ORIGINAL RESEARCH ARTICLE

CYP3A4*22 Genotype-Guided Dosing of Kinase Inhibitors in Cancer Patients

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Abstract

Introduction A genetic variant explaining a part of the exposure of many kinase inhibitors (KIs) is the single nucleotide polymorphism (SNP) CYP3A4*22, resulting in less CYP3A4 enzyme activity. The primary aim of this study was to investigate if the systemic exposure is non-inferior after a dose reduction of KIs metabolized by CYP3A4 in CYP3A4*22 carriers compared to patients without this SNP (i.e., wildtype patients) receiving the standard dose.

Methods In this multicenter, prospective, non-inferiority study, patients were screened for the presence of CYP3A4*22. Patients with the CYP3A4*22 SNP received a 20–33% dose reduction. At steady state, a pharmacokinetic (PK) analysis was performed and compared to the PK results from wildtype patients treated with the registered dose using a two-stage individual patient data meta-analysis approach.

Results In total, 207 patients were included in the final analysis. The CYP3A4*22 SNP was found in 16% of the patients in the final analysis (n = 34). Most of the included patients received imatinib (37%) or pazopanib (22%) treatment. The overall geometric mean ratio (GMR) comparing the exposure of the CYP3A4*22 carriers to the exposure of the wildtype CYP3A4 patients was 0.89 (90% confidence interval: 0.77–1.03).

Conclusion Non-inferiority could not be proven for dose reduction of KIs metabolized by CYP3A4 in CYP3A4*22 carriers compared to the registered dose in wildtype patients. Therefore, an up-front dose reduction based upon the CYP3A4*22 SNP for all KIs does not seem an eligible new way of personalized therapy.

Trial Registration International Clinical Trials Registry Platform Search Portal; number NL7514; registered 11/02/2019.

Key Points

The SNP CYP3A4*22 results in a decreased CYP3A4 enzyme activity, which is involved in the metabolism of several drugs.

CYP3A4*22 carriers receiving an up-front 20–33% dose reduction of a CYP3A4 metabolized KI did not have a non-inferior exposure compared to wildtype patients receiving the registered dose.

CYP3A4*22 genotype-guided dosing does not seem a suitable dosing strategy for all CYP3A4 metabolized KIs.

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1 Introduction

Kinase inhibitors (KIs) are important in various cancer treatments. Kinase inhibitors can inhibit kinases, which are responsible for many cell functions, including cell signaling, growth, and division. Although KI treatment has improved the survival of many cancer types over the last decades, side effects are common and severe toxicity still raises the concern of patients and their treating physicians.

For many KIs, the incidence and severity of the toxicity are related to drug exposure [1]. For example, higher imatinib exposure has been associated with anemia, neutropenia, leukopenia, and thrombocytopenia [2], while a higher pazopanib exposure has been associated with hypertension [3], and a high sunitinib exposure has been associated with more dose-limiting and grade ≥ 3 toxicities [4, 5]. Besides, KI exposure has a high inter- and intra-patient variability [6, 7]. This high variability is caused by

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several factors. Variability in the rate of drug metabolism is one of the factors [8].

Most KIs are predominantly metabolized by the CYP3A4 enzyme [9]. The CYP3A4 iso-enzyme forms a major part of the cytochrome P450 enzyme family [10]. The activity of CYP3A4 is highly variable between patients [11, 12]. A genetic variant explaining a part of the variability in the CYP3A4 activity is the *CYP3A4* intron 6 single nucleotide polymorphism (SNP) (rs35599367C >T or *CYP3A4*22*) [13, 14]. Due to this SNP, the formation of a non-functional splice variant of CYP3A4 is increased and consequently CYP3A4 protein levels and enzyme activity are decreased. The minor allelic frequency (MAF) for *CYP3A4*22* is 5.0% in Europeans, 2.6% in the admixed American population and < 1% for the Asian and African population [15]. The phenotypic prevalence of *CYP3A4*22* carriers in Europeans is thereby estimated to be around 10%.

As a result of the reduced CYP3A4 enzyme activity, the drug exposure of *CYP3A4*22* carriers who are treated with a CYP3A4 metabolized KI is higher compared to patients without this SNP (i.e., wildtype patients). This was acknowledged in several previous studies. For example, in a retrospective study including 114 patients treated with sunitinib, a 22.5% decrease in clearance of sunitinib was found for *CYP3A4*22* carriers [16]. Furthermore, in a population-pharmacokinetic model with data from 97 patients treated with pazopanib, a substantial lower clearance of 35% was found for *CYP3A4*22* carriers [17]. Moreover, the performed simulations estimated that trough concentrations at steady state are 50% higher in *CYP3A4*22* carriers [17]. A higher KI exposure caused by *CYP3A4*22* could possibly lead to more toxicity given the frequently observed exposure-toxicity relationships in anticancer drugs with a narrow therapeutic index [18].

