *PDGFRA* exon 18 in the GIST tumors and found no differences between smokers and non-smokers (data not shown). In this study, we explored multiple outcomes that might introduce the risk for chance findings. The outcomes of this study are highly correlated (e.g., anemia and fatigue and time to progression and overall survival); however, they should be interpreted as hypothesis-generating and need confirmation.

Currently, little is known of the effect of smoking on the metabolism of most anticancer

drugs. Since imatinib is a substrate for CYP1A2 and CYP1A1 *in vitro*[7,8], it was anticipated that smoking might have an effect on imatinib exposure comparable to erlotinib[6]. Recently, a significant effect of smoking was observed in irinotecan exposure, a chemotherapeutic drug not primarily metabolized by CYP1A1 and CYP1A2[32]. This result emphasizes the importance of studying the effect of smoking on the pharmacokinetics of anticancer drugs, including those for which clearance by CYP1A1 and CYP1A2 is a minor metabolic route, given that ~ 30% of both female and male cancer patients smoke[33].

In conclusion, this exploratory study suggests that smoking is not associated with altered pharmacokinetics of imatinib and more specifically with reduced systemic drug exposure. However, it does show that smokers have an increased risk for grade ≥ 2 anemia and fatigue. GIST patients who smoke may experience a shorter overall survival and a shorter time to progression on treatment with imatinib, but this observation is hypothesis generating and warrants further exploration.

References

1. Center for Disease Control and Prevention, United States 2. Tobacco use among adults. MMWR Morb Mortal Wkly Rep 55 2006;1145-1148.
2. Zevin S, Benowitz NL. Drug interactions with tobacco smoking. An update. Clin Pharmacokinet 1999; 36(6):425-438.
3. Seppala NH, Leinonen EV, Lehtonen ML, Kivisto KT. Clozapine serum concentrations are lower in smoking than in non-smoking schizophrenic patients. Pharmacol Toxicol 1999; 85(5):244-246.
4. van der Weide J, Steijns LS, van Weelden MJ. The effect of smoking and cytochrome P450 CYP1A2 genetic polymorphism on clozapine clearance and dose requirement. Pharmacogenetics 2003; 13(3):169-172.
5. Bondolfi G, Morel F, Crettol S, Rachid F, Baumann P, Eap CB. Increased clozapine plasma concentrations and side effects induced by smoking cessation in 2 CYP1A2 genotyped patients. Ther Drug Monit 2005; 27(4):539-543.
6. Lu JF, Eppler SM, Wolf J et al. Clinical pharmacokinetics of erlotinib in patients with solid tumors and exposure-safety relationship in patients with non-small cell lung cancer. Clin Pharmacol Ther 2006; 80(2):136-145.
7. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005; 44(9):879-894.
8. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.
9. van Oosterom AT, Judson I, Verweij J et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 2001; 358(9291):1421-1423.
10. van Oosterom AT, Judson IR, Verweij J et al. Update of phase I study of imatinib (STI571) in advanced soft tissue sarcomas and gastrointestinal stromal tumors: a report of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 2002; 38 Suppl 5:S83-S87.
11. Verweij J, van OA, Blay JY et al. Imatinib mesylate (STI-571 Glivec, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target. Results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study. Eur J Cancer 2003; 39(14):2006-2011.
12. Judson I, Ma P, Peng B et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005; 55(4):379-386.
13. Faber MS, Fuhr U. Time response of cytochrome P450 1A2 activity on cessation of heavy smoking. Clin Pharmacol Ther 2004; 76(2):178-184.
14. Bakhtiar R, Lohne J, Ramos L, Khemani L, Hayes M, Tse F. High-throughput quantification of the anti-leukemia drug STI571 (Gleevec) and its main metabolite (CGP 74588) in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 768(2):325-340.
15. Dutreix C, Peng B, Mehring G et al. Pharmacokinetic interaction between ketoconazole and imatinib mesylate (Glivec) in healthy subjects. Cancer Chemother Pharmacol 2004; 54(4):290-294.
16. Bolton AE, Peng B, Hubert M et al. Effect of rifampicin on the pharmacokinetics of imatinib mesylate (Gleevec, STI571) in healthy subjects. Cancer Chemother Pharmacol 2004; 53(2):102-106.
17. Frye RF, Fitzgerald SM, Lagattuta TF, Hruska MW, Egorin MJ. Effect of St John's wort on imatinib mesylate pharmacokinetics. Clin Pharmacol Ther 2004; 76(4):323-329.
18. Gardner ER, Burger H, van Schaik RH et al. Association of enzyme and transporter genotypes with the pharmacokinetics of imatinib. Clin Pharmacol Ther 2006; 80(2):192-201.
19. Pell JP, Haw SJ, Cobbe SM et al. Validity of self-reported smoking status: Comparison of patients admitted to hospital with acute coronary syndrome and the general population. Nicotine Tob Res 2008; 10(5):861-866.
20. Holtzman JL, Weeks CE, Kvam DC et al. Identification of drug interactions by meta-analysis of premarketing trials: the effect of smoking on the pharmacokinetics and dosage requirements for flecainide acetate. Clin Pharmacol Ther 1989; 46(1):1-8.
21. Walle T, Walle UK, Cowart TD, Conradi EC, Gaffney TE. Selective induction of propranolol metabolism by smoking: additional effects on renal clearance of metabolites. J Pharmacol Exp Ther 1987; 241(3):928-933.
22. Hunt SN, Jusko WJ, Yurchak AM. Effect of smoking on theophylline disposition. Clin Pharmacol Ther 1976; 19(5 Pt 1):546-551.
23. Carrillo JA, Herraiz AG, Ramos SI, Gervasini G, Vizcaino S, Benitez J. Role of the smoking-induced cytochrome P450 (CYP)1A2 and polymorphic CYP2D6 in steady-state concentration of olanzapine. J Clin Psychopharmacol 2003; 23(2):119-127.
24. O'Brien SG, Meinhardt P, Bond E et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. Br J Cancer 2003; 89(10):1855-1859.
25. Delbaldo C, Chatelut E, Re M et al. Pharmacokinetic-pharmacodynamic relationships of imatinib and its main metabolite in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 2006;

12(20 Pt 1):6073-6078.
26. Schmidli H, Peng B, Riviere GJ et al. Population pharmacokinetics of imatinib mesylate in patients with chronic-phase chronic myeloid leukaemia: results of a phase III study. Br J Clin Pharmacol 2005; 60(1):35-44.
27. Widmer N, Decosterd LA, Csajka C et al. Population pharmacokinetics of imatinib and the role of alpha-acid glycoprotein. Br J Clin Pharmacol 2006; 62(1):97-112.
28. Abernethy DR, Kerzner L. Age effects on alpha-1-acid glycoprotein concentration and imipramine plasma protein binding. J Am Geriatr Soc 1984; 32(10):705-708.
29. Blain PG, Mucklow JC, Rawlins MD, Roberts DF, Routledge PA, Shand DG. Determinants of plasma alpha 1-acid glycoprotein (AAG) concentrations in health. Br J Clin Pharmacol 1985; 20(5):500-502.
30. Van GM, Verweij J, Casali PG et al. Predicting toxicities for patients with advanced gastrointestinal stromal tumours treated with imatinib: a study of the European Organisation for Research and Treatment of Cancer, the Italian Sarcoma Group, and the Australasian Gastro-Intestinal Trials Group (EORTC-ISG-AGITG). Eur J Cancer 2006; 42(14):2277-2285.
31. Tungtrongchitr R, Pongpaew P, Soonthornruengyot M et al. Relationship of tobacco smoking with serum vitamin B12, folic acid and haematological indices in healthy adults. Public Health Nutr 2003; 6(7):675-681.
32. van der Bol JM, Mathijssen RH, Loos WJ et al. Cigarette smoking and irinotecan treatment: pharmacokinetic interaction and effects on neutropenia. J Clin Oncol 2007; 25(19):2719-2726.
33. Spitz MR, Fueger JJ, Eriksen MP, Newell GR. Cigarette smoking patterns of cancer patients. Prog Clin Biol Res 1990; 339:73-82.

5

Is rectal administration an alternative route for imatinib?

Nielka P. van Erp, Roos L. Oostendorp, Henk-Jan Guchelaar,
Jan H. M. Schellens and Hans Gelderblom
Cancer Chemotherapy and Pharmacology 2007, 60: 623-624

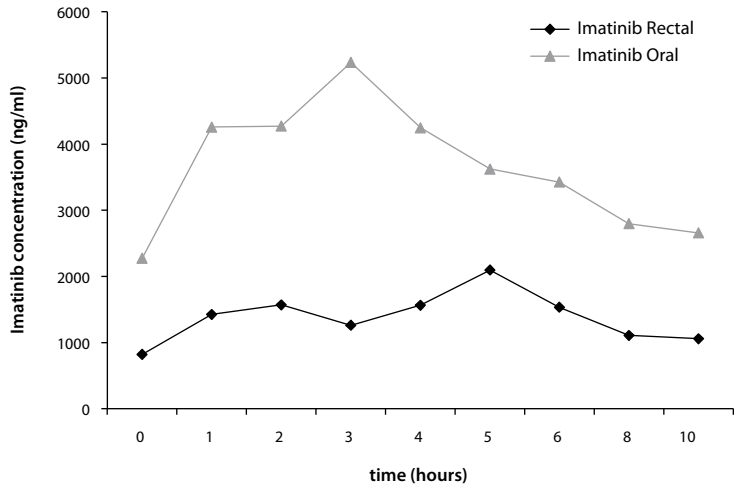
A 52 year old woman with metastatic gastro-intestinal stromal tumor (GIST) presented herself in March 2006 with tumor-related intra-abdominal obstructions and diffuse intra-abdominal bleeding. Priorly, the metastatic GIST was successfully treated with 400mg imatinib since 2002 but now appeared to be progressive again. The patient underwent palliative resection of multiple bleeding peritoneal tumor deposits. When confronted with GIST progression, as seen in this patient, the dose of imatinib should be elevated from 400 mg /day to 800 mg / day [1]. However, a major limitation for treatment in this patient was that, due to the gastro-intestinal obstructions, she was unable to take anything orally, including the imatinib tablets for 8 days prior to surgery. Unfortunately, imatinib is available as a tablet formulation only. Therefore, in this patient we tested the rectal route of administration as an alternative way to administer the drug.

The day following surgery, the patient received imatinib 400 mg b.i.d. with the imatinib oral tablets being administered rectally. After the fourth dose of imatinib given rectally we collected blood samples at t = 0, 1, 2, 3, 4, 5, 6, 8 and 10 hours. The patient volunteered in a pharmacokinetic study a year before [2]. In the study, after informed consent, we collected steady state blood levels of imatinib at the same time points as described above, but after an oral dose of 400 mg imatinib. This enabled us to compare the area under the concentration-time (AUC) curve following oral and rectal administration of imatinib in this patient. Plasma concentrations of imatinib were analyzed at The Netherlands Cancer Institute by a validated HPLC-UV assay with a variation coefficient within the generally accepted 15% range and a lower limit of quantification of 10ng/ml. $AUC_{0\text{-}10hr}$ after the oral administration of 400 mg imatinib was 35,508 and it was 14,243 ng/ml *h after rectal administration (Figure 1) calculated by the trapezoidal method. Assuming relatively small intraindividual variation in pharmacokinetics, comparison of the AUCs indicates that at least 40% of the oral imatinib levels are reached by rectal administration. About 40% will be a slight underestimation because steady-state conditions were not fully reached. The AUC after the fourth rectal dose was estimated at 80-90% of the steady-state AUC.

The $t_{1/2}$ of imatinib is ~18 hours [3]. In the 9 days before rectal administration of imatinib the body is cleared from imatinib. Therefore, the AUC measured after the fourth rectal dose of imatinib is solely produced by absorbance of imatinib from the rectum and is not influenced by the oral dose used before.

The lack of alternative dosing forms of imatinib sometimes causes problems in clinical practice. Patients with GIST may show obstruction or narrowing of the gastro-intestinal tract causing problems to take food and drugs orally. These patients are unable to take imatinib treatment. Based on our observation, in these circumstances, rectal administration of a double dose

Figure 1 Representative plasma concentrations versus time profile of imatinib after rectal or oral administration of 400 mg



of imatinib could be a good alternative. Imatinib mesylate is a highly water soluble drug with a bioavailability of nearly 100% when taken orally [4]. This characteristic readily predicts absorption from the rectal mucosa. Indeed, in the patient presented here, we demonstrated by plasma level measurement that imatinib could be administered rectally resulting in a 40% drug exposure. Therefore, doubling the dose is anticipated to reach a similar drug exposure compared to when given orally.

References

1. Verweij J, Casali PG, Zalcberg J et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet 2004; 364(9440):1127-1134.
2. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.
3. Judson I, Ma P, Peng B et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005; 55(4):379-386.
4. Peng B, Dutreix C, Mehring G et al. Absolute bioavailability of imatinib (Glivec) orally versus intravenous infusion. J Clin Pharmacol 2004; 44(2):158-162.

Part 2 | Clinical Pharmacology of Sunitinib

Relationship between CYP3A4 phenotype and sunitinib exposure in cancer patients

Nielka P. van Erp, Sharyn D. Baker, Alex Sparreboom, Zhaoyuan Chen, Jan den Hartigh, Jacqueline M.C. König-Quartel, Bart A. Ploeger, Anthe S. Zandvliet, Henk-Jan Guchelaar and Hans Gelderblom

Abstract

Purpose: The objective of this study was to explore the feasibility of CYP3A4 phenotyping for dose individualization of sunitinib. The relationship between CYP3A4 activity and sunitinib exposure was assessed. Moreover, the impact of sunitinib exposure on CYP3A4 activity was evaluated.

Patients and Methods: Sunitinib and midazolam pharmacokinetics were evaluated in cancer patients receiving sunitinib monotherapy in a "four weeks on - 2 weeks off" regimen. Serial blood samples for pharmacokinetic analysis of midazolam and sunitinib were collected on two separate days. On both PK days the patients received a single oral dose of 7.5 mg midazolam as a CYP3A4 phenotyping probe. The first PK day was at steady-state sunitinib pharmacokinetics (between day 14-20), the second PK day was after two weeks wash out of sunitinib (day 42). The influence of sunitinib on midazolam exposure was assessed by comparing midazolam exposure on the both PK days. For the phenotyping study, midazolam exposure at the second PK day, after the wash out of sunitinib, and sunitinib exposure at the first PK day were associated.

Results: A linear correlation between midazolam exposure and 1] steady-state sunitinib exposure (R^2= 0.56; P = .021) and 2] steady state sunitinib plasma trough levels (R^2= 0.51; P = .030) were found. Additionally a strong linear relation was found between sunitinib plasma trough levels and sunitinib exposure (R^2= 0.90; P < .0001). Co-administration of sunitinib reduced the exposure to midazolam. However this reduction was not significant (P = .113).

Conclusion: Steady-state sunitinib exposure and sunitinib trough levels are strongly related to CYP3A4 activity. Therefore, CYP3A4 phenotyping could be useful for individualization of the sunitinib starting dose. In addition, sunitinib exposure relates well to sunitinib plasma trough levels and sunitinib appears to be a mild inducer of CYP3A4.

Introduction

Sunitinib malate (Sutent®; SU11248) is an oral, multi-targeted tyrosine kinase inhibitor that specifically inhibits vascular endothelial growth factor receptors 1, 2 and 3 (VEGFR1, -2 and -3, respectively), platelet derived growth factor receptor alpha and beta (PDGFR-α and -β), KIT, Fms-like tyrosine kinase-3 receptor (FLT3), colony stimulating factor receptor type 1 (CSF-1R) and the receptor encoded by the ret proto-oncogene (RET)[1, 2]. Sunitinib is approved for first line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic gastrointestinal stromal tumors (GIST)[3-5]. Sunitinib is metabolized by cytochrome P450 (CYP) 3A4 to an equally active metabolite SU12662, which is further metabolized to inactive moieties by CYP3A4[6].

Clinical pharmacokinetics of sunitinib show high interpatient variability (~ 40%) which is mainly unexplained[5]. This could result in supra- or sub-therapeutic sunitinib levels leading to toxicity or inefficacy, respectively. Since sunitinib is predominantly metabolized by CYP3A4, variability in the activity of this enzyme may explain a considerable proportion of the observed interpatient variability in sunitinib pharmacokinetics. A noninvasive and inexpensive phenotypic probe to measure the CYP3A4 activity might be useful for therapeutic optimization of the dosage of sunitinib. The probe could also be used to evaluate the potential impact of sunitinib on CYP3A4 activity. In this study, midazolam was used for CYP3A4 phenotyping. The design of the study was not only suitable to assess the activity of CYP3A4, but also to evaluate the potential impact of sunitinib on the pharmacokinetics of midazolam in cancer patients. It is thought that sunitinib is neither an inhibitor nor an inducer of CYP-enzymes and therefore the drug is considered not prone to drug-drug and drug-food interactions, while other tyrosine kinase inhibitors (e.g. imatinib, erlotinib, gefitinib) appear to be substrates and/or inhibitors of several CYP-enzymes *in vivo* and *in vitro*[7-12]. For sunitinib, *in vivo* confirmatory studies to define an effect of sunitinib on CYP-enzymes are lacking. Moreover, recently it was shown in an *in vitro* study that sunitinib is a substrate for and an inhibitor of the transporter proteins ABCG2 and to some extent ABCB1, which may also lead to drug-drug interactions[13].

Therefore, in this study the relationship is determined between CYP3A4 activity and sunitinib exposure in cancer patients, using the phenotypic probe midazolam. In addition, the relationship between sunitinib plasma trough levels and sunitinib exposure is investigated and the effect of sunitinib on CYP3A4 activity *in vivo* is assessed.

Methods

Patients

Eligibility for study entry included adult cancer patients that were on sunitinib treatment for palliative treatment of various tumors, mainly mRCC and GIST, at a dose level of 25 – 50 mg once daily in a "four weeks on – two weeks off" schedule. 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 four weeks before entering the study was not allowed. Concurrent use of substances known or likely to interfere with the pharmacokinetics of sunitinib or with CYP3A4 activity, such as ketoconazole, fluconazole, rifampicin, St. John's wort etc., were not allowed within 14 days before study entry. 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.

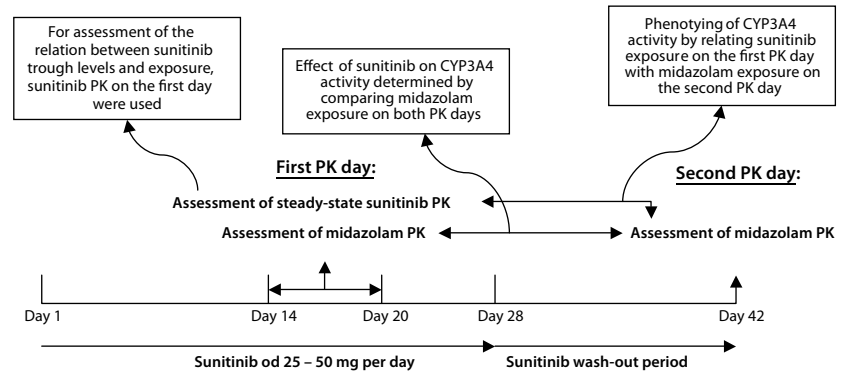
Study design

In this study, midazolam was used as a probe to assess CYP3A4 activity. The study was designed to determine the relationship between CYP3A4 activity and sunitinib exposure in cancer patients and additionally to evaluate the effect of sunitinib on CYP3A4 activity by studying its effect on midazolam pharmacokinetics.

All patients were treated in a "four weeks on – two weeks off" dosing schedule, with commercially available sunitinib malate hard capsules (Pfizer, Kent, United Kingdom) at an oral once daily dose ranging between 25 and 50 mg. The study was performed during one sunitinib treatment cycle of six weeks. Patients were admitted to the hospital on two separate days. The first PK day was at steady-state sunitinib pharmacokinetics (between day 14 - 20), the second PK day was on day 42, the last day of the two weeks "off period" after the wash out of sunitinib. On both PK days the patients were given one midazolam 7.5 mg tablet of a single batch (Roche, Woerden, The Netherlands) either with (first PK day) or without sunitinib (second PK day).

CYP3A4 phenotyping for dose individualization of sunitinib would be performed prior to treatment with sunitinib in clinical practice. Hence, exposure to midazolam without concomitant exposure to sunitinib (midazolam, second PK day) was related to sunitinib exposure at steady-state (suntinib, first PK day) to explore the feasibility of CYP3A4 phenotyping for dose individualization of sunitinib. Additionally midazolam exposures on the first and second PK day were compared to assess the effect of sunitinib on CYP3A4 activity (Figure 1).

Figure 1 Study design



Abbreviations: PK = pharmacokinetics; CYP3A4 = cytochrome P450 3A4; od = once daily

Sunitinib Pharmacokinetics

Blood samples were collected on the first PK day of the study for assessing sunitinib pharmacokinetics at steady-state. The samples were collected into heparin-containing tubes at 0, 10, 20, 40 minutes; 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hours after the sunitinib dose. Blood samples were centrifuged at 3,000 rpm for 10 minutes and plasma was divided into two aliquots and stored at –80˚C until the day of analysis. Total sunitinib plasma concentrations were determined in plasma using a validated liquid chromatographic tandem mass spectrometric (LC/MS/MS) assay, as described previously[14].

Midazolam Pharmacokinetics

Blood samples to assess midazolam pharmacokinetics were collected on the first and second PK day after a single dose of midazolam. The samples were collected into heparin-containing tubes at the following time points: 0, 10, 20, 40 minutes; 1, 2, 3, 4, 5, 7 hours after the midazolam dose. Blood samples were centrifuged at 3,000 rpm for 10 minutes and plasma was stored at –80˚C until the day of analysis. Midazolam was 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 (4µg/L) as the internal standard, followed by vortex mixing and centrifugation at 13,000 rpm for 5 minutes at ambient temperature. The supernatant was collected and 10 µL was separated on an Atlantis T3 C18 analytical column (2.1 x 50 mm, i.d 3 µm) and eluted with the following

gradient [flow rate (ml/min)/ time (minutes)/ percentage of solvent A (formic acid 0.1% in water)/ percentage of solvent B (formic acid 0.1% in acetonitril)]: 0.3/0.5/85/15/, 0.3/1/10/90, 0.3/4.3/10/90, 0.5/0.01/10/90, 0.5/0.39/85/15, 0.5/3.3/85/15, 0.3/0.05/85/15, 0.3/0.05/85/15. The effluent was monitored with a Micromass Quattro LC triple-quadrupole mass-spectrometric detector (Waters, Milford, MA, USA) using the electrospray positive ionization mode. The calibration curve of midazolam was linear over the range of 1 – 100 ng/mL. The within day and between day precision and accuracy were less than 5%. The lower limit of quantification (LLQ) of midazolam was 0.3 ng/mL.

Pharmacokinetic analysis

Sunitinib and midazolam plasma concentrations were analyzed by non-compartmental methods using WinNonlin (version 5.2.1) (Pharsight Corporation, Mountain View, CA, USA). Pharmacokinetic parameters assessed for midazolam were: AUC over the sampling period (0-7h), $AUC_{0-\infty}$ over an extrapolated time interval calculated as: $AUC_{0-\infty} = AUC_{(0-7hr)} + C_{(last)}/\lambda_z$, peak plasma concentration ($C_{l,max}$), time to reach peak concentration ($T_{l,max}$).

For sunitinib the following pharmacokinetic variables were assessed: AUC over the dosing interval (0-24h); apparent oral clearance (CL/F), calculated as dose/AUC_{0-24}; $C_{ss,min}$ = average trough plasma concentration; $T_{l,max}$ = time to reach peak plasma concentration; $C_{l,max}$ = peak plasma concentration. To account for the sunitinib dose differences (37.5mg and 50mg) between the patients, the $C_{ss,min}$, $C_{l,max}$, and AUC_{0-24hr} were normalized to a sunitinib dose of 50 mg.

Statistical analyses

The relationship between midazolam exposure and sunitinib exposure was studied by linear regression analysis. The Pearson R square (R^2) was used to asses the percentage of the variability in sunitinib exposure that could be explained by CYP3A4 activity. Midazolam exposures on the first and second PK day were compared by a two-tailed paired Student's *t*-test. For all tests *P* values < .05 were considered to be statically significant. Statistical calculations were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Patients

Nine patients were included in this pharmacokinetic study; 7 patients were treated with 50 mg and 2 patients were treated with 37.5 mg sunitinib once daily. Patient characteristics are summarized in Table 1. No unexpected side effects were observed on the day of midazolam administration or during sunitinib treatment.

Table 1 Patient characteristics

Characteristic	Value
Number of patients	9
Sex (female / male)	2/7
Age, years*	56 (41 - 78)
Baseline renal and liver function parameters	
Creatinine, µM*	76 (56 - 122)
Total bilirubin, µM*	8 (6 - 15)
ALT, units/L*	33 (14 - 68)
Baseline bone marrow function parameters	
Hb, mM*	8.7 (7 - 9.4)
WBC, x 10^9/L*	5.5 (3.5 - 38.2)
Thrombocytes, x 10^9/L*	193 (122 - 318)

* median values (range)

Sunitinib pharmacokinetics

Dose normalized sunitinib exposure (AUC_{0-24hr}) and trough levels ($C_{ss,min}$) at steady-state pharmacokinetics varied 8 to 9.5-fold (geometric mean = 1,105 ng·hr/mL, range 267 – 2,119 ng·hr/mL and geometric mean = 43.6 ng/mL, range 7.2 – 68.7 ng/mL, respectively) (Table 2). The interpatient variabilities (defined as the coefficient of variation (CV%)) in sunitinib exposure and sunitinib trough levels were large: 51% and 56%, respectively (Figure 2). Pharmacokinetic parameters of sunitinib are listed in Table 2. Sunitinib trough levels ($C_{ss,min}$) and sunitinib exposure (AUC_{0-24hr}) were found highly related (R^2 = .90, $P < 0.0001$) (Figure 3A).

Midazolam pharmacokinetics related to sunitinib exposure

Midazolam exposure (AUC_{0-7hr}), Cmax and Tmax are listed in Table 2. Midazolam exposure (AUC_{0-7hr}) was highly correlated to sunitinib trough levels ($C_{ss,min}$) (R^2 = .51, *P* = .030) and sunitinib exposure (AUC_{0-24hr}) (R^2 = .56, *P* = .021) and could thereby reduce both the interpatient variability in sunitinib trough levels and sunitinib exposure to 29% (Figure 3B, 3C).

Midazolam pharmacokinetics to evaluate the effect of sunitinib on CYP3A4

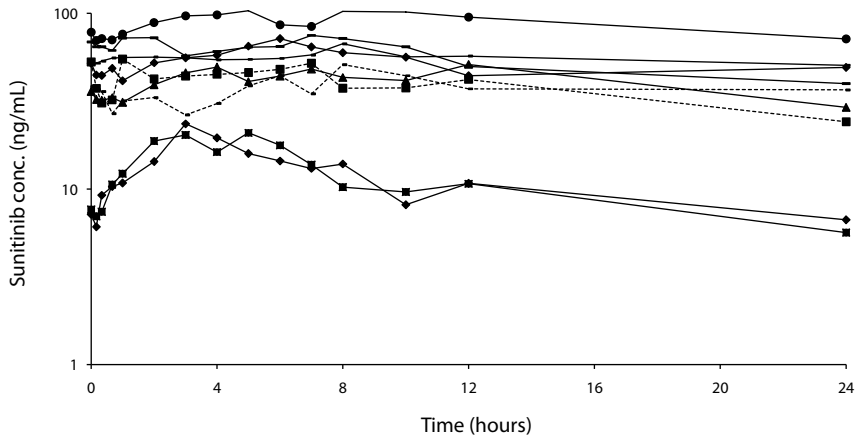
The mean midazolam exposure (AUC_{0-7hr}) with and without sunitinib were 91.0 µg*hr/L and 130.4 µg*hr/L, respectively (*P* = .113; Table 3).

