OPTIMIZING EXPOSURE OF ORAL TARGETED THERAPIES IN ONCOLOGY

TOWARDS PRECISION DOSING

Steffie Groenland

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Optimizing exposure of oral targeted therapies in oncology

towards precision dosing

Optimaliseren van de blootstelling aan orale doelgerichte therapieën binnen de oncologie

op weg naar doseren op maat

(met een samenvatting in het Nederlands)

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La médecine c’est guérir parfois, soulager souvent, consoler toujours

Ambroise Paré

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Preface

PREFACE

A major breakthrough in the treatment of cancer has been the development of imatinib, which has widely been praised as the magic anticancer bullet.[1] In the era of targeted therapies that we have come to know ever since, the number of oral targeted therapies that is approved each year increases exponentially. With the advent of these small molecules, that inhibit specific driver proteins in growth signal transduction pathways, the focus has increasingly shifted towards precision medicine, by selecting the right drug for individual patients based on molecular characteristics of their tumor. However, nowadays these drugs are still administered using a one-size-fits-all fixed dosing approach, while convincing arguments advocate for precision dosing instead.

First, there is a weak basis for the registered dose, as the recommended dose is determined in phase I studies, that typically include only a small number of patients and mainly focus on toxicity instead of efficacy.[2] Second, compared with intravenously administered classical cytotoxics, this new class of orally administered drugs shows a higher variability in pharmacokinetic (PK) exposure, due to differences in drug absorption. Additionally, many oral targeted therapies are characterized by complex pharmacological profiles, due to their poor formulation and complex metabolism, which adds to the high interindividual variability in exposure as well.[3] Consequently, fixed dosing results in a wide range of drug concentrations between patients.[4] Third, and most importantly, many of these new oral targeted therapies have a narrow therapeutic window.[5]

As a result, the problem with the currently used fixed dosing regimen is that ± 30% of patients are being underdosed and thus at risk of suboptimal treatment efficacy, whereas another ± 15% of patients are currently being overdosed, potentially resulting in unnecessary treatment-related toxicities.[5,6] Hence, rational precision medicine would not only include selecting the right drug, but also selecting the right dose. Precision dosing can be achieved by selecting the right starting dose for each individual (group of) patient(s), i.e. “right-dose-first-time” paradigm, after which this dose can be further optimized by PK-guided dosing, i.e. adjusting the dose based on measured drug concentrations.

THESIS OUTLINE

This thesis focuses on the rationale (**Part I**), the evidence (**Part II**) and the clinical application (**Part III and IV**) of precision dosing of oral targeted therapies in oncology.

Part I outlines the rationale for precision dosing of oral anticancer drugs. In **Chapter 1**, seven prerequisites for the rational application of PK-guided dosing are discussed for oral targeted therapies. In **Chapter 2**, it is discussed how the clinical relevance of precision

dosing in oncology can be demonstrated, and the need for randomized confirmatory trials is questioned.

Part II reports on exposure-response analyses in real-life patient cohorts. **Chapter 3** for abiraterone and its active metabolites in patients with metastatic castration-resistant prostate cancer, **Chapter 4** for first- and second-generation anaplastic lymphoma kinase inhibitors crizotinib and alectinib in patients with non-small-cell lung cancer, and **Chapter 5** for the combination of BRAF- and MEK-inhibitor dabrafenib plus trametinib in patients with melanoma.

Part III focuses on the application of precision dosing in clinical practice. First of all, the study protocol of the Dutch Pharmacology Oncology Group – Therapeutic Drug Monitoring (DPOG-TDM) study is described in **Chapter 6**. The first results of this prospective nationwide study on PK-guided dosing in 600 patients with 24 different oral targeted therapies are presented in **Chapter 7**. In **Chapter 8**, the results of PK-guided dose increases of imatinib in patients with gastrointestinal stromal tumors are described, for whom PK-guided dosing is performed as part of daily clinical practice at our institute for several years now. In **Chapter 9** it is illustrated how PK-guided dosing could support treatment decisions in case of toxicity by reporting on two patients who received an extremely low dose of pazopanib. **Chapter 10** provides practical recommendations for pharmacokinetic targets to guide dosing of oral anti-hormonal drugs.

Part IV of this thesis highlights alternative strategies for precision dosing. **Chapter 11** provides more detailed data on the first patients in the abiraterone cohort of the DPOG-TDM study, by describing how food interventions can be used to optimize abiraterone exposure in patients with trough concentrations below the target. In **Chapter 12**, a pharmacokinetic crossover study investigating the effect of splitting intake moments of pazopanib from 800 mg once daily into 400 mg twice daily is described. **Chapter 13** reports on the pazopanib cohort of the DPOG-TDM study in more detail, focusing on cost-neutral PK-guided interventions to optimize exposure. **Chapter 14** focuses on cyclin-dependent kinase inhibitors and provides an overview of the clinical pharmacokinetics and pharmacodynamics of these drugs, including exposure-response relationships and their potential for drug-drug interactions via cytochrome P450 3A4 (CYP3A4). In **Chapter 15**, the effect of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetic exposure of palbociclib is investigated in a randomized crossover study, aiming to provide guidelines for clinicians on dose modifications. An important factor in the high inter- and intra-individual variability of oral targeted therapies is the bioavailability.[3] In **Chapter 16**, a clinical study is presented in which the absolute bioavailability of imatinib is determined using an innovative design.

Finally, the main conclusions of the presented work are discussed and placed in a broader perspective, followed by a detailed summary of the most important findings described in each chapter.

In summary, this thesis describes the rationale and opportunities for precision dosing of oral targeted therapies in oncology, with the aim of optimizing exposure for individual patients.

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PART I

Rationale for precision dosing

Individualized dosing of oral targeted therapies in oncology is crucial in the era of precision medicine

Eur J Clin Pharmacol 2019; **75**: 1309–1318

Stefanie L. Groenland, Ron H.J. Mathijssen, Jos H. Beijnen, Alwin D.R. Huitema, Neeltje Steeghs

ABSTRACT

Purpose

While in the era of precision medicine, the right drug for each patient is selected based on molecular tumor characteristics, most novel oral targeted anticancer agents are still being administered using a one-size-fits-all fixed dosing approach. In this review, we discuss the scientific evidence for dose individualization of oral targeted therapies in oncology, based on therapeutic drug monitoring (TDM).

Methods

Based on literature search and our own experiences, seven criteria for drugs to be suitable candidates for TDM will be addressed: 1) absence of an easily measurable biomarker for drug effect; 2) long-term therapy; 3) availability of a validated sensitive bioanalytical method; 4) significant variability in pharmacokinetic exposure; 5) narrow therapeutic range; 6) defined and consistent exposure-response relationships; 7) feasible dose-adaptation strategies.

Results

All of these requirements are met for most oral targeted therapies in oncology. Also, prospective studies have already shown TDM to be feasible for imatinib, pazopanib, sunitinib, everolimus and endoxifen.

Conclusions

In order to realize the full potential of personalized medicine in oncology, patients should not only be treated with the right drug, but also at the right dose. TDM could be a suitable tool to achieve this.

INTRODUCTION

Many new oral targeted therapies have become available in oncology over the past two decades. As a result, the treatment paradigm has partly shifted from a one-size-fits-all approach into precision medicine, in which the right drug is selected based on molecular characteristics of the tumor.

Dose finding of these new oral targeted therapies, however, has simply been copied from classical intravenous cytotoxic drugs. In traditional phase I dose escalation studies, which generally enroll only few patients (median sample size of 26 patients[1]), doses are increased until dose-limiting toxicities occur. This maximum tolerated dose (MTD), at which typically only 3-6 patients have been treated, is then used in all further studies, leading to a one-size-fits-all fixed dosing strategy.[2] However, pharmacokinetic characteristics of these new oral targeted therapies suggest individualized dosing would be far more rational.

Although one might think drug selection based on molecular diagnoses makes any further dose individualization superfluous, it seems logical to combine these two approaches to realize the full potential of personalized medicine (**Figure 1**). Currently, all patients are treated at a standard fixed dose, resulting in low pharmacokinetic exposure and thus suboptimal treatment in a substantial proportion of patients. This subtherapeutic treatment is senseless, especially with these expensive drugs. Therefore, we believe that the current fixed dosing paradigm should be left. Therapeutic drug monitoring (TDM), which is individualized dosing based on measured plasma concentrations of the drug, can be used to select the right dose for each individual patient. In case of pharmacokinetic exposure below the predefined efficacy threshold and acceptable toxicities, pharmacokinetically guided interventions will be performed. These could include absolute dose increments or alternative interventions to increase pharmacokinetic exposure (i.e. concomitant intake with food in case of a clinically relevant food effect[3] or splitting intake moments in case of saturable absorption[4]). Although TDM is widely applied in clinical practice for many drug classes, such as antibiotics, anticonvulsants, and immunosuppressants, it is still being very limitedly applied in oncology.

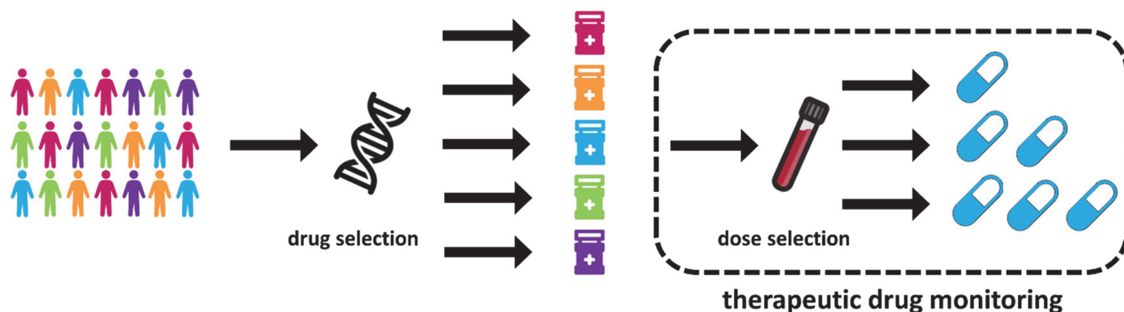


Figure 1 – Schematic overview of how precision medicine can be combined with dose individualization by therapeutic drug monitoring

Previous reviews have summarized the literature for TDM of individual oral anticancer drugs.[5–9] In this review, we will address the scientific evidence for dose individualization by TDM of oral targeted therapies in general. **Table 1** provides an overview of approved oral targeted therapies in oncology.

Table 1 – Overview of oral targeted therapies in oncology (2019)

Group	Drugs
ALK inhibitors	alectinib, brigatinib, ceritinib, crizotinib, lorlatinib
Anti-hormonal drugs	abiraterone, anastrozole, apalutamide, enzalutamide, exemestane, letrozole, tamoxifen
Bcr-Abl inhibitors	bosutinib, dasatinib, imatinib, nilotinib, ponatinib
BRAF inhibitors	dabrafenib, encorafenib, vemurafenib
BTK inhibitors	ibrutinib
CDK 4/6 inhibitors	abemaciclib, palbociclib, ribociclib
EGFR inhibitors	dacomitinib, erlotinib, gefitinib, osimertinib
EGFR / Her2 inhibitors	afatinib, neratinib, lapatinib
FLT3 inhibitors	gilteritinib, midostaurin
HDAC inhibitors	panobinostat, vorinostat
JAK inhibitors	ruxolitinib
MEK inhibitors	binimetinib, cobimetinib, trametinib
mTOR inhibitors	everolimus
NTRK inhibitors	larotrectinib
PARP inhibitors	olaparib, niraparib, rucaparib, talazoparib
PI3K inhibitors	copanlisib, duvelisib, idelalisib
VEGFR inhibitors	axitinib, cabozantinib, lenvatinib, nintedanib, pazopanib, regorafenib, sorafenib, sunitinib, tivozanib, vandetanib

ALK = anaplastic lymphoma kinase; Bcr-Abl = breakpoint cluster region-Abelson fusion protein; BRAF = serine/threonine-protein kinase B-Raf; BTK = Bruton's tyrosine kinase; CDK = cyclin dependent kinase; EGFR = epidermal growth factor receptor; Her2 = human epidermal growth factor receptor 2; FLT3 = FMS-like tyrosine kinase 3; HDAC = histone deacetylase; JAK = Janus-associated kinase; MEK = mitogen-activated protein kinase; mTOR = mammalian target of rapamycin; NTRK = neurotrophic tyrosine kinase; PARP = poly ADP ribose polymerase; PI3K = phosphoinositide 3 kinase; VEGFR = vascular endothelial growth factor receptor

CRITERIA FOR RATIONAL USE OF THERAPEUTIC DRUG MONITORING

For drugs to be suitable candidates for TDM, the following requirements have previously been proposed[10–14]:

1. absence of an easily measurable biomarker for drug effect;
2. long-term therapy;
3. availability of a validated sensitive bioanalytical method;
4. significant variability in pharmacokinetic exposure;
5. narrow therapeutic range;

6. defined and consistent exposure-response relationships;
7. feasible dose-adaptation strategies.

In the following paragraphs, each of these requirements will be discussed and it will be assessed whether they are met in the case of oral targeted therapies in oncology.

1. Absence of an easily measurable biomarker for drug effect

If more convenient, accurate, and precocious biomarkers for drug response would be available, these would make TDM superfluous. However, while toxicity can easily be measured, for efficacy, these biomarkers are generally not available (yet) and response evaluations with regard to antitumor efficacy are often based on radiological assessments, which are not performed timely enough to be a good biomarker. Imaging is usually performed every 8 to 12 weeks, while ideally dose adjustments should be made at an early stage (i.e. within 14 days). Also, once tumor progression is observed on radiological scans, resistant clones of tumor cells have already emerged and dose adjustments will probably be too late at this moment. Although for some tumor types blood-based tumor markers exist (e.g. cancer antigen 125 in ovarian cancer or carcinoembryonic antigen in colorectal cancer), these are not accurate enough to predict treatment response and to base treatment decisions upon.[15] The same holds true for other potential biomarkers available for oral targeted therapies including diastolic blood pressure for axitinib and skin rash for epidermal growth factor receptor inhibitors such as erlotinib and gefitinib.[16–18] Complete cytogenetic response in case of hematologic malignancies and prostate-specific antigen (PSA) in the case of prostate cancer are the only examples of biomarkers that can accurately predict response to treatment and that are used in clinical practice for this purpose.[19,20] Apart from these exceptions, the first requirement for TDM is met for most combinations of targeted therapies and tumor types.

2. Long-term therapy

Treatment should be long enough to allow sufficient time for dose adjustments to be made. As the mean treatment duration of targeted therapies is several months, while only few days to weeks are needed to reach steady-state concentrations, there is sufficient time to perform TDM. The time to steady-state concentrations depends on the elimination half-life ($t_{1/2}$) of a drug, which is typically around 20-30 hours for most oral anticancer drugs, although for some compounds this is markedly longer (e.g. enzalutamide (± 6 days[21]) and endoxifen, which is the active metabolite of tamoxifen (± 2 weeks[22])). After four to five times the $t_{1/2}$, steady-state concentrations have been attained.

3. Availability of a validated sensitive bioanalytical method

In order to perform dose individualization based on pharmacokinetic exposure, bioanalytical assays to measure plasma concentrations should be available. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is usually applied for

quantification of these drugs and validated assays are available for almost all oral anticancer drugs at a reasonable price. Since LC-MS/MS is a labor-intensive method and many different targeted therapies will be used in routine clinical practice, combining multiple drugs into one bioanalytical assay might be useful.[23–25] To implement TDM into routine clinical practice, an adequate infrastructure for sample collection and shipment should be in place, with a short turn-over and reporting time. In addition, dried blood spot (DBS) sampling could offer a more patient friendly sampling approach, as patients can obtain their blood samples themselves at home instead of having to visit the hospital. Whole blood samples can be collected by a finger prick at a paper DBS card, which can then be send to the laboratory by regular mail. DBS assays are already available for several oral anticancer drugs.[26–33] Also, commercial automated immunoassays could facilitate measurement in routine clinical practice, for example of imatinib.[34]

4. Significant variability in pharmacokinetic exposure

The fourth requirement for TDM comprises a marked variability in pharmacokinetic exposure. Otherwise, when pharmacokinetic exposure would be predictable and similar for all patients, there would be no need for dose individualization.

Oral targeted therapies typically exhibit a large interindividual variability in pharmacokinetic exposure in the range of 24-84%, providing a strong rationale for TDM.[35–40] Reasons for this high interindividual variability include differences in absorption, which could be influenced by the poor bioavailability of these drugs, potential food effects, or the use of drugs that alter the stomach pH (i.e. proton pump inhibitors and H_2 receptor antagonists); interactions with concomitant medication (e.g. via cytochrome P450 enzymes such as CYP3A4); pharmacogenetics (i.e. patients harboring polymorphisms of cytochrome P450 enzymes or ABC-transporters); hepatic and renal function; body composition and patient adherence.[2,41]

While data on interindividual variability are widely available, reports on intra-individual variability are sparse.[42] Poor formulations of most oral targeted therapies result in a low bioavailability and thus a high inter- and intra-individual variability.[43,44] The intra-individual variability should be judged taking into account the interindividual variability as well. For example, abiraterone has an intra-individual variability of 33%, while the interindividual variability is higher (i.e. 46%).[37] The same holds true for vemurafenib, which has an intra- and interindividual variability of 28% and 41%, respectively.[38] Unfortunately, these data are not available for all oral targeted therapies. Therefore, Chatelut *et al.* advocate intra-individual variability should ultimately be characterized before registration of new drugs.[42] Although assessment of the intra-individual variability for registration purposes might be challenging, especially in the context of oncological patients with potentially fluctuating (patho-) physiological states, we do propose that efforts should be made to quantify the intra-individual variability.

It is important to take the source of variability into account when deciding on the interval of sampling. When the major source of variability is interindividual variability, a single measurement or rare measurements would be sufficient. When the main source of variability is intra-individual variability, it depends on the origin. If the origin of intra-individual variability is random from dose to dose (e.g. due to poor formulation), TDM might not be useful, as a single sample would then be of limited value. If the intra-individual variability is caused by an identifiable reason (e.g. concomitant medication or fluctuations in (patho-)physiological conditions), more frequent sampling might be needed. In this case, the sampling interval should be oriented at the change of the condition (e.g. new concomitant medication). Regardless of the source of variability, it is important to continue sampling throughout therapy, since many factors that can influence pharmacokinetic exposure may vary over time (e.g. drug-drug interactions and compliance).

To conclude, variability in pharmacokinetic exposure of oral targeted therapies is definitely sufficiently high to meet the requisite.

5. Narrow therapeutic range

When the window between therapeutic and toxic concentrations is small, dose titration is important to minimize the risk of either ineffective treatment or unnecessary toxicities. The fact that > 50% of the oral targeted therapies have a recommended dose equal to the maximum tolerated dose (MTD), indicates these drugs have a narrow therapeutic index.[45] An exception to this is drugs with a plateau in the exposure-response curve that are dosed at the flat end of this curve, as might be the case for cabozantinib and pazopanib.[46,47] At the currently used fixed doses, ± 30% of patients are being under dosed (e.g. for abiraterone, imatinib, pazopanib, sunitinib, and vemurafenib), associated with decreased efficacy, while ± 15% of patients are being over dosed, causing unnecessary toxicities.[35,37,38,48–50] These numbers illustrate the significant proportion of patients being treated outside the therapeutic window in the absence of dose titration.

6. Defined and consistent exposure-response relationships

TDM is only rational if defined and consistent exposure-response relationships have been demonstrated for both efficacy and toxicity. For this purpose, exposure can be interpreted as minimum plasma concentration (C_{min}), maximum plasma concentration (C_{max}), or area under the plasma concentration-time curve (AUC). Extensive reviews summarizing the available literature on exposure-response relationships for each specific oral targeted therapy have previously been published.[5–9] For many of these drugs exposure-response relationships have been demonstrated and pharmacokinetic targets could be identified (e.g. imatinib, pazopanib, and sunitinib[51–54]). For other drugs, pharmacokinetic targets based on exposure-efficacy analyses are not well established yet (e.g. dabrafenib, lenvatinib, and palbociclib[5]), but based on their mechanism of action,

exposure-response relationships are to be expected. In these cases, the mean or median exposure could be taken as a reference. In previous analyses, we have demonstrated that targets based on exposure-efficacy analyses amounted to 82% (± 17%) and 85% (± 19%) of the average exposure in the population for kinase inhibitors and oral anti-hormonal drugs, respectively.[5–7] Therefore, targeting the mean or median exposure generally leads to efficacious concentrations (as the real exposure-efficacy threshold is expected to be lower). The fact that the exposure-efficacy threshold is generally lower than the average exposure in the population is not a surprising finding, since the efficacy of these drugs has been proven in phase III trials indicating that the mean exposure should be sufficient to generate an antitumor response.

The magnitude of exposure-response relationships can be illustrated by pazopanib, for which a clear exposure-efficacy relationship exists, with progression-free survival (PFS) being significantly longer in patients with C_{min} ≥ 20.5 mg/L (52.0 weeks versus 19.6 weeks[53]). The PFS of patients with an exposure below this target is even comparable with placebo (4.2 months)[55], making treatment at an inadequate pharmacokinetic exposure as ineffective as no treatment at all.

To overcome resistance, newer generation kinase inhibitors have been designed that block their target irreversibly (e.g. osimertinib, ibrutinib, and afatinib).[56–58] It still has to be elucidated how this affects exposure-response relationships, but based on their irreversible mechanism of action, it could be expected that these agents are relatively overdosed due to the MTD paradigm currently still used in dose finding studies. Since these drugs bind their target covalently, inhibition endures even after the drug has been cleared from the systemic circulation. Therefore, the efficacy threshold could be lower than the mean pharmacokinetic exposure at the recommended dose. So far, for none of these agents clear exposure-response relationships have been identified. For example, for osimertinib, a retrospective analysis of 780 subjects showed no association between exposure and response.[59]

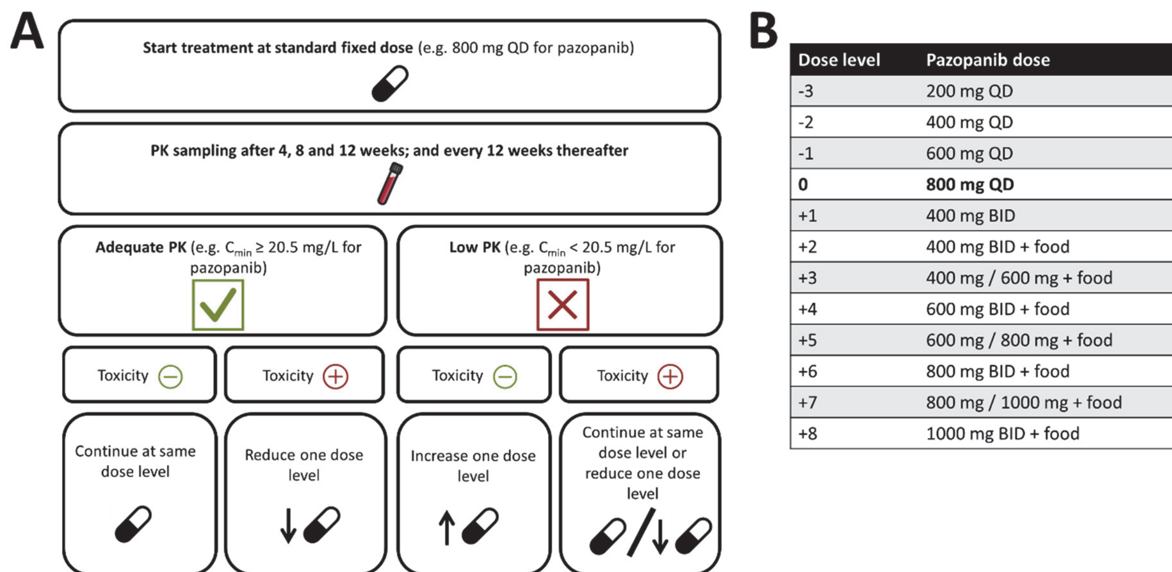
At the time new oral targeted therapies are approved, in most cases, insufficient data is available to draw conclusions on exposure-response relationships, while typically hundreds of patients have been treated with these drugs in the dose-finding and pivotal studies. However, data on pharmacokinetic exposure is often not structurally being collected in all patients. It is of great value to incorporate these pharmacokinetic analyses in the early stages of clinical development of these new drugs to ensure patients can be treated at a dose giving them adequate exposure.

Thus, for many oral targeted therapies defined and consistent exposure-response relationships exist, for others it can be reasonably expected while awaiting conclusive data, while for some exposure-response relationships might not be expected based on their irreversible mechanism of action and relatively high dose administered.

7. Feasible dose-adaptation strategy

For drugs to be suitable candidates for TDM, feasible dose-adaptation strategies should exist, leading to target attainment without additional toxicities. Prospective clinical studies have already shown TDM to be feasible for pazopanib[48,49], sunitinib[48,50], imatinib[48,60], everolimus[61], and endoxifen[62]. **Table 2** provides a summary of the results of these studies. In clinical practice, the dose-adaptation strategies used in these prospective studies could be applied (i.e. the same pharmacokinetic target and dose levels could be used). Also, algorithms describing dose-adaptation schedules for other oral targeted therapies have been published previously.[6]

Figure 2 provides a schematic overview of pharmacokinetically guided dose individualization, in which pazopanib is used as an example. Patients start treatment at the standard fixed dose. At regular time intervals, pharmacokinetic sampling is performed (e.g. 4, 8, and 12 weeks after start of treatment, and every 12 weeks thereafter). In case of pharmacokinetic exposure below the predefined target and acceptable toxicities, the dosage can be increased with one dose level.



Dose level	Pazopanib dose
-3	200 mg QD
-2	400 mg QD
-1	600 mg QD
0	**800 mg QD**
+1	400 mg BID
+2	400 mg BID + food
+3	400 mg / 600 mg + food
+4	600 mg BID + food
+5	600 mg / 800 mg + food
+6	800 mg BID + food
+7	800 mg / 1000 mg + food
+8	1000 mg BID + food

Figure 2 – Schematic overview of PK-guided dose adaptation strategy for oral anticancer drugs
A: Schematic overview of how therapeutic drug monitoring could be applied in clinical practice.
B: Example of proposed dose levels for pazopanib.
BID = twice daily; PK = pharmacokinetics; QD = once daily

Dose-adaptation strategies should take into account the MTD of the drug, or – when the MTD has not been reached – the highest dose tested in phase I dose escalation trials, when deciding on the maximum dose level. Although it could be argued that pharmacokinetically guided dose escalation above the MTD could be safe as well (since this dose escalation will only be done in patients with a low pharmacokinetic exposure), this should only be considered with careful monitoring of side effects.

Table 2 – Summary of results of prospective studies on the feasibility of TDM for oral targeted therapies in oncology

	Fox *et al.*[62]	Lankheet *et al.*[48]	Verheijen *et al.*[49]	Lankheet *et al.*[50]	Gotta *et al.*[60]	Krueger *et al.*[61]	de Wit *et al.*[72]
Drug(s)	endoxifen	imatinib sunitinib pazopanib	pazopanib	sunitinib	imatinib	everolimus	pazopanib
Evaluable patients (no.)	122	109	30	29	28	28	13
Inadequate PK exposure (no. (%))	78 (63%)	68 (62%)	17 (57%)	15 (52%)	17 (61%)	NR	1 (8%)
PK-guided intervention (no. (%))	68 (87%)	41 (60%)	10 (59%)	14 (93%)	NR	NR	1 (100%)
Successful PK-guided intervention (i.e. target attainment with acceptable toxicity) (no. (%))	65 (96%)	35 (85%)	7 (70%)	5 (36%)	NR	NR	NR

NR = not reported, PK = pharmacokinetic, TDM = therapeutic drug monitoring

Pharmacokinetically guided interventions do not necessarily have to include absolute dose escalations, as for some oral targeted therapies, other options to increase pharmacokinetic exposure are available as well. For oral targeted therapies with a clinically significant food effect (e.g. abiraterone, lapatinib, and pazopanib), careful concomitant intake with food could be used as a first step in case of low pharmacokinetic exposure.[3] Besides, for drugs with a saturable absorption profile (e.g. pazopanib and everolimus), splitting intake moments could provide a cost-neutral solution to attain adequate pharmacokinetic exposure.[4,63]

Another important consideration is the fact that progressive disease is irreversible. Therefore, it is important to attain an adequate pharmacokinetic exposure in each individual patient as soon as possible. In addition, it could be argued that dose reductions should only be made in case of intolerable toxicities, and not solely based on pharmacokinetic exposure (i.e. patients with high pharmacokinetic exposure, but without any side effects). On this aspect, TDM in oncology differs significantly from other disciplines, where it is often aimed at preventing toxicities as well due to its small therapeutic window.

To summarize, the feasibility of dose-adaptation strategies has been prospectively studied for several oral targeted therapies.[48–50,60–62] All of these studies have shown TDM to be feasible, at least for a subset of patients. For other oral targeted therapies, possible dose-adaptation strategies have been described in literature or could be set up taking into account the mentioned considerations, while awaiting additional prospective studies.[6]

DISCUSSION

In this concise review article, we discussed the conditions that should be fulfilled for oral targeted therapies in oncology to be suitable candidates for TDM. Apart from some exceptions (e.g. osimertinib or cabozantinib), for most oral targeted therapies all of these requirements are met, providing a strong rationale for TDM.

A practical advantage is that most oral anticancer drugs are administered at a once or twice daily basis, making the timing of sampling more convenient compared with intermittent dosing (e.g. classical chemotherapy or immunotherapy). TDM targets are generally based on trough concentrations (C_{min}). While ideally trough samples would be drawn, this is not always possible in routine clinical practice. In this case, samples could be drawn at a random time point and C_{min} can be estimated using several algorithms like the method proposed by Wang *et al.*[64] or by Bayesian forecasting. In addition, a number of powerful pharmacokinetic computer tools are available for this purpose.[65]

Even though convincing evidence supports dose individualization of oral targeted therapies, TDM is still being scarcely applied in daily clinical care. One of the reasons for this is that randomized controlled trials (RCTs), demonstrating the added value of TDM on

clinical treatment outcomes, are lacking. However, it is highly unlikely that these RCTs could ever be performed. First, high numbers of patients would be needed, while most oral targeted therapies are indicated for rare tumor types or for a small subset of patients. For example, a randomized phase 3 study of TDM in patients with gastro-intestinal stromal tumours (GIST) treated with imatinib has been terminated prematurely due to slow accrual.[66] Second, it is difficult to secure (sufficient) funding for these types of studies. Furthermore, it is questionable whether it is ethical to fail to perform dose adjustments for some patients, when clear exposure-response relationships exist. Only a few RCTs of fixed dosing versus PK-guided dosing have ever been completed in oncology, all with chemotherapy.[67–70]

Therefore, we are currently performing a large multi-center prospective study, in which we investigate the feasibility and efficacy of TDM for 23 different oral targeted therapies in more than 600 patients (www.trialregister.nl; NTR 6866[71]). Patients starting regular treatment with one of these drugs can be included in this study. For each drug, pharmacokinetic targets and dose levels have been defined and are described in the protocol. Pharmacokinetic sampling and dose adaptations are performed according to the strategy depicted in **Figure 2**. Primary outcome is to halve the proportion of patients with pharmacokinetic exposure below the target after 12 weeks (compared with historical data). Secondary outcomes are the safety, feasibility, and efficacy of pharmacokinetically guided dosing and physician adherence to the tailored treatment recommendations. If this study underscores the results of previous retrospective studies and prospective feasibility studies, this will further support the implementation of pharmacokinetically guided dose optimization as the new standard.[48–50,60–62,72]

As can be seen in **Table 2**, results of PK-guided dose individualization studies are currently not being reported in a uniform way, making mutual comparisons difficult. Therefore, we propose that future studies should at least report the following:

- Proportion of patients with low pharmacokinetic exposure;
- Proportion of patients in whom PK-guided interventions were applied;
    - Reasons why these were not applied in other patients (e.g. toxicity, physician adherence);
- Proportion of patients in which PK-guided interventions were successful, thus in which adequate PK-exposure was attained without intolerable toxicities.

In this way, study results could be compared more easily and potentially be combined in a meta-analytical approach.

It is essential to convince treating physicians of the importance of TDM, as they need to implement the treatment recommendations into clinical practice. Unwillingness of treating physicians to follow these treatment recommendations was the main reason that

a previous randomized controlled trial could not demonstrate the benefit of TDM for imatinib.[60]

Apart from the apparent advantages of TDM in optimizing pharmacokinetic exposure to improve treatment outcomes, TDM could serve several other purposes as well. First, it could play a role in detecting nonadherence to therapy. This is especially important in the case of long-term therapy, as compliance drastically decreases over time (e.g. for tamoxifen adherence was only 50% after four years of therapy[73]). Second, TDM could be helpful in the management of drug-drug interactions, since pharmacokinetic exposure to many oral targeted therapies is affected by concomitant use of CYP3A4 inhibitors/inducers or gastric acid-suppressive agents.[74] Last, measuring plasma drug concentrations could also support dose titration in patients with renal or hepatic impairment.

CONCLUSION

The pharmacokinetic characteristics of (most) oral targeted therapies in oncology support dose individualization by therapeutic drug monitoring. To realize the full potential of personalized medicine, we should not only treat each patient with the right drug, but also at the right dose.

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Demonstrating clinical relevance of individualized dosing of oral targeted therapies in oncology – the path to precision dosing

Submitted

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PART II

Exposure-response analyses

Exposure-response analyses of abiraterone and its metabolites in real-world patients with metastatic castration-resistant prostate cancer

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ABSTRACT

Background

Abiraterone acetate is an oral 17α-hydroxylase/C17,20-lyase (CYP17) inhibitor approved for the treatment of metastatic castration-resistant prostate cancer (mCRPC) patients. Previously, a prospective observational trial demonstrated a relationship between abiraterone trough concentrations (C_{min}) in plasma and treatment efficacy. The aim of our study was to investigate the exposure-response relationship of abiraterone and its metabolites, and to study if the proposed target for abiraterone of 8.4 ng/mL is feasible in a "real-world" patient cohort.

Patients and methods

mCRPC patients who had at least one abiraterone plasma concentration at steady-state were included in this study. Plasma abiraterone and its metabolites levels were analyzed using a validated liquid chromatography-mass spectrometry method. Using calculated C_{min} values of abiraterone and its active metabolite Δ(4)-abiraterone (D4A), univariate, and multivariable Cox regression analyses were performed.

Results

Sixty-two patients were included in this retrospective analysis, of which 42% were underexposed (mean abiraterone C_{min} < 8.4 ng/mL). In multivariable analysis, C_{min} ≥ 8.4 ng/mL was associated with longer prostate-specific antigen (PSA) independent progression-free survival (16.9 vs. 6.1 months; p=0.033), which resulted in a hazard ratio of 0.44 (95% confidence interval: 0.23-0.82, p=0.01). D4A C_{min} did not show a relationship with treatment efficacy.

Conclusion

Our study shows that mCRPC patients with an abiraterone C_{min} ≥ 8.4 ng/mL have a better prognosis compared with patients with low C_{min}. Monitoring C_{min} of abiraterone can help to identify those patients at risk of suboptimal treatment for whom treatment optimization may be appropriate.

INTRODUCTION

Abiraterone is an inhibitor of 17α-hydroxylase/C17,20-lyase (CYP17), an enzyme involved in the intra- and extragonadal biosynthesis of androgens, including testosterone. Initially, abiraterone acetate was approved for treatment of metastatic castration-resistant prostate cancer (mCRPC) as it improves overall survival (OS) and progression-free survival (PFS) in this patient population compared with placebo.[1,2]

Following oral ingestion, abiraterone acetate is rapidly deacetylated to form the active substance abiraterone. Further metabolism into its major inactive metabolites abiraterone sulfate and abiraterone N-oxide sulfate is facilitated by cytochrome P450 family 3A member 4 (CYP3A4) and sulfotransferase family 2A member 1 (SULT2A1).[3] More recently, an active metabolite of abiraterone was discovered named Δ(4)-abiraterone (D4A), which is formed by the enzyme 3β-hydroxysteroid-dehydrogenase.[4,5] D4A blocks CYP17, several steroidogenic enzymes, and the androgen receptor.[5,6] Conversely, D4A is further metabolized to 3-keto-5-α-abiraterone, which stimulates the androgen receptor.[7,8] The net result of these pharmacologic actions on therapeutic outcome remains to be elucidated.

Abiraterone acetate is administered in a fixed dose of 1000 mg once daily (QD). Mean steady-state trough concentrations (C_{min}) at this approved dose are 11.1 ng/mL for abiraterone and 1.6 ng/mL for D4A.[3,9] In a prospective observational trial, abiraterone C_{min} has been associated with treatment response in mCRPC patients. In this study, plasma trough concentrations of abiraterone were significantly higher in prostate-specific antigen (PSA) responders (n=38) compared with non-responders (n=23) (12.0 vs. 8.0 ng/mL, p=0.0015).[10] Furthermore, a threshold of 8.4 ng/mL has been identified, above which patients had a longer PFS compared with patients with C_{min} below this target (12.2 vs. 7.4 months, p=0.044).[10] The same research group reported that higher D4A C_{min} is related to shorter OS, but not PFS (n=30).[9]

Abiraterone acetate has a large interpatient variability in C_{min} of 46%.[10] Part of this variability may be accounted for by the food-effect, causing a sevenfold increase in maximum plasma concentration (C_{max}) with a low-fat meal and a 17-fold increase in C_{max} with a high-fat meal, compared with overnight fasting in healthy volunteers.[11] A prospective clinical trial has shown that abiraterone acetate 250 mg QD taken with a low-fat meal was non-inferior to abiraterone acetate at a standard dose of 1000 mg QD in modified fasting state, in terms of PSA response and PFS (n=72).[12] Furthermore, Stover *et al.* show that some men may benefit from taking abiraterone acetate concomitant with food.[13]

Previous studies clearly show an exposure-efficacy relationship between plasma trough concentrations of abiraterone and PFS. Yet, abiraterone acetate is still administered at

fixed doses, which could lead to suboptimal treatment for some patients. Therapeutic drug monitoring (TDM), the clinical practice of measuring drug concentrations in biological fluids to individualize drug dosing, could be used to improve patient care. Based on the current data, TDM of abiraterone may be implemented with a C_{min} threshold of 8.4 ng/mL. This threshold was established in a restrictive clinical study and needs to be confirmed with real-life data from daily clinical practice. The aim of our study was to assess the exposure-efficacy relationship of abiraterone and its major metabolites for the purpose of TDM in a "real-world" patient cohort. We hypothesized that patients with abiraterone $C_{min} \geq 8.4$ ng/mL will have a longer PFS compared with patients with a $C_{min} < 8.4$ ng/mL. A retrospective study was conducted to test this hypothesis.

METHODS

Patients and sampling

This was an observational study in the outpatient clinic of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam. Abiraterone concentrations were monitored in all mCRPC patients using abiraterone acetate as part of routine clinical care. As authorized by the institute, data from clinical care were used retrospectively. Clinical characteristics were collected from medical records, including demographic data, medical history, abiraterone acetate dose, treatment duration, reason for discontinuation, concomitant medication, and PSA levels. Furthermore, testosterone and androstenedione concentrations were determined during treatment using a validated liquid chromatography-mass spectrometry (LC-MS/MS) assay.[14]

Pharmacokinetics

Blood samples were drawn as part of routine clinical care every 3 months on average. The date and time of blood withdrawal, and date and time of drug intake were recorded. Patients with at least one available abiraterone plasma concentration at steady-state were included in this study. Steady-state was considered to be reached after 1 week of treatment, taken into account the 15-hour half-life.[3] Abiraterone and its metabolites D4A, abiraterone sulfate, and abiraterone N-oxide sulfate were quantified using a validated LC-MS/MS method.[15,16] Plasma samples were collected at random time points during a dosing interval at routine patient visits to the outpatient clinic, and therefore, C_{min} values were calculated from the measured concentrations. As abiraterone shows clear distribution pharmacokinetics, log-linear extrapolation was not feasible. Furthermore, the use of Bayesian estimates from a population pharmacokinetic model was considered, but this was complicated by high shrinkage. Therefore, we used the ratio of the observed concentration and median concentration as tool to calculate C_{min}. First, we simulated a full population concentration-time curve of abiraterone with the pharmacokinetic model published by Stuyckens *et al.*[17] Second, measured concentrations were divided by the

simulated concentrations of the population curve at the recorded time points. Third, the ratio between measured concentrations and simulated concentrations was multiplied by the simulated C_{min} of the population curve to obtain the final calculated C_{min}. Our data show that the shape of the D4A concentration-time curve is similar to that of abiraterone, and therefore, it is suggested that metabolite formation is rate-limiting in the clearance of D4A. As there is no pharmacokinetic model available for D4A, C_{min} was calculated in the same manner as the C_{min} of abiraterone. Measured concentrations of abiraterone sulfate and abiraterone N-oxide sulfate were divided into three groups based on the time of sampling after dosing (TAD), being 0-4, 4-10, and 10-24 hours after drug intake. Samples taken before steady-state was reached or more than 24 hours after the last dose were excluded from further analysis.

Outcome measures

Three clinical end points regarding treatment response were evaluated separately in this study; PSA response, PSA independent PFS, and time to PSA progression (TTPP). PSA response was defined as ≥ 50% decrease in PSA from baseline, both according to the Prostate Cancer Working Group 2 (PCWG2) criteria.[18,19] PSA independent PFS was defined as the time from treatment start to the first event of progression, being either radiographic progression, symptomatic progression (start of radiotherapy, samarium treatment, increase of analgesic dose, or a WHO performance level increase of at least 2), onset of next treatment or death from any cause. Radiographic progression was evaluated according to modified Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).[20] TTPP was defined as the time from treatment start to a 25% or greater PSA increase from the nadir, with an absolute increase in PSA levels of at least 2 ng/mL[20], and had to be confirmed by a subsequent PSA value, also according to PCWG2 criteria. Toxicity was defined as discontinuation due to adverse events, dose reductions due to adverse events or temporary treatment interruption.

Statistics

For the purpose of exposure-response analyses, the mean of all available abiraterone and metabolite levels per patient was used as parameter for exposure. The association between abiraterone plasma concentrations and metabolite concentrations was determined using the Spearman correlation test. Mann-Whitney U tests were used for univariable analysis of PSA response and plasma concentrations of abiraterone and its metabolites. Using the abiraterone C_{min} target of 8.4 ng/mL as a cut-off value, patients were divided into two groups (adequate vs. low C_{min}) for PFS analyses. As no exposure target is known for D4A, D4A plasma concentrations were divided into quartiles for further analyses. PFS functions were estimated using the Kaplan-Meier method and predictive factors were assessed using the univariable model (log rank-test). A stepwise logistic regression was performed for the determination of a predictive score of PFS. Variables significantly associated with outcome in univariate analysis were used in the

multivariate analysis. Ultimately, in multivariable analysis, PSA levels at baseline, WHO performance status, number of previous lines of treatment and whether patients switched from prednisone to dexamethasone during treatment were included as covariates. The following variables were tested but not included in the final model: age, weight, testosterone levels, androstenedione levels, prior treatment with docetaxel, hemoglobin, alkaline phosphatase, kidney, and liver function. All statistical analyses were performed in R (version 3.6.0, package "survival"). A post hoc power analysis was conducted to evaluate the statistical power of this study.

RESULTS

Evaluable patients

From June 2016 to June 2018, 62 patients on treatment with abiraterone acetate were included in this study. A full overview of patient characteristics is provided in **Table 1**. The median time of treatment was 13.6 months (range: 1.1-73.0 months). At data cut-off on 13 May 2019, 12 patients were still on abiraterone treatment. No relevant CYP-inhibiting or inducing co-medication was used during this treatment period. The Spearman correlation test showed that abiraterone and metabolite concentrations were statistically correlated, meaning that plasma samples with high abiraterone levels also contained high metabolite concentrations. Testosterone and androstenedione levels were below the lower limit of quantification of 0.01 ng/mL in all patients.

Pharmacokinetics

In total, 244 plasma samples were included. The distribution of time of sampling after dosing is shown in **Supplementary Figure 1**. Overall, a median (range) of 4 (1-11) samples were available per patient. In aggregate, the median ± SD abiraterone C_{min} concentration was 9.3 ± 10 ng/mL, and median ± SD metabolite plasma concentrations were 1.0 ± 0.9 ng/mL for D4A, 8.7 ± 7.2 $\cdot 10^3$ ng/mL for abiraterone sulfate and 7.8 ± 3.9 $\cdot 10^3$ ng/mL for abiraterone N-oxide sulfate. Interpatient variability (coefficient of variation; CV%) of mean plasma concentrations at a 1000 mg QD was 70% for abiraterone and 61% for D4A. Furthermore, mean intrapatient variability (CV%) at a 1000 mg QD was 53% for abiraterone and 45% for D4A.

An overview of the distribution of mean abiraterone and D4A C_{min} concentrations per patient is provided in **Figure 1**. Twenty-six (42%) patients had an abiraterone C_{min} below the target of 8.4 ng/mL. Four patients received a dose reduction to 500 mg QD (n=2) or 750 mg QD (n=2) due to adverse events, including hepatotoxicity and fatigue. Two of these patients had an abiraterone C_{min} below the target of 8.4 ng/mL after dose reduction. Of all explored clinical parameters, none were found to be significantly predictive of abiraterone plasma concentrations, except for body weight at baseline. Linear regression

indicated that patients with a higher body weight at baseline had a lower plasma concentration (p=0.014).

Exposure-response analyses of abiraterone

Among 62 included patients, 35 (56%) patients were considered PSA responders, vs. 27 (44%) patients without a PSA response. **Figure 2** shows the relationship between C_{min} of abiraterone and PSA response. Mean plasma trough concentrations of abiraterone were 11.4 ng/mL in PSA responders compared with 7.2 ng/mL in non-responders (p=0.18). The maximal change in PSA from baseline (%) after start of treatment is shown for each patient in **Figure 3**. Plasma concentrations of the inactive metabolites abiraterone N-oxide sulfate and abiraterone sulfate are depicted in **Supplementary Figure 2**. As no trough concentrations could be calculated for these metabolites, plasma levels are given in three groups based on the time after dosing. Median plasma concentrations were higher in PSA responders compared with non-responders in all groups but one.

Table 1 – Patient characteristics

		Abiraterone C_{min}	
	Total	**≥ 8.4 ng/mL**	**< 8.4 ng/mL**
Number of patients (n (%))	62 (100%)	36 (58%)	26 (42%)
Age (mean, range)	72 (60-87)	72 (60-87)	71 (61-83)
Weight (mean, range)	89 (57-175)	91 (57-175)	85 (68-117)
WHO performance status (n (%))			
0	22 (36%)	12 (33%)	10 (38%)
1	36 (58%)	22 (61%)	14 (54%)
2	4 (6%)	2 (5%)	2 (8%)
Dose reduction (n (%))	4 (6%)	2 (6%)	2 (8%)
Previous lines of therapy (n (%))			
0	33 (53%)	23 (64%)	10 (38%)
1	13 (21%)	7 (19%)	6 (23%)
2	10 (16%)	3 (8%)	7 (27%)
3	4 (7%)	2 (5%)	2 (8%)
4	2 (3%)	1 (3%)	1 (4%)
Previous chemotherapy (n (%))	26 (42%)	9 (25%)	17 (65%)
Switch to dexamethasone (n (%))	33 (53%)	25 (69%)	8 (69%)
Number of samples (n)	244	165	79
Samples per patient (mean (range))	4 (1-11)	5 (1-10)	3 (1-8)
Median (range) C_{min} (ng/mL)			
Abiraterone	9.3 (2.0-49.8)	14.9 (8.5-49.8)	6.3 (2.0-8.4)
D4A	1.0 (0.3-4.4)	1.3 (0.4-4.4)	0.7 (0.3-1.8)
Median testosterone levels (ng/mL)	< 0.010[a]	< 0.010[a]	< 0.010[a]
Median androstenedione levels (ng/mL)	< 0.010[a]	< 0.010[a]	< 0.010[a]

Demographic data and androgen levels are values at baseline.
[a] Data points below the lower limit of quantification of the bioanalytical method.
D4A = Δ(4)-abiraterone

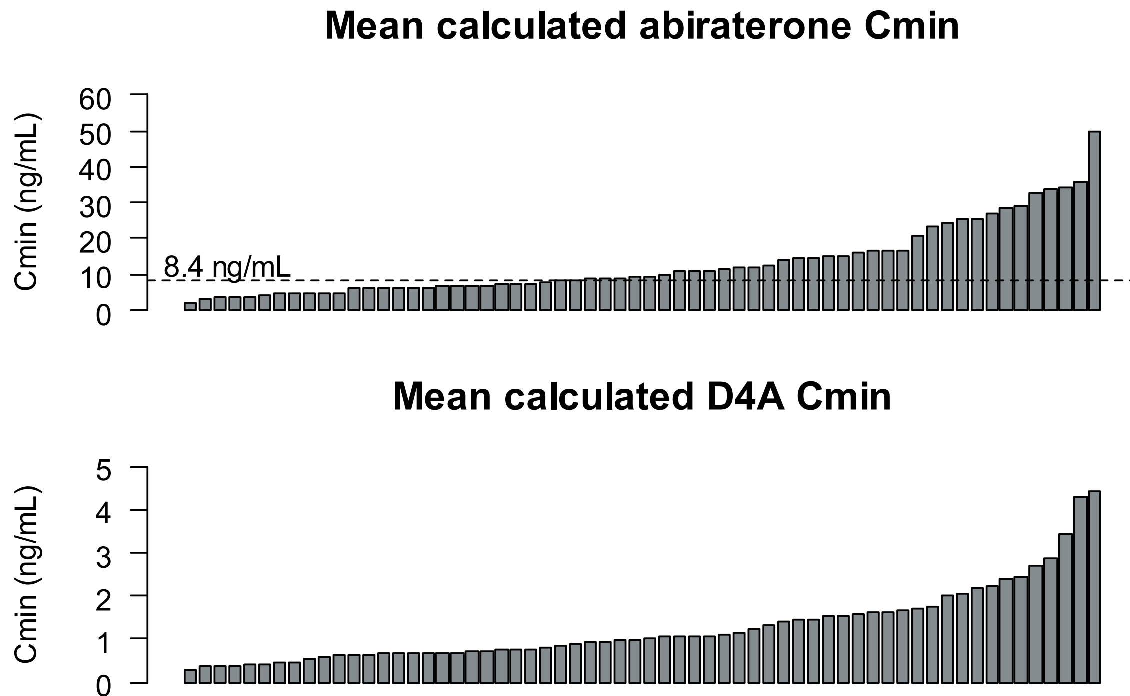

Figure 1 – Distribution of plasma concentrations of abiraterone and Δ(4)-abiraterone (D4A) in patients with metastatic castration-resistant prostate cancer (mCRPC), including the proposed target concentration for abiraterone of 8.4 ng/mL
Each bar represents one patient.

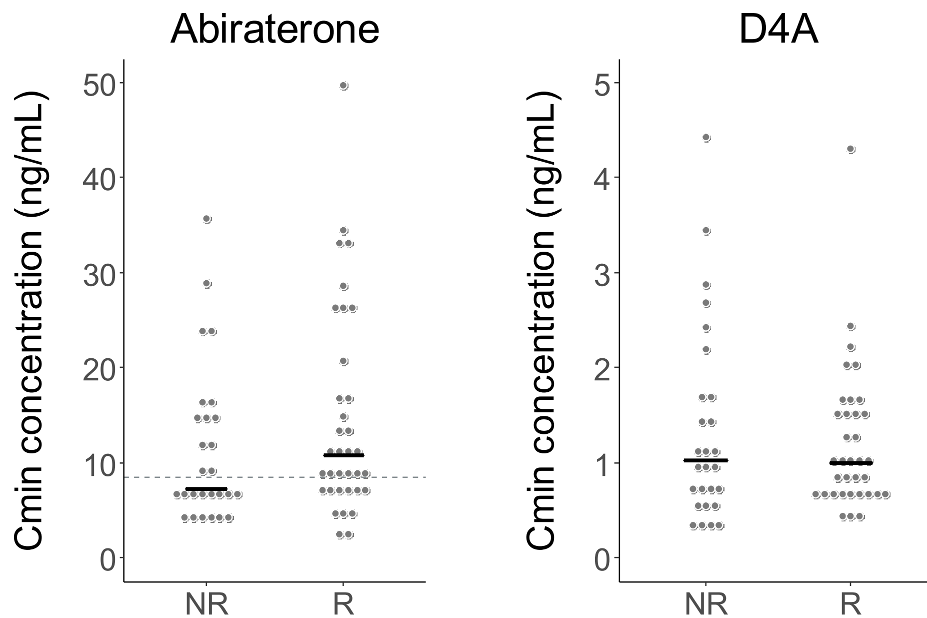

Figure 2 – Relationship between prostate-specific antigen response and the calculated trough concentration of abiraterone (left), Δ(4)-abiraterone (D4A) (right)
Horizontal lines represent the median concentration for PSA responders (R, n=35) and non-responders (NR, n=27), and the dotted lines represent the proposed target for abiraterone of 8.4 ng/mL. Mean plasma trough concentrations of abiraterone were 11.4 ng/mL in PSA responders compared with 7.2 ng/mL non-responders (p=0.18) and D4A plasma concentrations were 1.0 ng/mL in both PSA responders and non-responders (p=0.88).

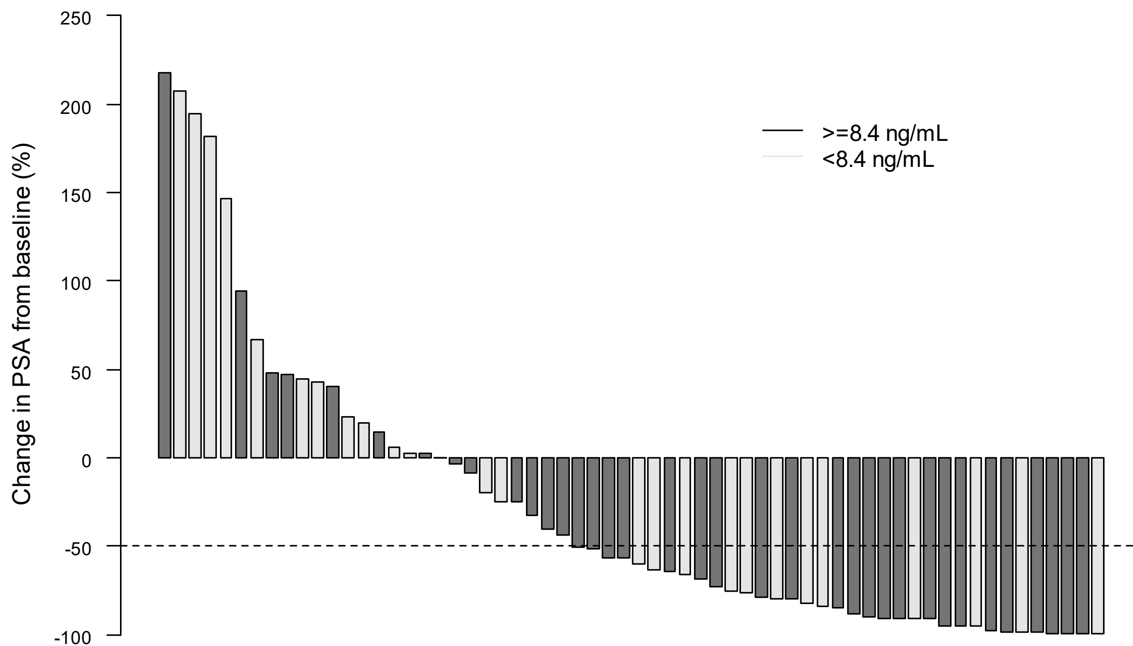

Figure 3 – Waterfall plot showing the PSA change from baseline (%) after start of abiraterone acetate treatment
Each bar represents one patient and the colors indicate if this patient had an abiraterone C_{min} above or below 8.4 ng/mL. The dotted line indicates a 50% PSA decrease from baseline, representing the cut-off for patients to be regarded PSA responders (> 50%) or non-responders.

For PSA independent PFS, 62 patients were included with 50 events (81% of patients) of progression. The remaining patients were still on treatment with abiraterone acetate. Median PSA independent PFS was 16.9 months in patients with an abiraterone $C_{min} \geq 8.4$ ng/mL compared with 6.1 months in patients with a C_{min} below the target (p=0.077, see **Figure 4**). The multivariable analysis resulted in a hazard ratio (HR) of 0.44 (95% CI 0.23-0.82, p=0.01).

For TTPP analysis, 62 patients were included with 53 events (85% of patients) of PSA progression. Three patients were still on treatment, 1 patient died prior to PSA progression, and 5 patients did not show PSA progression but discontinued treatment due to radiographic progression. These patients were censored for TTPP analysis. Median TTPP in patients with an abiraterone $C_{min} \geq 8.4$ ng/mL was 19.8 months compared with 3.7 months in patients with a C_{min} below the target (p=0.062, see **Figure 4**). In multivariable analysis, $C_{min} \geq 8.4$ ng/mL resulted in a HR of 0.52 (95% CI 0.29-0.97, p=0.038).

A post hoc power analysis was conducted using the above described results. The power to detect a difference in PFS from 16.1 to 6.1 months (with a hazard ratio of 0.44) between patients with $C_{min} \geq 8.4$ ng/mL vs. < 8.4 ng/mL, when there are 36 subjects in the first group and 26 in the second, using a two-sided log rank-test with alpha=0.05, was 80%.

Exposure-response analyses of D4A

Figure 2 shows the relationship between C_{min} of D4A and PSA response. Plasma concentrations were 1.0 ng/mL in both PSA responders and non-responders (p=0.88).

Patients were divided into quartiles based on plasma concentrations of D4A, and PFS analyses were performed using these groups. There was no significant difference in the four quartiles regarding PSA independent PFS (7.7 vs. 22 vs. 13 vs. 11 months, p=0.47).