The aim of the current study was to demonstrate that a dose reduction of KIs metabolized by CYP3A4 in *CYP3A4*22* carriers does not result in a lower exposure compared to wildtype patients receiving the standard dose. The secondary aim was to compare the incidence of toxicity and dose modifications after pharmacokinetic assessment between *CYP3A4*22* carriers and wildtype patients.

2 Methods

2.1 Study Drugs

Oral targeted anti-cancer drugs were included in the study protocol if drugs were primarily metabolized by CYP3A4, defined as an increase of at least 25% on area under the curve (AUC) when the KI was co-administered with a strong CYP3A4 inhibitor, like ketoconazole or itraconazole, according to the Summary of Product Characteristics

(SmPC) of the drug. Eventually, 19 drugs were included in this study (Table 1).

2.2 Patient Selection and Study Procedures

All adult cancer patients with an indication to start treatment (or have started treatment less than 7 days ago) with one of the study drugs described in Table 1 with a WHO performance status ≤ 2, not using medication or (herbal) supplements, which are known or suspected to strongly inhibit or induct the CYP3A4 enzymes, and who were able and willing to undergo blood sampling for pharmacokinetic (PK) and pharmacogenetic (PG) analysis, met the study selection criteria.

The study procedures are depicted in Fig. 1. All included patients were screened for the presence of the *CYP3A4*22* SNP using PG analysis. Due to the available formulations, it was not possible to apply a uniform dose reduction for all drugs. Therefore, depending on available tablet/capsule forms, a dose reduction between 20 and 33% was chosen per drug. Within the group of *CYP3A4*22* carriers treated with the same drug the same dose reduction was applied. Since no in vivo data regarding differences in CYP3A4 activity between heterozygous and homozygous CYP3A4*22 carriers is available, the same dose reduction was applied for these groups. The drug dose was not adjusted based on other

Table 1 Overview of all study drugs and number of included patients per drug

Study drug	Total patients	*CYP3A4*22* carriers
Brigatinib	1	0
Cabozantinib	15	3
Cobimetinib	4	0
Dabrafenib	40	5
Dasatinib	0	0
Encorafenib	5	0
Erlotinib	0	0
Everolimus	17	0
Imatinib (400 mg starting dose only)	92	11
Lapatinib	0	0
Nilotinib	0	0
Olaparib	45	1
Osimertinib	22	3
Palbociclib	3	0
Pazopanib	58	12
Ponatinib	0	0
Regorafenib	7	0
Ruxolitinib	1	0
Sunitinib	41	3
Total	351	38

CYP3A4 SNPs. The dose reduction lasted for 2–4 weeks (or at least five times the drug elimination half-life) after reporting of the SNP status to guarantee a steady-state exposure. Wildtype patients were treated with the standard dose in accordance with the SmPC of the European Medicine Agency (EMA). At steady state, a blood sample was collected for the PK analysis. Patients were instructed to obtain a PK sample directly prior to drug intake (i.e., C_{trough}) at steady state. If withdrawal of the PK sample at C_{trough} was impossible for logistical reasons, a blood sample for PK analysis was collected after reaching the time of maximum drug concentration (i.e., T_{max}). Samples taken after T_{max} were extrapolated to a calculated C_{trough} using the time after dose, drug elimination half-life reported in the SmPC, and dose interval, by log-linear extrapolation [19]. Patient characteristics, toxicity data, and laboratory results were collected at baseline and during the study period. The study ended after withdrawal of blood for the PK analysis and patients continued treatment with the standard dose or reduced dose according to physicians' choice. The 4 participating centers were Erasmus MC Cancer Institute, the Netherlands Cancer Institute, Leiden University Medical Centre and ADRZ Goes. Ethical approval was obtained from the Medical Ethical Committee of the Erasmus Medical Center and the study protocol was also approved by the board of directors of each participating center. The trial was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, applicable regulations, and the Declaration of Helsinki. The trial was registered in the Netherlands Trial Register (International Clinical Trials Registry Platform Search Portal [trialsearch. who.int]; number NL7514). All patients provided written informed consent.

2.3 Pharmacogenetic and Pharmacokinetic Analysis

All PG analyses were executed at the Erasmus University Medical Center. A blood sample of 4 mL was collected and isolated with the Nucleic Acid Isolation Kit I on the MagNA Pure Compact Instrument (Roche Diagnostics, Almere, The Netherlands). Genotyping was performed with the INFINITI® CYP450 3A4-3A5 Assay on the INFINITI® High Throughput System (HTS) (AutoGenomics Inc., Vista, CA USA). For confirmation, a 5′ nuclease assay qPCR (TaqMan™) using a Real-Time Quantstudio PCR System (ThermoFisher, Waltham, MA, US) was executed.

For the PK analysis, a 4 mL blood sample was collected after reaching steady state. Pharmacokinetic samples were processed to plasma or serum depending on the bioanalytical assay and stored at $T < -70$ °C until the time of analysis. Samples were analyzed in the local laboratory or transferred to the laboratory of another study site, depending on the study drug and availability of a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assay [20–24]. All bioanalytical methods were validated according to US FDA guidelines [25]. Pharmacokinetic measurements were performed in the laboratory of Translational Pharmacology, Department of Medical Oncology at the Erasmus Medical Center, the laboratory of Clinical Pharmacy and Toxicology at the Leiden University Medical Centre or the Bioanalytical Laboratory of the Pharmacy at the Netherlands Cancer Institute. Inter-laboratory comparisons were performed to guarantee interchangeability of the PK measurements.

