European Journal of Drug Metabolism and Pharmacokinetics (2024) 49:407–418
https://doi.org/10.1007/s13318-024-00896-2

REVIEW ARTICLE

The Use of Microdosing for In vivo Phenotyping of Cytochrome P450 Enzymes: Where Do We Stand? A Narrative Review

Lisa T. van der Heijden[1,2,3] · Frans L. Opdam[4] · Jos H. Beijnen[1,2,5] · Alwin D. R. Huitema[1,2,6,7]

Accepted: 27 March 2024 / Published online: 30 April 2024
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Abstract

Cytochrome P450 (CYP) enzymes play a central role in the elimination of approximately 80% of all clinically used drugs. Differences in CYP enzyme activity between individuals can contribute to interindividual variability in exposure and, therefore, treatment outcome. In vivo CYP enzyme activity could be determined with phenotyping. Currently, (sub)therapeutic doses are used for in vivo phenotyping, which can lead to side effects. The use of microdoses (100 µg) for in vivo phenotyping for CYP enzymes could overcome the limitations associated with the use of (sub)therapeutic doses of substrates. The aim of this review is to provide a critical overview of the application of microdosing for in vivo phenotyping of CYP enzymes. A literature search was performed to find drug–drug interaction studies of CYP enzyme substrates that used microdoses of the respective substrates. A substrate was deemed sensitive to changes in CYP enzyme activity when the pharmacokinetics of the substrate significantly changed during inhibition and induction of the enzyme. On the basis of the currently available evidence, the use of microdosing for in vivo phenotyping for subtypes CYP1A2, CYP2C9, CYP2D6, and CYP2E1 is not recommended. Microdosing can be used for the in vivo phenotyping of CYP2C19 and CYP3A. The recommended microdose phenotyping test for CYP2C19 is measuring the omeprazole area-under-the-concentration-time curve over 24 h ($AUC_{0–24}$) after administration of a single 100 µg dose. CYP3A activity could be best determined with a 0.1–75 µg dose of midazolam, and subsequently measuring AUC extrapolated to infinity (AUC_{∞}) or clearance. Moreover, there are two metrics available for midazolam using a limited sampling strategy: AUC over 10 h ($AUC_{0–10}$) and AUC from 2 to 4 h ($AUC_{2–4}$).

✉ Lisa T. van der Heijden
l.vd.heijden@nki.nl

1 Department of Pharmacology and Pharmacy, Antoni van Leeuwenhoek/The Netherlands Cancer Institute, Amsterdam, The Netherlands

2 Division of Pharmacology, Antoni van Leeuwenhoek/The Netherlands Cancer Institute, Amsterdam, The Netherlands

3 Department of Clinical Pharmacy, OLVG Hospital, Amsterdam, The Netherlands

4 Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

5 Division of Pharmaco-Epidemiology and Clinical Pharmacology, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands

6 Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

7 Department of Pharmacology, Princess Maxima Center, Utrecht, The Netherlands

Key Points

Information about sensitivity to changes in enzyme activity at the microdose level was available for five different probes at five cytochrome P450 enzymes.

The use of microdosing for in vivo phenotyping of CYP1A2, CYP2C9, CYP2D6, and CYP2E1 is not recommend on the basis of the current available evidence.

Microdoses of omeprazole (100 µg) and midazolam (0.1–75 µg) can be used for the determination of in vivo CYP2C19 and CYP3A activity.

1 Introduction

Cytochrome P450 (CYP) enzymes are a large superfamily of heme-containing enzymes that are involved in the metabolism of both endogenous and exogenous compounds [1, 2]. CYP enzymes play a role in the metabolism of approximately 80% of all clinically used drugs [3]. The activity of CYP enzymes is influenced by many factors, such as genetics, age, co-administration of exogenous compounds (e.g., drugs or food), and disease state [4–6]. Owing to these factors there is a large variability in CYP enzyme activity between individuals, which might contribute to heterogeneous therapy outcomes [7]. Dosing of drugs on the basis of CYP enzyme activity can result in more predictable therapy outcome [8–10].