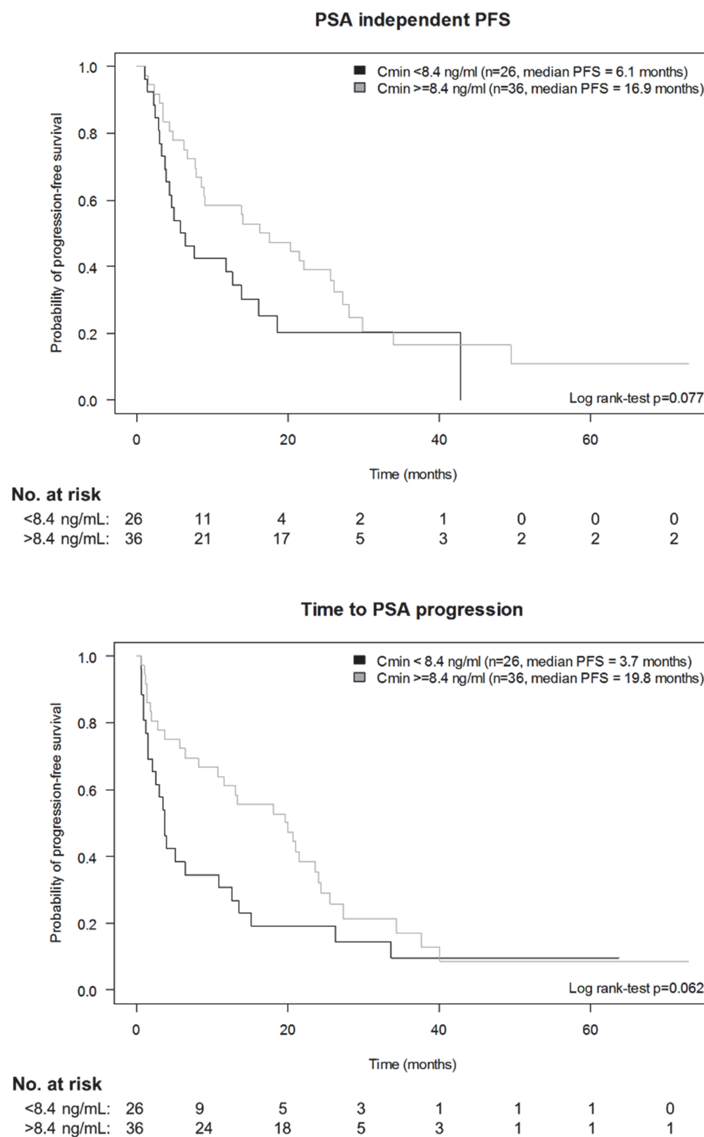

Figure 4 – Kaplan-Meier plots of PSA independent progression-free survival (PFS) in metastatic castration-resistant prostate cancer (mCRPC) patients with a mean abiraterone C_{min} above (n=36, grey line) or below (n=26, black line) the exposure target of 8.4 ng/mL
Prostate-specific antigen (PSA) independent PFS is shown in the upper panel, and time to PSA progression is shown in the lower panel.

Furthermore, there was no significant difference in the four quartiles regarding TTPP (8.2 vs. 15 vs. 5.1 vs. 11 months, p=0.57). Kaplan-Meier curves are shown in **Supplementary Figure 3**. Both univariable and multivariable analysis did not support a relationship between D4A plasma concentrations and PFS.

Exposure-toxicity analysis

Of 62 included patients, four patients received a dose reduction and three patients temporarily discontinued treatment due to the presence of adverse events. Reasons for dose reduction or treatment interruption included fatigue, hepatotoxicity and abdominal pain. Median abiraterone C_{min} was 9.0 ng/mL for patients experiencing clinically relevant adverse events, compared with 9.3 ng/mL in those who did not (p=1.0). Moreover, median D4A C_{min} concentrations were 1.1 vs. 1.0 for patients with and without adverse events, respectively (p=0.60).

DISCUSSION

In this study, plasma concentrations of abiraterone and its metabolites were monitored in a clinical setting. To our knowledge, this is the first study to evaluate the correlation between abiraterone C_{min} and response in a real-world patient cohort, including D4A and other metabolite data. Obtaining real-life data is relevant for clinical practice, as this better reflects daily practice than data derived from clinical trials.[21] Abiraterone acetate is administered at a fixed dose of 1000 mg QD. Our data show that patients with an abiraterone C_{min} ≥ 8.4 ng/mL have a longer PFS compared with patients with a pharmacokinetic exposure below this threshold. Furthermore, this study shows that 42% of patients with mCRPC may be underdosed with this standard fixed dosing regimen and could benefit from an individualized dosing strategy, which is in line with the previously reported 35% of patients having a C_{min} below the target.[10]

D4A was included in PFS analyses as it shows anti-androgen activity. However, it may be further converted to an androgen-stimulating metabolite and, therefore, the net contribution of D4A to the anti-tumor effect of abiraterone is ambiguous.[7,8] Although a previous study has shown that a higher D4A C_{min} was associated with shorter OS (HR 1.54, 95% CI 1.06-2.22, p=0.022) but not with PFS[9], our study did not reveal a relationship between D4A C_{min} and treatment response, PSA independent PFS or TTPP. Moreover, abiraterone and D4A concentrations are correlated, which indicates that abiraterone C_{min} may serve as a proxy for the total antitumor effect of abiraterone and its metabolites.

The exposure target for abiraterone of 8.4 ng/mL was based on a prospective observational study.[10] The CYP17 inhibitory concentrations 50% (IC50) value of abiraterone is 0.07 ng/mL. After correcting for plasma protein binding (99%), a minimum concentration of 7.0 ng/mL should be reached to inhibit 50% of CYP17 in plasma. The exposure target is close to this corrected IC50 value, which biologically substantiates the

threshold. Moreover, the CYP17 IC50 of D4A is 0.035 ng/mL. Given a protein binding of 99%, a minimum concentration of 3.5 ng/mL should be achieved to inhibit 50% of the CYP17 enzyme.[4,5] Only three patients reached this threshold, which could explain why no association was found between D4A plasma levels and response in this population.

Although we believe our study provides relevant information on exposure-response of abiraterone in real-life patients, our analysis does have some limitations. First, in this study not actual C_{min} but calculated (from measured) plasma concentrations were used. Although actual C_{min} may be more accurate than calculated C_{min}, the practical implementation of TDM is more feasible if samples can be drawn at random times during the dosing interval as it can be combined with routine visits to the outpatient clinic. Second, the extent of adherence to abiraterone acetate was not available due to the retrospective nature of this analysis. Although treating physicians provided instructions on drug intake and usage, this may be a potential source of variability in abiraterone C_{min}.

Based on our study and previously published data, an exposure target for abiraterone of 8.4 ng/mL seems appropriate for TDM. Patients with a C_{min} below this target may be advised to take the drug concomitant with food, thereby avoiding expensive dose increments. A single-dose study of abiraterone in healthy volunteers has shown that the area under the plasma concentration-time curve (AUC) and C_{max} increase 10- and 17-fold after intake with a high-fat meal, respectively, and sevenfold and fivefold after intake with a low-fat meal compared with overnight fasting, respectively.[11] The same study showed a less pronounced effect in mCRPC patients when comparing a modified fasting state with food intake (similar exposure with low-fat meals and a twofold increase with high-fat meals).[11] Furthermore, previous research has shown that some men may benefit from concomitant intake of abiraterone acetate with food in terms of PSA progression.[13] This may be attributed to a lower percentage of patients with C_{min} < 8.4 ng/mL. Based on this information, concomitant intake of abiraterone with a low-fat meal may increase plasma levels up to fivefold, which would be sufficient for the majority of included patients with $C_{min} \leq 8.4$ ng/mL to reach plasma levels above the target. Treatment optimization by individualized dosing strategies could lead to better efficacy of abiraterone and higher treatment response. Furthermore, the lack of a relationship between exposure and toxicity suggests that increasing plasma levels will, in these ranges, not result in additional toxicity. Although more research is needed to confirm our findings and to further study the 8.4 ng/mL threshold, we advise clinicians to consider integrating TDM of abiraterone into standard treatment of mCRPC patients. Currently, a study is performed in our institute to investigate the feasibility of TDM with abiraterone using a food intervention[22] by which we hope to improve outcome for mCRPC patients treated with abiraterone acetate.

CONCLUSION

Our study shows that patients with an abiraterone trough level above 8.4 ng/mL have a longer PFS compared with patients with a pharmacokinetic exposure below this threshold. Exposure to the active metabolite D4A did not show a relationship with treatment efficacy and therefore may not add to the prognostic value of abiraterone plasma levels. Monitoring abiraterone C_{min} can identify those patients who are underdosed and we advise clinicians to consider integrating TDM of abiraterone into standard treatment of mCRPC patients.

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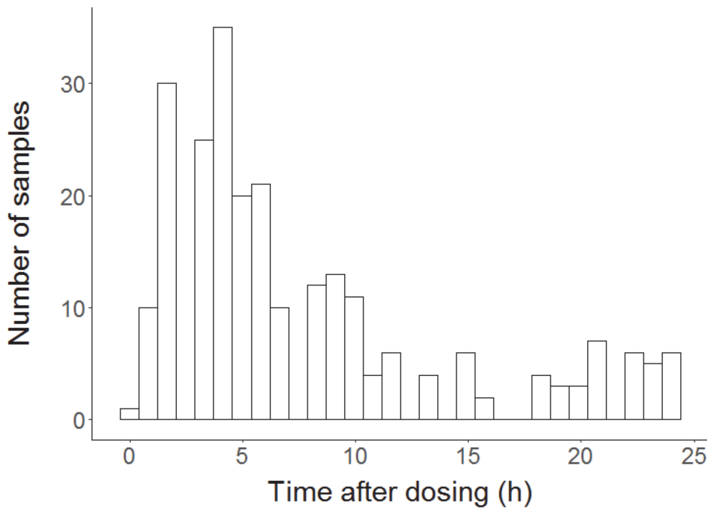
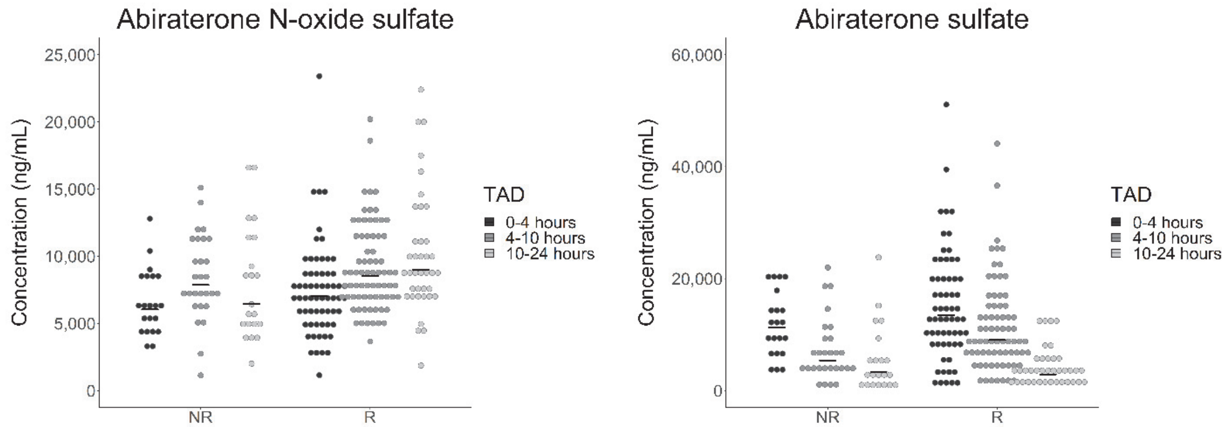
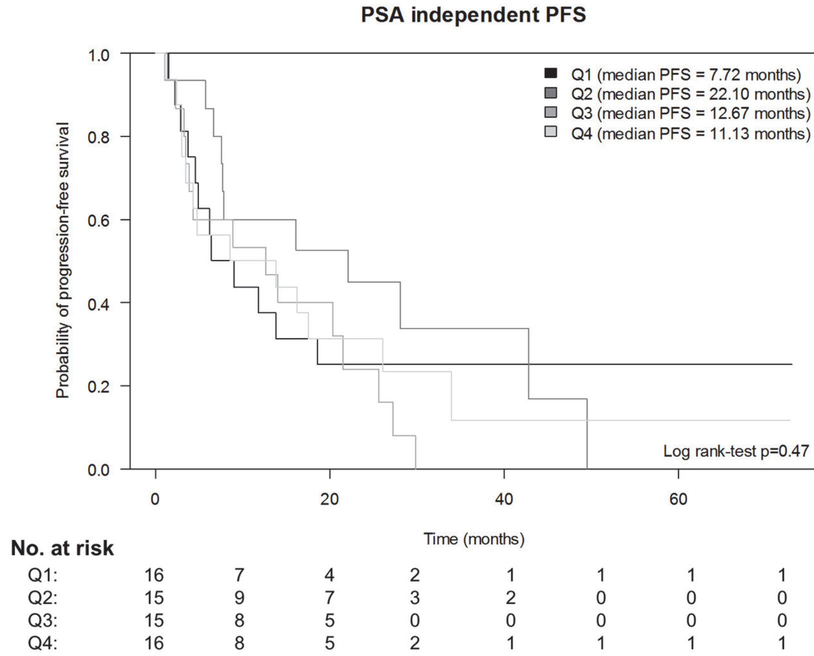
SUPPLEMENTARY DATA

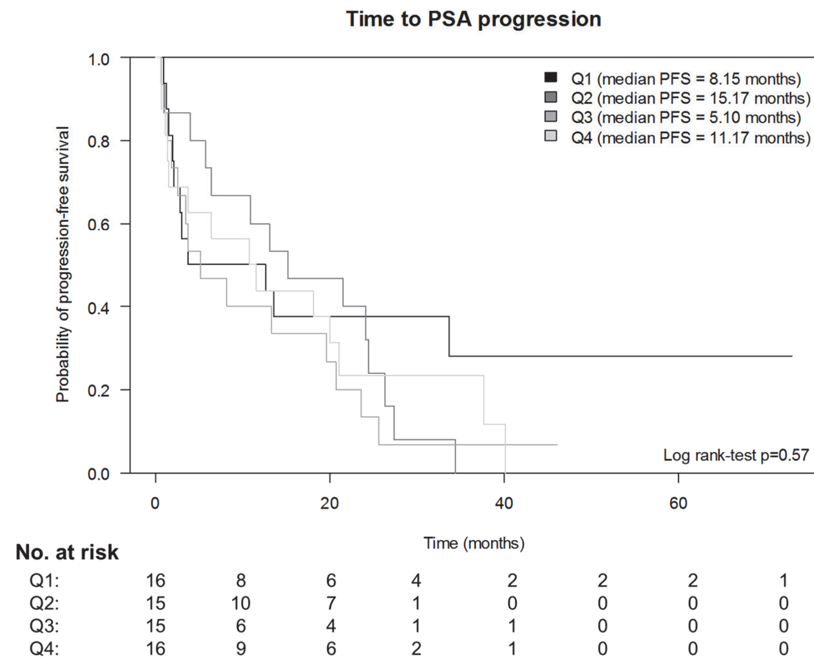


Supplementary Figure 1 – Histogram showing the number of samples taken at a certain time after dosing (TAD)



Supplementary Figure 2 – Relationship between prostate-specific antigen response and plasma concentrations of abiraterone N-oxide sulfate (left) and abiraterone sulfate (right)
Plasma concentrations are divided into three groups, based on the time of sampling after dosing (TAD), being 0-4, 4-10, and 10-24 hours. Horizontal lines represent the median concentration for PSA responders (R) and non-responders (NR). Median plasma concentrations of abiraterone N-oxide sulfate per group were $6.0 \cdot 10^3$, $7.9 \cdot 10^3$ and $6.4 \cdot 10^3$ ng/mL for the non-responders vs. $7.0 \cdot 10^3$, $8.5 \cdot 10^3$ and $9.0 \cdot 10^3$ ng/mL for PSA responders, respectively. Median plasma concentrations of abiraterone sulfate were $11 \cdot 10^3$, $5.4 \cdot 10^3$ and $3.4 \cdot 10^3$ ng/mL vs. $14 \cdot 10^3$, $9.1 \cdot 10^3$ and $2.9 \cdot 10^3$ ng/mL per group, respectively.





Supplementary Figure 3 – Kaplan-Meier plots of PSA independent progression-free survival (PFS) in metastatic castration-resistant prostate cancer (mCRPC) patients for each quartile of Δ(4)-abiraterone (D4A) concentrations

Prostate-specific antigen (PSA) independent PFS is shown in the upper panel, and time to PSA progression is shown in the lower panel.

Exposure-response analyses of anaplastic lymphoma kinase inhibitors crizotinib and alectinib in non-small cell lung cancer patients

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ABSTRACT

Crizotinib and alectinib are anaplastic lymphoma kinase (ALK)-inhibitors indicated for the treatment of ALK-positive metastatic non-small cell lung cancer (NSCLC). At the currently used fixed doses, interindividual variability in exposure is high. The aim of this study was to investigate whether minimum plasma concentrations (C_{min}) of crizotinib and alectinib are related to efficacy and toxicity. An observational study was performed, in which ALK-positive NSCLC patients who were treated with crizotinib and alectinib and from whom pharmacokinetic samples were collected in routine care, were included in the study. Exposure-response analyses were performed using previously proposed C_{min} thresholds of 235 ng/mL for crizotinib and 435 ng/mL for alectinib. Forty-eight crizotinib and 52 alectinib patients were included. For crizotinib, median progression-free survival (mPFS) was 5.7 vs. 17.4 months for patients with C_{min} < 235 ng/mL (48%) and ≥ 235 ng/mL, respectively (p=0.08). In multivariable analysis, C_{min} < 235 ng/mL resulted in a hazard ratio (HR) of 1.79 (95% confidence interval (CI) 0.90-3.59, p=0.100). In a pooled analysis of all crizotinib patients (not only ALK-positive, n=79), the HR was 2.15 (95% CI 1.21-3.84, p=0.009). For alectinib, mPFS was 12.6 months vs. not estimable (95% CI 19.8-not estimable) for patients with C_{min} < 435 ng/mL (37%) and ≥ 435 ng/mL, respectively (p=0.04). Multivariable analysis resulted in an HR of 4.29 (95% CI 1.33-13.90, p=0.015). In conclusion, PFS of crizotinib and alectinib treated NSCLC patients is prolonged in patients with C_{min} ≥ 235 ng/mL and 435 ng/mL, respectively. Therefore, therapeutic drug monitoring should be part of routine clinical management for these agents.

INTRODUCTION

Anaplastic lymphoma kinase (ALK) rearrangements are identified as oncogenic drivers in 3-7% of non-small cell lung cancer (NSCLC) patients.[1-3] Over the past decade, several ALK tyrosine kinase inhibitors have become available and have resulted in significant improvements in overall survival, which is now extended to a median of close to four years in the metastatic setting.[4] Crizotinib and alectinib are first and second generation ALK-inhibitors, respectively, with alectinib being inherently more effective.[5]

Pharmacokinetic exposure to crizotinib and alectinib may be related to toxicity and efficacy. Currently, both crizotinib and alectinib are administered as oral fixed doses of 250 mg twice daily (BID) and 600 mg BID, respectively, exhibiting a high interindividual variability in pharmacokinetic exposure of 40-45%.[6,7] Nevertheless, for both crizotinib and alectinib, no relation between exposure and toxicity could be established based on the currently available data.[6,7] However, in the registration study of crizotinib (n=114) it was shown that patients with a minimum plasma concentration (C_{min}) in the lowest quartile (i.e. < 235 ng/mL) had a significantly lower objective response rate and shorter progression-free survival (PFS) as compared with the upper three quartiles.[6] Similarly, it has been shown that patients treated with alectinib (n=46) in the lowest tertile of C_{min} (i.e. < 435 ng/mL) had less reduction in tumor size compared with the upper two tertiles.[7]

Therefore, personalized dosing based on measured drug levels (i.e. therapeutic drug monitoring) may be more rational, as a subgroup of patients might benefit from a higher dose of both crizotinib and alectinib.

However, before deciding whether individualized dosing might be appropriate, exposure-efficacy relationships need to be confirmed in an independent patient cohort. Furthermore, clinical trial populations differ from real-life patients in several aspects.[8] Therefore, exposure-efficacy analyses should preferably be performed in real-life patients, instead of in highly selected patients in clinical trials. For this reason, the aim of the observational study reported here was to investigate whether pharmacokinetic exposure to crizotinib and alectinib is related to efficacy and toxicity in a real-life patient cohort.

METHODS

Patient population and data collection

A retrospective observational cohort study was performed at The Netherlands Cancer Institute, Amsterdam, The Netherlands. Consecutive patients with ALK-positive NSCLC who were treated with crizotinib or alectinib were included. Pharmacokinetic (PK) samples of these patients were collected as part of routine care. An additional pooled analysis with all patients treated with crizotinib was performed (i.e. ALK-positive, c-ros oncogene 1

(ROS1)-positive and mesenchymal epithelial transition growth factor (cMET) dysregulation). Of these patients, demographic data, prior lines of treatment, crizotinib and alectinib dose, treatment duration, reason for discontinuation, clinically relevant toxicities, and progression-free survival were retrospectively collected from medical records. Following initiation of treatment, imaging was performed twice every 6 weeks, thereafter every 12 weeks.

Pharmacokinetic exposure

Plasma samples were collected during routine follow-up visits to the outpatient clinic. Date and time of last drug intake and plasma sampling were recorded in order to calculate the time after dose. Crizotinib and alectinib concentrations were quantified using validated liquid chromatography-tandem mass spectrometry assays.[9,10] As samples were collected at random time points during the dosing interval, C_{min} was calculated using the following algorithm[11]:

$$C_{min} = C_{measured} * 0.5^{\frac{dosing\ interval - TAD}{t_{1/2}}}$$

in which C_{min} is the calculated minimum plasma concentration, $C_{measured}$ is the measured plasma concentration, dosing interval is the time between two consecutive administrations of the drug (i.e. 12 hours for crizotinib and alectinib), TAD is the time after dose (i.e. the time between last intake of the drug and collection of the PK sample), and $t_{1/2}$ is the elimination half-life of the drug (i.e. 42 hours for crizotinib[6] and 32 hours for alectinib[7]). As crizotinib and alectinib have a longer elimination half-life than imatinib (i.e. 18 hours[12]), this algorithm should perform at least similarly to imatinib.

Samples drawn before steady-state was reached or more than one $t_{1/2}$ after the last dose were excluded from the analysis. The median of all available C_{min} levels per patient was taken as a measure of pharmacokinetic exposure.

Exposure-response analyses

Exposure-efficacy analyses were performed using previously proposed thresholds of 235 ng/mL for crizotinib and 435 ng/mL for alectinib.[6,7,13] PFS of patients with a median C_{min} above and below these thresholds was compared using univariable and multivariable Cox regression analyses. A two-sided p-value < 0.05 was considered statistically significant. Statistical analyses were performed using R version 3.3.6 (R Project, Vienna, Austria).[14]

Exposure-toxicity analyses were performed by comparing median C_{min} between patients with and without clinically relevant toxicities, which was defined as toxicities leading to treatment interruption, dose reduction, or treatment discontinuation.

Median follow-up time was determined using the reverse Kaplan-Meier method.[15]

Ethical regulations

The institutional review board authorized the study on 15 November 2018. The need for written informed consent was waived.

RESULTS

Patient characteristics

In total, 100 consecutive patients were included (48 treated with crizotinib and 52 treated with alectinib) between 2012 and 2019. Baseline characteristics of these patients are provided in **Table 1 and 2** for crizotinib and alectinib, respectively. At the time of data cut-off (26 April 2019), 11 and 34 patients were still on treatment for crizotinib and alectinib, respectively. For crizotinib, 23 patients (48%) had a median C_{min} < 235 ng/mL. For alectinib, 19 patients (37%) had a median C_{min} < 435 ng/mL.

Table 1 – Baseline characteristics of patients treated with crizotinib (n=48)

Patient characteristic	Median C_{min} < 235 ng/mL (n=23)	Median C_{min} ≥ 235 ng/mL (n=25)	All patients (n=48)
Gender, female	9 (39%)	14 (56%)	23 (48%)
Age (years)	48 [21-86]	60 [25-75]	53 [21-86]
Weight (kg)	79 [61-126]	74 [54-96]	77 [54-126]
Tumor stage			
III_A	1 (4%)	0	1 (2%)
III_B	3 (13%)	2 (8%)	5 (10%)
IV	19 (83%)	23 (92%)	42 (88%)
Brain metastases, yes	3 (13%)	2 (8%)	5 (10%)
Previous lines of systemic therapy			
0	11 (48%)	17 (68%)	28 (58%)
1	8 (35%)	6 (24%)	14 (29%)
≥ 2	4 (17%)	2 (8%)	6 (13%)
Crizotinib dose[a]			
250 mg BID	16 (70%)	21 (84%)	37 (77%)
200 mg BID	3 (13%)	2 (8%)	5 (10%)
250 mg QD	3 (13%)	2 (8%)	5 (10%)
250 mg QAD	1 (4%)	0	1 (2%)
WHO performance status			
0	16 (70%)	14 (56%)	30 (63%)
1	7 (30%)	11 (44%)	18 (38%)

Data are expressed as no. (%) or median [range], as appropriate.
[a] Lowest dose per patient
BID = twice daily; C_{min} = minimum plasma concentration; QAD = every other day; QD = once daily; WHO = World Health Organization

Table 2 – Baseline characteristics of patients treated with alectinib (n=52)

Patient characteristic	Median C_{min} < 435 ng/mL (n=19)	Median C_{min} ≥ 435 ng/mL (n=33)	All patients (n=52)
Gender, female	8 (42%)	20 (61%)	28 (54%)
Age (years)	54 [21-70]	60 [34-88]	57 [21-88]
Weight (kg)	76 [54-123]	79 [49-117]	78 [49-123]
Tumor stage			
III_A	0	1 (3%)	1 (2%)
III_B	0	2 (6%)	2 (4%)
IV	19 (100%)	30 (91%)	49 (94%)
Brain metastases, yes	10 (53%)	10 (30%)	20 (39%)
Previous lines of systemic therapy			
0	3 (16%)	13 (39%)	16 (31%)
1	8 (42%)	12 (36%)	20 (39%)
≥ 2	8 (42%)	8 (24%)	16 (31%)
Prior treatment with ALK-inhibitor(s), yes	16 (84%)	19 (58%)	35 (67%)
Alectinib dose[a]			
600 mg BID	16 (84%)	15 (45%)	31 (60%)
450 mg BID	1 (5%)	8 (24%)	9 (17%)
300 mg BID	2 (11%)	10 (30%)	12 (23%)
WHO performance status			
0	11 (58%)	6 (18%)	17 (33%)
1	8 (42%)	21 (64%)	29 (56%)
≥ 2	0	4 (12%)	4 (8%)
missing	0	2 (6%)	2 (4%)

Data are expressed as no. (%) or median [range], as appropriate
[a] Lowest dose per patient
ALK = anaplastic lymphoma kinase; BID = twice daily; C_{min} *= minimum plasma concentration; WHO = World Health Organization*

In general, patients with a low exposure tended to be younger and had a more favourable World Health Organization (WHO) performance status (**Table 1 and 2**). For alectinib, patients with a low exposure were more often pre-treated with ALK-inhibitor(s) (i.e. crizotinib and/or ceritinib, **Table 2**).

Of the patients who were treated with crizotinib and alectinib sequentially and were included in both datasets (n=17), seven patients had a low alectinib exposure, of whom five patients also had a low crizotinib exposure. Median follow-up was 43.6 months (range: 0.8-58.7 months) for crizotinib and 14.4 months (range: 2.2-24.6 months) for alectinib. Baseline characteristics of all patients treated with crizotinib (i.e. ALK-positive, ROS1-positive and cMET dysregulation) are provided in **Supplementary Table 1**.

Pharmacokinetic measurements

Of the 100 consecutively included patients, a median of 3 samples per patient (range: 1-15) were available. In total, 376 PK samples were eligible for analysis (235 crizotinib and 141 alectinib). **Figure 1** provides an overview of the median C_{min} per patient. Median crizotinib C_{min} per patient was 244 ng/mL (range: 103-688 ng/mL), with an interindividual and intra-individual variability of 45% and 20%, respectively, at the standard dose of 250 mg BID. Median alectinib C_{min} per patient was 517 ng/mL (range: 141-1944 ng/mL), with an interindividual and intra-individual variability of 57% and 27%, respectively, at the standard dose of 600 mg BID.

Exposure-efficacy analysis

Of the ALK-positive patients treated with crizotinib (n=48), 37 patients (77%) progressed, i.e. 20 patients (87%) in the group with median C_{min} < 235 ng/mL and 17 patients (68%) in the group with median C_{min} ≥ 235 ng/mL. Intracranial progression occurred in 17 patients, i.e. 8 patients in the group with median C_{min} < 235 ng/mL and 9 patients in the group with median C_{min} ≥ 235 ng/mL. Median PFS in patients with crizotinib C_{min} < 235 ng/mL was 5.7 months (95% confidence interval (CI): 5.0-26.8 months), compared with 17.4 months (95% CI: 16.9-not estimable months) in patients with C_{min} ≥ 235 ng/mL (p=0.08, log-rank test, **Figure 2A**). In multivariable analysis, C_{min} < 235 ng/mL resulted in a hazard ratio (HR) of 1.79 (95% CI: 0.90-3.59, p=0.10) when WHO performance status and the number of prior lines of treatment were taken into account (**Table 3**). A swimmer plot is shown in **Supplementary Figure 2**, which illustrates the treatment duration, dose reductions, and resistance mutations (when available) for each individual patient.

In the pooled analysis of all patients treated with crizotinib (i.e. ALK-positive, ROS1-positive, and cMET dysregulation, n=79), median PFS in patients with crizotinib C_{min} < 235 ng/mL was 5.3 months (95% CI: 4.9-15.9 months), compared with 11.8 months (95% CI: 8.7-18.1 months) in patients with C_{min} > 235 ng/mL (p=0.04, log-rank test, **Supplementary Figure 1**). In multivariable analysis, C_{min} > 235 ng/mL resulted in an HR of 2.15 (95% CI: 1.21-3.84, p=0.009) when mutation status, WHO performance status, and the number of prior lines of treatment were taken into account (**Supplementary Table 2**).

Of the patients treated with alectinib, 18 patients (35%) progressed, i.e. 10 patients (53%) in the group with median C_{min} < 435 ng/mL and 8 patients (24%) in the group with median C_{min} ≥ 435 ng/mL. Intracranial progression occurred in four patients, i.e. three patients in the group with median C_{min} < 435 ng/mL and one patient in the group with median C_{min} ≥ 435 ng/mL. Median PFS in patients with alectinib C_{min} < 435 ng/mL was 12.6 months (95% CI: 9.2-not estimable months), compared with not estimable (95% CI: 19.8-not estimable months) in patients with C_{min} ≥ 435 ng/mL (p=0.04, log-rank test, **Figure 2B**). In multivariable analysis, C_{min} ≥ 435 ng/mL resulted in an HR of 4.29 (95% CI: 1.33-13.90, p=0.015) when WHO performance status and prior treatment with ALK-inhibitor(s) were

taken into account (**Table 4**). A swimmer plot is shown in **Supplementary Figure 3**, which illustrates the treatment duration, dose reductions, and resistance mutations (when available) for each individual patient.

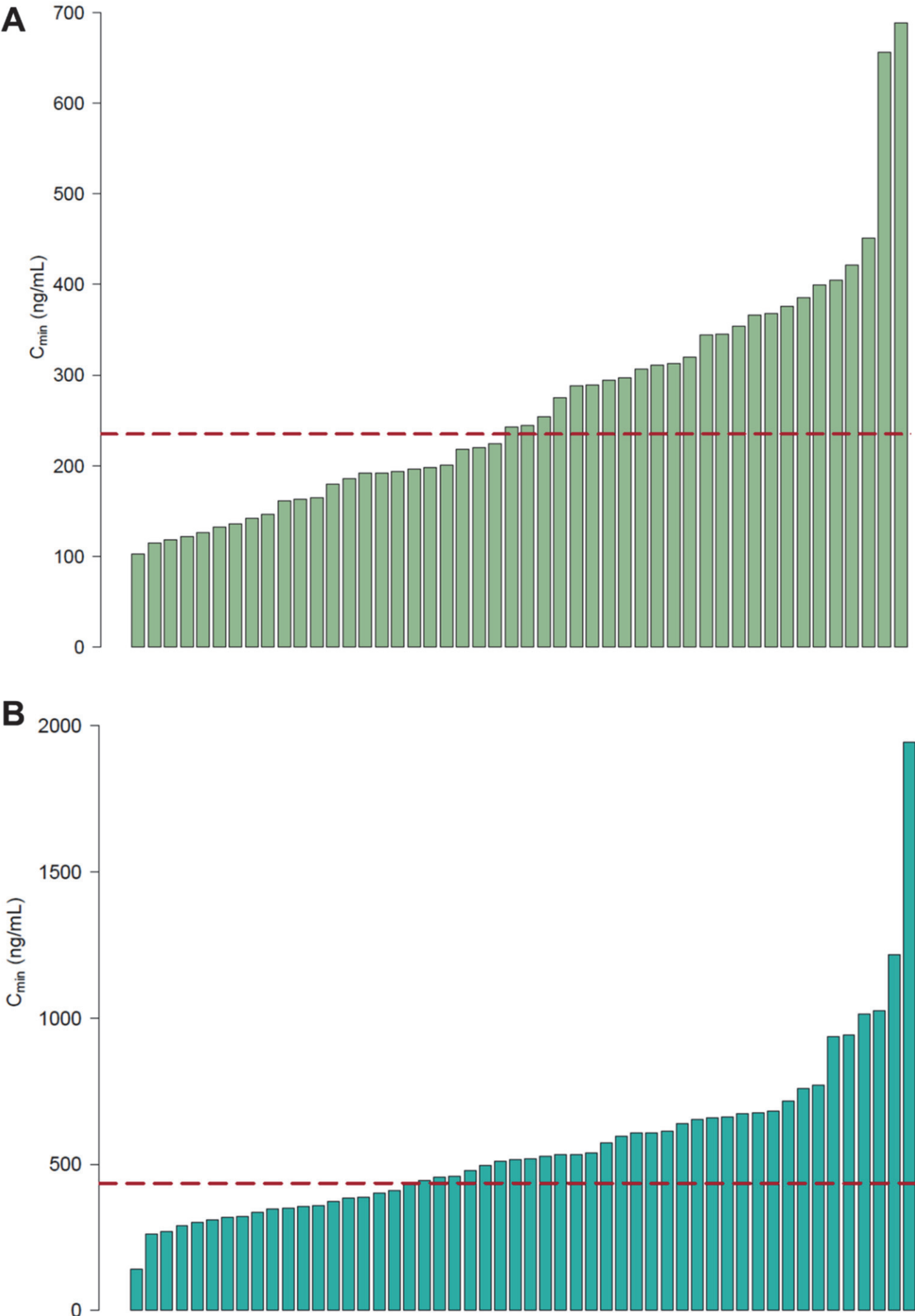


Figure 1 – Bar plots of median crizotinib and alectinib C_{min} per patient
A: Median crizotinib C_{min} per patient. Each bar represents one patient. The dotted line indicates the threshold of 235 ng/mL. Twenty-three patients (48%) have a pharmacokinetic exposure below this threshold. Interindividual and intra-individual variability were 45% and 20%, respectively.
B: Median alectinib C_{min} per patient. Each bar represents one patient. The dotted line indicates the threshold of 435 ng/mL. Nineteen patients (37%) have a pharmacokinetic exposure below this threshold. Interindividual and intra-individual variability were 57% and 27%, respectively.
C_{min} = minimum plasma concentration

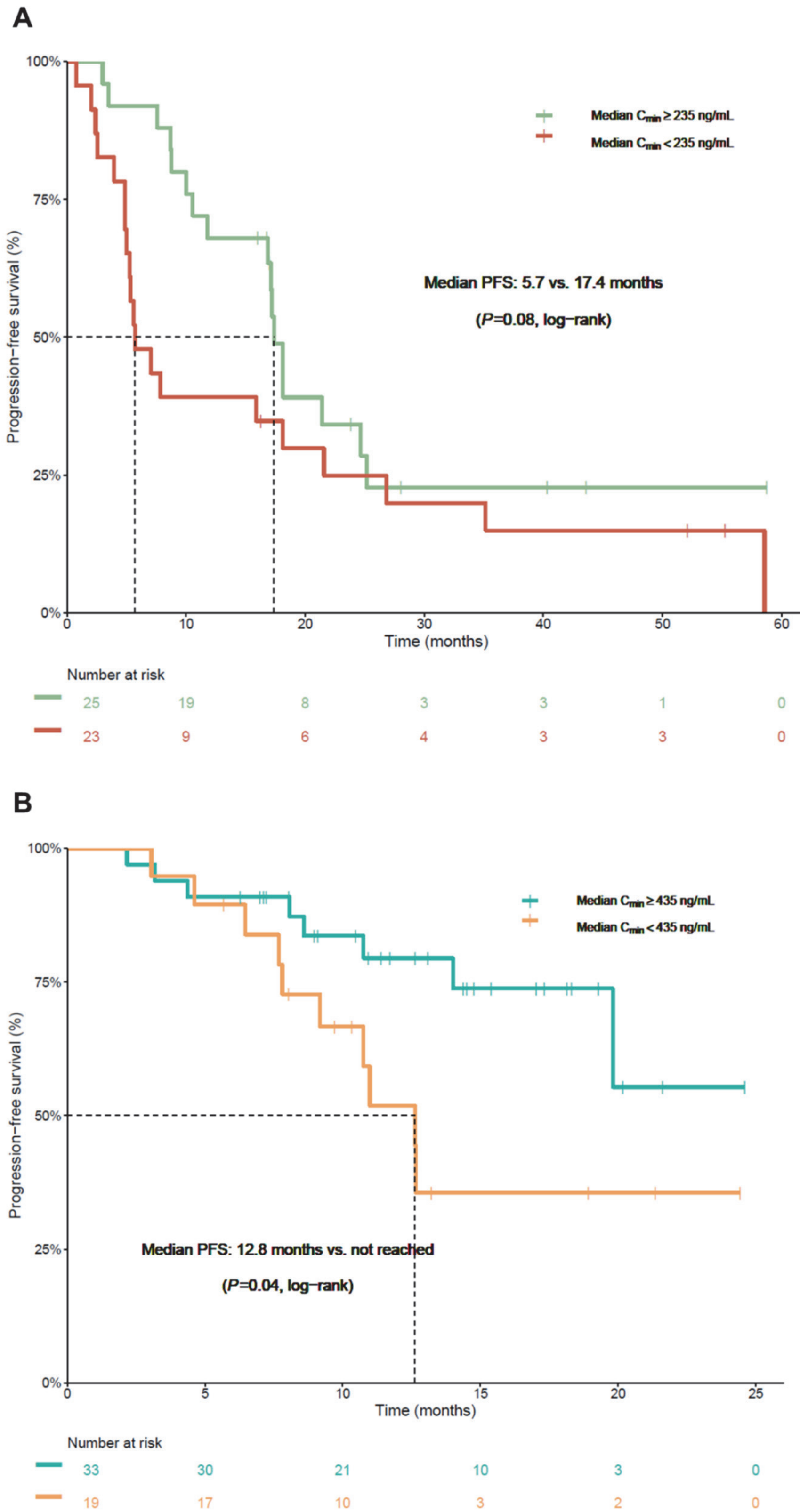


Figure 2 – Progression-free survival in patients treated with crizotinib and alectinib with an exposure above and below the proposed efficacy thresholds

A: Kaplan-Meier curve indicating the progression-free survival in patients treated with crizotinib with a median C_{min} above and below the threshold of 235 ng/mL.

B: Kaplan-Meier curve indicating the progression-free survival in patients treated with alectinib with a median C_{min} above and below the threshold of 435 ng/mL.

C_{min} = minimum plasma concentration

Table 3 – Univariable and multivariable Cox regression analysis for progression-free survival in patients treated with crizotinib

	Univariable			Multivariable		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
C_{min} < 235 ng/mL	1.76	0.92-3.39	0.089	1.79	0.90-3.59	0.100
Age (years)	0.99	0.97-1.01	0.295			
Gender, female	0.65	0.34-1.27	0.212			
WHO performance status	1.34	0.68-2.66	0.398	1.97	0.93-4.15	0.076
Number of prior lines of treatment	1.57	1.00-2.48	0.052	1.61	1.01-2.58	0.046

CI = confidence interval; C_{min} = minimum plasma concentration; HR = hazard ratio; WHO = World Health Organization

Table 4 – Univariable and multivariable Cox regression analysis for progression-free survival in patients treated with alectinib

	Univariable			Multivariable		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
C_{min} < 435 ng/mL	2.58	1.01-6.59	0.047	4.29	1.33-13.90	0.015
Age (years)	0.99	0.95-1.02	0.388			
Gender, female	0.35	0.13-0.93	0.035			
WHO performance status	1.28	0.69-2.38	0.428	2.35	1.07-5.16	0.034
Number of prior lines of treatment	1.65	1.05-2.61	0.030			
Prior treatment with ALK-inhibitor(s)	3.08	0.70-13.51	0.136	2.81	0.57-13.94	0.205

Data were missing for two patients regarding WHO performance status.

ALK = anaplastic lymphoma kinase; CI = confidence interval; C_{min} = minimum plasma concentration; HR = hazard ratio, WHO = World Health Organization

Exposure-toxicity analysis

For crizotinib, 13 patients experienced clinically relevant toxicities (10 dose reductions, 7 dose interruptions, and 1 treatment discontinuation), including liver toxicity (n=5), gastrointestinal toxicity (n=2), pneumonitis (n=1), neuropathy (n=1), renal insufficiency (n=1), neutropenia (n=1), and fatigue (n=1). In six of these patients, the toxicity event occurred before the first PK sample was collected. Median C_{min} before the toxicity event was 338 ng/mL (range: 185-678 ng/mL), compared with 264 ng/mL (range: 118-688 ng/mL) in patients without clinically relevant toxicities (p=0.281). The two patients with the highest median C_{min} did not have clinically relevant toxicities with the currently used definition. However, the first patient (median C_{min} 656 ng/mL) discontinued treatment due to cerebral progression, while at the same time she experienced symptoms possibly related to crizotinib (i.e. muscle weakness, ground glass opacities in the lungs and progression of kidney cysts). The second patient had a median C_{min} of 688 ng/mL and died at the intensive

care unit with an unknown cause of death, possibly due to cardiac arrhythmia, a recognised crizotinib toxicity.

For alectinib, 16 patients experienced clinically relevant toxicities (15 dose reductions, 7 dose interruptions), including edema (n=6), fatigue (n=4), myalgia (n=3), gastrointestinal toxicity (n=3), bradycardia (n=2), liver toxicity (n=2), skin rash (n=1), anemia (n=1), and renal insufficiency (n=1). In addition, six patients started at a lower dose, due to severe toxicity during prior treatment with crizotinib (n=4), elevated liver enzymes (n=1), and miscommunication between patient and physician (n=1). Median C_{min} in patients with and without clinically relevant toxicities was 539 ng/mL and 431 ng/mL, respectively ($p=0.205$).

DISCUSSION

In this observational study we investigated whether pharmacokinetic exposure to ALK-inhibitors crizotinib and alectinib is related to treatment efficacy and toxicity. Patients with a median alectinib $C_{min} \geq 435$ ng/mL had a significantly longer median PFS compared with patients with an exposure below this threshold (12.6 months vs. not reached yet, **Figure 2B**, **Table 4**)). For crizotinib, median PFS was also numerically longer in patients with a median $C_{min} \geq 235$ ng/mL (5.7 vs. 17.4 months), which is a clinically relevant difference, although this difference was not statistically significant (**Figure 2A**, **Table 3**). In the pooled analysis, which also included ROS1-positive and cMET-dysregulated patients, statistical significance was reached (**Supplementary Figure 1**, **Supplementary Table 2**). Exposure to both crizotinib and alectinib was not significantly related to clinically relevant toxicities.

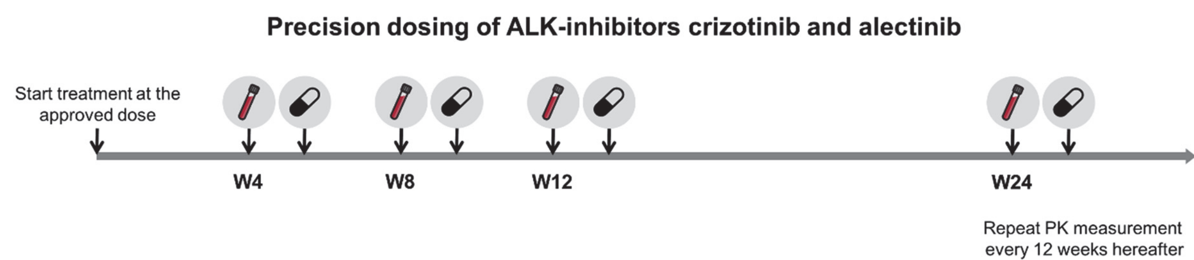
Interindividual variability in pharmacokinetic exposure was found to be considerable (i.e. 45-57%), which is in line with previous literature.[6,7] As a consequence, 48% of the patients treated with crizotinib and 37% of the patients treated with alectinib had an exposure below the efficacy threshold, and were found at risk of decreased treatment efficacy. This implies that individualized dosing is indicated in this subgroup of patients with a low exposure to improve treatment outcomes, for which we provide practical recommendations in **Figure 3**.

Apart from the high interindividual variability, intra-individual variability in alectinib exposure was also found to be large (i.e. 27%). This could be caused by the substantial food effect of alectinib, as its exposure increases more than fourfold when administered with a high-fat meal compared with fasting conditions.[16] According to the label, alectinib is administered concomitantly with food, but the content and volume of these meals could vary substantially within patients over time.

Many patients needed a dose reduction due to toxicity or started treatment at a lower dose (i.e. 23% for crizotinib and 40% for alectinib, **Table 1 and 2**). It is notable that, especially for alectinib, many of these patients still had an adequate exposure. Of the patients with a low exposure to crizotinib and alectinib, 30% and 16%, respectively,

received a prior dose reduction due to toxicity. This means that 70% and 84% of the low exposed patients, respectively, in the absence of toxicity, would potentially benefit from dose escalation.

Notably, patients with a low exposure to alectinib were more often pre-treated with other ALK-inhibitors (84% compared with 58% of patients with adequate exposure). Similar underlying factors (i.e. increased clearance or decreased absorption) could have caused a low exposure to both alectinib and the previous ALK-inhibitor(s). These patients may, therefore, have failed treatment with the previous ALK-inhibitor(s) earlier due to their low exposure and needed subsequent treatment with alectinib sooner. In addition, an inherently lower treatment adherence in these patients could have played a role.



PK-guided dosing recommendations

In case of (calculated) C_{min} below the TDM target of 235 ng/mL for crizotinib or 435 ng/mL for alectinib:

1. Check compliance and drug-drug interactions, adjust concomitant medication if necessary
2. If manageable toxicity: increase crizotinib or alectinib dose by one dose level

Dose level	Crizotinib dose
-3	200 mg QD
-2	250 mg QD
-1	200 mg BID
0	250 mg BID
+1	200 mg – 400 mg

Dose level	Alectinib dose
-2	300 mg BID
-1	450 mg BID
0	600 mg BID
+1	750 mg BID
+2	900 mg BID

Figure 3 – Practical recommendations for precision dosing of crizotinib and alectinib
Patients start treatment at the approved dose of 250 mg BID for crizotinib and 600 mg BID for alectinib. PK samples will be collected 4, 8 and 12 weeks after start of treatment and every 12 weeks thereafter. In case of (calculated) C_{min} below the TDM target of 235 ng/mL for crizotinib or 435 ng/mL for alectinib and manageable toxicity, the dose will be increased by one dose level (after checking treatment adherence and drug-drug interactions). Maximum dose levels are based on data from phase I dose finding studies.
BID = twice daily, C_{min} = minimum plasma concentration, PK = pharmacokinetic(ally), QD = once daily, TDM = therapeutic drug monitoring, W = week

While alectinib is indicated only for the treatment of ALK-positive NSCLC, crizotinib is also approved for ROS1-positive NSCLC and used off-label in the treatment of patients with cMET dysregulation (i.e. amplification or exon 14 skipping). Due to the similarity of ALK and ROS1 kinase domains, crizotinib has similar half maximal inhibitory concentrations values of 40-60 nM and 60 nM against ALK and ROS1, respectively, while the half maximal inhibitory concentration against cMET was notably lower (i.e. 8 nM).[17] It could, therefore, be hypothesized that the efficacy threshold of $C_{min} \geq 235$ ng/mL, that was established in

ALK-positive patients, will also hold true for ROS1-positive patients, while a lower threshold might be sufficient for patients with cMET dysregulation. Since these subgroups have a different prognosis and the efficacy threshold might be different, exposure-efficacy analyses should preferably be performed separately for each subgroup. However, to further increase our sample size, we did perform a pooled analysis, in which we accounted for mutation status, resulting in a statistically significant exposure-response relationship.

In a previously performed exposure-response analysis in patients treated with alectinib (n=207), no association has been identified between median C_{min} of alectinib and its active metabolite M4 and overall survival.[18] Although overall survival is regarded as the gold standard metric of treatment outcome, an exposure-efficacy relationship can easily be diluted by the effects of successive treatment lines.[4] In this study, a potential relationship with PFS has not been evaluated. Furthermore, no threshold was tested and adjustments to measured concentrations were performed to account for analytical bias (due to cross-validation issues).[18] More recent analyses across the phase III studies did demonstrate a relationship between exposure and PFS. They identified an optimal pharmacokinetic threshold of 1040 nM for the sum of alectinib and M4, corresponding to an alectinib C_{min} of approximately 370 ng/mL.[19] Since only limited data on this analysis is currently available in the literature, we did not use this cut-off value in our analyses.

An important strength of the current study is that data were collected from real-life patients, instead of from highly selected patients in a clinical trial. This is the first time that an exposure-response relationship is described outside the context of a clinical trial for ALK-inhibitors, which is relevant, since treatment in a real-life setting may differ considerably from treatment in a clinical trial setting (i.e. no drug accountability, more complex patients who would not be eligible for a trial). In addition, multiple samples over time were available for most patients, providing an adequate reflection of pharmacokinetic exposure during treatment.

On the other hand, limitations of this study include that the applied method to estimate C_{min} assumes an equal alectinib clearance in all patients, not taking into account interindividual differences in the elimination half-life. In addition, part of the samples (up to 59%, **Supplementary Figure 4**) were collected during the absorption or distribution phase (i.e. before the time to maximum concentration), resulting in an underestimation of C_{min}. However, due to the long $t_{1/2}$ (i.e. 42 hours for crizotinib and 32 hours for alectinib) in respect to the dosing interval (i.e. 12 hours), differences between C_{min} and C_{max} are small with a peak-to-trough ratio of approximately 1.3.[20,21] Therefore, the deviations from the actual trough levels can be considered acceptable. These imprecisions could have been circumvented by drawing actual trough levels, but this is less convenient for patients as the collection of PK samples is usually combined with regular visits to the outpatient clinic. Another approach would be to use population PK models to estimate C_{min}, which would allow for taking into account the interpatient variability in PK parameters. However,

disadvantages of this approach are its increased complexity and the fact that Bayesian estimation based on limited sampling will result in shrinkage towards the typical value.[22] Furthermore, another limitation is that selection bias could have played a role, as some patients may have discontinued treatment before the first sample was drawn. These patients may have had early progression due to low exposure. Finally, C_{min} might not be the most appropriate PK parameter to assess exposure-toxicity relationships. Although trough levels are critical to ensure maximal target engagement during the complete dosing interval at the tumor level, other PK parameters may better reflect the potential relationship between exposure and side effects (i.e. AUC or C_{max}).

It is known that the emergence of resistance mutations causes treatment failure.[23] An interesting concept that needs to be further elucidated is whether the prevalence of these resistance mutations is equally high in patients with a low pharmacokinetic exposure compared with patients with an adequate pharmacokinetic exposure. In **Supplementary Figure 2 and 3** we report the identified resistance mutations in our patient cohorts. However, as resistance mutation analysis was only performed in a small subset of patients (n=17), we have insufficient data to answer this question.

Future steps will be to evaluate the feasibility, tolerability, and efficacy of individualized dosing of crizotinib and alectinib, which will be studied in an ongoing prospective study on therapeutic drug monitoring (i.e. adjusting the dose based on measured drug levels) of oral anticancer drugs.[24] Although a randomized controlled trial comparing a therapeutic drug monitoring strategy to a flat dosing strategy could be considered the gold standard, this also assumes an equipoise between treatment arms. Therefore, it could be questioned if performing a randomized controlled trial is ethical where a clear exposure-response relationship exists.

In addition, it would be interesting to investigate the role of the active metabolite of alectinib (i.e. M4). This metabolite has a similar potency as alectinib itself, but a much lower abundance (± 30% of the parent).[7] Concentrations of M4 follow the alectinib concentrations, although data are very limited. Concomitant administration of alectinib with cytochrome P450 3A4 isozyme inhibitors or inducers results in inverse changes in alectinib and M4 concentrations, without affecting the total exposure to a clinically relevant degree.[25] This should be kept in mind when therapeutic drug monitoring of alectinib is applied without measuring M4. It should be noted that all patients in the current study were carefully monitored for drug-drug interactions in clinical practice and, therefore, strong or moderate cytochrome P450 3A4 isozyme inhibitors or inducers were not used in this cohort. A combined threshold of the sum concentration of alectinib plus M4 may further improve precision dosing of alectinib. But given the above considerations, we think the addition of M4 will not relevantly change the finding of this study.