2.4 Sample Size and Statistical Analyses

The aim of the study was to demonstrate that the trough level of *CYP3A4*22* carriers with a reduced dose is non-inferior to the trough level of non-carriers with a standard dose. The trough level was assumed to follow a log-normal distribution. With a non-inferiority margin of − 20% in relative difference (RD) (i.e., a difference of − 0.2231 on log-transformed data) and an average coefficient of variation of 40% (translated to a standard deviation [SD] of approximately
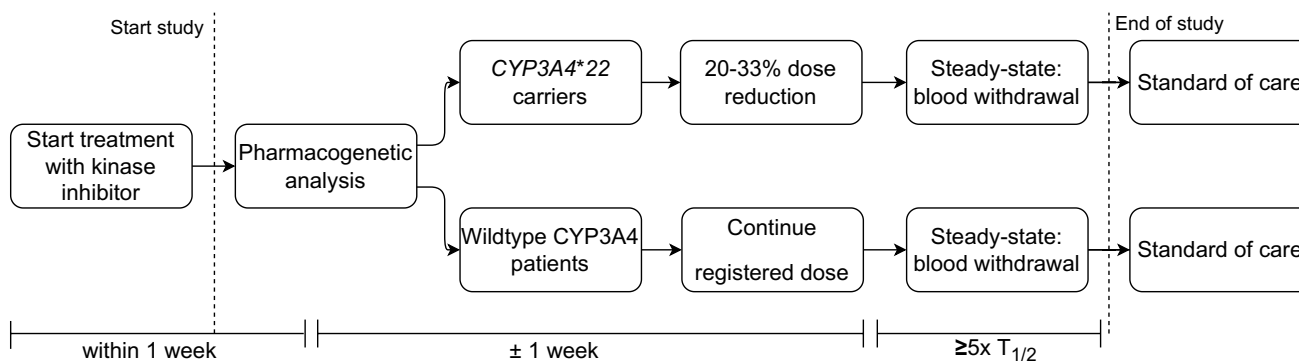

Fig. 1 Timeline of the study procedures

0.40 on the log-scale), at least 198 patients were required for this study of whom at least 33 had to be a *CYP3A4*22* carrier (power of 90% and one-sided alpha of 5%) [26]. Only study drugs with at least two *CYP3A4*22* carriers per drug were included in the final analysis. Inclusion of patients stopped when all these three criteria were met (i.e., at least 33 *CYP3A4*22* carriers, at least two *CYP3A4* carriers per drug, at least 165 CYP3A4 wildtype patients).

The two-stage individual patient data (IPD) meta-analysis was applied considering that several drugs were included. Each study drug was seen as a separate study within this analysis. Hereto, the 'ipdmetan' package in Stata was used [27]. Non-inferiority of the trough level was evaluated by looking at whether the lower bound of the two-sided 90% confidence interval (CI) for difference between carriers and non-carriers was higher than the non-inferiority boundary of −0.2231 on the log-scale (this boundary is equivalent to a − 20% relative difference and a geometric mean ratio of 0.80).

Non-inferiority regarding drug exposure was also analyzed for the two study drugs with the most *CYP3A4*22* carriers separately (i.e., pazopanib and imatinib). Pharmacokinetic data were log normalized before performing an independent sample t-test. Geometric mean ratio and corresponding 90% CI, interpreted as the RD in percentage, were calculated by exponentiation of the mean difference and 90% CI of this difference derived from the independent sample t-test. Exposure was regarded as non-inferior if the lower boundary of the CI of the geometric mean ratio was higher than 0.8 (i.e., equivalent to a −20% relative difference). For these compounds, the incidence of patients with an exposure above TDM targets proposed by Verheijen et al were compared between the *CYP3A4*22* carriers and the wildtype patients by the Fisher's exact test [28].

For the Hardy-Weinberg equilibrium, the distribution of genotypes was tested by a chi-squared test. Severe or medically significant toxicities (i.e., Grade ≥ 3 based on Common Terminology Criteria for Adverse Events [CTCAE] version 4.03) were described with numbers of patients and percentages for *CYP3A4*22* carriers and wildtype patients separately. Incidence of dose modifications at the end of study period was described using descriptive statistics.