Table 2 Pharmacokinetic parameters for evaluation of the relation between sunitinib and midazolam

Parameter[a]	Sunitinib at steady-state
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	1105 ± 189
Cl/F (L/hr)	71.8 ± 21.8
$C_{ss,min}$ (ng/mL)	43.6 ± 8.1
$T_{l,max}$ (hr)	6.1 ± 1.1
$C_{l,max}$ (ng/mL)	57.6 ± 8.6
	Midazolam after a single dose without sunitinib
$AUC_{0\text{-}7hr}$ (ng·hr/mL)	130.4 ± 22.9
$AUC_{0\text{-}\infty}$ (ng·hr/mL)	162.1 ± 34.7
$T_{l,max}$ (hr)	1.0 ± 0.3
$C_{l,max}$ (ng/mL)	51.9 ± 5.8

Results are presented as mean values ± the standard error of the mean (SEM).
[a] Sunitinib $C_{ss,min}$, $C_{l,max}$ and $AUC_{0\text{-}24hr}$ values are normalized to a sunitinib dose of 50 mg.
Abbreviations: $AUC_{0\text{-}24hr}$ = area under the plasma concentration-time curve at steady-state over a dose interval of sunitinib; $AUC_{0\text{-}7hr}$ = area under the plasma concentration-time curve over the observed interval after a single midazolam dose; $AUC_{0\text{-}\infty}$ = area under the plasma concentration-time curve over a time interval 0 - infinity; CL/F = apparent oral clearance; $C_{ss,min}$ = average trough plasma concentration; $T_{l,max}$ = time to reach peak plasma concentration; $C_{l,max}$ = peak plasma concentration.

Figure 2 Individual observed sunitinib plasma concentrations versus time profiles



Seven solid lines represent patients with sunitinib 50 mg/day. The two dotted lines represent the patients with sunitinib 37.5 mg/day

Figure 3 Correlation between **A**: sunitinib trough levels ($C_{ss,min}$) and sunitinib exposure ($AUC_{0\text{-}24hr}$), **B**: Midazolam exposure ($AUC_{0\text{-}7hr}$) and sunitinib trough levels ($C_{ss,min}$) **C**: Midazolam exposure ($AUC_{0\text{-}7hr}$) and sunitinib exposure ($AUC_{0\text{-}24hr}$)

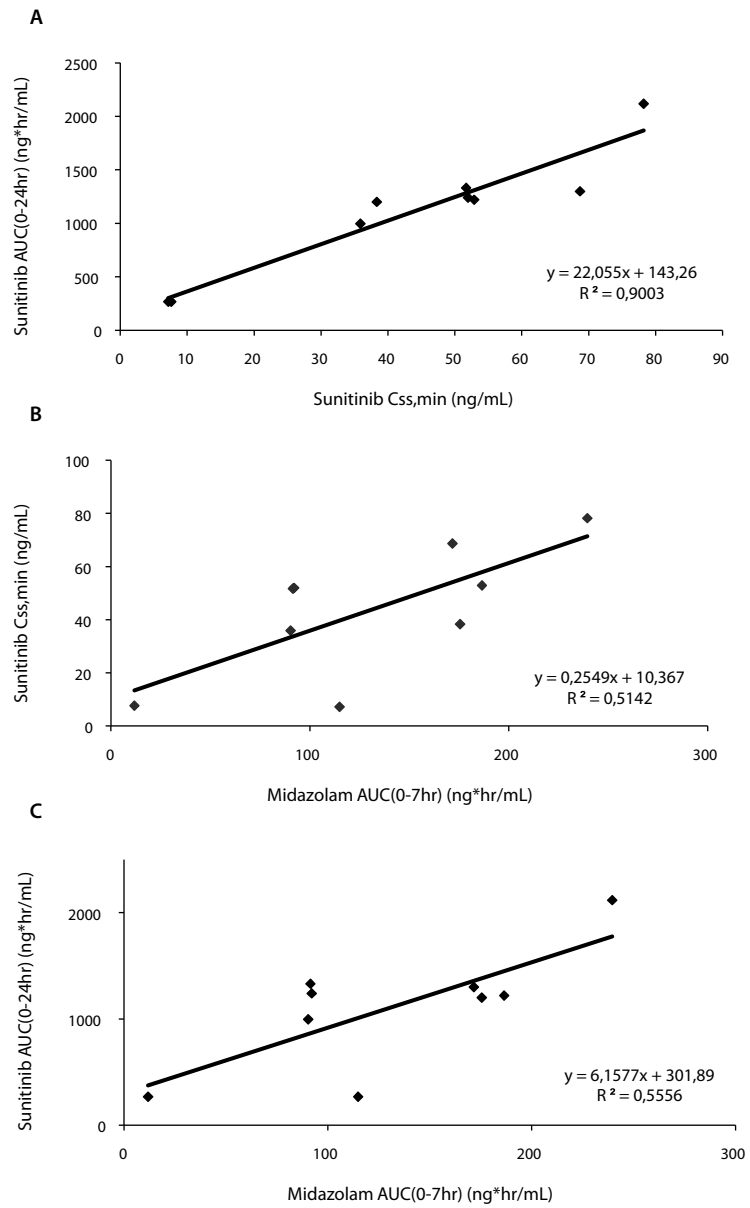


Table 3 Midazolam pharmacokinetics with and without sunitinib

Parameters	Midazolam alone	Midazolam with sunitinib	*P* value
$AUC_{0\text{-}7hr}$ (ng·hr/mL)	130.4 ± 22.9	91.0 ± 21.3	.113
$AUC_{0\text{-}\infty}$ (ng·hr/mL)	162.1 ± 34.7	118.8 ± 28.4	.092
$T_{l,max}$ (hr)	1.0 ± 0.3	1.0 ± 0.5	.895
$C_{l,max}$ (ng/mL)	51.9 ± 5.8	50.6 ± 12.2	.926

Results are presented as mean values ± the standard error of the mean (SEM)
Abbreviations: $AUC_{0\text{-}7hr}$ = area under the plasma concentration-time curve over the observed interval after a single midazolam dose; $AUC_{0\text{-}\infty}$ = area under the plasma concentration-time curve over a time interval 0 - infinity; $T_{l,max}$ = time to reach peak plasma concentration; $C_{l,max}$ = peak plasma concentration

Discussion

This study shows that sunitinib exposure is highly related to CYP3A4 activity. Also a strong relationship between sunitinib trough levels and sunitinib exposure is observed. Moreover, sunitinib appears to show a trend towards CYP3A4 induction, however this was not found significant.

Sunitinib is metabolized by cytochrome P450 3A4 (CYP3A4)[5]. No other enzymes are known to be involved in sunitinib metabolism[5, 6]. In addition, sunitinib appears to be an *in vitro* substrate and inhibitor for the ATP-binding cassette transporters ABCG2 and ABCB1 and these transporters may, therefore, also contribute to sunitinib disposition *in vivo*[13]. Similarly, midazolam is extensively metabolized by CYP3A4 with less affinity for CYP3A5[15]. It, however, appears to be a poor substrate of ABCB1 (P-glycoprotein; MDR1) and ABCG2 (BCRP; MXR)[16, 17]. Oral midazolam is widely accepted and used as a probe for evaluating (hepatic and intestinal) CYP3A4 activity, without influencing the activity of this enzyme[18-21].

Former studies have shown that sunitinib pharmacokinetics comprises high interpatient variability (~ 40%) with respect to drug exposure. Until now, this high interpatient variability was only marginally explained by the studied variables; tumor type, race, sex, body weight, and Eastern Cooperative Oncology Group score[22, 23]. The relationship between sunitinib exposure and clinical efficacy or toxicity has not yet been elucidated but substantial pharmacokinetic variability is likely to impact treatment outcome. Phenotyping patients for CYP3A4 activity may not only help to understand variability in sunitinib pharmacokinetics but it may also be a future clinical tool to individualize and optimize sunitinib treatment.

In the presented study the interindividual variability in the sunitinib exposure was large (51%) and partially explained by midazolam exposure. Therefore, CYP3A4 activity as assessed by oral midazolam phenotyping adds to the variables identified in explaining the variability in sunitinib exposure[23]. In fact, CYP3A4 activity explained a large part (51%) of the total interpatient variability in sunitinib exposure and might therefore help to identify patients predisposed to relatively high sunitinib exposure or those that are potentially underdosed.

When plasma drug concentrations are monitored to guide individual therapy, drug exposure is typically assessed by estimating the area under the plasma concentration time curve after taking blood samples at different time points. However, this strategy is difficult and time consuming in clinical practice and the use of surrogate parameters, such as drug trough levels, are favorable to determine drug exposure. To our knowledge, this is the first study that shows that sunitinib trough levels are highly correlated to sunitinib exposure. Therefore, through drug level measurement could be interesting especially since for imatinib, another tyrosine kinase inhibitor, a relation between elevated drug exposure and toxicities as well as minimal exposure levels and efficacy has been demonstrated[24, 25]. Our finding implicates that a relationship between sunitinib exposure and efficacy or toxicity could be studied by measuring sunitinib trough levels instead of sunitinib exposure, which is a more feasible approach than monitoring total exposure curves in treated patients. Before therapeutic drug monitoring is considered in clinical practice additional information on concentration-effect relationship of sunitinib for the different tumor subtypes is warranted[1, 26, 27].

Sunitinib shows a trend towards induction of CYP3A4 metabolism resulting in a reduced midazolam exposure while co-administered. Only gefitinib showed a similar interaction with midazolam in an *in vitro* study, however this effect has not been confirmed *in vivo* yet[10]. All other tyrosine kinase inhibitors are either CYP3A4 inhibitors (imatinib, dasatinib, and nilotinib) or show no influence on CYP3A4 metabolism, however caution with concomitant administered CYP3A4 substrates is still warranted[8, 9, 28].

The relatively small number of patients in this study may be considered as a limitation especially for regarding accurate estimation of interpatient variability in sunitinib PK and assessment of relationships. However, the dense sampling results in a reliable determination of the exposure to sunitinib and midazolam. Moreover, the interpatient variability in our study is consistent with the interpatient variability of ~ 40% reported so far[5, 23].

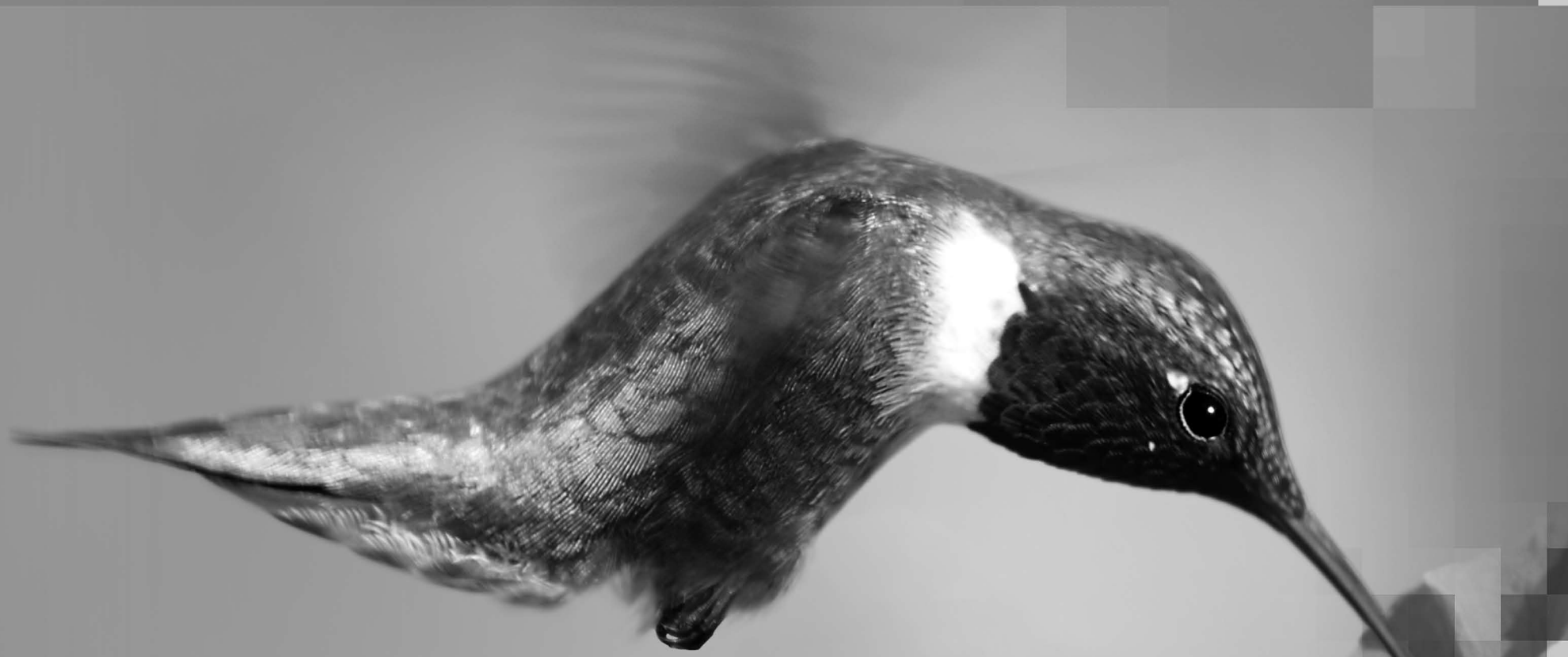
In conclusion, variability in sunitinib PK is strongly related to CYP3A4 activity and therefore CYP3A4 phenotyping could be useful for individualizing the sunitinib starting dose. Sunitinib trough levels relate well to sunitinib exposure, making this more assessable approach suitable for studying the exposure-efficacy and exposure-toxicity relations.

References

1. Mendel DB, Laird AD, Xin X et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 2003; 9(1):327-337.
2. Motzer RJ, Rini BI, Bukowski RM et al. Sunitinib in patients with metastatic renal cell carcinoma. JAMA 2006; 295(21):2516-2524.
3. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356(2):115-124.
4. Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006; 368(9544):1329-1338.
5. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
6. Adams VR, Leggas M. Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Ther 2007; 29(7):1338-1353.
7. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.
8. Tanaka C, Smith T, Kantarjian H et al. Clinical pharmacokinetics (PK) of AMN107, a novel inhibitor of Bcr-Abl, in healthy subjects and patients with imatinib resistant or intolerant chronic myelogenous leukemia (CML) or replapsed/refractory Ph+ acute lymphocytic leukemia (Ph+ALL). J Clin Oncol, 2006 ASCO Annual Meeting Proceedings Part I Vol. 24, No. 18S, 3095. 2006.
9. O'Brien SG, Meinhardt P, Bond E et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. Br J Cancer 2003; 89(10):1855-1859.
10. Li J, Zhao M, He P, Hidalgo M, Baker SD. Differential metabolism of gefitinib and erlotinib by human cytochrome P450 enzymes. Clin Cancer Res 2007; 13(12):3731-3737.
11. Swaisland HC, Ranson M, Smith RP et al. Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. Clin Pharmacokinet 2005; 44(10):1067-1081.
12. Hazarika M, Jiang X, Liu Q et al. Tasigna for chronic and accelerated phase Philadelphia chromosome--positive chronic myelogenous leukemia resistant to or intolerant of imatinib. Clin Cancer Res 2008; 14(17):5325-5331.
13. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. Drug Metab Dispos 2009; 37(2):359-365.
14. Minkin P, Zhao M, Chen Z, Ouwerkerk J, Gelderblom H, Baker SD. Quantification of sunitinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 874(1-2):84-88.
15. Fuhr U, Jetter A, Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the „cocktail" approach. Clin Pharmacol Ther 2007; 81(2):270-283.
16. Kim RB, Wandel C, Leake B et al. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. Pharm Res 1999; 16(3):408-414.
17. Kurnik D, Wood AJ, Wilkinson GR. The erythromycin breath test reflects P-glycoprotein function independently of cytochrome P450 3A activity. Clin Pharmacol Ther 2006; 80(3):228-234.
18. U.S.Food and Drug Administration, Center for Drug evaluation and Research, Clinical Pharmacology, Drug Interaction Studies--Study Design. http://www.fda.gov/cder/guidance/#clinical%20pharmacology .
19. Kirby B, Kharasch ED, Thummel KT, Narang VS, Hoffer CJ, Unadkat JD. Simultaneous measurement of in vivo P-glycoprotein and cytochrome P450 3A activities. J Clin Pharmacol 2006; 46(11):1313-1319.
20. Wyen C, Fuhr U, Frank D et al. Effect of an antiretroviral regimen containing ritonavir boosted lopinavir on intestinal and hepatic CYP3A, CYP2D6 and P-glycoprotein in HIV-infected patients. Clin Pharmacol Ther 2008; 84(1):75-82.
21. Magnusson MO, Dahl ML, Cederberg J, Karlsson MO, Sandstrom R. Pharmacodynamics of carbamazepine-mediated induction of CYP3A4, CYP1A2, and Pgp as assessed by probe substrates midazolam, caffeine, and digoxin. Clin Pharmacol Ther 2008; 84(1):52-62.
22. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006; 24(1):25-35.
23. Houk BE, Bello CL, Kang D, Amantea M. A population pharmacokinetic meta-analysis of sunitinib malate (SU11248) and its primary metabolite (SU12662) in healthy volunteers and oncology patients. Clin Cancer Res 2009; 15(7):2497-2506.
24. Larson RA, Druker BJ, Guilhot F et al. Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. Blood 2008; 111(8):4022-4028.
25. Picard S, Titier K, Etienne G et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood 2007; 109(8):3496-3499.
26. Hu S, Niu H, Minkin P et al. Comparison of antitumor effects of multitargeted tyrosine kinase inhibitors in acute myelogenous leukemia. Mol Cancer Ther 2008; 7(5):1110-1120.
27. Ikezoe T, Yang Y, Nishioka C et al. Effect of SU11248 on gastrointestinal stromal tumor-T1 cells: enhancement of growth inhibition via inhibition of 3-kinase/Akt/mammalian target of rapamycin signaling. Cancer Sci 2006; 97(9):945-951.
28. Brave M, Goodman V, Kaminskas E et al. Sprycel for chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant to or intolerant of imatinib mesylate. Clin Cancer Res 2008; 14(2):352-359.

7

Pharmacogenetic Pathway Analysis for determination of Sunitinib-induced Toxicity



Nielka P. van Erp, Karel Eechoute, Astrid A. van der Veldt,
John B. Haanen, An K.L. Reyners, Ron H.J. Mathijssen,
Epie Boven, Tahar van der Straaten, Renée F. Baak-Pablo,
Judith A.M. Wessels, Henk-Jan Guchelaar and Hans Gelderblom
Journal of Clinical Oncology 2009
(published online ahead of print, August 10, 2009)

Abstract

Purpose: To identify genetic markers in the pharmacokinetic and pharmacodynamic pathways of sunitinib that predispose for development of toxicities; thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any toxicity according to National Cancer Institute Common Toxicity Criteria higher than grade 2.

Patients and Methods: A multicenter pharmacogenetic association study was performed in 219 patients treated with single-agent sunitinib. A total of 31 single nucleotide polymorphisms in 12 candidate genes, together with several nongenetic variants, were analyzed for a possible association with toxicity. In addition, genetic haplotypes were developed and related to toxicity.

Results: The risk for leukopenia was increased when the G-allele in *CYP1A1* 2455A/G (odds ratio [OR], 6.24; *P* = .029) or the T-allele in *FLT3* 738T/C (OR, 2.8; *P* = .008) were present or CAG in the *NR1I3* (5719C/T, 7738A/C, 7837T/G) haplotype (OR, 1.74; *P* = .041) was absent. Any toxicity higher than grade 2 prevalence was increased when the T-allele of *VEGFR-2* 1191C/T (OR, 2.39; *P* = .046) or a copy of TT in the *ABCG2* (-15622C/T, 1143C/T) haplotype (OR, 2.63; *P* = .016) were present. The risk for mucosal inflammation was increased in the presence of the G-allele in *CYP1A1* 2455A/G (OR, 4.03; *P* = .021) and the prevalence of hand-foot syndrome was increased when a copy of TTT in the *ABCB1* (3435C/T, 1236C/T, 2677G/T) haplotype (OR, 2.56; *P* = .035) was present.

Conclusion: This exploratory study suggests that polymorphisms in specific genes encoding for metabolizing enzymes, efflux transporters, and drug targets are associated with sunitinib-related toxicities. A better understanding of genetic and nongenetic determinants of sunitinib toxicity should help to optimize drug treatment in individual patients.

Introduction

The oral, multitargeted tyrosine kinase inhibitor sunitinib (sunitinib malate; Sutent; Pfizer Pharmaceuticals Group, New York, NY) is known to inhibit vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3, platelet-derived growth factor receptor (PDGFR) α and β, KIT, Fms-like tyrosine kinase 3 receptor (FLT3), and the receptor encoded by the ret proto-oncogene (RET).[1-4] Sunitinib is approved for first-line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic gastrointestinal stromal tumors (GIST).[4-6] Targeted cancer therapies are generally considered to be less toxic than conventional chemotherapy since they specifically inhibit tyrosine kinase receptors that are frequently overexpressed or mutated in various types of tumor cells.[7] Tyrosine kinases, however, are also present in normal tissues and toxic effects are therefore difficult to eliminate. The 4 weeks on 2 weeks off dosing schedule of sunitinib was selected for the first phase I study on request of the health authorities to allow patients to recover from potential bone marrow and adrenal toxicity observed in animal models, indicating that toxicity was regarded as a serious problem.[3, 8] Although the proportion of patients with grade 3 to 4 adverse events was relatively low in the recent phase III studies, a dose interruption appeared to be necessary in 38% of patients with mRCC and in 28% of patients with GIST whereas a dose reduction was required in 32% and 11%, respectively. Similar percentages were reported in other studies.[2, 4, 9] Disease- and sunitinib-related toxicities can be distinguished based on results of a phase III trial in which the toxicity profile of sunitinib-treated patients has been compared with events in the placebo-treated patients.[2] Adverse events that preferentially occurred in the group treated with sunitinib were diarrhea, hand-foot syndrome, mucositis, vomiting, hypertension, leukopenia, neutropenia, and thrombocytopenia.[2-4, 9-13] Less common, but specific toxicities related to sunitinib were cardiotoxicity and hypothyroidism.[5, 14, 15]

Sunitinib is used as palliative therapy with no standard therapeutic options available after failure of the therapy. It is therefore relevant for patients to adhere to sunitinib therapy while their quality of life is not unnecessarily reduced by drug toxicity. To date, it is not completely clear which patient characteristics render an individual patient at risk for sunitinib-induced toxicity. The aim of the present study is to identify genetic markers in sunitinib disposition, metabolism, and mechanism of action that predispose for development of common sunitinib related toxicities: thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any higher than grade 2 National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) toxicity.

Patients and methods

A total of 219 patients from five Dutch medical centers were analyzed in this study. The study was approved by the medical ethics review board. Patients were treated at the Erasmus University Medical Center (n=74), the Netherlands Cancer Institute (n=51), Leiden University Medical Center (n=37), VU University Medical Center (n=36), and the University Medical Center Groningen (n=21). The collection of DNA and patient data was performed between June 2004 and May 2008. A total number of 159 mRCC, 50 GIST, and 10 patients with other tumors were included in this study. Of them, 77 patients with mRCC and 26 patients with GIST were treated according to an expanded access programme of sunitinib. Eligible patients were those treated with single agent sunitinib for at least one treatment cycle (4 consecutive weeks of 50 mg per day followed by a two-week period of rest).

Study design

Sunitinib toxicity was evaluated during the first treatment cycle by CTCAE version 3.0.[16] Toxicity scores were assessed by analysis of adverse events, physical examination and laboratory assessments carried out at baseline (before starting sunitinib), after 4 weeks of sunitinib therapy, and after 6 weeks (just before starting the second cycle). Demographic and clinical data of patients were reported on case record forms designed for data collection in this study. Patient characteristics considered relevant for experiencing toxicity were: age, gender, ethnicity, body-surface area (BSA), Eastern Cooperative Oncology Group (ECOG) performance status, tumor type, renal, liver and bone marrow function (serum creatinine, total bilirubin, albumin, ALT, AST, hemoglobin, leukocytes and thrombocytes). Residual blood or serum samples taken for routine patient care were stored at -20ºC at the local hospital laboratory. Of each patient one whole blood or serum sample was collected from the participating centers. All samples were anonymized by a third party, according to the instructions stated in the Codes for Proper Use and Proper Conduct in the Self-Regulatory Codes of Conduct (www.federa.org).

Definition of toxicity

All adverse events were graded by independent physicians of the participating medical centers. Four- and 6-week reported toxicities were compared to baseline conditions. The primary outcome measures of this study were thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any toxicity higher than grade 2. Toxicities were selected based on objectivity, clinical relevance and manageability of the symptoms. Thrombocytopenia and leukopenia were scored from blood cell counts and are thus objective endpoints. In case of any toxicity higher than grade 2, a dose interruption and, depending on the kind of toxicity, a resumed treatment with 25% dose reduction is advised in the drug label of sunitinib. Moreover, mucosal inflammation and hand-foot syndrome are frequently reported and poorly manageable and therefore dose reduction is relatively soon considered. In addition, dose reduction of at least 25% according to the drug label (data complete for 187 patients) which is applied because of safety or tolerability issues, after cycle 1 to 3 was related to the toxicity outcomes.

Genetic Polymorphisms

Nineteen polymorphisms in seven genes involved in the pharmacokinetics and 12 polymorphisms in five genes involved in the pharmacodynamics of sunitinib were selected. Selection criteria for the polymorphisms were an allelic frequency higher than 0.2 in whites and an assumed clinical relevance based on previously reported associations or the assumption that nonsynonymous amino acid change leads to changed protein functionality. The selected polymorphisms are listed in Table 1.

Genotyping of selected polymorphisms

Germline DNA was isolated from 1 ml of serum or EDTA-blood with the Magnapure LC (Roche Diagnostics, Almere, The Netherlands). DNA concentrations were quantified on the nanodrop (Isogen, IJsselstein, The Netherlands). Taqman assays were obtained from Applied Biosystems (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). All Single Nucleotide Polymorphisms (SNPs) were initially determined on the Biomark 48.48 Dynamic Array (Fluidigm, San Fransisco, CA, USA) according to the manufacturer's protocol. Failed samples were repeated on the TaqMan 7500 (Applied Biosystems), according to standard procedures. For serum samples, a pre-amplification step was necessary. Briefly, a dilution of all TaqMan assays in a total volume of 1.25 μL and 2.5 μL of pre-amplification mastermix (Applied Biosystems) was added to 1.25 μL of serum-DNA, and subsequently amplified by polymerase chain reaction. This mixture was 20 times diluted and 2.5 μL was used in the Biomark array according to the protocol.

Genotyping assay validity

The overall average success rate of the assays and the individual samples was 98%. The lowest success rate in our study was 93.5%. As a quality control, all DNA samples were genotyped in duplicate for 12 of 31 SNPs, and three DNA samples were genotyped in duplicate for all 31 SNPs. No inconsistencies were observed. In addition negative controls (water) were used. The allelic frequencies of the 31 single nucleotide polymorphisms were tested for Hardy-Weinberg equilibrium (HWE). Six genotype assay results did not meet HWE. However, of four of these, frequencies were compared with allelic frequencies as reported on the National Center for Biotechnology Information website (NCBI) for white population and found similar to the reported frequencies. Of the two remaining SNPs no frequencies were available on the

Table 1 Polymorphisms genotyped in the pharmacokinetic and pharmacodynamic pathway of sunitinib

	Gene	Polymorphism	rs-number
Pharmacokinetic pathway	NR1I2	-25385C/T	rs3814055
		-24113G/A	rs2276706
		7635A/G	rs6785049
		8055C/T	rs2276707
		10620C/T	rs1054190
		10799G/A	rs1054191
	NR1I3	5719C/T	rs2307424
		7738A/C	rs2307418
		7837T/G	rs4073054
	CYP3A5	6986A/G	rs776746
	CYP1A1	2455A/G	rs1048943
	CYP1A2	-163A/C	rs762551
	ABCG2	421C/A	rs2231142
		34G/A	rs2231137
		-15622C/T	*
		1143C/T	rs2622604
	ABCB1	3435C/T	rs1045642
		1236C/T	rs1128503
		2677G/T	rs2032582
Pharmacodynamic pathway	PDGFRα	1580T/C	rs35597368
		-1171C/G	rs1800810
		-735G/A	rs1800813
		-573G/T	rs1800812
	VEGFR2 (=KDR)	-604T/C	rs2071559
		-92G/A	rs1531289
		54T/C	rs7692791
		1191C/T	rs2305948
		1718T/A	rs1870377
	VEGFR3 (=FLT4)	1501A/G	rs307826
	RET	2251G/A	rs1799939
	FLT3	738T/C	rs1933437

* No rs-number assigned yet

NCBI website (www.ncbi.nlm.nih.gov). The homozygotic wildtype frequencies of both SNPs exceed the HWE and were therefore allowed for the analysis.