In conclusion, here we demonstrated that exposure to crizotinib and alectinib is related to efficacy in a real-life patient cohort, providing a strong rationale for therapeutic drug monitoring. Individualized dosing based on therapeutic drug monitoring may improve treatment outcomes for the subgroup of patients with a C_{min} below the efficacy thresholds of 235 ng/mL for crizotinib and 435 ng/mL for alectinib.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY DATA

Supplementary Table 1 – Baseline characteristics of all patients treated with crizotinib (i.e. ALK-positive, ROS1-positive and cMET-dysregulated patients)

Patient characteristic	Median C_{min} < 235 ng/mL (n=34)	Median C_{min} ≥ 235 ng/mL (n=45)	All patients (n=79)
Gender, female	11 (32%)	24 (53%)	35 (44%)
Age (years)	54 ± 15	60 ± 12	57 ± 14
Mutation			
ALK	23 (68%)	25 (56%)	48 (61%)
ROS1	4 (12%)	10 (22%)	14 (18%)
cMET amplification	7 (21%)	7 (16%)	14 (18%)
cMET exon 14 skipping	0	3 (7%)	3 (4%)
Tumor stage			
III_A	1 (3%)	0	1 (1%)
III_B	3 (9%)	2 (4%)	5 (6%)
IV	30 (88%)	43 (96%)	73 (92%)
Brain metastases, yes	7 (21%)	6 (13%)	13 (17%)
Previous lines of systemic therapy			
0	14 (41%)	23 (51%)	37 (47%)
1	12 (35%)	19 (42%)	31 (39%)
≥ 2	7 (21%)	3 (7%)	10 (13%)
WHO performance status			
0	20 (59%)	19 (42%)	39 (49%)
1	13 (38%)	22 (49%)	35 (44%)
2	0	3 (7%)	3 (4%)

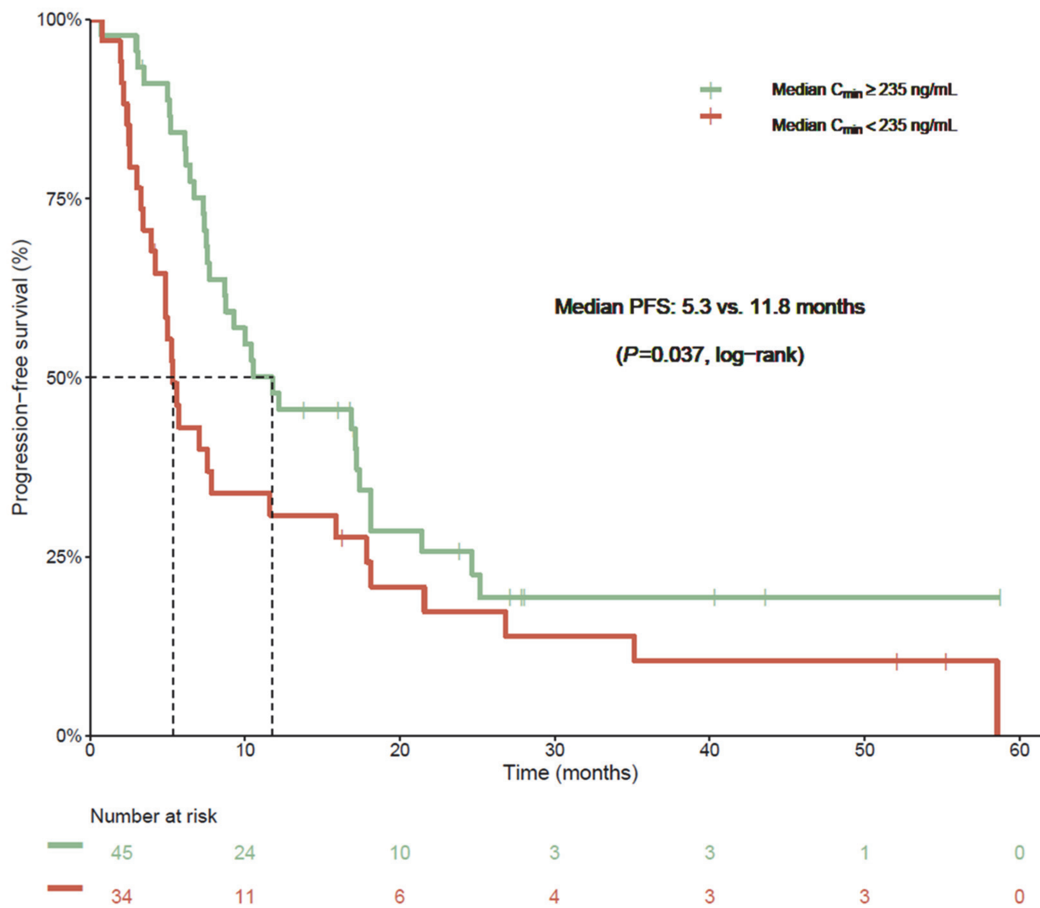
ALK = anaplastic lymphoma kinase; cMET = mesenchymal epithelial transition growth factor; C_{min} = minimum plasma concentration; ROS1 = c-ros oncogene 1; WHO = World Health Organization

Supplementary Table 2 – Univariable and multivariable Cox regression analysis for progression-free survival in all patients treated with crizotinib (i.e. ALK-positive, ROS1-positive and cMET-dysregulated patients)

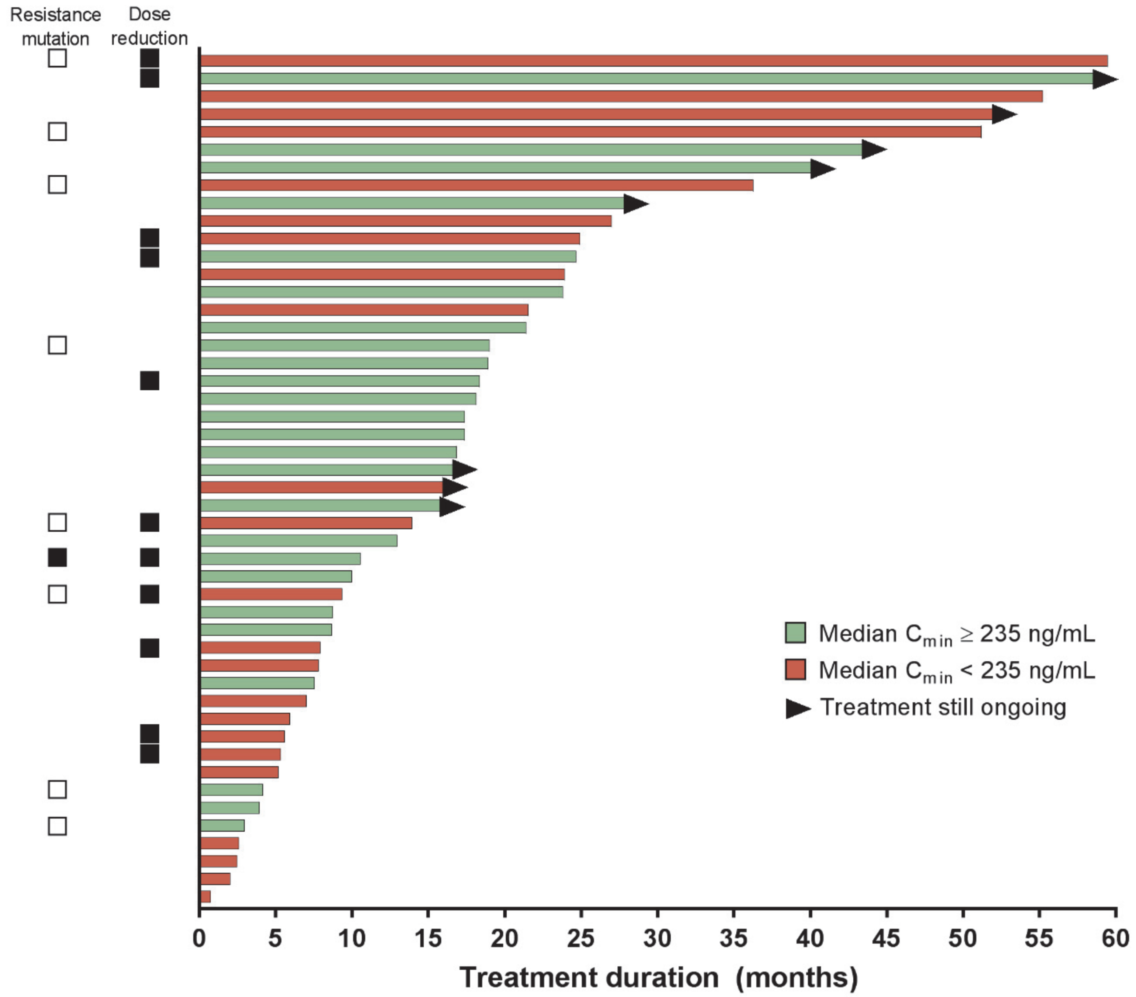
	Univariable			Multivariable		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
C_{min} < 235 ng/mL	1.69	1.03-2.78	0.039	2.15	1.21-3.84	0.009
Mutation status[a]						
ROS1	1.61	0.79-3.29	0.188	1.11	0.45-2.78	0.818
cMET amplification	2.80	1.46-5.37	0.002	2.45	1.22-4.91	0.011
cMET exon 14 skipping	2.93	0.87-9.93	0.083	2.99	0.83-10.68	0.093
Age (years)	1.00	0.98-1.01	0.588			
Gender, female	0.79	0.48-1.31	0.365			
WHO performance status	1.71	1.07-2.74	0.026	1.88	1.08-3.27	0.025
Number of prior lines of treatment	1.29	0.99-1.68	0.063	1.11	0.82-1.51	0.502

[a] hazard ratio compared to ALK-positive patients

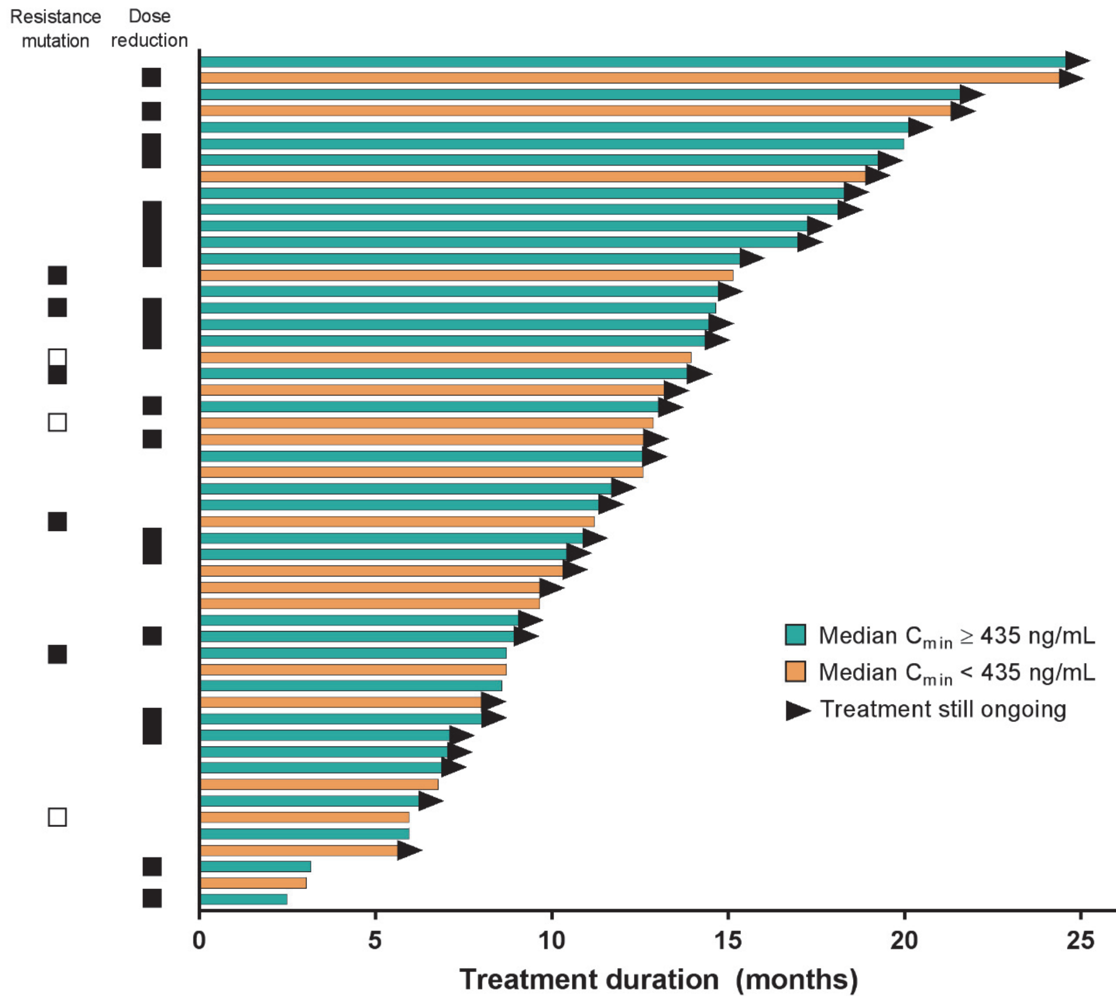
ALK = anaplastic lymphoma kinase; CI = confidence interval; cMET = mesenchymal epithelial transition growth factor; C_{min} = minimum plasma concentration; HR = hazard ratio; ROS1 = c-ros oncogene 1; WHO = World Health Organization



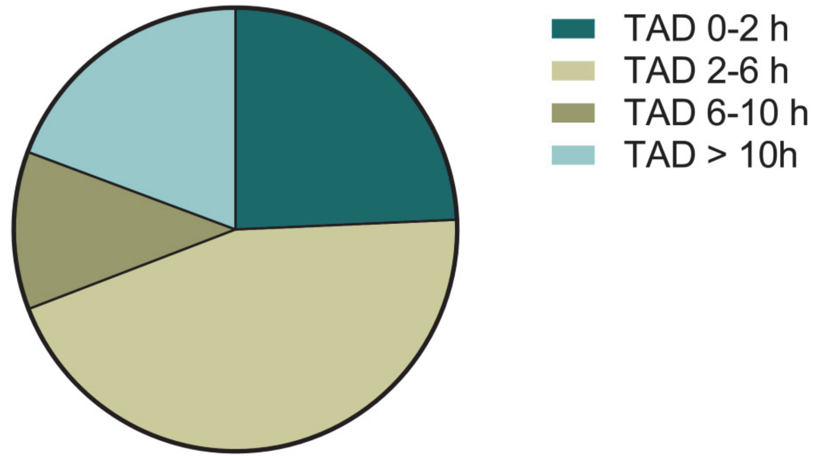
Supplementary Figure 1 – Kaplan-Meier curve of progression-free survival in all patients treated with crizotinib (i.e. ALK-positive, ROS1-positive and cMET-dysregulated patients)
Kaplan-Meier curve indicating the progression-free survival in all patients treated with crizotinib (i.e. ALK-positive, ROS1-positive and cMET-dysregulated patients) with a median C_{min} above and below the threshold of 235 ng/mL.
ALK = anaplastic lymphoma kinase; cMET = mesenchymal epithelial transition growth factor; ROS1 = c-ros oncogene 1

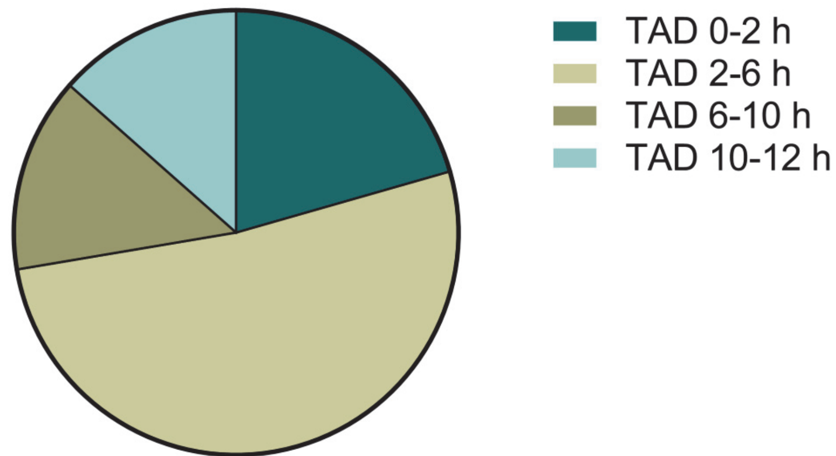


Supplementary Figure 2 – Swimmer plot of patients treated with crizotinib
Each bar represents one patient. White boxes indicate patients in whom the presence of resistance mutations was evaluated at disease progression, but in whom none were identified. The black box indicates the one patient in whom a resistance mutation was identified (i.e. ALK G1202R).
ALK = anaplastic lymphoma kinase; C_{min} = minimum plasma concentration



Supplementary Figure 3 – Swimmer plot of patients treated with alectinib
Each bar represents one patient. White boxes indicate patients in whom the presence of resistance mutations was evaluated at disease progression, but in whom none were identified. Black boxes indicate the patients in whom a resistance mutation was identified (i.e. ALK G1202R (n=3) and ALK I1171N (n=2)).
ALK = anaplastic lymphoma kinase; C_{min} *= minimum plasma concentration*





Supplementary Figure 4 – Pie charts of the distribution of time after dose for crizotinib and alectinib samples
TAD = time after dose

Exposure-response analyses of BRAF- and MEK-inhibitors dabrafenib plus trametinib in melanoma patients

Manuscript in preparation

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PART III

Precision dosing in clinical practice

Therapeutic drug monitoring of oral anticancer drugs: the DPOG-TDM protocol for a prospective study

Ther Drug Monit 2019; **41**: 561–567

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ABSTRACT

Background

Oral anticancer drugs show a high interpatient variability in pharmacokinetics (PK), leading to large differences in drug exposure. For many of these drugs, exposure has been linked to efficacy and toxicity. Despite this knowledge, these drugs are still administered in a one-size-fits-all approach. Consequently, individual patients have a high probability to be either underdosed, which can lead to decreased antitumor efficacy, or overdosed, which could potentially result in increased toxicity. Therapeutic drug monitoring (TDM), personalized dosing based on measured drug levels, could be used to circumvent underdosing and overdosing and thereby optimize treatment outcomes.

Methods

In this prospective clinical study (www.trialregister.nl; NL6695), the feasibility, tolerability, and efficacy of TDM of oral anticancer drugs will be evaluated. In total, at least 600 patients will be included for (at least) 23 different compounds. Patients starting regular treatment with one of these compounds at the approved standard dose can be included. PK sampling will be performed at 4, 8, and 12 weeks after start of treatment and every 12 weeks thereafter. Drug concentrations will be measured, and trough concentrations (C_{min}) will be calculated. In case where C_{min} falls below the predefined target and acceptable toxicity, a PK-guided intervention will be recommended. This could include emphasizing compliance, adapting concomitant medication (due to drug-drug interactions), instructing to take the drug concomitantly with food, splitting intake moments, or recommending a dose increase.

Discussion

Despite a strong rationale for the use of TDM for oral anticancer drugs, this is currently not yet widely adopted in routine patient care. This prospective study will be a valuable contribution to demonstrate the additional value of dose optimization on treatment outcome for these drugs.

BACKGROUND

Although in the past century intravenously administered chemotherapy has always formed the backbone of cancer therapy, this paradigm has shifted in the past two decades toward personalized treatment in which oral anticancer drugs are indispensable. Despite the high interpatient variability in pharmacokinetic (PK) exposure and the fact that for most of these oral anticancer drugs, an exposure-response relationship has been identified, these drugs are still administered at fixed doses. As a consequence, individual patients have a high probability to be either underdosed (> 30% of patients) or overdosed (> 15% of patients), leading to decreased antitumor efficacy and increased toxicity, respectively.[1–6] Therapeutic drug monitoring (TDM), personalized dosing based on measured drug levels, can be used to address these problems and thereby optimize treatment.

Practical guidelines for TDM of kinase inhibitors and oral antihormonal drugs have been developed and published previously.[4,5] Also, feasibility studies have been performed for several anticancer drugs, and they showed TDM to be feasible and safe.[1,2,6–8]

Despite the strong rationale for TDM, it has not yet been implemented as the standard of care in clinical practice. Reasons for this include reimbursement and regulatory issues for higher than approved doses of these expensive drugs and reimbursement of drug level measurement. In addition, clinicians might be reluctant to increase the dose in fear of toxicity, although several studies have shown TDM to be feasible and safe.[1,2,6–8] Furthermore, indisputable evidence on the efficacy of TDM, demonstrated in prospective studies, is lacking. Although randomized controlled trials (RCTs) are considered the gold standard in evidence-based medicine, it would be challenging to perform a RCT on TDM of oral anticancer drugs. This is mainly because a high number of patients with mostly rare cancers would be needed and it would be challenging to secure funding for this. These difficulties are illustrated by the premature termination of a randomized trial of TDM in imatinib patients.[9] Also, it could be argued that not performing dose increments in part of the patients is unethical when clear exposure-response relationships exist.[10]

Therefore, it is necessary to obtain prospective clinical data on the feasibility, tolerability, and efficacy of TDM of oral anticancer drugs. In this study, we aim to implement TDM for these drugs in multiple large medical centers across the Netherlands, assembled in the Dutch Pharmacology Oncology Group (DPOG, www.dpog.nl) and to build a prospective registry to structurally collect data on patients' clinical outcome and the effectiveness of the interventions.

METHODS

The DPOG-TDM study is a multicenter investigator-initiated prospective clinical study. Patients with a regular indication for selected oral anticancer agents start treatment at the standard approved dose according to the label, which includes regular monitoring on drug-drug interactions, contra-indications, and other treatment-specific parameters. Then, drug levels will be measured at 4, 8, and 12 weeks after start of treatment and every 12 weeks thereafter, except for compounds with intermittent dosing schedules or a long elimination half-life ($t_{1/2}$). An overview of the PK sampling schedule per compound can be found in **Table 1**. For each of these agents, detailed drug-specific TDM and dosing guidelines have been formulated based on the currently available evidence and best practice (see Supplemental Data File, http://links.lww.com/TDM/A341). According to the (calculated) trough levels (C_{min}) of the drug and the reported toxicities, treatment recommendations will be provided to the treating physician. This could include PK-guided interventions such as emphasizing compliance, adapting concomitant medication (due to drug-drug interactions), instructing the patients to take the drug concomitantly with food, splitting intake moments, or recommending a dose increase.

In total, at least 600 patients will be included for 23 different oral anticancer drugs, with a possibility to extend with additional agents (and patients) when additional funding is secured.

Figure 1 presents a schematic overview of the study design. **Table 1** summarizes the PK sampling schedules, TDM targets, and dose levels per drug.

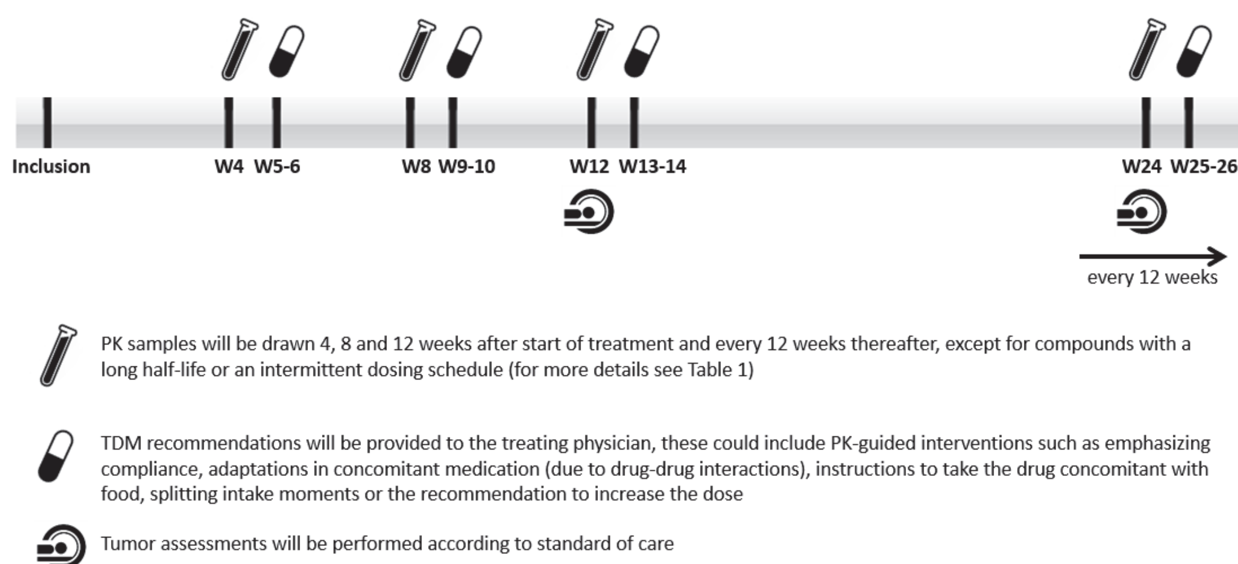

Figure 1 – Study schedule
PK = pharmacokinetic; TDM = therapeutic drug monitoring; W = week

Objectives

The primary objective of this study is to halve the proportion of patients with a drug exposure below the TDM target after two potential PK-guided interventions, which for most compounds will be after 12 weeks. **Table 2** provides the historically presented fraction of patients with an exposure below the TDM target, which will be used as comparison.

The secondary objectives of this study are to determine the tolerability and feasibility of PK-guided dosing, to determine the objective response rate (according to the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1), to determine the time to tumor progression and progression-free survival, to determine the proportion of patients with drug exposure below the TDM target after one potential PK-guided intervention, and to have a physician adherence of > 90% in following the provided treatment recommendations. Objective response rate will be defined as the proportion of patients with confirmed complete response or confirmed partial response according to RECIST version 1.1. Time to tumor progression will be defined as the time from start of treatment to the first documentation of objective tumor progression. Progression-free survival will be defined as the time from the start of treatment to the first documentation of objective tumor progression or to death due to any cause, whichever occurs first.

Inclusion criteria

Patients are eligible for this study if they are aged 18 years or older, have a diagnosis of cancer, an indication to start treatment with one of the oral anticancer drugs included in the study protocol, have a World Health Organization (WHO) performance status of 0, 1, or 2, have a life expectancy of at least 3 months, allowing adequate follow-up of toxicity and antitumor efficacy, and are willing to provide written informed consent.

Exclusion criteria

Patients are excluded if they start treatment at a reduced dose, are known with alcoholism, drug addiction, and/or a psychiatric or physiological condition, which, in the opinion of the investigator, would impair treatment compliance, have any other disease, neurological or metabolic dysfunction, physical examination finding or laboratory finding giving reasonable suspicion of a disease or condition that contra-indicates the use of the drug or puts the patient at high risk of treatment-related complications, or are legally incapable.

TDM targets

The TDM targets used in this study are based on previously published practical guidelines on TDM of kinase inhibitors and oral antihormonal drugs and are shown in **Table 1**.[4,5] For most drugs (16 of 23), this TDM target is based on exposure-efficacy

Table 1 – Summary of TDM targets, PK parameters, and defined dose levels per oral anticancer drug

Drug	Indication	TDM target[a] (ng/mL)	$t_{1/2}$ (h)	t_{max} (h)	Starting dose	Stepwise increases in daily dose per dose level (mg)	Maximum dose	Remarks
Abiraterone	PC	8.4	12	2	1000 mg QD	250	1500 mg QD	First step is intake concomitant with light meal or snack
Alectinib	NSCLC	435	32	4	600 mg BID	300	900 mg BID	
Axitinib	RCC	5	4	3	5 mg BID	4 - 6	10 mg BID	
Bosutinib	CML	147	34	6	500 mg QD	100	600 mg QD	
Cobimetinib[b]	Mel	127	44	2	60 mg QD	20	100 mg QD	
Crizotinib	NSCLC	235	42	5	250 mg BID	100	200 mg – 400 mg	
Dasatinib	CML	2.61	6	1	100 mg QD	40	180 mg QD	
Enzalutamide[c]	PC	5000	139	1	160 mg QD	40	240 mg QD	
Erlotinib	NSCLC	500	36	4	150 mg QD	50	300 mg QD	
Everolimus	RCC, NET, BC	10	38	1	10 mg QD	2.5	15 mg QD	
Gefitinib	NSCLC	200	41	5	250 mg QD	250	750 mg QD	
Imatinib	GIST, CML	1100[d]	19	3	400 mg QD	200	400 mg BID	In case of very low PK (C_{min} < 550 ng/mL), an increase of two dose levels will be recommended
Nilotinib	CML	469	17	3	300 mg BID	200	600 mg BID	
Olaparib	OC	1290	12	2	400 mg BID	200	600 mg BID	
Palbociclib[b]	BC	61	27	4	125 mg QD	25	200 mg BID	
Pazopanib	RCC, STS	20500	31	4	800 mg QD	200	1000 mg BID	First step is to split intake moments into 400 mg BID, second step is intake concomitant with food
Regorafenib[b]	CRC, GIST	1400	28	4	160 mg QD	40	200 mg QD	
Sorafenib	HCC, TC	3750	26	8	400 mg BID	200	800 mg BID	

Sunitinib[e]	GIST, NET, RCC	50[f]	50/ 95[g]	5	50 mg QD	12.5	75 mg QD	
Tamoxifen[h]	BC	5.97	336	2	20 mg QD	10	60 mg QD	In case of very low PK ($C_{steady\text{-}state}$ < 3 ng/mL), an increase of two dose levels will be recommended
Trametinib[i]	Mel	10.6	96	2	2 mg QD	0.5	3 mg QD	
Vemurafenib	Mel	42000	34	5	960 mg BID	480	1440 mg BID	
Vismodegib[h]	BCC	11.4	96	4	150 mg QD	150	450 mg QD	

[a] TDM target concentrations are all C_{min}, except for tamoxifen, for which the TDM target refers to the steady-state concentration of its active metabolite endoxifen

[b] Because these drugs have an intermittent dosing schedule, PK samples will be drawn 3, 7, and 11 weeks after the start of treatment

[c] Because enzalutamide has a long half-life (± 6 days), PK samples will be drawn 4, 10, and 16 weeks after the start of treatment

[d] For CML patients, the TDM target is C_{min} ≥ 1000 ng/mL

[e] For patients receiving sunitinib in an intermittent dosing schedule, PK samples will be drawn 4, 10, and 16 weeks after the start of treatment

[f] TDM target for intermittent dosing schedule is C_{min} ≥ 50 ng/mL (sum of concentrations of both sunitinib and its active metabolite N-desethylsunitinib), whereas for continuous dosing schedule, TDM target is C_{min} ≥ 37.5 ng/mL

[g] $t_{1/2}$ is different for sunitinib (50 hours) and its active metabolite N-desethylsunitinib (95 hours)

[h] Because (active metabolites of) these drugs have a very long half-life, PK samples will be drawn each 12 weeks

[i] For dabrafenib, drug concentrations will only be measured, no dose adaptations will be recommended because this might not be the ideal drug for TDM

BC = breast cancer; BCC = basal cell carcinoma; BID = twice daily; C_{min} = minimum plasma concentration / trough concentration; $C_{steady\text{-}state}$ = steady-state concentration; CML = chronic myelogenous leukemia; CRC = colorectal cancer; GIST = gastrointestinal stromal tumor; HCC = hepatocellular carcinoma; Mel = melanoma; NET = neuro-endocrine tumor; NSCLC = non-small-cell lung cancer; OC = ovarian cancer; PK = pharmacokinetics; QD = once daily; PC = prostate cancer; RCC = renal cell carcinoma; STS = soft tissue sarcoma; TC = thyroid cancer; TDM = therapeutic drug monitoring; t_{max} = time to maximum concentration; $t_{1/2}$ = elimination half-life

analyses. If these analyses were not available (yet), the mean or median exposure of the drug was taken as a reference. For the compounds with TDM targets based on exposure-efficacy analyses, the PK targets amounted to 81-85% of the average population exposure.[4,5] Therefore, targeting the mean or median concentration will generally lead to an efficacious exposure. In the meantime, thorough exposure-efficacy analyses will be awaited, which can provide a definitive target for TDM.

Dose levels

Levels for dose adjustments have been defined for each drug, indicating the maximum dose of the drug and the steps with which the dose should be increased in case of low exposure or decreased in case of toxicity. The highest dose level is based on the maximum tolerated dose found in the phase I study. If the maximum tolerated dose was not

reached, the highest dose tested in the phase I study was taken as the maximum dose. In case of saturated absorption, concomitant intake with food (abiraterone and pazopanib) or splitting intake moments (pazopanib) will be recommended, based on findings from previous studies.[32,33] In **Table 1,** the maximum dose levels and stepwise increases are reported for each drug. All dose levels per drug are described in the Supplemental Data File (http://links.lww.com/TDM/A341).

Table 2 – Historical data of the percentage of patients below TDM target per oral anticancer drug

Drug	Patients below TDM target at standard dose (%)	Reference
Abiraterone	35	[11]
Alectinib	33	[12]
Axitinib	38	[13]
Bosutinib	50	[14]
Cobimetinib	50	[15]
Crizotinib	25	[16]
Dasatinib	50	[17]
Enzalutamide	2	[18]
Erlotinib	11	[3]
Everolimus	37	[19]
Gefitinib	26	[20]
Imatinib	73	[3]
Nilotinib	25	[21]
Olaparib	50	[22]
Palbociclib	50	[23]
Pazopanib	16-20	[24,25]
Regorafenib	50	[26]
Sorafenib	50	[27]
Sunitinib	49	[3]
Tamoxifen	20	[28]
Trametinib[a]	27	[29]
Vemurafenib	52	[30]
Vismodegib	50	[31]

[a] Data reported only for trametinib, as for dabrafenib, no dose adjustments will be recommended because little evidence for an exposure-response relationship for dabrafenib is available.
TDM = therapeutic drug monitoring

Pharmacokinetic measurements

Concentrations of the drug will be measured using validated liquid chromatography-tandem mass spectrometry assays.[34–39] Quality of measurements will be secured by interlaboratory comparison. Patients will be instructed to let the blood sample be drawn after the time to maximum concentration (t_{max}) of the drug has been reached. Each time

a PK sample is drawn, the patient will be asked the date and time of the last drug intake, and this will be recorded, as well as the time of blood sampling, to calculate the time after dose. Trough concentrations will then be calculated based on the time after dose and the $t_{1/2}$ of the drug using the following formula:

$$C_{min} = C_{measured} * 0.5^{\frac{dosing\ interval - TAD}{t_{1/2}}}$$

where C_{min} is the minimum drug concentration, $C_{measured}$ is the measured drug concentration, TAD is the time after dose, and $t_{1/2}$ is the average elimination half-life of the drug.[40]

Table 1 shows the PK sampling schedule per drug and the corresponding t_{max} and $t_{1/2}$ values, which will be used to calculate the trough levels.

PK-guided interventions

If the estimated trough concentration is below the predefined TDM target and the patient does not show any treatment-related ≥ grade 3 toxicity, a PK-guided intervention will be recommended to the treating physician within 1-2 weeks. This could include emphasizing compliance, adapting concomitant medication (due to drug-drug interactions), instructing to take the drug concomitantly with food, splitting intake moments, or recommending a dose increase. If patients show any ≥ grade 3 toxicity, the dose will be interrupted until the toxicity is ≤ grade 1. If the toxicity was treatment-related, the dose will be reduced with one dose level.

In case of concentrations below the TDM target, compliance will be checked directly with the patient. If compliance seems to be the cause of low PK exposure, no dose increments will be performed. Instead, compliance will be emphasized, and a new PK sample will be drawn after steady-state concentrations have been reached again. In this way, compliance will be assessed before making dose increases.

Tolerability and efficacy assessments

Toxicity will be evaluated during routine visits to the outpatient clinics. Tumor assessments according to RECIST version 1.1 will be performed at least every 12 weeks as part of standard care.

Statistics: sample size

The primary objective of this study is to halve the proportion of patients with a PK exposure below the predefined TDM target after two PK-guided interventions. If we consider the percentages reported in the literature as historical controls (**Table 2**), then using an exact binomial test with a nominal 0.05 two-sided significance level will provide the power as indicated in **Table 3** assuming different levels of the null and alternative

hypothesis and various sample sizes. Obviously, if a higher proportion of patients have a low PK exposure, fewer patients are needed to provide a reasonable power. Sample size calculations were performed using the power.binom.test function of the pwr package in R.[41]

Table 3 – Sample size calculation showing power at different levels of null and alternative hypothesis and three examples of sample size

Proportion of patients with a drug exposure below TDM target		Number of patients		
		30	60	90
Null	**Alternative**		**Power** (%, 1-β)	
0.10	0.05	1	19	33
0.20	0.10	18	43	71
0.30	0.15	32	71	92
0.40	0.20	60	92	98
0.50	0.25	80	97	
0.60	0.30	91		

TDM = therapeutic drug monitoring

Regarding the secondary outcome of evaluating the tolerability and feasibility of TDM, generally ± 25-30% of the total patient group will be eligible for dose escalation. To assess the feasibility of PK-guided interventions in at least 8 patients, about 3-4 times as many patients need to be included. Therefore, the aim is to include at least 30 patients per compound. For abiraterone, imatinib, pazopanib, sunitinib, and trametinib, patient inclusion will be expanded to be able to evaluate the influence of TDM on efficacy as well.

Statistics: analysis

The full analysis set will include all patients who received at least one dose of the oral anticancer drug. Patients will only be considered evaluable for the primary endpoint if they have completed the first three PK measurements. An exact binomial test will be performed for each drug. In some situations of drug and target combinations, it may prove difficult to obtain a sufficient number of patients for an acceptable level of power. Therefore, an additional meta-analytic approach will be applied to test the "proof-of-principle" of TDM. For each drug, the standardized change in percentage of patients with a concentration below the predefined target at the third PK measurement after the start of treatment will be calculated. Secondary endpoints will be described using descriptive statistics.

Logistic and administrative arrangements

The DPOG-TDM study was assessed by the accredited Medical Ethics Committee of the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital (NKI-AVL) on May 3, 2017, and it was decided that the study did not fall under the Dutch Medical Research Involving Human Subjects Act because no additional procedures are required for the

participants. The institutional review board authorized the study on August 7, 2017. Patients do need to give written informed consent because data will be collected and shared. The study protocol follows the principles of the Declaration of Helsinki and the code of conduct of the Dutch FEDERA guidelines.

The NKI-AVL is the coordinating center. Other participating centers are the Erasmus Medical Center (EMC), Radboud University Medical Center (Radboud UMC), Leiden University Medical Center (LUMC), and University Medical Center Groningen (UMCG). Additional participating centers of the study are currently being recruited.

Data on baseline characteristics, measured blood concentrations, TDM recommendations, dose adjustments, toxicity, efficacy, and survival will be collected in the electronical case report form. Members of the study team will have access to the final dataset.

DISCUSSION

Currently, all patients treated with oral anticancer drugs receive a standard fixed dose (i.e. all patients receive exactly the same dose, independent of their weight or body surface area), although it is well known that these drugs show a large interpatient variability, and for many of them, exposure has been linked to efficacy and adverse events, providing a strong rationale for TDM. Also, other strategies such as body surface area-based dosing do not lead to an improvement in PK exposure.[42] This study aims to demonstrate the added value of TDM in collaborating hospitals.

In the development of the study design, several choices had to be made. Ideally, one would choose to perform a RCT. However, this would require an even larger sample size. Also, it could be considered unethical to fail to increase the dose in case of measured low exposure when a clear relationship between exposure and efficacy exists. Therefore, we decided to perform this prospective intervention trial.

Furthermore, we decided to only recommend dose increments in case of low PK exposure, whereas it could be argued that dose reductions in case of high PK exposure might be beneficial as well, as this could be associated with less toxicity and lower costs. However, in oncology, cautions are warranted regarding dose reductions based on PK exposure because disease progression is an irreversible event. Of course, dose reductions would be made in case of toxicity, as this is regular routine patient care, which is also included in the labels.

In addition, we chose to calculate C_{min} using the above-mentioned formula based on time after dose and the average elimination half-life of the drug, assuming a one-compartment model and that distribution has largely been completed by the time the sample is drawn. Alternative methods could be to draw actual trough levels or to estimate C_{min} using

existing population PK models. However, these methods are not feasible in clinical practice because the timing of actual trough samples would be inconvenient for patients and the use of population PK models would be time consuming and would seriously delay the report of PK results and treatment advice to the treating physician and patient. Furthermore, Bayesian estimates of trough concentration based on a single sample suffer from shrinkage (regression to the mean), which will result in the misclassification of patients with low trough levels. Therefore, we chose to use this method, as it is easy to use, relatively precise, and thereby suitable to implement in routine care.

Many factors could contribute to low PK exposure, including drug-drug interactions, absorption problems (e.g. caused by poor bioavailability, food effects, or altered stomach pH), pharmacogenetics, and compliance.[43] Compliance can be defined as the extent to which the patient follows the dosing schedule as intended by the prescriber. Especially in case of long-term treatment, compliance is known to decrease over time, potentially leading to low PK exposure and thereby decreased efficacy.[44] For example, poor adherence to imatinib has been related to suboptimal treatment outcomes.[45] TDM could play a role in detecting poor compliance to oral anticancer drugs.

Previous attempts to evaluate the efficacy of TDM have failed to do so because of the unwillingness of treating physicians to follow treatment recommendations.[8] We realize it is important that treatment recommendations should be followed to adequately evaluate the feasibility, tolerability, and efficacy of TDM. This is one of the reasons why physician adherence was chosen as one of the secondary objectives. We hope to achieve a high physician adherence by providing treating physicians with the available scientific evidence on exposure-efficacy relationships. Also, we summarize for them the number of patients previously treated at the proposed dose level (e.g. in the phase I study) and the tolerability in these patients.

We believe that the current fixed dosing paradigm should be changed. Subtherapeutic treatment with these expensive drugs due to low PK exposure at the standard dose is senseless. It is our opinion that personalized dosing based on individual drug levels is far more rational. If this large prospective study underscores the results of previous retrospective studies and prospective feasibility studies,[1,2,6–8] this will support the implementation of PK-guided dose optimization as the new standard, although we realize that classical endpoints such as improvement of survival and/or quality of life will not be explored in this study. Because reimbursement of drug level measurement and administration of higher than approved doses of these expensive drugs could remain a challenge in the implementation of TDM as the standard of care in oncology, next steps would be to perform cost-effectiveness analyses and to address this with the concerning healthcare authorities.

The DPOG-TDM study protocol has been developed to be a dynamic protocol, meaning that future oral anticancer drugs could be added to the protocol. Also, when new literature on exposure-efficacy relationships becomes available, the TDM targets could be updated. The guidance provided in this protocol could also be used outside this study for the implementation of TDM of oral anticancer drugs in the rest of the world.

In conclusion, this prospective clinical trial evaluating the feasibility, tolerability, and efficacy of TDM of oral anticancer drugs will be a valuable contribution to the fields of clinical pharmacology and oncology and holds promise to optimize treatment outcomes for patients treated with these agents.

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Therapeutic drug monitoring based precision dosing of oral targeted therapies in oncology: a prospective multicentre study

Interim analysis

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On behalf of the Dutch Pharmacology Oncology Group

The results described in this chapter have not been published yet and include preliminary data that could deviate from the final publication

Therapeutic drug monitoring (TDM) of imatinib in patients with gastrointestinal stromal tumours (GIST) – results from daily clinical practice

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ABSTRACT

Aim

Higher imatinib exposure is correlated with longer time to progression, while the variability in exposure is high. This provides a strong rationale for therapeutic drug monitoring, which has therefore been implemented in routine clinical practice in our institute. The aim of this study is to evaluate whether pharmacokinetically (PK)-guided dose increases are feasible in daily clinical practice and result in an improved exposure ($C_{min} \geq$ 1100ng/mL) and longer progression-free survival (PFS).

Methods

This retrospective study included all patients with a gastrointestinal stromal tumour (GIST) in the Netherlands Cancer Institute who started imatinib treatment at a dose of 400 mg and of whom PK plasma samples were available. Of these patients, minimum plasma concentrations (Cmin) of imatinib, frequency and successfulness of PK-guided dose increases and PFS in the palliative treatment setting were analysed.

Results

In total, 169 consecutive patients were included, of whom 1402 PK samples were collected. In 126 patients (75%), C_{min} was below the efficacy threshold of 1100 ng/mL. In 78 of these patients (62%), a PK-guided dose increase was performed, which was successful in 49 patients (63%). PFS was similar in patients with and without imatinib dose increase. However, due to the small number of patients with progressive disease, no definite conclusions on the effect on PFS could yet be drawn.

Conclusion

This is the largest cohort evaluating PK-guided dose increases of imatinib in patients with GIST in routine clinical practice and demonstrating its feasibility. PK-guided dose increases should be applied to optimise exposure in the significant subset of patients with a low C_{min}.

INTRODUCTION

Gastrointestinal stromal tumour (GIST) is a rare type of soft tissue sarcoma treated with the tyrosine kinase inhibitor imatinib in neoadjuvant, adjuvant and palliative treatment setting. Since the introduction of imatinib, the survival in advanced GIST has improved drastically.[1] Nevertheless, therapeutic failures are still common and survival could be further improved.

In a previous study by Demetri *et al.* the time to progression was almost three times longer for patients with imatinib trough levels (C_{min}) ≥ 1100 ng/mL compared with those with a C_{min} below this threshold (30 months vs. 11 months).[2] These results are in line with the exposure-response relationship that has been identified for patients with chronic myeloid leukaemia (CML) treated with imatinib.[3-5] There is, however, a large inter- and intra-individual variability (47-75% and 19-26%, respectively) in imatinib exposure.[2,6-10] Furthermore, decreased imatinib exposure of 30% after long-term treatment has been described.[8] The high variability in imatinib exposure combined with the clear exposure-response relationship provides a strong rationale for adjusting the dose based on measured drug levels, also known as therapeutic drug monitoring (TDM).

Previously, our group has published a report describing the pharmacokinetics (PK) of imatinib in routine clinical care, showing that 32-73% of the patients was systematically underexposed.[9,11] In addition, TDM of imatinib in patients with GIST has shown promising results. PK-guided dose interventions in 20 patients treated with imatinib resulted in adequate C_{min} (1100-3200ng/mL) in 95% of patients in a previous study.[10] In addition, Zuidema *et al*. described that TDM-guided dosing is a cost-effective intervention for patients with advanced GIST.[12] Based on the previous fact, TDM of imatinib has been implemented as standard of care in the Netherlands Cancer Institute (NKI).

In our routine TDM program, drug levels are measured regularly in the out-patient clinic and dose regimens are adjusted accordingly, striving for a C_{min} ≥ 1100ng/mL.[2,3,7] Data on the state of affairs of TDM of imatinib in patients with GIST are not widely available. Therefore, the aim of this retrospective study was to evaluate whether PK-guided dose increases are feasible in daily clinical practice and result in an increased proportion of patients with adequate exposure (as defined as C_{min} ≥ 1100ng/mL) and longer progression-free survival (PFS).

METHODS

Patient selection

Data were collected from the Dutch GIST Registry (DGR), a database containing information on all patients with GIST treated in one of the five Dutch GIST centres since 2009. The DGR was approved by the local independent ethics committee. All patients in

the NKI, one of these centres, who started treatment with imatinib at the standard dose of 400 mg once daily (QD) and of whom PK samples were available were included. Of these patients, demographic data, tumour characteristics, imatinib treatment details, imatinib trough levels, PFS (in case of metastatic disease) and clinically relevant toxicities (i.e. toxicities requiring a dose reduction, treatment interruption or discontinuation) were obtained from the database. In the neoadjuvant setting, imatinib was continued until maximum tumour response (in general around 6 months).[13,14] The duration of imatinib treatment in the adjuvant setting was 12 months before 2012 and was expanded to 36 months thereafter.[15,16] Palliative treatment was continued until progressive disease or unacceptable toxicity.

PK analyses

Plasma samples were collected during routine follow-up visits (in general, monthly during the first three months and once every three months thereafter). Date and time of last imatinib ingestion and plasma collection were recorded. Imatinib concentrations were measured using a validated liquid chromatography-tandem mass spectrometry assay.[17] C_{min} was estimated using the following formula, which has previously been validated by Wang *et al.*[18]:

$$C_{min} = C_{measured} * 0.5^{\frac{dosing\ interval - TAD}{t_{1/2}}}$$

In this formula, the C_{min} is the calculated minimum plasma concentration (trough level, in ng/mL), $C_{measured}$ is the measured plasma concentration (in ng/mL), dosing interval is the time in hours between two consecutive ingestions of the drug, TAD is the time after dose, that is, the time between last administration of imatinib and collection of the plasma sample in hours and $t_{1/2}$ is the elimination half-life of the drug (18 hours for imatinib[19]).

If samples were collected within 0.5 hour after imatinib intake, they were interpreted as C_{min}. When samples were collected before t_{max} (i.e. 0.5-2.5 hours after intake), C_{min} was calculated by multiplying the ratio of the measured concentration and the typical population concentration (at the corresponding time after dose) with the typical population value of C_{min}.[20]

TDM subgroups

Patients were retrospectively divided into group 1 (low PK) if they had a C_{min} below the threshold of 1100 ng/mL on at least two occasions or if they received a PK-guided dose increase based on one C_{min} below this threshold. Patients in group 1 were further subdivided based on the experienced toxicity and whether or not they received a dose increase (group 1A: no toxicity, dose increase; group 1B: toxicity, no dose increase; group 1C: no toxicity, no dose increase). Dose increases were considered successful if the median C_{min} at the increased dose was ≥ 1100 ng/mL and if the patient tolerated this dose for at least six months. Patients were divided into group 2 (adequate PK) if all C_{min} were ≥

1100 ng/mL. Patients in group 2 were further subdivided based on experienced toxicity (group 2A: no toxicity; group 2B: toxicity). For the comparison of exposure and PFS, group 1B+1C (low PK, no dose increase) and group 2A+2B (adequate PK) were combined and compared with group 1A (low PK, dose increase).

Statistical analysis

Descriptive statistics were performed using IBM SPSS Statistics, version 25, and PK results were analysed using R, version 3.6.1 (R Project, Vienna, Austria). Survival was estimated with the Kaplan-Meier method. To compare treatment outcomes, PFS was compared for metastatic patients with *KIT* exon 11 mutations using Cox regression analyses. Factors with a known correlation to outcome (according to National Institutes of Health risk assessment: tumour size, primary tumour location and mitotic rate[21] and World Health Organisation performance status[22]) and with an apparent unequal distribution among the subgroups were included in the multivariable analysis. A two-sided *P*-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

In total, 202 patients were diagnosed with GIST between January 2009 and July 2019, registered in the DGR and treated in the NKI. PK data were available for 169 patients (84%). Sixteen patients were treated in the beginning of the implementation and received no TDM, thirteen patients just started treatment with no PK samples yet and four patients were lost to follow-up before PK data were collected. Patient characteristics are shown in **Table 1**. The median age at diagnosis was 63 years (interquartile range (IQR): 53-70) and a slight majority was men (58%). Most frequent GIST location was the stomach (50%) or ileum/jejunum (34%). The most common mutation was *KIT* exon 11 (82%) but also 13 patients had a known imatinib insensitive mutation: 11 *KIT* exon 9 and 2 *PDGFR* D842V. At baseline, 39% of the tumours had a high mitotic rate and 27% of the patients already had metastatic disease. Patients were treated in different treatment settings: neoadjuvant (n=75), adjuvant (n=75) and palliative (n=83). Patients who received a dose increase (group 1A) appeared to be younger, and patients with a low exposure (group 1) were relatively more often men (68% and 54% vs. 44% in group 2) and had *KIT* exon 11 mutated GIST (86% and 90% vs. 65% in group 2).

PK samples

In total, 1402 PK samples were collected with a median of 7 samples per patient (IQR: 4-12). The median time after dose was 15.3 hours (IQR: 10.0-18.5 hours). The median total time on imatinib was 23 months (IQR: 10-36 months). The median C_{min} per patient was 1074 ng/mL (IQR: 946-1247 ng/mL, **Figure 1**). Inter- and intra-individual variability was 49%

and 26%, respectively, at the standard dose of 400 mg QD. **Table 2** provides an overview of all PK samples.

Table 1 – Patient characteristics

	All patients	Group 1A	Group 1B+1C	Group 2A+2B
No. of patients	169	78	48	43
Age at diagnosis	63 [53-70]	58 [50-68]	65 [57-73]	64 [60-75]
Primary tumour size (in mm)	100 [62-150]	102 [64-135]	96 [60-150]	108 [63-168]
Gender, male	98 (58%)	53 (68%)	26 (54%)	19 (44%)
Primary GIST location				
Gastric	84 (50%)	34 (44%)	26 (54%)	24 (56%)
Ileum/jejunum	57 (34%)	29 (37%)	16 (33%)	12 (28%)
Duodenum	13 (8%)	8 (10%)	2 (4%)	3 (7%)
Rectum	7 (4%)	4 (5%)	1 (2%)	2 (5%)
Esophagus	3 (2%)	1 (1%)	2 (4%)	0
Colon	3 (2%)	1 (1%)	1 (2%)	1 (2%)
Other[a]	2 (1%)	1 (1%)	0	1 (2%)
Mutation status				
KIT exon 11	138 (82%)	67 (86%)	43 (90%)	28 (65%)
KIT exon 9	11 (7%)	5 (6%)	0	6 (14%)
KIT other[b]	2 (1%)	1 (1%)	1 (2%)	0
PDGFR non-D842V	6 (4%)	3 (4%)	2 (4%)	1 (2%)
PDGFR D842V	2 (1%)	0	2 (4%)	0
SDH deficient	1 (1%)	0	0	1 (2%)
NF1 related	2 (1%)	0	0	2 (5%)
Wildtype	4 (2%)	1 (1%)	0	3 (7%)
No mutation analysis	3 (2%)	1 (1%)	0	2 (5%)
Baseline mitotic rate				
Low (≤ 5/5 mm[b])	84 (50%)	39 (50%)	27 (56%)	18 (42%)
High (> 5/5 mm[b])	66 (39%)	34 (44%)	16 (33%)	16 (37%)
Unknown	19 (11%)	5 (6%)	5 (10%)	9 (21%)
Tumour status at diagnosis				
Localised disease	55 (33%)	27 (35%)	17 (35%)	11 (26%)
Locally advanced	64 (38%)	30 (39%)	17 (35%)	17 (40%)
Multiple locations	4 (2%)	1 (1%)	1 (2%)	2 (5%)
Metastatic disease	46 (27%)	20 (26%)	13 (27%)	13 (30%)
Performance status[c]				
WHO 0	117 (69%)	55 (71%)	32 (67%)	30 (70%)
WHO 1	31 (18%)	10 (13%)	14 (29%)	7 (16%)
WHO ≥ 2	3 (2%)	2 (2%)	0	1 (2%)
Unknown	18 (11%)	11 (14%)	2 (4%)	5 (12%)
Treatment objective[d]				
Neoadjuvant	75 (32%)	33 (30%)	22 (33%)	20 (36%)
Adjuvant	75 (32%)	41 (37%)	22 (33%)	12 (22%)
Palliative	83 (36%)	37 (33%)	23 (34%)	23 (42%)

Treatment duration[e]				
Neoadjuvant	6 [5-7]	6 [5-7]	7 [6-8]	6 [4-8]
Adjuvant	34 [31-36]	35 [34-36]	30 [29-31]	23 [10-36]
Palliative	30 [23-37]	35 [13-57]	30 [28-32]	12 [8-16]

Data are expressed as no. (%) or median [interquartile range], as appropriate. Group 1A: low exposure, PK-guided dose increase, group 1B+1C: low exposure, no PK-guided dose increase, group 2A+2B: adequate exposure all the time.
[a] One in the liver, one deposition in mesentery
[b] One KIT exon 13 and one KIT exon 17
[c] WHO Performance status at start of first treatment or if palliative treatment at start of palliative treatment
[d] Several patients had imatinib in multiple treatment settings
[e] Median treatment duration (in months, [95% confidence interval]) was estimated using the Kaplan-Meier method, censoring patients who were still on treatment.

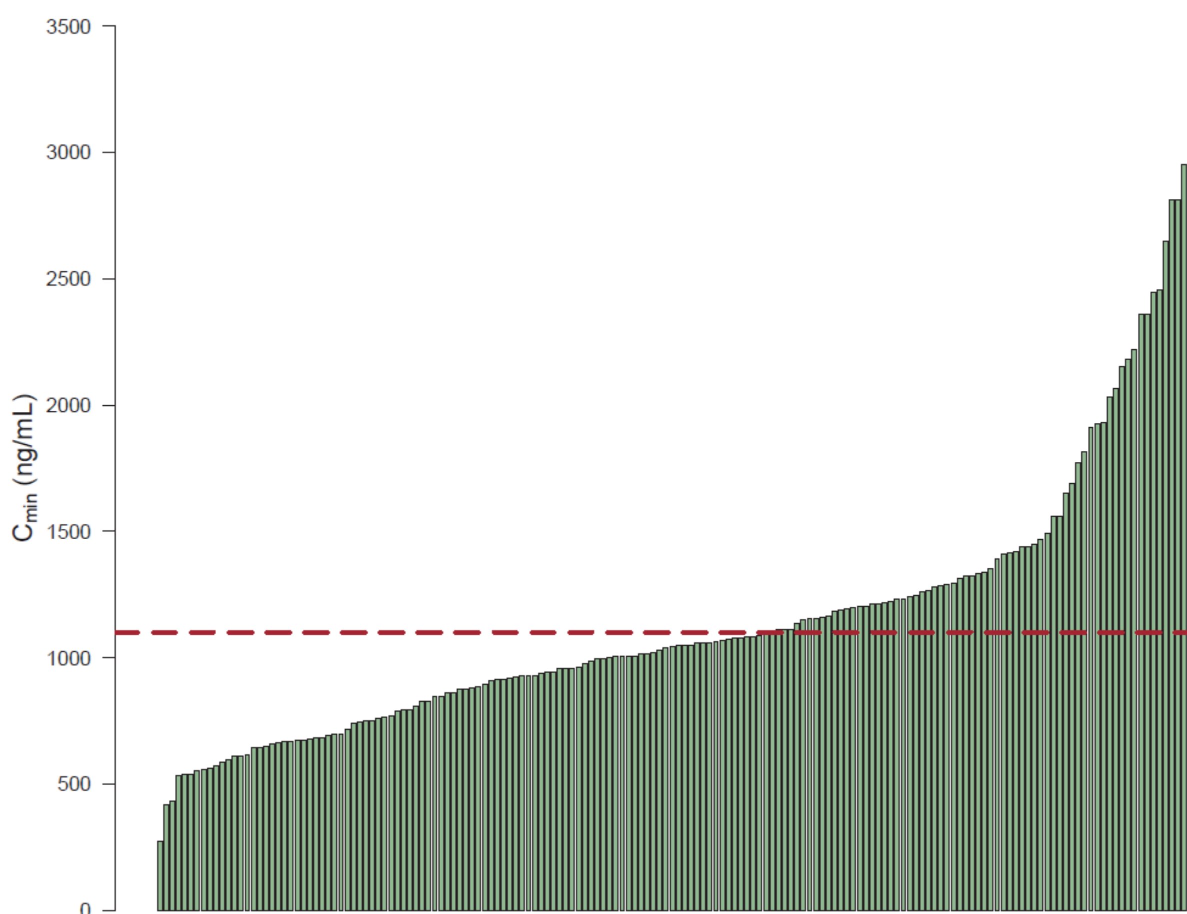


Figure 1 – Median imatinib C_{min} per patient at the standard dose
Each bar represents one patient. The dashed line indicates the efficacy threshold of 1100 ng/mL. Only PK samples at the standard dose of 400 mg QD were included. Ninety-eight patients (58%) had a median C_{min} below this threshold. Inter- and intra-individual variability were 49% and 26%, respectively.
C_{min} = minimum plasma concentration; PK = pharmacokinetic; QD = once daily

TDM recommendations

In total, 126 patients (75%) had a low imatinib exposure according to the used definition (group 1: ≥ 2 C_{min} < 1100 ng/mL or a dose increase based on a single C_{min} below this threshold), while in the other 43 patients (25%), C_{min} was ≥ 1100 ng/mL all the time (group 2). **Figure 2** demonstrates the distribution of patients among the different groups.

In 78 of the patients with a low PK exposure (66%), a PK-guided dose increase was performed (group 1A) after a median of two low PK samples (IQR: 1-3). The daily dose of imatinib was escalated to 600 mg in 43 patients, while in 35 patients, the dose was further increased to 800 mg (either administered as 800 mg QD or 400 mg twice daily). This PK-guided dose increase was successful (i.e. target attainment without additional toxicities) in 49 of these patients (63%). The C_{min} increased by 55% (IQR: 35-76%) and 86% (IQR: 65-127%) with dose increases to 600 mg and 800 mg, respectively. Reasons why this was not successful in other patients were emerging toxicity (n=13), PK (i.e. C_{min} remained below target, n=7) or both (n=2). In addition, in seven patients, the effect has not been evaluated (yet). The median imatinib C_{min} increased from 864 ng/mL (IQR: 698-1089 ng/mL) before to 1198 ng/mL (IQR: 1081-1428 ng/mL) after the PK-guided dose increase ($p<0.001$). **Figure 3** visualizes imatinib exposure in the different subgroups in box plots.

In 23 patients with a low exposure (18%), it was not feasible to perform a PK-guided dose increase, because they already experienced toxicity at the standard dose of 400 mg QD (group 1B).

The remaining 25 patients with a low exposure (20%) experienced no toxicities and a PK-guided dose increase could thus theoretically have been implemented in these patients (group 1C). Reasons why PK-guided dose increases were not performed were physician adherence (n=9; either due to borderline low C_{min} (n=2) or unknown reasons (n=7)), treatment discontinuation (n=7), inclusion in a study where imatinib dose escalation was not allowed (n=3, control arm (i.e. imatinib only) of a clinical trial investigating alternating treatment with imatinib and regorafenib) or the C_{min} was reported as adequate by the hospital pharmacist (n=6).

In group 2, 26 patients (60%) did not experience toxicities and continued treatment at the standard dose (group 2A), while 17 patients (40%) did experience clinically relevant toxicities (group 2B). These toxicities resulted in imatinib dose reduction (n=8), treatment interruption (n=5) and treatment discontinuation (n=4). The median C_{min} was comparable in group 2A and 2B.