The actual enzyme activity of an individual person could be obtained through in vivo phenotyping. In vivo phenotyping would consist of two steps: (1) the administration of a selective substrate of the enzyme (the probe), and (2) the quantification of the metric representing enzyme activity. This metric is often a pharmacokinetic metric representing clearance (Cl) or a metabolic pathway [11, 12]. The value of the metric would give an indication of the activity of the enzyme. For example, high enzyme activity would correspond in a high clearance value, while low enzyme activity would be associated with a low clearance value. There are currently no guidelines regarding the application of in vivo phenotyping, but recommendations regarding probe and metric selection could be acquired from the drug–drug interaction guidelines of the European Medicines Agency (EMA) and US Food and Drug Administration (FDA) [13, 14]. The major disadvantage of the currently used phenotyping tests is the use of therapeutic or sub-therapeutic doses. Fuhr et al. formulated a list of validated phenotyping procedures of which all are used in the milligram range [11]. These (sub)therapeutic doses can result in side effects or therapeutic effects, such as (mild) sedation for low dose midazolam (a sedative drug and a selective substrate of CYP3A) or hypoglycaemic effect for low dose tolbutamide (an antihyperglycemic drug and a selective substrate of CYP2C9) [11, 15–17].

The use of microdosing within the field of in vivo phenotyping could overcome the above described limitations. A microdose has been defined as 1/100th of the anticipated therapeutic dose with a maximum of 100 µg [14]. Owing to their low dose, microdoses are assumed to be nontoxic and nonpharmacologically active [18]. Microdoses differ from subtherapeutic doses in the amount of drug administered. While microdoses are dosed at a maximum of 100 µg, subtherapeutic doses refers to any dose below the registered therapeutic dose. Originally microdosing has been used to quickly assess the pharmacokinetics of a new drug prior to phase I clinical trials [19]. In recent years, the application of microdosing has been extended toward drug–drug interaction studies, site of action studies [20], and recently the application in in vivo phenotyping studies has been proposed [20, 21]. However, there is still much unknown about the application of microdosing for in vivo phenotyping.

The aim of this narrative review is to provide a comprehensive overview of the use of microdosing for in vivo phenotyping of CYP enzymes. Two aspects of in vivo microdose phenotyping tests will be discussed. Firstly, the sensitivity of the test to detected changes in the activity of the CYP enzyme will be reviewed. Before a phenotyping test can be used for individualized dosing, the test needs to be validated at the microdose level to ensure that it reflects the enzyme activity in many different settings [11]. Second, the linearity of the phenotyping metric over the dosing range from a microdose to the currently used (sub-) therapeutic dose (µg to mg) is discussed. Scalability over this dosing regimen would allow the extrapolation of the currently established knowledge on in vivo phenotyping at the (sub)therapeutic level to the microdose level. This could enable the application of microdosing for in vivo phenotyping since the current knowledge at the (sub)therapeutic level could be extrapolated to microdose level and only the necessary clinical studies need to be conducted to complete the necessary evidence for clinical application. Lastly, recommendations regarding the use of microdosing for the in vivo phenotyping of CYP enzymes will be formulated.

2 Methods and Literature Search

2.1 Literature Search

The literature search was performed in PubMed and Embase using the following terms: microdose*, phase 0, microgram dose, phenotyp*, drug–drug interactions, drug interactions, human microdosing trials, and cytochrome P-450 enzyme. The search was performed on 23 June 2023. Results were restricted to the English language and studies in humans. Additional studies were selected from review articles and reviewing the reference section of each article (citation snowballing). Initial selection was based on title and abstract, while inclusion was determined by full-text assessment. Continuing, studies were included when pharmacokinetic outcomes of phenotyping and/or drug interactions were available. Microtracer trials were excluded from this review as the total administrated dose exceeds the maximum dose definition of a microdose (> 100 µg) [22].

2.2 Sensitivity of In vivo Phenotyping Methods

A frequently used method to determine the sensitivity of a phenotyping method is to measure the phenotyping metric under three conditions: (1) baseline (or control) where the metric is quantified in the absence of any factors influencing the enzyme, (2) inhibition where an inhibitor of the enzyme is co-administered with the probe, and (3) induction where an inductor of the enzyme is co-administered with the probe. The phenotyping method is considered sensitive to the respective enzyme activity when the metric significantly changes during inhibition and induction enzyme compared with baseline.