Haplotype estimation

Polymorphisms within a gene were tested to detect linkage disequilibrium (LD). If LD between SNPs was present, haploblocks (with several haplotypes) were determined. The uncertainty measure R_h^2 was calculated. R_h^2 gives us information on the uncertainty in the prediction of common haplotypes from unphased SNP genotypes [17]. A haplotype was considered to be present if the haplotype uncertainty measure R_h^2 was greater than 0.98 as tested with the software program CHAPLIN [18]. Haplotypes with an uncertainty measure $R_h^2 \leq 0.7$ in CHAPLIN were not considered for further analysis since the data provided no information on haplotypes in our population. All haplotypes with uncertainty ($0.7 < R_h^2 \leq 0.98$) and without uncertainty ($R_h^2 > 0.98$) were computed and assigned per individual using gPLINK [19]. Rare haplotypes (< 2%) were combined into one group of other haplotypes in the association analysis. The haplotypes used in this study had no phase uncertainty ($R_h^2 > 0.98$). The *VEGFR-2* gene had a large phase uncertainty ($R_h^2 \leq 0.7$) indicating that in our population *VEGFR-2* polymorphisms could not be defined as a haplotype. The following SNPs were combined for further analysis: *ABCG2*; 1143C/T and -15622C/T; *PDGFR*α; -573G/T, -1171C/G, -735G/A, 1580T/C; *NR1I3*; 5719C/T, 7738A/C, 7837T/G; *NR1I2 ;* 10620C/T, 10799G/A and *ABCB1*; 3435C/T, 1236C/T, 2677G/T.

Statistical design and data analysis

For the analysis of toxicity, we used dichotomous end points expressed as increased toxicity (yes or no) or any toxicity higher than grade 2 (yes or no). All demographic and clinical variables were tested univariately against the selected primary outcomes using *t* test, the Mann-Whitney *U* test or the χ^2 test, depending on the tested variables. A χ^2 test was also used to detect linkage disequilibrium (LD). The polymorphisms were initially tested with 2 *df*. If the initial 2 *df* tests resulted in $P \leq .1$, the polymorphisms were fitted and the most appropriate model (multiplicative, dominant, or recessive) was selected. The number of copies of each haplotype was used as parameter in the analysis. The polymorphisms and haplotypes were tested univariately against the selected primary outcomes using a χ^2 test. Candidate variables with $P \leq .1$ were selected for the multiple logistic regression analysis with toxicity as depending variable. All multivariate logistic regression analyses were corrected for age, gender and ECOG performance status. Additional patient characteristics were introduced in the multivariate analyses based on univariate tested results if $P \leq .1$. Missing data were kept as missing data except for BSA and ECOG performance status. Missing BSA values (n=15) were replaced for the median BSA ($1.93m^2$) and missing ECOG performance status (n=7) were replaced for the median ECOG performance status (1). To test this action, the multivariate analyses were performed with and without the replacement of the patients with missing BSA and ECOG performance status. Similar results were generated, indicating that the replacement was legitimate. All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL, USA). With the sample size of our study, an increase in toxicity of 17% could be measured between two groups with a power of 80% and a confidence interval of 99%. All results from the multivariate analyses with P less than .05 were considered significant. Since this was an exploratory study, no correction for multiple testing was done.

Results

Patients

Nineteen out of 219 patients had to be excluded from analysis for several reasons including progressive disease (PD) during the first treatment cycle resulting in early death (n=4), discontinuation of sunitinib in the first treatment cycle due to adverse events (hypertension grade 3, headache grade 3 and rash grade 3, respectively; n=3) and no acceptable genotyping success rate due to poor DNA quality (n=12). For toxicity analyses, a total of 200 patients were evaluable (Table 2). For the endpoint any toxicity higher than grade 2, the three patients who stopped therapy due to adverse events were included (n=203).

Table 2 Patient characteristics (N=203)

Characteristic		Value
Age (years)		
Median (range)		60 (20-84)
Sex		
Male		129 (63.5%)
Female		74 (36.5%)
Body Surface Area (square meters)		
Median (range)		1.93 (1.47-2.51)
ECOG performance status		
0		81 (39.9%)
1		90 (44.3%)
2		17 (8.4%)
3		8 (3.9%)
Missing		7 (3.4%)
Ethnicity		
Caucasian		190 (93.6%)
Blacks		6 (3.0%)
Asian		2 (1.0%)
Latin-American		2 (1.0%)
Middle East		3 (1.5%)
Tumor types		
Renal cell carcinoma		152 (74.9%)
Gastrointestinal stromal tumor		46 (22.7%)
Other		5 (2.5%)
Previous medical treatments		
Yes*		116 (57.1%)
No		87 (42.9%)
First treatment regimen (N=116)*		
Interferon-alpha (INF-α)		46 (39.7%)
Imatinib		46 (39.7%)
Sorafenib		5 (4.3%)
Others		19 (16.4%)
Dose reduction after sunitinib cycle 1 – 3		
Yes	Renal cell carcinoma	58 (28.6%)
	GIST	14 (6.9%)
	Other tumor	1 (0.5%)
No	Renal cell carcinoma	94 (46.3%)
	GIST	32 (15.8%)
	Other tumor	4 (2.0%)
Baseline chemistry and hematology		
Creatinine (μM)		
Median (range)		96.0 (40-176)
Total bilirubin (μM)		
Median (range)		7 (3-32)
Albumine (gram/L)		
Median (range)		40 (23-52)
ALT (units/L)		
Median (range)		18 (3-210)
AST (units/L)		
Median (range)		24 (9-190)
Hemoglobin (mM)		
Median (range)		7.6 (5.2-10.4)
Leukocytes (*10^9/L)		
Median (range)		7.5 (3.6-56.5)
Thrombocytes (*10^9/L)		
Median (range)		284.0 (92-864)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECOG, Eastern Cooperative Oncology Group. CR, complete response ; PR, partial response; SD stable disease ; PD, progressive disease.

Toxicities

The hematological toxicities scored in this analysis were thrombocytopenia (40% any grade), leukopenia (59%, any grade). Non-hematological toxicities were primarily any toxicity higher than 2 (22%), mucosal inflammation (44%) and hand-foot syndrome (19%; Table 3). Dose reduction after cycle 1 to 3 was related to mucosal inflammation ($P = .002$) and any toxicity higher than grade 2 ($P < .001$)

Table 3 Number (No) of patients (%) according to the distribution of increased toxicity grades

Toxicity	No/Yes	Grade	No (%)
Thrombocytopenia (n=198)	No		118 (59.0)
	Yes	1	58 (29.0)
		2	14 (7.0)
		3	7 (3.5)
		4	1 (0.5)
Leukopenia (n=198)	No		81 (40.5)
	Yes	1	91 (45.5)
		2	22 (11.0)
		3	4 (2.0)
Any toxicity > 2 (n=203)		0, 1, 2	158 (77.8)
		3, 4	45 (22.2)
Mucosal inflammation (n=199)	No		112 (56.0)
	Yes	1	57 (28.5)
		2	25 (12.5)
		3	5 (2.5)
Hand-foot syndrome (n=199)	No		162 (81.0)
	Yes	1	27 (13.5)
		2	8 (4.0)
		3	2 (1.0)

Pharmacogenetic risk factors for sunitinib-induced toxicity

The results of the multivariate logistic regression analysis for the selected endpoints thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any toxicity higher than grade 2 are summarized in Table 4. For thrombocytopenia, an increase in age (P = .030) and ECOG performance status (P = .050) were independently significant in the multivariate logistic model. The factors associated with development of leukopenia were: *CYP1A1* 2455A/G; the presence of the G allele in an additive model was related to a 6.2-fold increase in the risk for leukopenia during the first treatment cycle (P = .029); the presence of the *FLT3* 738C allele (dominant model) was related to a 2.8-fold reduction in the risk for leukopenia (P = .008); the absence of the *NR1I3* CAG haplotype was related to a 1.7-fold increased risk for leukopenia (P = .041) and 4); one grade increase in ECOG performance status, implicating a worse clinical condition, was related to a 1.8-fold reduction in the risk of leukopenia (P = .016). The presence of the *VEGFR-2* 1191T-allele (additive model) was related to an increased risk of 2.4-fold for the development of any toxicity higher than grade 2 (P = .046), while the risk for this toxicity was 2.6-fold higher when 1 or 2 copies of TT in the *ABCG2* haplotype were present (P = .016). For mucosal inflammation only *CYP1A1* 2455A/G was independently related; the G-allele (additive model) resulted in a 4.0-fold higher risk for mucosal inflammation (P = .021). The occurrence of hand-foot syndrome was related to the *ABCB1* haplotype; the absence of copies of the TTT haplotype was protective and was related to a 2.6-fold lower risk to experience hand-foot syndrome as compared to patients with copies of the TTT haplotype (P=.035). The explained variance (R^2) of the patient characteristics, without taking the polymorphisms into account, in the multivariate analyses was between the 2 to 10% of the total variance. After adding the selected polymorphisms the explained variance increased to 10 to 23% of the total variance.

Discussion

To the best of our knowledge, this is the first study exploring the relationship between drug-induced toxicity and genetic polymorphisms in genes encoding for enzymes, efflux transporters and targets involved in the pharmacokinetics and pharmacodynamics of sunitinib.

Sunitinib is metabolized by cytochrome P450 3A4 (CYP3A4) and CYP3A5. In addition, affinity of sunitinib for the ATP-binding cassette transporters ABCG2 and ABCB1 has also recently been reported.[20] The transcription of CYP3A4 is regulated by members of the NR1I nuclear receptor subfamily.[21] Metabolism through CYP1A1 and CYP1A2 is hypothesized since these enzymes appear to be involved in the metabolism of multiple tyrosine kinase inhibitors (eg, imatinib, erlotinib).[22, 23] Both genes encoding the sunitinib targets, as well as genes encoding the enzymes (except for CYP3A4, in which no functional polymorphisms have been identified) and efflux transporters involved in sunitinib's disposition and metabolism are highly polymorphic and may be related to the differential toxicity response in patients treated with sunitinib.

Although the nature and incidence of adverse events related to sunitinib are currently well recognized and described, data regarding determinants of toxicity are still scarce.[2, 4, 5, 14, 24, 25] So far, only one study has described factors (low BSA, high age, female gender) that are associated with the development of severe toxicities, defined as dose reduction or permanent discontinuation of sunitinib therapy.[9] That study, however, was limited to patient characteristics and no genetic determinants were investigated. In our study, these patient characteristics, and another (performance status), were included as covariates in the data analysis. We should emphasize, however, that the definition of the endpoint severe toxicity is different in both studies as well as the observed study period (whole sunitinib treatment period *v* first treatment cycle in our study).

Table 4 Factors relevant for sunitinib-induced toxicity, defined as thrombocytopenia, leukopenia, any toxicity > grade 2, mucosal inflammation or hand-foot syndrome

	Data corrected for patient characteristics[1]				Uncorrected data[1]	
	Factor	**Genotype**	**OR (95% CI)**	***P-value***	**OR (95% CI)***	***P-value***
Thrombocytopenia (n=177)	Age		1.04 (1.00–1.07)	**.030**		
	Gender		1.93 (0.97–3.85)	.063		
	ECOG		0.60 (0.37–1.00)	**.050**		
	NR1I2 -24113 G/A	GG *vs*	1		1	
		AG + AA	0.60 (0.28–1.28)	.187	0.51 (0.25–1.05)	0.067
	CYP1A2 -163 A/C	AA *vs*	1		1	
		AC + CC	0.67 (0.34–1.32)	.251	0.65 (0.34–1.23)	0.186
	ABCG2 421 C/A	CC *vs*	1		1	
		CA + AA	2.09 (0.88–4.96)	.096	1.93 (0.85–4.42)	0.118
	ABCG2 haplotype ξ	TT-TT + TT-other *vs*	1		1	
		Other-other	1.93 (0.96–3.86)	.065	1.77 (0.91–3.46)	0.093
	PDGFRα haplotype ψ	TGAT-TGAT + TGAT-other	1		1	
		vs Other-other	0.44 (0.17–1.18)	.103	0.36 (0.14–0.92)	**0.033**
Leukopenia (n=188)	Age		1.01 (0.98–1.04)	.423		
	Gender		0.81 (0.41–1.60)	.536		
	ECOG		0.57 (0.36–0.90)	**.016**		
	VEGFR-2 -92G/A	GG → GA → AA	0.74 (0.44–1.23)	.241	0.74 (0.45–1.22)	.235
	VEGFR-2 1718 T/A	TT → AT → AA	1.49 (0.84–2.66)	.172	1.47 (0.83–2.60)	.188
	CYP1A1 2455A/G	AA → AG → GG	6.24 (1.20–32.42)	**.029**	4.87 (1.06–22.29)	**.042**
	FLT 3 738T/C	TT *vs*	1		1	
		CT + CC	0.36 (0.17–0.77)	**.008**	0.41 (0.20–0.85)	**.016**
	NR1I3 haplotype Ω	CAG-CAG → CAG-other	1.74 (1.02–2.96)	**.041**	1.81 (1.07–3.04)	**.026**
		→ other-other				
	NR1I3 haplotype Ω	TCT-TCT → TCT-other	0.54 (0.27–1.06)	.074	0.55 (0.28–1.06)	.075
		→ Other-other				
Any toxicity > grade 2 (n=183)	Age		1.03 (0.99–1.07)	.140		
	Gender		1.71 (0.69–4.26)	.248		
	ECOG		1.31 (0.79–2.19)	.299		
	BSA		0.22 (0.02–2.49)	.220		
	VEGFR-2 1191 C/T	CC → TC → TT	2.39 (1.02–5.60)	**.046**	2.31 (1.07–4.99)	**.033**
	ABCG2 haplotype ξ	TT-TT + TT-other *vs*	1		1	
		Other-other	0.38 (0.17–0.83)	**.016**	0.40 (0.19–0.84)	**.016**
Mucosal Inflammation (n=193)	Age		1.00 (0.97–1.03)	.956		
	Gender		1.54 (0.82–2.88)	.177		
	ECOG		1.31 (0.86–1.99)	.212		
	NR1I2 -24113G/A	GG *vs*	1		1	
		AG + AA	0.58 (0.30–1.13)	.110	0.55 (0.29–1.04)	.064
	CYP1A1 2455A/G	AA → AG → GG	4.03 (1.24–13.09)	**.021**	4.15 (1.29–13.36)	**.017**
	ABCG2 34G/A	GG → AG	2.45 (0.74–8.17)	.144	2.41 (0.75–7.76)	.140
	NR1I3 haplotype Ω	TCT-TCT → TCT-other	0.78 (0.42–1.44)	.420	0.77 (0.42–1.42)	.404
		→ Other-other				
Hand-Foot Syndrome (n=182)	Age		0.99 (0.96–1.02)	.563		
	Gender		1.22 (0.56–2.68)	.612		
	ECOG		0.76 (0.43–1.33)	.336		
	ABCB1 haplotype ∞	TTT-TTT + TTT-other	1		1	
		vs Other-other	0.39 (0.16–0.94)	**.035**	0.39 (0.16–0.92)	**.032**
	ABCB1 haplotype ∞	CTT-CTT → CTT-other	0.38 (0.11–1.32)	.126	0.36 (0.10–1.27)	.114
		→ Other-other				

Abbreviations: OR, odds ratio; CI, confidential interval; ECOG, Eastern Cooperative Oncology Group. Multiplicative model is indicated with (→) between the genotypes. Dominant and recessive models are indicated with (vs) between the two groups of genotypes.

1: All toxicity outcomes in the corrected analysis are corrected for; age, gender and ECOG performance status. Additional correction with body surface area (BSA) was done for Any toxicity > grade 2. Under the uncorrected data only the genotypes are included in the multivariate analysis. Description haplotypes: ξ = ABCG2 -15622C/T and 1143C/T; ψ = PDGFRα -573G/T, -1171C/G, -735G/A and 1580T/C; Ω = NR1I3 5719C/T, 7738A/C and 7837T/G; ∞ = ABCB1 3435C/T, 1236C/T and 2677G/T.

P-value < .05 is regarded as significant and printed bold.

Description of polymorphisms and rs-numbers: See table

To our knowledge, we report for the first time herein that the *ABCB1* TTT haplotype was related to hand-foot syndrome. The TTT haplotype as well as the T genotype in 3435C/T and the T polymorphism in 1236C/T separately have been associated with higher exposures to drugs transported by ABCB1 due to a decreased expression of the ABCB1 transporter.[26-31] Also, for the other ABC-transporter investigated, ABCG2, the TT haplotype was related to the development of increased toxicity (eg, any toxicity > grade 2). This haplotype has been associated with increased erlotinib exposure, a tyrosine kinase inhibitor that uses metabolic and predisposition pathways similar to those of sunitinib.[32] Thus, our results concerning *ABCB1* and *ABCG2* are in line with previously reported functional consequences of the studied genetic variants and might lead to an increased systemic exposure to sunitinib resulting in dose-limiting toxicities. Certainly, to confirm our findings, further studies that relate pharmacogenetics to pharmacokinetics and pharmacodynamics are required.

Thus far, the extrahepatic CYP1A1 enzyme has not been described as being involved in the metabolism of sunitinib. For other receptor tyrosine kinase inhibitors, such as erlotinib, imatinib and gefitinib affinity for CYP1A1 has been demonstrated in in vitro studies.[22, 23] Therefore, we also included genetic variants of *CYP1A1* in the present study. The polymorphism studied in *CYP1A1* resulting in an amino acid change of isoleucine 462 Valine was found to be related to the occurrence of mucosal inflammation and leukopenia. This suggests that CYP1A1 may also play a role in the metabolism of sunitinib *in vivo*.

In addition, we investigated genetic polymorphisms in the *NR1I3* gene, encoding the constitutive androstane receptor. This nuclear receptor plays an important role in the regulation of multiple drug detoxification genes, such as *CYP3A4*. The functionality of polymorphisms in *NR1I3* is not yet fully elucidated, however we found a relationship between the absence of the CAG haplotype in this gene and an increased risk for leukopenia[33]. Obviously, it would be interesting to relate this polymorphism with sunitinib exposure levels in future studies.

The *VEGFR-2* 1191CT and TT genotypes were found to be predictive for the development of coronary heart disease due to a lower binding efficiency of VEGF to the polymorphic VEGFR-2.[34] In our study, these genotypes were related to the development of any toxicity higher than 2, which predominantly included fatigue, thrombocytopenia, and hypertension. The polymorphic receptor might therefore be involved in sunitinib-induced cardiac toxicity and the development of hypertension.

The importance of the FLT3 receptor has been described in relation to the development of several subtypes of leukemia such as acute myeloid leukemia, acute lymphocytic leukemia, and chronic myeloid leukemia, in which FLT3 is frequently overexpressed and/or mutated.[35, 36] However, the association between *FLT3* 738T/C polymorphism and a reduction in the risk of leukopenia has not previously been described. Since sunitinib-induced leukopenia could be regulated strongly by this polymorphic receptor the clinical relevance should be further investigated.

In our study, a large number of candidate polymorphic loci were evaluated and multiple analyses of each genetic polymorphism were performed. This introduces the potential problem of multiple testing which increases the risk to find false-positive relations. However, our study was designed to explore associations that should be confirmed in an independent group of patients. The presented odds ratios and CIs facilitate comparisons of replicate studies with our data.

The ECOG performance status was not consistently related to the occurrence of toxicities in our study. The quantified performance status is multifactorial and is dependent on subjective interpretation of the physician. Moreover, in our study patients with poor performance status had relatively high baseline thrombocyte and leukocyte counts resulting in a small number of reported leukopenia and thrombocytopenia in this group in the first treatment cycle.

Toxicities in the first treatment cycle of sunitinib were used as outcome measure. The rationale was that signs of clinical deterioration from disease progression in later cycles could be misinterpreted and would interfere with the drug-induced toxicity outcome. We hypothesized that patients that suffer from relatively mild (grade 1 or 2) toxicities in the first treatment cycle were at risk for developing more severe toxicity during further treatment cycles because the two weeks of rest would not be sufficient for patients to recover to baseline conditions. This cumulative effect is underscored by measured blood cell counts and the observed dose reduction after cycle 1 to 3. Indeed, we found for leukocyte count and to a lesser extent also for thrombocyte count, that 91% and 73%, respectively, of the patients had not returned to baseline values (defined as > 90% of baseline counts) at cycle 2 day 1 (data not shown). In addition, we found that mucosal inflammation and any toxicity higher than grade 2 were strongly related to a dose reduction after cycle 1 to 3, indicating that these toxicities are regarded as clinically relevant to the treating physicians.

Together, the genetic, clinical and demographic determinants in this exploratory study explain between 10 and 23% of the total variance in toxicity response. Although it indicates that the major part of the variability is left unexplained, it also shows that pharmacogenetics may make a greater contribution to explaining variability in sunitinib toxicity as compared to the nongenetic determinants in our study. From this study we cannot conclude whether the genetic variants are prognostic or predictive markers, due to the absence of a placebo-treated control group of patients. However in the future, pharmacogenetics may help to select patients which need a priori dose reduction to prevent toxicities.

In conclusion, this study suggests a relationship between polymorphisms in the genes *CYP1A1*, *ABCB1*, *ABCG2*, *NR1I3*, *VEGFR-2* and *FLT3* and the development of sunitinib toxicity. The next step will be to validate our data with the aim to better understand the determinants of sunitinib toxicity.

Acknowledgement

We thank L.Vroling, R.R. de Haas, C. Tillier, J. Ouwerkerk, M.A.G. den Hollander, P. de Bruijn, A. Nanninga for their help with sample and data collection. We thank T. Korse and Dr. J. Bonfrer for their cooperation.

References

1. Mendel DB, Laird AD, Xin X et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 2003; 9(1):327-337.
2. Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006; 368(9544):1329-1338.
3. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006; 24(1):25-35.
4. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356(2):115-124.
5. Chu TF, Rupnick MA, Kerkela R et al. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet 2007; 370(9604):2011-2019.
6. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
7. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005; 353(2):172-187.
8. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov 2007; 6(9):734-745.
9. van der Veldt AAM, Boven E, Helgason HH et al. Predictive factors for severe toxicity of sunitinib in unselected patients with advanced renal cell cancer. Br J Cancer 2008; 99(2):259-265.
10. Adams VR, Leggas M. Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Ther 2007; 29(7):1338-1353.
11. Motzer RJ, Rini BI, Bukowski RM et al. Sunitinib in patients with metastatic renal cell carcinoma. JAMA 2006; 295(21):2516-2524.
12. Saltz LB, Rosen LS, Marshall JL et al. Phase II trial of sunitinib in patients with metastatic colorectal cancer after failure of standard therapy. J Clin Oncol 2007; 25(30):4793-4799.
13. Motzer RJ, Michaelson MD, Redman BG et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol 2006; 24(1):16-24.
14. Rini BI, Tamaskar I, Shaheen P et al. Hypothyroidism in patients with metastatic renal cell carcinoma treated with sunitinib. J Natl Cancer Inst 2007; 99(1):81-83.
15. Schmidinger M, Zielinski CC, Vogl UM et al. Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. J Clin Oncol 2008; 26(32):5204-5212.
16. Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events, Version 3.0, August 9, 2006. http://ctep.cancer.gov .
17. Stram DO, Haiman CA, Hirschhorn JN et al. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. Hum Hered 2003; 55(1):27-36.
18. Epstein MP, Satten GA. Inference on haplotype effects in case-control studies using unphased genotype data. Am J Hum Genet 2003; 73(6):1316-1329.
19. Purcell S, Neale B, Todd-Brown K et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81(3):559-575.
20. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. Drug Metab Dispos 2009; 37(2):359-365.
21. Harmsen S, Meijerman I, Beijnen JH, Schellens JH. Nuclear receptor mediated induction of cytochrome P450 3A4 by anticancer drugs: a key role for the pregnane X receptor. Cancer Chemother Pharmacol 2009; 64(1):35-43.
22. Li J, Zhao M, He P, Hidalgo M, Baker SD. Differential metabolism of gefitinib and erlotinib by human cytochrome P450 enzymes. Clin Cancer Res 2007; 13(12):3731-3737.
23. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.
24. Bhojani N, Jeldres C, Patard JJ et al. Toxicities associated with the administration of sorafenib, sunitinib, and temsirolimus and their management in patients with metastatic renal cell carcinoma. Eur Urol 2008; 53(5):917-930.
25. Hutson TE, Figlin RA, Kuhn JG, Motzer RJ. Targeted therapies for metastatic renal cell carcinoma: an overview of toxicity and dosing strategies. Oncologist 2008; 13(10):1084-1096.
26. Aarnoudse AJ, Dieleman JP, Visser LE et al. Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. Pharmacogenet Genomics 2008; 18(4):299-305.
27. Hoffmeyer S, Burk O, von RO et al. Functional

polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000; 97(7):3473-3478.
28. Saitoh A, Sarles E, Capparelli E et al. CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. AIDS 2007; 21(16):2191-2199.
29. Fukui N, Suzuki Y, Sawamura K et al. Dose-dependent effects of the 3435 C>T genotype of ABCB1 gene on the steady-state plasma concentration of fluvoxamine in psychiatric patients. Ther Drug Monit 2007; 29(2):185-189.
30. Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. Pharmacol Ther 2006; 112(2):457-473.
31. Mathijssen RH, Marsh S, Karlsson MO et al. Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 2003; 9(9):3246-3253.
32. Rudin CM, Liu W, Desai A et al. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. J Clin Oncol 2008; 26(7):1119-1127.
33. Lamba J, Lamba V, Schuetz E. Genetic variants of PXR (NR1I2) and CAR (NR1I3) and their implications in drug metabolism and pharmacogenetics. Curr Drug Metab 2005; 6(4):369-383.
34. Wang Y, Zheng Y, Zhang W et al. Polymorphisms of KDR gene are associated with coronary heart disease. J Am Coll Cardiol 2007; 50(8):760-767.
35. Carow CE, Levenstein M, Kaufmann SH et al. Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. Blood 1996; 87(3):1089-1096.
36. Nakao M, Yokota S, Iwai T et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 1996; 10(12):1911-1918.

Clinically irrelevant effect of Grapefruit Juice on the steady-state sunitinib exposure

Nielka P. van Erp, Sharyn D. Baker, Anthe S. Zandvliet,
Bart A. Ploeger, Margaret den Hollander, Zhaoyuan Chen,
Jan den Hartigh, Jacqueline M.C. König-Quartel,
Henk-Jan Guchelaar and Hans Gelderblom

Submitted

Abstract

Purpose: To determine the effect of grapefruit juice, a potent intestinal cytochrome P450 3A4 (CYP3A4) inhibitor, on steady-state sunitinib pharmacokinetics (PK).

Methods: Sunitinib PK were evaluated in cancer patients receiving sunitinib monotherapy in a "four weeks on - 2 weeks off" dose regimen. Serial blood samples for PK analysis of sunitinib were collected on two separate days. On both PK days patients received a single oral dose of 7.5 mg midazolam as a phenotypic probe for intestinal CYP3A4 activity. The first PK day was at steady-state sunitinib PK (between days 14-20), the second PK day was on day 28. On day 25, 26 and 27, 200 mL grapefruit juice was consumed three times a day. The effect of grapefruit juice on sunitinib exposure was assessed by comparing sunitinib pharmacokinetics on both PK days.