3 Results

3.1 Patient Characteristics

In total, 351 patients were included in the study (Fig. 2), 88 of whom were excluded from the analysis. The most common reasons for exclusion were preliminary stop with the study drug due to toxicity (*n* = 28: 26 wildtype patients, two *CYP3A4*22* carriers) or progressive disease (*n* = 10;

9 wild-type patients, one unknown), a dose reduction not according to protocol (*n* = 20; 20 wildtype patients) and incorrect timing of the blood draw for PK analysis (*n* = 10). Furthermore, another 56 patients did complete the study successfully but were not evaluable for the primary endpoint because fewer than two *CYP3A4*22* carriers treated with the same drug were included in the study group (i.e., brigatinib, cobimetinib, encorafenib, everolimus, olaparib, palbociclib, regorafenib, ruxolitinib). Therefore, 207 patients were eventually included in the final analysis.

The median age of the patients included in the final analysis (*n* = 207) was 64 years and 54% were male (Table 2). Most patients had a WHO performance status of 0 (46%) or 1 (43%), 72% had metastatic disease, and 58% had not received systemic treatment before. More than half of the patients included in the final analysis received imatinib (37%) or pazopanib (22%), followed by dabrafenib (15%), sunitinib (12%), osimertinib (10%) and cabozantinib (4%). The majority of the *CYP3A4*22* carriers were treated with pazopanib (32%) or imatinib (29%). Table 2 depicts an extensive summary of the baseline characteristics.

3.2 Pharmacogenetics

From all 351 patients who were included in the study, 38 *CYP3A4*22* carriers were identified (11%) (Table 1). The *CYP3A4*22* SNP was in Hardy–Weinberg equilibrium (*p* = 0.08). The MAF of the SNP in all these patients was 6%. From the 207 patients included in the final analysis, 34 patients were *CYP3A4*22* carrier (16%) (Table 2). Most of these patients had a heterozygote *CYP3A4*22* genotype (15%), but three patients were homozygote for the SNP *CYP3A4*22* (1%) who were treated with a dose conform the heterozygote *CYP3A4*22* carriers. Exact genotypes observed in the patients included in the primary analysis are depicted in Table 2. Notably, from the nine included cabozantinib patients, three were *CYP3A4*22* carrier (33%) and from the 46 pazopanib patients, 11 were *CYP3A4*22* carrier (24%), both of which are considerably higher proportions than those reported in the other study drugs (osimertinib 3/21 [14%], dabrafenib 4/30 [13%], imatinib 10/76 [13%], sunitinib 3/25 [12%]).

3.3 Results of Pharmacokinetic Analysis

True C_{trough} measurements were taken in 35% of all PK samples. For the other 65% of the samples, the C_{trough} was estimated [19, 29]. An overview of the geometric mean C_{trough} and the 95% CI of the geometric mean per drug is given in Table 3. The overall geometric mean ratio comparing the exposure of the *CYP3A4*22* carriers to the exposure of the wildtype CYP3A4 patients is 0.89 (90% CI 0.77–1.03). This is equal to a RD in C_{trough} exposure (*CYP3A4*22* vs wildtype

Fig. 2 Study flowchart
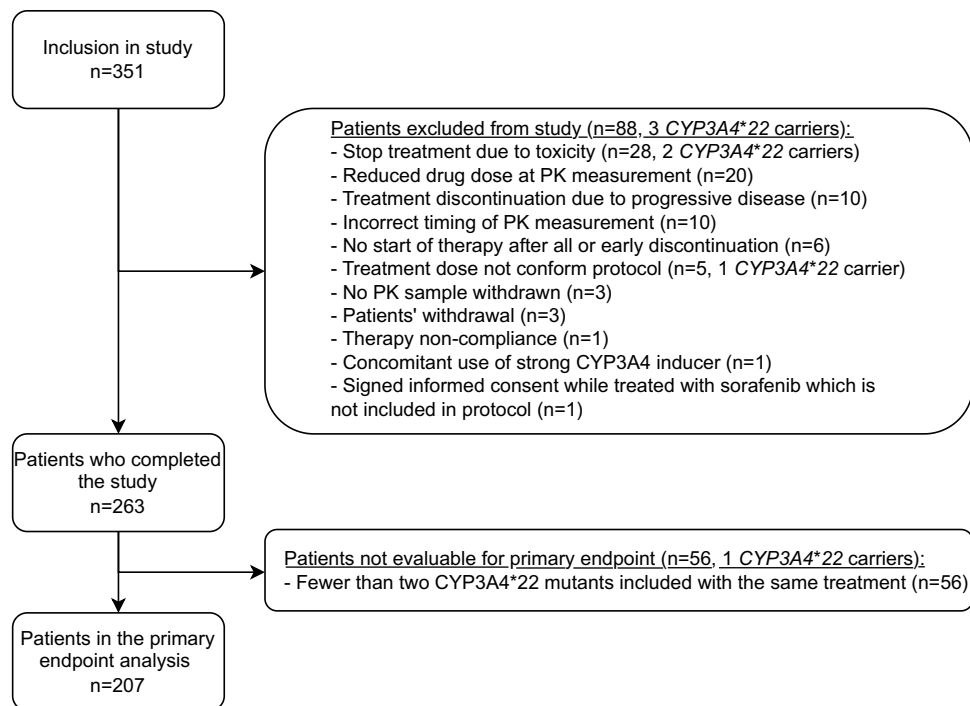
CYP3A4) of − 11.0% (90% CI RD: − 23 to 3.3%). Figure 3 represents a forest plot of the two-stage IPD meta-analysis approach with the effect sizes of the individual drugs (i.e., geometric mean ratios of C_{trough} and their 90% CIs), the weight of each drug on the overall effect and the overall effect size.