Table 2 – Pharmacokinetic characteristics of imatinib trough levels

	All patients	Group 1A	Group 1B+1C	Group 2A+2B
No. of patients	169	78	48	43
C_{min} per patient (ng/mL)	1074 [946-1247]	1051 [902-1191]	1050 [950-1158]	1418 [1237-2813]
C_{min} per patient before PK-guided dose increase (ng/mL)	NA	864 [698-1089][a]	NA	NA
C_{min} per patient after PK-guided dose increase (ng/mL)	NA	1198 [1081-1428][a]	NA	NA
Number of samples per patient	7 [4-12]	10 [6-13]	8 [5-13]	4 [2-7]

Data are expressed as no. (%) or median [interquartile range], as appropriate.
Group 1A: low exposure, PK-guided dose increase, group 1B+1C: low exposure, no PK-guided dose increase, group 2A+2B: adequate exposure all the time.
[a] C_{min} after PK-guided dose increase was significantly higher than C_{min} before PK-guided dose increase ($p<0.001$, Wilcoxon signed rank test). Patients in whom the effect of the PK-guided dose increase was not evaluated (yet) were excluded for this analysis.
C_{min} = minimum plasma concentration; NA = not applicable; PK = pharmacokinetic

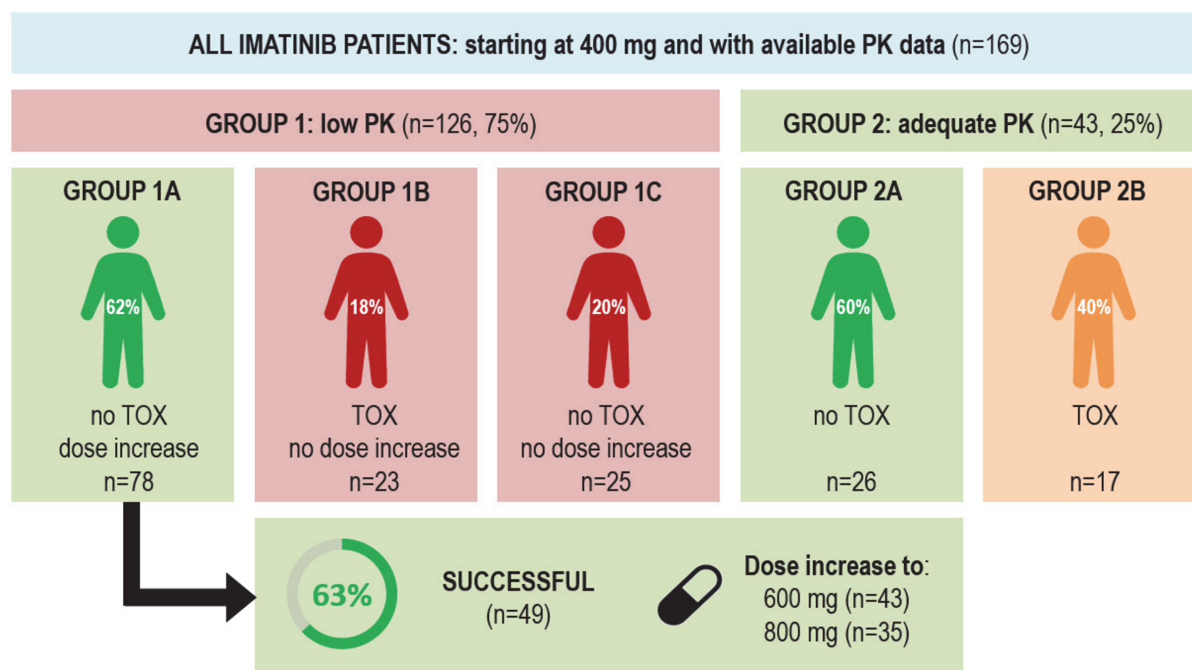


Figure 2 – Schematic overview of results
Patients were classified in group 1 if they had ≥ 2 C_{min} below the efficacy threshold of 1100 ng/mL or if they received a PK-guided dose increase based on a single C_{min} below this threshold.
Patients in group 1 were further divided based on the experienced toxicity and whether or not they received a dose increase (group 1A: no toxicity, dose escalation; group 1B: toxicity, no dose increase; group 1C: no toxicity, no dose increase).
Dose increases were considered successful if the median C_{min} at the increased dose was ≥ 1100 ng/mL and if the patient tolerated this dose for at least six months.
Patients were classified in group 2 (adequate PK) if they had all C_{min} ≥ 1100 ng/mL.
Patients in group 2 were further divided based on the experienced toxicity (group 2A: no toxicity; group 2B: toxicity).
TOX = toxicity; C_{min} = minimum plasma concentration; PK = pharmacokinetic

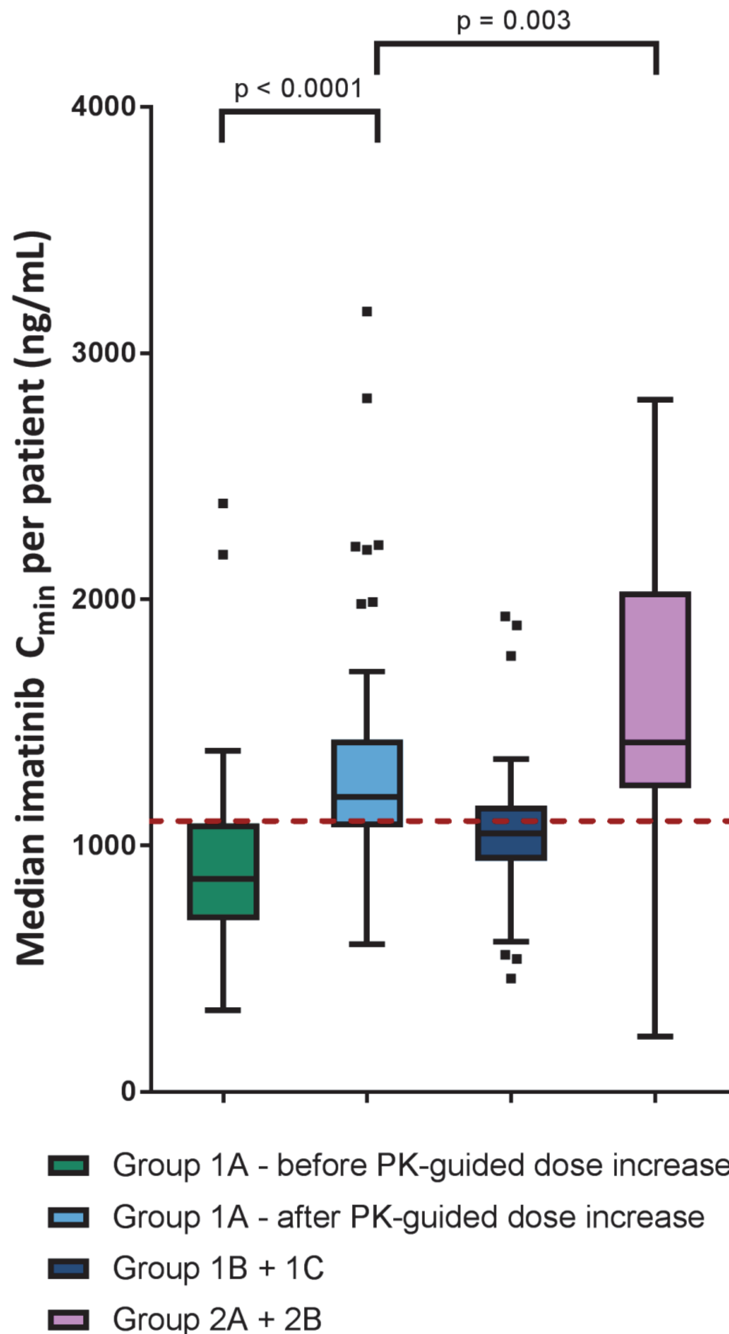
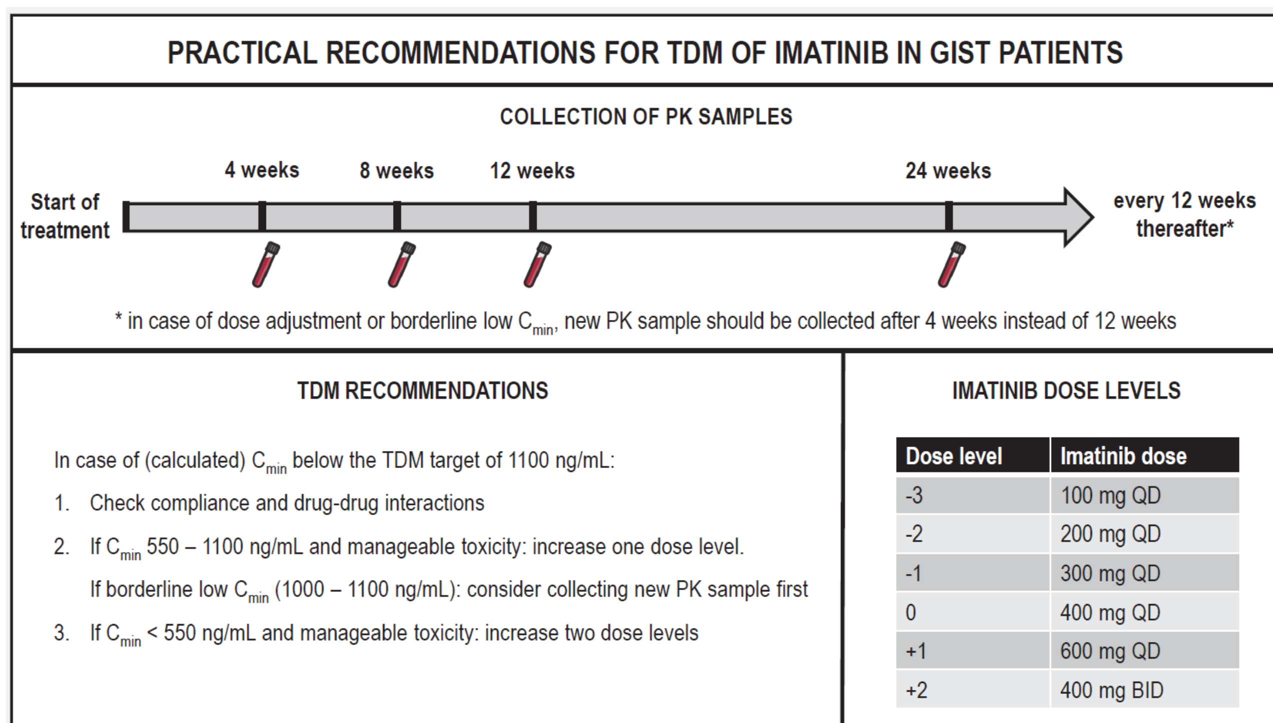


Figure 3 – Boxplots of imatinib C_{min} in patients with adequate and low exposure, before and after dose increase

Group 1A: low exposure, PK-guided dose increase, group 1B: low exposure, no PK-guided dose increase due to toxicity, group 1C: low exposure, no toxicity, no PK-guided dose increase for other reasons.

Group 2: adequate exposure with (2B) or without (2A) toxicity.

C_{min} = minimum plasma concentration; PK = pharmacokinetic



Dose level	Imatinib dose
-3	100 mg QD
-2	200 mg QD
-1	300 mg QD
0	400 mg QD
+1	600 mg QD
+2	400 mg BID

Figure 4 – Practical recommendations for TDM of imatinib in patients with GIST
BID = twice daily; C_{min} = minimum plasma concentration; GIST = gastrointestinal stromal tumour; PK = pharmacokinetic; QD = once daily; TDM = therapeutic drug monitoring

Treatment outcome

The median follow-up time was 48 months (95% confidence interval (CI) 40.5-55.5). Sixty-nine *KIT* exon 11 mutated patients received imatinib with palliative intent, of which 32 (46%) had progressive disease. The median progression-free survival (mPFS) in these patients was 31 months (95% CI: 24.6-37.4) and did not differ between the groups (log-rank test: p=0.149, **Supplementary Figure 1**). The mPFS was not reached for group 1A, was 30 months for group 1B+1C (95% CI: 27.8-32.2) and 30 months for group 2A+2B (95% CI: 4.8-55.3). Multivariable Cox regression yielded similar results (**Supplementary Table 1**).

DISCUSSION

In this manuscript, we described the largest cohort of patients with GIST in whom PK-guided dose increases of imatinib were performed in daily clinical practice. In total, 75% of patients had a low exposure. In 62% of these patients, a PK-guided dose increase was performed, which was successful in 63% of them. Patients with initial low PK who received PK-guided dose increases, levelled the outcome of patients with all adequate PK. Hence, TDM of imatinib is feasible in clinical practice and leads to an increased proportion of patients with adequate exposure.

It is remarkable that in our cohort, patients with a low imatinib exposure appeared to be more often men. Pharmacologically this might be explained by a lower absorption or a higher clearance of imatinib in men than in women. In a population PK model, imatinib clearance was indeed found to be higher in men[23], while other studies did not report an influence of gender on imatinib PK.[6,9] In addition, PK-guided dose increases tended to be more often feasible in younger patients.

No differences in PFS were found between the three subgroups. Moreover, looking at the Kaplan-Meier curve (**Supplementary Figure 1**), it seems that group 2A+2B (adequate PK) has a similar PFS to 1B+1C (low PK, no intervention), while one would expect a longer PFS for group 2A+2B due to the known exposure-response relationship.[2] However, these results are difficult to interpret for several reasons. First, subgroups were small and the number of events was still limited. Second, PFS in group 1B+1C (low PK, no intervention) was longer than expected from the literature (i.e. 30 months vs. 11.3 months[2]), possibly explained by the fact that exposure was only slightly below the target in this group (1050 ng/mL). Finally, patients who are longer on imatinib treatment have a higher chance of being classified as group 1, as the chance of detecting a low C_{min} increases with an increasing number of PK samples per patient.

Theoretically, PK-guided dose increases could have been performed for patients in group 1C as well, as these patients had low PK without reported toxicity. In part of these patients, toxicity might still have played a role in the decision not to increase the dose but may not have been documented in the patient files. In addition, for some patients, C_{min} was initially reported to be adequate, since at that time the described algorithm to estimate C_{min} was not used yet and a slightly lower threshold was used for reporting of the results ($C_{min} \geq$ 1000 ng/mL, based on the target for CML[4,5]).

In a previous randomized controlled trial on TDM of imatinib between 2009-2011 by Gotta *et al.*, dose recommendations were not followed up upon in almost half of the patients.[24] In contrast, in our study, the lack of physician adherence was only encountered in nine patients (8%). The higher physician adherence in our study compared with that of Gotta *et al.* may be explained by the passage of time and TDM becoming more accepted as routine clinical practice meanwhile.

The percentage of patients in whom a PK-guided dose increase is considered successful does depend on the applied definition of successful. In our opinion, a definition including tolerability and median exposure over time after the dose increase reflects the concept of successful. With this definition, the relatively high intra-individual variability of imatinib is taken into account. In addition, if treatment at a higher imatinib dose is only feasible for a short period of time due to toxicity, this should not be considered successful.

The majority of patients (75%) had a low exposure according to the applied definition. This could raise the question whether the threshold of 1100 ng/mL is a valid target. Because

imatinib has been demonstrated to be an effective treatment for GIST, it would be unlikely that the majority of patients are underdosed. In another exposure-response analysis, C_{min} ≥ 760 ng/mL was identified as the optimal threshold.[7] It has to be noted though, that this cohort seems not to be representative of the general GIST population, as PK samples were collected randomly instead of consecutively for all patients, and PFS was unexpectedly long compared with that of the literature. As the cohort of Demetri *et al.* is a better reflection of clinical practice, we decided to use their proposed threshold of C_{min} ≥ 1100 ng/mL in our routine TDM.

Strengths of the current study include the accurate reflection of daily clinical practice and the measurement of multiple PK samples over time. In addition, to the best of our knowledge, this is the largest cohort of patients with GIST in whom PK-guided dose increases were performed in daily clinical practice. From our current cohort, 38% of patients were also described in a previously published article on imatinib PK in patients with GIST.[25] In that article, no PK-guided dose increases were described, as we do in the present article. Therefore, new to the present article is the focus on feasibility of TDM in daily clinical practice. Limitations could be that the reported C_{min} was not accompanied with a specific dose recommendation, which could have impaired the physician adherence. In addition, the methods used to estimate C_{min} are accompanied by some inherent limitations, for example, the formula does not correct for inter-individual variability in imatinib clearance. In addition, although data collection in the GIST registry is prospective, the current research questions were answered retrospectively, with the inherent limitations. Furthermore, the number of PK samples and treatment duration differ among patients, which could have influenced the results, and data on patient compliance are lacking. Finally, due to the small number of patients with metastatic disease, no definite conclusions on the effect on PFS could yet be drawn.

To conclude, these results emphasise the feasibility of TDM of imatinib in clinical practice. Although this has been routine clinical practice for patients with GIST in our hospital for several years now, this has not yet been applied worldwide. Efforts should be made to implement TDM as part of routine care for every patient. Real-life data of these patients should continuously be collected to further investigate the effects on treatment efficacy.

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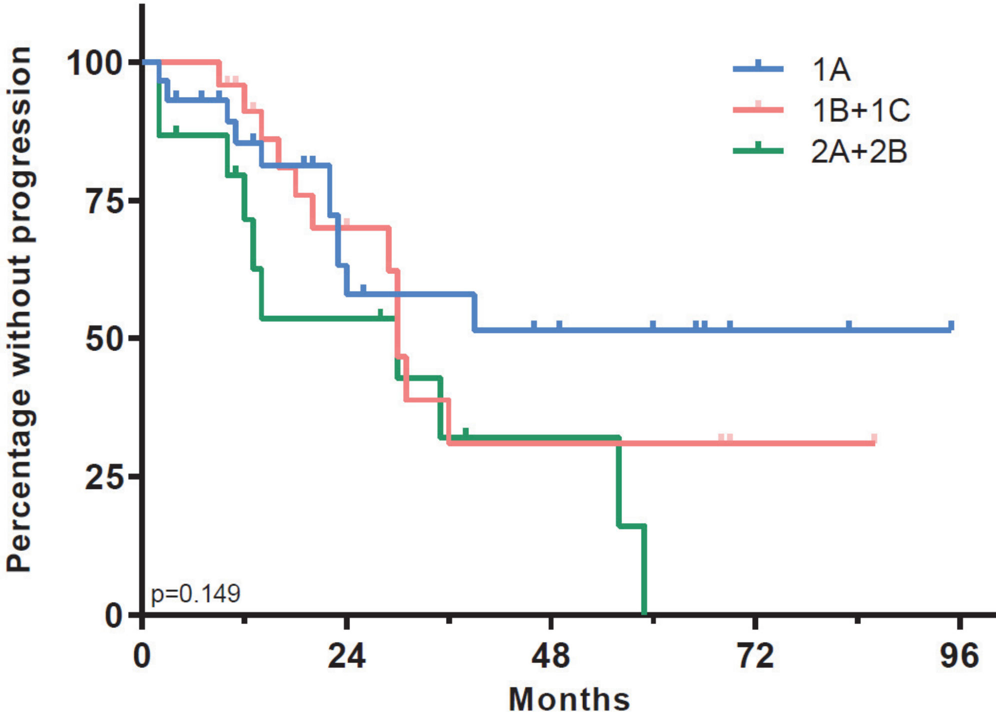
SUPPLEMENTARY DATA



No. at risk	O	N				
1A:	11	30	11	7	2	0
1B+1C:	11	24	9	3	1	0
2A+2B:	10	15	6	2	0	0

Supplementary Figure 1 – Progression-free survival of *KIT* exon 11 mutated patients in palliative treatment setting
Group 1A: low exposure, PK-guided dose increase, group 1B+1C: low exposure, no PK-guided dose increase, group 2A+2B: adequate exposure.
O = number of observed events; N = number of patients at risk

Supplementary Table 1 – Cox regression analysis for progression-free survival in *KIT* exon 11 mutated patients treated in the palliative setting

	Univariable analysis			Multivariable analysis		
	HR	**95% CI**	**p–value**	**HR**	**95% CI**	**p–value**
Subgroup						
1A	0.439	0.186-1.040	0.061	0.389	0.088-1.719	0.213
1B+1C	0.590	0.250-1.393	0.228	0.517	0.143-1.868	0.314
2A+2B	Reference					
Primary tumor size	1.003	0.995-1.010	0.485	1.008	0.999-1.017	0.086
Primary GIST location						
Gastric	Reference					
Small bowel	1.329	0.629-2.808	0.456	1.689	0.573-4.978	0.342
Other	0.837	0.272-2.574	0.757	0.968	0.229-4.095	0.965
Baseline mitotic rate						
Low	Reference					
High	1.688	0.699-4.077	0.244	1.762	0.576-5.386	0.321
Age at diagnosis	1.040	1.009-1.071	0.010	1.027	0.982-1.074	0.240
Gender						
Male	Reference					
Female	1.095	0.505-2.372	0.818	0.880	0.259-2.992	0.838
Performance status						
WHO 0	Reference					
WHO ≥ 1	0.749	0.337-1.666	0.479	0.799	0.242-2.636	0.713

All selected univariable confounders were included in the multivariable model.
Group 1A: low exposure, PK-guided dose increase, Group 1B+1C: low exposure, no PK-guided dose increase, Group 2A+2B: adequate exposure all the time.

Harnessing soft tissue sarcoma with low-dose pazopanib – a matter of blood levels

BMC Cancer 2020; **18**: 1200

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// ABSTRACT

Background

Pazopanib is a tyrosine kinase inhibitor indicated for the treatment of renal cell carcinoma and soft tissue sarcoma. Despite the high inter-patient variability in pharmacokinetic exposure, pazopanib is administered at a fixed dose of 800 mg once daily (QD). Pharmacokinetic exposure is linked to both efficacy and toxicity. In this case report, we illustrate the value of therapeutic drug monitoring by describing two patients with adequate pazopanib trough concentrations (C_{min}) at an eight times lower than standard dose.

Case presentation

Patient A is a 69-year-old woman with metastatic leiomyosarcoma who had significant toxicities and a high C_{min} on the standard dose. While dose reductions to 200 mg QD and later 200 mg every other day were made, pazopanib C_{min} remained above the efficacy threshold. Patient B is a 50-year-old male with metastatic angiosarcoma and a history of Gilbert syndrome. Pazopanib treatment was initiated at the standard dose of 800 mg QD, but was reduced to 200 mg QD 1-week-on – 1-week-off due to total bilirubin elevation. Pazopanib C_{min} was adequate in this patient as well.

Conclusion

It could be valuable to measure pazopanib levels in case of dose reductions due to toxicity, as exposure could still be adequate at considerably lower than standard doses.

BACKGROUND

Pazopanib is a tyrosine kinase inhibitor mainly targeting the vascular endothelial growth factor receptor and is indicated for the treatment of advanced renal cell carcinoma and soft tissue sarcoma.[1] Despite the high inter-patient variability in exposure (40-70%), pazopanib is administered at a fixed oral dose of 800 mg once daily (QD).[1,2] Suttle *et al.* reported a clear exposure-response relationship, with patients with a pazopanib trough concentration (C_{min}) above 20.5 mg/L having a significantly longer progression-free survival (PFS). Also, an exposure-toxicity relationship has been demonstrated, with an increasing incidence of toxicities such as hypertension, diarrhea, elevated alanine aminotransferase levels, stomatitis and hand-foot syndrome with increasing pazopanib plasma concentrations.[3] It has been shown that patients are unlikely to tolerate $C_{min} \geq 50$ mg/L for a prolonged period of time.[4] In this case report, we illustrate the value of therapeutic drug monitoring (TDM) for pazopanib by describing two patients with pazopanib C_{min} above the efficacy threshold of 20.5 mg/L at an eight times lower than standard dose.

CASE PRESENTATION

Case A

We present a 69-year-old woman with a history of metastatic leiomyosarcoma, for which pazopanib treatment was initiated at the standard dose of 800 mg QD, after she progressed upon first-line chemotherapy with doxorubicin. During the first month of treatment pazopanib was temporarily withheld twice due to significant toxicities, including fatigue, nausea, vomiting and syncope. Pazopanib plasma concentrations were measured and C_{min} was calculated using the formula proposed by Wang *et al.*[5], showing high pazopanib trough levels (36.1 mg/L and 41 mg/L). Pazopanib treatment was resumed after sequential dose reductions to 600 mg QD and 200 mg QD. The last dose was well tolerated despite mild liver enzyme disorders and hypertension. During the following months, the patient developed diarrhea and hypothyroidism, after which pazopanib was further reduced to 200 mg every other day. Pazopanib C_{min} remained adequate at this eight times lower than standard dose at first, although the last two measurements were below the efficacy threshold (**Figure 1A**). Unfortunately, 14 months after start of treatment, progressive disease was observed, after which chemotherapy with trabectedin was started.

Case B

The second case is a 50-year-old male with metastatic angiosarcoma and a history of Gilbert syndrome, previously treated with 6 cycles of doxorubicin in combination with ifosfamide. Pazopanib treatment was started at the standard dose of 800 mg QD. Shortly

hereafter, total bilirubin increased to twice the upper limit of normal with only minimal elevation of direct bilirubin, after which pazopanib was halted. Upon normalization of bilirubin, pazopanib treatment was resumed at a reduced dose of 400 mg QD and later 200 mg QD 1-week-on – 1-week-off. At the end of the on-treatment week pazopanib C_{min} was 29.9 mg/L (**Figure 1B**). The patient is still on treatment now, nine months after pazopanib initiation, with a partial remission.

DISCUSSION AND CONCLUSION

It is remarkable that even these unusually low doses of pazopanib lead to an adequate exposure, defined by $C_{min} \geq 20.5$ mg/L.[3] In the absence of TDM, when doses would be reduced according to the Summary of Product Characteristics (SPC), treatment would probably have been discontinued in these patients, as the SPC considers the exposure at 200 mg QD as markedly reduced and insufficient to obtain a clinically relevant effect.[1] However, we observed an adequate C_{min} at considerably lower than standard doses and treatment could have been continued for 14 and 9 months (ongoing response), respectively. This is a relatively long duration of response taking into account the median PFS of 4.6 months for sarcoma patients found in the phase 3 trial.[6] Although the efficacy threshold of $C_{min} \geq 20.5$ mg/L has been established in patients with renal cell carcinoma[3], a similar trend has been demonstrated in sarcoma patients.[7]

Gilbert's syndrome, uridine diphosphoglucuronate glucuronosyltransferase 1A1 (UGT1A1) polymorphism, has been associated with pazopanib-induced hyperbilirubinemia, which was probably the case in the second patient.[8] Given the benign etiology of this condition, continuing pazopanib treatment was possible, although in reduced dose.

With regard to obtaining the most adequate exposure, the dosing regimen of 200 mg every other day is most rational, as with the 200 mg 1-week-on – 1-week-off regimen patients will probably not have an adequate exposure in the off-treatment week. Unfortunately, we do not have any trough concentrations in the off-treatment week of patient B available.

As we illustrated with these two cases, it could be valuable to measure pazopanib levels in case of dose reductions due to toxicity, as C_{min} could still be above the efficacy threshold at considerably lower than standard doses.

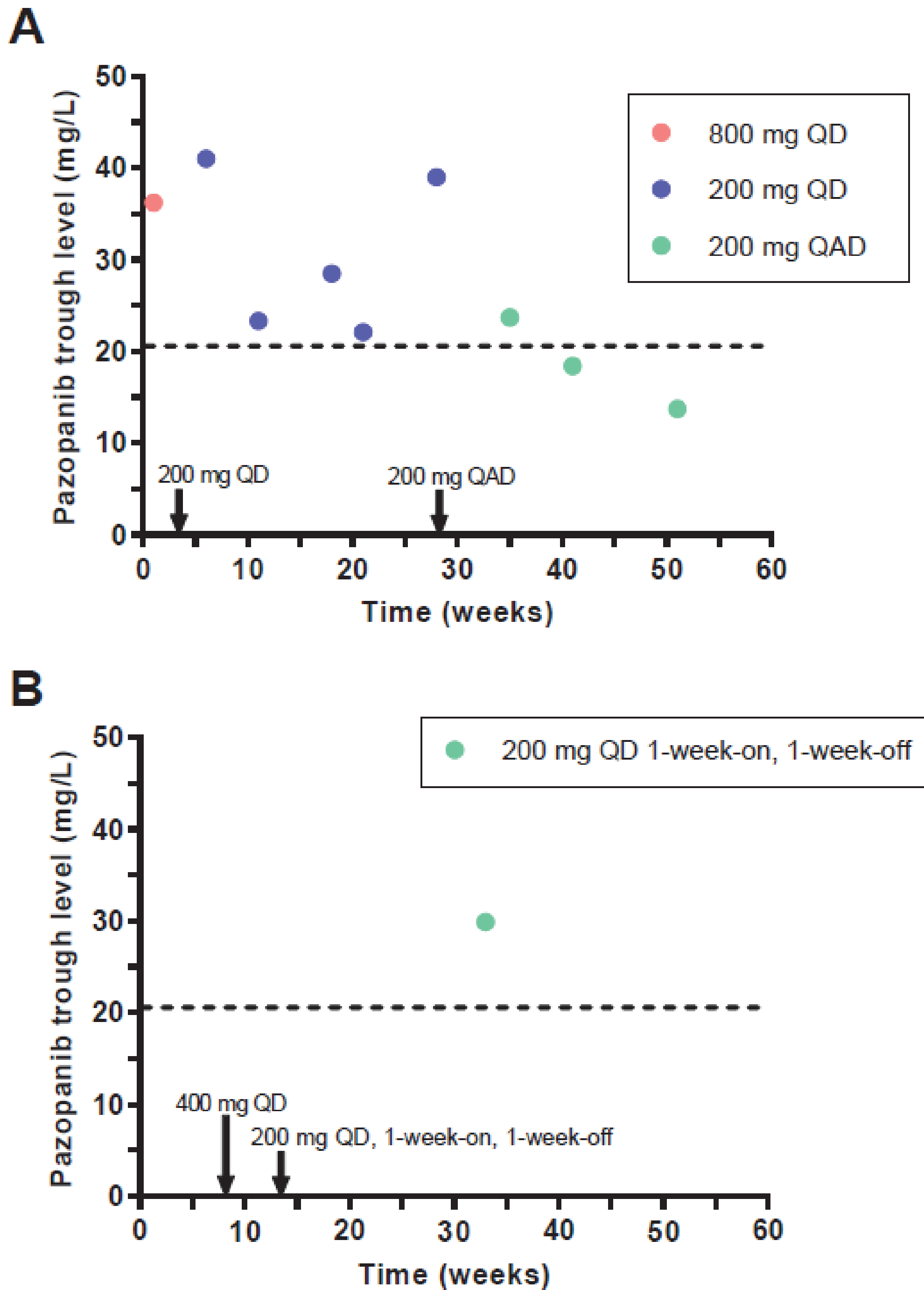

Figure 1 – Calculated pazopanib trough levels
A: For patient A, pazopanib dose was rapidly reduced to 200 mg QD in the first three weeks and further reduced to 200 mg every other day in week 28. Corresponding doses of pazopanib were: week 0-1 800 mg QD, week 1-2 stop, week 2-2.5 600 mg QD, week 2.5-3 stop, week 3-11.5 200 mg QD, week 11.5-13 stop, week 13-28 200 mg QD, week 28-59 200 mg every other day.
B: For patient B, pazopanib dose was reduced to 400 mg QD after two months and to 200 mg QD 1-week-on, 1-week-off after three months. Corresponding doses of pazopanib were: week 0-6 800 mg QD, week 6-8 stop, week 8-12 400 mg QD, week 12-13 stop, week 13-39 200 mg QD 1-week-on, 1-week-off.
The dashed line indicates the pharmacokinetic target of $C_{min} \geq 20.5$ mg/L. The arrows indicate the time at which doses were changed.
C_{min} = minimum blood concentration; QAD = every other day; QD = once daily

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Therapeutic drug monitoring of oral anti-hormonal drugs in oncology

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ABSTRACT

Oral anti-hormonal drugs are essential in the treatment of breast and prostate cancer. It is well known that the interpatient variability in pharmacokinetic exposure is high for these agents and exposure-response relationships exist for many oral anti-hormonal drugs. Yet, they are still administered at fixed doses. This could lead to under dosing and thus suboptimal efficacy in some patients, while other patients could be overdosed resulting in unnecessary side effects. Therapeutic drug monitoring (TDM), individualized dosing based on measured blood concentrations of the drug, could therefore be a valid option to further optimize treatment. In this review, we provide an overview of relevant clinical pharmacokinetic and pharmacodynamic characteristics of oral anti-hormonal drugs in oncology and translate these into practical guidelines for TDM.

For some agents, TDM targets are not well established yet and as a reference the median pharmacokinetic exposure could be targeted (exemestane: minimum plasma concentration (C_{min}) 4.1 ng/mL and enzalutamide: C_{min} 11.4 mg/L). However, for most drugs, exposure-efficacy analyses could be translated into specific targets (abiraterone: C_{min} 8.4 ng/mL, anastrozole: C_{min} 34.2 ng/mL, and letrozole: C_{min} 85.6 ng/mL). Moreover, prospective clinical trials have shown TDM to be feasible for tamoxifen, for which the exposure-efficacy threshold of its active metabolite endoxifen is 5.97 ng/mL. Based on the available data, we therefore conclude that individualized dosing based on drug concentrations is feasible and promising for oral anti-hormonal drugs and should be developed further and implemented into clinical practice.

INTRODUCTION

Breast and prostate cancer are both highly prevalent malignancies, with breast cancer being the most commonly diagnosed malignancy in women and prostate cancer in men in the Western world. Breast and prostate cancer represent the second leading cause of cancer deaths in women and men, respectively.[1] As these tumors are often dependent on estrogens and androgens for their growth, anti-hormonal drugs are imperative in their treatment.

Even though many oral anti-hormonal drugs show exposure-response relationships and the interpatient variability in pharmacokinetic (PK) exposure is high (up to 141% for abiraterone)[2], they are still administered at fixed doses. As a result, some patients may be underdosed, which could lead to suboptimal efficacy, while other patients might be overdosed, causing unnecessary toxicity. Treatment could be optimized by therapeutic drug monitoring (TDM), which is individualized dosing based on measured blood concentrations of the drug.[3–8]

Use of TDM in oncology has been previously advocated, and for other targeted therapies, such as kinase inhibitors, TDM targets have been described previously.[3,4,6,7,9] The aim of this review is to summarize the available PK and pharmacodynamic (PD) data on oral anti-hormonal drugs, to discuss exposure-toxicity and exposure-efficacy relationships and to propose PK targets, which can be used for TDM.

Table 1 provides a summary of selected (steady state) PK parameters of these drugs. The proposed targets and TDM recommendations have been summarized in **Table 2**.

METHODS

Although this is not a systematic review, the literature was searched as comprehensively as possible. For all oral anti-hormonal drugs, the US FDA Clinical Pharmacology & Biopharmaceutics Review and the Committee for Medicinal Products for Human Use European Public Assessment Report were consulted. Furthermore, PubMed searches were performed using the term “pharmacokinetics” in combination with the different oral anti-hormonal drugs. In addition, citation snowballing was used to find other relevant studies.

Table 1 – Overview of oral anti-hormonal drugs in oncology with selected (steady-state) pharmacokinetic parameters

Drug	Indication	(Primary) target	Approved dose (mg QD)	t_{max} (h)	C_{min} (ng/mL ± SD or [range])	Inter-patient variability (CV%)	Intra-patient variability (CV%)	$t_{1/2}$ (h)	Time to steady-state plasma concentration (days)	References
Anastrozole	BC	Aromatase	1	2	33.2 [0.0-132.1]	41-44[e]	7-12[f]	50	7	12–18
Exemestane	BC	Aromatase	25	2	4.1 [1.3-38.1][b]	33-39[f]	40[f]	24	7	19–21
Letrozole	BC	Aromatase	2.5	1-2	88.4 [0.0-349.2]	43-55[e]	10-21[f]	48	60	18,22–24
Tamoxifen[a]	BC	ER	20	2	9.72 [1.73-30.8][c]	40-49[g]	11[g]	336	70	25–28
Abiraterone	PC	CYP17	1000	2	10.9[d] ± 4.8	46[e]	33[e]	12	3	10,29
Enzalutamide	PC	AR	160	1	11,400 ± 2,950	26[e]	not reported	139	29	30

[a] Pharmacokinetic data shown for the active metabolite endoxifen

[b] Calculated C_{min} based on median C_{max} (7.7 ng/mL, range 2.5-72.0) and $t_{1/2}$, calculated with the formula proposed by Wang *et al.*[31]

[c] Steady-state concentration

[d] Weighted average

[e] Variability in C_{min}

[f] Variability in AUC

[g] Variability in steady-state concentration

AR = androgen receptor; AUC = area under the plasma concentration-time curve; BC = breast cancer; C_{min} = minimum plasma concentration; C_{max} = maximum plasma concentration; CYP17 = 17α-hydroxylase; CV = coefficient of variation, ER = estrogen receptor; PC = prostate cancer; QD = once daily; SD = standard deviation; t_{max} = time to C_{max}; $t_{1/2}$ = elimination half-life

Table 2 – Overview of practical therapeutic drug monitoring (TDM) recommendations for oral anti-hormonal drugs in oncology

Drug	TDM recommendation[a]	Proposed target (ng/mL)	Mean/ median exposure (C_{min} in ng/mL)	Outcome parameter associated with TDM target	References
Anastrozole	Exploratory	$C_{min} \geq 34.2$	33.2	Estradiol suppression	[12]
Exemestane	Exploratory		4.1[b]		[20]
Letrozole	Promising	$C_{min} \geq 85.6$	88.4	Increased time to tumor progression	[22]
Tamoxifen	Viable	$C_{min} \geq 5.97$	9.72	Lower recurrence rate	[25,34]
Abiraterone	Promising	$C_{min} \geq 8.4$	10.9	PSA reduction, progression-free survival	[29,32]
Enzalutamide	Exploratory	$C_{min} \geq 5000$	11,400	16β-^{18}F-5α-dihydrotestosterone imaging	[36]

[a] The provided TDM recommendation is considered promising if a pharmacokinetic TDM target is available, or viable if a prospective TDM study has been conducted. Otherwise, the recommendations are considered exploratory.
[b] Calculated C_{min} based on median C_{max} and $t_{1/2}$, calculated with the formula proposed by Wang *et al.*[31]
C_{min} = minimum plasma concentration; C_{max} = maximum plasma concentration; PSA = prostate-specific antigen; $t_{1/2}$ elimination half-life

PRACTICAL RECOMMENDATIONS FOR THERAPEUTIC DRUG MONITORING OF ORAL ANTI-HORMONAL DRUGS IN ONCOLOGY

Anti-androgens

Abiraterone

Abiraterone acetate (Zytiga®) is the prodrug of abiraterone, which is a steroidal irreversible inhibitor of 17α-hydroxylase (cytochrome P450 (CYP) 17), thereby blocking the androgen synthesis. Abiraterone acetate is currently indicated for metastatic castration-resistant prostate cancer.[10] In the near future, this indication might be expanded to patients with locally advanced or metastatic prostate cancer who are naive to anti-hormonal treatment.[11]

According to the Summary of Product Characteristics, abiraterone acetate should be ingested in modified fasting state, which means no food 2 h before or 1 h after drug

intake. Chi *et al.* studied the food effect of the pharmacokinetics of abiraterone acetate and found a seven- and five-fold increase in maximum plasma concentration (C_{max}) and area under plasma concentration-time curve (AUC), respectively, with a low-fat meal and a 17- and 10-fold increase in C_{max} and AUC, respectively, with a high-fat meal, compared to overnight fasting in healthy subjects. However, in patients with metastatic castration-resistant prostate cancer, the food effect was compared to a modified fasting state, showing a less pronounced effect (similar exposure with low-fat meals and a twofold increase with high-fat meals). Adverse events (all grade ≤ 3) were similar in the different groups (mainly hot flashes, fatigue, and hypokalemia).[32]

Abiraterone acetate has a high interpatient variability of 41-141% for AUC from time zero to infinity and 46% for C_{min}, with an intrapatient variability of 33%.[2,29] In the population-PK model described by Stuyckens *et al.* food and hepatic impairment appeared to be relevant covariates that influence abiraterone exposure, while 70% of the interpatient variability remained unexplained.[33]

Unfortunately, insufficient PK data have been collected in the pivotal trial and phase II trials to evaluate the exposure-toxicity relationships.[10] Abiraterone acetate was generally well tolerated, and no dose-limiting toxicities were reported for doses up to 2000 mg once daily (QD).[37]

A model has been developed in which a relationship between pharmacokinetics and prostate-specific antigen (PSA) reduction has been established.[2,38] Additionally, in a prospective observational study in patients with castration-resistant prostate cancer (n=61), higher abiraterone trough concentrations (C_{min}) were found in PSA responders compared to non-responders (12.0 vs. 8.0 ng/mL, p=0.0015), in which PSA response was defined as a PSA decline of at least 50% after 3 months of treatment.[29] The most predictive C_{min} cut-off for PSA response was 8.4 ng/mL according to a receiver operating characteristic curve. Using this threshold, exposure-survival analysis found a progression-free survival, defined as the time from treatment initiation to the first progression event (either PSA or radiologic progression), of 7.4 months in patients with a C_{min} below 8.4 ng/mL and 12.2 months in patients with a C_{min} above 8.4 ng/mL (p=0.044). Nineteen of the 55 patients (35%) in this study had a C_{min} below 8.4 ng/mL.[29]

Abiraterone is converted into the active metabolite Δ^4-abiraterone (D4A) by the enzyme 3β-hydroxysteroid-dehydrogenase. Although the conversion ratio of abiraterone to D4A is low (± 5%), it targets multiple steps of the androgen receptor signaling pathway, some of them more potently than abiraterone. An exposure-efficacy relationship has not been established for D4A hitherto, but given the dual mechanism of action of D4A (both inhibition of CYP17 and blockage of the androgen receptor), it is to be expected that such a relationship exists and may be identified. Therefore, measuring this metabolite could be interesting to refine TDM-guided dosing in the future.[39,40]

Given the clear exposure-efficacy relationship, a target C_{min} of ≥ 8.4 ng/mL can be recommended for abiraterone. At the currently used fixed dose of 1000 mg QD, 35% of patients do not reach this threshold, with the potential to increase progression-free survival by 4.8 months for this subpopulation. Although it is advised to administer abiraterone in a modified fasting state, in clinical practice, patients often take it after an overnight fast. Since a clinically significant food effect has been shown when compared to overnight fasting, a first step in case of low exposure could be to administer abiraterone concomitantly with a light meal or a snack, before escalating the dose.

Enzalutamide

Enzalutamide (Xtandi®) is an androgen-receptor antagonist indicated for the treatment of metastatic castration-resistant prostate cancer.[30] Enzalutamide is metabolized by CYP2C8 and CYP3A4/5 to an inactive carboxylic acid (M1) and an active N-desmethyl metabolite (M2). The mean ± standard deviation C_{min} of the approved 160-mg QD dose was 11.4 ± 3.0 mg/L for enzalutamide, 13.0 ± 3.8 mg/L for M2, and 8.4 ± 6.8 mg/L for M1.[41] Since M2 has a high abundancy and similar potency to enzalutamide, and concentrations of these two compounds can differ between patients (±25%), it could be scientifically interesting to measure both enzalutamide and its M2 metabolite.[30] Future studies should clarify the role of this M2 metabolite in TDM.

No clinically significant exposure-toxicity relationships have been found so far.[30]

An exposure-efficacy analysis has been executed for enzalutamide in the pivotal phase III study, using the sum of the C_{min} for enzalutamide and M2 with overall survival as the endpoint. All quartiles performed significantly better than placebo ($p < 0.0001$), yet no differences between the exposure quartiles could be found ($p \geq 0.5499$).[41]

In the phase I/II trial, PSA decreases at 12 weeks were comparable in the different dose levels (range: 60-600 mg).[36] Although enzalutamide targets the androgen receptor and could therefore cause a PSA decline without reflecting tumor response, it has been shown that PSA decline after 12 weeks is still associated with progression-free survival and overall survival.[42]

In the phase I trial, 16β-^{18}F-5α-dihydrotestosterone positron emission tomography imaging in 22 patients suggested androgen receptor binding was higher in the 150-mg (corresponding to a median C_{min} of 11.4 mg/L) than in the 60-mg dose group (corresponding to a median C_{min} of approximately 5 mg/L). No additional effect was seen at higher doses, suggesting a plateau at a dose of 150 mg and C_{min} of 12 mg/L.[36]

Given the lack of an exposure-toxicity relationship, the limited evidence for an exposure-efficacy relationship, and the small interpatient variability in exposure (26% for C_{min}), enzalutamide may not be the ideal drug for TDM. In absence of an exposure-efficacy target, the mean C_{min} of 11.4 mg/L at the standard dose of 160 mg QD could be used as a

reference. As this is the mean exposure, approximately 50% of patients will have concentrations below this reference. Based on the 16β-^{18}F-5α-dihydrotestosterone data, dose increments could be considered in patients with a very low (e.g. < 5 mg/L) C_{min}. Taking into account the mean exposure and standard deviation, less than 2.5% of patients will have trough concentrations < 5 mg/L.

Anti-estrogens

Tamoxifen

Tamoxifen (Nolvadex®) is an estrogen receptor antagonist indicated for the treatment of estrogen receptor-positive breast cancer. Tamoxifen is extensively metabolized mainly by CYP2D6 and CYP3A4 into a range of active and inactive metabolites.[43] Endoxifen is one of the most potent and abundant metabolites and, therefore, TDM of tamoxifen has focused on measuring endoxifen concentrations. Endoxifen shows a large interpatient variability in steady-state concentrations of 40-49% , while the intrapatient variability is only 11%.[25,27,28]

No clear relationship between endoxifen concentration and toxicity has been reported in the literature. A retrospective study (n=109) could not find evidence for an association between exposure and hot flashes, a major side effect of tamoxifen treatment.[44] In another prospective trial (n=122), no significant correlation was found between tamoxifen metabolites and hot flash score (p=0.07).[45]

A retrospective analysis of 1370 patients with estrogen receptor-positive breast cancer receiving tamoxifen in the adjuvant setting, found that patients in the lowest endoxifen exposure quintile (0-5.9 ng/mL) had a higher risk of recurrence than patients above this threshold (hazard ratio 0.74; 95% confidence interval 0.55-1.00). The recurrence rate was 16% for patients in the lowest quintile vs. 10.1-14.7% in the higher exposure quintiles. The investigators also explored dichotomized optimal cut-off points for the association between endoxifen concentrations and additional breast cancer events, in which an endoxifen concentration ≥ 5.97 ng/mL was the best threshold. This threshold corresponds closely to the lowest quintile.[34]

With the same dataset, an anti-estrogenic activity score was developed taking into account the IC_{50}-corrected concentrations of tamoxifen, endoxifen, 4-hydroxytamoxifen, and N-desmethyltamoxifen.[46] An anti-estrogenic activity score threshold of 1798 was associated with a hazard ratio of 0.69 (95% confidence interval 0.48-0.99). It should be noted that this anti-estrogenic activity score was dominated by endoxifen, suggesting that endoxifen can serve as a proxy for the overall anti-estrogenic effect of tamoxifen and its metabolites.

While a clear exposure-efficacy relationship has been demonstrated in the adjuvant setting, Neven *et al.* did not find this relationship in the neo-adjuvant and metastatic setting.[47]

In a recent prospective clinical trial (n=122), tamoxifen doses were tailored based on endoxifen concentrations.[45] Breast cancer patients with an endoxifen concentration < 5.6 ng/mL (corresponding to 15 nmol/L) received a 20-mg dose increase, while patients with endoxifen concentrations between 5.6 and 11.2 ng/mL (or 15-30 nmol/L) were recommended a dose increase of 10 mg. All patients with endoxifen concentrations ≥ 11.2 ng/mL continued treatment at the fixed dose of 20 mg of tamoxifen. In total, 68 of 122 patients had at least one dose increment, after which 96% of patients achieved an endoxifen concentration ≥ 5.6 ng/mL, compared to only 76% at baseline.[45]

Although it is known that CYP2D6 intermediate and poor metabolizer phenotypes are associated with lower endoxifen concentrations, the CYP2D6 phenotype only accounts for 18-43% of the interpatient variability in endoxifen concentrations.[34,47–49] Twenty-four percent of the poor metabolizers and 58% of the intermediate metabolizers still have an endoxifen concentration above the efficacy threshold, while 12% of the normal metabolizers do not reach this threshold[34] As endoxifen concentrations cannot be adequately predicted by the CYP2D6 phenotype, we advocate endoxifen-guided dosing instead of genotype-guided dosing.

At the currently used fixed dose of 20 mg QD, 20% of patients do not reach the proposed efficacy threshold of 5.97 ng/mL, with the potential to lower the recurrence rate by 26% in this subpopulation. The presence of a large retrospective exposure-efficacy study and prospective dose individualization study support the conclusion that it is feasible to dose tamoxifen based on measured endoxifen concentrations, using ≥ 5.97 ng/mL as a threshold, although no unequivocal evidence from a prospective trial is available yet, which demonstrates that TDM increases tamoxifen treatment efficacy.

Aromatase inhibitors

Estrogens are synthesized from androgens by the aromatase enzyme complex. This enzyme system is inhibited by aromatase inhibitors (AIs). After previous use of the first- and second-generation AIs (e.g. aminoglutethimide and formestane), the third-generation AIs currently used in clinical practice are anastrozole, letrozole, and exemestane. These drugs are indicated for the treatment of postmenopausal patients with estrogen receptor-positive breast cancer, either in the (neo)adjuvant or metastatic setting.[13,19,22] Anastrozole and letrozole are non-steroidal AIs that bind reversibly to aromatase while exemestane is a steroidal AI that binds irreversibly to aromatase.[50]

Since AIs inhibit the synthesis of estrogens, measuring circulating estrogen levels would be a good biomarker for efficacy. However, the sensitivity of the currently most commonly used estrogen assays is insufficient to measure the low concentrations of circulating estradiol in postmenopausal women, especially in those receiving AI treatment.[51,52] Patients receiving anastrozole, letrozole, and exemestane treatment have median estradiol concentrations of 1.26, 0.63, and 0.63 pg/mL, respectively.[53,54] In daily clinical

practice, circulating estradiol is measured using immunoassays (optimized to measure concentrations between 40-2000 pg/mL[52]), while mass spectrometry would be a more sensitive method, although this method is more costly and labor intensive. Even mass spectrometry assays are not always sensitive enough to measure the low circulating concentrations of estradiol in patients receiving AI treatment, for which assays with a lower limit of quantification (LLOQ) of 0.1-0.2 pg/mL are needed.[52] A recently published article suggested measuring gonadotrophins as a possible surrogate marker for estrogen activity.[55] Future studies are needed to confirm the feasibility of gonadotrophins as a biomarker for efficacy of AIs.

Hypothetically, one could imagine the dosing of AIs could be personalized using a pharmacodynamic biomarker, such as measured estradiol concentrations or gonadotrophin levels. In absence of these data, individualized dosing based on pharmacokinetics is more within reach.

Anastrozole

Ingle *et al.* reported a high variability in anastrozole concentrations at the standard dose of 1 mg QD, with a median of 33.2 ng/mL, interquartile range 23.5-44.8 ng/mL, and a range from LLOQ (0.1 ng/mL) to 132.1 ng/mL (n=649)[12], while the intra-patient variability is small (7-12%).[18]

To our knowledge, no exposure-toxicity relationship has been described for anastrozole. In phase I studies patients received repeated doses up to 10 mg QD and single doses up to 60 mg QD, which were well tolerated and did not cause any serious adverse events.[14] A linear dose-exposure relationship was found for doses of 0.5 up to 10 mg.[14]

Dose-efficacy and exposure-efficacy relationships have only been studied with estrogen suppression as a surrogate marker of effect. Although previous studies showed estradiol suppression to below the limits of detection (LLOQ 2 pg/mL) for doses of 1 mg or higher[14,56], it could still be possible that higher doses suppress estradiol to a greater extend, which could not have been quantified with these assays.

In a prospective study (n=649), Ingle *et al.* reported significantly lower anastrozole concentrations in patients with stable or increased estradiol concentrations compared with patients with decreased estradiol concentrations (LLOQ 0.625 pg/mL) after the start of anastrozole treatment (26.7 vs. 34.2 ng/mL, $p < 0.001$).[12] This indicates that TDM could be of value for anastrozole. However, because not all patients with decreased estradiol concentrations compared to baseline necessarily have sufficient estrogen suppression, higher anastrozole concentrations might be needed to attain adequate estrogen suppression.

No definitive exposure-efficacy target has been proposed yet for anastrozole. However, based on the available data, dose escalation could be considered for patients with a C_{min}

< 34.2 ng/mL.[12] Since the median exposure is 33.2 ng/mL, approximately 50% of patients will have a C_{min} below this threshold at the currently used fixed dose of 1 mg QD. Future studies should further investigate the relationship of anastrozole plasma concentrations with both circulating estrogen levels and progression-free and recurrence-free survival.

Exemestane

Although exemestane is extensively metabolized, 17-hydroxy-exemestane is the only active metabolite. However, because the 17-hydroxy-exemestane concentration is ten times lower than the exemestane concentration and 17-hydroxy-exemestane is 2.6 times less potent than exemestane, its additional anti-estrogenic effect is limited.[19]

Estradiol and exemestane share the same steroidal backbone. This structural resemblance can lead to falsely elevated estradiol concentrations in immunoassays. Therefore, measuring estradiol concentrations with liquid chromatography-tandem mass spectrometry instead of immunoassays therefore is the preferred option.[57]

Hertz *et al.* reported a median C_{max} of exemestane of 7.7 ng/mL (range: 2.5-72.0, n=246) at the standard daily dose of 25 mg. Higher exemestane concentrations have been associated with the CYP3A4*22 variant, white race, elevated liver enzymes, renal insufficiency, lower body mass index (BMI), and not having received prior chemotherapy (all $p < 0.05$). However, these factors explained less than 10% of the overall interpatient variability in exemestane concentrations.[20]

No exposure-toxicity relationship has been shown for exemestane. In general, exemestane is well tolerated, with single doses up to 800 mg and multiple doses up to 200 mg administered in phase I studies.[19]

Exposure increases proportionally with increasing dose. Estrogen suppression was maximal at a dose of 25 mg (used assay is not mentioned).[19] However, exemestane concentrations were not significantly different in patients who did and did not achieve estradiol suppression to undetectable concentrations (LLOQ 1.25 pg/mL).[58]

No exposure-efficacy analyses have been reported yet for exemestane. Future studies need to explore any relationship between exemestane exposure and clinical response. In the absence of an exposure-efficacy target, the median C_{max} of 7.7 ng/mL could be used as a reference for TDM, corresponding to a calculated trough concentration of 4.1 ng/mL.[31] As this is the median exposure, approximately 50% of patients will have trough concentrations below this proposed reference.

Letrozole

Desta *et al.* reported a high interpatient variability, with a median steady-state exposure of 88.4 ng/mL (range: LLOQ (7 ng/mL)-349.2 ng/mL) at the standard dose of 2.5 mg QD.[23] Higher exposure was associated with increasing age, lower BMI, and CYP2A6 genetic variations. The lower exposure with increasing BMI can be explained by the fact that

letrozole is a highly lipophilic drug with a large volume of distribution (183L), which increases with increasing BMI. These three variables explain only 32.3% of the interpatient variability, thus a large proportion remains to be elucidated.[23]

In phase I studies, single doses up to 30 mg and repeated doses up to 5 mg were well tolerated. Higher exposure did not cause increased toxicity.[22] Exposure increases approximately linearly with doses up to the standard dose of 2.5 mg QD, while at higher doses the exposure increases non-linearly.[22]

No significant relationship was found between dose (range 0.5-5.0 mg) and estrogen suppression, albeit the assay used may not have been sensitive enough (LLOQ 2.5 pg/mL).[22] Furthermore, Hertz *et al.* found that median steady-state concentrations of letrozole were comparable in patients who did and did not achieve E2 suppression to undetectable concentrations (LLOQ 1.25 pg/mL, 88.8 vs. 105.7 ng/mL, respectively, p=0.63).[58]

In an exposure-efficacy analysis, patients were divided into groups reaching different letrozole plasma concentrations. This analysis showed a tendency to an increase in the time to tumor progression for those patients with a letrozole plasma concentration ≥ 85.6 ng/mL.[22] Future studies need to confirm this exposure-efficacy relationship. Until then, the most appropriate target for TDM of letrozole is 85.6 ng/mL. Because the median exposure is 88.4 ng/mL, slightly less than 50% of patients will not reach this target at the currently used fixed dose of 2.5 mg QD.

DISCUSSION

The data presented in this review highlight clear opportunities to improve and to optimize treatment with anti-hormonal agents in oncology through TDM. However, the evidence for this is not equally strong for all agents. Because of this, we evaluated the available evidence and proposed TDM recommendations, which we considered either *exploratory, promising*, or *viable,* as presented in **Table 2**. The provided TDM recommendation is considered *promising* if a PK TDM target is available or *viable* if a prospective TDM study has been conducted. Otherwise the recommendations are considered *exploratory*.

Future studies are needed to explore exposure-efficacy relationships for those oral anti-hormonal drugs that are classified as *exploratory* (anastrozole, enzalutamide, and exemestane). In addition, prospective clinical studies should be performed to demonstrate the safety and feasibility of TDM for those oral anti-hormonal drugs that are classified as *promising* (abiraterone and letrozole). Ideally, for those drugs for which TDM is *viable* (tamoxifen), randomized controlled trials comparing TDM and fixed dosing with regard to relevant clinical efficacy endpoints such as progression-free survival and overall survival would be needed. Then, TDM could be fully integrated in clinical practice and become the *standard of care*. However, given the large patient numbers needed to

conduct adequately powered randomized controlled trials, especially in the adjuvant setting, this will be a major challenge. Instead, future research could focus on prospective clinical studies strengthening the evidence of the PK target and confirming the safety and feasibility of TDM.[59]

Currently, exposure-efficacy and exposure-toxicity analyses are pivotal parts of the drug development process.[60] However, in the era in which most of the older oral anti-hormonal drugs were registered, this was uncommon, resulting in a paucity of PK-PD data for these agents. Nonetheless, a PK target could be identified for four of the seven included agents. Overall, these targets amounted to 85% (± 19%) of the mean population exposure (**Figure 1**). This is in accordance with the data for kinase inhibitors, as reported previously of 82% (± 17%).[3,4] Thus, targeting the mean exposure, in the absence of exposure-efficacy analyses, generally leads to adequate concentrations.

While awaiting a TDM target based on exposure-response analyses, measuring drug concentrations and collecting data on the efficacy and toxicity in routine patient care can provide us with valuable data on exposure-efficacy and exposure-toxicity relationships, comparable to safety monitoring as part of post-marketing surveillance.

To measure drug concentrations of oral anti-hormonal drugs, validated bio-analytical assays are needed. Our methods for the quantification of abiraterone, enzalutamide, and endoxifen have been previously published.[61–63] Additionally, methods on the quantification of anastrozole, letrozole, and exemestane have been published by other investigators.[64–66] Currently, we are validating an assay for the simultaneous measurement of all mentioned oral anti-hormonal drugs, which makes this a suitable assay for TDM in clinical practice.

Apart from the apparent advantages of TDM, another potential advantage for anti-hormonal drugs could be the monitoring of medication adherence, as it is has been shown that compliance decreases with long-term treatment.[67] Additionally, TDM could help in detecting drug-drug interactions.

Since many of the anti-hormonal drugs have considerably long elimination half-lives, PK sampling for TDM should be timed appropriately to ensure that steady-state concentration has been achieved. In **Table 1**, the time until steady-state concentration has been reached is specified for the different compounds.

In disciplines other than oncology, TDM is being broadly applied, for example in patients using antibiotics, antiretroviral drugs, and immunosuppressants. An important difference, however, is the fact that in oncology we are reluctant to reduce the dose in the case of high drug concentrations because tumor progression is irreversible. For this reason, we advise to increase the dose in the case of low drug concentrations, while reducing doses only in the case of toxicity.

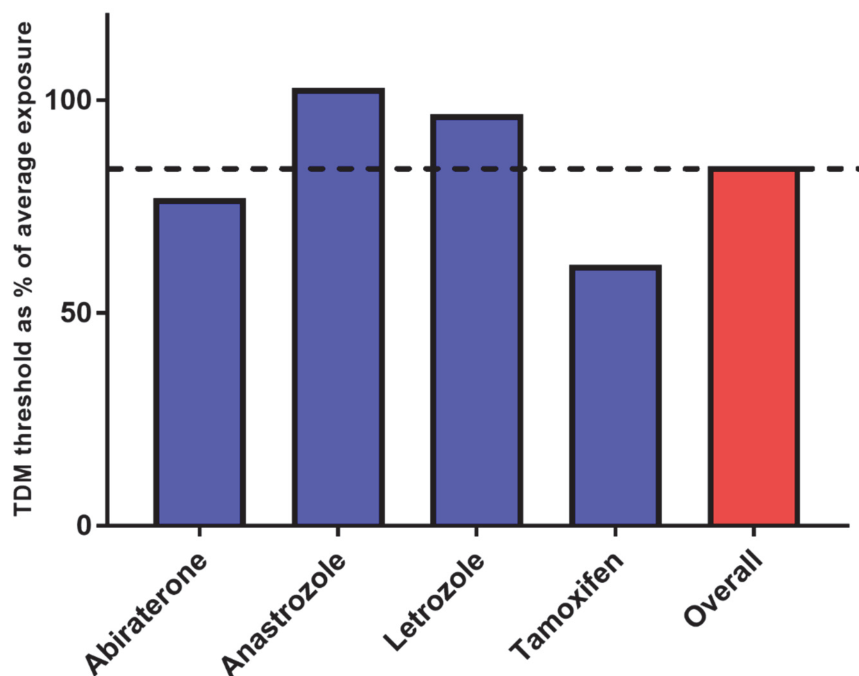

Figure 1 – Proposed therapeutic drug monitoring (TDM) thresholds as percentage of the average exposure
On average the threshold amounted to 85% (± 19%) of the population average (indicated by the dotted line).