Results: In 8 patients the effect of grapefruit juice on sunitinib exposure was evaluated. Concomitant use of grapefruit juice and sunitinib resulted in an 11% increase of the relative bioavailability of sunitinib ($P < .05$). The effect of grapefruit juice on CYP3A4 activity was confirmed by an approximate increase of 50% in mean midazolam exposure ($AUC_{0\text{-}24hr}$) from 122.1 ng*hr/mL to 182.0 ng*hr/mL ($P = .034$).

Conclusion: Grapefruit juice consumption results in a marginal increase in sunitinib exposure which was not considered clinically relevant. Therefore, the warning in the sunitinib drug label for concomitant use of grapefruit juice should be reconsidered.

Introduction

Sunitinib malate (Sutent®; SU11248) is a multitarget tyrosine kinase inhibitor registered for the first line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic gastrointestinal stromal tumors (GIST).[1-3] The approved dosing regimen for sunitinib is a "four weeks on – two weeks off" schedule.[4] Sunitinib is absorbed from the gastrointestinal tract to an unknown extent. The intake of food does not affect the pharmacokinetics of sunitinib.[5] Sunitinib is *in vitro* extensively protein bound, has a long half-life of ~50 hours and a large apparent volume of distribution of ~2000 liters.[3, 6] Cytochrome P450 3A4 (CYP3A4) metabolizes sunitinib into an active metabolite, SU12662, which is further metabolized by CYP3A4 into inactive moieties.[3, 7, 8] Sunitinib has not been described to be a substrate of any other metabolizing enzymes besides CYP3A4. It was identified *in vitro* as a moderate substrate of the ATP-binding cassette (ABC) drug transporters ABCG2 and ABCB1 and showed no affinity for organic anion transporting polypeptides (OATPs). However, the clinical relevance of these transporters on the disposition *in vivo* needs to be addressed.[9, 10]

Co-administration of ketoconazol, a potent CYP3A4 inhibitor, resulted in a 51% increase of the combined area under the concentration time curve (AUC) of sunitinib and SU12662 after a single dose of sunitinib in healthy volunteers.[3] This observation was extrapolated to warnings for the potential effect of strong CYP3A4 inhibitors including grapefruit juice in the drug label of sunitinib[8].

Grapefruit juice contains a rich mixture of several hundred ingredients which may be responsible for the grapefruit juice – drug interaction effect.[11-14] By administering the purified forms of the different compounds to human volunteers, the furanocoumarins (mostly bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB)) were confirmed to result in a significant CYP3A4 inhibiting effect.[15-17] Grapefruit juice is an inhibitor of intestinal CYP3A4, with little effect on hepatic CYP3A4 activity.[18] Grapefruit juice also appears to be an inhibitor of ABCB1 and possibly of OATP located in the intestines.[17-20]

Recently, multiple oral anticancer therapies, mainly tyrosine kinase inhibitors, were introduced and since most of them are substrates of CYP3A4, their drug label contains a warning against the consumption of grapefruit juice. Sofar, only one study has determined the effect of grapefruit juice on an oral anticancer drug (etoposide).[21] In this study an opposite effect of grapefruit juice was observed. Since more patients will be treated with oral anticancer therapy in the future, it is relevant to better understand and determine the clinical relevance of an effect of grapefruit juice on oral anticancer therapy exposure. Therefore, in this study the effect of grapefruit juice on the steady-state sunitinib exposure in cancer patients was determined.

Methods

Patients

Patients eligible for study entry were treated with sunitinib at a dose level of 25 – 50 mg once daily in a "four weeks on – two weeks off" regimen. All 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 four weeks before entering the study protocol was not allowed. Concurrent use of substances known or likely to interfere with the pharmacokinetics of sunitinib and with CYP3A4 activity, such as ketoconazol, fluconazol, rifampicin and St. John's wort, were not allowed within 14 days before study entry and during the study. All patients had adequate bone marrow, renal and hepatic functions as defined by hemoglobin ≥ 6.0 mmol/L, WBC ≥ 3.0 × 10^9/L, platelets ≥ 100 × 10^9/L, creatinine clearance ≥ 60 mL/min and bilirubin ≤ 1.75 × the upper limit of institutional normal range. Prior to commencing the study, a sample size of 8 patients was determined as sufficient for a paired, two sided analysis to detect a difference of 25% in sunitinib exposure with a power (1-β) of 0.8 (80%), and a two-sided significance level (α) of 0.05 (5%). 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.

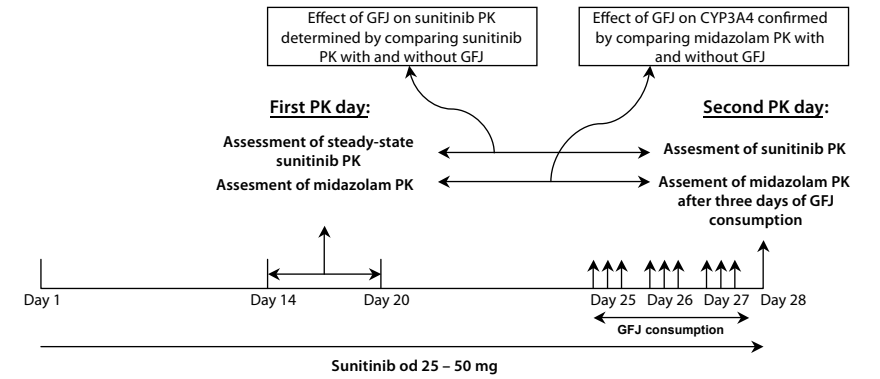
Study design

The study was designed to evaluate the effect of grapefruit juice on steady-state sunitinib pharmacokinetics. All patients were treated with commercially available sunitinib malate hard capsule (Pfizer, Kent, United Kingdom) at an oral dose of 25 – 50 mg once daily in a "four weeks on followed by two weeks off" dose regimen. The study was performed during one sunitinib treatment cycle of six weeks. Patients were admitted to the hospital on two separate PK days. The first PK day was at steady-state sunitinib PK (between day 14 - 20) and the second PK day was on day 28. On days 25, 26, and 27, the patients took 200 ml grapefruit juice of a preselected lot of commercially available grapefruit juice three times daily. On these three days, sunitinib was simultaneously used with the morning consumption of the grapefruit juice. On both PK days patients were given one midazolam 7.5 mg tablet (Roche, Woerden, The Netherlands) as a phenotypic probe to confirm the inhibitory effect of grapefruit juice on intestinal CYP3A4 activity (Fig. 1).

Selection of a grapefruit juice batch

Different batches of grapefruit juice show a considerable variability in BG (~35 fold) and DHB (~200 fold) concentration.[22] Therefore selecting a batch with a sufficient amount of BG and DHB to induce a clinically relevant effect on CYP3A4 substrates was necessary before the interaction study was conducted.[15]

Figure 1 Study design



Abbreviations: GFJ = grapefruit juice; PK = pharmacokinetics; od = once daily

Concentrations of BG and DHB were quantified in various batches of grapefruit juice from different brands using a validated high pressure liquid chromatography – ultraviolet detection (HPLC-UV) method. This assay was based on a previously published method with minor modifications.[22] Briefly, the juice was homogenized by shaking. Grapefruit juice (0.5mL) was mixed with 10 μL internal standard fenprocoumon (100 μg/mL, in methanol) and 2 mL ethyl acetate. Calibration standards contained 0.2 – 4 μg/mL BG and 0.1 – 2 μg/mL DHB were prepared at the start of each analytical run. The standard stock solution contained BG and DHB (100 and 50 μg/mL in DMSO:methanol(1:3)). The residue from the organic phase was reconstituted with 100 μL of DMSO/acetonitril solution (1:3 v/v) and applied to a HPLC separation system (Unexas 2104, Knauer, Berlin, Germany). The compounds of interest were separated on a Hypersil ODS RP analytical column (4.6 x 100 mm, i.d 3 μm) using the following gradient [time scale (minutes - minutes)/ percentage of solvent A (water 2500/ phosphoric acid 1.25)/ percentage of solvent B (acetonitril)]: 0-7/70/30, 7-17 70/30 → 0/100, 17-18/0/100, 18-19 0/100 → 70/30, 19-22/70/30. DHB, fenprocoumon and BG eluted at 10.9, 12.8 and 16.5 minutes, respectively. Linearity was confirmed over the range of 0.2 – 24 μg/mL for BG and 0.1 – 12 μg/mL for DHB. The within day and between day precision and accuracy were < 15%.

Pharmacokinetic sampling

Blood samples were collected on the first and second PK day of the study for assessing sunitinib and midazolam plasma concentrations. Blood was collected in heparin-containing

tubes at the following time points: pre-dose, 10, 20, 40 minutes; 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours after simultaneous intake of sunitinib and midazolam. Blood samples were centrifuged at 3,000 rpm for 10 minutes and plasma was stored at –80˚C until the day of analysis.

Bioanalysis of sunitinib and midazolam

Sunitinib was measured using a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay, which has been described earlier.[23] The calibration curve of sunitinib is linear over the range of 0.2 – 500 ng/mL. The within day and between day precision and accuracy were < 8%. The LLQ of the sunitinib assay was 0.2 ng/mL.

Midazolam was measured using a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay. Briefly, 200 μl plasma was extracted by adding 500 μl of acetonitril containing midazolam D4 (4μg/L) as the internal standard, followed by vortex mixing and centrifugation at 13,000 rpm for 5 minutes at ambient temperature. The supernatant was collected and 10 μL was separated on an Atlantis T3 C18 analytical column (2.1 x 50 mm, i.d 3 μm) and eluted with the following gradient [flow rate (ml/min)/ time (minutes)/ percentage of solvent A (formic acid 0.1% in water)/ percentage of solvent B (formic acid 0.1% in acetonitril)]: 0.3/0.5/85/15/, 0.3/1/10/90, 0.3/4.3/10/90, 0.5/0.01/10/90, 0.5/0.39/85/15, 0.5/3.3/85/15, 0.3/0.05/85/15, 0.3/0.05/85/15. The effluent was monitored with a Micromass Quattro LC triple-quadrupole mass-spectrometric detector (Waters, Milford, MA, USA) using the electrospray positive ionization mode. The calibration curve of midazolam was linear over the range of 1 – 100 ng/mL. The within day and between day precision and accuracy were < 5%. The LLQ of the midazolam assay was 0.3 ng/mL.

Pharmacokinetic analysis of midazolam

Midazolam plasma concentrations were analyzed by non-compartimental methods using WinNonlin (version 5.2.1) (Pharsight Corporation, Mountain View, CA, USA). The midazolam area under the concentration time curve ($AUC_{0\text{-}24hr}$) was calculated and was compared between the first and second PK days. Statistical analysis included the two-tailed paired Student's *t*-test, and *P* values < 0.05 were considered statistically significant. The statistical calculations were performed using SPSS 16.0 (SPSS Inc. headquarters, Chicago, Illinois, USA)

Pharmacokinetic analysis of sunitinib

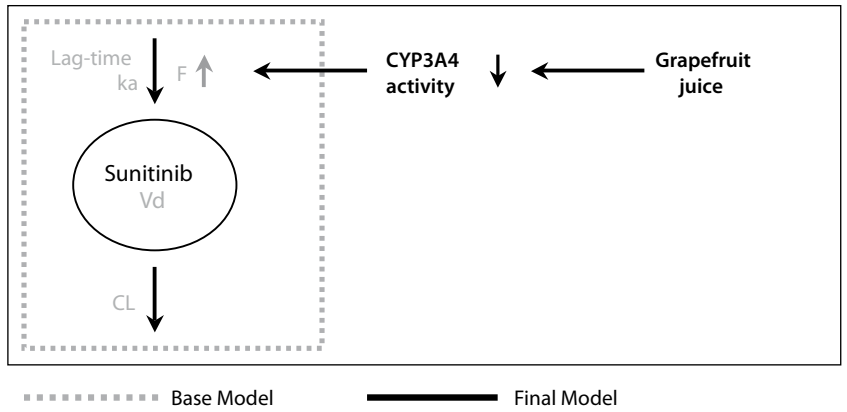
Sunitinib plasma concentrations were evaluated by a population pharmacokinetic method using NONMEM (version VI, level 1.0) (Globomax, Hanover, MD, USA). The First-Order Conditional Estimation (FOCE) method of NONMEM with interaction (INTER) between the interindividual and residual random effects was used.[24] Discrimination between hierarchical models was based on comparison of the objective function values (OFV) of NONMEM using the likelihood ratio test. A decrease in ΔOFV of 3.84 (=$P < .05$) was considered statistical significant.

A base model was developed to describe sunitinib pharmacokinetics, using sunitinib concentrations obtained on the first and second PK day. Next, a final model was developed by the introduction of a grapefruit juice effect on the relative bioavailability of sunitinib, resulting in an effect on the apparent clearance and apparent volume of distribution and thereby exposure to sunitinib, since it was hypothesized that grapefruit juice exerts its effect only by irreversible inhibition of intestinal CYP3A4 and possibly by inhibition of ABCB1 (Fig. 2). The recovery half life of CYP3A4 activity after grapefruit juice consumption was set to 23 hours.[25]

The model was evaluated by goodness of fit plots, case deletion diagnostics and a numerical predictive check. Moreover, a log-likelihood profile was generated for the effect size of grapefruit juice to determine the confidence interval.

The effect of grapefruit juice on sunitinib bioavailability was evaluated for various scenarios: 1) simultaneous intake of sunitinib and grapefruit juice, 2) sunitinib intake 7 hours, 3) 24 hours, 4) 72 hours and 5) one week after the last grapefruit juice consumption.

Figure 2 Sunitinib pharmacokinetic model



Results

Patients

Eight patients were enrolled into the study. All were evaluable for PK analysis. Patient characteristics are summarized in Table 1. No severe or unexpected side effects were observed during the three days of grapefruit juice co-administration or by midazolam co-administration on both PK days.

Table I Patient characteristics

Characteristic	Value
Number of patients	8
Sex (female / male)	1/7
Age, years*	54 (41 - 78)
Baseline serum renal and liver function parameters	
Creatinine, μM*	77 (56 - 122)
Total bilirubin, μM*	9 (6 - 15)
ALT, units/L*	39 (18 - 68)
Baseline bone marrow function parameters	
Hb, mM*	8.7 (7 - 9.4)
WBC, x 10^9/L*	5.5 (3.5 - 38.2)
Thrombocytes, x 10^9/L*	196 (149 - 318)

* Median values (range)

Selection of grapefruit juice

The concentration of BG and DHB was measured in 6 different lots of grapefruit juice. BG and DHB concentrations among the lots tested varied with ~4.5 fold and ~20 fold, respectively. The concentration of BG and DHB in the selected lot of grapefruit juice was 33.1 μmol/L and 2.7 μmol/L, respectively, corresponding with 2.2 mg/200mL BG and 0.2 mg/200mL DHB. Due to the expiration date a second lot of the same brand was selected for the last two patients of the study. The concentrations in the second lot selected were 23.5 μmol/L BG and 5.7 μmol/L DHB, corresponding with 1.6 mg/200mL BG and 0.4 mg/200mL DHB. The concentration of BG in both lots was sufficient to induce a significant drug interaction.[15]

Pharmacokinetic analysis of midazolam

Midazolam exposure ($AUC_{0\text{-}24hr}$) increased after prior intake of grapefruit juice. The midazolam exposure expressed as $AUC_{0\text{-}24hr}$ (± standard error of the mean (SEM)) with and without grapefruit juice were 122.1 (± 32.9) ng*hr/mL and 182.0 (± 52.2) ng*hr/mL, respectively (*P*-value = .034). Thereby, midazolam exposure increased with ~50% in the presence of grapefruit juice. These results confirm the inhibitory effect of grapefruit juice on intestinal CYP3A4 activity.

Pharmacokinetic analysis of sunitinib

A one-compartment model with linear elimination and first-order absorption adequately described the time profile of sunitinib concentrations. The data did not contain sufficient information to support a two-compartment model[6]. Inclusion of an absorption lag time significantly improved the base model of sunitinib. Between-subject variabilities of the absorption rate and clearance were large (60-70%). The base model of sunitinib is graphically presented in Figure 2 (left side).

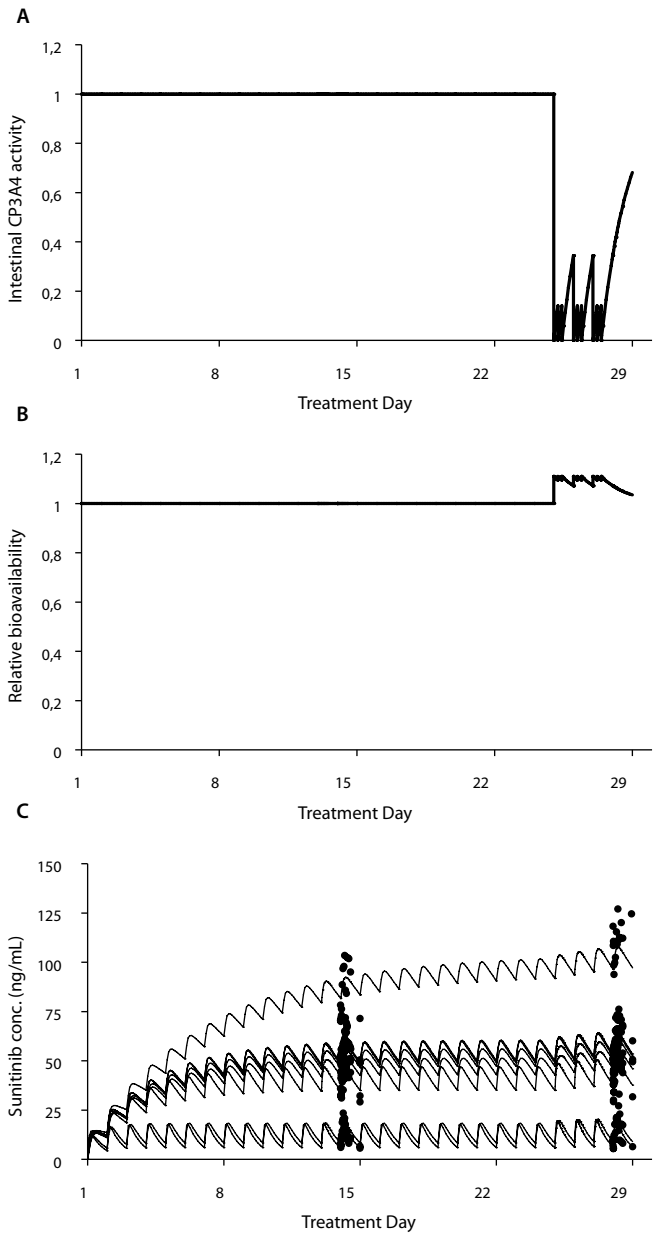
In the final model, CYP3A4 activity was depleted by each grapefruit juice consumption (9 in total) and the activity was restored with a half life of 23 hours (Fig. 3A).[25] Inhibition of CYP3A4 activity resulted in an increase in the relative bioavailability of sunitinib (Fig. 3B). The individual predicted and measured sunitinib concentrations are depicted for all patients (Fig. 3C). Introduction of the grapefruit juice effect on the relative bioavailability of sunitinib significantly improved the model (ΔOFV = -10.01, $P < .05$) and resulted in the final model (Fig. 2).

The estimated pharmacokinetic parameters in the final model are listed in Table 2. The derived parameters are calculated with the estimated pharmacokinetic parameters and represent the data when grapefruit juice and sunitinib are used simultaneously. Goodness-of-fit plots demonstrated that the final model adequately described the time profile of sunitinib concentrations. Case deletion diagnostics demonstrated that the estimated grapefruit juice effect was not highly dependent on the data from a single patient (range in relative F = 1.05 – 1.14). Moreover, suitability of the final model was confirmed by the results from a numerical predictive check.[26] Out of 268 observed sunitinib concentrations, 21.6% were below the P25-P75 (interquartile) prediction interval, 57.1% were within the interval and 21.3% was above the P25-P75 prediction interval.

Based on the final model it is determined that simultaneous intake of sunitinib and grapefruit juice results in a decrease of intestinal CYP3A4 activity and a consequent increase of sunitinib exposure of 11% (as a result of the increased relative bioavailability 1.11, 95%CI: 1.042-1.082).

Since the intestinal CYP3A4 activity is restored with a half-life of 23 hours, the relative bioavailability of sunitinib is also restored with a half-life of 23 hours. The different time interval evaluations resulted in the following estimates: when grapefruit juice is consumed 7 hours before the sunitinib dose, the exposure is still increased by ~8.9%, after 24 hours the effect is diminished to ~5.3% and after 72 hours to ~1.3%. If sunitinib therapy starts one week after the last grapefruit juice consumption the effect of grapefruit juice on the exposure to sunitinib is negligible (~0.07%).

Figure 3 Effect of grapefruit juice on sunitinib pharmacokinetics



A: Depletion of CYP3A4 activity by grapefruit juice consumption. **B**: Increase in relative bioavailability of sunitinib by grapefruit juice consumption. **C**: Individual predicted (lines) and measured (solid marks) sunitinib concentrations

Table 2 Estimated and derived sunitinib pharmacokinetic parameters in the final model

Estimated Parameters	Estimate	Standard Error of Estimate (RSE%)	Interindividual variability (IIV)(CV%)	Standard Error of IIV (RSE%)
Cl/F (L/hr)	50.5	28.5	67.9	42.7
Vd/F (L)	3210	7.8	nd	nd
ka (hr^{-1})	0.468	29.1	63.9	42.9
Relative F	1.11	70	nd	nd
Absorption lag time (hr)	0.487	7.3	nd	nd
Proportional residual error (%)	16.3	22.9	nd	nd

Derived Parameters*	Sunitinib without grapefruit juice	Sunitinib with grapefruit juice when simultaneously taken
$AUC_{0\text{-}24hr}$ (ng*hr/mL)	1122 (277 – 2399)	1245 (308 – 2663)
C_{max} (ng/mL)	13.0 (10.0 – 14.6)	14.4 (11.1 – 16.2)
$t_{1/2}$ (hr)	53 (12 – 107)	
T_{max} (hr)	8.2 (2.8 – 12.4)	

Abbreviations: RSE = relative standard error; Cl/F = apparent clearance; Vd/F = apparent volume of distribution; ka = absorption rate constant; F = bioavailability; nd = not determined; $AUC_{0\text{-}24hr}$ = area under the plasma concentration-time curve over the dose interval 0-24hr at steady-state pharmacokinetics; $t_{1/2}$ = elimination half-life; T_{max} = time to reach peak plasma concentration. Between-subject variability was assessed using exponential models.

* Derived parameters are calculated from estimated parameters and are demonstrated as mean values (range).

Discussion

This study shows that inhibition of the intestinal CYP3A4 activity by grapefruit juice results in a significant but not clinically relevant increase in the sunitinib exposure. The drug label of sunitinib includes the advice to avoid the consumption of grapefruit juice during sunitinib treatment. This warning is based upon an extrapolation of the effect of ketoconazol on sunitinib exposure after single dose administration. Our study is the first to directly investigate the effect of grapefruit juice on sunitinib exposure in cancer patients under steady-state conditions and shows that there is no scientific basis for the warning in the sunitinib's drug label.

Moreover, this is the second study investigating an interaction of grapefruit juice with oral anticancer therapy and both studies show an irrelevant effect of grapefruit juice which contrasts the warning in the drug label[21]. All eight registered tyrosine kinase inhibitors are substrates of CYP3A4 and therefore include the warning for consuming grapefruit juice in their drug label. This is the first study that shows a clinically irrelevant effect of grapefruit juice on one of the tyrosine kinase inhibitors, sunitinib, which could also be relevant for the other TKIs.

Grapefruit juice is a potent inhibitor of intestinal CYP3A4 with little effect on the activity of hepatic CYP3A4. The affinity for only intestinal CYP3A4 was concluded from the significant effect grapefruit juice has on the exposure to CYP3A4 substrates (e.g. simvastatin, felodipine, triazolam) after oral administration, while the effect was only limited after intravenous administration of these drugs.[15, 27-29] Grapefruit juice is also an inhibitor of the drug transporters ABCB1, OATP1A2 and OATP2B1, which could contribute to the effect of grapefruit juice on the exposure of co-administered drugs.[13, 30-35]

Midazolam is extensively metabolized by CYP3A4 with less affinity for CYP3A5, and is not transported by ABCB1, ABCG2 and OATPs[36-39]. In previous studies, grapefruit juice showed a pronounced effect on the exposure of orally administered midazolam[25, 29, 40]. In this study, midazolam was co-administrated on both PK days as a phenotypic probe to confirm the decreased activity of intestinal CYP3A4 by the selected batch of grapefruit juice.

The patients in our study consumed grapefruit juice three times a day for three days (25, 26, and 27) at steady-state. On the last sunitinib treatment day (day 28) in the six week treatment cycle, the sunitinib PK was determined and compared to the data obtained without the exposure to grapefruit juice. The effect of grapefruit juice was estimated on the relative bioavailability of sunitinib, since grapefruit juice is a potent intestinal CYP3A4 inhibitor and therefore, only an effect on the sunitinib uptake is expected rather than on sunitinib clearance, volume of distribution, absorption rate constant and lag time. Indeed the concomitant use of grapefruit juice results in a significant increase of 11% in sunitinib exposure. However, since the reported interpatient variability in sunitinib clearance is large ~ 40% the effect of grapefruit juice on sunitinib exposure is negligible and should not be regarded as clinically relevant.[6] Moreover, the marginal 11% increase in sunitinib exposure is unlikely to result in a different toxicity profile or treatment efficacy, although data on the drug exposure – treatment outcome and toxicity response relation are not available yet.

Grapefruit juice irreversibly inhibits CYP3A4 and it therefore takes time to restore CYP3A4 functionality since new enzymes needs to be formed. The recovery half-life of CYP3A4 activity after consuming grapefruit juice was set to 23 hours according to the recovery study of Greenblatt et al.[14] The recovery half-life was confirmed by several interaction studies between midazolam and grapefruit juice over different time intervals[29, 40, 41].

The half-life of sunitinib is long (~50 hours). Steady-state sunitinib PK is therefore achieved within ~ 8 days. After starting grapefruit juice consumption is takes ~ 8 days to achieve new steady-state sunitinib PK. At the second PK day, after three days co-administration of grapefruit juice, a new steady-state was not reached yet. Since a large effect, and thereby potential toxicity, was hypothesized it was considered unethical to continue the co-administration until steady-state sunitinib PK was reached. Due to this study design the effect of grapefruit juice on sunitinib pharmacokinetics could only be estimated by a compartmental approach. The estimated apparent clearance and volume of distribution are similar to the described parameters of an earlier published compartmental approach.[6] Conversely, a non-compartimental approach was used for determining midazolam exposure after a single dose of 7.5mg. Since, only an exposure difference in midazolam was required to determine the effect of grapefruit juice, which could adequately be determined by a non-compartimental approach due to extensive sampling from start until undetectable levels of midazolam were measured.

The lack of a clinically relevant effect of grapefruit juice on sunitinib exposure was not related to the batch of grapefruit juice that was used in this study. First, the grapefruit juice selected had a sufficient content of BG (2.2mg/ 1.6mg) to induce a significant effect on CYP3A4 activity.[15] Secondly, even after the recovery of a proportion of the intestinal CYP3A4 enzymes on the second PK day, a significant effect ~50% was observed on the phenotypic drug midazolam, which is comparable to the effect of grapefruit juice on midazolam exposure explored in earlier interaction studies[25, 40]. No effect of sunitinib on midazolam exposure is expected since midazolam exposure is similar to earlier published data[40, 42]. The increase in midazolam exposure due to grapefruit juice co-administration confirms the significant effect that grapefruit juice has on intestinal CYP3A4 activity. Hence, the marginal effect observed on sunitinib bioavailability is likely to be the result of the limited efficiency of sunitinib metabolism by intestinal CYP3A4. The limited effect of grapefruit juice is in contrast with the large effect (51% increase) observed after the co-administration of ketoconazol.[3] This could be the result of a change in enzymes that play a dominant role after prolonged exposure to the drug as was seen for imatinib in earlier studies.[43] The interaction with ketoconazol was studied after a single dose, while the interaction with grapefruit juice was determined at steady-state sunitinib exposure. Another explanation could be that ketoconazol is a strong intestinal and hepatic CYP3A4 inhibitor while grapefruit juice is only capable of inhibiting intestinal CYP3A4.