3.4 Pharmacokinetics of Pazopanib

In total, 46 patients treated with pazopanib were included in the final analysis (22%). Ten patients were genotyped *CYP3A4*1/*22* while one patient was *CYP3A4*22/*22*. The other 35 patients were wildtype patients.

The geometric mean C_{trough} of the *CYP3A4*22* carriers treated with 600 mg once daily (OD) was 28.3 mg/L (95% C: 20.6–39.0 mg/L) while the geometric mean C_{trough} of the wildtype patients treated with 800 mg OD was 27.1 mg/L (95% CI 21.9–33.6 mg/L). The geometric mean ratio comparing the C_{trough} of *CYP3A4*22* carriers versus wildtype patients was 1.05 (90% CI 0.74–1.48).

In the group of *CYP3A4*22* carriers, four patients (36%) had a C_{trough} exposure below the earlier proposed efficacy threshold of 20.5 mg/L [28], compared to 34% ($n = 12$) of the wildtype pazopanib patients ($p = 1.00$).

3.5 Pharmacokinetics of Imatinib

In total, 76 patients treated with imatinib were included in the final analysis (37%). Ten patients were genotyped

as heterozygote *CYP3A4*22* carriers and 66 as wildtype patients.

The geometric mean C_{trough} of the *CYP3A4*22* carriers treated with imatinib 300 mg OD was 792 ng/mL (95% CI 636–986 ng/mL). The geometric mean C_{trough} of the wildtype patients treated with imatinib 400 mg OD was 1201 ng/mL (95% CI 1071–1345 ng/mL). The geometric mean ratio comparing the C_{trough} of *CYP3A4*22* carriers versus wildtype patients was 0.66 (90% CI 0.51–0.85).

In the group of *CYP3A4*22* carriers, nine out of ten patients (90%) had a C_{trough} exposure below the adopted efficacy threshold of 1100 ng/mL [28], while the incidence of patients with a exposure below the target was 45% ($n = 30$) in the wildtype patients ($p = 0.014$).

3.6 Toxicity and Incidence of Dose Modifications

Overall, during the study period (i.e., after the baseline screening until the end of study) eight (24%) *CYP3A4*22* carriers presented with grade ≥ 3 adverse events compared to 30 (17%) patients with grade ≥ 3 adverse events in the wildtype group. An overview of the grade ≥ 3 adverse events occurring during the study period (i.e., after the baseline screening until the end of study) is presented in Table 4.

For the patients treated with imatinib, grade ≥3 adverse events were observed in 12% ($n = 8$) of the wildtype patients compared to 10% ($n = 1$) of the *CYP3A4*22* carriers. Of the wildtype patients treated with pazopanib, 29% ($n = 10$) reported grade ≥3 adverse events while 36% ($n = 4$) of the

Table 2 Baseline characteristics of patients included in final analysis

Characteristic	All patients (*n* = 207)	Wildtype CYP3A4 patients (*n* = 173)	*CYP3A4*22* carriers (*n* = 34)
Sex			
Female	95 (46%)	75 (43%)	20 (59%)
Male	112 (54%)	98 (57%)	14 (41%)
Ethnic origin			
White	188 (91%)	155 (90%)	33 (97%)
Asian	6 (3%)	6 (3%)	–
Arabic	6 (3%)	6 (3%)	–
Black	3 (2%)	3 (2%)	–
Other	4 (2%)[a]	3 (2%)[b]	1 (3%)[c]
Age (at inclusion)			
Median [IQR] in years	64 [55–73]	65 [56–73]	62 [55–72]
WHO performance score			
0	96 (46%)	79 (46%)	17 (50%)
1	89 (43%)	75 (43%)	14 (41%)
2	22 (11%)	19 (11%)	3 (9%)
*CYP3A4*22* status			
No *CYP3A4 *22* carrier	173 (84%)	173 (100%)	NA
Heterozygote *CYP3A4 *22* carrier	31 (15%)	NA	31 (91%)
Homozygote *CYP3A4 *22* carrier	3 (1%)	NA	3 (9%)
Other *CYP3A4* SNPs			
*CYP3A4 *1/*1B*	14 (6.8%)	14 (8%)	–
*CYP3A4 *1B/*1B*	1 (0.5%)	1 (0.6%)	–
*CYP3A4 *1/*3*	1 (0.5%)	1 (0.6%)	–
*CYP3A4 *1/*12*	1 (0.5%)	1 (0.6%)	–
*CYP3A4 *3/*22*	5 (2.4%)	NA	5 (15%)
No other SNPs	185 (89%)	156 (90%)	29 (85%)
Drug			
Cabozantinib	9 (4%)	6 (4%)	3 (9%)
Dabrafenib	30 (15%)	26 (15%)	4 (12%)
Imatinib (400 mg starting dose only)	76 (37%)	66 (38%)	10 (29%)
Osimertinib	21 (10%)	18 (10%)	3 (9%)
Pazopanib	46 (22%)	35 (20%)	11 (32%)
Sunitinib	25 (12%)	22 (13%)	3 (9%)
Primary malignancy			
GIST	85 (41%)	75 (43%)	10 (29%)
Sarcoma	46 (22%)	35 (20%)	11 (32%)
Melanoma	26 (13%)	23 (13%)	3 (9%)
Lung cancer	25 (12%)	21 (12%)	4 (12%)
Renal cell carcinoma	24 (12%)	18 (10%)	6 (18%)
Neuroendocrine tumor	1 (1%)	1 (1%)	0 (0%)
Metastatic disease			
Yes	148 (71%)	125 (72%)	23 (68%)
No	59 (29%)	48 (28%)	11 (32%)
Prior systemic treatment			
Yes	87 (42%)	71 (41%)	16 (47%)
No	120 (58%)	102 (59%)	18 (53%)