Three different levels of evidence level were defined: (A) significant difference in the metric at the microdose level between baseline and inhibition/induction, (B) difference in metric at the microdose level between baseline and inhibition/induction but no statistics were performed, (C) no significant difference in metric at the microdose level between baseline and inhibition/induction. An evidence level of A for probe sensitivity indicates that the phenotyping method can be used for in vivo phenotyping at the microdose level, level B indicates that there is a potential use for the phenotyping method at the microdose level, and level C indicates that the probe-metric combination is not suitable for in vivo phenotyping at the microdose level.

2.3 Pharmacokinetic Scalability

The scalability of microdose pharmacokinetics were determined by comparing pharmacokinetic parameters of the microdose with the pharmacokinetic parameters of the therapeutic dose. For the area-under-the-concentration-time-curve (AUC) the value extrapolated to infinity in ng × h/mL was used, unless otherwise denoted, and presented dose-adjusted to 100 µg. The maximum observed concentration (C_{max}) is also presented as dose-adjusted to 100 µg. Furthermore, the clearance (Cl) was reported in L/h (apparent Cl for oral administration), volume of distribution (V_d) in L (apparent V_d for oral administration), and half-life ($t_{1/2}$) in h. Pharmacokinetic data from single microdose studies were completed with literature data from therapeutic pharmacokinetic studies. Microdose pharmacokinetics were considered predictive if the mean observed values of the microdose and of the therapeutic dose were within a twofold of each other, which is the generally accepted criterion for the predictive value of microdose pharmacokinetics [22–25].

In this review, four evidence levels of scalability were defined. (A) High scalability: all pharmacokinetic parameters of parent and metabolite fell within the twofold criterion; (B) good scalability: all pharmacokinetic parameters of either parent or metabolite fell within the twofold criterion; (C) moderate scalability: the metric used for phenotyping fell within the twofold criterion; (D) poor scalability: none of the pharmacokinetic parameters fell within the twofold criterion or the metric used for phenotyping falls outside the twofold criterion. Evidence level A and B indicate that results of in vivo phenotyping at the microdose level could be extrapolated to therapeutic dose for data interpretation, level C indicates that the results of in vivo phenotyping might be extrapolated from the microdose level to the therapeutic dose level if the respective metric is sensitive to changes in CYP enzyme activity, and D indicates that the results of in vivo phenotyping at the microdose level could not be extrapolated to therapeutic dose level.

3 Results of Literature Search

3.1 Included Literature

A total of 23 out of 827 screened articles were included in the current review. The selection process is depicted in Fig. 1. Of the 23 articles, 13 articles reported the sensitivity of microdose probes [21, 26–30]. These articles are summarized in Supplementary Table 1. Continuing, 17 articles were included for the evaluation of scalability of microdose pharmacokinetics to the therapeutic level [28, 29, 31–39]. Taken together, this review describes the application of in vivo phenotyping with microdoses of CYP1A2 with caffeine, CYP2C9 with glibenclamide, losartan, tolbutamide, and warfarin, CYP2C19 with lansoprazole and omeprazole, CYP2D6 with yohimbine, CYP2E1 with chlorzoxazone, and CYP3A4 with midazolam, quinidine, and verapamil. Details of the comparison between microdose and pharmacokinetics of therapeutic doses are described in Supplementary Table 2 for studies containing both microdose and therapeutic data and Supplementary Table 3 for studies describing only microdose pharmacokinetics. The latter studies were completed with studies reporting pharmacokinetics of therapeutic doses. The evidence levels for sensitivity and scalability for each microdose phenotyping test are reported in Table 1.

3.2 Cytochrome P450 Enzymes

3.2.1 CYP1A2

3.2.1.1 Caffeine Caffeine is recommended as a probe for CYP1A2 activity by the EMA owing to its predominant metabolism by CYP1A2 to paraxanthine at therapeutic levels [13, 40]. At a microdose of 25 µg, the AUC extrapolated to infinity (AUC_∞) of caffeine was sensitive to CYP1A2 inhibition, but this effect was smaller compared with the effect at a therapeutic dose of 250 mg (8.1-fold versus 13.7-fold, respectively) [21, 41]. The authors did not report any hypothesis regarding the observed difference, but it could