In conclusion, grapefruit juice only marginally increases the sunitinib exposure which is not regarded clinically relevant. Therefore, the warning in the drug label for the concomitantly use of grapefruit juice should be reconsidered.

Acknowledgement

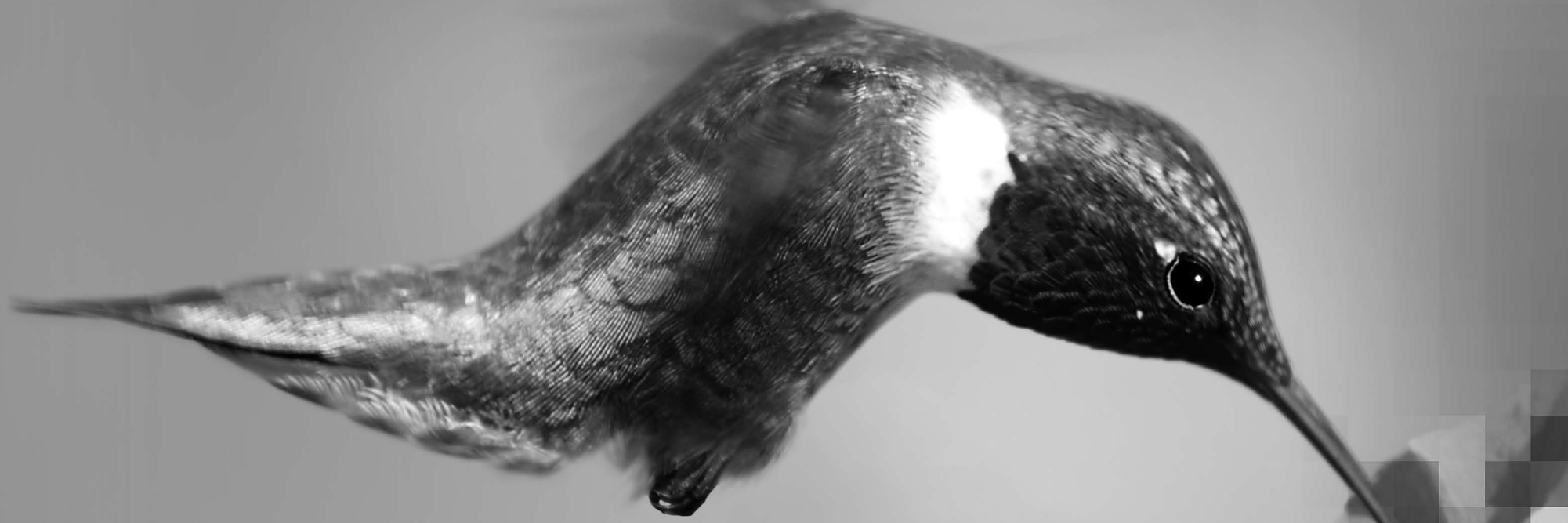
We thank Alex Sparreboom for the discussion on trial design.

References

1. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356(2):115-124.
2. Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006; 368(9544):1329-1338.
3. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
4. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006; 24(1):25-35.
5. Bello CL, Sherman L, Zhou J et al. Effect of food on the pharmacokinetics of sunitinib malate (SU11248), a multi-targeted receptor tyrosine kinase inhibitor: results from a phase I study in healthy subjects. Anticancer Drugs 2006; 17(3):353-358.
6. Houk BE, Bello CL, Kang D, Amantea M. A population pharmacokinetic meta-analysis of sunitinib malate (SU11248) and its primary metabolite (SU12662) in healthy volunteers and oncology patients. Clin Cancer Res 2009; 15(7):2497-2506.
7. Adams VR, Leggas M. Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Ther 2007; 29(7):1338-1353.
8. Pfizer. Drug label Sutent approved 01/26/2006. http://www.accessdata fda.gov/scripts/cder/drugsatfda/ Accessed on 02/18/2009. 2006. Ref Type: Electronic Citation
9. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. Drug Metab Dispos 2009; 37(2):359-365.
10. Hu S, Chen Z, Franke R et al. Interaction of the multikinase inhibitors sorafenib and sunitinib with solute carriers and ATP-binding cassette transporters. Clin Cancer Res 2009; In press.
11. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998; 46(2):101-110.
12. Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME et al. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. Drug Metab Dispos 1997; 25(11):1228-1233.
13. Wang EJ, Casciano CN, Clement RP, Johnson WW. Inhibition of P-glycoprotein transport function by grapefruit juice psoralen. Pharm Res 2001; 18(4):432-438.
14. Paine MF, Criss AB, Watkins PB. Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetic and binding properties. Drug Metab Dispos 2004; 32(10):1146-1153.
15. Goosen TC, Cillie D, Bailey DG et al. Bergamottin contribution to the grapefruit juice-felodipine interaction and disposition in humans. Clin Pharmacol Ther 2004; 76(6):607-617.
16. Rashid J, McKinstry C, Renwick AG, Dirnhuber M, Waller DG, George CF. Quercetin, an in vitro inhibitor of CYP3A, does not contribute to the interaction between nifedipine and grapefruit juice. Br J Clin Pharmacol 1993; 36(5):460-463.
17. Paine MF, Widmer WW, Hart HL et al. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. Am J Clin Nutr 2006; 83(5):1097-1105.
18. Saito M, Hirata-Koizumi M, Matsumoto M, Urano T, Hasegawa R. Undesirable effects of citrus juice on the pharmacokinetics of drugs: focus on recent studies. Drug Saf 2005; 28(8):677-694.
19. Paine MF, Widmer WW, Pusek SN et al. Further characterization of a furanocoumarin-free grapefruit juice on drug disposition: studies with cyclosporine. Am J Clin Nutr 2008; 87(4):863-871.
20. Mertens-Talcott SU, Zadezensky I, De Castro WV, Derendorf H, Butterweck V. Grapefruit-drug interactions: can interactions with drugs be avoided? J Clin Pharmacol 2006; 46(12):1390-1416.
21. Reif S, Nicolson MC, Bisset D et al. Effect of grapefruit juice intake on etoposide bioavailability. Eur J Clin Pharmacol 2002; 58(7):491-494.
22. De Castro WV, Mertens-Talcott S, Rubner A, Butterweck V, Derendorf H. Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. J Agric Food Chem 2006; 54(1):249-255.
23. Minkin P, Zhao M, Chen Z, Ouwerkerk J, Gelderblom H, Baker SD. Quantification of sunitinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 874(1-2):84-88.
24. Beal SL, Boeckman AJ, Sheiner LB. NONMEM user's guides. 1988. University of California at San Francisco, San Francisco CA.
25. Greenblatt DJ, von Moltke LL, Harmatz JS et al. Time course of recovery of cytochrome p450 3A function after single doses of grapefruit juice. Clin Pharmacol Ther 2003; 74(2):121-129.

26. Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther 2007; 82(1):17-20.
27. Lilja JJ, Kivisto KT, Neuvonen PJ. Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. Clin Pharmacol Ther 2000; 68(4):384-390.
28. Culm-Merdek KE, von Moltke LL, Gan L et al. Effect of extended exposure to grapefruit juice on cytochrome P450 3A activity in humans: comparison with ritonavir. Clin Pharmacol Ther 2006; 79(3):243-254.
29. Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, Krahenbuhl S. Interaction between grapefruit juice and midazolam in humans. Clin Pharmacol Ther 1995; 58(1):20-28.
30. Satoh H, Yamashita F, Tsujimoto M et al. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. Drug Metab Dispos 2005; 33(4):518-523.
31. Dresser GK, Bailey DG, Leake BF et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther 2002; 71(1):11-20.
32. Glaeser H, Bailey DG, Dresser GK et al. Intestinal drug transporter expression and the impact of grapefruit juice in humans. Clin Pharmacol Ther 2007; 81(3):362-370.
33. Hermann M, Asberg A, Reubsaet JL, Sather S, Berg KJ, Christensen H. Intake of grapefruit juice alters the metabolic pattern of cyclosporin A in renal transplant recipients. Int J Clin Pharmacol Ther 2002; 40(10):451-456.
34. Bailey DG, Dresser GK, Leake BF, Kim RB. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. Clin Pharmacol Ther 2007; 81(4):495-502.
35. Eagling VA, Profit L, Back DJ. Inhibition of the CYP3A4-mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by grapefruit juice components. Br J Clin Pharmacol 1999; 48(4):543-552.
36. Franke RM, Baker SD, Mathijssen RH, Schuetz EG, Sparreboom A. Influence of solute carriers on the pharmacokinetics of CYP3A4 probes. Clin Pharmacol Ther 2008; 84(6):704-709.
37. Fuhr U, Jetter A, Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. Clin Pharmacol Ther 2007; 81(2):270-283.
38. Kim RB, Wandel C, Leake B et al. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. Pharm Res 1999; 16(3):408-414.
39. Kurnik D, Wood AJ, Wilkinson GR. The erythromycin breath test reflects P-glycoprotein function independently of cytochrome P450 3A activity. Clin Pharmacol Ther 2006; 80(3):228-234.
40. Farkas D, Oleson LE, Zhao Y et al. Pomegranate juice does not impair clearance of oral or intravenous midazolam, a probe for cytochrome P450-3A activity: comparison with grapefruit juice. J Clin Pharmacol 2007; 47(3):286-294.
41. Veronese ML, Gillen LP, Burke JP et al. Exposure-dependent inhibition of intestinal and hepatic CYP3A4 in vivo by grapefruit juice. J Clin Pharmacol 2003; 43(8):831-839.
42. Mueller SC, Majcher-Peszynska J, Mundkowski RG et al. No clinically relevant CYP3A induction after St. John's wort with low hyperforin content in healthy volunteers. Eur J Clin Pharmacol 2009; 65(1):81-87.
43. van Erp NP, Gelderblom H, Karlsson MO et al. Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 2007; 13(24):7394-7400.

Mitotane has a strong inducing effect on CYP3A4 activity



Nielka P. van Erp, Henk-Jan Guchelaar, Johannes A. Romijn,
Jan den Hartigh and Hans Gelderblom

Submitted

Abstract

Context: The effects of mitotane on pharmacokinetics of co-administered drugs are unknown. The aim of the present study was to describe the effects of mitotane on the pharmacokinetics of the phenotypic probe midazolam and of sunitinib.

Patient and Methods: Sunitinib and midazolam pharmacokinetics were evaluated in 9 patients during sunitinib therapy. Two of these patients had adrenocortical carcinoma (ACC) and were treated with mitotane. Serial blood samples for pharmacokinetic analysis of midazolam, 1-hydroxy-midazolam and sunitinib were collected at steady-state sunitinib pharmacokinetics (between days 14-20). To assess CYP3A4 activity the patients received a single dose of oral midazolam 7.5mg concomitantly with sunitinib at the day of PK assessment.

Results: Both mitotane treated patients showed highly induced CYP3A4 activity reflected by decreased midazolam exposure compared to the other 7 patients (mean $AUC_{0\text{-}12hr}$ ± SD = 7.8 ± 2.6 μg*hr/L *vs.* 139.6 ± 59.7 μg*hr/L, resp), increased 1-hydroxy-midazolam exposure (mean $AUC_{0\text{-}12hr}$ ± SD = 341.8 ± 69.6 μg*hr/L *vs.* 35.2 ± 11.5 μg*hr/L, resp) and a decreased sunitinib exposure (mean $AUC_{0\text{-}24hr}$ ± SD= 268 ± 0.3 μg*hr/L *vs* 1344 ± 358 μg*hr/L, resp).

Conclusions: Mitotane is associated with a strong inducing effect on CYP3A4 activity which will result in clinically relevant interactions since many drugs are metabolized by this enzyme.

Introduction

Mitotane (o,p'-DDD) is used to treat patients with adrenocortical carcinoma (ACC)[1, 2]. Careful monitoring of serum drug levels is important, because mitotane has a narrow therapeutic window, Mitotane levels > 14 mg/L are required for the therapeutic effects, whereas serum drug levels >20mg/L correlate with considerable side-effects especially neurologic toxicity[3]. Since mitotane accumulates in adipose tissue, the plasma elimination half-life is extremely long (18-159 days). Consequently, it can take months to reach steady-state pharmacokinetics and, conversely, it takes also months to observe a decrease in plasma levels after discontinuation of mitotane[3]. Unfortunately, many patients show progressive disease despite treatment with mitotane. Therefore, more effective additional treatment modalities are warranted, including polychemotherapy.

Surprisingly, there is hardly any information available on the metabolic pathways of mitotane, nor on the potential influence of mitotane on the metabolism of co-administered drugs. However, organochlorine insecticides, to which mitotane is chemically closely related, induce microsomal liver enzymes[4]. In accordance, a case report described an interaction between mitotane and the anticoagulant warfarin which resulted in increased warfarin requirements, suggesting induction of metabolizing enzymes by mitotane[5].

In the present report the pharmacokinetic effects of mitotane on cytochrome P450 (CYP) 3A4 activity is described using the phenotypic probe midazolam[6]. Midazolam is extensively metabolized by CYP3A4 and to a lesser extent by CYP3A5[7]. It is used as a phenotypic probe to determine the activity of CYP3A4[7] . In addition, we describe the effect of mitotane on the exposure to a relatively new oral anticancer drug sunitinib. Sunitinib is also metabolized by CYP3A4 to an equally active metabolite SU12662, which is further metabolized to inactive moieties by CYP3A4[8]. These studies were performed in 9 patients with different malignancies who participated in a sunitinib pharmacokinetic study designed to determine the relation between CYP3A4 activity and sunitinib exposure. Two of these patients showed a very different pharmacokinetic profile. Both patients were treated with mitotane for ACC.

Patients and Methods

Patients

Nine patients were included in the pharmacokinetic study. Two patients (1 male; 46 years old, 72kg, Eastern Cooperative Oncology Group (ECOG) performance status = 1 and 1 female; 42 years old, 65kg, ECOG performance status = 1) with metastatic ACC showed progressive disease despite mitotane therapy and were treated with sunitinib as an experimental therapy. The other 7 patients (1 female, 6 male; 2 gastrointestinal stromal tumors, 2 metastatic renal

cell carcinoma, 1 prostate carcinoma, 1 chordoma and 1 osteosarcoma; median (range) age = 60 (41 – 77); weight = 82kg (68 – 98); ECOG performance status = 1 (0 – 1)) used sunitinib without mitotane therapy. 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 with sunitinib 37.5 – 50 mg once daily in a "four weeks on – two weeks off" dosing schedule. Pharmacokinetic assessment of midazolam and sunitinib at steady-state was performed between days 14 - 20. A single dose of midazolam 7.5 mg was administered concomitantly with the regular dose of sunitinib. Blood samples were collected pre-dose, and 0, 10, 20, 40 minutes; 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hours after midazolam and sunitinib administration.

Measurement

The mitotane concentrations in both patients were measured by gas chromatographic – electron capture detection assay[9]. Sunitinib concentrations were quantified by liquid chromatographic tandem mass spectrometric (LC/MS/MS) assay[10]. Midazolam and 1-hydroxy-midazolam levels were determined by LC/MS/MS assay.

Pharmacokinetic analysis

Sunitinib, midazolam and 1-hydroxy-midazolam plasma exposure was assessed by non-compartimental methods using WinNonlin (version 5.2.1) (Pharsight Corporation, Mountain View, CA, USA). Midazolam and 1-hydroxy-midazolam exposure ($AUC_{0\text{-}12hr}$) was assessed over 12 hours since midazolam and 1-hydroxy-midazolam have a short half-life (1.0-3.5 hr and 0.8-1.0 hr, respectively), and therefore the elimination was nearly completed at 12 hour post-dose. Sunitinib exposure ($AUC_{0\text{-}24hr}$) was assessed over 24 hours. The mitotane concentrations of both mitotane users were determined in the pre-dose blood sample.

Results

Clinical characteristics of patients with ACC

Patient 1, a 44 year old man, was diagnosed in March 2007 with ACC in his right adrenal gland of 3.5 inches. The primary tumor was extirpated. However, in May 2007 there was local and distal recurrence of ACC. Mitotane therapy was started in May 2007. After failed standard systemic anti-tumour therapies he started with sunitinib as an experimental therapy in December 2007 in addition to mitotane (3.5 g tid). Although there was progressive disease during mitotane treatment the patient continued this therapy since it was hypothesized that some cells might still be sensitive to mitotane. The patient was treated with sunitinib for three months (2 treatment cycles of 6 weeks) and stopped since no response to sunitinib was observed. In January 2008, the patient volunteered in the pharmacokinetic study.

Patient 2, a 44 year old woman, was diagnosed in June 2005 with ACC in her right adrenal gland with two hepatic metastases with a total tumor radius of ~ 6.3 inches. The adrenal gland was extirpated and in addition a segmental resection of the liver was performed. In September 2006 there was recurrence of the tumor. Mitotane therapy started in June 2005 and continued until August 2007. After failed standard systemic anti-tumour therapies she started with sunitinib as an experimental therapy in October 2007 and was treated with sunitinib for three months (2 treatment cycles) and stopped since no response to sunitinib therapy was observed. In October 2007, the patient volunteered in the pharmacokinetic study.

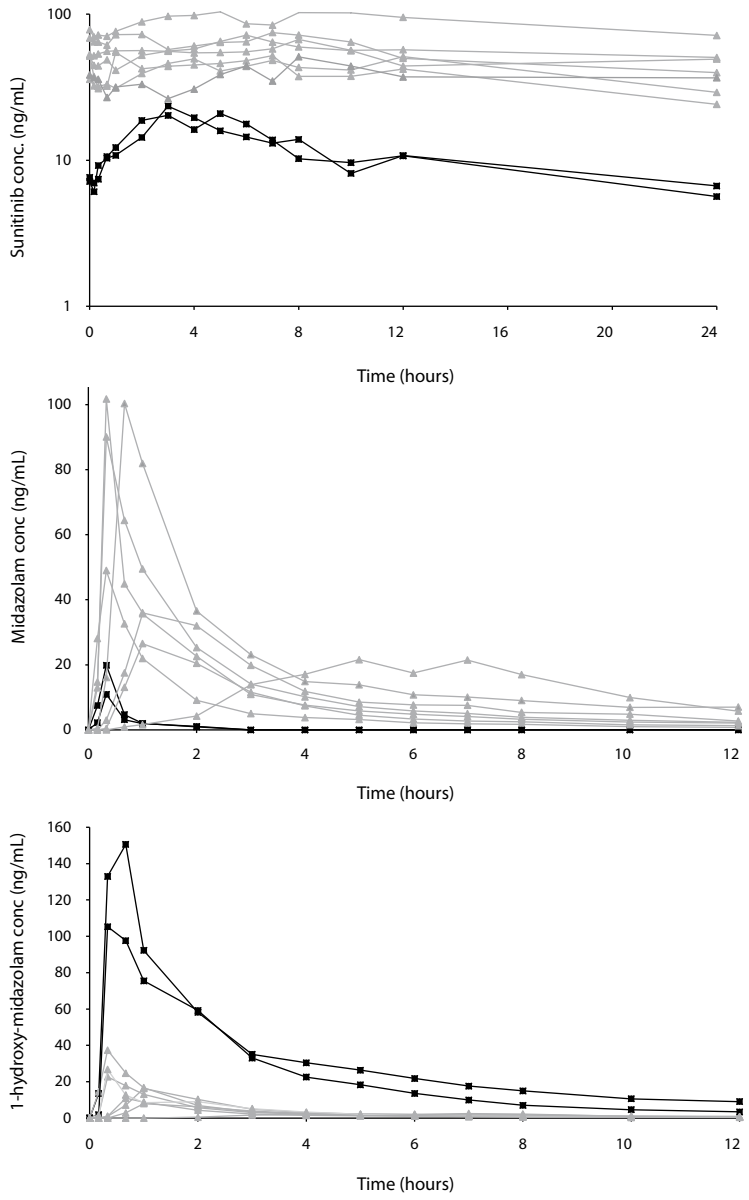
Pharmacokinetic data

Mitotane has an extremely long elimination half life (18-159 days) and therefore an effect of mitotane on co-administered drugs could still be present although mitotane therapy stopped several months before (patient 2). Indeed, the mitotane serum concentrations were 8.1 mg/L in patient 1 and 4.9 mg/L in patient 2. Both mitotane exposed patients showed highly induced CYP3A4 activity resulting in decreased sunitinib, and midazolam exposure (including increased 1-hydroxy-midazolam exposure) (Fig. 1).

The two mitotane treated patients showed markedly reduced sunitinib exposure ($AUC_{0\text{-}24hr}$) compared to the other 7 patients (mean $AUC_{0\text{-}24hr} \pm SD = 268 \pm 0.3$ μg*hr/L versus 1344 ± 358 μg*hr/L, respectively, Fig. 1A) as well as compared to sunitinib exposure levels reported in literature (mean $AUC_{0\text{-}24hr} \pm SD = 965 \pm 367$ μg*hr/L[11] and 1296 ± 358 μg*hr/L[12]).

In addition, mitotane treatment was associated with strikingly reduced midazolam exposure ($AUC_{0\text{-}12hr}$) compared to the exposure measured in the other patients (mean $AUC_{0\text{-}12hr} \pm SD = 7.8 \pm 2.6$ μg*hr/L versus 139.6 ± 59.7 μg*hr/L, respectively, Fig. 1B). Examples of dose normalized (7.5 mg) midazolam exposure levels reported in literature are: ($AUC_{0\text{-}12hr} \pm SD$) 116 ± 57.4 μg*hr/L[13] and ($AUC_{0\text{-}\infty} \pm SE$) 120.6 ± 15.7 μg*hr/L[14]. Midazolam is metabolized by CYP3A4 into 1-hydroxy-midazolam and to a lesser extent into 4-hydroxy-midazolam. Both patients treated with mitotane showed highly elevated 1-hydroxy-midazolam exposure levels ($AUC_{0\text{-}12hr}$) compared to the other patients in the sunitinib pharmacokinetic study (mean $AUC_{0\text{-}12hr} \pm SD = 341.8 \pm 69.6$ μg*hr/L versus 35.2 ± 11.5 μg*hr/L, respectively, Fig. 1C), indicative of increased CYP3A4 activity.

Figure 1 Individual plasma concentrations of **A**) sunitinib, **B**) midazolam and **C**) 1-hydroxy-midazolam.
The 2 black curves represent the mitotane treated patients, the gray curves the 7 non-mitotane treated patients.



Discussion

Mitotane treatment was associated with induced metabolism of midazolam as well as of sunitinib in these 2 patients. Since midazolam is mainly metabolized by CYP3A4 with little affinity for CYP3A5, ABCB1 and ABCG2 our observation supposedly is the result of a strong inducing effect of mitotane on CYP3A4 activity[7, 15, 16].This observation is clinically relevant, since many drugs are metabolized through CYP3A4 e.g. simvastatin, clarithromycin, cyclosporine etc[17]. Consequently, co-administration of mitotane is likely to result in drug-drug interactions, as observed with midazolam and sunitinib. This inducing effect of mitotane on CYP3A4 is extremely potent even in comparison with the CYP3A4 inducing effects of rifampicin. The CYP3A4 inducing effect of mitotane in our study (17.8-fold decrease in midazolam exposure) is much stronger than the effect described for rifampicin on midazolam exposure (8.0-fold decrease in midazolam exposure)[18].

In conclusion, in this pharmacokinetic study we observed a very strong CYP3A4 inducing effect of mitotane which led to a significant drug-drug interaction with sunitinib even after 2 month of cessation of mitotane therapy. This CYP3A4 inducing effect of mitotane will also affect the pharmacokinetics of other drugs which are metabolized by CYP3A4 and can thus cause considerable drug-drug interactions. We can not exclude additional effects of mitotane on other metabolizing enzymes. Therefore, physicians who treat ACC patients with mitotane should be aware of these potential drug interactions which can result in inadvertent therapeutic failure of the co-administered drug.

References

1. Bergenstal DM, Lipsett MB, Moy RH, Hertz R. Regression of Adrenal Cancer and Suppression of Adrenal Function in Man by O,P' Ddd. Transactions of the Association of American Physicians 1959; 72:341-350.
2. Allolio B, Hahner S, Weismann D, Fassnacht M. Management of adrenocortical carcinoma. Clin Endocrinol (Oxf) 2004; 60(3):273-287.
3. Baudin E, Pellegriti G, Bonnay M et al. Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'DDD) levels on the treatment of patients with adrenocortical carcinoma. Cancer 2001; 92(6):1385-1392.
4. Street JC. VI. Biochemical and pathological effects. Organochlorine insecticides and the stimulation of liver microsome enzymes. Ann N Y Acad Sci USA 1969; 160(1):274-290.
5. Cuddy PG, Loftus LS. Influence of mitotane on the hypoprothrombinemic effect of warfarin. South Med J 1986; 79(3):387-388.
6. Magnusson MO, Dahl ML, Cederberg J, Karlsson MO, Sandstrom R. Pharmacodynamics of carbamazepine-mediated induction of CYP3A4, CYP1A2, and Pgp as assessed by probe substrates midazolam, caffeine, and digoxin. Clin Pharmacol Ther 2008; 84(1):52-62.
7. Fuhr U, Jetter A, Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. Clin Pharmacol Ther 2007; 81(2):270-283.
8. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
9. Moolenaar AJ, Niewint JW, Oei IT. Estimation of o,p'-DDD in plasma by gas-liquid chromatography. Clin Chim Acta 1977; 76(2):213-218.
10. Minkin P, Zhao M, Chen Z, Ouwerkerk J, Gelderblom H, Baker SD. Quantification of sunitinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 874(1-2):84-88.
11. Houk BE, Bello CL, Kang D, Amantea M. A population pharmacokinetic meta-analysis of sunitinib malate (SU11248) and its primary metabolite (SU12662) in healthy volunteers and oncology patients. Clin Cancer Res 2009; 15(7):2497-2506.
12. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006; 24(1):25-35.
13. Mueller SC, Majcher-Peszynska J, Mundkowski RG et al. No clinically relevant CYP3A induction after St. John's wort with low hyperforin content in healthy volunteers. Eur J Clin Pharmacol 2009; 65(1):81-87.
14. Farkas D, Oleson LE, Zhao Y et al. Pomegranate juice does not impair clearance of oral or intravenous midazolam, a probe for cytochrome P450-3A activity: comparison with grapefruit juice. J Clin Pharmacol 2007; 47(3):286-294.
15. Kim RB, Wandel C, Leake B et al. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. Pharm Res 1999; 16(3):408-414.
16. Kurnik D, Wood AJ, Wilkinson GR. The erythromycin breath test reflects P-glycoprotein function independently of cytochrome P450 3A activity. Clin Pharmacol Ther 2006; 80(3):228-234.
17. The Trustees of Indiana University. P450 Drug Interaction Table. http://medicine.iupui.edu/clinpharm/ddis/table.asp accessed june 2009.
18. Chung E, Nafziger AN, Kazierad DJ, Bertino JS, Jr. Comparison of midazolam and simvastatin as cytochrome P450 3A probes. Clin Pharmacol Ther 2006; 79(4):350-361.

10

Absorption of cytochrome P450 3A4 inhibiting furanocoumarins from grapefruit juice after oral administration

Nielka P. van Erp, Jan den Hartigh, Hans Gelderblom
and Henk-Jan Guchelaar

Submitted

Abstract

Purpose: Furanocoumarins in grapefruit juice are potent cytochrome P450 3A4 (CYP3A4) inhibitors, however it appears that this only effects intestinal CYP3A4. The reason for an absent effect on hepatic CYP3A4 is yet unknown and we hypothesize that this might be the result of limited absorption of these substrates after oral administration.