GIST gastro-intestinal stromal tumor, *IQR* interquartile range, *NA* not applicable, *SNP* single nucleotide polymorphism, *WHO* World Health Organization

[a]Mixed racial parentage (*n* = 2), latino (*n* = 1), unknown ethnic origin (*n* = 1)

[b]Mixed racial parentage (*n* = 1), latino (*n* = 1), unknown ethnic origin (*n* = 1)

[c]Mixed racial parentage (*n* = 1)

Table 3 Overview of the geometric mean C_{trough} and 95% CI of the GM per drug

Drug	Wildtype CYP3A4		CYP3A4*22 carriers	
	GM C_{trough}	95% CI GM	GM C_{trough}	95% CI GM
Cabozan-tinib	1063 ng/mL	728–1550	1,117 ng/mL	796–1568
Dabrafenib	120 ng/mL	74–195	101 ng/mL	39–262
Imatinib	1200 ng/mL	1072–1345	792 ng/mL	636–985
Osimertinib	219 ng/mL	177–270	235 ng/mL	72–766
Pazopanib	27 mg/L	22–34	28 mg/L	21–39
Sunitinib	61 ng/mL	51–73	66 ng/mL	40–107

CI confidence interval, GM geometric mean

CYP3A4*22 carriers reported grade ≥3 adverse events. Supplementary Tables 1 and 2 summarize the grade ≥3 adverse events observed during the study period (i.e., after the baseline screening until the end of study) of the patients treated with imatinib and pazopanib, respectively.

Of the patients enrolled in the study ($n = 351$), 10 patients (9 wildtype patients, one unknown) discontinued treatment prematurely due to progressive disease. Furthermore, 48 patients (2 CYP3A4*22 carriers and 46 wildtype patients) received a dose reduction or discontinued treatment due to toxicity prior to PK assessment. These patients were excluded from the final analysis (Fig. 2). The two CYP3A4*22 carriers who stopped their treatment due to toxicity, stopped their treatment before their CYP3A4 genotype was known and were therefore treated with the full dose at the time of toxicity/end of therapy. From the patients included in the final analysis ($n = 207$), the dose was lowered due to toxicity after the pharmacokinetic analysis in three CYP3A4*22 carriers (9%) and 30 wildtype patients (17%). A dose increase was performed in 14 CYP3A4*22 carriers (41%) after the PK analysis in the study: ten based on C_{trough} (imatinib $n = 8$, pazopanib $n = 2$), three preference patient/physician (osimertinib $n = 2$, pazopanib $n = 1$) and

one because of disappointing efficacy (dabrafenib $n = 1$). In 16 wildtype patients (9%) the dose was increased due to low C_{trough} and for one patient the dose was increased because of no toxicity.

4 Discussion

In this prospective study, CYP3A4*22 carriers receiving an up-front 20–33% dose reduction of a CYP3A4 metabolized KI did not have a non-inferior exposure compared to wildtype patients receiving the registered dose. Although a trend towards comparable exposure was seen for patients treated with cabozantinib, pazopanib, sunitinib and osimertinib, the exposure of imatinib was significantly lower in CYP3A4*22 carriers treated with the reduced dose, exposing them to a risk of under-dosing. In terms of toxicity, wildtype patients more often received a dose reduction due to toxicity, but the incidence of grade ≥ 3 adverse events was comparable between CYP3A4*22 carriers and wildtype patients. Based on the results from the current study, standard dose reductions based on CYP3A4*22 genotyping for patients treated with CYP3A4 metabolized KI should not be recommended.