CONCLUSION

This review has summarized the clinical PK and PD properties of oral anti-hormonal drugs used in daily oncology practice and aimed to translate these data into practical guidelines for TDM.

For abiraterone, anastrozole, and letrozole, PK targets for TDM could be identified. Furthermore, for tamoxifen, a prospective clinical trial has already demonstrated the feasibility of individualizing the dose based on the endoxifen concentration. However, prospective studies to correlate individualized dosing with tumor response or outcome parameters, such as progression-free survival and overall survival, are lacking.

To conclude, the data presented in this review highlight clear opportunities to study and improve the treatment with oral anti-hormonal agents in oncology via TDM.

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PART IV

Alternative strategies for precision dosing

Concomitant intake of abiraterone acetate and food to increase pharmacokinetic exposure: real life data from a therapeutic drug monitoring program

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ABSTRACT

Aim

Abiraterone acetate is approved for the treatment of metastatic prostate cancer. At the currently used fixed dose of 1000 mg once daily in modified fasting state, 40% of patients do not reach the efficacy threshold of a minimum plasma concentration (C_{min}) ≥ 8.4 ng/mL and are thereby at risk of decreased treatment efficacy. This study aims to evaluate whether pharmacokinetically (PK) guided abiraterone acetate dosing with a food intervention is feasible and results in an increased percentage of patients with concentrations above the target.

Methods

Patients starting regular treatment with abiraterone acetate in modified fasting state were included. Pharmacokinetic analysis was performed 4, 8 and 12 weeks after start of treatment and every 12 weeks thereafter. In case of C_{min} < 8.4 ng/mL and acceptable toxicity, a PK-guided intervention was recommended. The first step was concomitant intake of abiraterone acetate with a light meal or a snack.

Results

In total, 32 evaluable patients were included, of which 20 patients (63%) had a C_{min} < 8.4 ng/mL at a certain time point during treatment. These patients were recommended to take abiraterone acetate concomitantly with food, after which C_{min} increased from 6.9 ng/mL to 27 ng/mL ($p < 0.001$) without additional toxicities. This intervention led to adequate exposure in 28 patients (87.5%).

Conclusion

Therapeutic drug monitoring of abiraterone was applied in clinical practice and proved to be feasible. Concomitant intake with food resulted in a significant increase in C_{min} and offers a cost-neutral opportunity to optimize exposure in patients with low C_{min}.

INTRODUCTION

Abiraterone acetate is an antihormonal prodrug, which is rapidly converted to its active form abiraterone after oral ingestion. Abiraterone inhibits 17α-hydroxylase/C17,20-lyase (CYP17) and thereby blocks the androgen biosynthesis. Initially, abiraterone acetate was approved for the treatment of metastatic castration-resistant prostate cancer, but recently, it has also been approved for the treatment of metastatic hormone-sensitive prostate cancer.[1]

Exposure-response analyses have shown that plasma concentrations of abiraterone are related to efficacy.[2–4] Carton *et al.* demonstrated that progression-free survival (PFS) was significantly longer in patients with a minimum plasma concentration (C_{min}) above 8.4 ng/mL compared with those below (12.2 vs. 7.4 months, p=0.044).[3] We have confirmed this exposure-efficacy threshold in a real-life patient cohort.[4]

Abiraterone acetate is currently administered using a one-size-fits-all approach, in which all patients receive a dose of 1000 mg once daily (QD) without food. This dosing strategy results in high interindividual variability in exposure to abiraterone, with a coefficient of variation (CV%) of 46-70% for C_{min}.[3,4] At the currently used fixed dose, 35-42% of patients do not reach the efficacy threshold of $C_{min} \geq 8.4$ ng/mL and are thus underdosed.[3,4] This provides a strong rationale for therapeutic drug monitoring (TDM) to intervene and to increase the number of patients having an adequate abiraterone exposure.

As food intake impacts the absorption of abiraterone, concomitant intake of abiraterone acetate and food could be applied in case of low exposure. According to the drug label[5], abiraterone acetate should be administered in a modified fasting state, which means no food 2 h before and 1 h after intake of the drug. However, concomitant intake with food has been shown to result in a clinically relevant increase in exposure in a previous food-effect study.[6]

The aim of this study was to evaluate whether TDM of abiraterone with a food intervention is feasible in clinical practice and results in an increased percentage of patients with efficacious exposure to abiraterone without additional toxicities.

METHODS

Patients

Patients starting regular treatment with abiraterone acetate at the registered dose of 1000 mg QD in a modified fasting state were included in an ongoing prospective study on TDM of oral anticancer drugs (www.trialregister.nl; NL6695).[7]

Objectives

The primary objective of this study was to halve the percentage of patients with an exposure below the target of 8.4 ng/mL after 12 weeks compared to historical data. The study of Carton *et al.* was taken as a reference, in which 35% of patients had a mean C_{min} < 8.4 ng/mL.[3] Secondary objectives were to evaluate the feasibility, tolerability and efficacy of TDM of abiraterone with a food intervention in clinical practice and to achieve a physician adherence > 90% (i.e. whether TDM recommendations were followed by the treating physician). Feasibility was defined as the percentage of successful pharmacokinetically (PK) guided interventions (i.e. target attainment without additional toxicities). Tolerability was evaluated by the incidence of clinically relevant toxicities, defined as toxicities leading to dose reduction, treatment interruption or discontinuation, as evaluated by the treating physician. Preliminary efficacy was assessed by comparing PFS and prostate-specific antigen (PSA) responses between patients who needed a PK-guided intervention and those who did not (i.e. all C_{min} ≥ 8.4 ng/mL). PFS was defined as the time from start of treatment to progression, as assessed by the treating physician based on either PSA increase, radiological progression or clinical progression. PSA response was defined as ≥ 50% decrease in PSA from baseline, according to the Prostate Cancer Working Group 2 criteria.[8,9]

PK samples

PK samples were collected 4, 8 and 12 weeks after start of treatment and every 12 weeks thereafter. **Figure 1** provides an overview of the study design. Abiraterone concentrations were measured using a validated liquid chromatography-tandem mass spectrometry assay.[10] C_{min} was estimated using the following formula:

$$C_{min} = C_{measured} * 0.5^{\frac{dosing\ interval - TAD}{t_{1/2}}}$$

in which $C_{measured}$ is the measured plasma concentration, dosing interval is the time between two consecutive administrations of the drug (i.e. 24 h), TAD is the time after dose (i.e. time between last intake of the drug and collection of the PK sample) and $t_{1/2}$ is the elimination half-life of the drug (i.e. 12 h[11]).

PK-guided interventions

In case of C_{min} < 8.4 ng/mL and acceptable toxicity, a PK-guided intervention was recommended. After compliance and drug-drug interactions were checked, the first step was concomitant intake of abiraterone acetate with a light meal or a snack. No specified meals were used. Patients were instructed to take abiraterone acetate for example with some bread, yoghurt or fruit, but not with food high in fat. If exposure remained below the target, dose increments of abiraterone acetate were recommended (to 1250 and 1500 mg, respectively). Dose reductions were solely based on toxicities, not on exposure.

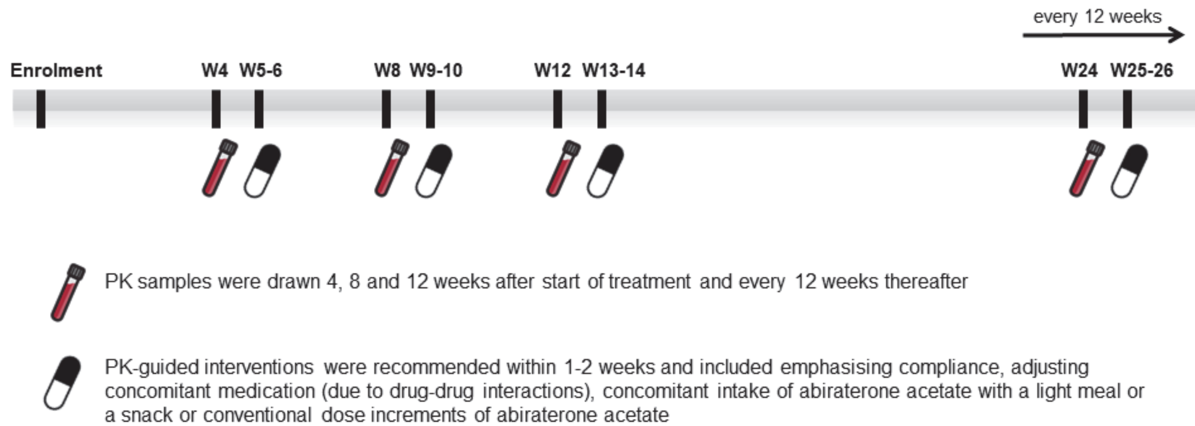

Figure 1 – Schematic overview of study design
PK = pharmacokinetic(ally); W = week

Statistical analyses

Patients were evaluable for the primary endpoint if they completed the first three PK measurements. The effect of concomitant intake of abiraterone acetate and food was evaluated by a Wilcoxon signed-rank test and a Mann-Whitney U test. Preliminary efficacy was evaluated using univariable and multivariable Cox regression and logistic regression analyses. Other data were analysed using descriptive statistics. Statistical analyses were performed using R version 3.3.2.[12]

Ethical regulations

This study was assessed by the accredited Medical Ethics Committee of the Netherlands Cancer Institute, Amsterdam, The Netherlands, in May 2017, and it was reviewed not to fall under the Dutch Medical Research Involving Human Subjects Act, because TDM is performed as standard care, and no additional procedures were required for participants. The study was authorized by the institutional review board. Patients provided written informed consent. The study protocol followed the principles of the Declaration of Helsinki.

RESULTS

Patient characteristics

In total, 32 evaluable patients were enrolled in the study between June 2017 and December 2018 (**Figure 2**). Baseline characteristics of these patients are provided in **Table 1**. Twenty-nine patients completed the first three PK measurements and were eligible for evaluation of the primary endpoint. Twenty patients (63%) had C_{min} < 8.4 ng/mL at a certain time point during treatment. In general, these patients tended to have received more prior lines of treatment, had a worse World Health Organisation (WHO) performance status and had a higher baseline PSA compared with patients with all C_{min} ≥

8.4 ng/mL. At the time of data cut-off (30 August 2019), 13 patients (41%) were still on treatment with a median duration of 11.4 months (range: 2.8-26.3 months).

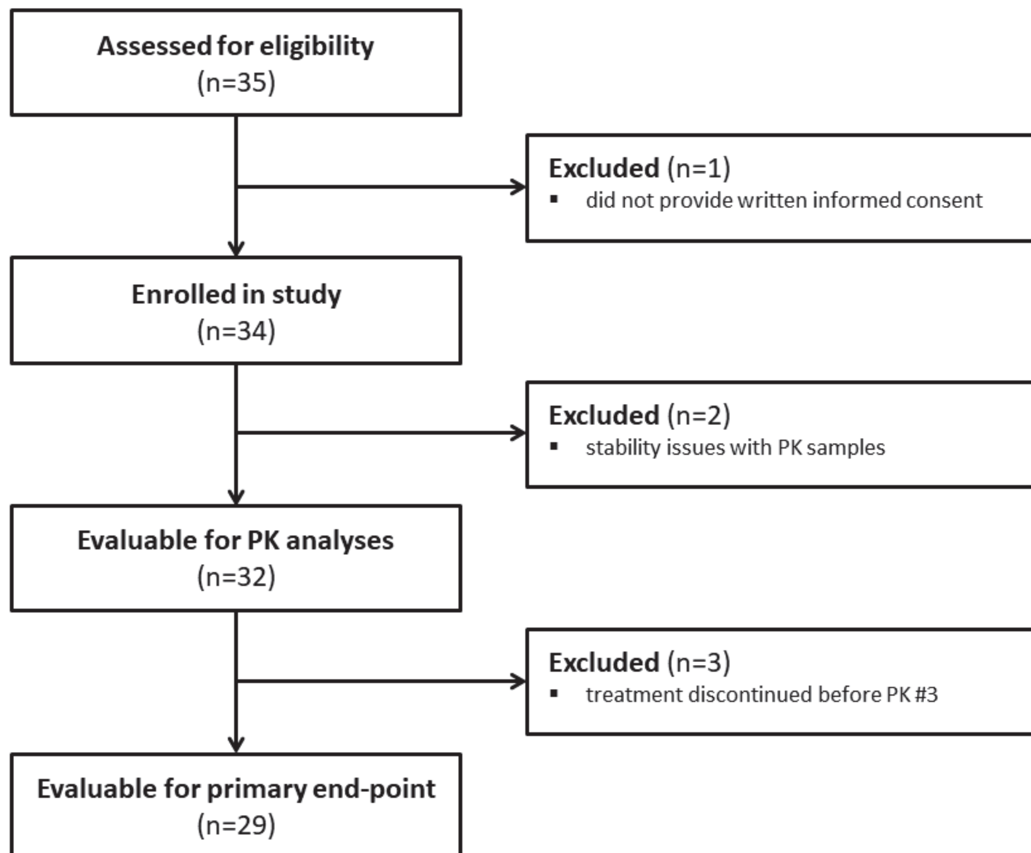

Figure 2 – Patient flow chart
PK = pharmacokinetic

Pharmacokinetically guided dosing

In total, 194 samples have been collected, with a median number of samples per patient of 6 (range: 1-13). First, the results with regard to the primary outcome will be described (i.e. to halve the percentage of patients with a low exposure after 12 weeks). An overview of the median C_{min} and the percentage of patients with C_{min} below the efficacy threshold at each time point can be found in **Table 2**. After 4 weeks of abiraterone acetate treatment at 1000 mg QD in modified fasting state, median abiraterone C_{min} was 12.5 ng/mL (range: 1.0-100 ng/mL), and 8 patients (25%) had a C_{min} < 8.4 ng/mL. After 12 weeks, median abiraterone C_{min} increased to 17 ng/mL (range: 6.7-126 ng/mL) after a food intervention was implemented in patients with a low exposure, with 10% of patients not reaching the target.

To evaluate the secondary objectives, all PK-guided interventions were taken into account, so also the interventions that were performed after 12 weeks. **Figure 3** provides an overview of the PK-guided interventions and its results. The 20 patients (63%) with C_{min} < 8.4 ng/mL at a certain time point during treatment were recommended to take

abiraterone acetate concomitantly with a light meal or a snack. In one patient, this PK-guided intervention could not be performed, because treatment was discontinued because of progression. The interventions resulted in adequate exposure (i.e. $C_{min} \geq 8.4$ ng/mL) in 16 patients (84%). In two patients, the effect could not be evaluated, as treatment was discontinued because of progression before the next PK measurement. In one patient, C_{min} remained below the target initially, and further dose escalation was not deemed feasible because of prior liver toxicity. Eventually, the target was reached with the initial recommended intake of food. Physician adherence to the recommendations was 100%. In total, 28 patients (87.5%) eventually had an adequate exposure. Only one patient (3%) had a median C_{min} < 8.4 ng/mL.

Table 1 – Baseline characteristics

Characteristic	Patients with ≥ 1 measurement of abiraterone C_{min} < 8.4 ng/mL (n=20)	Patients with all measurements of abiraterone $C_{min} \geq$ 8.4 ng/mL (n=12)	All evaluable patients (n=32)
Age (years)	73 [52-87]	73 [63-83]	73 [52-87]
WHO performance status			
0	3 (15%)	5 (42%)	8 (25%)
1	11 (55%)	6 (50%)	17 (53%)
2	5 (25%)	1 (8%)	6 (19%)
3	1 (5%)	0	1 (3%)
Treatment setting			
Castration-resistant	19 (95%)	12 (100%)	31 (97%)
Hormone-sensitive	1 (5%)	0	1 (3%)
Previous lines of systemic treatment[a]			
0	11 (55%)	9 (75%)	20 (63%)
1	4 (20%)	2 (17%)	6 (19%)
≥ 2	5 (25%)	1 (8%)	6 (19%)
Previous systemic treatment[a]			
Docetaxel	9 (45%)	2 (17%)	11 (34%)
Enzalutamide	3 (15%)	1 (8%)	4 (13%)
Radium-223	3 (15%)	1 (8%)	4 (13%)
Cabazitaxel	4 (20%)	0	4 (13%)
Gleason score			
≤ 7	10 (50%)	7 (58%)	17 (53%)
8-10	9 (45%)	5 (42%)	14 (44%)
Missing	1 (5%)	0	1 (3%)
Baseline PSA (ng/mL)	83 [6-1036]	32 [6-282]	48 [6-1036]

Data are expressed as no. (%) or median [range], as appropriate.
[a] in castration-resistant setting
C_{min} = minimum plasma concentration; PSA = prostate specific antigen

Table 2 – Abiraterone C_{min} and percentage of patients with low pharmacokinetic exposure after 4, 8 and 12 weeks

Parameter	Result
Abiraterone C_{min}	**in ng/mL [range]**
PK sample #1 (week 4)	13 [1.0-100]
PK sample #2 (week 8)	17 [5.8-114]
PK sample #3 (week 12)	17 [6.7-126]
Patients with C_{min} below the target of 8.4 ng/mL	**n (%)**
PK sample #1 (week 4)	8 (25%)
PK sample #2 (week 8)	6 (19%)
PK sample #3 (week 12)	3 (10%)
Any time point during treatment	20 (63%)

Data are expressed as median [range] or number (%), as appropriate.

PK#1: 32 patients; PK#2: 31 patients; PK#3: 29 patients.

C_{min} = minimum plasma concentration; PK = pharmacokinetic

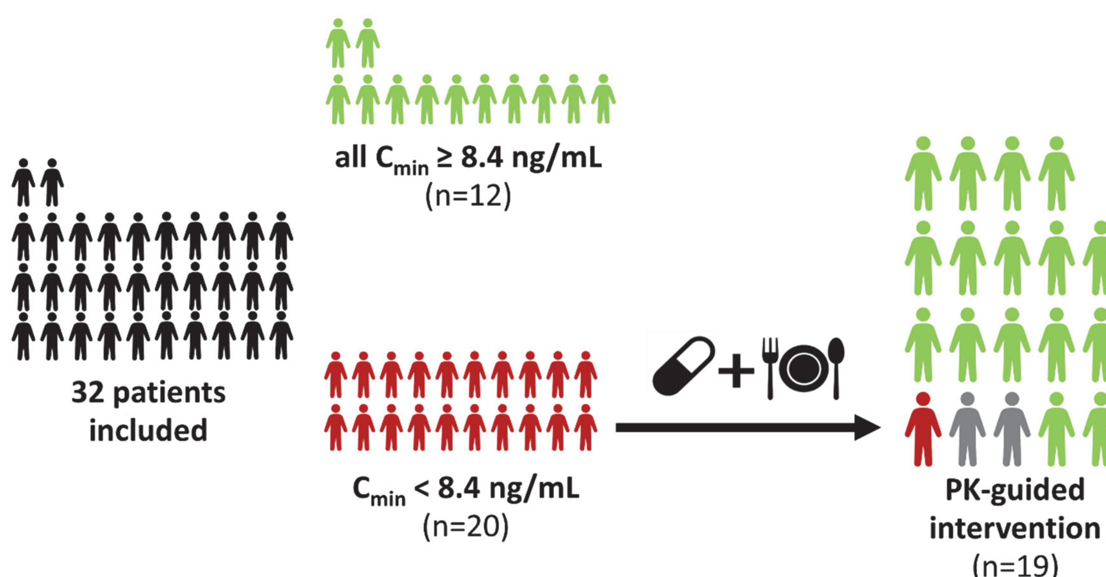

Figure 3 – Schematic overview of study results

The group of patients with C_{min} < 8.4 ng/mL (n=20) had one or more PK-samples with a calculated C_{min} < 8.4 ng/mL at a certain time point during their treatment. In one patient, a PK-guided intervention could not be performed, because treatment was discontinued because of progressive disease. In two patients, the effect of the PK-guided intervention could not be evaluated, because treatment was discontinued because of progressive disease before the next PK measurement.

In one patient, the PK-guided intervention did not result in C_{min} ≥ 8.4 ng/mL, further dose escalation was not deemed feasible because of prior liver toxicity.

C_{min} = minimum plasma concentration; PK = pharmacokinetically

Figure 4 shows boxplots of abiraterone C_{min} in patients with adequate and low exposure, before and after concomitant intake with food. In the group of patients with adequate exposure (i.e. all C_{min} ≥ 8.4 ng/mL), in which no PK-guided intervention was needed, median abiraterone C_{min} was 23 ng/mL (range: 15-70 ng/mL). In the group with low exposure (i.e. C_{min} < 8.4 ng/mL), median abiraterone C_{min} before the PK-guided

intervention was 6.9 ng/mL (range: 1.0-8.2 ng/mL). Concomitant intake of abiraterone acetate and food resulted in an increase in C_{min} to 27 ng/mL (range: 4.3-94 ng/mL, $p < 0.001$), which was comparable to the patients with all C_{min} above the target.

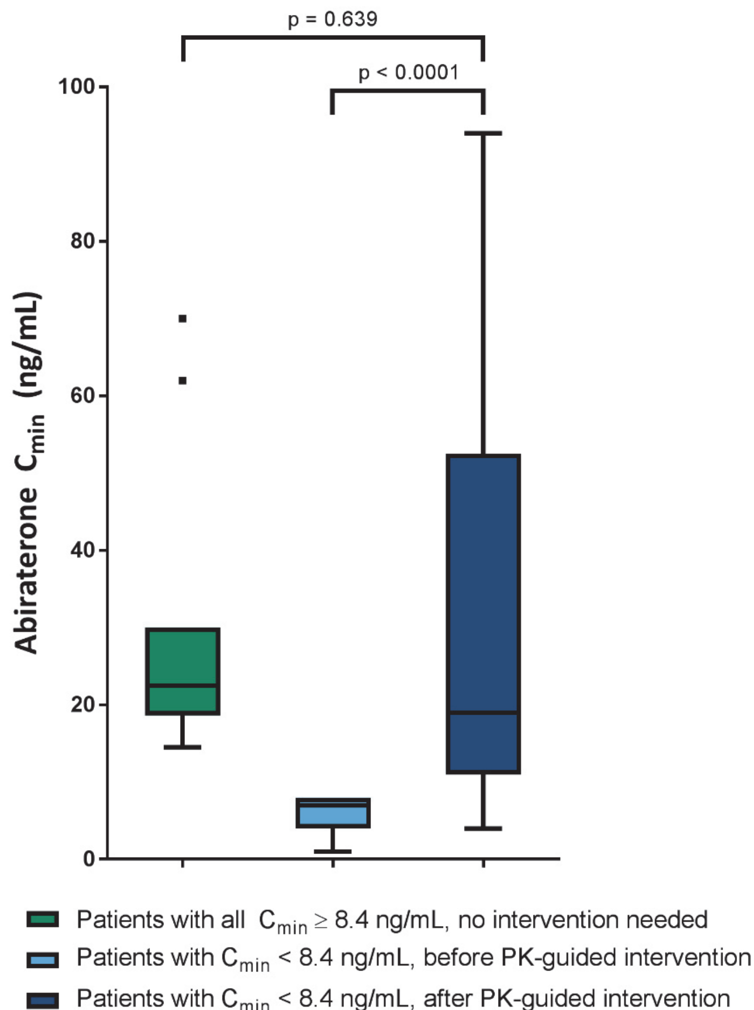

Figure 4 – Box plots of abiraterone C_{min} in patients with adequate and low pharmacokinetic exposure, before and after concomitant intake with food
C_{min} = minimum plasma concentration; PK = pharmacokinetically

For the patients who had a C_{min} < 8.4 ng/mL at a later time point during treatment, median C_{min} in previous PK samples was 14 ng/mL (range: 9.0-77 ng/mL) with a median intra-individual variability (CV%) of 23%.

In **Supplementary Figure 1**, individual graphs of the patients who received a food intervention are shown, depicting all measured abiraterone concentrations before and after concomitant intake with a light meal or a snack.

Toxicity

Three patients needed a dose reduction to 500 mg QD because of toxicity (elevated liver enzymes (n=2) and fatigue (n=1)). None of these patients had received a PK-guided

intervention when toxicity emerged. Median C_{min} at presentation was 33 ng/mL (range: 11-48 ng/mL). After dose reduction, exposure remained adequate in two patients. In one patient, C_{min} dropped below the target, after which the dose was carefully increased to 1000 mg QD concomitant with food, and the target was reached eventually.

In the patients who did receive a PK-guided intervention, this did not lead to additional toxicities.

Efficacy

Median PFS was 9.3 months (95% confidence interval (CI): 6.8-NA) in patients with one or more C_{min} < 8.4 ng/mL, compared with not reached yet (95% CI: 15.8-NA) in patients with all C_{min} ≥ 8.4 ng/mL (hazard ratio: 2.59, 95% CI: 0.84-7.97, p=0.097). However, in multivariable Cox regression, C_{min} < 8.4 ng/mL resulted in a hazard ratio of 1.14 (95% CI: 0.34-3.85, p=0.834), when WHO performance status and number of prior lines of treatment were taken into account. In five patients, the last C_{min} before progression was < 8.4 ng/mL (three of them received successful PK-guided interventions before).

In the initially low C_{min} cohort, 10 patients had a PSA response (50%), whereas in the group with all C_{min} ≥ 8.4 ng/mL, 11 patients (92%) had a PSA response. Multivariable logistic regression resulted in an odds ratio of 0.15 for C_{min} < 8.4 ng/mL (p=0.154).

DISCUSSION

In this prospective study we evaluated the feasibility of PK-guided abiraterone acetate dosing. At the authorized dose of 1000 mg QD in modified fasting state, 63% of patients had a C_{min} < 8.4 ng/mL at a certain time point during treatment. Concomitant intake with a light meal or a snack in these patients resulted in a 3.8-fold increase in C_{min} without additional toxicities (**Figures 3 and 4**; **Table 2**). Hence, TDM of abiraterone is feasible and concomitant intake with food offers a strategy to optimize exposure in patients with a low C_{min}. For the small proportion of patients in whom the target is not attained with this food intervention, a dose increase can be recommended, although this has not been the case in this study.

In our study, 63% of patients had a low exposure at a certain time point during treatment, which is notably higher than the 35-42% reported in literature.[3,4] However, these values refer to the mean or median value of multiple abiraterone C_{min} measurements, whereas in our study, it represents every patient with a single measurement below the target. In our cohort, only one patient (3%) had a median C_{min} below 8.4 ng/mL because of the successful PK-guided interventions. It is remarkable that especially patients with more prior lines of treatment appear to be at risk of low exposure, which was also seen in our previous exposure-response analysis for abiraterone.[4] It would be of interest to further

investigate the mechanism behind the lower exposure in this subgroup (e.g. higher clearance because of enzyme induction or decreased absorption).

Concomitant intake with food not only resulted in an increased exposure but also led to a considerably higher interindividual variability (**Figure 4**). As a result, some patients attained very high C_{min} levels. This may be attributed to the fact that meals were not specified and that the composition could thus differ between patients and time points. However, no additional toxicities were experienced by these patients, which is in line with previous literature where no exposure-toxicity relationship was found either.[1,3,4] Therefore, the increased interindividual variability in exposure is considered acceptable, as long as C_{min} levels are above 8.4 ng/mL.

Since abiraterone also shows a high intra-individual variability, many patients had a C_{min} below 8.4 ng/mL at a later time point during treatment. From that moment, patients were recommended to take abiraterone acetate concomitantly with food, whereas it was uncertain if this would have been necessary all the time. However, owing to the absence of an exposure-toxicity relationship, long-term implementation of this PK-guided intervention does not appear to be harmful.

The magnitude of the food effect in our study is not in line with the previous study by Chi *et al*.[6] While they found a similar exposure (i.e. area under the concentration-time curve (AUC)) for a low-fat meal compared with modified fasting state, our study shows a 3.8-fold increase in C_{min} after concomitant intake with a light meal or a snack. A possible explanation for this could be that many patients (65%) took abiraterone acetate early in the morning, which was probably after an overnight fast. In that case, the results would be more consistent with the study of Chi *et al.*, who reported a five-fold increase in AUC for a low-fat meal compared with overnight fasting in healthy volunteers.[6]

Compared with conventional dose increments, concomitant intake with food offers a cost-neutral strategy to increase pharmacokinetic exposure, although a longer treatment duration could result in higher total treatment costs. Additional costs for a 250 mg or 500 mg dose increase would be €862 or €1782, respectively, per patient per month in The Netherlands. Furthermore, concomitant intake with food is more patient-friendly because patients do not have to take into account the modified fasting conditions.

This prospective study provides real-life data on a TDM programme. Advantages of this study design include the fact that data are representative for the abiraterone population in clinical practice and that our findings can easily be implemented in routine care. On the other hand, this is simultaneously a limitation of our study because compliance could not be guaranteed (i.e. no drug accountability has been performed, and no patient diaries were used).

Although this study demonstrated that an adequate exposure could be attained in the majority of patients by the support of TDM, the ultimate goal is to improve treatment efficacy. Preliminary data on efficacy in this small group of patients indicate that patients who needed a PK-guided intervention still have a shorter PFS than patients with all adequate C_{min}. However, patients with a low C_{min} had a less favourable prognosis at baseline, as they received more prior lines of treatment, had a worse WHO performance status and a higher baseline PSA. We have statistically shown that the adverse results in this cohort are influenced by the adverse patient characteristics in the initially low C_{min} cohort. To evaluate whether TDM actually improves treatment outcomes, a larger cohort of patients will be needed. Therefore, patient inclusion in this study will continue to investigate the effect on treatment efficacy as well.

The significant food effect of abiraterone raises two other interesting concepts. The first is a cost-saving approach: treating patients at a lower dose with food, as has been evaluated by Szmulewitz *et al*.[13] Ideally, this would be investigated using a two-step procedure. To start, it should be proven that adding food to efficiently raise C_{min} is associated with better treatment outcomes for the standard dose of abiraterone. Then, the same should be proven for lower doses of abiraterone. The other concept is a more pragmatic approach: to recommend concomitant intake with food to all patients, regardless of pharmacokinetic exposure.

In conclusion, we demonstrated that TDM of abiraterone is feasible in clinical practice. Furthermore, concomitant intake of abiraterone acetate and food resulted in a significant increase in C_{min} and thereby offers a safe and cost-neutral opportunity to optimize exposure in patients with a low C_{min}. Therefore, we recommend to implement TDM of abiraterone for all patients in routine care.

ACKNOWLEDGEMENTS

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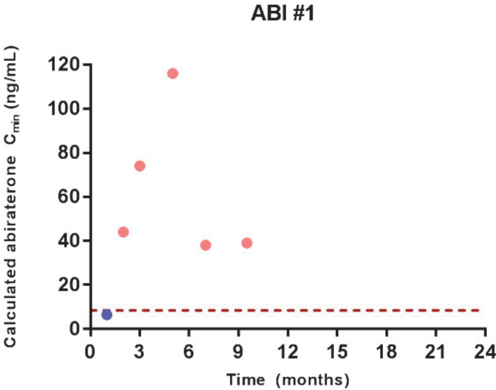
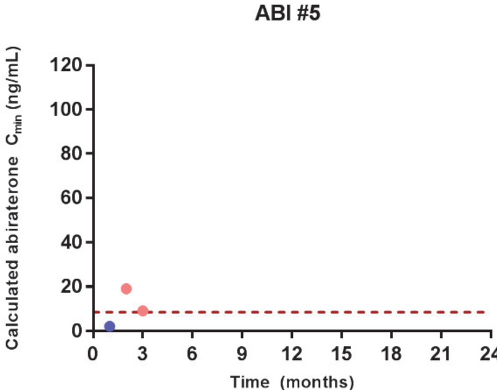
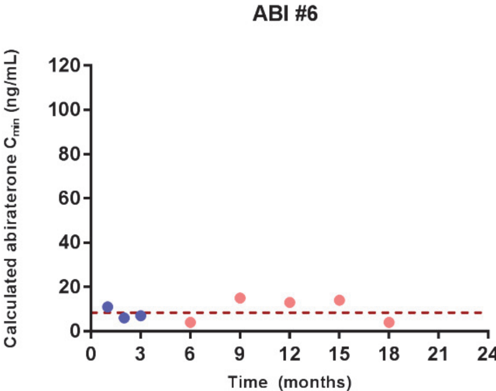
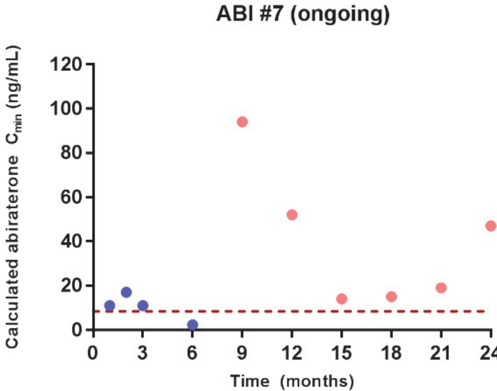
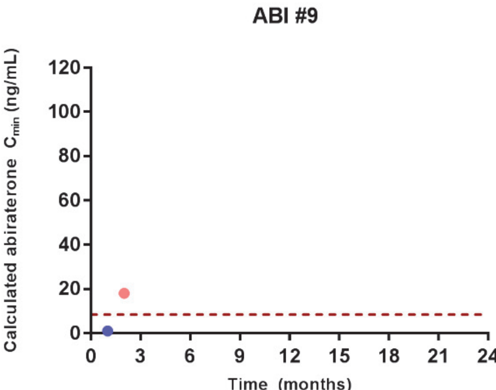
SUPPLEMENTARY DATA

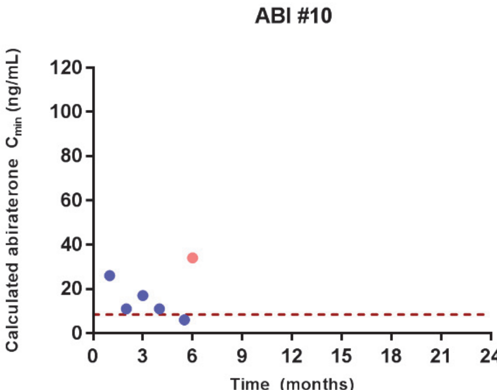












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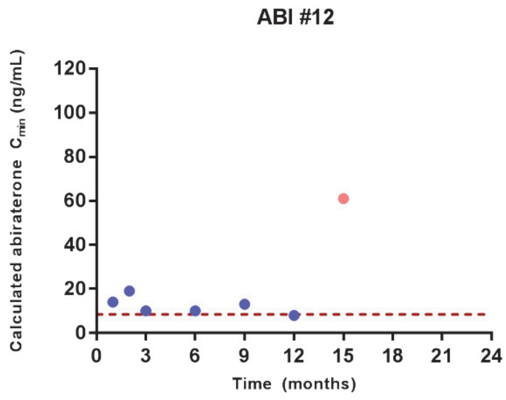

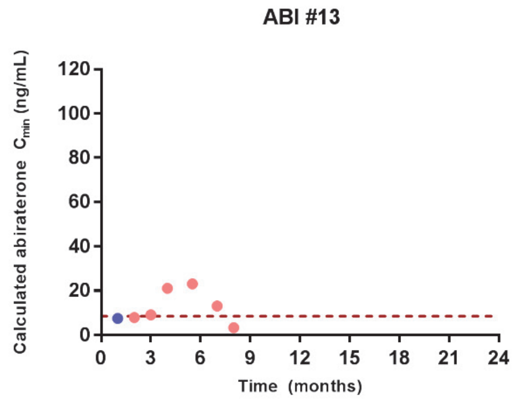

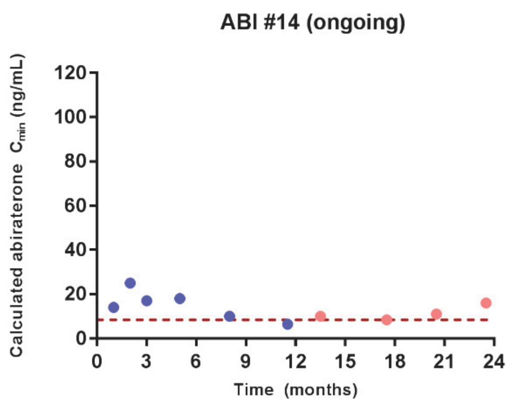

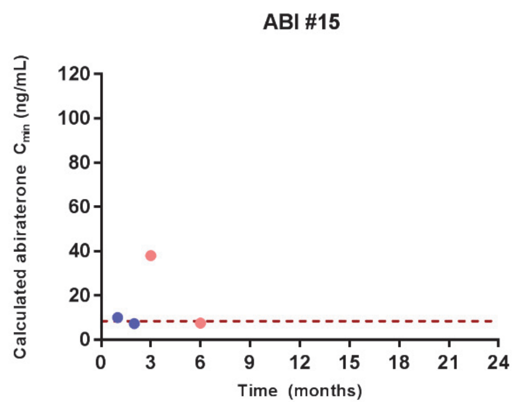

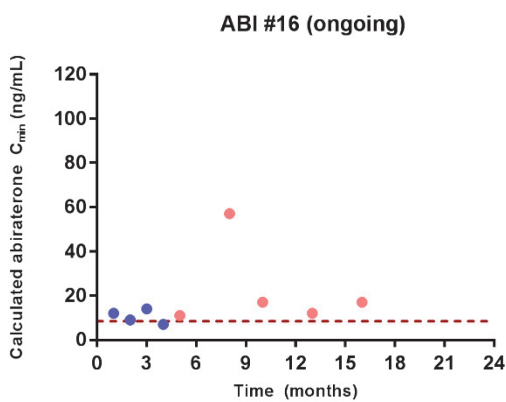

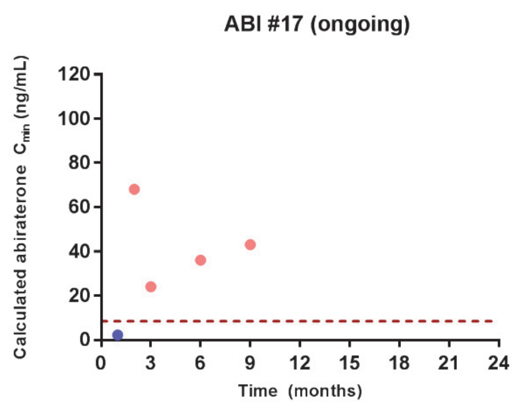

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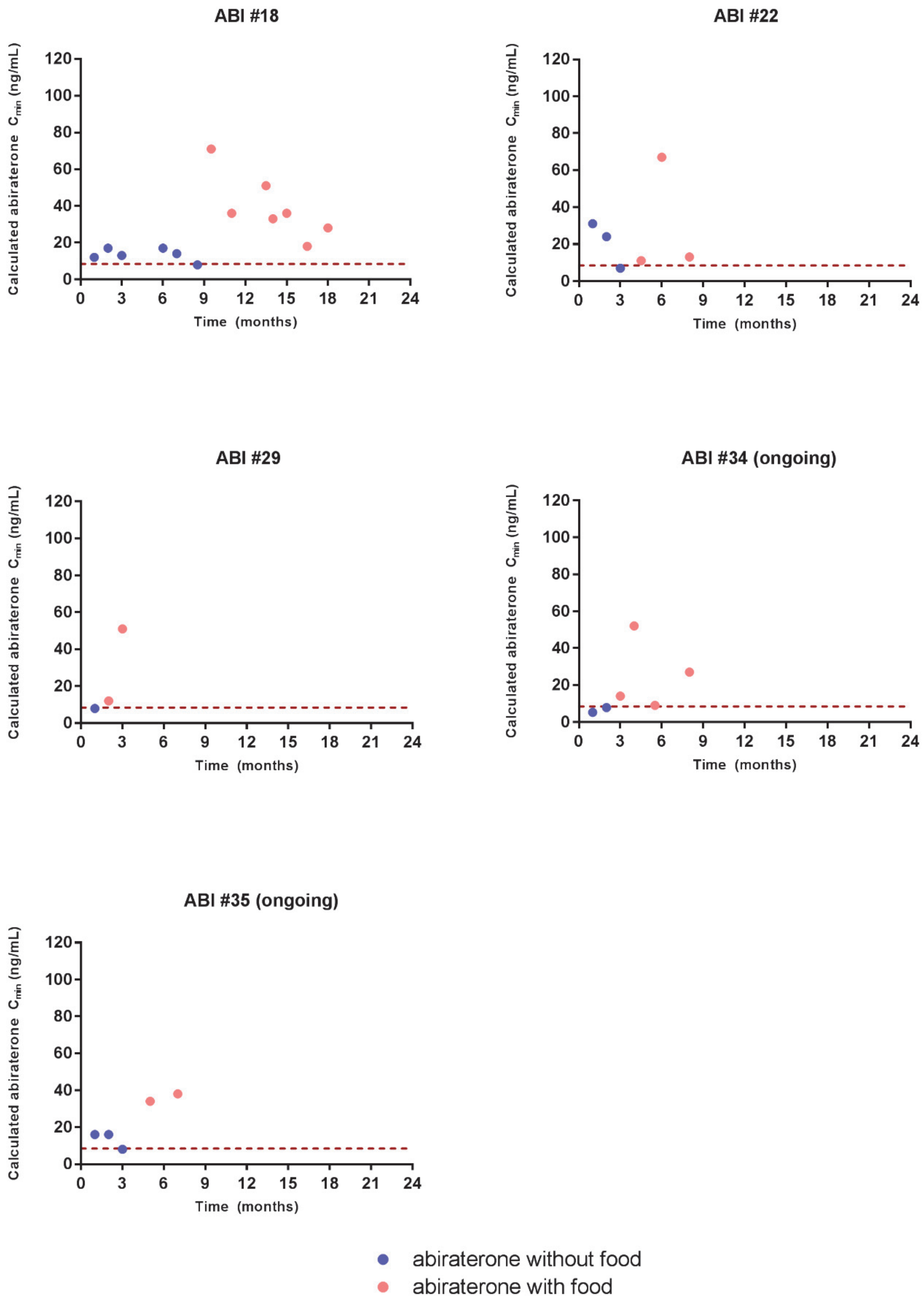

Supplementary Figure 1 – Individual patient graphs of the patients who received a food intervention and in whom the effect was evaluated (n=17), depicting all calculated abiraterone C_{min}, before and after concomitant intake with a light meal or a snack.

The dashed line indicates the pharmacokinetic target of $C_{min} \geq 8.4$ ng/mL. Blue dots indicate calculated abiraterone C_{min} in modified fasting state. Pink dots indicate calculated abiraterone C_{min} concomitant with a light meal or a snack.

C_{min} = minimum plasma concentration

12

Cost-neutral optimization of pazopanib exposure by splitting intake moments: a prospective pharmacokinetic study in cancer patients

Clin Pharmacokinet 2020; **59**: 941–948

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ABSTRACT

Background and objective

Pazopanib is an oral tyrosine kinase inhibitor used in the treatment of renal cell carcinoma and soft-tissue sarcoma. At the approved dose of 800 mg once daily (QD), 16-20% of patients are being underdosed and at risk of decreased efficacy. This study aimed to show whether splitting intake moments, as a cost-neutral alternative to a dose increase, leads to an increased exposure.

Methods

We performed a cross-over trial comparing the pharmacokinetics of pazopanib 800 mg QD with pazopanib 400 mg twice daily. Pharmacokinetic sampling was performed at steady-state for both dosing schedules.

Results

Nine evaluable patients were included. At the 800 mg QD dosing schedule, median minimum plasma concentration (C_{min}), area under the concentration-time curve from zero to 24 h (AUC_{0-24h}), and maximum plasma concentration (C_{max}) were 23.2 mg/L (interquartile range 18.5-27.6), 773 mg h/L (557-1009) and 40.6 mg/L (36.4-56.4) compared with 41.6 mg/L (30.5-55.8, p=0.004), 942 mg h/L (885-1419, p=0.027) and 50.2 mg/L (46.8-72.5, p=0.074) at 400 mg twice daily. One patient experienced a grade 3 event (i.e. diarrhea).

Conclusions

This study demonstrates that splitting intake moments of pazopanib leads to a 79% increase in C_{min}, with acceptable tolerability. Therefore, this new dosing schedule offers a cost-neutral opportunity to optimize treatment in patients with low exposure.

Clinical Trial Registration

NL6137 (https://www.trialregister.nl)

INTRODUCTION

Pazopanib is an oral tyrosine kinase inhibitor approved for the treatment of advanced renal cell carcinoma (RCC) and soft-tissue sarcoma (STS). Pazopanib is targeted at the vascular endothelial growth factor receptors-1, -2 and -3, platelet-derived growth factor receptors-α and -β, fibroblast growth factor receptor, and stem cell factor receptor (c-Kit).[1] In the phase III trial in patients with RCC, pazopanib prolonged progression-free survival from 4.2 to 9.2 months compared to placebo.[2]

Exposure-response analyses by Suttle *et al.* revealed that patients with RCC with a minimum plasma concentration (C_{min}) ≥ 20.5 mg/L had a significantly longer progression-free survival than patients with a C_{min} below this threshold (19.6 vs. 52.0 weeks, p=0.004).[3] This exposure-efficacy threshold has been confirmed in the adjuvant setting and in a real-life patient cohort of patients with RCC.[4,5] A similar trend was found for patients with STS as well, although not statistically significant.[5]

At the currently used fixed dose of 800 mg once daily (QD), interindividual variability in pharmacokinetic exposure is high (40-70%)[5–7] and about 16-20% of patients do not reach the efficacy threshold of C_{min} ≥ 20.5 mg/L.[3,5] These patients are thus underdosed and potentially at risk of decreased antitumor efficacy. This provides a strong rationale for therapeutic drug monitoring, which is individualized dosing based on measured drug concentrations.[8] In a previous prospective clinical trial (n=30) by Verheijen *et al.*, it has been demonstrated that pharmacokinetically guided pazopanib dosing is feasible and results in an increased proportion of patients with adequate pharmacokinetic exposure. To achieve this, pazopanib dosages needed to be increased up to 1800 mg QD in some cases.[9] However, because of the non-linear absorption of pazopanib, which is plateauing at dosages above 800 mg, absolute dose increments are not an efficient strategy to increase pharmacokinetic exposure for pazopanib.[10] Furthermore, it leads to an increase in treatment costs. Previously, we have developed a population pharmacokinetic model based on three clinical trials (n=96) and have shown that the relative bioavailability of pazopanib dosed at 400 mg is estimated to be 59% higher than at 800 mg.[10] Therefore, we hypothesized that splitting intake moments would be a convenient and cost-neutral option for dose optimization.

The aim of this pharmacokinetic cross-over trial was to demonstrate whether switching patients from an 800 mg QD to a 400 mg twice daily (BID) dosing schedule leads to a significant increase in pharmacokinetic exposure, in particular C_{min}.

METHODS

Study design

We performed a prospective multi-center clinical trial with a cross-over design. **Figure 1** provides a schematic overview of the study design. First, pharmacokinetic exposure was determined at the 800 mg QD dosing schedule. Subsequently, patients switched to a 400 mg BID schedule for 7 days, after which the pharmacokinetic exposure was determined again at this new dosing schedule. As the elimination half-life ($t_{1/2}$) of pazopanib is 31 h, 7 days was accepted to be sufficient to attain steady-state concentrations at the 400 mg BID dosing schedule (i.e. more than four to five times $t_{1/2}$). Patients were instructed to take pazopanib at approximately 8.00 a.m., and 8.00 p.m. at the BID dosing schedule, in a modified fasting state, meaning no food 2 h before and 1 h after drug intake. Patients requiring a dose interruption or dose reduction or who discontinued treatment during the study were considered non-evaluable for pharmacokinetic analysis and were replaced. At the end of the trial, pazopanib treatment was continued as part of standard care.

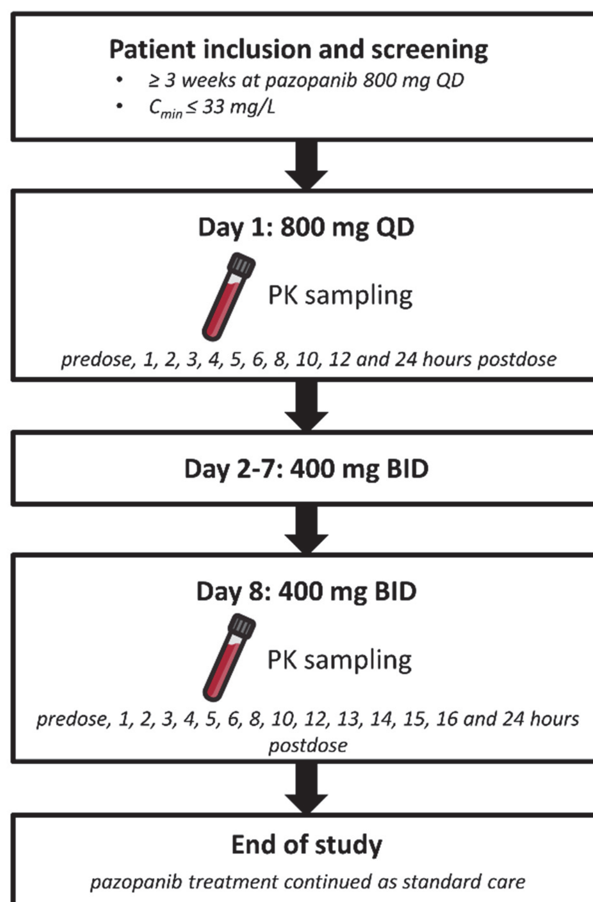

Figure 1 – Schematic overview of clinical trial design
At the 400 mg BID dosing schedule, the second dose of pazopanib was taken 12 hours after the first dose. Sampling time points are relative to the first dose.
BID = twice daily; C_{min} = minimum plasma concentration; PK = pharmacokinetic; QD = once daily

Patient population

Patients with histological or cytological proof of cancer with an indication for treatment with pazopanib (i.e. advanced RCC or STS) were eligible for inclusion. Since evidence suggests pazopanib exposure may drop during the first weeks of treatment, all patients needed to be on pazopanib 800 mg QD treatment ≥ 3 weeks prior to start of the study.[10] Further inclusion criteria were age ≥ 18 years, World Health Organization performance status of 0, 1 or 2, and adequate organ function per judgement of the treating physician.

Patients were excluded in case of a (calculated) C_{min} > 33 mg/L at screening, as by expecting an increase in C_{min} of at least 50% after splitting intake moments based on previous simulations[10], C_{min} is expected to rise above 50 mg/L in these patients, which is associated with an increased risk of toxicity.[9] Another exclusion criterion was concomitant use of medication that could influence the pharmacokinetics of pazopanib within 14 days or five half-lives of the drug (whichever was shorter) before the start of the study, consisting of (but not limited to) gastric acid suppressing agents, cytochrome P450 3A4-inhibitors/inducers, P-glycoprotein, and/or breast cancer resistance protein modulators.

Pharmacokinetics

At screening, either an actual trough concentration was drawn or C_{min} was calculated using the following formula[11]:

$$C_{min} = C_{measured} * 0.5^{\frac{dosing\ interval - TAD}{t_{1/2}}}$$

where C_{min} is the calculated minimum plasma concentration, $C_{measured}$ is the measured plasma concentration, dosing interval is the time between two consecutive administrations of the drug (i.e. 24 h for pazopanib), TAD is the time after dose (i.e. the time between the last drug intake and collection of the pharmacokinetic sample), and $t_{1/2}$ is the average elimination half-life of the drug (i.e. 31 h for pazopanib[1]).

At day 1 and day 8 of the study, patients were admitted to the hospital and blood samples were collected for pharmacokinetic analysis. Time points at day 1 (800 mg QD) were pre-dose and 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h post-dose. Time points at day 8 (400 mg BID) were pre-dose and 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 14, 15, 16, and 24 h post-dose. Sampling time points were relative to the first dose, the second dose was taken after collection of the 12 h post-dose sample. At each time point, blood samples were collected in 3-mL K_2 EDTA tubes and centrifuged directly after collection (1500G, 5 minutes, 4°C). Plasma was stored at -20°C until analysis. Plasma pazopanib concentrations were measured using a validated liquid chromatography-tandem mass spectrometry method.[12]

Study endpoint

The primary endpoint of this study was to evaluate whether switching patients from an 800 mg QD to a 400 mg BID dosing schedule would lead to an increase in pharmacokinetic exposure, measured as C_{min} and area under the concentration-time curve from zero to 24 h (AUC_{0-24h}). The secondary endpoint was to compare adverse events between the two dosing schedules. As an exploratory endpoint, the cost effectiveness of this intervention compared with QD dose increments was evaluated.

Safety assessments

Recording of adverse events (AEs), vital signs, and hematology and blood chemistry assessments was performed at day 1 and day 8 of the study. The incidence, severity, and start and end dates of all AEs were recorded and graded according to the Common Terminology Criteria for Adverse Events, version 4.03. Toxicity at the 800 mg QD dosing schedule was assessed at screening and at day 1. Only toxicities that were present at that time, were taken into account. Toxicity at the 400 mg BID dosing schedule was assessed at day 8.

Statistics

Splitting intake moments was considered to result in an increase in C_{min} and AUC_{0-24h} of at least 50%, based on previous simulations.[10] By assuming an intra-individual standard deviation of the difference between the two dosing schedules of 50%, ten evaluable patients had to be included to obtain 80% power (two-sided $\alpha=0.05$) to detect this increase of $\geq$ 50%. Pharmacokinetic parameters were calculated using non-compartmental analysis. C_{min} was defined as the median value of the pre-dose and 24 h post-dose sample for the 800 mg QD dosing schedule, and of the pre-dose, 12 and 24 h post-dose sample for the 400 mg BID dosing schedule. AUC_{0-24h} was calculated using the linear/log trapezoidal method. C_{max} was defined as the highest measured concentration for each dosing schedule. Minimum plasma concentration, AUC_{0-24h}, and C_{max} of the two dosing schedules were compared using two-sided Wilcoxon signed rank tests. All statistical analyses were performed using R version 3.3.2 (R Project, Vienna, Austria).[13]

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of The Netherlands Cancer Institute-Antoni van Leeuwenhoek. Participating centers were The Netherlands Cancer Institute-Antoni van Leeuwenhoek and the Erasmus MC Cancer Institute. Local approval was obtained in each participating center. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent prior to enrollment in the study. This trial was registered in the Netherlands Trial Register (https://www.trialregister.nl, NL6137) and the EudraCT

database (2016-005252-21). The full trial protocol can be accessed upon reasonable request by contacting the corresponding author.

RESULTS

Patient characteristics

In total, 11 patients were enrolled in the study from June 2017 until January 2019, of which nine patients were evaluable for pharmacokinetic analyses. In one patient, pazopanib treatment was interrupted because of toxicity before all pharmacokinetic measurements were completed and one patient did not take pazopanib according to the protocol. Both of these patients were excluded. Initially, unevaluable patients were replaced according to the protocol. However, the last patient was unevaluable after the study had been closed. As the study was already positive on its primary endpoint of change in C_{min}, it was decided not to replace this patient. Baseline characteristics of all patients are provided in **Table 1**. The majority of patients were female (73%), and the median age was 61 years. Six patients were diagnosed with RCC and five patients with STS. Median time on pazopanib treatment before enrollment in the study was 4.5 months.

Table 1 – Baseline characteristics of all patients (n=11) and evaluable patients (n=9)

Characteristic	**All patients** (n=11)	**Evaluable patients** (n=9)
Gender, female	8 (73%)	7 (78%)
Age (years)	61 [42-78]	55 [42-78]
Tumor type		
Renal cell carcinoma	6 (55%)	6 (67%)
Soft tissue sarcoma	5 (45%)	3 (33%)
WHO performance status		
0	6 (55%)	5 (56%)
1	4 (36%)	4 (44%)
2	1 (9%)	0 (0%)
Previous lines of systemic treatment (number)	0 [0-2]	0 [0-2]
Previous systemic treatment		
Chemotherapy	4 (36%)	3 (33%)
Targeted therapy	1 (9%)	0 (0%)
Time on pazopanib (months)	4.5 [0.7-28.7]	4.5 [0.7-23.7]

Data are expressed as no. (%) or median [range], as appropriate

Pharmacokinetics

Figure 2 shows the pazopanib concentration-time curves at both dosing schedules. An overview of the pharmacokinetic parameters for each of the dosing schedules is provided in **Table 2**. In **Figure 3**, plots of C_{min}, AUC_{0-24h}, and C_{max} at both dosing schedules are shown. Using the 800 mg QD dosing schedule, median C_{min}, AUC_{0-24h}, and C_{max} were 23.2

mg/L (interquartile range (IQR) 18.5-27.6), 773 mg h/L (IQR 557-1009), and 40.6 mg/L (IQR 36.4-56.4), respectively. Switching to the 400 mg BID dosing schedule resulted in an increase in C_{min}, $AUC_{0\text{-}24h}$, and C_{max} to 41.6 mg/L (IQR 30.5-55.8, 79% increase, p=0.004), 942 mg h/L (IQR 885-1419, 22% increase, p=0.027), and 50.2 mg/L (IQR 46.8-72.5, 19% increase, p=0.074), respectively.

Adverse events

An overview of all treatment-related AEs is provided in **Table 3**. All but one patient experienced treatment-related AEs. No patients discontinued treatment and none required a dose reduction because of an adverse event. A single patient experienced a grade 3 event of diarrhea at the 400 mg BID dosing schedule, for which pazopanib treatment was interrupted at day 6 of the study. Calculated C_{min} at this time was high (72.9 mg/L). Treatment was resumed after five days at 800 mg QD, without toxicity. This patient was excluded from the pharmacokinetic analysis, because no PK samples were available at the 400 mg BID dosing schedule.