Therefore, the absorption of bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB) was studied in healthy volunteers after drinking grapefruit juice. Additionally, the concentration of BG and DHB in different brands and lots of grapefruit juice was analyzed.

Patients and Methods: Six Caucasian healthy volunteers consumed 3 times 400 mL (equal to a BG and DHB dose of 3 x 2.45 mg and 3 x 3.22 mg respectively) grapefruit juice at t= 0, 3 and 6 hour. The serum concentrations of BG and DHB were determined at t= 1, 7 and 8 hour using a validated HPLC-UV method.

Results: BG and DHB levels were undetectable in all volunteers both after single and after multiple dosing, indicating that these inhibitors can only act via the intestinal and not via the hepatic CYP3A4. The variability of BG and DHB concentrations between the different brands and lots of grapefruit juice is substantial. However, the variability within one lot is small.

Conclusion: Since the furanocoumarins BG and DHB are not absorbed after a single or multiple consumptions of grapefruit juice, they are intestinal CYP3A4 inhibitors rather than hepatic CYP3A4 inhibitors. The large variability in concentration BG and DHB between different brands and lots of grapefruit juice necessitates quantification of these ingredients for selecting grapefruit juice for interaction studies.

Introduction

In 1991 the first report was published describing the potential interaction between grapefruit juice and felodipine[1]. Grapefruit juice resulted in an increased felodipine plasma concentration, which led to a decrease in blood pressure. In the following years the prominent role of the metabolic enzyme cytochrome P450 3A4 (CYP3A4) underlying this drug interaction became clear. The effect of grapefruit juice on CYP3A4 appears to be the result of a irreversible inactivation of this enzyme[2, 3]. The pronounced effect of grapefruit juice on the oral availability of multiple drugs that are substrates of CYP3A4, has since the first serendipitous observation, been described thoroughly in many studies[4-10]. Additionally, an inhibitory effect of grapefruit juice on the ATP binding pocket B1 transporter (P-glycoprotein) and on the organic anion transporting polypeptide 1A2 (OATP1A2) and OATP2B1 was postulated[8, 11-15].

In contrast to orally administered drugs, grapefruit juice appears to have only a little effect on intravenously administered drugs[16-19]. Grapefruit juice acts by inhibiting intestinal CYP3A4 activity during uptake of the drug from the intestinal lumen to the systemic circulation and it is thought that hepatic CYP3A4 is largely unaffected, but this has never been studied in detail. Possible explanations for this divergent effect of grapefruit juice on intestinal and hepatic CYP3A4 could be poor absorption of the CYP3A4 inhibiting ingredients or dilution of these substances to concentrations below their effective enzyme inhibitory concentrations[20].

Grapefruit juice is a complex and rich mixture of several hundred ingredients. Much effort has been invested to identify the chemical substance responsible for the inhibiting effect on CYP3A4. The flavonoids; naringin, naringenin, quercetin, kaempferol, and the furanocoumarins; bergamottin, 6',7'-dihydroxybergamottin and its dimers bergapten, bergaptol, 6',7'-epoxybergamottin have been suggested to contribute to the grapefruit juice – drug interactions[2, 12, 20, 21]. The administration of the purified forms of these different compounds to human volunteers, pointed into the direction of the furanocoumarins as being the group of substances most responsible for the CYP3A4 inhibiting effect[10, 22, 23]. The most abundant furanocoumarins present in grapefruit juice are bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB). A complicating factor is that among grapefruit juices brands the concentrations of these furanocoumarins exhibit substantial variability, potentially resulting in a more or less pronounced effect on CYP3A4[24]. To find an explanation for the pronounced effect of BG and DHB on the intestinal but absent effect on the hepatic CYP3A4 enzyme, we investigated whether BG and DHB are absorbed after drinking grapefruit juice with a predetermined dose of BG and DHB. Additionally, BG and DHB were quantified in different brands and batches of grapefruit juice.

Material and methods

BG and DHB in grapefruit juice

Materials

Five different brands and different lots of a single brand of commercially available grapefruit juices were obtained from local grocery stores in The Netherlands. BG and DHB were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standard fenprocoumon was kindly supplied by F. Hoffmann-La Roche (Basel, Switzerland).

Analysis of bergamottin and 6', 7'-dihydroxybergamottin in grapefruit juice

The concentrations BG and DHB were determined using a validated high pressure liquid chromatography – ultraviolet detection (HPLC-UV) method. The used assay is based on a previously published method with minor modifications[24]. Briefly, the juice was homogenized by shaking. Grapefruit juice (0.5mL) was mixed with 10 µL internal standard (100 µg/mL, in methanol) and 2 mL ethyl acetate. Calibration standards contained 0.2 – 4 µg/mL BG and 0.1 – 2 µg/mL DHB were prepared at the start of each analytical run. The standard stock solution contained BG and DHB (100 and 50 µg/mL in DMSO:methanol(1:3)). The extraction was performed by shaking for 30 minutes and separation by centrifugation; 4,000 rpm for 3 minutes. The organic phase was collected and evaporated (40°C; N_2). The residue was reconstituted with 100 µL of DMSO/acetonitril solution (1:3 v/v). A volume of 30 µL of each sample was applied to a HPLC separation system (Unexas 2104, Knauer, Berlin, Germany). The compounds of interest were separated on a Hypersil ODS RP analytical column (4.6 x 100 mm, i.d 3 µm) and eluted over 22 minutes with a flow rate of 1 mL/min and the following gradient [time scale (minutes - minutes)/ percentage of solvent A (water 2500/phosphoric acid 1.25)/ percentage of solvent B (acetonitril)]: 0-7/70/30, 7-17 70/30 → 0/100, 17-18/0/100, 18-19 0/100 → 70/30, 19-22/70/30. The effluent was monitored with a diode array detector (Dionex, UVD340U, Germering, Germany). The UV absorption profile was monitored between 210 – 350 nm. DHB, fenprocoumon and BG eluted at 10.9, 12.8 and 16.5 minutes, respectively. Linearity was confirmed over the range of 0.2 – 24 µg/mL for BG and 0.1 – 12 µg/mL for DHB. The within day and between day precision and accuracy were within 15%.

Study in healthy volunteers

Study design

The study was designed to evaluate the absorption of BG and DHB from the gastrointestinal tract after consuming volumes of grapefruit juice concordant to the volumes described to cause significant drug interactions. Six Caucasian healthy volunteers (4 females, 2 males; age 26 – 40) consumed 3 times 400 mL of a preselected grapefruit juice batch at 0, 3 and 6 hour. To determine serum concentrations of BG and DHB, blood samples were collected at 1, 7 and 8 hour. Blood samples were centrifuged at 4,000 rpm for 5 min and serum was divided into two tubes and stored at –20˚C until the day of analysis. The study was approved by the institutional ethics committee (Leiden University Medical Center, Leiden, The Netherlands).

Analysis of bergamottin and 6', 7'-dihydroxybergamottin in serum

The analytical method used to determine BG and DHB in serum is identical to the method used to determine BG and DHB in juice. Sample preparation was moderately adjusted; 0.5mL serum, 0.5mL phosphate buffer pH 3.0, 0.5M, 10 µL internal standard (10 µL/mL in methanol) and 4mL ethyl acetate were mixed and processed similar to the method described for grapefruit juice. Calibration standards contained 0.02 – 0.4 µg/mL BG and 0.01 – 0.2 µg/mL DHB were prepared at the start of each analytical run. The standard stock solution contained BG and DHB (10 and 5 µg/mL in DMSO:methanol(1:3)). Linearity was confirmed over the range of 0.04 – 1.60 µg/mL for BG and 0.02 – 0.80 µg/mL for DHB. The within day and between day precision and accuracy were within 15%. Stability was studied over a period of 30 days at four conditions; room temperature, refrigerated, frozen and after 3 freeze-thaw cycles and accuracy and precision remained within 15%. The LLQ levels for BG and DHB were 0.04 µg/mL and 0.02 µg/mL, respectively. The LLQ easily met the criteria of accuracy and precision of < 20% and the BG and DHB response at the LLQ was at least 5 times the blank response[25].

Results

Bergamottin and 6',7'-dihydroxybergamottin concentration in grapefruit juice

The BG and DHB concentrations as determined in different brands and lots of grapefruit juice are summarized in Table 1 and showed considerable variability. Moreover, the variation in BG and DHB concentrations within one lot (analyzed in three packets) was relatively small (< 10%). For the performance of the study in healthy volunteers Brand B2 was selected.

Amount of grapefruit juice consumed by healthy volunteers

We aimed to investigate the absorption of a BG dose in the range of at least 1.7-2 mg BG from the intestines, which is a dose capable of inducing a significant effect on felodipine exposure[10]. To administer a sufficient amount of BG in our experiment 400mL (= 2.45 mg BG) of grapefruit juice was administered.

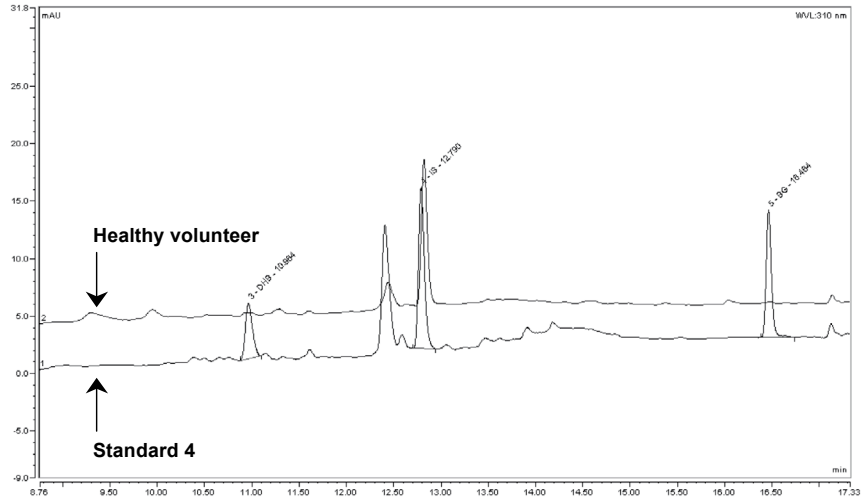
Serum bergamottin and 6', 7'-dihydroxybergamottin concentration

The serum concentrations BG and DHB after a single dose and after multiple doses of 400mL grapefruit juice were not detectable (< 0.04 µg/mL BG and <0.02 µg/mL DHB =LLQ).

Table 1 BG and DHB concentration in different batches grapefruit juice

Product	Bergamottin (mg/L)	6',7'-dihydroxybergamottin (mg/L)
Brand A lot 1	11.9	2.0
Brand A lot 2	8.0	2.1
Brand A lot 3	8.4	2.4
Brand B lot 1	10.0	7.5
Brand B lot 2	6.1	8.1
Brand C	2.6	1.0
Brand D	5.5	1.1
Brand E	4.3	0.4

Figure 1 Chromatograms DHB, IS and BG in Standard and Healthy volunteer



Discussion

In this study the absorption of the two major furanocoumarins in grapefruit juice, capable of inhibiting CYP3A4, was investigated in order to clarify their effect on intestinal and hepatic CYP3A4 activity. Indeed, the effect of grapefruit juice on CYP3A4 is thought to be the result

of an irreversible inactivation of the intestinal and not hepatic CYP3A4 enzyme but the reason for this divergent effect is yet unclear[20]. Our study shows for the first time that the furanocoumarins BG and DHB are not absorbed after single or multiple consumptions of grapefruit juice which could explain their inhibiting effects on CYP3A4 located in the gastrointestinal tract but the absent effect on the same enzymes located in the liver. In addition, a large variability in furanocoumarin concentrations was observed in different brands and in different lots of one brand of grapefruit juice.

Besides inhibition of intestinal CYP3A4, grapefruit juice also inhibits the transporters ABCB1, OATP1A2 and OATP2B1 and this may additionally contribute to the effect that grapefruit juice has on the exposure of co-administered drugs[8, 11-15, 26]. Our results also implicate that interaction of BG and DHB with these transporters located outside the intestinal wall, such as in the kidney, will be limited[16, 17].

We investigated the absorption of BG and DHB after a single dose but also after multiple dosing since one may argue that BG and DHB is only absorbed after saturation of CYP3A4 e.g. after the first dose. Indeed, one study has reported an effect of grapefruit juice on the elimination half life of midazolam and the production of $^{14}CO_2$ after intravenous erythromycin administration and therefore an effect on hepatic CYP3A4 activity after consuming double strength grapefruit juice 240 mL tid for three days[27]. In contrast, an effect of grapefruit juice on hepatic CYP3A4 activity studied with other compounds (lovastatin and simvastatin) was not observed with similar amounts of double strength grapefruit juice[28, 29]. In all three studies the concentration of BG and DHB in the juices was not quantified. Contrastingly, in the our study for the first time the quantity of BG and DHB administered was measured and related to the amount of BG and DHB absorbed. Double quantities of grapefruit juice (400mL) were used to simulate the double strength used in the described studies, which is approximately double the amount normally used in interaction studies[10, 16, 27, 30]. Additionally, 1.7mg and 2mg bergamottin causes a significant drug interaction with felodipine. The 400mL used in this study equalizes 2.45mg BG and 3.22mg DHB. Repeated doses were administered with short time intervals (3hours) to saturate the intestinal CYP3A4 and prevent the formation of new CYP3A4 (CYP3A4 $t_{½} \cong 7 – 23$hours)[4, 5]. The sampling times, 1 hour after de first dose and 1 and 2 hours after the third dose of grapefruit juice, were selected based on the time to maximal BG concentration after consumption of BG capsules; ~1 hour[10]. However, also after multiple dosing non-detectable serum levels of BG and DHB were found. The lower limit of quantification of the validated HPLC-UV method was 0.02 µg/mL for BG and 0.04 µg/mL for DHB, which makes the method suitable for detecting clinically relevant BG and DHB concentrations. Indeed, BG and DHB were able to inhibit CYP3A4 mediated testosterone hydroxylation by 50% and 87.5% at 0.17 – 0.04 µg/mL and 0.15 – 0.04 µg/mL in *in vitro* experiments, respectively[20]. Serum BG and DHB levels below the lower limit of quantitation of the assay are therefore very unlikely to result in any clinical effect on hepatic CYP3A4.

These results indicate that these grapefruit juice compounds are not or only in a very limited amount absorbed after oral consumption and therefore only result in a local effect on the transporters and enzymes in the intestinal wall.

Our study confirms earlier findings regarding high variability of furanocoumarins concentrations in different brands and lots of grapefruit juices[24]. The concentrations BG and DHB measured in grapefruit juices are in the same range as earlier reported. The variability was postulated to be the result of the kind of grapefruit used (white, pink or red) and the storage conditions of the juices[24].

The variable concentrations in the different juices have important implications both clinically and experimentally. Drug-interactions with grapefruit juice could be strongly influenced by the juice that is used since higher concentrations of BG and DHB would logically result in a more pronounced inhibition of intestinal CYP3A4. Therefore, in pharmacological interaction studies a standardized quantity of BG and DHB should be administered in order to interpret the results and make the comparison with other studies possible. An international standardized quantity of 2mg BG could be used since this amount has demonstrated to result in a significant drug interaction in humans[10]. DHB has always demonstrated to exhibit a greater potency as BG *in vitro*, however the magnitude of the difference varied from ~2 - >20-fold[20]. An international standardized quantity of 1mg DHB could therefore be safely suggested for interaction studies.

In the current study we have focused on the most abundant furanocoumarins, BG and DHB and therefore we can not totally exclude an effect of other active compounds in grapefruit juice on hepatic CYP3A4 activity. However, interaction studies with the purified form of the different compounds of grapefruit juice make the furanocoumarins the group that most likely results in an inhibitory effect of CYP3A4[10, 22, 23]. Theoretically, by the design of our study we can not exclude the absorption of BG and DHB across the intestinal wall followed by an extremely high extraction ratio for these components, which could result in undetectable levels of BG and DHB in serum due to a complete first pass effect. However this theoretical large effect of BG and DHB on hepatic enzymes has not been confirmed in interaction studies so far.

In conclusion, BG and DHB are not absorbed in clinically relevant amounts after oral administration of grapefruit juice. This explains why grapefruit juice has an effect on orally administered CYP3A4 substrates whereas it has no effect on CYP3A4 substrates when administered intravenously. The observation that the contents of BG and DHB are highly variable among different brands and lots of grapefruit juices has important consequences for both the interpretation of clinical grapefruit juice – drug interactions and the design of interaction studies.

References

1. Bailey DG, Spence JD, Munoz C, Arnold JM. Interaction of citrus juices with felodipine and nifedipine. Lancet 1991; 337(8736):268-269.
2. Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME et al. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. Drug Metab Dispos 1997; 25(11):1228-1233.
3. Lown KS, Bailey DG, Fontana RJ et al. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. J Clin Invest 1997; 99(10):2545-2553.
4. Lilja JJ, Kivisto KT, Neuvonen PJ. Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. Clin Pharmacol Ther 2000; 68(4):384-390.
5. Greenblatt DJ, von Moltke LL, Harmatz JS et al. Time course of recovery of cytochrome p450 3A function after single doses of grapefruit juice. Clin Pharmacol Ther 2003; 74(2):121-129.
6. Culm-Merdek KE, von Moltke LL, Gan L et al. Effect of extended exposure to grapefruit juice on cytochrome P450 3A activity in humans: comparison with ritonavir. Clin Pharmacol Ther 2006; 79(3):243-254.
7. Benton RE, Honig PK, Zamani K, Cantilena LR, Woosley RL. Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. Clin Pharmacol Ther 1996; 59(4):383-388.
8. Eagling VA, Profit L, Back DJ. Inhibition of the CYP3A4-mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by grapefruit juice components. Br J Clin Pharmacol 1999; 48(4):543-552.
9. Fuhr U, Muller-Peltzer H, Kern R et al. Effects of grapefruit juice and smoking on verapamil concentrations in steady state. Eur J Clin Pharmacol 2002; 58(1):45-53.
10. Goosen TC, Cillie D, Bailey DG et al. Bergamottin contribution to the grapefruit juice-felodipine interaction and disposition in humans. Clin Pharmacol Ther 2004; 76(6):607-617.
11. Bailey DG, Dresser GK, Leake BF, Kim RB. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. Clin Pharmacol Ther 2007; 81(4):495-502.
12. Wang EJ, Casciano CN, Clement RP, Johnson WW. Inhibition of P-glycoprotein transport function by grapefruit juice psoralen. Pharm Res 2001; 18(4):432-438.
13. Hermann M, Asberg A, Reubsaet JL, Sather S, Berg KJ, Christensen H. Intake of grapefruit juice alters the metabolic pattern of cyclosporin A in renal transplant recipients. Int J Clin Pharmacol Ther 2002; 40(10):451-456.
14. Glaeser H, Bailey DG, Dresser GK et al. Intestinal drug transporter expression and the impact of grapefruit juice in humans. Clin Pharmacol Ther 2007; 81(3):362-370.
15. Satoh H, Yamashita F, Tsujimoto M et al. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. Drug Metab Dispos 2005; 33(4):518-523.
16. Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, Krahenbuhl S. Interaction between grapefruit juice and midazolam in humans. Clin Pharmacol Ther 1995; 58(1):20-28.
17. Kharasch ED, Walker A, Hoffer C, Sheffels P. Intravenous and oral alfentanil as in vivo probes for hepatic and first-pass cytochrome P450 3A activity: noninvasive assessment by use of pupillary miosis. Clin Pharmacol Ther 2004; 76(5):452-466.
18. Lundahl J, Regardh CG, Edgar B, Johnsson G. Effects of grapefruit juice ingestion--pharmacokinetics and haemodynamics of intravenously and orally administered felodipine in healthy men. Eur J Clin Pharmacol 1997; 52(2):139-145.
19. Ducharme MP, Warbasse LH, Edwards DJ. Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. Clin Pharmacol Ther 1995; 57(5):485-491.
20. Paine MF, Criss AB, Watkins PB. Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetic and binding properties. Drug Metab Dispos 2004; 32(10):1146-1153.
21. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998; 46(2):101-110.
22. Rashid J, McKinstry C, Renwick AG, Dirnhuber M, Waller DG, George CF. Quercetin, an in vitro inhibitor of CYP3A, does not contribute to the interaction between nifedipine and grapefruit juice. Br J Clin Pharmacol 1993; 36(5):460-463.
23. Paine MF, Widmer WW, Hart HL et al. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. Am J Clin Nutr 2006; 83(5):1097-1105.
24. De Castro WV, Mertens-Talcott S, Rubner A, Butterweck V, Derendorf H. Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. J Agric Food Chem 2006; 54(1):249-255.
25. U.S.Food and Drug Administration. Guidance for industry; Bioanalytical Method Validation, May 2001. http://www.fda.gov/cder/guidance/ #Biopharmaceutics .
26. Dresser GK, Bailey DG, Leake BF et al. Fruit juices

inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther 2002; 71(1):11-20.
27. Veronese ML, Gillen LP, Burke JP et al. Exposure-dependent inhibition of intestinal and hepatic CYP3A4 in vivo by grapefruit juice. J Clin Pharmacol 2003; 43(8):831-839.
28. Lilja JJ, Kivisto KT, Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. Clin Pharmacol Ther 1998; 64(5):477-483.
29. Kantola T, Kivisto KT, Neuvonen PJ. Grapefruit juice greatly increases serum concentrations of lovastatin and lovastatin acid. Clin Pharmacol Ther 1998; 63(4):397-402.
30. Bailey DG, Dresser GK, Kreeft JH, Munoz C, Freeman DJ, Bend JR. Grapefruit-felodipine interaction: effect of unprocessed fruit and probable active ingredients. Clin Pharmacol Ther 2000; 68(5):468-477.

Part 3 | Appendix

General Discussion and Future Perspectives

11

Chapter 11

Introduction

Tyrosine kinases regulate cellular proliferation, survival, differentiation, function, and motility[1]. In the 1980s the first aberrant protein (BCR-ABL) leading to uncontrolled tyrosine kinase activity was discovered[2]. This fusion protein was the product of the minute chromosome, later known as the Philadelphia chromosome, discovered in chronic myelogenous leukemia (CML) by Nowell and Hungerford in 1960[3]. Since the discovery of BCR-ABL, several tyrosine kinases have been associated with development of cancer. For example, human epithelial growth factor (HER2) is expressed in ~25% of all breast cancers[4], BCR-ABL is expressed in ~90% of Philadelphia chromosome positive CML[5] and cKIT is expressed in ~85% of gastrointestinal stromal tumors (GIST)[6]. The tyrosine kinases are deregulated as a result of protein fusion, mutations or increased/aberrant expression of a receptor tyrosine kinase, its ligand, or both[1]. Because tyrosine kinases appear to be important in cancer biology they were interesting proteins for targeted anticancer therapy. Since 2001, eight tyrosine kinase inhibitors (TKIs) are approved for the treatment of specific malignancies. In this thesis the clinical pharmacology of two TKIs, imatinib and sunitinib, were studied and described. Imatinib is the first licensed TKI and is approved for the treatment of Philadelphia chromosome positive (Ph+) CML and for cKIT positive unresectable and/or metastatic malignant GIST[7-9]. The second drug studied, sunitinib, is approved for the treatment of GISTs after failure of imatinib therapy as well as for the treatment of advanced and/or metastatic renal cell carcinoma (mRCC)[10-12].

Pharmacokinetic aspects

TKIs appear to have very similar pharmacokinetic profiles (**chapter 2**). However many pharmacokinetic aspects remain to be studied because most of these drugs received accelerated approval before completing all intended studies, since they are used for serious life-threatening diseases with poor treatment options available. For example, imatinib was introduced onto the marked for CML after one phase I and three phase II trials[7, 13-16]. The applicant committed e.g. to provide complete follow-up safety and efficacy, to conduct a dose finding study in children, to study imatinib pharmacokinetics in patients with liver impairment and to study the influence of cytochrome P450 (CYP) 3A4 inducers on imatinib exposure after drug approval[17, 18]. Sunitinib was also approved under accelerated approval regulations for the treatment of mRCC with the commitment to provide additional information on e.g. the efficacy and safety after complete follow-up, provide additional information on the adverse effect 'left-ventricular ejection fraction', provide an analysis on the relation between exposure and efficacy outcomes and report the pharmacokinetics

of sunitinib in liver impaired patients[i]. After drug approval, case reports and investigator driven interactions and drug disposition studies are published that provide additional insight in involvement of enzymes and drug transporters important in drug disposition. For instance the influence of the adenosine-5'-triphosphate (ATP) binding-cassette (ABC) drug transporters B1 and G2 on imatinib and sunitinib disposition was discovered after approval of the drug by independent researchers[19-21]. The clinical relevance of the affinity and inhibition capacity of sunitinib and imatinib for these transporters needs to be further addressed in additional research.

The clinical relevance of the principal metabolic pathways is typically investigated in healthy volunteers after a single dose of the drug of interest in pharmacological studies before drug approval. However, the clinical relevance of these apparently important enzymes at steady-state pharmacokinetics is usually unknown. In **chapter 3** an absent effect of ritonavir, a potent CYP3A4 inhibitor, was observed on steady-state imatinib pharmacokinetics while CYP3A4 is claimed to be the dominant metabolic route of imatinib. For imatinib some extra studies have been dedicated to the effect of the less dominant enzymes[22-24]. CYP1A2 is one of these minor enzymes in imatinib metabolism. However an absent effect of CYP1A2 induction, by cigarette smoking, on imatinib pharmacokinetics was observed in the study described in **chapter 4**. Still many metabolic pathways in imatinib metabolism need to be explored and additional research is required to better define important enzymes at steady-state imatinib pharmacokinetics in cancer patients. Additionally, the uptake of imatinib from the rectum was measured and described in **chapter 5**. It appears that imatinib is moderately absorbed from the rectum and this route of administration could be considered when oral intake is impossible.