The rationale of performing a dose reduction for CYP3A4*22 carriers was based on results from previous literature. Due to the CYP3A4*22 SNP, the formation of a nonfunctional CYP3A4 alternative splice variant was 2-fold higher, while the CYP3A4*22 genotype resulted in a CYP3A4 mRNA level and CYP3A4 protein level of 58% and 28% of the levels produced with a wildtype CYP3A4 genotype [13, 14, 30]. In CYP3A4*22 carriers the clearance of erythromycin (a CYP3A4 probe drug), sunitinib, and pazopanib, was reduced by 40%, 22.5%, and 35%, respectively [16, 17, 31]. There are several possible explanations why the hypothesis of the study could not be confirmed. First, the current study included many different KIs introducing statistical challenges. It could be that up-front

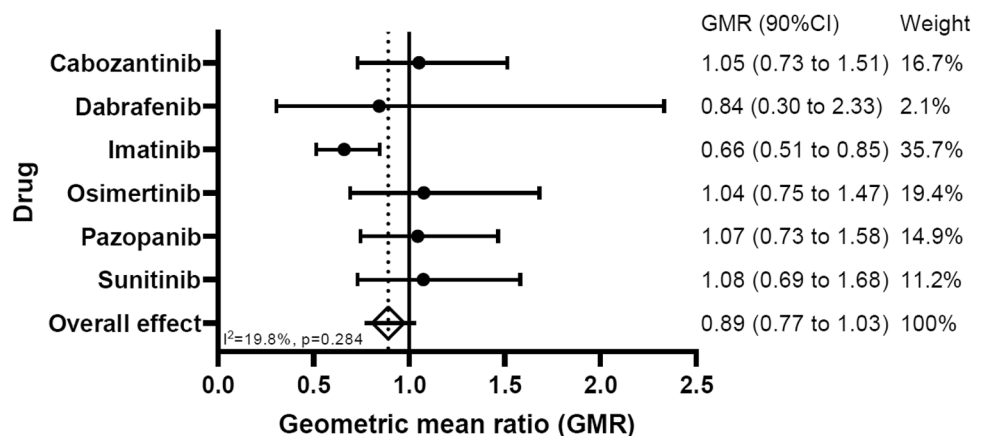**Fig. 3** Forest plot of the two-stage individual patient data meta-analysis

Drug	GMR (90%CI)	Weight
Cabozantinib	1.05 (0.73 to 1.51)	16.7%
Dabrafenib	0.84 (0.30 to 2.33)	2.1%
Imatinib	0.66 (0.51 to 0.85)	35.7%
Osimertinib	1.04 (0.75 to 1.47)	19.4%
Pazopanib	1.07 (0.73 to 1.58)	14.9%
Sunitinib	1.08 (0.69 to 1.68)	11.2%
Overall effect	0.89 (0.77 to 1.03)	100%

$I^2 = 19.8\%$, $p = 0.284$

Table 4 Overview of grade ≥3 toxicity

Grade ≥ 3 toxicity	Wildtype CYP3A4 patients (n = 173)	CYP3A4*22 carriers (n = 34)	All patients (n = 207)
Hyperglycemia	3 (1.7%)	1 (2.9%)	4 (1.9%)
eGFR decrease	1 (0.6%)	–	1 (0.5%)
Hyponatremia	4 (2.3%)	1 (2.9%)	5 (2.4%)
Hypomagnesemia	1 (0.6%)	–	1 (0.5%)
Hypoalbuminemia	1 (0.6%)	–	1 (0.5%)
AST increase	3 (1.7%)	–	3 (1.4%)
ALT increase	3 (1.7%)	–	3 (1.4%)
GGT increase	6 (3.5%)	1 (2.9%)	7 (3.4%)
ALP increase	3 (1.7%)	–	3 (1.4%)
Bilirubin increase	2 (1.2%)	1 (2.9%)	3 (1.4%)
Anemia	2 (1.2%)	–	2 (1%)
Thrombocytopenia	–	1 (2.9%)	1 (0.5%)
Leukocytopenia	1 (0.6%)	–	1 (0.5%)
Neutropenia	1 (0.6%)	–	1 (0.5%)
Nausea	1 (0.6%)	–	1 (0.5%)
Vomiting	1 (0.6%)	–	1 (0.5%)
Mucositis	1 (0.6%)	–	1 (0.5%)
Diarrhea	1 (0.6%)	–	1 (0.5%)
Sensory neuropathy	1 (0.6%)	1 (2.9%)	2 (1.0%)
Motoric neuropathy	–	1 (2.9%)	1 (0.5%)
Fatigue	1 (0.6%)	–	1 (0.5%)
Fever	1 (0.6%)	–	1 (0.5%)
Rash	1 (0.6%)	–	1 (0.5%)
Pain	2 (1.2%)	1 (2.9%)	3 (1.4%)
Hypertension	6 (3.5%)	3 (8.8%)	9 (4.3%)
Vasculitis	1 (0.6%)	–	1 (0.5%)