Table 2 – Pharmacokinetic parameters of pazopanib at the 800 mg QD and 400 mg BID dosing schedule (n=9)

PK parameter	800 mg QD	400 mg BID	Percentage change	p-value
$\mathbf{C_{min}}$ (mg/L)[a]	23.2 (18.5-27.6)	41.6 (30.5-55.8)	+ 79%	**0.004**
$\mathbf{AUC_{0\text{-}24h}}$ (mg*h/L)[b]	773 (557-1009)	942 (885-1419)	+ 22%	**0.027**
$\mathbf{C_{max}}$ (mg/L)[c]	40.6 (36.4-56.4)	50.2 (46.8-72.5)	+ 19%	0.074

Bold values indicate statistically significant p values.
Data expressed as median (IQR).
[a] C_{min} was defined as the median value of the predose and 24 hours postdose sample for the 800 mg QD dosing schedule, and of the predose, 12 and 24 hours postdose sample for the 400 mg BID dosing schedule.
[b] $AUC_{0\text{-}24h}$ was calculated using the linear/log trapezoidal method.
[c] C_{max} was defined as the highest measured concentration for each dosing schedule.
$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time zero to 24 hours; BID = twice daily, C_{max} = maximum plasma concentration; C_{min} = minimum plasma concentration; IQR = interquartile range; QD = once daily

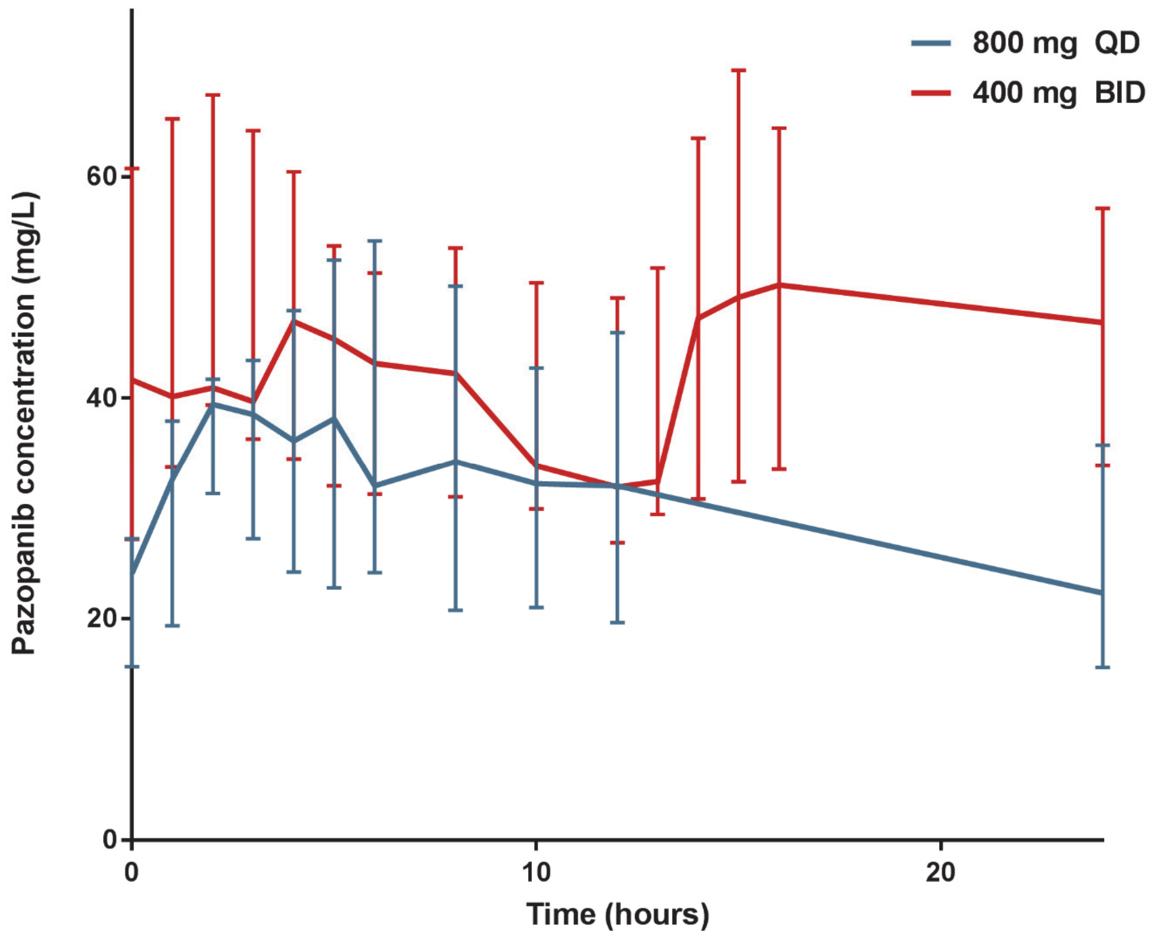

Figure 2 – Pazopanib plasma concentration-time curves (median + IQR) of the 800 mg QD and 400 mg BID dosing schedule (n=9)

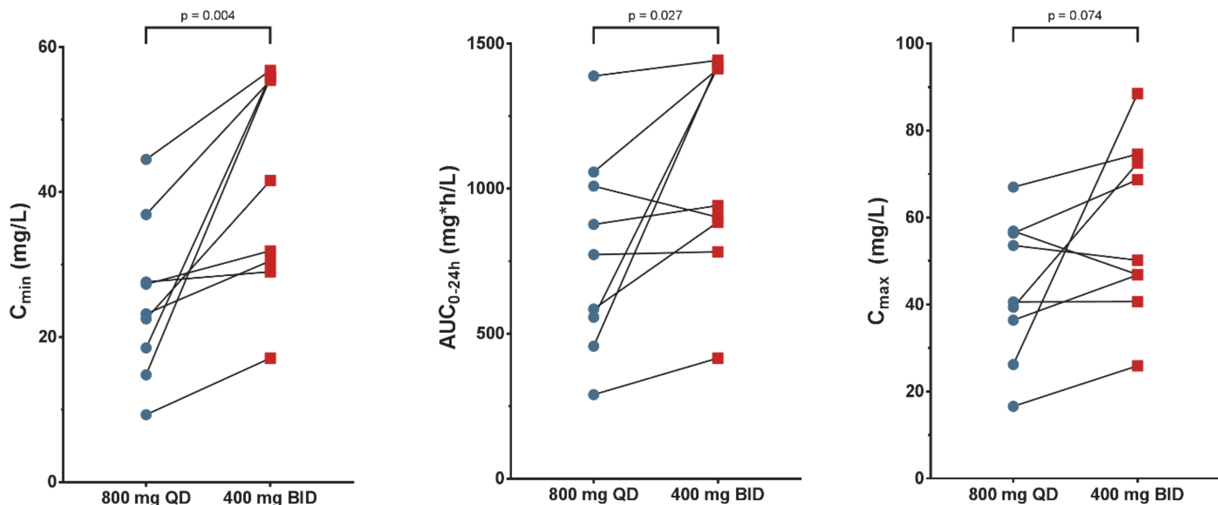

Figure 3 – Plots of pazopanib C_{min}, AUC_{0-24h} and C_{max} for both dosing schedules (n=9)

C_{min} was defined as the median value of the predose and 24 hours postdose sample for the 800 mg QD dosing schedule, and of the predose, 12 and 24 hours postdose sample for the 400 mg BID dosing schedule. AUC_{0-24h} was calculated using the linear/log trapezoidal method. C_{max} was defined as the highest measured concentration for each dosing schedule.

AUC_{0-24h} = area under the plasma concentration-time curve from time zero to 24 hours; BID = twice daily; C_{max} = maximum plasma concentration; C_{min} = minimum plasma concentration; QD = once daily

Table 3 – Treatment-related adverse events (all patients, n=11), according to CTCAE v4.03

Adverse event	800 mg QD		400 mg BID	
	Any grade (n)	**Grade ≥ 3** (n)	**Any grade** (n)	**Grade ≥ 3** (n)
Diarrhea	4	0	6	1
Fatigue	5	0	8	0
Hypertension	4	0	4	0
Nausea	3	0	4	0
Hypothyroidism	2	0	3	0
Anorexia	2	0	3	0
Dysgeusia	1	0	1	0
Vomiting	0	0	2	0
Myalgia	1	0	1	0
Thrombocytopenia	1	0	1	0
Bilirubin increase	1	0	1	0
Localized edema	1	0	1	0
Hair change	1	0	1	0
Skin hypopigmentation	1	0	1	0
Abdominal pain	1	0	1	0
Headache	1	0	1	0
Generalized muscle weakness	1	0	1	0
Weight loss	1	0	1	0
Total number of patients experiencing AEs	7	0	10	1

AE = adverse event; BID = twice daily; CTCAE = Common Terminology Criteria for Adverse Events; QD = once daily

DISCUSSION

In this prospective, multi-centre, cross-over trial we evaluated the effect of splitting intake moments of pazopanib from 800 mg QD into 400 mg BID on the pharmacokinetic exposure. This intervention resulted in a significant increase in C_{min} and $AUC_{0\text{-}24h}$ of 79% and 22%, respectively, with acceptable tolerability (**Figure 2, Figure 3, Table 2**). C_{max} was also numerically higher (19%), although this difference was not statistically significant. Thereby, splitting intake moments offers a convenient strategy to optimize pazopanib treatment for patients with a low pharmacokinetic exposure.

As the number of doses increases, C_{min} is expected to increase as well, even if the bioavailability would remain equal. However, the increased bioavailability is reflected by the significant increase in AUC of 22%, which demonstrates the proof of principle of splitting intake moments for pazopanib. The efficacy threshold of C_{min} ≥ 20.5 mg/L is determined for a once daily dosing schedule and reflects a certain total exposure (i.e. AUC). As AUC does not increase to the same extent as C_{min} when splitting intake moments,

the same C_{min} value at a twice daily dosing schedule reflects a lower total exposure. In general, if a certain total exposure is needed for efficacy, using the same C_{min} target for a twice daily dosing schedule could result in a potential risk of underdosing. Therefore, for pazopanib it should be further investigated if the target of $C_{min} \geq 20.5$ mg/L also applies for the twice daily dosing schedule or that a higher threshold should be used. Since exposure-efficacy analyses were only performed for C_{min} and not for AUC, it is unknown which parameter most accurately predicts clinical response. However, C_{min} is the most pragmatic predictor, as only a single plasma sample is needed.

Pazopanib shows a complex absorption profile, which has been described by Yu *et al.* and consists of a sequential fast and slow absorption phase. This is explained by the fact that pazopanib is only water soluble at pH < 4, resulting in a fast absorption at the first part of the intestine (i.e. the duodenum), when pazopanib is still in solution. As the pH rises sharply > 4 in the small intestines, pazopanib precipitates (i.e. speculation based on the above mentioned physiological considerations[14]) and further absorption becomes dissolution rate limited, which occurs much slower. The poor solubility of pazopanib also explains the previously observed dose dependency in relative bioavailability, which was estimated to be 59% higher at 400 mg compared with 800 mg.[10]

In fact, the oral bioavailability of pazopanib at the approved dose of 800 mg is only 14-39%, due to its suboptimal pharmaceutical formulation.[15] This results in both a high inter- and intra-individual variability in pharmacokinetic exposure. In a previous study, we have shown that with an improved pharmaceutical formulation with a much better dissolution profile, only 300 mg QD is needed to attain a similar pharmacokinetic exposure as with 800 mg QD of the current formulation.[16] However, this improved formulation is not available in clinical practice.

As the bioavailability increases by splitting intake moments of pazopanib, variability was expected to decline.[17] However, in this study we still observed a substantial interindividual variability at 400 mg BID. Hence, plasma concentrations and toxicity should still be carefully monitored. In addition, relatively large individual differences in increases were seen between patients (**Figure 3**), which could be explained by the relatively high intra-individual variability of pazopanib (i.e. 24.7%[18]) and differences in drug absorption between patients.

In a previous pharmacokinetically guided dosing study of pazopanib, dose increments to dosages ranging from 1000 mg QD to 1800 mg QD were needed to attain adequate exposure.[9] The costs of these additional 200-1000 mg of pazopanib are €873 - €4342 per patient per month in The Netherlands, part of which could theoretically be saved when splitting intake moments is used to increase pharmacokinetic exposure. Because dose increments in the case of low exposure are currently not implemented as standard of care, this calculation does not represent an actual cost saving. Instead, it represents a

comparison of the costs between absolute dose increments and splitting intake moments. As splitting intake moments does not lead to any additional costs compared to the standard dose of 800 mg QD, it can be concluded that this is a cost-neutral strategy.

A strength of the current study is that full pharmacokinetic curves instead of only trough samples were obtained at both dosing schedules, enabling comparison of C_{max} and AUC_{0-24h} as well. Limitations of this study include the fact that toxicity of the QD and BID schedule could not be reliably compared because of the short duration of BID dosing (i.e. only seven days) and the fact that only patients who already tolerated the 800 mg QD dose for multiple weeks were eligible for enrollment. Although this was sufficient to reach steady-state concentrations, more time might be needed for adverse events to emerge. Furthermore, only nine evaluable patients were included instead of ten. A post hoc power calculation indicated that nine patients provided 75% power to detect an increase in pharmacokinetic exposure of 50%.

A drawback of switching to a twice daily dosing schedule could be the inconvenience for patients with regard to the modified fasting state in which pazopanib should be administered. An alternative strategy to increase pharmacokinetic exposure to pazopanib could be concomitant intake with food.[7,19] Both strategies could be applied to reach the predefined target. We are currently performing a prospective study on therapeutic drug monitoring of oral anticancer drugs, including pazopanib, in which we split intake moments as a first step to optimize pazopanib treatment in case of low pharmacokinetic exposure. As a second step, concomitant intake with food is recommended.[8,20]

The current study demonstrated that previous simulations using a population pharmacokinetic model of pazopanib adequately predicted the effect of splitting intake moments on C_{min} (i.e. 75% vs. 79% increase), validating a population pharmacokinetic simulation approach for changes in pazopanib dosing schedules.[10] It has to be noted, though, that the increase in AUC_{0-24h} was less pronounced than the simulations predicted (i.e. 22% vs. 59%). However, pazopanib pharmacokinetics shows wide variability, and consequently, comparisons based on a relatively small sample size are difficult. Furthermore, this study illustrates the relevance of population pharmacokinetic simulations in general, which could be applied more often in oncology. These simulations could provide a rationale for proof-of-concept pharmacokinetic studies. The pharmacokinetic data of these clinical studies could then be added to the original population pharmacokinetic model, to further optimize its predictions.

Implications of this study for clinical practice are that patients with pazopanib $C_{min} < 20.5$ mg/L could be switched from an 800 mg QD to a 400 mg BID dosing schedule to improve pharmacokinetic exposure. The feasibility, tolerability, and efficacy of this strategy will now be further studied in a prospective clinical study on therapeutic drug monitoring of oral anticancer drugs (https://www.trialregister.nl; NL6695).[8] Furthermore, data of the

current study could be added to the existing population pharmacokinetic model to better characterize the non-linear pharmacokinetics of pazopanib and to further optimize the dosing schedule.

CONCLUSIONS

This study demonstrates that pharmacokinetic exposure to pazopanib can be boosted by splitting intake moments from 800 mg QD to 400 mg BID, leading to a significant increase in C_{min} of 79% with acceptable tolerability. This is relevant for the 16-20% of patients who are currently underdosed and therefore have a risk of decreased efficacy. As the observed variability in pazopanib pharmacokinetics is large, also after splitting the dose, this strategy should only be applied for those patients where follow-up blood concentration monitoring is in place. Hence, splitting intake moments offers a simple, effective, and cost-neutral strategy to optimize treatment in the significant subset of patients with a low pazopanib exposure.

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Individualized dosing of pazopanib – using cost-neutral interventions to optimize exposure

Interim analysis

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On behalf of the Dutch Pharmacology Oncology Group

The results described in this chapter have not been published yet and include preliminary data that could deviate from the final publication

Clinical pharmacokinetics and pharmacodynamics of the cyclin-dependent kinase 4 and 6 inhibitors palbociclib, ribociclib and abemaciclib

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ABSTRACT

Palbociclib, ribociclib and abemaciclib are inhibitors of the cyclin-dependent kinases 4 and 6 which have been approved for the treatment of locally advanced or metastatic breast cancer. In this review, we provide an overview of the available clinical pharmacokinetic and pharmacodynamic characteristics of these novel drugs, summarize the results of food-effect and drug-drug interaction studies and highlight exposure-response and exposure-toxicity relationships. All three drugs exhibit a large interindividual variability in exposure (coefficient of variation ranging from 40-95% for minimum plasma concentration (C_{min})), are extensively metabolized by cytochrome P450 3A4 and have their brain penetration limited by efflux transporters. Abemaciclib has three active metabolites with similar potency that are clinically relevant (i.e. M2, M20, M18), whereas the metabolites of palbociclib and ribociclib are not of clinical significance. Pharmacokinetic exposure increases in a dose-proportional manner for palbociclib, whereas exposure increases under- and over-proportionally with increasing dose for abemaciclib and ribociclib, respectively. High exposure is associated with an increased risk of neutropenia, and for ribociclib also to QTc prolongation. For abemaciclib, a clear exposure-efficacy relationship has been described, while for palbociclib and ribociclib exposure-response analyses remain inconclusive. Future studies are needed to address exposure-efficacy relationships to further improve dosing.

INTRODUCTION

Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors have emerged as important targeted therapies in the treatment of patients with advanced breast cancer. CDK4/6 inhibitors act on the cell cycle and prevent G1-to-S-phase progression. In order for cells to proceed past this G1-to-S-phase checkpoint, retinoblastoma protein (Rb) needs to be phosphorylated, which is effectuated by CDK4/6.[1] Aberrations in this pathway are often involved in carcinogenesis, resulting in persistent cell proliferation.[2] Treatment with CDK4/6 inhibitors prevents phosphorylation of Rb and thereby causes a G1 cell cycle arrest, blocking cell division (**Figure 1**).

As of today, three CDK4/6 inhibitors are available in the clinic (i.e. palbociclib, ribociclib and abemaciclib), and many more are in (pre)clinical development (**Table 1**). Although all three CDK4/6 inhibitors are approved for treatment in combination with endocrine therapies (i.e. aromatase inhibitors or fulvestrant), only abemaciclib is registered to use as monotherapy. In general, the efficacy of CDK4/6 inhibitors is strikingly consistent between endocrine partners and clinical settings with respect to improved progression-free survival (PFS), and emerging evidence of overall survival (OS) benefit, but their toxicity differs. The aim of this review is to summarize the available clinical pharmacokinetic and pharmacodynamic data on the currently approved CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib. In addition, we will focus on exposure-response relationships and the potential for pharmacokinetically guided dose individualization.

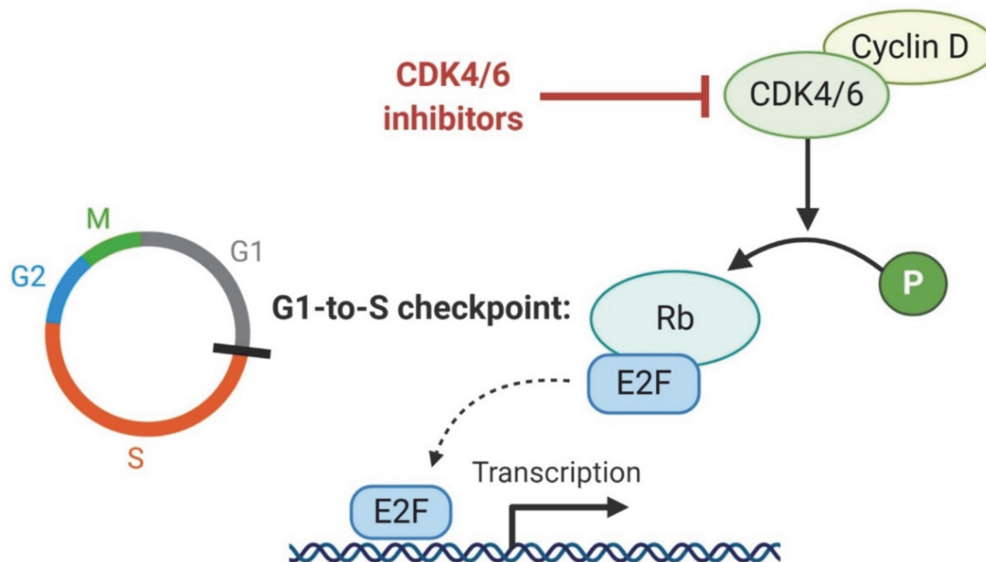

Figure 1 – Mechanism of action of CDK4/6 inhibitors
For cells to progress from G1 to S phase in the cell cycle, Rb needs to get phosphorylated, which is catalyzed by the complex formed by CDK4/6 and cyclin D. Upon phosphorylation of Rb, the transcription factor E2F is released, ultimately resulting in cells proceeding to S phase. CDK4/6 inhibitors prevent Rb from getting phosphorylated and thereby block cell cycle progression. Created with BioRender.com.
CDK = cyclin-dependent kinases; P = phosphoryl (PO_3-); Rb = retinoblastoma

Table 1 – Overview of CDK4/6 inhibitors that are approved for clinical use or in clinical development

CDK4/6 inhibitor	Indication	Dose	Year of approval
Palbociclib (PD0332991)	BC (HR+ HER2-)[a]	125 mg QD 3/1	2015
Ribociclib (LEE011)	BC (HR+ HER2-)[a]	600 mg QD 3/1	2017
Abemaciclib (LY2835219)	BC (HR+ HER2-)[b]	150 mg BID 200 mg BID[c]	2018
Trilaciclib (G1T28)	SCLC[d] TNBC[e]	240 mg/m^2 [f]	clinical development (phase II)
Lerociclib (G1T38)	BC (HR+ HER2-)[g] NSCLC[h]	150 mg BID 200 mg BID[i]	clinical development (phase II)
SHR-6390	BC (HR+ HER2-)[a] BC (HER2+)[j] GC (HER2+)[j]	150 mg QD	clinical development (phase II)
PF-06873600	BC (HR+ HER2-)[b] TNBC ovarian cancer	dose finding ongoing, starting dose not reported	clinical development (phase I/II)
FN-1501	advanced solid tumors	dose finding ongoing, starting at 2.5 mg QD	clinical development (phase I)
BPI-16350	advanced solid tumors	dose finding ongoing, 50-500 mg QD	clinical development (phase I)
FCN-437	advanced solid tumors	dose finding ongoing, starting dose not reported, QD 3/1	clinical development (phase I)

All compounds are administered orally, unless indicated otherwise.
[a] in combination with an aromatase inhibitor or fulvestrant
[b] in combination with an aromatase inhibitor or fulvestrant, or as monotherapy
[c] 150 mg BID is the recommended dose for combination therapy, 200 mg BID for monotherapy
[d] in combination with topotecan; carboplatin and etoposide; or carboplatin, etoposide and atezolizumab
[e] in combination with gemcitabine and carboplatin
[f] administered intravenously
[g] in combination with fulvestrant
[h] in combination with osimertinib, in patients with EGFR-mutated tumors
[i] not decided yet which dose will be selected for phase III trial
[j] in combination with pyrotinib (EGFR/HER2/HER4 inhibitor)
3/1 = 3-weeks-on/1-week-off; BC = breast cancer; BID = twice daily; CDK = cyclin dependent kinase; EGFR = epithelial growth factor receptor; GC = gastric cancer; HER = human epithelial growth factor receptor 2; HR = hormone receptor; NSCLC = non-small-cell lung cancer; QD = once daily; SCLC = small cell lung cancer; TNBC = triple negative breast cancer

PALBOCICLIB

Palbociclib was the first CDK4/6 inhibitor to obtain approval by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2015. In the pivotal PALOMA-2 study, the addition of palbociclib to letrozole as first-line treatment for hormone receptor (HR) positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer patients resulted in a median progression-free survival (mPFS) of 24.8 months compared with 14.5 months for letrozole alone (HR 0.58 (95% confidence interval (CI): 0.46-0.72), $p < 0.001$).[3] Similarly, the PALOMA-3 study demonstrated that palbociclib and fulvestrant were superior to fulvestrant alone in patients who progressed on ≥1 prior lines of treatment (mPFS 9.2 vs. 3.8 months, HR 0.42 (95% CI: 0.32-0.56), $p < 0.001$).[4]

The approved dose of palbociclib is 125 mg once daily (QD) in a 3-weeks-on/1-week-off dosing schedule. This was also the maximum tolerated dose (MTD), with neutropenia being the only dose-limiting toxicity (DLT).[5]

Physiochemical properties and formulation

Palbociclib is a synthetic 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, which belongs to the class of pyridopyrimidines (**Figure 2**).[7] Palbociclib is a weak base with two pK_a values of 3.9 and 7.4, and a calculated log octanol-water partition coefficient (cLogP, which is an indicator of lipophilicity) of 2.7.[8,9] Palbociclib is highly soluble at pH < 4, but its solubility rapidly decreases at higher pH.[9] For drugs to be classified as high solubility compounds, their highest approved dose needs to be soluble in ≤ 250 mL of aqueous media (i.e. ≥ 0.5 mg/mL for palbociclib) over the entire pH range of 1.0-6.8.[10] Therefore, palbociclib is considered a low solubility compound. Together with its high permeability, palbociclib is classified as a class II compound, according to the Biopharmaceutics Classification System (BCS).[9] Initially, palbociclib free base was formulated in capsules, but recently a bioequivalent tablet formulation was approved, containing the free base as well.[11]

In vitro, palbociclib bound reversibly to its targets and the half-maximal inhibitory concentrations (IC_{50}) were 0.011 and 0.016 µM for CDK4 and CDK6, respectively, corresponding to plasma concentrations of 33.5-48.7 ng/mL when corrected for protein binding.[12]

Drug transporters

In vitro assays demonstrated that palbociclib is a substrate of the efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein

(BCRP).[13,14] Although this only marginally affected the oral bioavailability in *in vivo* experiments with P-gp and/or BCRP knock-out mice, it has been demonstrated that the brain penetration was drastically restricted by these transporters.[13]

In addition, *in vitro* and *in vivo* studies have shown that palbociclib inhibits the organic cation transporter 2 (OCT2)[15], which is involved in the renal tubular secretion of creatinine. Although this has not been studied in patients treated with palbociclib, inhibition of the OCT2 transporter has been associated with an increase in creatinine levels without affecting glomerular filtration.[16]

Clinical pharmacokinetics

Table 2 provides and overview of selected steady-state pharmacokinetic parameters of palbociclib. The bioavailability of palbociclib is low (46%).[9] Palbociclib has a large volume of distribution of ~2800 L and the total plasma protein binding is 85.3%, with similar binding to albumin and α1-acid glycoprotein.[5,9] Metabolism mainly takes place by cytochrome P450 3A4 (CYP3A4) and sulfotransferase 2A1 (SULT2A1) and results in the formation of many metabolites, of which M22 (i.e. palbociclib glucuronide) is the most abundant (14.8%) and M17 (i.e. a lactam of palbociclib) is pharmacologically active with a similar potency as palbociclib, but accounting for less than 10% of total plasma exposure **(Figure 2).**[9] Hepatic metabolism is the main route of elimination, as in the mass-balance study 74.1% of palbociclib was excreted in feces compared with 17.5% in urine, including both unchanged palbociclib and metabolites.[9]

Pharmacokinetics in special populations

Pediatric cancer patients

As of to date, palbociclib is not approved for the treatment of pediatric cancer and hence no pharmacokinetic data is available in this subgroup.[17] Several phase I-II studies in pediatric patients are currently ongoing.[18-22]

Patients with renal impairment

In a clinical study, subjects with mild (estimated glomerular filtration rate (eGFR) 60-90 mL/min/1.73m^2), moderate (eGFR 30-60 mL/min/1.73m^2) and severe (eGFR < 30 mL/min/1.73m^2) renal impairment showed an increase in palbociclib $AUC_{0-\infty}$ of 39%, 42% and 31%, respectively, compared with patients with a normal renal function. Similarly, C_{max} was 17%, 12% and 15% higher, respectively.[23] In a population pharmacokinetic analysis (n=183, of whom n=73 and n=29 with mild and moderate renal impairment, respectively), creatinine clearance did not significantly affect palbociclib exposure, which is consistent with renal clearance being a minor route of elimination.[9] No data are available for patients

requiring hemodialysis, but based on the large fraction of palbociclib bound to plasma proteins (i.e. 85.3%), hemodialysis is expected to have limited effect on palbociclib exposure.[9] In conclusion, no dose adjustments are needed for patients with an eGFR ≥ 15 mL/min/1.73m^2 [23], but it should be kept in mind that exposure is 30-40% higher in patients with renal impairment.

Patients with hepatic impairment

Palbociclib unbound $AUC_{0-\infty}$ was 17% lower in subjects with mild hepatic impairment (Child-Pugh class A) and 34% and 77% higher in patients with moderate (Child-Pugh class B) and severe (Child-Pugh class C) hepatic impairment, respectively, compared with subjects with a normal hepatic function. Unbound C_{max} was increased by 7%, 38% and 72%, respectively.[23] These findings are in line with the fact that hepatic clearance is the major route of elimination, and

were also supported by population pharmacokinetic analyses.[9] Based on the above, no dose adjustments are needed for patients with mild or moderate hepatic impairment, while a dose reduction from 125 mg (standard dose) to 75 mg QD is recommended for patients with severe hepatic impairment.[23] It has to be noted that interpretation of palbociclib plasma concentrations in this subgroup could be complicated by the increasing fraction unbound with worsening hepatic function, because this might not be reflected in the total concentration, which is usually measured.[23] In addition, caution is warranted when using the Child Pugh score in patients with cancer, as this score has not been developed nor validated for this population.[24]

Other factors influencing palbociclib pharmacokinetics

The effect of other intrinsic factors on palbociclib exposure was investigated using a population pharmacokinetic model. Age and body weight were significant covariates on palbociclib clearance, which was higher in younger patients and in patients with a higher body weight (i.e. compared with a typical patient of 61 years and 73.7 kg, clearance was increased by 14.7% and decreased by 8.33% in a 45-year-old and a 97-year-old subject, respectively, while for body weight clearance was decreased by 13.2% at a weight of 55 kg and increased by 14.2% at a weight of 97 kg), although these small differences are not expected to be clinically relevant. Gender had no effect on palbociclib exposure.[9]

In a subgroup analysis of the PALOMA-2 study, palbociclib exposure was higher in Japanese and other Asian patients compared to non-Asian patients (geometric mean C_{min} 95.4 ng/mL and 90.1 ng/mL vs. 61.7 ng/mL), whereas in a similar analysis of the PALOMA-3 study no difference was found.[25,26] In another study (n=25), $AUC_{0-\infty}$ and C_{max} were 30% and 35% higher, respectively, in Japanese subjects.[27] No dose adjustments are recommended based on ethnicity.[9]

Food effect

Food-effect studies of capsule and tablet formulations of palbociclib are summarized in **Table 3.** In a previous pooled analysis it has been demonstrated that palbociclib exposure is substantially lower in a subset of patients (i.e. 13%), possibly due to a decreased absorption caused by an elevated stomach pH. This subgroup is classified as low-liers, defined as C_{max} < 21.4 ng/mL.[9] In the food-effect study, when the patients who met the low-lier criteria were excluded, 90%CIs were within the bioequivalence margins, implying no food-effect in patients with adequate absorption.[28]

Concomitant intake with food resulted in a reduced interindividual variability, because the small subset of low-liers now leveled up to the exposure of the rest of the population, supporting the recommended ingestion of palbociclib capsules together with a meal.[28]

While palbociclib capsules need to be administered with food, the recently approved tablet formulation can be taken with or without food, offering more flexibility to patients. Palbociclib exposure was not significantly altered due to food intake using the tablet formulation, showing to be more robust to pH differences.[11]

Drug-drug interactions

In **Table 4**, results of drug-drug interaction studies and recommendations for dose adjustments are shown. Overall, no (clinically relevant) interactions with fulvestrant, goserelin or aromatase inhibitors were found. In contrast, palbociclib exposure was significantly altered by strong CYP3A4 modulators.

No clinical studies have been executed for moderate CYP3A4 inhibitors, but simulations predicted that they would increase palbociclib C_{max} and $AUC_{0-\infty}$ by approximately 23% and 40%, respectively.[29] According to the label, no dose reduction is warranted although these results suggest that a dose reduction from 125 mg (standard dose) to 100 mg QD might be advised. To further substantiate this finding, we are currently performing a clinical study to investigate the effect of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of Palbociclib.[30] In addition, relevant interactions between palbociclib and CYP3A4 substrates with a narrow therapeutic index could occur, since palbociclib can weakly inhibit CYP3A4.[31]

As the solubility of palbociclib is pH-dependent, it could be expected that acid-reducing agents would decrease its exposure. Although palbociclib exposure was substantially reduced when administered concomitantly with rabeprazole under fasted conditions, this effect was only modest under fed conditions (**Table 4**).[32] Therefore, no dose adjustments are indicated when palbociclib capsules are co-administered with acid-reducing agents, as they have to be administered under fed conditions. Exposure of palbociclib tablets was not affected by acid-reducing agents.[11]

Pharmacokinetic-pharmacodynamic relationships

Exposure-response

Initial exposure-response analyses based on data of the PALOMA-1 study were inconclusive due to limited data (n=81). Although a trend for prolonged PFS was observed in patients with an average palbociclib concentration (C_{avg}) above the median of 60 ng/mL (median PFS estimated from Kaplan Meier curves were 17 months vs. 24.5 months, p-value not reported), multivariable analyses yielded inconsistent results.[9]

In the PALOMA-3 study, PFS was similar in patients with C_{avg} above and below the median of 78 ng/mL. It has to be noted, though, that exposure in this trial appeared to be higher than in PALOMA-1 (at the same dose, but with fulvestrant instead of aromatase inhibitors). Even in the group with low exposure, median C_{avg} was 63 ng/mL, which is higher than the cut-off value used in the PALOMA-1 study. Time-varying C_{avg} as a continuous variable was a significant predictor of PFS in univariable analysis, although this did not remain significant in multivariable analysis.[27,33]

In the PALOMA-2 study, no exposure-response relationship has been identified.[34,35]

As exposure-response analyses have thus far not resulted in a clear answer and optimal data to perform them were not available, this needs to be further elucidated. Lower thresholds of C_{min} may be related to efficacy. Preferably, these additional analyses should include palbociclib plasma concentrations measured at regular intervals throughout treatment and use median C_{min} as a measure of exposure. Previously, it has been suggested that individual concentrations could be compared to the mean C_{min} of 61 ng/mL of the PALOMA-1 study.[36]

Exposure-toxicity

In phase I studies higher AUC values were related to a greater reduction in absolute neutrophil count and platelet levels, with a wide range in EC_{50} values (estimated plasma exposure resulting in 50% decrease from baseline) varying from 253-716 ng/mL*h for neutropenia and 184-1370 ng/mL*h for thrombocytopenia.[5,6]

A semi-mechanistic pharmacokinetic-pharmacodynamic model has been developed to quantify the relationship between palbociclib exposure (i.e. plasma concentration) and neutropenia.[37] In this model, the maximum anti-proliferative effect on neutrophil precursor cells (E_{max}) was notably lower than for cytotoxic drugs (e.g. docetaxel and etoposide), and the reported EC_{50} value was 40.1 ng/mL.

Interestingly, patients with grade 3 or 4 neutropenia had a significantly longer PFS compared with patients with lower grade or no neutropenia (p=0.0046). Multivariable analysis resulted in a hazard ratio of 0.502 (95% CI 0.26-0.98, p=0.046). This could be explained by either the hypothesis that tumor cells in patients with neutropenia are more

sensitive to palbociclib or an underlying higher exposure in these patients.[9] As higher drug exposure causes more neutropenia and more neutropenia is related to a prolonged PFS, this suggests that an exposure-response relationship exists.

Population pharmacokinetic models

In a population pharmacokinetic model palbociclib pharmacokinetics was best described by a two-compartment model with first-order absorption.[9] Two additional models have been developed to predict drug-drug interactions with CYP3A4 inhibitors and to quantify the exposure-response relationship for neutropenia.[29,37]

RIBOCICLIB

In 2017, ribociclib has been approved by the FDA and EMA based on the results of a preplanned interim analysis of the MONALEESA-2 study.[38] In the second interim analysis of this randomized placebo-controlled phase III trial (n=668) comparing first-line treatment with letrozole with or without ribociclib, mPFS was significantly longer in the ribociclib group compared with the control group (25.3 vs. 16 months, HR 0.57 (95% CI: 0.46-0.70), $p < 0.001$).[39] In 2018, the indication was extended to combination treatment with fulvestrant, based on the MONALEESA-3 study. This study revealed that addition of ribociclib to the treatment of fulvestrant, improved mPFS from 12.8 to 20.5 months (HR 0.60 (95% CI: 0.48-0.73), $p < 0.001$) and resulted in a prolonged median OS (40.0 months vs. not reached yet (HR 0.72 (95% CI: 0.57-0.92), p=0.005).[40,41]

The approved dose of ribociclib is 600 mg QD in a 3-weeks-on/1-week-off dosing schedule. In the phase I dose escalation study, doses ranging from 50-1200 mg QD were explored.[42] The MTD was established as 900 mg QD, with neutropenia and thrombocytopenia being the most common DLTs.[42] The lower dose level of 600 mg QD was selected for further development, as this resulted in a lower rate of QTc prolongation, and clinical activity was already observed at this dose level.[42]

Physiochemical properties and formulation

Ribociclib is a 7-cyclopentyl-*N,N*-dimethyl-2-{[5-(piperazin-1-yl)pyridine-2-yl]amino}-7*H*-pyrrolo[2,3-*d*]pyrimidine produced by chemical synthesis. It is formulated as a succinate salt in film-coated tablets containing 200 mg of ribociclib free base. Although initially a capsule formulation was used in clinical trials, the equivalence of both dosage forms was demonstrated in a bioequivalence study.[43] Ribociclib is a weak base with two pK_a values of 5.5 and 8.6, with its succinate salt exhibiting high solubility at pH ≤ 4.5 (solubility > 2.4 mg/mL), but it decreases at higher pH. Thus, ribociclib succinate was classified as a low solubility compound.[43,44] The ribociclib Log P was reported to be 1.95, and its estimated effective human permeability (hP_{eff}) was $0.9*10^{-4}$ cm/s. Based on these data, it was categorized in BCS class IV (low solubility, low permeability).[43,45]

In vitro, IC_{50} values for ribociclib were 8 and 39 nM for CDK4 and CDK6, respectively, corresponding to plasma concentrations of 11.6-56.5 ng/mL when corrected for protein binding.[46]

Drug transporters

In vitro and *in vivo* studies have demonstrated that ribociclib is a transport substrate of P-gp.[47,48] Interestingly, pharmacokinetic and tissue distribution studies in mouse models showed that this efflux transporter is responsible for limiting the ribociclib penetration into the brain, since the brain-to-plasma concentration ratio increased by at least 23-fold when the P-gp was knocked out or inhibited. Plasma pharmacokinetic parameters were not significantly affected, except for $AUC_{0\text{-}24h}$, which increased 2.3-fold in mice lacking the P-gp and BCRP. This increase is likely due to P-gp activity, since ribociclib has not shown noticeable transport by BCRP.[47]

Besides interacting as a substrate, ribociclib also inhibited P-gp.[48] Moreover, at clinically relevant concentrations it also inhibited BCRP, OCT2, multidrug and toxin extrusion protein (MATE) 1, and bile salt export pump (BSEP).[15,46,48,49] In a retrospective study in patients treated with ribociclib, creatinine levels increased 37% compared to baseline, probably due to OCT2 inhibition.[50]

Clinical pharmacokinetics

Selected steady-state pharmacokinetic parameters of ribociclib are displayed in **Table 2**. Exposure of ribociclib increased over-proportionally with dose in the range of 50-1200 mg, possibly caused by a lower clearance at higher doses.[51]

The absolute oral bioavailability has not been determined, but using a physiologically-based pharmacokinetic (PBPK) model it was predicted that 90% of the standard dose of ribociclib (600 mg) would be absorbed mainly in the small intestine.[44] Ribociclib has a moderate human protein binding (± 70%), and is equally distributed in plasma and red blood cells. The apparent volume of distribution was estimated at 1090 L, using a population pharmacokinetic analysis.[46,49]

Ribociclib is metabolized primarily by CYP3A4 with the formation of the active metabolite M4 (**Figure 2**). It is also metabolized to a minor extent by flavin-containing monooxygenase (FMO) 3 and FMO1, the last being involved in the formation of the metabolite M13. These two metabolites may be reactive by forming covalent adducts in hepatocytes. M4, M13 and M1 (a secondary glucuronide of ribociclib) were the major circulating metabolites, accounting for, respectively, 8.6%, 9.4% and 7.8% of total radioactivity in a mass balance study. Considering these data, the contribution of the active metabolite M4 to the clinical activity was considered negligible.[46,49,52]

Feces was the major route of excretion compared to urine, accounting for, respectively, 69.1% and 22.6% of the dose recovered, where ribociclib was the major entity found in excreta.[46,49]

Pharmacokinetics in special populations

Pediatric cancer patients

Ribociclib was the first CDK4/6 inhibitor studied in pediatric patients in a phase I clinical trial, where patients with neuroblastoma, rhabdoid tumors or solid tumors with alterations in the cyclin D-CDK4/6-INK4-Rb pathway were included. The MTD and recommended phase II dose (RP2D) were 470 and 350 mg/m^2, respectively. The RP2D dose was selected based on overall safety and pharmacokinetic considerations, since the exposure at 350 mg/m^2 was equivalent to that observed at 600 mg in adults. Pharmacokinetic parameters, including t_{max}, C_{max}, $AUC_{0-\tau}$ and $t_{1/2}$, were similar in adults and pediatric patients.[53]

Patients with renal impairment

The effect of renal impairment on the pharmacokinetics of ribociclib was assessed in a population pharmacokinetic analysis, which included patients with normal renal function (n=438), mild renal impairment (n=488) and moderate renal impairment (n=113). In this analysis, mild and moderate renal impairment had no effect on the exposure and clearance of ribociclib, therefore no dose adjustments are recommended for patients with mild or moderate renal impairment.[46,49,52]

Furthermore, a clinical trial showed that for patients with severe renal impairment and end stage renal disease (eGFR < 15 mL/min/1.73 m^2), $AUC_{0-\infty}$ increased 281% and 137%, and C_{max} 168% and 110%, respectively, compared to subjects with normal renal function.[54] Based on these results, a starting dose of 200 mg daily is recommended by the FDA for patients with severe renal impairment, while the EMA recommends a starting dose of 400 mg in these cases.[52,55]

Patients with hepatic impairment

In a clinical study (n=28), mild hepatic impairment had no effect on ribociclib exposure. In contrast, for moderate hepatic impairment, $AUC_{0-\infty}$ and C_{max} increased 28% and 44%, respectively, while for severe hepatic impairment they increased 29 and 32%. A population pharmacokinetic analysis (n=160 with normal hepatic function and n=47 with mild hepatic impairment) further supported that ribociclib exposure was unaffected by mild hepatic impairment. Based on these results, a reduction of the starting dose to 400 mg is recommended for patients with moderate or severe hepatic impairment.[46,49]

Other factors influencing ribociclib pharmacokinetics

The effect of other intrinsic factors on ribociclib pharmacokinetics was evaluated by population pharmacokinetic analyses (n=208). Body weight and age were statistically significant covariates for ribociclib clearance. Based on simulations, it was predicted that a change of body weight from the reference value of 70 kg to 50 or 100 kg would change steady state C_{max}, C_{min}, and $AUC_{0\text{-}24h}$ of ribociclib up to 22%, which was considered a small effect relative to the inherent pharmacokinetic variability. Age was predicted to have only a mild effect on exposure. Race and gender were statistically insignificant parameters.[46]

Furthermore, a cross-study comparison exhibited that, on average, the exposure of ribociclib in Japanese patients was higher than in Caucasian patients, but the individual values were within the same range. In summary, the effects of body weight, age, gender and race on ribociclib pharmacokinetics were considered not clinically relevant, and therefore, no dose adjustment is required.[46,49]

Food effect

Table 3 summarizes the food-effect studies that have been performed for the capsule and tablet formulation, of which the latter is more relevant since this is the marketed formulation. Since the geometric mean ratios were ≈1 for $AUC_{0\text{-}\infty}$ and C_{max}, no effect of food intake was observed on the pharmacokinetics of ribociclib.[44]

Additionally, the *in vitro* solubility of ribociclib was evaluated in biorelevant media, including simulated fed (pH 5.0) and fasted (pH 6.5) intestinal fluid, where the maximum dose (600 mg) was dissolved in 250 mL. This suggests that ribociclib absorption is unlikely to be affected by changes in the gastric pH due to food intake, among others. PBPK models also predicted that the exposure of ribociclib was independent of the gastric pH in the range of 1.0-8.0.[44] Altogether, this information supports that ribociclib can be administered either with or without food.[46,49,52]

Drug-drug interactions

An overview of all drug-drug interaction studies is provided in **Table 4**. Ribociclib had no (clinically relevant) interactions with fulvestrant and the aromatase inhibitors.[46,49,52,55] Ribociclib is extensively metabolized via CYP3A4, therefore its pharmacokinetics is strongly affected by strong inhibitors or inducers of this enzyme. Ribociclib can reversibly inhibit CYP3A4 and CYP1A2.[48] Altogether, it is recommended that drugs with narrow therapeutic index that are sensitive substrates of these drug-metabolizing enzymes or transporters which are inhibited by ribociclib (section 3.2) should be cautiously monitored in concomitant treatments with ribociclib.[46,49]

Since ribociclib shows a pH-dependent solubility, drugs that alter the gastric pH could be expected to affect its exposure. However, ribociclib exposure was similar in patients with

and without a proton pump inhibitor, and these drugs could thus be administered concomitantly.[44]

Pharmacokinetic-pharmacodynamic relationships

Exposure-response

Due to very limited data, exposure-response analyses for ribociclib remain inconclusive. In the MONALEESA-2 study only 44 out of 334 patients had progressive disease and available pharmacokinetic data. No indication for an exposure-efficacy relationship was found. Data on confirmed best response were available for 72 patients with pharmacokinetic data, and showed similar ribociclib exposure in responders vs. non-responders. No exposure-response analyses have been performed for the MONALEESA-3 study.[46,56] Future studies should establish exposure-efficacy relationships and identify an optimal threshold concentration.

Exposure-toxicity

Although higher C_{min} levels were related to a greater decrease in absolute neutrophil count and platelet count in the phase I study, ribociclib is dosed at the flat ends of these plateauing curves.[42] Pooled data from four clinical studies (n=196) were used to develop a logistic regression model for ≥ grade 2 neutropenia. Although a trend was found for an increased risk of neutropenia at higher ribociclib exposure, this was not statistically significant. For each 100 ng/mL increase in C_{min} the odds ratio for ≥ grade 2 neutropenia was 1.05 (95%CI: 0.99-1.11, p=0.087).[46] In addition, a pharmacokinetic-pharmacodynamic model for neutropenia using data of 1052 patients from six clinical trials showed that the relationship between exposure and neutropenia was not influenced by age, race, or the use of anastrozole, letrozole, tamoxifen or fulvestrant.[56]

Furthermore, a relationship between ribociclib exposure and QTc prolongation has been established, which was described by a log-linear mixed effect model. Mean QTc prolongation was 22.87 ms at the mean steady-state C_{max} of 2237 ng/mL. No exposure-toxicity relationship could be demonstrated for hepatotoxicity, due to the limited number of grade 3 or 4 events.[46]

Population pharmacokinetic models

A population pharmacokinetic model has been developed based on pooled data of 208 patients of whom 4731 pharmacokinetic samples were available. The model was validated using a dataset consisting of 175 pharmacokinetic samples from 93 patients in the MONALEESA-2 study. A two-compartment model with delayed zero-order absorption and linear clearance best fitted the data, with dose and body weight being significant covariates on clearance. Clearance decreased with increasing dose, which is in line with the observed more than dose-proportional increase in exposure.[46]

ABEMACICLIB

Abemaciclib was the third CDK4/6 inhibitor approved by the FDA and EMA in 2018. In the MONARCH-3 study, abemaciclib increased mPFS compared to placebo (14.7 months vs. not reached, HR 0.54 (95% CI: 0.41-0.72), $p < 0.001$) in the first-line setting combined with anastrozole or letrozole.[57] In the same way, the MONARCH-2 study demonstrated that abemaciclib was superior to placebo in the second-line setting in combination with fulvestrant.[58]

In contrast to palbociclib and ribociclib, abemaciclib is dosed twice daily (BID) and in a continuous dosing schedule. In the dose escalation part of the phase I study, doses up to 275 mg BID have been evaluated with 200 mg BID being identified as MTD.[59] This is the recommended dose for abemaciclib monotherapy, whereas 150 mg BID is the recommended dose for combination therapy (i.e. with aromatase inhibitors or fulvestrant), because of better tolerability. Although fatigue was the most common DLT, gastrointestinal and hematological toxicities were also frequently observed.[59]

Physiochemical properties and formulation

Abemaciclib is a synthetic *N*-(5-((4-ethylpiperazin-1-yl)methyl)pyrididin-2-yl)-5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1*H*-benzo[*d*]imidazole-6-yl)pyrimidin-2-amine. It is formulated in tablets containing 50, 100, 150 or 200 mg of the free base. Since capsules were used in the pivotal MONARCH-1 and MONARCH-2 trials, bioequivalence between both formulations was tested and confirmed. Abemaciclib is a tribasic compound with pK_a values of 3.80, 4.48 and 7.95, and a log P of 3.36. It is soluble over the pH range of 1.0-6.8 (solubility > 0.8 mg/mL), and classified as a highly soluble drug. Considering that abemaciclib showed a moderate permeability (predicted $hP_{eff} = 2.46*10^{-4}$ cm/s), it was classified as a BCS class 3 (high solubility, low permeability) drug.[60,61]

Abemaciclib is a potent, ATP-competitive, reversible inhibitor of CDK4 and CDK6, with IC_{50} values of 2 and 10 nM, respectively, corresponding to plasma concentrations of 27.4-136.9 ng/mL after correcting for protein binding.[62] Abemaciclib has three active metabolites with similar potency: N-desethylabemaciclib (M2), hydroxyabemaciclib (M20) and hydroxy-N-desethylabemaciclib (M18) (**Figure 2**). Their IC_{50} values (nM) for CDK4 and CDK6 are 1.2 and 1.3 for M2, 1.5 and 1.9 for M20 and 1.5 and 2.7 for M18.[63,64]

Drug transporters

Abemaciclib is a substrate of efflux transporters P-gp and BCRP. *In vivo* studies showed that abemaciclib penetration through the blood-brain barrier improved in P-gp deficient mice.[14] The abemaciclib metabolite M2 is also a substrate of P-gp and BCRP, and its exposure increased significantly around 5.3-fold in P-gp/BCRP deficient mice with respect to the wild-type. Also, in this mouse model the brain penetration of both abemaciclib and

M2 increased 25- and 4-fold, respectively, compared to the wild-type.[65] Additionally, abemaciclib itself inhibits P-gp and BCRP.[66,67]

The renal transporters OCT2, MATE1 and MATE2-K are reversibly inhibited by abemaciclib and its active metabolites M2 and M20 at clinically relevant concentrations.[67,68] *In vitro* studies have shown that OCT2, MATE1 and MATE-K metformin transport is inhibited in the presence of abemaciclib, M2 or M20. The clinical implications of this interaction were also determined (**Table 4**).[68] This reversible inhibition of renal transporters has been related to elevated creatinine levels, without renal function being affected.[68]

Clinical pharmacokinetics

Abemaciclib pharmacokinetics is summarized in **Table 2**, and is characterized by a high variability. It showed a relatively modest absolute oral bioavailability of 45%.[59,69,70] Abemaciclib and its active metabolites showed a high protein binding of 96.3% for abemaciclib, 93.4% for M2, 96.8% for M18 and 97.8% for M20. The mean volume of distribution is 750 L.[67,71] Abemaciclib is cleared mainly by hepatic metabolism, primarily by CYP3A4 with the formation of M2, M20 and M18 (**Figure 2**). The AUC of these metabolites represent 25%, 26% and 13%, respectively, of the total circulating entities in plasma.[67] In a mass balance study, abemaciclib was excreted as metabolites mainly in feces, with 81% of the administered dose recovered in feces, and ≈3% in urine.[67,71]

Pharmacokinetics in special populations

Pediatric cancer patients

Information on abemaciclib pharmacokinetics in the pediatric population is not available hitherto.[72] Currently, two phase I studies are ongoing.[70,71]

Patients with renal impairment

No dedicated study has evaluated the effect of renal impairment on the pharmacokinetics of abemaciclib. However, a population pharmacokinetic analysis, including patients with normal renal function (n=483), mild (n=381) and moderate (n=126) renal impairment, showed no significant differences in abemaciclib exposure. Therefore, no dose adjustment is required in patients with mild or moderate renal impairment. This was expected since the renal clearance of abemaciclib and its active metabolites is minor. The effect of severe renal impairment has not been determined yet.[64,67,71,75]

Patients with hepatic impairment

In a clinical trial, the total exposure of abemaciclib plus M2, M20 and M18 was similar in participants with mild and moderate hepatic impairment, showing an increase of 20% and 10%, respectively, compared to participants with normal hepatic function. In contrast, severe hepatic impairment resulted in a 140% increase in exposure of abemaciclib active entities. Furthermore, the mean plasma half-life of abemaciclib was prolonged ($t_{1/2}$=55 h

vs. 24 h in healthy subjects), absorption was slower (t_{max}=24 h vs. 7 h in healthy subjects) and protein binding decreased. Consequently, it is recommended to reduce the dose frequency to a once daily administration for patients with severe hepatic impairment (i.e. Child-Pugh class C).[64,67,71,75]

Other factors influencing abemaciclib pharmacokinetics

The influence of intrinsic factors on the abemaciclib pharmacokinetics was evaluated in a population pharmacokinetic analysis (n=994), in which sex, age, race and body weight were found to be insignificant covariates for the abemaciclib exposure.[71,76] As a result, no special dose adjustments are required.

Food effect

An overview of food-effect studies for abemaciclib using a capsule or tablet formulation is provided in **Table 3**. The food-effect study with the tablet formulation is the most relevant since abemaciclib is marketed in this formulation. The exposure of abemaciclib increased with concomitant administration of a high-fat meal, but this was deemed not clinically relevant considering the high inter-subject variability and the fact that changes in exposure were within the abemaciclib therapeutic window.[77,78] Therefore, abemaciclib can be administered with or without food.

Drug-drug interactions

Drug-drug interaction studies for abemaciclib are summarized in **Table 4**. The potential pharmacokinetic interaction between abemaciclib and fulvestrant or aromatase inhibitors was not formally evaluated. However, historical comparisons indicated that these drugs had no clinically relevant effect on the pharmacokinetics of abemaciclib, or vice versa.[67,71,75]

Due to the extensive metabolism of abemaciclib via CYP3A4, the exposure of abemaciclib plus its active metabolites M2, M20 and M18 is substantially affected when co-administered with strong CYP3A4 modulators.

Additionally, interactions with abemaciclib as a perpetrator could occur with substrates of transporters inhibited by abemaciclib (i.e. P-gp and renal transporters, **Table 4**).

Pharmacokinetic-pharmacodynamic relationships

Exposure-response

In a preclinical pharmacokinetic-pharmacodynamic model of xenograft tumors, $C_{min} \geq 200$ ng/mL has been identified as a potential efficacy threshold. Simulations with this model indicated that a maximum decrease in phosphorylated Rb levels was attained at a dose of 50 mg/kg, corresponding to a C_{min} of 200 ng/mL. A limitation of this study is that concentrations of the active metabolites M2 and M20 were not taken into account.[79]

In all three MONARCH-studies, exposure-response relationships were demonstrated. Although abemaciclib in the MONARCH-1 study (n=132) could not be linked to objective response rate (ORR) and PFS, simulations with a pharmacokinetic-pharmacodynamic model found a positive relationship between exposure and tumor shrinkage. Also, these simulations suggested that the ORR would be higher at an abemaciclib dose of 200 mg BID compared with 150 mg BID (31% vs. 25%, respectively).[64] Using a similar approach, higher abemaciclib exposure was related to an increased tumor shrinkage in the MONARCH-2 study (n=477) as well, with the effect being most pronounced in the first months after start of treatment.[64] Finally, in the MONARCH-3 study (n=393) an exposure-response relationship was not only established for tumor size reduction, but also for PFS.[63]

In summary, abemaciclib exposure was related to efficacy in several clinical trials. Therefore, it has been suggested that from an efficacy point of view, 200 mg BID would be a better starting dose than 150 mg BID. However, this higher starting dose is not deemed feasible, since 50% of patients needs a dose reduction due to toxicity. Based on the available data, no specific target for TDM can be proposed yet, but the optimal target might probably be somewhere between 169-197 ng/mL (i.e. the median exposure at 150 mg and 200 mg, respectively). Future exposure-response analyses need to identify the optimal threshold for efficacy, which could be performed using data from the MONARCH-studies or from a real-world patient cohort. Preferably, it should also be investigated whether it has additional value to include the concentrations of the active metabolites in this threshold, or that abemaciclib concentrations alone could serve as a proxy.

Exposure-toxicity

Higher abemaciclib concentrations were related to an increased incidence and severity of neutropenia. Dynamic pharmacokinetic-pharmacodynamic models for neutrophil count have been developed using data of the MONARCH-2 (n=593) and MONARCH-3 (n=477) studies. In these models, higher total C_{max} of abemaciclib and its active metabolites was related to a greater decrease in neutrophil production rate, and thus an increased risk of neutropenia.[63,64]

Population pharmacokinetic models

In a population pharmacokinetic model based on data obtained from the phase I study (n=224), abemaciclib pharmacokinetics were best described by a linear one-compartment model with time- and dose-dependent bioavailability. Relative bioavailability decreased with an increasing dose, being 10% lower at a dose of 200 mg compared with 150 mg. This could be attributed to a saturable absorption, which is in line with preclinical data.[79] Plasma exposure of abemaciclib also decreased over time with steady-state concentrations being attained after 70 days.[76,79]

DISCUSSION

By providing an overview of the clinical pharmacokinetics and pharmacodynamics of the three licensed CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib, it becomes apparent that they share several characteristics. Similarities include the high interindividual variability in exposure, the predominant metabolism by CYP3A4, the brain penetration being limited by efflux transporters and the exposure-toxicity relationship for neutropenia. On the other hand, there are also substantial differences. First, abemaciclib has a divergent dosing schedule, as it is dosed twice daily and continuously, instead of once daily and intermittently for palbociclib and ribociclib. Second, dose-proportionality of pharmacokinetics varies between compounds. Palbociclib exposure increases linearly with increasing dose, whereas ribociclib exhibits a more than dose-proportional dose-exposure relationship, and abemaciclib exposure, in contrast, increases less than proportionally with increasing dose, due to the lower fraction absorbed at higher doses. Third, abemaciclib has active and significantly abundant metabolites that should be taken into account when assessing its exposure (i.e. M2, M20 and M18), while this is not the case for palbociclib and ribociclib. Fourth, a clear exposure-efficacy relationship has been described for abemaciclib, while for palbociclib and ribociclib exposure-response analyses remain inconclusive. This might be explained by the applied methodologies and sample sizes that were used in these exposure-response analyses. It is important to further elucidate exposure-response relationship for all three CDK4/6 inhibitors. And finally, ribociclib frequently prolongs the QTc interval, in an exposure-related manner, whereas this, to our current knowledge, has not been reported for palbociclib and abemaciclib. These particular characteristics may support in selecting the most appropriate CDK4/6 inhibitor for individual patients.

Interestingly, the incidence of neutropenia is much lower for abemaciclib than for palbociclib and ribociclib. This is possibly caused by the greater selectivity of abemaciclib for CDK4 compared with CDK6, its twice daily dosing schedule, or the conversion to metabolites with less hematologic toxicity.[59,62] In general, the effect of CDK4/6 inhibitors on neutrophil progenitor cells is cytostatic rather than cytotoxic, and associated with a notably low incidence of febrile neutropenia, in contrast to chemotherapy.[37]

Many patients require dose reductions due to neutropenia, which can remain problematic even at the lowest doses according to the label (i.e. 75 mg QD for palbociclib, 200 mg QD for ribociclib and 50 mg BID for abemaciclib). If exposure in these patients is low, switching to an alternative treatment might be preferred, whereas in patients with adequate exposure prolonging the dose interval to every other day (QAD) for palbociclib and ribociclib, or QD for abemaciclib, could be an option, as has previously been described for pazopanib.[80] Alternatively, the time off-treatment could be prolonged (i.e. 2-weeks-on/2-

weeks-off treatment, as was allowed in the PALOMA-3 study). From a pharmacological point of view, though, prolonging the dose interval would be more rational.