Only little information on the metabolism of sunitinib is available[25, 26]. Since the TKIs appear to have a very similar pharmacokinetics profile many pathways known for other TKIs could be of interest for sunitinib. Additionally, the effect of sunitinib on drug disposition of co-administered drugs has not been investigated in cancer patients at steady-state pharmacokinetics. Therefore, the effect of sunitinib on midazolam exposure and the effect of grapefruit juice on sunitinib exposure have been studied at steady-state sunitinib pharmacokinetics in cancer patients. Sunitinib appears to have an inducing effect on CYP3A4 activity (**chapter 6**) which needs confirmation. Coincidently, a very potent inducing effect on CYP3A4 by mitotane was observed, resulting in decreased sunitinib and midazolam exposure (**chapter 9**). Grapefruit juice increases the relative bioavailability of sunitinib to a clinically non-relevant extent (**chapter 8**), and therefore no scientific evidence was found for the warning in the sunitinib label regarding grapefruit juice consumption.

i Pfizer Inc., Sutent (sunitinib malate): Letter action date 01/26/2006 [accessed 2009 February 24]. Available from: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/

Interpatient variability in drug exposure

The interpatient variability in drug exposure is large, ~40% for both imatinib and sunitinib, similar to the reported variability of all TKIs[18, 26-29]. The study of van Glabbeke et al. demonstrated that imatinib related toxicities are highly dose dependent and thus associated with imatinib exposure[30]. On the other hand, lower trough levels of imatinib appeared to be associated with a decreased efficacy to imatinib therapy[31, 32]. Although little data is available on the relation between sunitinib exposure and toxicity or efficacy, a similar relationship is hypothesized. Sunitinib dose escalation results in a proportional increase in sunitinib trough levels. At increasing dose levels more dose limiting toxicities were observed[33]. An association between sunitinib trough levels and treatment response has not been published yet. The large interpatient variability can result in either unintended toxicity response as well as in decreased therapeutic response. Hence identification of factors affecting the pharmacokinetic profile of TKIs could aid in predicting and adjusting the individual doses to prevent toxic response or therapeutic failure[22]. In the population pharmacokinetic approach of Widmer et al. multiple variables that might explain for the large interpatient variability of imatinib such as age, body weight, gender, disease and α-1 acid glycoprotein were explored. Only α-1 acid glycoprotein explained a substantial part of the interpatient pharmacokinetic variability[28]. For sunitinib, only recently, a study has been described in which variables were explored that could explain for the large interpatient variability in pharmacokinetics. Body weight, gender, race, elevated ECOG performance status, and tumor type explained a substantial part of the interpatient variability in the apparent clearance; body weight and gender explained a part of the interpatient variability in the volume of distribution. However, the major part of the interpatient variability in sunitinib pharmacokinetics remains unexplained[34]. Besides the patient characteristics and the physiological parameters, also the activity of both the enzymes and transporters might be of great influence on the large interpatient variability. Both imatinib and sunitinib are substrates of ABCB1 and ABCG2[19, 21]. The genes encoding these transporters are highly polymorphic which could significantly influence drug absorption[35]. Additionally, functional polymorphisms in enzymes can decrease or increase the metabolic capacity. Genotyping as well as phenotyping of enzymes and transporters might help us to explain a large part of the interpatient variability. Several studies have investigated the effect of transporter polymorphisms on imatinib exposure. *ABCB1 1236T>A*, *ABCB1 2677T>A* and *ABCG2 421C>A* polymorphisms appear to effect imatinib trough levels[36-38]. Similarly the CYP2D6*4 polymorphism results in an increase in imatinib exposure[22]. For sunitinib no studies are available associating genetic polymorphisms in transporters or enzymes and drug exposure. However, an effect of polymorphisms in enzymes and transporters is hypothesized since sunitinib is also a substrate for CYP3A4, ABCB1 and ABCG2 and exploring such associations seems interesting to investigate. In **chapter 7** the relation between sunitinib-induced toxicity and polymorphisms in genes encoding

metabolizing enzymes, drug transporters, targets are described. Polymorphisms in the genes *CYP1A1*, *ABCB1*, *ABCG2*, *NR1I3*, *VEGFR-2* and *FLT3* appear to be associated with the development of sunitinib-induced toxicity. Both imatinib and sunitinib are extensively metabolized by CYP3A4[18, 26]. CYP3A4 is also highly polymorphic; however clinically significant polymorphisms are very uncommon and therefore only a limited role for CYP3A4 pharmacogenetics is predicted[39]. A CYP3A4 phenotypic approach to predict the systemic exposure to imatinib and sunitinib might instead very well explain the large interpatient variability in pharmacokinetics[40]. In **chapter 6** the relation between CYP3A4 activity, determined by midazolam exposure, and sunitinib were investigated and a good relation between the activity of CYP3A4 and sunitinib exposure as well as with sunitinib trough levels was found.

Mechanisms of resistance

Resistance to imatinib and sunitinib therapy can be subdivided into two separate mechanisms: tyrosine kinase reactivation in the presence of a TKI by for example gene amplification or point mutations or the development of resistance which is independent of the tyrosine kinase activity[41].

Point mutations in the tyrosine kinase are the most common reason for the development of TKI resistance. Indeed, for imatinib resistance, various secondary mutations in BCR-ABL have been characterized. Mutations in the adenosine triphosphate (ATP) binding loop (P-loop) of BCR-ABL are frequently observed and associated with a poor response[42]. Most GISTs harbor (primary) mutant c-KIT (~80%) or platelet-derived growth factor receptor α (~5-7%). ~14% of the GISTs exhibit primary resistance to imatinib, additionally another 40-50% develop resistance within 2 years of therapy[43]. GIST responsiveness to imatinib varies for the different primary *c-KIT* genotype; exon 11- mutant GISTs are more sensitive than exon 9-mutant or wild-type GISTs. In contrast, progression free and overall survival on sunitinib therapy were significantly longer for primary *c-KIT* exon 9 mutations and the wild type genotype compared to exon 11 mutations. Secondary point mutations are common in GISTs that show secondary resistance but not in those that exhibit primary resistance. Secondary point mutations are usually located in the drug/ATP binding pocket of the receptor (exon 13 and 14) or in the activation loop (exon 17). In patients that exhibit resistance to imatinib because of secondary point mutations the progression free and overall survival for sunitinib were longer for patients who had secondary *c-KIT* exon 13 or 14 mutations than those with secondary *c-KIT* exon 17 or 18 mutations. Secondary mutations in the activation loop (exon 17 and 18) are insensitive to imatinib and sunitinib therapy[43]. Associations between primary and secondary mutations in tyrosine kinases, important in renal cell carcinoma, and response to sunitinib therapy have not been discovered yet.

Besides the specific tyrosine kinase related resistance, it is thought that exposure levels (pharmacokinetics) also may play a role in the initial or secondary resistance. Recently, a correlation was observed between clinical effect in CML (defined as major molecular response and complete cytogenetic response) and a minimal trough level of imatinib, indicating that inadequate drug exposure levels could also result in initial or secondary imatinib resistance[31]. A minimal exposure to imatinib and sunitinib is also suggested for the effective treatment of GIST and mRCC, although studies supporting this hypothesis have not been performed yet. Several possible mechanisms have been described resulting in an inadequate drug exposure; i) increased levels of the acute phase binding protein (α acid glycoprotein (AAG)) resulting in a reduced free fraction of the drug[28, 44], ii) increased functionality of the highly polymorphic efflux transporters ABCB1 and ABCG2[21, 35-37], iii) upregulated drug clearance by increased activity of metabolizing enzymes[22, 40]. Additionally, the exposure to the drug can decrease over time due to increased drug clearance[27].

Future research perspectives

In the recent years important progress has been made in unraveling the pathophysiology of cancer. With this gaining insight, targeted therapies, that can specifically inhibit deregulated cellular processes important for maintenance of the malignancies, have been and are being developed. Ultimately, this may lead to an approach of cancer as being a chronic disease instead of a life threatening disease. A major challenge to address in the treatment of chronic cancerous disease is how to circumvent antitumor drug resistance.

With the better characterization of tumor biology and the somatic mutations resulting in tumor progression, the disease could be treated on a more individualized and targeted basis. For example, GIST tumors harboring specific mutations in the cKIT receptor that respond better to either imatinib or sunitinib might better be treated based upon somatic tumor characteristics rather that the first line, "one size fits all" approach. Drug development of anticancer drugs for tumor subtypes harboring specific somatic mutations rather than for anatomic or histological tumor subtypes may lead to more effective therapies and less tumor resistance. However, this approach may be in conflict with the study design of pharmaceutical industry at this moment in which antitumor drugs are developed for large groups of patients and it is therefore questionable whether we can expect this somatic mutation driven approach from industry studies. For GIST tumors the role of *cKIT* mutations for imatinib and sunitinib sensitivity are thoroughly investigated and better understood. However, for mRCC and many other tumors these investigations for tumor subtype specific drug sensitivity should be performed in the nearby future.

Additionally, genotypic features in drug targets, enzymes and transporters might predispose

for development of side effects to antitumor therapy. The drug targets are not solely expressed on tumor tissues but also on "healthy" cells responsible for physiological processes in our body. Affinity differences, due to genetic alteration, of the drug for the targets could result in a more or less pronounced effect on normal cells and thereby results in toxicity. By better characterization of factors that result in toxicity, therapies can be selected that have a favorable toxicity profile which will result in a better adherence to and acceptance of the therapy and less required dose adjustments. Dose adjustments due to toxicity could be harmful since subtherapeutic exposure levels for an adequate antitumor response might be generated. Also polymorphisms in genes encoding enzymes and transporters important for drug metabolism and disposition can lead to toxicity or inefficacy as a result of higher or lower exposure levels.

Enzymes and transporters claimed to be important at time of drug approval are typically identified in *in vitro* studies and confirmed in single dose interaction studies in healthy volunteers. The warnings for co-administered drugs and food in the drug label are based upon extrapolations from these single dose interactions studies. Since TKIs are administered on a daily basis and some appear to be substrates as well as inhibitors of their own metabolic and disposition pathway, the enzymes and transporters that are important at steady-state pharmacokinetics in cancer patients could be very different from those identified just after starting therapy as we have demonstrated in several studies described in this thesis. Therefore, pharmacological studies at steady-state pharmacokinetics using phenotypic probes should be done to identify the enzymes and transporters that are important in drug metabolism and disposition. This will result in better scientifically based warnings in the drug label for drugs and food that should not be co-administered. This may ultimately result in more reliable medication surveillance by physicians and pharmacists resulting in less sub- and supratherapeutic exposure levels in patients treated with these drugs.

For all TKIs, except for imatinib in CML treatment, minimal exposure levels or minimal trough levels required for a therapeutic response are unknown. A complicating factor is that different tumors (depending on different TKs) and tumor subtypes (with different somatic mutations) will require different concentration levels due to sensitivity differences. Inadequate drug concentrations could result in either tumor progression or drug related toxicities. I would like to hypothesize that subtherapeutic concentrations results in the selection of less sensitive cells which, by generating secondary mutations, results in drug resistance. Based on data from dose limiting toxicity studies (phase I trails), initially a fixed dose is used for the treatment with TKIs, regardless of the sensitivity of the tumor or the individual drug concentration. Only during phase I studies pharmacokinetics data are collected while the therapeutic response is monitored during phase II and III trails. A better determination of the relation between drug concentrations and disease response during phase II and III trials could help us in individualizing treatment aimed at preventing therapeutic failure and toxicities. The TKIs are generally administered in a daily regimen and thereby suppress tumor growth continuously. Interesting parallels between the therapy with TKIs and antiretroviral therapy (used in HIV infections) which also encounters resistance can be drawn. The interindividual variability is large for all TKIs and determinants for this large variability are at least partly unknown. The "fixed dose for all tumors approach" that is applied will not result in the aimed exposure level or the aimed trough level in all patients due to the large interpatient variability. Therefore therapeutic drug monitoring (TDM) could become important in the treatment of cancer for this group of drugs. Initially, it needs to be established which PK parameter associates best with therapeutic response. Limiting sampling makes TDM more feasible. Therefore after establishing the most suitable PK parameter effort should be invested in determining the minimal amount of samples needed to obtain the parameter. For sunitinib for example we have investigated that trough levels correspond well with exposure levels, which makes trough level monitoring suitable for both concentration threshold as well as exposure determination. The monitoring just after starting therapy is required to adjust the dose until the aimed drug concentration is reached. However, since the drug concentration can decrease over time, repeatedly monitoring would be required. For most TKIs the correct PK parameter that relates to therapeutic response needs to be identified and additionally a limiting sampling approach needs to be defined.

Although a promising group of new drugs have been discovered and are used in the treatment of malignancies still great profit can be achieved by a better understanding of important pharmacogenetic and pharmacokinetic features of these drugs which could result in a more individualized approach with less toxicity and more efficacy.

References

1. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005; 353(2):172-187.
2. Konopka JB, Watanabe SM, Witte ON. An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell 1984; 37(3):1035-1042.
3. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960; 132:1497.
4. Dean-Colomb W, Esteva FJ. Her2-positive breast cancer: herceptin and beyond. Eur J Cancer 2008; 44(18):2806-2812.
5. Stam K, Heisterkamp N, Grosveld G et al. Evidence of a new chimeric bcr/c-abl mRNA in patients with chronic myelocytic leukemia and the Philadelphia chromosome. N Engl J Med 1985; 313(23):1429-1433.
6. Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. Mod Pathol 1998; 11(8):728-734.
7. Druker BJ, Talpaz M, Resta DJ et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001; 344(14):1031-1037.
8. Novartis. Drug label Gleevec approved on 05/20/2003. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ Accessed on 01/19/2009.
9. van Oosterom AT, Judson I, Verweij J et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 2001; 358(9291):1421-1423.
10. Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006; 368(9544):1329-1338.
11. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356(2):115-124.
12. Pfizer. Drug label Sutent approved 01/26/2006. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ Accessed on 02/18/2009.
13. Druker BJ, Sawyers CL, Kantarjian H et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001; 344(14):1038-1042.
14. Talpaz M, Silver RT, Druker BJ et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 2002; 99(6):1928-1937.
15. Kantarjian H, Sawyers C, Hochhaus A et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 2002; 346(9):645-652.
16. Sawyers CL, Hochhaus A, Feldman E et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 2002; 99(10):3530-3539.
17. Johnson JR, Bross P, Cohen M et al. Approval summary: imatinib mesylate capsules for treatment of adult patients with newly diagnosed philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase. Clin Cancer Res 2003; 9(6):1972-1979.
18. Cohen MH, Williams G, Johnson JR et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res 2002; 8(5):935-942.
19. Burger H, Nooter K. Pharmacokinetic resistance to imatinib mesylate: role of the ABC drug pumps ABCG2 (BCRP) and ABCB1 (MDR1) in the oral bioavailability of imatinib. Cell Cycle 2004; 3(12):1502-1505.
20. Shukla S, Sauna ZE, Ambudkar SV. Evidence for the interaction of imatinib at the transport-substrate site(s) of the multidrug-resistance-linked ABC drug transporters ABCB1 (P-glycoprotein) and ABCG2. Leukemia 2008; 22(2):445-447.
21. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. Drug Metab Dispos 2009; 37(2):359-365.
22. Gardner ER, Burger H, van Schaik RH et al. Association of enzyme and transporter genotypes with the pharmacokinetics of imatinib. Clin Pharmacol Ther 2006; 80(2):192-201.
23. van Erp NP, Gelderblom H, Van GM et al. Effect of cigarette smoking on imatinib in patients in the soft tissue and bone sarcoma group of the EORTC. Clin Cancer Res 2008; 14(24):8308-8313.
24. Wang Y, Zhou L, Dutreix C et al. Effects of imatinib (Glivec) on the pharmacokinetics of metoprolol, a CYP2D6 substrate, in Chinese patients with chronic myelogenous leukaemia. Br J Clin Pharmacol 2008; 65(6):885-892.
25. Adams VR, Leggas M. Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Ther 2007; 29(7):1338-1353.
26. Goodman VL, Rock EP, Dagher R et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007; 13(5):1367-1373.
27. Judson I, Ma P, Peng B et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005; 55(4):379-386.
28. Widmer N, Decosterd LA, Csajka C et al. Population pharmacokinetics of imatinib and the role of alpha-acid glycoprotein. Br J Clin Pharmacol 2006; 62(1):97-112.
29. Schmidli H, Peng B, Riviere GJ et al. Population pharmacokinetics of imatinib mesylate in patients with chronic-phase chronic myeloid leukaemia: results of a phase III study. Br J Clin Pharmacol 2005; 60(1):35-44.
30. Van GM, Verweij J, Casali PG et al. Predicting toxicities for patients with advanced gastrointestinal stromal tumours treated with imatinib: a study of the European Organisation for Research and Treatment of Cancer, the Italian Sarcoma Group, and the Australasian Gastro-Intestinal Trials Group (EORTC-ISG-AGITG). Eur J Cancer 2006; 42(14):2277-2285.
31. Larson RA, Druker BJ, Guilhot F et al. Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. Blood 2008; 111(8):4022-4028.
32. Picard S, Titier K, Etienne G et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood 2007; 109(8):3496-3499.
33. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006; 24(1):25-35.
34. Houk BE, Bello CL, Kang D, Amantea M. A population pharmacokinetic meta-analysis of sunitinib malate (SU11248) and its primary metabolite (SU12662) in healthy volunteers and oncology patients. Clin Cancer Res 2009; 15(7):2497-2506.
35. Lepper ER, Nooter K, Verweij J, Acharya MR, Figg WD, Sparreboom A. Mechanisms of resistance to anticancer drugs: the role of the polymorphic ABC transporters ABCB1 and ABCG2. Pharmacogenomics 2005; 6(2):115-138.
36. Dulucq S, Bouchet S, Turcq B et al. Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood 2008; 112(5):2024-2027.
37. Gurney H, Wong M, Balleine RL et al. Imatinib disposition and ABCB1 (MDR1, P-glycoprotein) genotype. Clin Pharmacol Ther 2007; 82(1):33-40.
38. Petain A, Kattygnarath D, Azard J et al. Population pharmacokinetics and pharmacogenetics of imatinib in children and adults. Clin Cancer Res 2008; 14(21):7102-7109.
39. van Schaik RH. CYP450 pharmacogenetics for personalizing cancer therapy. Drug Resist Updat 2008; 11(3):77-98.
40. Li J, Karlsson MO, Brahmer J et al. CYP3A phenotyping approach to predict systemic exposure to EGFR tyrosine kinase inhibitors. J Natl Cancer Inst 2006; 98(23):1714-1723.
41. Ramirez P, DiPersio JF. Therapy options in imatinib failures. Oncologist 2008; 13(4):424-434.
42. Branford S, Rudzki Z, Walsh S et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003; 102(1):276-283.
43. Heinrich MC, Maki RG, Corless CL et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J Clin Oncol 2008; 26(33):5352-5359.
44. Larghero J, Leguay T, Mourah S et al. Relationship between elevated levels of the alpha 1 acid glycoprotein in chronic myelogenous leukemia in blast crisis and pharmacological resistance to imatinib (Gleevec) in vitro and in vivo. Biochem Pharmacol 2003; 66(10):1907-1913.

Summary

Every year approximately 3.2 million Europeans are diagnosed with cancer and with ~1.7 million deaths from cancer per year it is the second most common cause of death[i]. In the Netherlands approximately 83 thousand persons are diagnosed with cancer each year and the mortality incidence is around 40 thousand persons a year[ii]. Although, multiple anticancer therapies have been developed in the recent years, the quest for novel therapies which harbors better efficacy and less toxicity is still an important topic.

In the 1980s a group of possibly interesting proteins, tyrosine kinases, in cancer biology were discovered. Tyrosine kinases (TKs) are enzymes that catalyze the transfer of phosphate from adenosine triphosphate (ATP) to other cellular proteins and thereby regulate several crucial processes regarding survival, proliferation and motility of cells. The activity of TKs is normally under tight control. However, in multiple cancers TKs appear to be deregulated which make them interesting targets for anticancer therapy. In 2001 the first tyrosine kinase inhibitor (TKI), imatinib, was registered for the treatment of BCR-Abl positive chronic myelogenous leukemia (CML). Since the introduction of imatinib, seven other TKIs have been registered and more TKIs will be introduced in the near future. Although these TKIs were initially introduced as the "magic bullets" that would be highly tumor-cell specific and thus highly antitumor effective with only minor toxicity towards normal cells, limitations were soon encountered. The development of resistance and the occasionally observed toxicities constitute the major challenge in the treatment with TKIs.

A better understanding of the pharmacokinetics of TKIs might help us to prevent sub- or supratherapeutic exposure to these drugs. Additionally, a better understanding of polymorphisms in the pharmacokinetic and pharmacodynamics pathways of the TKIs might also help us to prevent toxicities and to optimize tumor response by individualizing the dose and choice of antitumor therapy. This thesis focuses on the pharmacokinetics of imatinib and sunitinib in cancer patients and on the use of different tools, phenotyping and pharmacog-enotyping, to optimize and individualize TKI therapy.

TKIs represent a relatively new and fast growing group of anticancer drugs developed as oral formulations which are administered in cancer patients in a daily regimen. Most of the current knowledge of the pharmacokinetic behavior of the TKIs is derived from *in vitro* experiments, animal studies, drug-drug interaction studies and mass balance studies in healthy volunteers with a single dose of the aimed TKI. However, since this group of drugs is administered in a daily schedule, other enzymes and drug transporters might become important at steady-state pharmacokinetics, which could result in adjusted warnings for co-administered drugs and food. In **chapter 2** an overview is provided of the current knowledge on the

i http://ec.europa.eu/health
ii http://www.ikcnet.nl

pharmacokinetic aspects; absorption, distribution, metabolism, excretion (ADME), drug transporters and drug-drug interactions of the eight registered TKIs: imatinib, gefitinib, erlotinib, sorafenib, sunitinib, dasatinib, lapatinib and nilotinib. Additionally, the similarities and differences between these apparently related TKIs are summarized.

Clinical pharmacology of imatinib

Imatinib is predominantly metabolized by the enzyme cytochrome P450 3A4 (CYP3A4) and is therefore prone to drug interactions with co-administered drugs, food, and herbal supplements. The warnings for CYP3A4 inducers or inhibitors are based on drug interaction studies with a single-dose of imatinib. However, it is unknown if similar drug interactions occur at steady-state imatinib pharmacokinetics. Therefore, the effect of ritonavir, a potent CYP3A4 inhibitor, on steady-state imatinib in cancer patients was investigated (**chapter 3**). Surprisingly, imatinib appears to be insensitive to potent CYP3A4 inhibition by ritonavir at steady-state. Since imatinib is a CYP3A4 inhibitor itself it is hypothesized that the drug relies on alternate elimination pathways after prolonged exposure due to autoinhibition of CYP3A4. For drugs with complex elimination pathways, such as imatinib, interaction studies that are performed after a single dose may not provide us with correct information applicable for clinical practice. Therefore, it is preferred to perform interaction studies at steady state pharmacokinetics which better represents the clinical situation since other enzymes, that only play a secondary role in *in vitro* experiments, could play a dominant role at steady-state.

Possible interesting cytochrome enzymes for imatinib metabolism are: CYP1A1, CYP1A2, CYP2C9, CYP2C19, and CYP2D6. CYP1A2 is induced by cigarette smoking and therefore smokers might be exposed to lower blood concentrations of imatinib than non smokers. In **chapter 4** the effect of smoking on imatinib pharmacokinetics, safety, and efficacy was investigated. The results of this study did not reveal a dominant role for CYP1A2 in imatinib metabolism since smoking did not alter the pharmacokinetics and thereby the exposure to imatinib. Interestingly, smoking was related to an increased risk for grade ≥ 2 anemia and fatigue and additionally showed a shorter overall survival and a shorter time to progression on treatment with imatinib. However, these last two observations warrants further confirmation.

Coincidently, one of the patients who volunteered in the imatinib pharmacokinetic study was admitted to the hospital a year later with tumor-related intra-abdominal obstructions and diffuse intra-abdominal bleedings. Due to gastro-intestinal obstruction the patient was unable to take the imatinib tablets orally; therefore the tablets were administered rectally. The uptake of imatinib after rectal administration is described in **chapter 5**. The imatinib exposure after rectal administration appeared to be approximately 40% of the exposure reached after oral administration. Therefore, rectal administration could be considered in situations were oral intake of the tablets is impossible.

Clinical pharmacology of sunitinib

Sunitinib is a multitargeted tyrosine kinase inhibitor, known to inhibit vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3, platelet-derived growth factor receptor (PDGFR) α and β, KIT, Fms-like tyrosine kinase 3 receptor (FLT3), and the receptor encoded by the ret proto-oncogene (RET). The drug is approved for the first line treatment of metastatic renal cell carcinoma (mRCC) and imatinib resistant metastatic GIST. The toxicity profile of sunitinib is pronounced and includes e.g.: fatigue, mucosal inflammation, cardiotoxicity and myelo-suppression. Approximately 30% of the patients treated with sunitinib need a dose reduction or interruption due to adverse events making toxicity a limiting factor in the successful treatment with this drug. In the following chapters several approaches have been explored with the aim to individualize sunitinib therapy and thereby reduce toxicity. Additionally, the effect of CYP3A4 inhibition on sunitinib exposure as well as the effect of sunitinib on CYP3A4 activity is studied in drug-interaction studies.

In **chapter 6** the possible use of the noninvasive CYP3A4 phenotypic probe, midazolam, to predict sunitinib exposure was explored. Additionally the relation between sunitinib plasma trough levels and sunitinib exposure was determined since monitoring sunitinib trough levels provide a more feasible and assessable approach to study exposure-effect and -toxicity relations. Moreover, the effect of sunitinib on CYP3A4 activity was evaluated. Since sunitinib is solely metabolized by CYP3A4, the activity of CYP3A4 might explain the large and yet unexplained interpatient variability (~40%) in sunitinib clearance. The activity of CYP3A4 can be determined by the phenotypic probe midazolam which is also mainly metabolized by CYP3A4 without exerting influence on the activity of this enzyme. It appears that midazolam exposure relates well to sunitinib exposure as well as sunitinib trough levels and explains a large part of the interpatient variability in sunitinib clearance. Also a strong relation was found between sunitinib trough levels and sunitinib exposure which legitimates the use of sunitinib trough levels instead of the multiple sampling approaches to determine exposure-effect and –toxicity relationships. Additionally, sunitinib appears to be a mild CYP3A4 inducer however this observation needs confirmation.

Both genes encoding the sunitinib targets (VEGFR1, -2 and -3, PDGFR-α and PDGFR-β, KIT, FLT3, and RET), as well as genes encoding the enzymes and efflux transporters involved in sunitinib's disposition and metabolism are highly polymorphic and may be related to the differential toxicity response in patients treated with sunitinib. The identification of genetic

markers related to toxicity outcomes in the pharmacokinetic and pharmacodynamic pathways of sunitinib are described in **chapter 7**. The selected toxicity outcomes; thrombocytopenia, leucopenia, mucosal inflammation, hand-foot syndrome and any toxicity > grade 2, were based on the results of a published placebo controlled study. We selected toxicities that appear to be causally related to sunitinib treatment. Thrombocytopenia was not associated with any of the genetic polymorphisms studied. Polymorphisms in *FLT3, NR1I3 and CYP1A1* were related to leucopenia. The same polymorphism in *CYP1A1* was related to mucosal inflammation. Hand-foot syndrome appeared to be related to a polymorphism in *ABCB1*. Finally any toxicity > grade 2 was associated with polymorphisms in *VEGFR2* and *ABCG2*. The polymorphisms identified in this study should be regarded as hypothesis generating and need to be confirmed in an independent group of patients.

Since CYP3A4 appears to be the most important enzyme in the metabolism of sunitinib the drug label warns for multiple drugs and food substrates known to interfere with the activity of this enzyme. However, most of these warnings are not based on study results but rather are extrapolations of the observed interaction with model drugs such as rifampicin (CYP3A4 inducer) and ketoconazol (CYP3A4 inhibitor). Grapefruit juice is a potent CYP3A4 inhibitor of the enzymes located in the intestines. The effect of grapefruit juice on the bioavailability of sunitinib has not been studied yet. Nevertheless, the drug label of sunitinib advises patients to avoid the consumption of this juice. In **chapter 8** the effect of grapefruit juice on steady-state sunitinib exposure was evaluated. The co-administration of grapefruit juice with sunitinib resulted in an 11% elevation of sunitinib bioavailability which is not regarded as clinically relevant.

Two patients in the sunitinib pharmacokinetic study described in chapter 8 showed aberrant pharmacokinetics of sunitinib and midazolam, the latter being used as a CYP3A4 phenotypic probe (**chapter 9**). Both patients were also treated with mitotane which appeared to be a very potent CYP3A4 inhibitor.

As described before, grapefruit juice is a potent inhibitor of intestinal CYP3A4 enzymes. However it has no effect on the same CYP3A4 enzymes located in the liver. An explanation for this unexpected effect is not found yet. A possible explanation could be that the active ingredients in grapefruit juice are not absorbed across the intestinal wall.

Chapter 10 describes the absorption of two active ingredients in grapefruit juice in healthy volunteers after consuming large quantities of the juice: bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB), which are held responsible for CYP3A4 inhibition. Additionally the amount of BG and DHB in different brands and lots of grapefruit juice was quantified. The two ingredients, BG and DHB, were undetectable both after single and multiple consumptions of grapefruit juice. Therefore, the lack of substantial absorption of BG and DHB probably explains why grapefruit juice has an inhibitory effect on intestinal CYP3A4 only and not on hepatic CYP3A4. The large variability in concentration BG and DHB between different brands and lots of grapefruit juice necessitates quantification of these ingredients in order to make the interpretation of the results and comparison between different interaction studies possible.

In the final **chapter** the results of this thesis are discussed and possible future directions are outlined. Future developed antitumor treatments will more specifically interact with the underlying mechanism responsible for deregulation of cellular growth control in tumor cells. With a better understanding of tumor biology, a more individualized approach will probably be reached resulting in the application of targeted drugs developed to inhibit specific tumor subtypes. Individualization will also result in the selection of the right individual patients that profit most an endurable toxicity profile. Additionally, monitoring the exposure to the drugs and adjusting the individual dose based on the exposure level measured will contribute to the optimization of antitumor response and limitation of drug related toxicities. Much effort will be needed to determine the exposure-effect and exposure-toxicity relation for the different tumor subtypes and patients. The use of predictive biomarkers and therapeutic drug monitoring will probably become more important in future antitumor treatment.