ALP alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *eGFR* estimated glomerular filtration rate, *GGT* gamma glutamyl transferase

genotyping of CYP3A4 is useful for specific agents, but not for all KIs that are primarily metabolized by CYP3A4. In the current study, the non-inferiority requirements were not met for the entire study group nor for the individual drugs. Imatinib exposure was even significantly lower after dose reduction in *CYP3A4*22* carriers in the current study, so initial dose reduction for this drug should not be recommend. The weight of the imatinib results is 35% and thereby has a considerable influence on the overall effect. However, looking at the results from cabozantinib, osimertinib, pazopanib and sunitinib, the lower boundary of the CI of the GMR for these drugs was just below 0.8 and the GMR point estimates above 1.0 (Fig. 3). Although conclusions for individual drugs based on the current analysis are underpowered, these results suggest that a non-inferior exposure might be found for cabozantinib, osimertinib, pazopanib, and sunitinib in a study with a larger sample size. Future studies are warranted to investigate this. Another possible explanation of why the hypothesis was not confirmed in the current study is that all included drugs are predominantly,

but not exclusively, metabolized by CYP3A4. For instance, cabozantinib is also partly metabolized by CYP2C9 and dabrafenib and pazopanib by CYP2C8, neither of which were measured or genotyped in this study [32–34]. Drug transporters such as P-glycoprotein or Breast Cancer Resistance Protein can also influence drug exposure of pazopanib and dabrafenib [33, 34]. Variation in activity of other CYP enzymes and drug transporters were not tested in our study but could have influenced the observed drug exposure. In previous studies, the exposure of pazopanib after 600 mg and 800 mg doses was almost comparable despite *CYP3A4* genotype [35], which can be attributed to its complex nonlinear pharmacokinetics with saturable absorption plateauing at doses above 600 mg. Of note, removal of the patients treated with pazopanib from our final analysis did not change the overall results of our study (results not shown). Contrary to our expectations and results from previous literature, the exposure of imatinib was significantly lower for *CYP3A4*22* carriers. Imatinib is a CYP3A4 inhibitor by itself. Due to this auto-inhibition of CYP3A4, the metabolism of imatinib

might not solely rely on CYP3A4 but relies on other metabolism routes at steady-state [36]. Therefore the influence of *CYP3A4*22* on the exposure of imatinib might be lower than expected.

Surprisingly, the incidence of grade ≥ 3 toxicity did not seem higher in the wildtype patients compared to *CYP3A4*22* carriers, but dose reductions due to toxicity after the PK analysis were more often performed in this group. This might partly be explained given that many patients who experienced toxicity received a dose reduction early in the study and were therefore excluded from the final analysis. Adverse events experienced by the excluded patients were not accounted for in the current toxicity analysis, which could have biased the overview of the toxicity in both groups.

This is the first prospective study investigating upfront *CYP3A4*22* genotyping and dosing of KIs in cancer patients. The prospective non-inferiority design of the study and the inclusion of multiple KIs are strengths of this study. At the same time, the inclusion of several KIs means that the study was underpowered for analysis of individual drugs. Therefore, it is hard to make statements about the effect of *CYP3A4*22* genotyping for each individual KI. Since we considered it very complicated to include a sufficient number of patients for each drug, a two-stage IPD meta-analysis approach was chosen. Besides, patients were not randomized in the current study because of the challenge to include a sufficient number of patients. Furthermore, comparing the toxicity between *CYP3A4*22* carriers and wildtype patients introduced a challenge. Every study drug has its own toxicity profile and a different number of patients was included per study drug, making it hard to extrapolate the toxicity data to the entire group. Finally, the proportion of *CYP3A4*22* carriers in some of the study drugs was remarkably high (e.g., 33% of the cabozantinib patients was *CYP3A4*22* carrier and 24% of the pazopanib patients) compared to the MAF of 5.0% in Europeans reported in previous literature [15].

Although *CYP3A4*22* genotype-guided initial dosing does not seem a suitable dosing strategy for all CYP3A4 metabolized KIs based on this study, it still has potential for specific scenarios. For example, for KIs with an established exposure-toxicity relationship but without exposure-response relationship, *CYP3A4*22* genotype-guided dosing seems an interesting approach to prevent (unnecessary) toxicity without the risk to clinically relevantly under-dose *CYP3A4*22* carriers. Furthermore, it could be useful to determine *CYP3A4*22* status for patients who experienced severe toxicity on a previous CYP3A4 metabolized cancer drug, since that might increase the a priori chance of being a *CYP3A4*22* carrier with a high exposure on previous therapy. Finally, future studies should focus on the role of *CYP3A4*22* genotype-guided dosing for cabozantinib, osimertinib and sunitinib, since the results of these individual drugs in the current study were promising and the non-inferiority requirements might be met for these drugs in future studies with a larger sample size.

Declarations

Conflicts of Interests The authors declare no potential conflicts of interest.

Availability of Data and Material The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval Ethical approval was obtained from the Medical Ethical Committee of the Erasmus Medical Center and the study protocol was also approved by the board of directors of each participating center. The trial was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, applicable regulations, and the Declaration of Helsinki. The trial was registered in the Netherlands Trial Register (International Clinical Trials Registry Platform Search Portal (trialsearch.who.int); number NL7514).

Consent to Participate All patients provided written informed consent.

Consent for Publication Not applicable

Code Availability Not applicable

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