Although it is known that CDK4/6 inhibitors combined with endocrine therapy provide an effective treatment strategy, it is currently unclear whether CDK4/6 inhibitors can best be added to first- or second-line treatment. This paramount question is currently being addressed in the SONIA study, a nationwide study in The Netherlands that will randomize 1000 patients between first- and second-line treatment with a CDK4/6 inhibitor.[81] In an additional side study, pharmacokinetic samples are collected to further elucidate exposure-response relationships.

The currently approved CDK4/6 inhibitors are predominantly metabolized by CYP3A4. Therefore, increased exposure, and hence an increased risk of toxicity, can be expected in patients harboring mutations as in CYP3A4*22, as a result of lower levels of functional CYP3A4 and thus a decreased clearance. The reported prevalence of these mutations is up to 10% [82], and it could be argued that this subset of patients may benefit from a lower starting dose. This is currently being investigated in the STAR22 study.[83]

Although CDK4/6 inhibitors are currently only approved for the treatment of breast cancer, they are in clinical development for many other indications**.**

CONCLUSIONS

CDK4/6 inhibitors are a new class of promising oral targeted therapies in oncology, with complex pharmacokinetic and pharmacodynamic profiles, which we summarized in this review. Future studies should focus on the further exploration of exposure-response relationships and the potential for pharmacokinetically guided dose individualization.

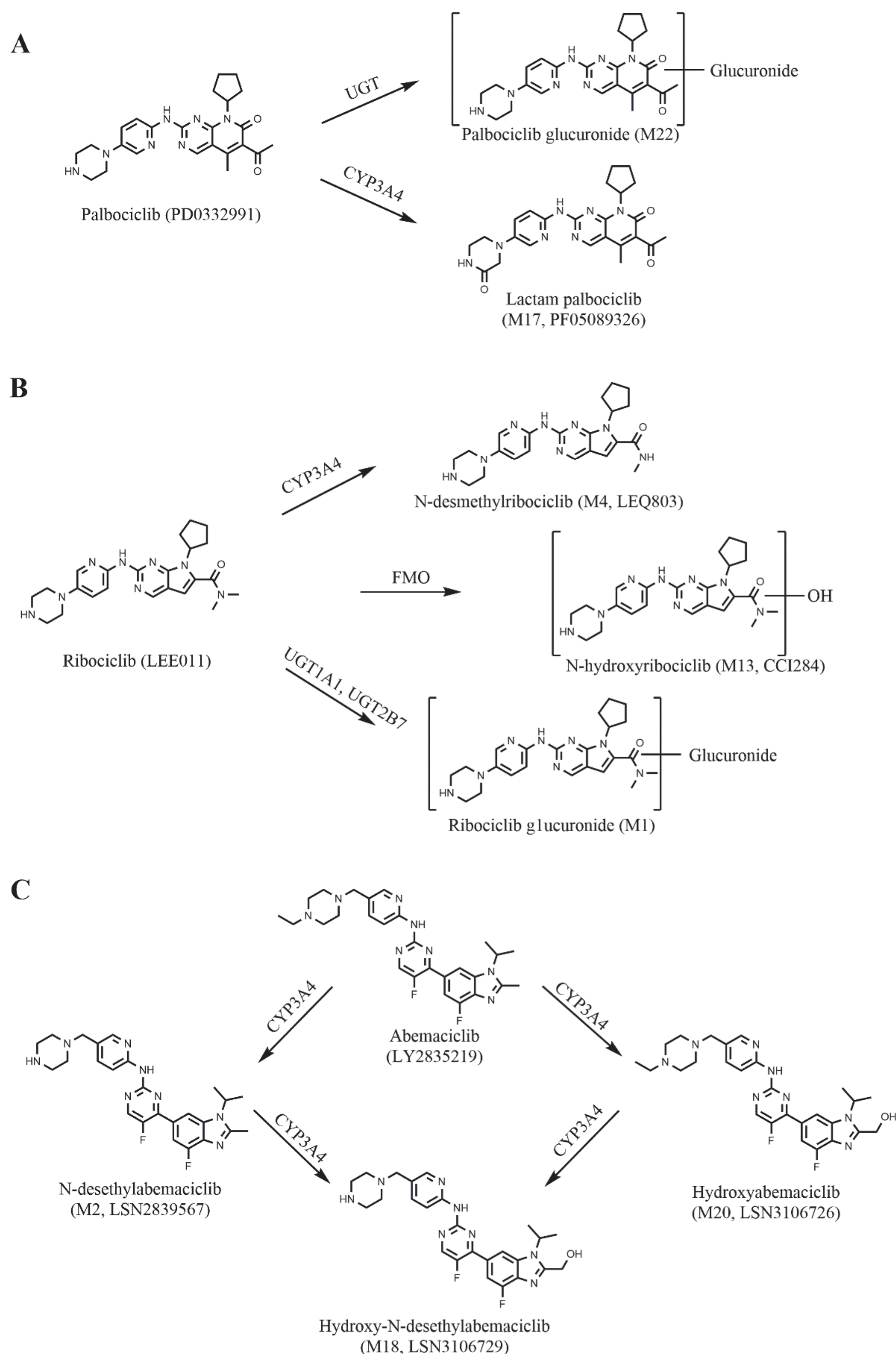

Figure 2 – Chemical structures of CDK4/6 inhibitors palbociclib (A), ribociclib (B) and abemaciclib (C) and their main metabolites

Chemical structures and metabolism were obtained from FDA and EMA reviews.[9,46,75] This figure was created using ChemDraw Professional 15.0.

UGT = Uridine 5'-diphospho-glucuronosyltransferase; CYP3A4 = cytochrome P450 3A4; FMO = Flavin-containing monooxygenase

Table 2 – Selected steady-state pharmacokinetic parameters of CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib

CDK4/6 inhibitor	Study	N	C_{min} (ng/mL)	C_{max} (ng/mL)	$AUC_{0-\tau}$ (ng/mL*h)	t_{max} (h)	$t_{1/2}$ (h)
Palbociclib	Flaherty *et al.*[5]	13	NR	86 (34%)	NR	4 (1-10)	NR
	Flaherty *et al.*[5]	4	47.0[a] (48%)	97.4[a] (41%)	1733[a] (42%)	5.5 (2.0-9.8)	25.9 (29%)
	Mukai *et al.*[c,25]	142	61.7[b] (59%)	NR	NR	NR	NR
	Tamura *et al.*[84]	6	88.5 (49%)	185.5 (27%)	2838 (43%)	4.0 (4.0-6.0)	23.2 (33%)
	PALOMA-1, A5481003[9]	12 (C_{min}: 71)	60.8[b] (42%)	116[b] (28%)	1982[b] (29%)	7.9 (2.2-8.2)	28.8 (17%)
	PALOMA-3, A5481023[27]	218	76.6[b] (41%)	NR	NR	NR	NR
Ribociclib	Curigliano *et al.*[85]	3	NR	3083[a] (31%)	38896[a,d] (43%)	2 (2-2)	NR
	Samant *et al.*[44]	13	NR	1620[b] (53%)	21100[b,d] (57%)	NR	NR
	Samant *et al.*[44]	48	NR	1870[b] (60%)	23700[b,d] (61%)	NR	NR
	Samant *et al.*[44]	36	711[b] (73%)	NR	NR	NR	NR
	Doi *et al.*[e,51]	8	NR	3280[b] (60%)	51600[b] (59%)	5.0 (4.0-7.6)	53.6 (45%)
	Infante *et al.*[h,42,46]	64	732 (80%)	2130 (59%)[d]	NR	NR (1-5)	32.6[a]
Abemaciclib	Patnaik *et al.*[59]	72 (150 mg) 52 (200 mg)	169[b] (95%) 197[b] (82%)	249 [b] (86%) 298 [b] (72%)	2390 (90%)[b,d] 3000 (69%)[b,d]	4 (0-10.2) 4 (0-10)	22.8 (8.9-60.8)[f] 21.3 (11.6-63.0)[f]
	Fujiwara *et al.*[69]	2 (150 mg) 5 (200 mg)	1176, 103[g] 210[b] (89%)	1381, 149[g] 298[b] (64%)	15500, 1,460[g] 3072[b] (73%)	4.0 (4.0-4.0) 4 (2.1-6.0)	21.9 (19.3-24.6)[f] 16.3 (14.2-22.6)[f]
	Kim *et al.*[86]	2 (150 mg) 9 (200 mg)	NR	146, 183[g] 483[b] (41%)	1060, 1,600[g] 3460[b] (49%)	4.0 (0-7.9) 4.0 (0-9.7)	NR
	Kim *et al.*[86]	4 (150 mg) 6 (200 mg)	NR	288[b] (71%) 304[b] (66%)	2060[b] (66%) 2100[b] (58%)	5.5 (4.0-8.0) 6.9 (0-7.9)	NR
	Kim *et al.*[86]	5 (150 mg) 8 (200 mg)	NR	492[b] (117%) 227[b] (17%)	3460[b] (125%) 1380[b] (144%)	1.0 (0-8.0) 5.0 (0-8.0)	NR

Reported pharmacokinetic parameters were determined at steady-state, at the approved dose and in patients. Pharmacokinetic parameters are reported as median, unless indicated otherwise. Variability is reported as (CV%) or [90% confidence interval].

[a] arithmetic mean
[b] geometric mean
[c] in non-Asian patients, C_{min} in Japanese patients (n=27) was 95.4 ng/mL (31.3%) and C_{min} in other Asian patients (n=11) was 90.1 ng/mL (36.0%)
[d] based on patient numbers < N
[e] in Japanese patients
[f] after a single dose
[g] individual values are reported if N < 3
[h] C_{min} and C_{max} values of study X2101 were reported in the FDA review, t_{max} and $t_{1/2}$ in the paper of Infante *et al.*

$AUC_{0-\tau}$ = area under the plasma-concentration time curve until next dose; C_{max} = maximum plasma concentration; C_{min} = minimum plasma concentration; CV = coefficient of variation; NA = not applicable; NR = not reported; SD = standard deviation; $t_{1/2}$ = terminal elimination half-life; t_{max} = time to maximum concentration

Table 3 – Overview of food-effect on the pharmacokinetics of CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib

CDK4/6 inhibitor	Study	Food-effect	Results	Conclusion
Palbociclib	Ruiz-Garcia *et al.*[28], n=28, capsules	low fat vs. fasted	↑ $AUC_{0-\infty}$ 12%[a] ↑ C_{max} 28%[a]	Concomitant intake with food resulted in higher exposure, while variability was substantially lower. Therefore, palbociclib capsules should be administered concomitant with food.
		moderate fat vs. fasted	↑ $AUC_{0-\infty}$ 12%[a] ↑ C_{max} 24%[a]	
		high fat vs. fasted	↑ $AUC_{0-\infty}$ 19%[a] ↑ C_{max} 37%[a]	
	A5481081[11], n=44, tablets	moderate fat vs. fasted	↑ $AUC_{0-\infty}$ 9%[a] ↑ C_{max} 10%[a]	No relevant food-effect. Therefore, palbociclib tablets could be administered with or without food.
		high fat vs. fasted	↑ $AUC_{0-\infty}$ 22%[a] ↑ C_{max} 26%[a]	
Ribociclib	Samant *et al.*[44], n=24, tablets	high fat vs. fasted	↑ $AUC_{0-\infty}$ 6%[a] ↓ C_{max} 0.3%[a]	No relevant food-effect. Therefore, ribociclib can be administered with or without food.
	CLEE011A2111[46], n=24, capsules	high fat vs. fasted	↓ $AUC_{0-\infty}$ 0.6% ↑ C_{max} 32%	No relevant food-effect. Therefore, ribociclib capsules can be administered with or without food.
Abemaciclib	Turner *et al.*[70,87], n=23, capsules	high fat vs. fasted	↑ $AUC_{0-tlast}$ 15%[a] ↑ C_{max} 24% ↑ t_{max} 2 h	No relevant food-effect. Therefore, abemaciclib capsules can be administered with or without food.
		standard meal vs. fasted	↑ $AUC_{0-tlast}$ 11%[a] ↑ C_{max} 25%	
	Turner *et al.*[70,88], n=29, capsules	high fat diet vs. fasted	↑ $AUC_{0-\infty}$ 26%[a] ↑ C_{max} 37%[a]	No relevant food-effect. Therefore, abemaciclib capsules can be administered with or without food.
	Turner *et al.*[77,78], n=24, tablets	high fat vs. fasted	↑ $AUC_{0-\infty}$ 13%[a] ↑ C_{max} 30%[a]	No relevant food-effect. Therefore, abemaciclib tablets can be administered with or without food.

All reported studies were randomized crossover studies in healthy volunteers, in which a single dose of the CDK4/6 inhibitor was administered.

[a] Calculated based on $AUC_{0-\infty}$ and C_{max} values

AUC = area under the plasma-concentration time curve; C_{max} = maximum plasma concentration; t_{max} = time to maximum concentration

Table 4 – Overview of drug-drug interactions of CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib

CDK4/6 inhibitor	Study	Interacting compound	Results	Conclusion
Palbociclib	Hoffman *et al.*[89], n=12	itraconazole *(strong CYP3A4 inhibitor)*	↑ $AUC_{0-\infty}$ 87% ↑ C_{max} 34%	Clinically relevant interaction, concomitant use with strong CYP3A4 inhibitors should be avoided, otherwise a dose reduction to 75 mg QD is recommended.
	Hoffman *et al.*[90], n=15	rifampicin *(strong CYP3A4 and SULT inducer)*	↓ $AUC_{0-\infty}$ 85% ↓ C_{max} 70%	Clinically relevant interaction, concomitant use with strong CYP3A4 inducers should be avoided.
	Hoffman *et al.*[91], n=14	modafinil *(moderate CYP3A4 inducer)*	↓ $AUC_{0-\infty}$ 32% ↓ C_{max} 11%	No clinically relevant interaction, could thus be used concomitantly with moderate CYP3A4 inducers.
	Hoffman *et al.*[31], n=26	midazolam *(CYP3A4 substrate)*	midazolam: ↑ $AUC_{0-\infty}$ 61% ↑ C_{max} 37%	Palbociclib is a moderate CYP3A inhibitor, caution is recommended if administered concomitantly with sensitive CYP3A substrates with narrow therapeutic index.
	PALOMA-1[9], n=12, patients	letrozole on palbociclib palbociclib on letrozole	↓ AUC_{0-24h} 2%[a] ↓ C_{max} 6%[a] ↓ AUC_{0-24h} 10%[a] ↓ C_{max} 9%[a]	No clinically relevant interaction.
	PALOMA-3[27], n=40, patients	fulvestrant on palbociclib[b] palbociclib on fulvestrant[c] goserelin on palbociclib[d] palbociclib on goserelin	↑ C_{min} 29%[a] ↑ C_{min} 22%[a] ↓ C_{min} 7%[a] ↑ C_{min} 10%[a]	No clinically relevant interaction.
	Sun *et al.*[32], n=26 (fasting), n=27 (fed), capsules	rabeprazole (fasting)	↓ $AUC_{0-\infty}$ 62%[a] ↓ C_{max} 80%[a]	No clinically relevant interaction under fed conditions, could thus be used concomitantly.

		rabeprazole (fed)	↓ $AUC_{0-\infty}$ 13%[a] ↓ C_{max} 41%[a]	
		famotidine	↓ $AUC_{0-\infty}$ 4%[a] ↓ C_{max} 5%[a]	
		local antacid	↑ $AUC_{0-\infty}$ 5-6%[a] ↓ C_{max} 4%[a]	
	A5481091[11], n=44, tablets	rabeprazole	↑ $AUC_{0-\infty}$ 6%[a] ↓ C_{max} 3%[a]	No clinically relevant interaction, could thus be used concomitantly.
	Yu *et al.*[29], PBPK simulations	diltiazem *(moderate CYP3A4 inhibitor)*	↑ AUC_{0-t} 42% ↑ C_{max} 23%	No clinically relevant interactions, no dose adjustments needed.
		verapamil *(moderate CYP3A4 inhibitor)*	↑ AUC_{0-t} 38% ↑ C_{max} 22%	
		fluoxetine *(weak CYP3A4 inhibitor)*	↑ AUC_{0-t} 3%	
		fluvoxamine *(weak CYP3A4 inhibitor)*	↑ AUC_{0-t} 4%	
		efavirenz *(moderate CYP3A4 inducer)*	↓ AUC_{0-t} 38% ↓ C_{max} 32%	
Ribociclib	CLEE011A2101[46,49], n=24	ritonavir *(strong CYP3A4 inhibitor)*	↑ $AUC_{0-\infty}$ 220% ↑ C_{max} 70%	Clinically relevant interaction, concomitant use with strong CYP3A4 inhibitors should be avoided, otherwise a dose reduction to 400 mg QD is recommended.
		rifampicin *(strong CYP3A4 inducer)*	↓ $AUC_{0-\infty}$ 89% ↓ C_{max} 81%	Clinically relevant interaction, concomitant use with CYP3A4 inducers should be avoided.
	CLEE011A2106[46,49], n=25, 400 mg[e]	midazolam *(CYP3A4 substrate)*	midazolam: ↑ AUC 280% ↑ C_{max} 110%	Ribociclib is a moderate CYP3A4 inhibitor, caution is recommended if administered concomitantly with sensitive CYP3A4 substrates with narrow therapeutic index.

		caffeine *(CYP1A2 substrate)*	caffeine: ↑ AUC 20% ↓ C_{max} 10%	Ribociclib is a weak CYP1A2 inhibitor, no dose adjustments are needed.
	CLEE011E2301, n=15-18 CLEE011X2106, n=11 CLEE011X2101[b,4,6], n=64, patients	letrozole, anastrozole, exemestane	concentrations of monotherapy and combination therapy overlapped	No clinically relevant interaction.
	Samant *et al.*[f,44], n=2-48	proton pump inhibitors	↑/↓ $AUC_{0-\tau}$ 9-23%[a] ↑/↓ C_{max} 10-23%[a] ↓C_{min} 17%[a]	No clinically relevant interaction, could thus be used concomitantly.
	PBPK simulations[46]	erythromycin *(moderate CYP3A4 inhibitor)*	↑ $AUC_{0-\tau}$ 93% ↑ C_{max} 29%	Clinically relevant interaction, no initial dose adjustment needed, but close monitoring for signs of toxicity
		ketoconazole *(strong CYP3A4 inhibitor)*	↑ $AUC_{0-\tau}$ 209% ↑ C_{max} 50%	Clinically relevant interaction, concomitant use with strong CYP3A4 inhibitors should be avoided, otherwise a dose reduction to 400 mg QD is recommended.
		fluvoxamine *(weak CYP3A4 inhibitor)*	↑ $AUC_{0-\tau}$ 2% ↑ C_{max} 1%	No clinically relevant interaction.
		carbamazepine *(strong CYP3A4 inducer)* efavirenz *(moderate CYP3A4 inducer)*	↓ $AUC_{0-\infty}$ 52% ↓ C_{max} 34% ↓ $AUC_{0-\infty}$ 60% ↓ C_{max} 37%	Clinically relevant interaction, concomitant use with CYP3A4 inducers should be avoided.
Abemaciclib	NCT02117648[67], n=26, patients	clarithromycin *(strong CYP3A4 inhibitor)*	↑ $AUC_{0-tlast}$ 237%/119%[g] ↑ C_{max} 30%/7%[g] ↑ $t_{1/2}$ 120%	Clinically relevant interaction, concomitant use with strong CYP3A4 inhibitors should be avoided, otherwise a dose reduction to 100 mg BID is recommended.

	NCT02256276[87], n=24	rifampicin *(strong CYP3A4 inducer)*	↓ $AUC_{0\text{-tlast}}$ 95%/77%[g] ↓ C_{max} 92%/45%[g]	Clinically relevant interaction, concomitant use with CYP3A4 inducers should be avoided.
	Chappell *et al.*[68], n=40	metformin *(OCT2, MATE1 and MATE2-k substrate)*	metformin: ↑ $AUC_{0\text{-}\infty}$ 37% ↑ C_{max} 22%	Abemaciclib inhibits the renal transport proteins OCT2, MATE1 and MATE2-k.
	NCT02677844[92], n=35	loperamide *(P-gp substrate)*	loperamide: ↑ $AUC_{0\text{-tlast}}$ 13% ↑ C_{max} 35%	No clinically relevant interaction, concomitant use is possible.
	Posada *et al.*[61], PBPK simulations	diltiazem *(moderate CYP3A4 inhibitor)* verapamil *(moderate CYP3A4 inhibitor)*	↑ $AUC_{0\text{-}\infty}$ 290%/137%[h] ↑ C_{max} 90% ↑ $AUC_{0\text{-}\infty}$ 127%/62%[h] ↑ C_{max} 63%	Clinically relevant interaction, no initial dose adjustment needed, but close monitoring for signs of toxicity.
		itraconazole *(strong CYP3A4 inhibitor)* ketoconazole *(strong CYP3A4 inhibitor)*	↑ $AUC_{0\text{-}\infty}$ 611%/278%[h] ↑ C_{max} 117% ↑ $AUC_{0\text{-}\infty}$ 1470%/615%[h] ↑ C_{max} 146%	Clinically relevant interaction, concomitant use with strong CYP3A4 inhibitors should be avoided (especially ketoconazole), otherwise a dose reduction to 100 mg BID is recommended.
		efavirenz *(moderate CYP3A4 inducer)* bosentan *(moderate CYP3A4 inducer)* modafinil *(weak CYP3A4 inducer)*	↓ $AUC_{0\text{-}\infty}$ 69%/52%[h] ↓ C_{max} 51% ↓ $AUC_{0\text{-}\infty}$ 68%/42%[h] ↓ C_{max} 60% ↓ $AUC_{0\text{-}\infty}$ 46%/29%[h] ↓ C_{max} 34%	

Reported studies were performed in healthy volunteers using a single dose of the CDK 4/6 inhibitor, unless indicated otherwise.
[a] calculated based on AUC and C_{max} values
[b] no intra-individual comparison, but interindividual comparison with historical data
[c] no intra-individual comparison, but interindividual comparison between treatment and placebo arm
[d] no intra- individual comparison, but interindividual comparison between pre- and postmenopausal patients
[e] simulations predicted that for 600 mg, midazolam C_{max} and AUC would increase 140% and 420%, respectively
[f] no intra-individual comparison, but interindividual comparison between patients with and without a proton pump inhibitor

[g] abemaciclib/total active species

[h] abemaciclib/potency-adjusted unbound active species

AUC = area under the plasma-concentration time curve; BID = twice daily; C_{max} = maximum plasma concentration; CYP = cytochrome P450; MATE = multidrug and toxin extrusion protein; OCT = organic cation transporter; PBPK = physiologically-based pharmacokinetic; QD = once daily; SULT = sulfotransferase; t_{max} = time to maximum concentration

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Effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib: a randomized cross-over trial in patients with breast cancer

Interim analysis

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The results described in this chapter have not been published yet and include preliminary data that could deviate from the final publication

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Determination of the absolute bioavailability of oral imatinib using a stable isotopically labeled intravenous imatinib-d8 microdose

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ABSTRACT

Purpose

The aim of this study was to ascertain whether the absolute bioavailability of oral imatinib (Glivec®) during steady state plasma pharmacokinetics in cancer patients could be determined through a concomitant intravenous administration of a single 100 µg microdose of deuterium labeled imatinib (imatinib-d8). Secondly, the usefulness of liquid chromatography-tandem mass spectrometry (LC-MS/MS) was investigated for simultaneous analysis of orally and intravenously administered imatinib.

Methods

Included patients were on a stable daily dose of 400 mg oral imatinib prior to study participation. On day 1, patients received a 100 µg intravenous imatinib-d8 microdose 2.5 h after intake of the oral dose. Plasma samples were collected for 48 h. Imatinib and imatinib-d8 concentrations were simultaneously quantified using a validated LC-MS/MS assay. The absolute bioavailability was calculated by comparing the dose-normalized exposure to unlabeled and stable isotopically labeled imatinib in plasma.

Results

A total of six patients were enrolled. All patients had a history of gastrointestinal stromal tumors (GIST). The median absolute bioavailability of oral imatinib at steady state was 76% (range 44-106%). Imatinib and imatinib-d8 plasma concentrations were quantified in all collected plasma samples, with no samples below the limit of quantification for imatinib-d8.

Conclusion

The absolute bioavailability of imatinib was successfully estimated at steady state plasma pharmacokinetics using the stable isotopically labeled microdose trial design. This study exhibits the use of a stable isotopically labeled intravenous microdose to determine the absolute bioavailability of an oral anticancer agent in patients with LC-MS/MS as the analytical tool.

INTRODUCTION

The last decade has shown an increasing number of anticancer drugs that are administered orally.[1–3] This so called "intravenous to oral switch" in oncology has resulted in an increased attention on the investigation of the absolute bioavailability during clinical drug development. Determining the absolute oral bioavailability of a new drug candidate facilitates the identification of potential developmental challenges such as absorption and first pass metabolism during the clinical development of a drug. Hence, the assessment of the absolute bioavailability is also crucial for the development of optimized oral formulations. As a result, data on the absolute bioavailability of novel oral drugs is now increasingly requested by the FDA and EMA.[4,5]

The conventional way to assess the absolute oral bioavailability is by using a two-period crossover study design, where an intravenous dose and an oral dose are administered to a study subject with a washout period in between. The absolute bioavailability is then calculated by dividing the plasma exposure after oral administration by the plasma exposure after intravenous administration. A limitation of this design is that for drugs that are poorly soluble in aqueous media, it might be impossible to develop an intravenous formulation at therapeutic strength. In addition, it assumes linear pharmacokinetics and constant clearance between the oral and intravenous dose event, which might not always be the case for drugs demonstrating a high intra-patient variability. This may result in a systemic error in the determination of the absolute bioavailability.[6]

A study design of co-administering an intravenous isotopically labeled microdose (≤ 100 µg, less than 1/100th of the therapeutic dose) with a therapeutic oral dose provides a solution to these problems. Because only a small amount of drug needs to be dissolved in an intravenous formulation, drug solubility issues can be circumvented. In addition, according to the current regulatory guidelines, clinical intravenous microdose studies could be carried out without additional toxicity investigations, saving costs, and time associated with intravenous drug development.[7] Furthermore, because the intravenous microdose is administered during the same dose event as the oral therapeutic dose, the study duration is shortened and intra-occasion variability is not an issue, resulting in a more accurate determination of the absolute bioavailability and increased patients convenience.

Absolute bioavailability microdose trials can be performed using either radiolabeled or stable isotopically labeled drug processed into an intravenous formulation. In recent years, accelerator mass spectrometry (AMS) to measure a radiolabeled microdose has been utilized to support several clinical absolute bioavailability studies.[8] A drawback of AMS is that sample analysis is labor and time intensive, expensive, and that AMS is only available in a limited number of places dedicated to biomedical research worldwide.[9] An alternative analytical approach for conducting microdose studies is using liquid

chromatography coupled to tandem mass spectrometry (LC-MS/MS) to quantitate both the intravenous and the oral drug. Because both labeled and unlabeled drug can be measured simultaneously with LC-MS/MS, it is an elegant and cost-effective alternative to AMS.[9,10]

For the group of tyrosine kinase inhibitors, an important class of novel oral anticancer agents, it has been demonstrated that for many drugs registered in the past years, the absolute bioavailability has not been assessed at the time of drug licensing.[3] One reason for this might be that poor drug solubility hampers the development of an aqueous intravenous dose at therapeutic strength, making it almost impossible to use the conventional crossover trial design. In this trial, we used imatinib, a tyrosine kinase inhibitor used for the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST), to demonstrate the potential of using a stable isotopically labeled 100 µg microdose in combination with LC-MS/MS to assess the absolute bioavailability.

The objective of this study was to ascertain whether the absolute bioavailability of oral imatinib (Glivec®) during steady state plasma pharmacokinetics in cancer patients could be determined through a concomitant intravenous administration of a single 100 µg microdose of deuterium labeled imatinib (imatinib-d8). Secondly, the usefulness of liquid chromatography-tandem mass spectrometry (LC-MS/MS) is investigated for simultaneous analysis of orally and intravenously administered imatinib.

MATERIALS AND METHODS

Study design and sample collection

This was a single center, open-label study in which the absolute bioavailability of imatinib (**Figure 1A**) was determined at steady state by concomitant administration of an intravenous microdose of stable isotopically labeled imatinib-d8 (**Figure 1B**). **Figure 2** provides a schematic overview of the study design. On day 1, patients received a single intravenous microdose of imatinib-d8, next to the standard treatment of imatinib 400 mg once daily (Glivec®). After intake of imatinib at approximately 08:30 a.m., a 100 µg imatinib-d8 microdose was administered intravenously as a bolus injection at the estimated maximum plasma concentration (t_{max}) of oral imatinib (2.5 h post oral dose). Oral imatinib intake was not interrupted for the duration of the study. The study (Netherlands Trial Register, NTR7642, www.nederlandstrialregister.nl) was approved by both the Medical Ethics Committee of The Netherlands Cancer Institute, Amsterdam, The Netherlands, as well as the competent authority (Centrale Commissie Mensgebonden Onderzoek, CCMO). The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to study assessments.

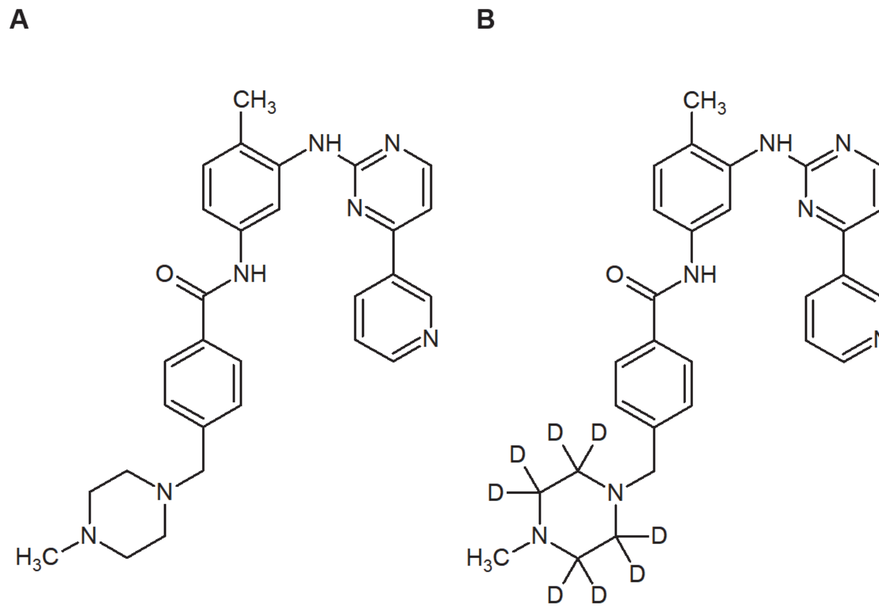

Figure 1 – Molecular structures
A: imatinib
B: imatinib-d8

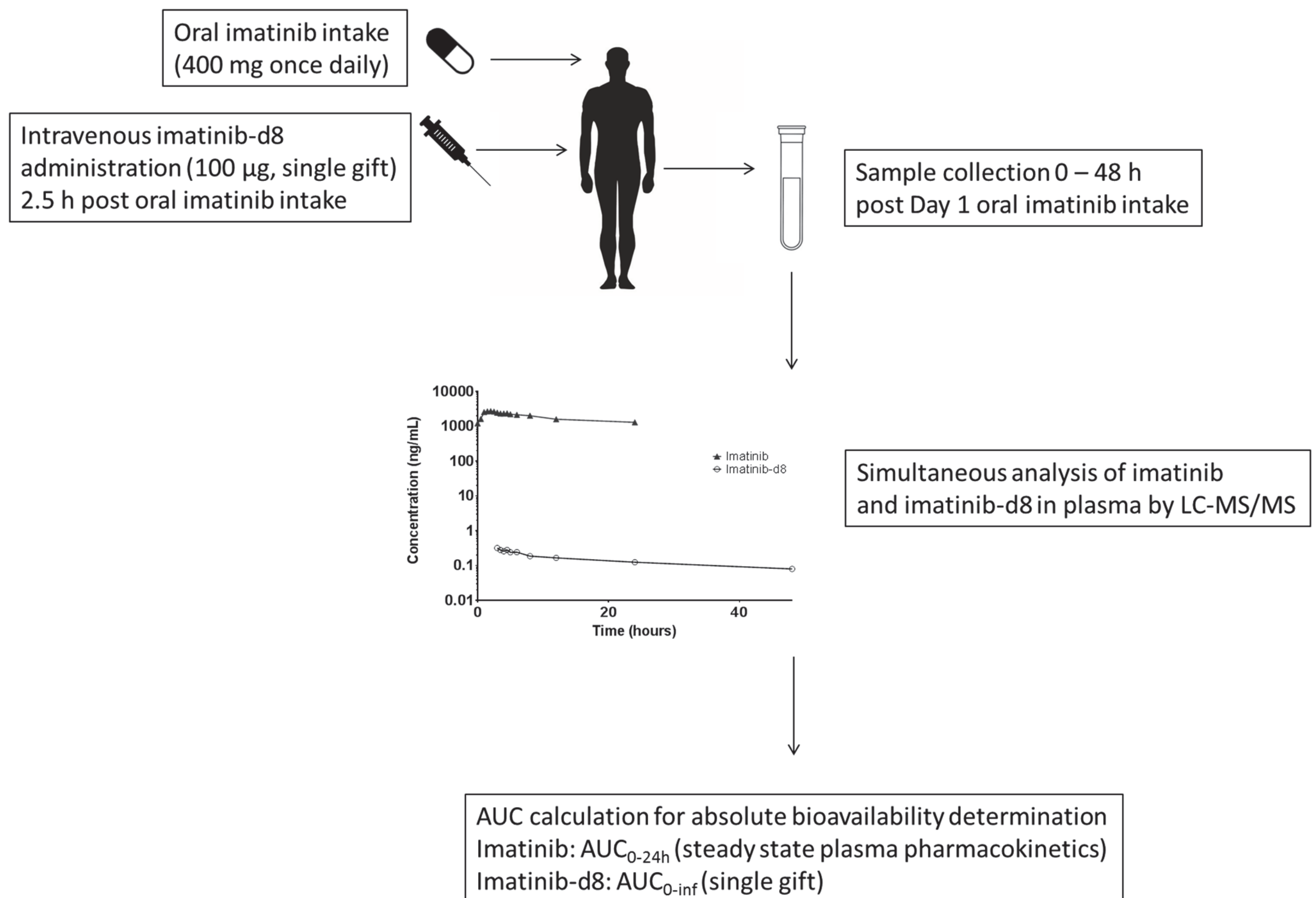

Figure 2 – Schematic overview of the imatinib absolute bioavailability microdose trial design
LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; AUC_{0-24h} = area under the plasma concentration-time curve up to 24 hours; AUC_{0-inf} = area under the plasma concentration-time curve extrapolated to infinity

Patients

Patients ≥ 18 years of age, treated with imatinib 400 mg once daily in the morning for at least 7 days (steady state plasma concentrations), were included. Subjects needed to have acceptable organ function, as evidenced by laboratory data: aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) ≤ 5x the upper limit of normal (ULN), total serum bilirubin ≤ 2x ULN, renal function as defined by glomerular filtration rate (GFR MDRD) > 40 mL/min/1.73m^2. Subjects who received treatment with inhibitors or inducers of CYP3A4 were excluded.

Treatment

Patients received 400 mg imatinib (Glivec®) tablets once daily in the morning as part of routine clinical care. According to the drug label, imatinib was ingested concomitant with a meal.[11] Meals were not standardized. The reference drug imatinib-d8 (Toronto Research Chemicals, ON, Canada) was formulated in the hospital pharmacy of The Netherlands Cancer Institute and was supplied as a 0.1 mg/mL in NaCl 0.9% solution for intravenous injection.

Sample collection, processing, and analysis

From day 1 to day 3, pharmacokinetic sampling was performed. Blood samples were collected at predose, 0.5, 1, 1.5, 2, 2.5 (pre intravenous microdose), 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24 (pre day 2 oral dose) and 48 h (pre day 3 oral dose), after oral imatinib intake.

Peripheral blood for quantification of imatinib and imatinib-d8 was drawn in 4-mL K_2 EDTA tubes and centrifuged directly after collection (1500 g, 10 min, 4°C). Plasma was stored at -80 °C until analysis. A validated LC-MS/MS assay was used for the simultaneous quantification of imatinib and imatinib-d8.[12] Routine sample analysis acceptance criteria for bioanalytical data according to FDA and EMA guidelines[13,14] were applied and results were reported using Analyst 1.6.2. software (Sciex, Framingham, MA, USA).

Pharmacokinetic analysis and absolute bioavailability calculation

Imatinib and imatinib-d8 plasma concentrations were used to determine the maximum observed plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time zero to 24 h ($AUC_{0\text{-}24h}$) for imatinib, and from time zero to infinity ($AUC_{0\text{-}inf}$) for imatinib-d8, the terminal phase half-life ($t_{½}$) and the elimination rate constant from the central compartment (k_e), the volume of distribution (V_d), and total plasma clearance (CL). Parameters were calculated using plasma concentration-time curves obtained from 0 to 24 h for imatinib, and from 0 to 48 h for imatinib-d8. Non-compartmental analysis was performed using R version 3.0.1.[15]

As the exposure at steady state plasma pharmacokinetics during the dose interval is equivalent to the exposure from zero to infinity following a single administration[16], the

$AUC_{0\text{-}24h}$ for imatinib and the $AUC_{0\text{-}inf}$ for imatinib-d8 could be used to calculate the absolute bioavailability without dose interruptions for the patients.

The absolute bioavailability (F) of oral imatinib was calculated as the ratio of dose-normalized exposures of the oral (po) imatinib and intravenous (iv) imatinib-d8 gift expressed as a percentage using the following formula:

$$F(\%) = \frac{[AUC_{0-24}]_{po}/Dose_{po}}{[AUC_{0-inf}]_{iv}/Dose_{iv}}\ x\ 100$$

RESULTS

A total of six patients have been included, with a median age of 65 years (range 52-72). Of these patients, 50% received adjuvant imatinib treatment for GIST and 50% were treated in the metastatic setting. An overview of patient baseline characteristics can be found in **Table 1**.

Table 1 – Patient baseline characteristics

Characteristic	Patients
Age, years	65 (52-72)
Gender, male	4 (67%)
Tumor type	
GIST	6 (100%)
Treatment setting	
Adjuvant	3 (50%)
Metastatic	3 (50%)
Previous surgery type	
Wedge partial resection of the stomach	3 (50%)
Partial small bowel resection	1 (17%)
Multiple resections[a]	2 (33%)
Time on imatinib treatment (in years)	3.2 (0.3-13.0)
Albumin (in g/L)	44 (42-47)
eGFR[b] (in mL/min)	69 (58-84)

Data are expressed as no. (%) or median (range), as appropriate.
[a] one patient with wedge partial resection of the stomach and partial colon resection, one patient with wedge partial resection of the stomach, splenectomy and partial pancreas resection
[b] eGFR was calculated using the MDRD-4 formula
eGFR = estimated glomerular filtration rate; GIST = gastrointestinal stromal tumour

All included patients were evaluable for pharmacokinetic analysis. Mean plasma concentration-time curves of imatinib and imatinib-d8 can be found in **Figure 3**. A summary of imatinib and imatinib-d8 pharmacokinetic parameters can be found in **Table 2**.

The absorption of imatinib after oral administration of tablets was rapid, with a median t_{max} of 2 h. The C_{max} of oral imatinib at steady state was 2.9 ± 0.8 μg/mL. The mean AUC_{0-24} for oral imatinib was 42.6 ± 12.9 μg·h/mL, and the mean AUC_{0-inf} for imatinib-d8 was 0.015 ± 0.007 μg·h/mL. The AUC_{0-inf} for imatinib-d8 normalized to a 400 mg imatinib dose was 60.5 ± 26.4 μg·h/mL. Individual plasma concentration-time curves demonstrated up to two secondary peaks after the C_{max}, with different profiles for oral imatinib and intravenous imatinib-d8 (**Supplementary Figure 1**). The ratios between the curves for oral imatinib and intravenous imatinib-d8 remained constant during the elimination phase, with a dose-normalized imatinib:imatinib-d8 ratio in plasma of 2.00 at *t* = 6 h and of 2.04 at *t* = 24 h. The $t_{½}$ and clearance of imatinib-d8 were 45.5 h and 7.6 L/h, respectively.

The absolute bioavailability (F) of oral imatinib at steady state was calculated for each individual subject. **Table 3** demonstrates that the median absolute bioavailability of oral imatinib in cancer patients was 76% (range 42-106%).

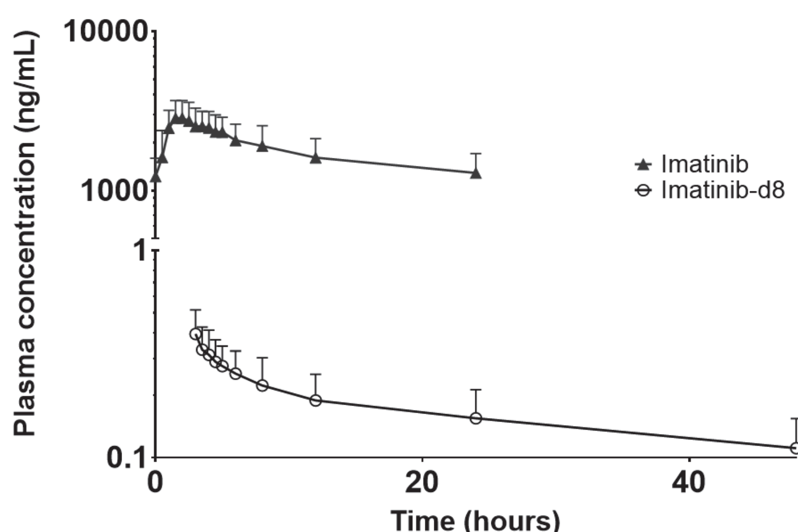

Figure 3 – Plasma concentration-time curves of imatinib and imatinib-d8 (mean ±SD) following oral administration of 400 mg imatinib dose at t=0h and intravenous administration of a 100 μg imatinib-d8 microdose at t=2.5h in patients (n=6) displaying steady state imatinib plasma pharmacokinetics

Table 2 – Summary of imatinib and imatinib-d8 steady state pharmacokinetic parameters following concomitant administration of an oral imatinib dose (400 mg) and an intravenous imatinib-d8 microdose (100 μg) in cancer patients (n=6)

Parameter		Imatinib	Imatinib-d8
C_{max} (μg/mL)	Mean	2.9	0.00051
	CV (%)	27.4	23.1
$C_{min, 0h}$ (μg/mL)	Mean	1.2	N/A
	CV (%)	27.4	N/A
t_{max} (h)	Median	2	N/A
	Range	1.5-2	N/A
AUC_{0-24} (μg·h/mL)	Mean	42.6	N/A
	CV (%)	30.2	N/A

AUC_{0-inf} (µg·h/mL)	Mean	N/A	0.015
	CV (%)	N/A	43.7
$t_{½}$ (h)	Mean	34.1	45.5
	CV (%)	46.7	37.9
k_e (h^{-1})	Mean	0.023	0.017
	CV (%)	27.5	28.1
V_d/F (L)	Mean	190	N/A
	CV (%)	26.7	N/A
V_d (L)	Mean	N/A	462
	CV (%)	N/A	28.2
CL/F (L/h)	Mean	4.2	N/A
	CV (%)	31.3	N/A
CL (L/h)	Mean	N/A	7.6
	CV (%)	N/A	36.1

AUC_{0-inf} = area under the plasma concentration-time curve from time zero to infinity; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 h; CL/F = apparent oral clearance; CL = apparent total body clearance; C_{max} = maximum observed plasma concentration; C_{min} = minimum observed plasma concentration at t = 0 h; CV = coefficient of variation; N/A = not applicable; t_{max} = time to reach maximum observed plasma concentration; $t_{½}$, = terminal half-life; V_d/F = apparent volume of distribution after oral administration; V_d = apparent volume of distribution.

Table 3 – Absolute bioavailability of oral imatinib at steady state plasma pharmacokinetics (n=6)

	Imatinib tablet (400 mg)	Intravenous imatinib-d8 (100 µg)
AUC_{0-24} (µg·h/mL)(CV%)	42.6 (30.2)	N/A
AUC_{0-inf} (µg·h/mL) (CV%)	N/A	0.015 (43.7)
Dose normalized AUC_{0-inf} (µg·h/mL) (CV%)	N/A	60.5 (43.7)
F (%)(median, range)	76 (42-106)	-

DISCUSSION

The current study describes results on the determination of the absolute bioavailability of oral imatinib following concomitant administration of a single intravenous stable isotopically labeled imatinib-d8 microdose.

Technically, the stable isotopically microdose trial design proved successful. For all patients, imatinib and imatinib-d8 concentrations could be simultaneously quantified in all collected plasma samples. The quantification of imatinib-d8 was not biased by high concentrations of unlabeled imatinib present in the same plasma sample. In theory, the use of deuterium as a label for the intravenous microdose may result in a kinetic isotope effect (KIE), caused by increased bond strength of the carbon-deuterium bond, as

compared with the carbon-hydrogen bond. The KIE may result in altered pharmacokinetics (e.g. altered metabolism) of the deuterium labeled drug, with an incorrect calculation of the absolute bioavailability as a result.[6] As the deuterium labels in the imatinib-d8 structure were not located at metabolic hot spots in the imatinib molecule[17], the KIE was assumed to be negligible. As seen in **Figure 3**, the curves for oral and intravenous imatinib demonstrate a parallel decline during the terminal elimination phase, with a constant mean dose-normalized imatinib:imatinib-d8 ratio in plasma of around 2.00, confirming that the KIE for the imatinib-d8 molecule was indeed negligible. The curves presented here demonstrate the validity of using the deuterium labeled imatinib-d8 drug molecule for intravenous microdose administration.

The median absolute bioavailability was calculated to be 76% which was less than the 98% (87-111% (90% confidence interval)) reported using a traditional two-period crossover design in healthy volunteers.[18] There might be different reasons for the lower absolute bioavailability found in this study as compared with the study in healthy volunteers. In the previous absolute bioavailability trial, healthy volunteers demonstrated considerable inter-subject variation in the absolute bioavailability of imatinib in twelve treated subjects.[1] The reasons for the high variability may be attributed to inter-subject variations in the activity of cytochrome P450 isoenzyme 3A4 (CYP3A4), a major enzyme in the biotransformation of imatinib.[1] It could be that the lower bioavailability found in our study may solely be a result of this interpatient variability, as both studies demonstrate a relatively large inter-subject variability in small study populations (6 and 12 subjects included for each trial, respectively). An alternative theory may be that the absolute bioavailability actually differs between healthy volunteers and GIST patients. If so, there might be a change present at baseline, or a change developed during prolonged treatment with imatinib. In theory, GIST disease status may negatively influence the absorption of drug into the systemic circulation, resulting in a lower absolute bioavailability at baseline. In a previous study, patients with a prior major gastrectomy had a significantly lower $C_{min,}$ while other types of surgery were not associated with decreased pharmacokinetic exposure.[19] However, in another observational study, type of surgery and extent of resection were not predictive of low imatinib concentrations.[21] Our study patient population consisted of patients without prior major gastrectomy (**Table 1**), and results were therefore not likely to be influenced by prior surgery.

Another explanation for the lower bioavailability might be a change developed during prolonged imatinib treatment. Imatinib pharmacokinetic parameters have been described to change from early to later treatment phase, with a trend towards increased imatinib clearance after long-term exposure[21,22], although this finding could not be reproduced in other studies.[20,23] In our study population, all patients were on imatinib treatment for several months or years (median 3.2 years, range 0.3-13.0 years), and the clearance was similar to the clearance observed during the first month of treatment as

described by Judson *et al.* (7.6 L/h vs. 9.2 L/h).[21] Since pharmacokinetic exposure to imatinib has been related to treatment efficacy[24], therapeutic drug monitoring has been implemented in our hospital. Therefore, in case of an increased clearance and thus a lower pharmacokinetic exposure, dose escalations have probably been performed. These patients were not eligible for inclusion in this trial, which might explain the absence of an observed increase in drug clearance as a result of selection bias. Furthermore, if a change in clearance was found, this would not have explained the lower value for absolute bioavailability, as the oral and intravenous dose are co-administered during a single-dose event, eliminating inter-dose variability.

In a prospective pharmacokinetic trial on imatinib plasma concentrations in GIST patients, a reduced exposure of approximately 30% to imatinib was observed after long-term treatment (> 90 days), most likely due to reduced drug absorption over time.[25] This reduced exposure may potentially be a result of the lower absolute bioavailability that we observed in our trial. Although different theories for this reduced absorption and/or bioavailability do exist (e.g. changed activity or expression of drug transporters involved in active transport, upregulation of CYP3A4)[25], none have been confirmed to date.

Finally, the lower bioavailability found in our study could potentially be explained by the fact that patients ingested imatinib concomitant with food (according to the label), while the previous absolute bioavailability study has been performed under fasted conditions. Although a previous food-effect study concluded that food did not affect imatinib pharmacokinetics to a clinically relevant extent, C_{max} and $AUC_{0\text{-}24h}$ decreased 15% and 9%, respectively, after concomitant intake with a high-fat meal compared to the fasted state.[26]

Interestingly, the individual plasma concentration-time curves demonstrated up to two secondary peaks after the C_{max}, with different profiles for oral imatinib and intravenous imatinib-d8 (**Supplementary Figure 1**). Previous research on imatinib has not demonstrated enterohepatic cycling of imatinib. Another explanation for these peaks might be bile secretion triggered by food intake, resulting in acceleration of drug solubility in the gastrointestinal lumen, although food has been described to have no relevant impact on the rate or extent of bioavailability.[27]

By using the stable isotopically labeled microdose trial design, the number of dose events and collected plasma samples were reduced by half, as compared with the previously performed absolute bioavailability trial using a conventional crossover design.[18] This reduction may aid to perform this trial in patients in the future, as it offers the possibility to be combined with a phase I/II trial in patients without adding a separate intravenous dose event. The microdose trial design using a stable isotopically labeled drug will only mildly increase patient burden by adding a single intravenous microdose administration to the study procedures. This minor adjustment may result in increased and more

relevant knowledge on the pharmacokinetics of a novel drug product in an early stage of clinical drug development.

CONCLUSION

The absolute bioavailability of oral imatinib in cancer patients during steady state pharmacokinetics was successfully determined using a stable isotopically labeled microdose trial. This study demonstrates the potential to use a stable isotopically labeled microdose in combination with LC-MS/MS for the assessment of absolute bioavailability. In addition, the potential added value of performing an absolute bioavailability study in the intended patient population for clinical use during steady state pharmacokinetics was demonstrated by comparing the results obtained with a previously performed absolute bioavailability trial in healthy volunteers.

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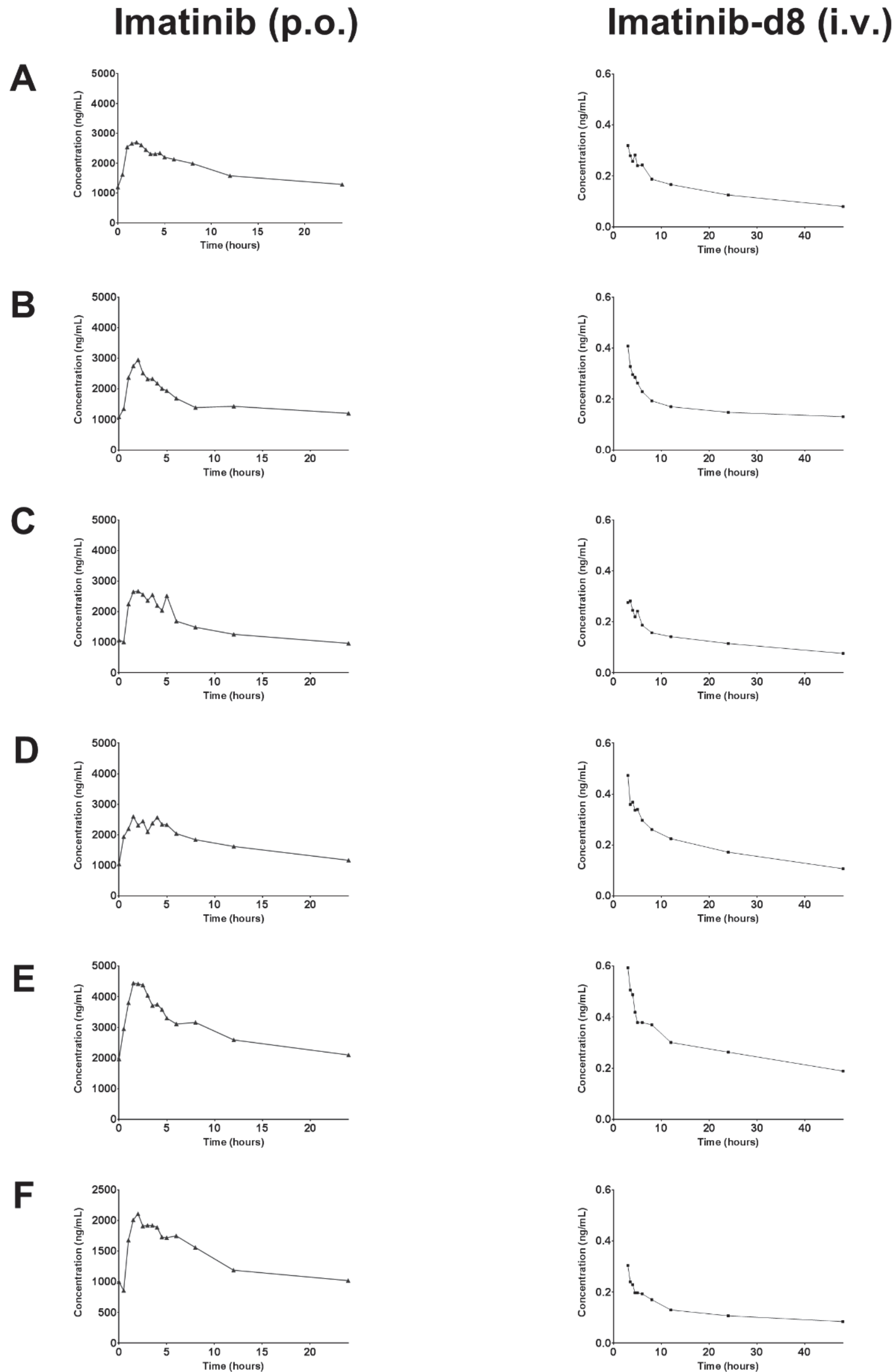
SUPPLEMENTARY DATA



Supplementary Figure 1 – Plasma concentration-time curves of imatinib and imatinib-d8 following oral (p.o.) administration of 400 mg imatinib dose at *t* = 0 h and intravenous (i.v.) administration of a 100 µg imatinib-d8 microdose at *t* = 2.5 h in patients (1-6, A-F) displaying steady state imatinib plasma pharmacokinetics

Conclusions and perspectives

CONCLUSIONS AND PERSPECTIVES

This thesis focuses on precision dosing of oral targeted therapies in oncology. Key points extracted from this thesis and the lessons learned are summarized in **Figure 1** and discussed in more detail in the following paragraphs.

In short, the path forward for precision dosing in oncology can be visualized as a double-edged sword, but with both sides of the blade contributing towards more rational precision medicine by optimizing exposure.

On the one side, we should gain a better understanding of the sources of variability in exposure. These factors should be controlled where possible in order to minimize the variability within and between patients. Selection of the right starting dose for each individual (group of) patient(s) will result in a decreased proportion of patients that are treated outside the therapeutic window in the first place, i.e. "right-dose-first-time" paradigm.

On the other side, we should accept that a certain degree of unexplained variability will always remain. Therefore, we should work around it by developing dose-adaptation strategies that result in exposures matching the target. In this way, we can titrate individual patients towards the therapeutic window by PK-guided dosing.

This paradigm is illustrated in the **Graphical summary** of this thesis, in which it is indicated how each chapter connects to it.

LESSONS (TO BE) LEARNED

Understanding and minimizing variability

Oral targeted therapies exhibit a large interindividual variability in pharmacokinetic exposure, caused by many factors. We should gain a better understanding of the sources of variability and minimize them where possible. In this way, fewer patients will be dosed outside the therapeutic window, i.e. "right-dose-first-time" paradigm.

Pharmacokinetic exposure is related to response

Pharmacokinetic exposure to many oral targeted therapies is related to both efficacy and toxicity. At the currently used fixed doses, a substantial subset of patients is under- or overexposed, providing a strong rationale for precision dosing.

Precision dosing is ready for prime time

PK-guided dosing was demonstrated to be feasible for many oral targeted therapies and resulted in more patients reaching the target exposure. Therefore, the stage is set for the implementation of PK-guided dosing in routine clinical practice. Cost-neutral interventions can be applied if possible.

Real-life data collection

Preferably, both exposure-response analyses and feasibility studies are performed in a real-life setting, rather than in highly selected patients included in clinical trials. After the implementation of precision dosing, the collection of real-life data in registries should continue to further optimize the precision dosing approach.

Precision dosing should be part of drug development

Phase I/II studies should aim to define a target exposure instead of a (maximum tolerated) fixed dose. The feasibility of precision dosing can then be investigated in phase II/III studies. In this way, precision dosing will be an integral part of drug development.

Figure 1 – Key points extracted from this thesis and the lessons (to be) learned
PK = pharmacokinetically

UNDERSTANDING AND MINIMIZING VARIABILITY

First of all, this thesis underlines that the variability in pharmacokinetic exposure between patients treated at the same dose is high for the oral targeted therapies that were studied, with coefficients of variation in minimum plasma concentration (C_{min}) of around 40-70% (**Chapter 3, 4, 7, 10, 11, 14)**. As illustrated in the hallmarks of variability in the **Graphical summary**, many factors contribute to this high inter- and intra-individual variability. Several of these sources of variability have been addressed in the work described in this thesis.

It starts with a proper pharmaceutical formulation, as a higher bioavailability results in a reduced inter- and intra-individual variability in pharmacokinetic exposure.[1,2] Currently, the absolute bioavailability is not determined for over half of the oral targeted therapies.[3] The innovative clinical trial design used in **Chapter 16** provides a more efficient way to conduct absolute bioavailability trials.[4] Notably, the low bioavailability of pazopanib (i.e. 14-39%) is a result of its poor solubility and its relative bioavailability was predicted to be higher at lower doses.[5,6] Therefore, splitting intake moments can be used to increase the relative bioavailability and thereby boost pazopanib exposure (**Chapter 12 and 13**).