Nederlandse Samenvatting

Kanker is een veelvoorkomende aandoening en vormt de op één na meest voorkomende doodsoorzaak in Europa evenals in Nederland. Ongeveer de helft van de patiënten met kanker overlijdt en medicamenteuze behandelingen zijn, uitzonderingen daargelaten, over het algemeen maar beperkt effectief en weinig specifiek. De vraag naar betere therapeutische mogelijkheden die gerichter de tumorcellen bestrijden en minder schadelijke effecten op gezond weefsel laten zien is daarom van groot belang. In de tachtiger jaren werd een groep eiwitten ontdekt, tyrosine kinasen, die mogelijk een cruciale rol vervullen in het ontstaan van kanker. Tyrosine kinasen (TKs) zijn enzymen die middels fosfaatoverdracht op andere eiwitten binnen de cel verschillende cruciale processen activeren en coördineren, waaronder de overleving en deling van cellen. De activiteit van deze TKs is normaliter streng gereguleerd. Echter bij verschillende vormen van kanker is vastgesteld dat de nauwe regulatie van deze TKs niet meer goed functioneert. Hiermee werden ze een geschikt aangrijpingspunt voor nieuw te ontwikkelen antikanker therapie. In 2001 werd de eerste TK remmer, imatinib, op de geneesmiddelenmarkt toegelaten. Het geneesmiddel werd geregistreerd voor twee types kanker die sterk afhankelijk bleken te zijn van een ontregelde TK activiteit. Na de toelating van imatinib zijn nog zeven andere TK remmers toegelaten voor verschillende tumoren.

Deze nieuwe groep TK remmers werden initieel gezien als het universele antwoord op de behandeling van kanker. Echter de beperkingen van deze geneesmiddelen zijn inmiddels ook duidelijk. Resistentie tegen TK remmers vormt een groot probleem en ook bijwerkingen (toxiciteit) blijken een grote belemmering in de behandeling te vormen. Een beter begrip over hoe het menselijke lichaam omgaat met deze TK remmers, de farmacokinetiek van deze geneesmiddelen, kan ons helpen bij het goed doseren van de TK remmers bij patiënten die behandeld worden met deze geneesmiddelen. Hiermee kan een te hoge blootstelling (geneesmiddelspiegel) worden voorkomen, waardoor het risico op toxiciteit afneemt alsmede een te lage blootstelling waardoor het geneesmiddel niet effectief is. Aanvullend kunnen genetische veranderingen (o.a. polymorfismen), die coderen voor de enzymen, de geneesmiddelen pompen (transporters) en de TKs voor een afwijkende reactie van de patient en of tumor op het geneesmiddel zorgen. Een beter inzicht op de invloed van deze polymorfismen kan dus ook resulteren in een betere individuele afstemming van de therapie. Het doel van het in dit proefschrift beschreven onderzoek was meer kennis te vergaren over de farmacokinetiek van imatinib en sunitinib in patiënten met kanker. Ook zijn verschillende mogelijkheden onderzocht om de therapie beter op de individuele patiënt af te stemmen en daarmee over- en onderdoseringen te voorkomen.

TK remmers vormen een relatieve nieuwe en snel groeiende groep geneesmiddelen. Het zijn geneesmiddelen die dagelijks, oraal moeten worden ingenomen in tegenstelling tot de meeste tot dusver gebruikte antikanker therapieën die vooral intraveneus worden gegeven. De kennis over de farmacokinetiek van deze geneesmiddelen is afgeleid van uitgevoerde *in vitro*

(regeerbuis / celonderzoek) experimenten, dierexperimenteel onderzoek, geneesmiddel interactiestudies en blootstellingstudies in gezonde vrijwilligers na de inname van een eenmalige dosis van het beoogde geneesmiddel. Echter de farmacokinetiek van een geneesmiddel kan behoorlijk veranderen als het dagelijks wordt ingenomen (continue blootstelling) en het zijn eigen chemische omzetting (metabolisme) mogelijk remt of stimuleert. In **hoofdstuk 2** wordt een overzicht gegeven wat tot dusver bekend is over de farmacokinetiek; opname, verdeling, metabolisme en uitscheiding alsmede het transport en de geneesmiddelinteracties van de acht tot dusver geregistreerde TK remmers: imatinib, gefitinib, erlotinib, sorafenib, sunitinib, dasatinib, lapatinib en nilotinib. Tevens wordt ingegaan op overeenkomsten en verschillen tussen deze geneesmiddelen die allen tot dezelfde familie behoren.

Klinische farmacologie van imatinib

Imatinib wordt vooral gemetaboliseerd door een enzym genaamd cytochroom P450 3A4 (CYP3A4). Dit enzym is betrokken bij het metabolisme van veel geneesmiddelen en wordt tevens door veel geneesmiddelen geremd of gestimuleerd (geinduceerd). Imatinib is daarom gevoelig voor geneesmiddelinteracties. De bijsluiter van imatinib waarschuwt dan ook voor het gebruik van remmers of inductoren (stimulatoren) van dit enzym aangezien dat de imatinib blootstelling kan beïnvloeden. In **hoofdstuk 3** hebben we onderzocht of bij continue blootstelling aan imatinib remming van CYP3A4 invloed heeft op deze blootstelling. Hiertoe is ritonavir, een krachtige CYP3A4 remmer, toegevoegd aan de behandeling met imatinib, nadat imatinib al geruime tijd gebruikt werd. Imatinib bleek in dit onderzoek ongevoelig voor de gelijktijdige toediening van een krachtige remmer van CYP3A4. Imatinib blijkt zelf ook in staat CYP3A4 te remmen. Het is daarom mogelijk dat imatinib na langere blootstelling zijn eigen metabolisme via CYP3A4 remt en omgezet wordt door enzymen die aanvankelijk minder belangrijk leken in *in vitro* experimenten. Enzymen die in staat zijn imatinib te metaboliseren zijn: CYP1A1, CYP1A2, CYP2C9, CYP2C19 en CYP2D6. In een volgend onderzoek, beschreven in **hoofdstuk 4**, is gekeken naar de invloed van CYP1A2 stimulatie op de imatinib blootstelling. Het is bekend dat het roken van sigaretten het enzym CYP1A2 stimuleert. Als CYP1A2 een belangrijk enzym vormt in de omzetting van imatinib zou roken resulteren in een lagere imatinib blootstelling vergeleken met niet-rokers. Dit effect hebben we echter niet kunnen waarnemen. Rokers kregen wel vaker anemie (bloedarmoede) en waren vaker vermoeid dan niet rokers. Tevens lieten rokers een kortere overleving en een kortere tijd tot tumorgroei op imatinib therapie zien. Deze laatste bevindingen moeten beschouwd worden als hypothese genererend en dienen bevestigd en verklaard te worden in aanvullend onderzoek.
Ongeveer een jaar na deelname aan het imatinib farmacokinetiek onderzoek, beschreven in hoofdstuk 3, werd een patiente opgenomen met darmobstructie veroorzaakt door

tumorgroei. Deze patiënte kon niets oraal innemen, waardoor besloten werd imatinib tabletten rectaal te geven. Resultaten van dit n = 1 onderzoek zijn beschreven in **hoofdstuk 5**. De opname van imatinib uit het rectum resulteert in ongeveer 40% blootstelling van de blootstelling waargenomen na orale inname. Rectale toediening kan daarom overwogen worden in noodsituaties waarbij uiteraard gecorrigeerd moet worden voor de gereduceerde opname.

Klinische farmacologie van sunitinib

Sunitinib is een TK remmer die meerdere TKs remt. Sunitinib vertoont een uitgesproken toxiciteitprofiel. Bijwerkingen die veelvuldig gerapporteerd worden zijn o.a.: vermoeidheid, slijmvliesontstekingen, cardiale afwijkingen en beenmergonderdrukking. Ongeveer 30% van de mensen die behandeld worden met sunitinib heeft een dosisreductie of –onderbreking nodig ten gevolge van bijwerkingen op het geneesmiddel. In dit proefschrift zijn meerdere benaderingen bekeken met tot doel de sunitinib bijwerkingen te verminderen. Tevens is gekeken naar de farmacokinetiek van sunitinib na chronische blootstelling in patiënten met kanker.
In **hoofdstuk 6** is een onderzoek beschreven dat de relatie bestudeert tussen CYP3A4 activiteit en sunitinib blootstelling. Tevens is gekeken naar de relatie tussen de sunitinib dalspiegels en de totale blootstelling aan sunitinib. Aanvullend is de invloed van sunitinib op de CYP3A4 activiteit bestudeerd. Sunitinib wordt voor zover bekend alleen via CYP3A4 omgezet. De variatie in sunitinib blootstelling tussen patiënten is groot ~40%. Het is goed voorstelbaar dat de activiteit van CYP3A4 sterk bepalend is voor de sunitinib blootstelling en een groot deel van de variatie tussen de patiënten verklaart. Het geneesmiddel midazolam wordt vaak gebruikt om de activiteit van CYP3A4 te bepalen omdat midazolam alleen via CYP3A4 gemetaboliseerd wordt en zelf geen invloed heeft op de activiteit van dit enzym. Uit ons onderzoek blijkt dat de midazolam blootstelling goed is gerelateerd aan de sunitinib blootstelling en aan de sunitinib dalspiegels. Midazolam blootstelling lijkt hiermee een groot gedeelte van de variatie tussen de patiënten te kunnen verklaren. Sunitinib dalspiegel en sunitinib blootstelling zijn sterk gecorreleerd waardoor in vervolgonderzoek de sunitinib dalspiegel in plaats van de vele male intensievere volledige sutent blootstellingbepaling kan worden gebruikt om de relatie tussen sunitinib blootstelling met effectiviteit en toxiciteit vast te stellen en eventueel dosisaanpassingen te doen. Sunitinib lijkt een stimulerend effect te hebben op de CYP3A4 activiteit deze bevinding moet echter bevestigd worden in een grotere groep patiënten.
De relatie tussen sunitinib toxiciteit en genetische veranderingen (polymorfismen) die coderen voor zowel enzymen, geneesmiddel pompen als de aangrijpingspunten voor

sunitinib is beschreven in **hoofdstuk 7**. Trombocytopenie (bloedplaatjes tekort), leukopenie (witte bloedcellen tekort), slijmvliesontsteking, hand-voet syndroom (rode en pijnlijke handen en voeten) en matig ernstige bijwerkingen in het algemeen (toxiciteit > graad 2) zijn als eindpunten genomen. Deze bijwerkingen zijn geselecteerd op basis van een studie waarin de toxiciteit in een placebo groep vergeleken werd met de toxiciteit in een sunitinib behandelde groep. Als de bijwerking veel voorkwam in de sunitinib behandelde groep en niet in de placebo groep dan werd een causaal verband verondersteld tussen het sunitinib gebruik en het optreden van de bijwerking. Aanvullend hebben we een selectie gemaakt op basis van objectiviteit en klinische relevantie. Trombocytopenie bleek niet gerelateerd aan een polymorfisme. Leukopenie kon geassocieerd worden met een polymorfisme in *FLT3*, *NR1I3* en *CYP1A1*. Hetzelfde polymorfisme in *CYP1A1* was gerelateerd aan het optreden van slijmvliesontstekingen. Hand-voet syndroom bleek gerelateerd aan *ABCB1* polymorfismen en toxiciteit > graad 2 bleek geassocieerd met een polymorfisme in *ABCG2*. De functionaliteit van enkele polymorfismen die gevonden zijn zouden de bijwerkingen goed kunnen verklaren. Van enkele andere polymorfismen is de functionaliteit nog niet opgehelderd. Deze studie moet gezien worden als een exploratief onderzoek waarin gezocht is naar genetische veranderingen die mogelijk gerelateerd zijn aan bijwerkingen. De relatie tussen de genen die wij gevonden hebben en de bijwerkingen die optreden moet in een onafhankelijk onderzoek bevestigd worden.

CYP3A4 speelt bij de omzetting van sunitinib een cruciale rol. Ook in de bijsluiter van sunitinib wordt gewaarschuwd voor de combinatie van sunitinib met sterke remmers en inductoren van CYP3A4. De geneesmiddelen waarvoor gewaarschuwd worden zijn niet allemaal in combinatie met sunitinib onderzocht. Er zijn parallellen getrokken tussen het onderzoek wat is uitgevoerd met een model remmer (ketoconazol) en inductor (rifampicine) van CYP3A4 en andere geneesmiddelen waarbij een vergelijkbaar effect bekend is. Zo is ook grapefruitsap opgenomen in de lijst met middelen die niet gecombineerd mogen worden met sunitinib. Grapefruitsap is een krachtige remmer van de CYP3A4 enzymen die in de darm aanwezig zijn. Het heeft echter geen invloed op de CYP3A4 enzymen in de lever. Voor sunitinib is het niet bekend hoeveel procent van de dosis van het geneesmiddel in de bloedcirculatie wordt opgenomen (wat de biologische beschikbaarheid is). De invloed van de CYP3A4 activiteit in de darm op de sunitinib blootstelling is hierom lastig te voorspellen. In **hoofdstuk 8** is een onderzoek beschreven waarin de invloed van grapefruitsap op de sunitinib blootstelling wordt onderzocht. Gelijktijdige consumptie van grapefruitsap met sunitinib inname resulteert in een 10% hogere sunitinib blootstelling wat niet als klinisch relevant wordt beschouwd aangezien de variatie tussen patiënten in sunitinib blootstelling al ~40% is.

Het viel op dat twee patiënten die behandeld werden met sunitinib een erg afwijkende blootstelling van zowel sunitinib als midazolam en zijn metaboliet lieten zien.

De overeenkomst tussen de patiënten bleek het gebruik aan mitotaan te zijn. Mitotaan is een geneesmiddel wat als sinds 1959 gebruikt wordt bij de behandeling van bijnierschorscarcinoom. Er is echter vrijwel niets bekend over de omzetting van mitotaan en over het effect wat mitotaan heeft op de activiteit van verschillende enzymen en daarmee op de blootstelling van andere geneesmiddelen. Mitotaan blijkt zich te gedragen als een krachtige CYP3A4 inductor wat veel consequenties heeft voor geneesmiddelen die naast mitotaan worden gebruikt (**hoofdstuk 9**).

In **hoofdstuk 10** wordt de absorptie van bergamottin (BG) en 6',7'-dihydroxybergamottin (DHB) bestudeerd in gezonde vrijwilligers. BG en DHB worden gezien als de verbindingen in grapefruitsap die verantwoordelijk zijn voor het remmen van het CYP3A4 enzym in de darm. Ook is onderzocht of in verschillende merken en in de verschillende charges van hetzelfde merk grapefruitsap de concentraties BG en DHB veel uiteen lopen. Beide verbindingen konden na ruime consumptie van het sap niet worden teruggevonden in het bloed van de vrijwilligers. Een beperkte absorptie van BG en DHB door de darm is daarom mogelijk de verklaring voor het krachtige effect van grapefruitsap op de darmenzymen maar niet op de enzymen in de lever. Bovendien blijkt de concentratie BG en DHB erg te variëren tussen de verschillende merken en charges grapefruitsap. Dit maakt het noodzakelijk om de hoeveelheid BG en DHB voor interactieonderzoeken te standaardiseren zodat vergelijking tussen en interpretatie van de resultaten van de studies met grapefruitsap mogelijk wordt.

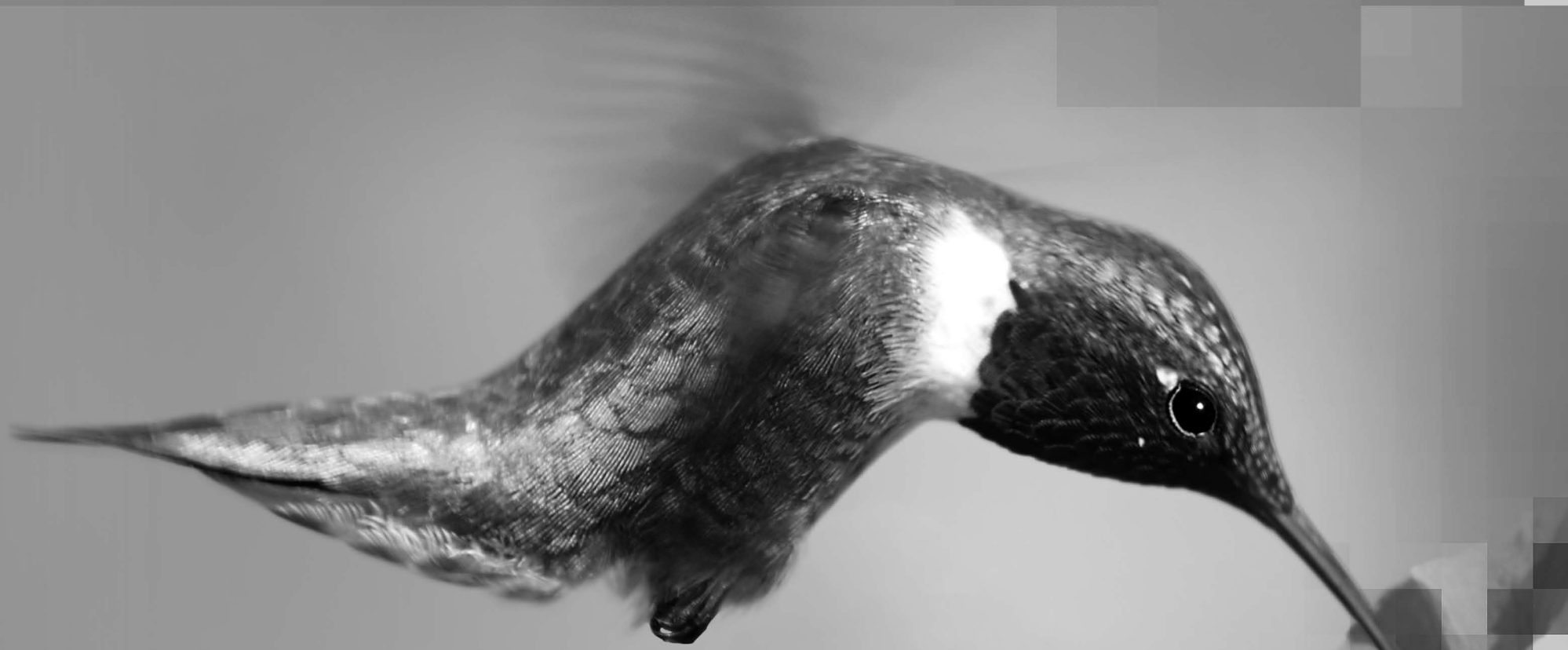
In het laatste hoofdstuk worden de resultaten van het uitgevoerde farmacologische onderzoek naar imatinib en sunitinib in perspectief geplaatst. Ook worden toekomstige ideeën belicht die voorvloeien uit het bescheven onderzoek.

Waarschijnlijk zullen tumoren door een beter inzicht in de mechanismen die ten grondslag liggen aan het ontstaan ervan anders behandeld gaan worden. Het uitgangspunt van de behandeling zal dan niet zozeer gestuurd worden door het orgaan waarin de tumor zich ontwikkeld maar meer door het eiwit / de eiwitten die voor de ontregelde groei van cellen zorgen. Hierdoor wordt de keuze voor behandeling meer per individu dan per patiënten groep bepaald waarbij de therapie wordt afgestemd op het type ontregeling, de gevoeligheid en het genetisch profiel van de tumor. Daarnaast zal ook gestreefd worden naar de meest ideale blootstelling aan het geneesmiddel waarbij effectiviteit zonder onnodige toxiciteit wordt bereikt. Met de introductie van deze nieuwe groep geneesmiddelen die door continue blootstelling de groei van de tumor onderdrukken zal in de komende jaren veel tijd besteed gaan worden aan het vaststellen van de relatie tussen geneesmiddelblootstelling - tumoreffect en –bijwerkingen. De rol van 'therapeutic drug monitoring' zal hiermee een belangrijke positie gaan innemen bij de behandeling van tumoren zoals dat nu al het geval is voor menig ander ziektebeeld.

Nawoord

List of Publications

Curriculum Vitae



Dit proefschrift is tot stand gekomen door de samenwerking met en de inzet van velen.
Op de eerste plaats wil ik de patiënten bedanken die bereid waren deel te nemen aan de niet altijd even eenvoudige farmacokinetiek onderzoeken. Een gezicht en hun verhaal achter de data die geanalyseerd zijn maakt de relevantie van het doen van onderzoek zoveel duidelijker.
Part of the research described in this thesis has been performed under the supervision of Sharyn Baker at the analytical pharmacology core laboratory of SKCCC at Johns Hopkins, Baltimore, Maryland, U.S.A.. Sharyn, thank you so much for giving me the opportunity to work at your laboratory. It has been a valuable and inspiring experience to work with you and your colleagues at an institute as educational as Johns Hopkins. Throughout the thesis we have worked together on several research projects and I would like to thank you for the pleasant and scientific valuable collaboration.
Alex Sparreboom, your innovative and enthusiastic approach towards new projects and your way to explain unexpected results has an accelerating effect on me. Thank you for giving me a glimpse of your scientific approach and for all your help with different research projects described in this thesis.
Opleiders, Irene, Juliette, Kirsten, Jan, Henk-Jan en de andere ziekenhuisapothekers, bedankt voor de mooie en afwisselende opleidingsjaren. Dankzij jullie is mijn enthousiasme voor ons prachtvak alleen maar groter geworden.
Werken aan onderzoek is soms frustrerend maar over het algemeen gewoonweg erg leuk. Een uit de hand gelopen hobby voor mij maar ook voor veel mensen om me heen is me opgevallen. Dat geldt zeker voor mijn kamergenoten, Rogier, Jan, Wouter, Jesse, Marloes en niet te vergeten de ex-roomies, Els, Judith en lang geleden Annemieke: mede dankzij de vele discussies en relativerende gesprekken met jullie ben ik het altijd leuk blijven vinden.
Zonder de steun van ons toxicologie laboratorium was mijn promotieonderzoek nooit voltooid. Maanden heb ik bij jullie doorgebracht waarin ik jullie heb bestookt met vragen om mijn analyses draaiende te krijgen. Ook hebben jullie me praktisch geholpen met het opzetten en valideren van de bepaling die voor dit proefschrift zo belangrijk was. Jan, Judith, Marco, Ed en in het bijzonder Jacqueline en Trees; geweldig dat jullie me zo hebben geholpen en bestand bleken tegen mijn soms ambitieuze en dwingende verzoeken.
Tot het laatste jaar van mijn promotie dacht ik dat farmacogenetica geen onderdeel van mijn proefschrift zou vormen. Het is echter zonde goede ideeën niet tot uitvoer te brengen. Zonder de wetenschappelijk en praktische hulp van Judith, Renée en Tahar was dit mij nooit gelukt. Bedankt voor de goede en mooi bekroonde samenwerking.
Jan en Margret bedankt voor jullie praktische en onmisbare hulp bij het afnemen van de bloedmonsters en bij het opstellen van de onderzoeksvoorstellen.
Coauteurs, bedankt voor het becommentariëren van de manuscripten, de samenwerking heeft tot een beter resultaat geleid.

Lieve Maris, ook al was het op dat moment nog niet kristalhelder - eigenlijk was jij erbij toen ik verliefd werd op de wetenschap.
Waarde paranimfen, Lieve Anthe, wat is er veel gesproken over onderzoek. Onze ultieme manier van ontspanning en vermaak kunnen weinigen om ons heen begrijpen.
Beste Jan, je bent een geweldige collega, die eigenlijk al in menig artikel van dit proefschrift bedankt had moeten worden voor de wetenschappelijke verscherping.
Mijn lieve familie dank ik voor het eeuwig vertrouwen in me en de heerlijke nuchterheid waarmee jullie alles voor me konden relativeren.
Tot slot de belangrijkste van allemaal, Bastiaan.

1. G. Los, F. Yang, G. Samimi, G. Manorek, I. M. Guerorguieva, S. Howell, **N. van Erp**, J. K. Breaux: Using mRNA expression profiling to determine anticancer drug efficacy. Cytometry 47: 66-71, 2002

2. **N. P. van Erp**, S. D. Baker, M. Zhao, M. A. Rudek, H. J. Guchelaar, J. W. Nortier, A. Sparreboom, H. Gelderblom: Effect of milk thistle (Silybum marianum) on the pharmacokinetics of irinotecan. Clin Cancer Res 11: 7800-6, 2005

3. **N. P. van Erp**, R. L. Oostendorp, H. J. Guchelaar, J. H. Schellens, H. Gelderblom: Is rectal administration an alternative route for imatinib? Cancer Chemother Pharmacol 60: 623-4, 2007

4. **N. P. van Erp**, H. Gelderblom, M. O. Karlsson, J. Li, M. Zhao, J. Ouwerkerk, J. W. Nortier, H. J. Guchelaar, S. D. Baker, A. Sparreboom: Influence of CYP3A4 inhibition on the steady-state pharmacokinetics of imatinib. Clin Cancer Res 13: 7394-400, 2007

5. **N. P. van Erp**, H. Gelderblom, Glabbeke M. Van, Oosterom A. van, J. Verweij, H. J. Guchelaar, M. biec-Rychter, B. Peng, J. Y. Blay, I. Judson: Effect of cigarette smoking on imatinib in patients in the soft tissue and bone sarcoma group of the EORTC. Clin Cancer Res 14: 8308-13, 2008

6. **N.P. van Erp**, K. Eechoute, A.A. van der Veldt, J.B. Haanen, A.K.L. Reynders, R.H.J. Mathijssen, E. Boven, T. van der Straaten, R.F. Baak-Pablo, J.A.M. Wessels, H.-J. Guchelaar, H. Gelderblom: Pharmacogenetic Pathway Analysis for determination of Sunitinib-Induced Toxicity. J Clin Oncol 2009 *(published online ahead of print, August 10, 2009)*

7. **N.P. van Erp**, H. Gelderblom, H. J. Guchelaar: Clinical Pharmacokinetics of Tyrosine Kinase Inhibitors. Cancer Treat Rev 2009 *(in press)*

Nielka van Erp werd op 4 april 1977 geboren te Liempde, waar ze samen met haar broers Twan en Bart opgroeide. Na het behalen van haar VWO diploma aan het Jacob-Roelands-lyceum, begon zij in 1996 met de studie Farmacie aan de Universiteit Utrecht. Tijdens de doctoraalfase, die ze in 2001 afrondde, heeft zij 9 maanden onderzoek gedaan naar verschillen in genexpressie profielen in cisplatine resistente en -gevoelige cellijnen (Dr. G. Los) aan The University of California, San Diego, Cancer Center, V.S. In 2003 behaalde ze haar apothekersdiploma, waarna ze in dienst trad als projectapotheker bij de afdeling Klinische Farmacie en Toxicologie van het Leids Universitair Medisch Centrum (LUMC). In 2005 startte ze met de opleiding tot ziekenhuisapotheker die in 2009 werd afgerond. Tijdens haar opleiding begon ze aan het promotieonderzoek naar geneesmiddelinteracties met tyrosine kinase remmers. In het eerste jaar van haar opleiding heeft ze gedurende drie maanden gewerkt aan haar onderzoek in het analytisch chemisch en farmacologisch laboratorium (S.D. Baker, PharmD, PhD) van het SKCCC, Johns Hopkins, Baltimore, Maryland, V.S.. Haar onderzoek werd begeleid door Prof. dr. H.-J. Guchelaar van de afdeling Klinische Farmacie en Toxicologie en Dr. A.J. Gelderblom van de afdeling Klinische Oncologie van het LUMC . Per januari 2010 zal ze werkzaam zijn in een nieuwe functie binnen de afdeling Klinische Farmacie en Toxicologie en Klinische Oncologie van het LUMC waarin ze mede verantwoordelijk is voor het oprichten van een faciliteit voor klinisch geneesmiddel-onderzoek voor fase I/II onderzoek met experimentele antikankermiddelen.