Other hallmarks of variability are drug-food and drug-drug interactions.[7,8] Pharmacokinetic exposure of many oral targeted therapies is dramatically affected by gastric acid-suppressive agents (as we show for three patients in **Chapter 13**) and cytochrome P 450 3A4 (CYP3A4) modulators (as we summarize for cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors in **Chapter 14**). It is important to quantify the effect sizes of these drug-drug interactions to inform on appropriate dose adjustments in routine clinical management, as we did in **Chapter 15**. As described in **Chapter 11 and 13**, the food-effect can sometimes be leveraged to optimize exposure in patients who are underdosed.

Moreover, (patho-)physiological differences between patients can affect drug absorption, distribution, metabolism and excretion. Another key determinant in the variability in pharmacokinetic exposure is treatment adherence.[9] In the prospective study on PK-guided dosing described in **Chapter 6, 7, 11 and 13**, compliance was first evaluated with the patient in case of low exposure, before any PK-guided interventions were implemented. Lastly, pharmacogenomics can explain part of the observed variability in pharmacokinetic exposure. As many oral targeted therapies are extensively metabolized by cytochrome P450 enzymes, polymorphisms in these enzymes, can result in an altered clearance.[10,11]

Taken together, it is essential to enhance our knowledge on how each of these factors contributes to the variability in pharmacokinetic exposure within and between patients. In this way, variability can be reduced and the fraction of patients that are treated outside

the therapeutic window can thereby be reduced. Nevertheless, unexplained variability will always remain, providing a strong rationale for PK-guided dosing (**Chapter 1**). In fact, measured drug concentrations are the translation of all known and unknown factors influencing variability.

PHARMACOKINETIC EXPOSURE IS RELATED TO RESPONSE

The presence of defined and consistent exposure-response relationships is the main reason to apply precision dosing, as described in **Chapter 1**. Pharmacokinetic exposure to many oral targeted therapies is related to both efficacy and toxicity.[12,13] In **Chapter 3-5** of this thesis, this is demonstrated for several of these compounds in real-life patient cohorts (i.e. abiraterone, crizotinib, alectinib and trametinib). In all three studies, a substantial subset of patients was underexposed (i.e. 37-63%) at the currently used fixed dosing regimens.

However, not for all oral targeted therapies clinically relevant exposure-response relationships were identified. For example, in **Chapter 5**, pharmacokinetic exposure to dabrafenib could not be related to either efficacy or toxicity. Also, previous studies did not demonstrate an exposure-response relationship for enzalutamide and osimertinib.[14,15] This may be explained by a plateau in the exposure-response curve for these drugs, that are dosed at the flat end of this curve. For some molecular targets (i.e. BRAF and EGFR) the therapeutic window appears to be wider than for others (i.e. ALK, MEK and VEGF). Also, newer generation kinase inhibitors may have a more robust formulation resulting in reduced variability.

It is thus essential to study for which oral targeted therapies precision dosing holds promise, and for which it might not be worthwhile. Before solid conclusions can be drawn on the absence of an exposure-response relationship, it should be ensured that the study has sufficient power to demonstrate this. Otherwise, absence of evidence is not evidence of absence, as is the case for several underpowered exposure-response analyses for endoxifen.[16–18]

Although dose reductions do not need to be actively recommended in case of high exposure and manageable tolerability, measuring drug concentrations can support dose reductions in case of toxicity. In **Chapter 9**, two patients are described who maintained adequate exposure at an eight times lower than standard dose of pazopanib. According to the drug label, this dose was considered insufficient to obtain a clinically relevant effect, and without PK-guided dosing, treatment would definitely have been discontinued in these patients. Adequate drug levels can thus support to continue treatment at a reduced dose, while low drug levels may justify switching therapy.

PRECISION DOSING IS READY FOR PRIME TIME

In this thesis, it is argued why the fixed dosing paradigm should be left (**Chapter 1 and 2**), and the stage is set for the implementation of precision dosing. For drugs with clearly proven exposure-efficacy relationships (**Chapter 3-5 and 10**), for which PK-guided dose adaptation was demonstrated to be feasible and resulted in more patients reaching the target exposure (**Chapter 7, 8, 11 and 13**), PK-guided dosing should be implemented as part of routine clinical management.

Notably, PK-based dose adjustments are already routinely made for many drugs and are recommended by health authorities, e.g. for patients with renal impairment or for drug-drug interaction management.[19–22] Precision dosing simply extrapolates this exposure matching paradigm from selected patient populations to each individual patient with a suboptimal exposure, irrespective of the underlying cause. If it has been demonstrated that exposure is related to a relevant clinical outcome (i.e. efficacy or toxicity) and that exposure can be improved by PK-guided dosing, it could be logically assumed that PK-guided dosing would result in better treatment outcomes. Therefore, as argued in **Chapter 2**, confirmatory randomized trials are not needed to demonstrate the clinical relevance of precision dosing.

Furthermore, the right starting dose can be selected for each individual (group of) patients, e.g. based on pharmacogenomics or by appropriate management of drug-drug interactions (as shown in **Chapter 15**), i.e. “right-dose-first-time” paradigm, after which this dose can be further optimized by PK-guided dosing.

Our focus should now be twofold. On the one hand, we should continue to establish exposure-response relationships and perform feasibility studies for PK-guided dosing of new oral targeted therapies. On the other hand, we should ensure that precision dosing will be implemented in routine clinical practice. As described in **Chapter 7**, the three-stage-design of the Dutch Pharmacology Oncology Group – Therapeutic Drug Monitoring (DPOG-TDM) study provides a framework to perform feasibility studies and subsequently enables further nationwide implementation using the existing infrastructure of the DPOG-TDM study. This systematic approach can also be used internationally for this purpose, within the same protocol or by setting up similar frameworks in other countries.

Unlike certain other classes of drugs for which TDM is advocated, that are aimed at exogenous targets (e.g. anti-infectious drugs), oral targeted therapies are aimed at targets originating from endogenous proteins. The inherently small therapeutic indices associated with some of these targets imply that the need for precision dosing will remain in the foreseeable future, despite the development of newer generation inhibitors with optimized pharmaceutical formulations. This further stresses the importance of investing

in a solid infrastructure for PK-guided dosing (i.e. sample collection, shipment and measurement, with a short turn-around time).

Increased treatment costs for higher than approved dosages will remain a concern. In **Chapter 11-13** of this thesis, we provide examples of cost-neutral interventions that could be performed as first steps in case of low exposure. Moreover, absolute dose increments have been proven cost-effective for several anticancer drugs, including imatinib and tamoxifen.[23–25] Ideally, though, prices would be determined per treatment, regardless of the administered dose.

REAL-LIFE DATA COLLECTION

Preferably, both exposure-response analyses and feasibility studies are performed in a real-life setting, as were the studies described in this thesis (**Chapter 3-9, 11-13, 15 and 16**), rather than in highly selected patients included in clinical trials. This will provide a more accurate reflection of clinical practice. Due to strict eligibility criteria, patients enrolled in clinical trials may differ from those in clinical practice, limiting the generalizability of these studies.[26,27] Mainly, interindividual variability in PK exposure is higher in real-life patients, as this is a far more diverse population with more comorbidities and concomitant medication. Also, treatment adherence is strictly monitored in clinical trials, while this may be monitored less stringently in clinical practice. Furthermore, treatment outcomes are often better in patients treated in clinical trials (i.e. efficacy), compared with patients receiving the same treatment under real-world circumstances (i.e. effectiveness), which is referred to as the efficacy-effectiveness gap.[28,29] Downsides of real-world studies, though, include the risk of confounding and the uncertainties about the quality of the collected data. These limitations should be adequately addressed to prevent that flawed conclusions are drawn, i.e. by taking known prognostic factors into account in multivariable analyses and by building registries to prospectively capture data.

Also after implementation of precision dosing in routine care, the collection of real-life data in registries should continue. In this way, the precision dosing strategy can be further improved with data of additional patients. The third-stage cohorts of the DPOG-TDM study can be used for this purpose. In addition, large international collaborations and pooled analyses will maximize what can be learned from these data. Especially in this setting, registries that are integrated with electronic patient files will enable timely and efficient data collection.

PRECISION DOSING SHOULD BE PART OF DRUG DEVELOPMENT

The work described in this thesis emphasizes the important role of the discipline of clinical pharmacology in (early) drug development. As proposed by Lewis Sheiner, cycles of

learning and confirming should be alternated throughout a drug's life cycle.[30] In fact, incentives by regulatory authorities have now resulted in a drug's pharmacokinetics and pharmacodynamics being more extensively studied. Yet, more action can be taken upon this knowledge to optimize treatment.

More attention should be paid to optimize pharmaceutical formulations, which will help to increase bioavailability and reduce variability. In addition, intra-individual variability needs to be characterized in more detail, as this increases our understanding of the pharmacokinetic behavior of a new drug.[31] Furthermore, efforts should be made to shorten the delay between the approval of a new drug and the identification of a suitable PK target for precision dosing. To illustrate this delay, imatinib was approved for the treatment of chronic myeloid leukemia in 2001, while the first publication on its exposure-response relationship appeared only in 2007.[32] Similarly, pazopanib was marketed in 2010, whereas it took until 2014 before its efficacy threshold was proposed.[33] A major stride can be made by defining a target exposure in phase I/II studies, instead of a (maximum tolerated) fixed dose for all patients, provided that enough data is available to allow for meaningful analyses. The feasibility of precision dosing can then be evaluated in phase II/III studies. In this way, precision dosing will be an integral part of drug development.

CONCLUDING REMARKS

In this thesis, the rationale (**Part I**), the evidence (**Part II**) and the clinical application (**Part III and IV**) of precision dosing of oral targeted therapies in oncology are described. Based on the high interindividual variability and exposure-response relationships, it is concluded that there is a strong rationale for precision dosing. Moreover, PK-guided dosing was feasible in clinical practice for several compounds and resulted in more patients reaching the target exposure. Taken together, precision dosing has the potential to further optimize treatment with oral targeted therapies. Therefore, efforts should be made to implement precision dosing in routine clinical practice. Furthermore, the path forward should focus on investigating exposure-response relationships of new oral targeted therapies, identify optimal thresholds for PK-guided dosing and study the feasibility of precision dosing for these new compounds. Ideally, target exposures should be defined in early phase clinical trials to make precision dosing an integral part of drug development.

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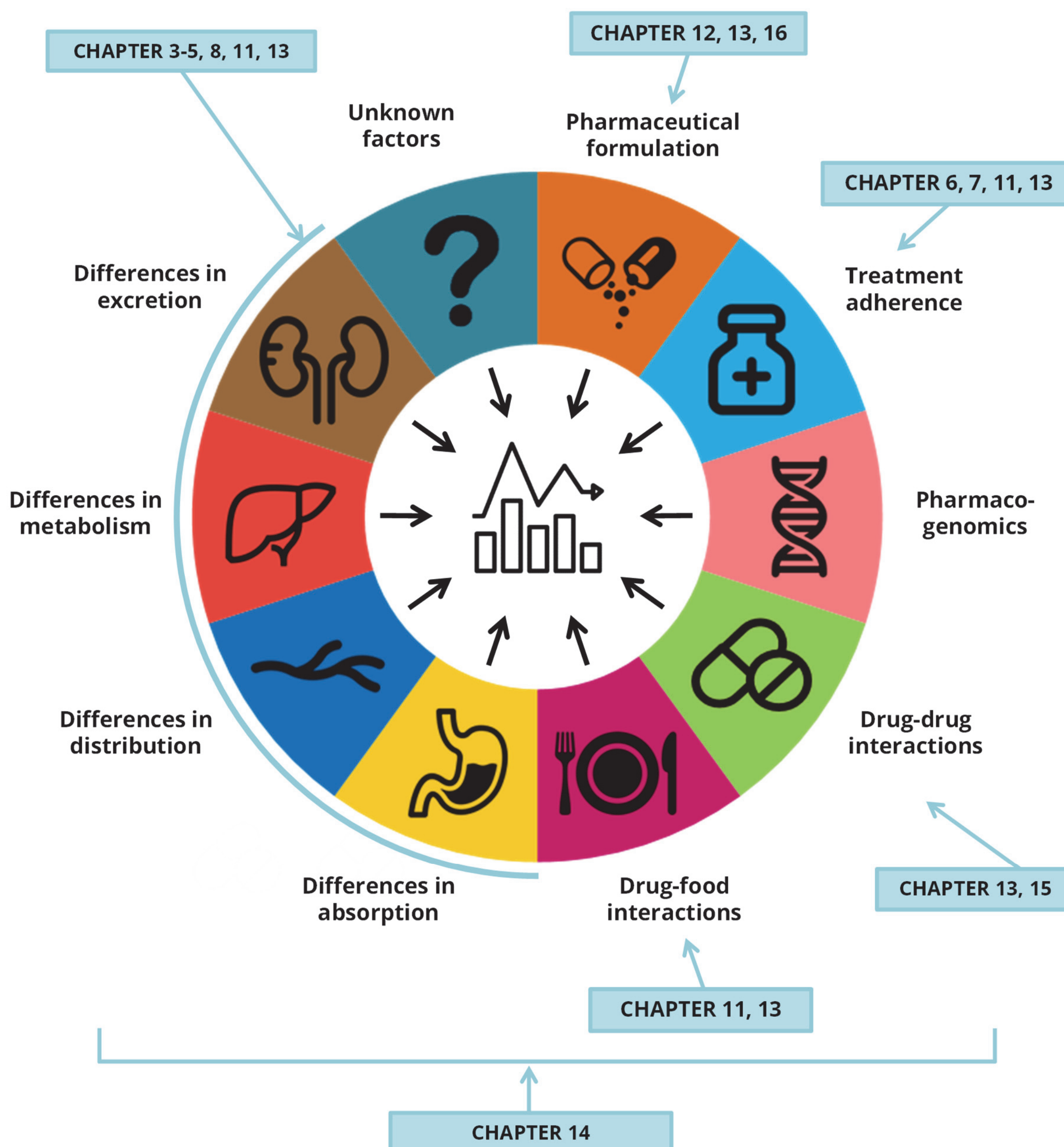
Graphical summary

UNDERSTANDING AND MINIMIZING VARIABILITY

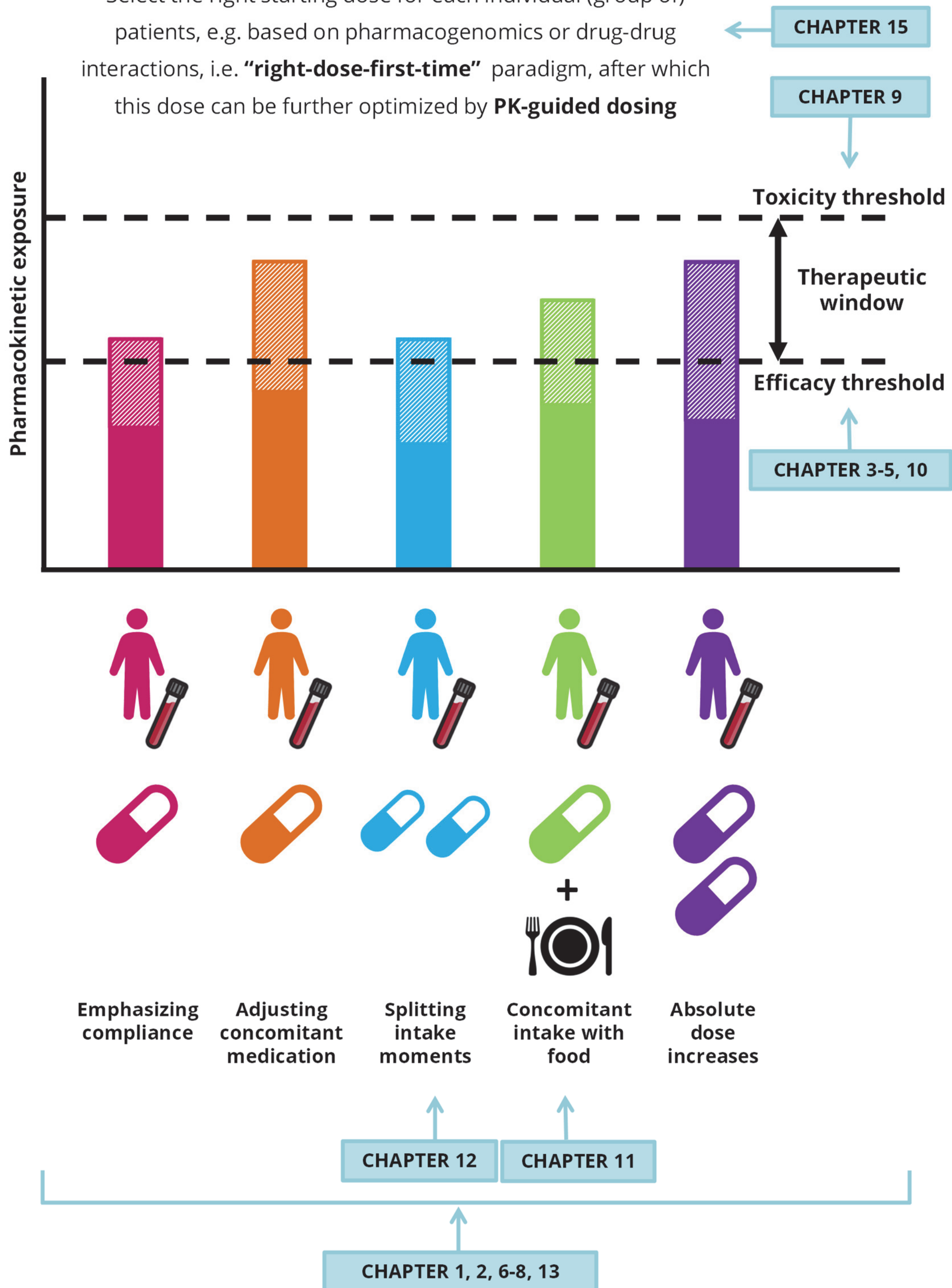
Gain a better understanding of the sources of variability in pharmacokinetic exposure. Control these factors where possible, in order to minimize the variability and reduce the proportion of patients treated outside the therapeutic window.

HALLMARKS OF VARIABILITY



Graphical representation of the precision dosing paradigm described in this thesis.
The hallmarks of variability were designed in analogy to the hallmarks of cancer as described by Hanahan & Weinberg.[1] The height of the bars on the right side of the figure were chosen at random and do not represent a certain pharmacokinetic exposure or effect size of the PK-guided intervention.

PRECISION DOSING TO TITRATE INDIVIDUAL PATIENTS TOWARDS THE THERAPEUTIC WINDOW



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Summary

SUMMARY

The advent of oral targeted therapies in oncology has widely been praised as a major breakthrough in the treatment of cancer. While our focus has increasingly shifted towards precision medicine, by selecting the right treatment based on molecular characteristics of the tumor, most of these novel anticancer drugs are still administered using a one-size-fits-all fixed dosing approach. However, pharmacokinetic (PK) characteristics of these new oral targeted therapies suggest precision dosing would be far more rational. Precision dosing can be achieved by selecting the right starting dose for each individual (group of) patient(s), i.e. "right-dose-first-time" paradigm, after which this dose can be further optimized by PK-guided dosing, i.e. adjusting the dose based on measured drug concentrations. This thesis focused on the rationale (**Part I**), the evidence (**Part II**) and the clinical application (**Part III and IV**) of precision dosing of oral targeted therapies in oncology.

PART I – RATIONALE FOR PRECISION DOSING

The aim of **Chapter 1** was to address the scientific evidence for PK-guided dosing of oral targeted therapies by discussing the following criteria for its rational use:

1. absence of an easily measurable biomarker for drug effect;
2. long-term therapy;
3. availability of a validated sensitive bioanalytical method;
4. significant variability in pharmacokinetic exposure;
5. narrow therapeutic range;
6. defined and consistent exposure-response relationships;
7. feasible dose-adaptation strategies.

These requirements are met for most oral targeted anticancer drugs. Hence, to realize the full potential of precision medicine, we should not only treat patients with the right drug, but also at the right dose.

Although PK-guided dosing is rational and supported by an increasing body of evidence, it is still limitedly applied in oncology. An argument frequently used against its implementation in routine cancer care is the absence of randomized controlled trials (RCTs). In **Chapter 2**, it was argued why randomized confirmatory studies are not needed to demonstrate the clinical relevance of precision dosing. First, the inadequacy of the currently used fixed dosing paradigm and the progress made thus far in implementing precision dosing in oncology were discussed. Then, compelling arguments were laid out why large confirmatory RCTs are not necessary for the implementation of PK-guided dosing when clear exposure-efficacy relationships have been demonstrated. Finally, a path forward was proposed to demonstrate the clinical relevance of PK-guided dosing in oncology.

PART II – EXPOSURE-RESPONSE ANALYSES

Before PK-guided dosing could be applied in clinical practice, sufficient evidence on exposure-efficacy relationships should be established and optimal thresholds for PK-guided dosing need to be identified. For this purpose, it is essential that exposure-response analyses are performed. In this part, exposure-response analyses were described for five oral targeted therapies: abiraterone, crizotinib, alectinib, dabrafenib and trametinib.

In **Chapter 3**, exposure-response analyses for abiraterone and its main metabolites were described. Abiraterone is an inhibitor of 17α-hydroxylase/C17,20-lyase (CYP17)and is used in the treatment of metastatic prostate cancer. Sixty-two patients were included in this observational study in which it was confirmed that the previously established threshold of abiraterone minimum plasma concentration (C_{min}) of ≥ 8.4 ng/mL is related to prolonged progression-free survival (PFS) in a real-life patient cohort (16.9 vs. 6.1 months, p=0.033). Interindividual variability in exposure at the currently approved fixed dosing regimen was high, with a coefficient of variation of 70% for C_{min}. As a result, a substantial subgroup of 42% of patients treated with abiraterone acetate is underexposed in routine care and at risk of suboptimal treatment efficacy.

Chapter 4 focused on anaplastic lymphoma kinase (ALK)-inhibitors crizotinib and alectinib, both indicated for the treatment of ALK-positive metastatic non-small-cell lung cancer. Exposure-response analyses were explored using previously proposed C_{min} thresholds of 235 ng/mL for crizotinib and 435 ng/mL for alectinib. In 52 patients treated with alectinib, PFS was significantly longer in patients with a median C_{min} ≥ 435 ng/mL compared to patients with an exposure below this threshold (not estimable (95% confidence interval (CI): 19.8-not estimable) vs. 12.6 months, p=0.04). For crizotinib, a clinically relevant difference in median PFS was found as well, although this difference was only statistically significant in a pooled analysis including 79 patients with ALK-positive, ROS1-positive and cMET-dysregulated tumors (11.8 vs. 5.3 months, p=0.04). At the standard fixed dose, interindividual variability in exposure was high, with coefficients of variation for C_{min} of 45% and 57% for crizotinib and alectinib, respectively. Notably, 37-48% of patients did not reach the efficacy thresholds and could potentially benefit from a higher dose of crizotinib or alectinib.

Chapter 5 described exposure-response analyses in patients with melanoma treated with the combination of BRAF- and MEK-inhibitor dabrafenib plus trametinib. In total, 140 patients were included. Although dabrafenib exposure was not related to efficacy, exposure to trametinib was related to survival, with trametinib C_{min} ≥ 15.6 ng/mL being identified as the optimal threshold. Median PFS in patients with trametinib C_{min} ≥ 15.6 ng/mL was 10.9 months, compared with 6.0 months for those with C_{min} below this threshold (p=0.06). Furthermore, OS was ten months longer in patients with trametinib C_{min} levels ≥ 15.6 ng/mL compared to patients with C_{min} below this threshold (22.8 vs. 12.6 months, p=0.003). These results implicate that a substantial subset of patients (i.e. 63%)

may potentially benefit from a higher dose of trametinib, provided that they would tolerate this dose increase.

Taken together, exposure was related to efficacy for four out of five studied oral targeted therapies. For these drugs, validated C_{min} thresholds were identified that can be used for PK-guided dosing. In general, interindividual variability in exposure was high. In all three studies, a substantial subset of patients was underexposed (i.e. 37-63%) at the currently used fixed dosing regimen. PK-guided dosing may optimize treatment outcomes in these patients.

PART III – PRECISION DOSING IN CLINICAL PRACTICE

This part focused on the clinical application of precision dosing for multiple oral targeted therapies in various settings.

First of all, **Chapter 6** described the protocol of the Dutch Pharmacology Oncology Group – Therapeutic Drug Monitoring (DPOG-TDM) study. This is an ongoing nationwide prospective protocol providing a framework to investigate the feasibility, tolerability and efficacy of PK-guided dosing for multiple oral targeted therapies simultaneously. Primary outcome of this trial is to halve the proportion of patients with a low exposure after 12 weeks compared with historical data. Patients can be enrolled in this study when they initiate regular treatment with one of the oral targeted therapies included in the protocol at the approved dose. PK samples are then collected 4, 8 and 12 weeks after start of treatment for most compounds, and every 12 weeks thereafter. In case of C_{min} below the predefined TDM target and manageable toxicity, a PK-guided intervention is recommended. This could include emphasizing compliance, adjusting concomitant medication due to drug-drug interactions, concomitant intake with food, optimizing the dosing schedule or dose increases.

In **Chapter 7**, the first (interim) results of the DPOG-TDM study were reported. Of the 386 patients that were evaluable for the primary outcome, 22.8% had a low exposure after 12 weeks, compared with 40.4% in historical data ($p < 0.001$). PK-guided dosing thus reduced the proportion of underexposed patients by 44% (95% CI: 32-54%). Of the 543 patients that were evaluable for the overall analyses, 48.1% had a C_{min} below the target at a certain time point during treatment. In 53.3% of these patients, PK-guided interventions were performed, which were successful in 76.6% of the patients in whom the effect was evaluated. Reasons why PK-guided interventions could not be performed in other patients were mainly toxicity (63.9%), lack of physician adherence (17.2%) or treatment discontinuation (13.1%). This study showed that PK-guided dose optimization of oral targeted therapies was feasible in clinical practice and reduced the proportion of underexposed patients. Therefore, these findings support the introduction of PK-guided dosing as standard of care.

Chapter 8 evaluated PK-guided dose increases of imatinib in patients with gastrointestinal stromal tumors (GIST) in routine clinical practice. Based on the strong rationale, PK-guided dosing of imatinib has been gradually implemented as standard of

care at the Netherlands Cancer Institute since 2009. In this chapter, 169 patients were described of whom 1402 PK samples were collected. In 75% of patients, C_{min} was below the efficacy threshold of 1100 ng/mL. PK-guided dose increases were performed in 62% of these patients and were successful in 63% of them. PFS was similar in patients with and without imatinib dose increases. However, due to the small number of patients with progressive disease, no definite conclusions on the effect of PFS could yet be drawn. This is the largest cohort in which PK-guided dose increases of imatinib in patients with GIST were performed in routine clinical practice. These findings highlight the feasibility of PK-guided dosing of imatinib in clinical practice.

Chapter 9 described two patients with soft tissue sarcoma who were treated at an eight times lower than standard dose of pazopanib due to toxicity, and still maintained a C_{min} above the efficacy threshold of 20.5 mg/L. In the absence of PK-guided dosing, when doses would have been reduced according to the drug label, treatment would definitely have been discontinued in these patients, as the label considers the exposure at a four times lower than standard dose insufficient to obtain a clinically relevant effect. Here, the strong value of measuring pazopanib concentrations in case of dose reductions due to toxicity is illustrated, as exposure could still be adequate at considerably lower than standard doses.

Lastly, the relevant clinical pharmacokinetic and pharmacodynamic characteristics of oral anti-hormonal drugs were outlined and translated into practical guidelines for PK-guided dosing in **Chapter 10**. For some compounds, targets for PK-guided dosing are not well established yet and as a reference the median pharmacokinetic exposure could be targeted (i.e. exemestane and enzalutamide). However, for most drugs, exposure-efficacy relationships could be translated into specific C_{min} thresholds (i.e. abiraterone: 8.4 ng/mL, anastrozole: 34.2 ng/mL, letrozole: 85.6 ng/mL). Moreover, prospective clinical trials have shown PK-guided dosing to be feasible for tamoxifen, for which the efficacy threshold of its active metabolite endoxifen is suggested to be 5.97 ng/mL. Based on the available data, it was therefore concluded that precision dosing based on drug concentrations is feasible and promising for oral anti-hormonal drugs and should be developed further and implemented into clinical practice.

PART IV – ALTERNATIVE STRATEGIES FOR PRECISION DOSING

In the last part of this thesis, alternative strategies that can be applied to optimize exposure were highlighted, focusing on cost-neutral interventions, drug-drug interactions and bioavailability.

Chapter 11 reported the results of the first 32 evaluable patients treated with abiraterone acetate in the DPOG-TDM study. According to the drug label, abiraterone acetate should be administered in modified fasting state, which means no food two hours before and one hour after drug intake. However, concomitant intake with food has been shown to result in a clinically relevant increase in exposure in previous food-effect studies. Therefore, the aim was to evaluate whether PK-guided dosing of abiraterone using a food

intervention is feasible in clinical practice and results in an increased percentage of patients with efficacious exposure to abiraterone without additional toxicities. Concomitant intake with a light meal or a snack resulted in a 2.9-fold increase in C_{min} and led to an adequate exposure in 87.5% of patients. Although concomitant intake with food also resulted in an increased interindividual variability, and thus very high C_{min} levels in some patients, no additional toxicities were experienced by these patients. It was thus concluded that concomitant intake with food offers a cost-neutral opportunity to optimize exposure in patients with low abiraterone C_{min}.

In **Chapter 12**, another cost-neutral strategy was applied to optimize pazopanib exposure. At the approved fixed dose of 800 mg once daily, 16-30% of patients treated with pazopanib are being underdosed and are at risk of decreased efficacy, providing a strong rationale for PK-guided dosing. PK-guided dosing of pazopanib has previously been proven feasible and resulted in an increased proportion of patients with adequate exposure. To achieve this, pazopanib dosages needed to be increased to 1000-1800 mg. However, due to the non-linear absorption of pazopanib, which is plateauing at dosages above 800 mg, absolute dose increments are not an efficient strategy to increase exposure. Furthermore, it leads to an increase in treatment costs. A previous population pharmacokinetic model estimated that the relative bioavailability of pazopanib dosed at 400 mg would be 59% higher than at 800 mg. Therefore, a prospective pharmacokinetic cross-over trial was performed comparing the pharmacokinetics of pazopanib 800 mg once daily with pazopanib 400 mg twice daily. In this study, splitting intake moments of pazopanib resulted in a 79% increase in C_{min}, with acceptable tolerability and thereby provides a promising cost-neutral strategy to optimize treatment in the substantial subset of patients with a low pazopanib exposure.

As a next step, splitting intake moments of pazopanib was implemented as a PK-guided intervention in the DPOG-TDM study. The results of this cohort were reported in **Chapter 13** of this thesis. In case of C_{min} below the efficacy threshold of 20.5 mg/L and manageable toxicity, intake moments were split into 400 mg twice daily as a first step. Secondly, concomitant intake with food was recommended. By applying this PK-guided dosing strategy, the proportion of patients with a low exposure after 12 weeks was 12.9% (95% CI: 3.6-29.8%), compared with 26.7% in historical data. Overall, 37% of patients had a C_{min} below the target at a certain time point during treatment. In 82% of these patients, PK-guided interventions could be performed, which were successful in 62% of them. Hence, it was concluded that PK-guided dose optimization of pazopanib using cost-neutral interventions is feasible in clinical practice and results in an increased proportion of patients with an adequate exposure.

Chapter 14 served as an introduction to the clinical drug-drug interaction study described in the next chapter. Here, an overview of the clinical pharmacokinetic and pharmacodynamic characteristics of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors

palbociclib, ribociclib and abemaciclib was provided. The results of food-effect and drug-drug interaction studies are summarized and the current knowledge on exposure-response and exposure-toxicity relationships is highlighted. All three CDK4/6 inhibitors are characterized by a high interindividual variability in exposure, have their brain penetration limited by efflux transporters, and are extensively metabolized by cytochrome P450 3A4 (CYP3A4). Their exposure is dramatically affected by strong CYP3A4 modulators. Higher exposure is associated with an increased risk of neutropenia for all CDK4/6 inhibitors. In addition, an exposure-efficacy relationship has been demonstrated for abemaciclib, whereas these remain inconclusive thus far for palbociclib and ribociclib.

Despite the well-established effects of strong CYP3A4 modulators on the pharmacokinetics of CDK4/6 inhibitors, the effect of moderate CYP3A4 inhibitors has thus far only been studied in physiologically based pharmacokinetic simulations. **Chapter 15** therefore described the preliminary results of a randomized pharmacokinetic cross-over trial in which the effect of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib is studied in breast cancer patients. It was found that the area under the plasma-concentration time curve (AUC) and maximum plasma concentration (C_{max}) were increased by 44% and 43%, respectively, suggesting that a dose reduction of palbociclib from 125 mg to 75 mg once daily would be rational, when used concomitantly with moderate CYP3A4 inhibitors. Moreover, this study could serve as a showcase for other oral targeted therapies metabolized by CYP3A4 and other moderate CYP3A4 inhibitors.

An important factor in the high variability in exposure of oral targeted therapies is a suboptimal bioavailability. Hence, the assessment of the absolute bioavailability is crucial in the development of optimized oral formulations. In the study described in **Chapter 16**, an innovative design was applied to determine the absolute bioavailability of oral imatinib using a stable isotopically labeled intravenous imatinib-d8 microdose. Median absolute bioavailability at steady-state was 76%, which was less than the 98% reported using a traditional two-period cross-over design in healthy volunteers. This study demonstrated the potential of using a stable isotopically labeled microdose for the assessment of the absolute bioavailability. Hopefully, this new approach will result in increased and more relevant knowledge on the pharmacokinetics of novel oral targeted therapies in an early stage of drug development.

In conclusion, this thesis described the results of several studies on precision dosing of oral targeted therapies in oncology. It was concluded that there is a strong rationale for precision dosing and that randomized trials may not be necessary to demonstrate its clinical relevance. In addition, exposure was related to efficacy for many oral targeted therapies. Furthermore, PK-guided dosing was feasible in clinical practice for most compounds and cost-neutral strategies can be applied to optimize exposure for some of them. Lastly, treatment with oral targeted therapies could be further optimized by

studying drug-drug interactions and bioavailability. When taken together, precision dosing has the potential to further optimize treatment with oral anticancer drugs and should, therefore, be implemented in routine clinical management.

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

De komst van orale doelgerichte therapieën binnen de oncologie wordt gezien als een belangrijke doorbraak in de behandeling van kanker. Terwijl we ons steeds meer richten op behandelen op maat, door het selecteren van de juiste behandeling op basis van moleculaire kenmerken van de tumor, worden de meeste van deze nieuwe antikanker medicijnen nog steeds toegediend in een vaste dosering die voor alle patiënten gelijk is. De farmacokinetische (PK) kenmerken van deze nieuwe orale doelgerichte therapieën suggereren echter dat doseren op maat veel logischer zou zijn. Doseren op maat kan worden bereikt door het selecteren van de juiste startdosering voor iedere individuele patiënt(engroep), het zogeheten *"right-dose-first-time"* paradigma, waarna deze dosering verder kan worden bijgesteld door middel van PK-gestuurd doseren, dat wil zeggen het aanpassen van de dosering op basis van gemeten geneesmiddelconcentraties. Dit proefschrift richtte zich op de rationale (**Deel I**), het bewijs (**Deel II**) en de klinische toepassing (**Deel III en IV**) van doseren op maat van orale doelgerichte therapieën binnen de oncologie.

DEEL I – RATIONALE VOOR DOSEREN OP MAAT

Het doel van **Hoofdstuk 1** was om het wetenschappelijke bewijs voor PK-gestuurd doseren van orale doelgerichte therapieën te bespreken door onderstaande criteria voor rationale toepassing ervan te bediscussiëren:

1. ontbreken van een eenvoudig meetbare *biomarker* voor het effect van een geneesmiddel;
2. langdurige therapie;
3. beschikbaarheid van een gevalideerde en sensitieve bio-analytische methode;
4. belangrijke mate van variabiliteit in farmacokinetische blootstelling;
5. smalle therapeutische breedte;
6. vastgestelde en consistente relaties tussen blootstelling en respons;
7. haalbare strategieën voor dosisaanpassingen.

Voor de meeste orale doelgerichte antikanker medicijnen wordt aan deze vereisten voldaan. Om het volledige potentieel van behandelen op maat te realiseren, zouden we patiënten daarom niet alleen met het juiste geneesmiddel moeten behandelen, maar ook met de juiste dosering.

Alhoewel PK-gestuurd doseren rationeel is en wordt ondersteund door een toenemende mate van bewijs, wordt het beperkt toegepast binnen de oncologie. Een argument dat vaak wordt gebruikt tegen de implementatie van PK-gestuurd doseren in de reguliere zorg is het ontbreken van gerandomiseerde gecontroleerde studies (RCT's). In **Hoofdstuk 2** werd beargumenteerd waarom RCT's niet nodig zijn om de klinische relevantie van doseren op maat aan te tonen. Allereerst werden de beperkingen van de huidige vaste

dosering en de vooruitgang tot nu toe met het implementeren van doseren op maat besproken. Vervolgens werden argumenten besproken waarom grote bevestigende RCT's niet noodzakelijk zijn voor de implementatie van PK-gestuurd doseren wanneer duidelijke relaties tussen blootstelling en effectiviteit zijn aangetoond. Tenslotte werd een plan van aanpak voorgesteld om het belang van PK-gestuurd doseren binnen de oncologie aan te tonen.

DEEL II – BLOOTSTELLING-RESPONS ANALYSES

Voordat PK-gestuurd doseren kan worden toegepast in de klinische praktijk, moeten relaties tussen blootstelling en effectiviteit worden vastgesteld en moeten optimale streefwaardes voor PK-gestuurd doseren worden geïdentificeerd. Daarom is het van essentieel belang dat blootstelling-respons analyses worden uitgevoerd. In dit deel van het proefschrift werden blootstelling-respons analyses beschreven voor vijf orale doelgerichte therapieën: abiraterone, crizotinib, alectinib, dabrafenib en trametinib.

In **Hoofdstuk 3** werden blootstelling-respons analyses voor abiraterone en de belangrijkste metabolieten beschreven. Abiraterone is een remmer van 17α-hydroxylase/C17,20-lyase (CYP17) en wordt gebruikt bij de behandeling van gemetastaseerd prostaatcarcinoom. In deze observationele studie zijn 62 patiënten geïncludeerd. Hierin werd bevestigd dat de eerder vastgestelde streefwaarde van een dalspiegel (C_{min}) ≥ 8.4 ng/mL gerelateerd is aan een langere progressievrije overleving in de klinische praktijk (16.9 vs. 6.1 maanden, p=0.033). De variabiliteit in blootstelling tussen patiënten op de huidige vaste dosering was groot, met een variatie coëfficiënt van 70% voor C_{min}. Als gevolg daarvan heeft 42% van de patiënten die behandeld worden met abiraterone acetaat in de klinische praktijk een te lage blootstelling en lopen daarmee risico op suboptimale effectiviteit van de behandeling.

Hoofdstuk 4 richtte zich op de anaplastisch lymfoom kinase (ALK)-remmers crizotinib en alectinib, die beide worden gebruikt bij de behandeling van ALK-positief gemetastaseerd niet-kleincellig longcarcinoom. Blootstelling-respons relaties werden onderzocht door gebruik te maken van eerder voorgestelde streefwaarden voor C_{min}, namelijk 235 ng/mL voor crizotinib en 435 ng/mL voor alectinib. Bij 52 patiënten die behandeld werden met alectinib was de progressievrije overleving significant langer in patiënten met een mediane C_{min} ≥ 435 ng/mL, vergeleken met patiënten met een blootstelling onder deze streefwaarde (niet bereikt (95% betrouwbaarheidsinterval: 19.8 – niet bereikt) vs. 12.6 maanden, p=0.04). Voor crizotinib werd ook een klinisch relevant verschil in progressievrije overleving gevonden, alhoewel dit verschil alleen statistisch significant was in een samengestelde analyse waarin 79 patiënten werden meegenomen met ALK-positieve, ROS1-positieve en cMET-gedysreguleerde tumoren (11.8 vs. 5.3 maanden, p=0.04). Op de standaarddosering was de variabiliteit in blootstelling tussen patiënten groot, met variatiecoëfficiënten voor C_{min} van 45% en 57% voor respectievelijk crizotinib

en alectinib. Het was opmerkelijk dat 37-48% van de patiënten de streefwaarden voor effectiviteit niet haalde. Deze patiënten zouden dus baat kunnen hebben bij een hogere dosering van crizotinib of alectinib.

Hoofdstuk 5 beschreef blootstelling-respons analyses in patiënten met melanoom die behandeld werden met de combinatie van de BRAF-remmer dabrafenib en de MEK-remmer trametinib. In totaal werden 140 patiënten geïncludeerd. Alhoewel de blootstelling aan dabrafenib niet gerelateerd was aan de effectiviteit, werd wel een relatie tussen blootstelling en overleving gevonden voor trametinib, waarbij een trametinib C_{min} ≥ 15.6 ng/mL werd vastgesteld als optimale streefwaarde. De mediane overleving was tien maanden langer in patiënten met een trametinib C_{min} ≥ 15.6 ng/mL vergeleken met patiënten met een C_{min} onder deze streefwaarde (22.8 vs. 12.6 maanden, p=0.003). De mediane progressievrije overleving in patiënten met een trametinib C_{min} ≥ 15.6 ng/mL was 10.9 maanden, vergeleken met 6.0 maanden in patiënten met een C_{min} onder deze streefwaarde (p=0.06). Deze resultaten impliceren dat een substantieel deel van de patiënten (namelijk 63%) mogelijk baat heeft bij een hogere dosering van trametinib, mits zij deze dosisverhoging verdragen.

Bij elkaar genomen was de blootstelling gerelateerd aan de effectiviteit voor vier van de vijf onderzochte orale doelgerichte therapieën. Voor deze geneesmiddelen zijn gevalideerde C_{min} streefwaarden vastgesteld die gebruikt kunnen worden voor PK-gestuurd doseren. In het algemeen was de variabiliteit in blootstelling tussen patiënten groot. In alle drie de studies had een substantieel deel van de patiënten een te lage blootstelling (namelijk 37-63%) op de huidige vaste dosering. PK-gestuurd doseren kan mogelijk de behandeluitkomsten optimaliseren in deze patiënten.

DEEL III – DOSEREN OP MAAT IN DE KLINISCHE PRAKTIJK

Dit deel richtte zich op de klinische toepassing van doseren op maat voor meerdere orale doelgerichte therapieën in verschillende situaties.

Allereerst werd in **Hoofdstuk 6** het protocol van de Dutch Pharmacology Oncology Group – Therapeutic Drug Monitoring (DPOG-TDM) studie beschreven. Dit is een lopende prospectieve studie in meerdere ziekenhuizen in Nederland, waarin de haalbaarheid, veiligheid en effectiviteit van PK-gestuurd doseren wordt onderzocht voor meerdere orale doelgerichte therapieën. Het primaire eindpunt van deze studie is om de fractie patiënten met een te lage blootstelling na 12 weken te halveren in vergelijking met historische data. Patiënten kunnen in deze studie worden geïncludeerd wanneer zij starten met reguliere behandeling met een van de orale doelgerichte therapieën die zijn opgenomen in het protocol op de geregistreerde dosering. PK samples worden vervolgens afgenomen 4, 8 en 12 weken na start van de behandeling voor de meeste geneesmiddelen, en daarna iedere 12 weken. In het geval van C_{min} onder de vooraf vastgestelde TDM streefwaarde en

dat de therapie goed verdragen wordt, wordt een PK-gestuurde interventie aangeraden. Dit kan bestaan uit het benadrukken van de therapietrouw, aanpassen van co-medicatie in het geval van geneesmiddelinteracties, gelijktijdig innemen met voedsel, optimaliseren van het doseerschema of verhogen van de dosering.

In **Hoofdstuk 7** werden de eerste (interim) resultaten van de DPOG-TDM studie gerapporteerd. Van de 386 patiënten die evalueerbaar waren voor het primaire eindpunt, had 22.8% een lage blootstelling na 12 weken, vergeleken met 40.4% in historische data ($p < 0.001$). PK-gestuurd doseren heeft het aandeel patiënten met een lage blootstelling dus teruggebracht met 44% (95% betrouwbaarheidsinterval: 32-54%). Van de 543 patiënten die evalueerbaar waren voor de algemene analyses, had 48.1% op enig moment tijdens de behandeling een C_{min} onder de streefwaarde. Bij 53.3% van deze patiënten werd een PK-gestuurde interventie toegepast, welke succesvol was bij 76.6% van de patiënten bij wie het effect was geëvalueerd. Redenen waarom PK-gestuurde interventies niet konden worden toegepast bij andere patiënten waren voornamelijk toxiciteit (63.9%), niet opvolgen van het doseeradvies door de behandelend arts (17.2%) of staken van de behandeling (13.1%). Deze studie heeft laten zien dat PK-gestuurde dosisoptimalisatie van orale doelgerichte therapieën haalbaar is in de klinische praktijk. Daarnaast leidde het tot een reductie van het aantal patiënten met een te lage blootstelling. Daarom ondersteunen deze bevindingen de introductie van PK-gestuurd doseren als standaardzorg.

Hoofdstuk 8 evalueerde PK-gestuurde dosisverhogingen van imatinib bij patiënten met gastro-intestinale stromale tumoren (GIST) in de reguliere patiëntenzorg. Op basis van de sterke rationale is PK-gestuurd doseren van imatinib geleidelijk geïmplementeerd als standaardzorg in het Antoni van Leeuwenhoek sinds 2009. In dit hoofdstuk werden 169 patiënten beschreven van wie 1402 PK samples zijn verzameld. Bij 75% van deze patiënten was de C_{min} onder de streefwaarde voor effectiviteit van 1100 ng/mL. PK-gestuurde dosisverhogingen zijn toegepast in 62% van deze patiënten en waren succesvol bij 63% van hen. De progressievrije overleving was vergelijkbaar tussen patiënten met en zonder dosisverhogingen van imatinib. Door het kleine aantal patiënten met progressieve ziekte konden echter geen definitieve conclusies worden getrokken over het effect op progressievrije overleving. Dit is het grootste cohort waarin PK-gestuurde dosisverhogingen van imatinib zijn beschreven bij patiënten met GIST. Deze bevindingen benadrukken de haalbaarheid van PK-gestuurd doseren van imatinib in de klinische praktijk.

Hoofdstuk 9 beschreef twee patiënten met een wekedelensarcoom die werden behandeld op een acht keer lagere dosering dan de standaard dosering van pazopanib vanwege toxiciteit en toch een C_{min} boven de streefwaarde van 20.5 mg/L behielden. Zonder PK-gestuurd doseren, wanneer de dosering gereduceerd zou zijn volgens de bijsluiter, zou de behandeling zeker gestaakt zijn bij deze patienten, omdat de

blootstelling op een vier keer lagere dosering dan de standaard dosering in de bijsluiter wordt beschouwd als onvoldoende om een klinisch relevant effect te bewerkstelligen. Hier werd de sterke meerwaarde geïllustreerd van het meten van pazopanib concentraties in het geval van dosisreducties vanwege toxiciteit, omdat de blootstelling toch adequaat kan zijn op aanzienlijk lagere doseringen dan de standaard dosering.

Tot slot werden de relevante klinisch farmacokinetische en farmacodynamische kenmerken van orale anti-hormonale geneesmiddelen samengevat en omgezet in praktische richtlijnen voor PK-gestuurd doseren in **Hoofdstuk 10**. Voor sommige geneesmiddelen zijn streefwaarden voor PK-gestuurd doseren nog niet goed vastgesteld en als referentie kan de mediane PK blootstelling worden nagestreefd (dit is het geval voor exemestaan en enzalutamide). Voor de meeste geneesmiddelen konden blootstelling-respons relaties echter worden omgezet in specifieke C_{min} streefwaarden (dit is het geval voor abiraterone: 8.4 ng/mL, anastrozol: 34.2 ng/mL en letrozol: 85.6 ng/mL). Bovendien hebben prospectieve klinische studies laten zien dat PK-gestuurd doseren haalbaar is voor tamoxifen, waarvoor een streefwaarde voor effectiviteit van de actieve metaboliet endoxifen van 5.97 ng/mL wordt voorgesteld. Op basis van de beschikbare data wordt daarom geconcludeerd dat doseren op maat op basis van geneesmiddelconcentraties haalbaar en veelbelovend is voor orale anti-hormonale geneesmiddelen. Dit moet nu verder ontwikkeld worden en geïmplementeerd worden in de klinische praktijk.

DEEL IV – ALTERNATIEVE STRATEGIEËN VOOR DOSEREN OP MAAT

In het laatste deel van dit proefschrift werden alternatieve strategieën uitgelicht die toegepast kunnen worden voor het optimaliseren van de blootstelling, toegespitst op kostenneutrale interventies, geneesmiddelinteracties en biologische beschikbaarheid.

Hoofdstuk 11 rapporteerde de resultaten van de eerste 32 patiënten die behandeld zijn met abiraterone binnen de DPOG-TDM studie. Volgens de bijsluiter moet abiraterone acetaat worden ingenomen in gemodificeerde nuchtere toestand, wat inhoudt dat er geen voedsel twee uur voor en één uur na inname van het geneesmiddel gebruikt mag worden. In eerdere voedsel-effect studies is echter aangetoond dat gelijktijdige inname met voedsel resulteert in een klinisch relevante toename van de blootstelling. Daarom was het doel van deze studie om te evalueren of PK-gestuurd doseren van abiraterone met behulp van voedsel interventies haalbaar is in de klinische praktijk en of dit resulteert in een hoger percentage patiënten met een effectieve blootstelling aan abiraterone zonder extra toxiciteit. Gelijktijdige inname met een lichte maaltijd of een tussendoortje resulteerde in een 2.9-voudige toename in C_{min} en leidde tot een adequate blootstelling bij 87.5% van de patiënten. Alhoewel gelijktijdige inname met voedsel ook resulteerde in een grotere variabiliteit tussen patiënten, en dus een erg hoge C_{min} in sommige patiënten, ervaarden deze patiënten niet meer bijwerkingen. Daarom werd geconcludeerd dat

gelijktijdige inname met voedsel een kostenneutrale mogelijkheid biedt om de blootstelling te optimaliseren voor patiënten met een lage abiraterone C_{min}.

In **Hoofdstuk 12** werd een andere kostenneutrale strategie toegepast om de blootstelling aan pazopanib te optimaliseren. Op de geregistreerde vaste dosering van eenmaal daags 800 mg heeft 16-30% van de patiënten die behandeld worden met pazopanib een te lage blootstelling en lopen dus risico op een verminderde effectiviteit. Daarom is er een sterke rationale voor PK-gestuurd doseren. Eerder is aangetoond dat PK-gestuurd doseren van pazopanib haalbaar is en resulteert in een toename van het aantal patiënten met een adequate blootstelling. Om dit te bereiken was het nodig om de pazopanib doseringen te verhogen tot 1000-1800 mg. Door de non-lineaire opname van pazopanib, die een plateau bereikt op doseringen boven 800 mg, zijn absolute dosisverhogingen geen efficiënte strategie om de blootstelling te verhogen. Bovendien leidt dit tot een toename van de behandelkosten. Een eerder populatie farmacokinetisch model voorspelde dat de relatieve biologische beschikbaarheid van pazopanib op een dosering van 400 mg 59% hoger zou zijn dan op 800 mg. Daarom werd een prospectieve farmacokinetische *cross-over* studie uitgevoerd waarin de farmacokinetiek van eenmaal daags 800 mg pazopanib werd vergeleken met tweemaal daags 400 mg. In deze studie resulteerde het spreiden van innamemomenten van pazopanib in een stijging van 79% in C_{min}, met acceptabele bijwerkingen. Daarmee biedt het een veelbelovende kostenneutrale strategie om de behandeling te optimaliseren voor het aanzienlijke deel van de patiënten met een lage blootstelling aan pazopanib.

De volgende stap was om het spreiden van innamemomenten van pazopanib te implementeren als PK-gestuurde interventie binnen de DPOG-TDM studie. De resultaten van dit cohort werden gerapporteerd in **Hoofdstuk 13** van dit proefschrift. In het geval van C_{min} onder de streefwaarde van 20.5 mg/L en acceptabele toxiciteit werden de inname momenten gesplitst in tweemaal daags 400 mg als eerste stap. Als tweede stap werd gelijktijdige inname met voedsel aangeraden. Door het toepassen van deze PK-gestuurde doseerstrategie, was het percentage patiënten met een lage blootstelling na 12 weken 12.9% (95% betrouwbaarheidsinterval: 3.6-29.8%), vergeleken met 26.7% in historische data. Daarnaast had 37% van de patiënten op een zeker moment tijdens de behandeling een C_{min} onder de streefwaarde. In 82% van deze patiënten kon een PK-gestuurde interventie worden toegepast, die succesvol was in 62% van hen. Daarom werd geconcludeerd dat PK-gestuurde dosisoptimalisatie van pazopanib door middel van kostenneutrale interventies haalbaar is in de klinische praktijk en resulteert in een toegenomen aantal patiënten met een adequate blootstelling.

Hoofdstuk 14 diende als introductie op de klinische geneesmiddelinteractie studie beschreven in het volgende hoofdstuk. Hierin werd een overzicht gegeven van klinisch farmacokinetische en farmacodynamische kenmerken van de cycline-afhankelijke kinasen 4 en 6 (CDK4/6) remmers palbociclib, ribociclib en abemaciclib. De resultaten van

voedsel-effect en geneesmiddelinteractie studies werden samengevat en de huidige kennis over blootstelling-respons en blootstelling-toxiciteit relaties werden besproken. Alle drie de CDK4/6 remmers worden gekenmerkt door een hoge variabiliteit in blootstelling tussen patiënten, een blootstelling in de hersenen die beperkt wordt door *efflux* transporters en uitgebreide metabolisatie door cytochroom P450 3A4 (CYP3A4). De blootstelling aan deze geneesmiddelen wordt in grote mate beïnvloedt door sterke CYP3A4 modulatoren. Hogere blootstelling is geassocieerd met een verhoogd risico op neutropenie voor alle CDK4/6 remmers. Daarnaast is een relatie tussen blootstelling en effectiviteit aangetoond voor abemaciclib, terwijl deze analyses tot nu toe niet eenduidig waren voor palbociclib en ribociclib.

Ondanks de welbekende effecten van sterke CYP3A4 modulatoren op de farmacokinetiek van CDK4/6 remmers, is het effect van matige CYP3A4 remmers tot nu toe alleen onderzocht in fysiologisch gebaseerde PK simulaties. **Hoofdstuk 15** beschreef daarom de voorlopige resultaten van een gerandomiseerde PK *cross-over* studie waarin het effect van de matige CYP3A4 remmer erythromycine op de PK van palbociclib wordt bestudeerd in patiënten met borstkanker. De oppervlakte onder de plasma concentratie-tijd curve (AUC) en de topspiegel (C_{max}) namen toe met respectievelijk 44% en 43%. Dit suggereert dat een dosisreductie van palbociclib van eenmaal daags 125 mg naar eenmaal daags 75 mg rationeel zou zijn bij gelijktijdig gebruik met matige CYP3A4 remmers. Bovendien kan deze studie als voorbeeld dienen voor andere orale doelgerichte therapieën die gemetaboliseerd worden door CYP3A4, en voor andere matige CYP3A4 remmers.

Een belangrijke factor in de grote variabiliteit in blootstelling van orale doelgerichte therapieën, is een suboptimale biologische beschikbaarheid. Daarom is het vaststellen van de absolute biologische beschikbaarheid cruciaal in de ontwikkeling van geoptimaliseerde orale formuleringen. In de studie beschreven in **Hoofdstuk 16** is een innovatieve studieopzet toegepast om de absolute biologische beschikbaarheid van oraal imatinib te bepalen door middel van een stabiele isotoop gelabelde intraveneuze imatinib-d8 microdosis. De mediane absolute biologische beschikbaarheid op *steady-state* was 76%. Dat is minder dan de 98% die gevonden is met de traditionele *two-period cross-over* opzet bij gezonde vrijwilligers. Deze studie toont de potentie van het gebruik van een stabiele isotoop gelabelde microdosis voor het beoordelen van de absolute biologische beschikbaarheid. Hopelijk leidt deze nieuwe benadering tot een toename van meer relevante kennis van de farmacokinetiek van nieuwe orale doelgerichte therapieën in een vroeg stadium van geneesmiddelenontwikkeling.

Concluderend beschreef dit proefschrift de resultaten van verschillende studies naar doseren op maat van orale doelgerichte therapieën binnen de oncologie. Er wordt geconcludeerd dat er een sterke rationale is voor doseren op maat en dat gerandomiseerde studies niet nodig zijn om de klinische relevantie daarvan aan te tonen. Daarnaast was de blootstelling gerelateerd aan de effectiviteit voor veel orale

doelgerichte therapieën. Bovendien was PK-gestuurd doseren haalbaar in de klinische praktijk voor de meeste geneesmiddelen en konden kostenneutrale strategieën worden toegepast om de blootstelling te optimaliseren voor sommige van deze geneesmiddelen. Tot slot kan de behandeling met orale doelgerichte therapieën nog verder worden geoptimaliseerd door onderzoek te doen naar geneesmiddelinteracties en de biologische beschikbaarheid. Bij elkaar genomen heeft doseren op maat de potentie om de behandeling met orale antikanker medicijnen verder te verbeteren, en daarom zou het geïmplementeerd moeten worden in de routine patiëntenzorg.

Appendices

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List of publications
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CURRICULUM VITAE

Steffie Groenland was born on October 20th 1991 in Obdam, The Netherlands. In 2010 she graduated from Gymnasium at the Trinitas College, location Han Fortmann in Heerhugowaard. Subsequently, she started studying Medicine at the VU Medical Center in Amsterdam. During this study, she participated in the student-run clinic and coordinated its pharmacovigilance project. As part of her master's program, she performed a research internship at the department of Nephrology of the Northwest Clinics in Alkmaar, focusing on the relation between duration of the prodromal phase and renal damage in ANCA-associated vasculitis. After graduating *cum laude* in 2016, she started as a PhD candidate at the department of Clinical Pharmacology at the Netherlands Cancer Institute – Antoni van Leeuwenhoek in Amsterdam under supervision of prof. dr. Alwin Huitema, prof. dr. Jos Beijnen and dr. Neeltje Steeghs. Her PhD research focused on precision dosing of oral targeted therapies in oncology and resulted in this thesis. Her main project was setting up and coordinating a large prospective clinical trial in ten hospitals throughout the Netherlands. In 2019 she received an ESMO Merit Award for an oral presentation on this work in Barcelona, Spain. During her PhD research, she also trained as a clinical pharmacologist. In December 2020, she started working as a resident in internal medicine at the Northwest Clinics in Alkmaar.