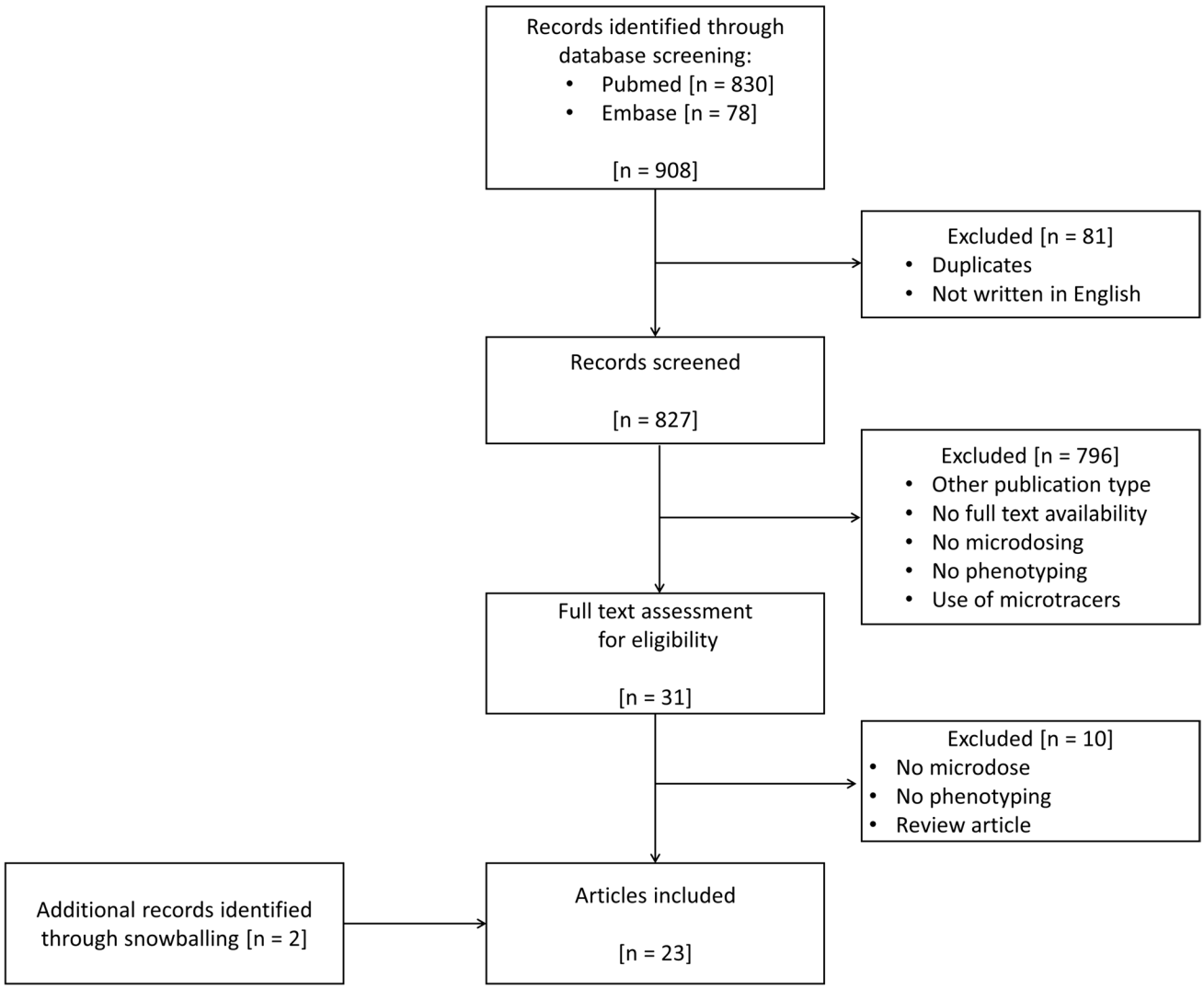

Fig. 1 Schematic overview of the selection process.

possibly be caused by difference in study design, participant populations, or a concentration-dependent contribution of CYP1A2 to the metabolism of caffeine. Currently, there is no information available about the sensitivity of CYP1A2 induction. The extrapolation of microdose metrics to therapeutic level is complicated owing to the difference in the above described effect size of CYP1A2 inhibition between the two dosing levels despite the dose-proportional pharmacokinetics of caffeine (0.25–250 mg) [21, 41–43]. Therefore, sensitivity data of the AUC_{∞} of a caffeine microdose to induction needs to be obtained before clinical application could be considered (Table 1).

3.2.2 CYP2C9

3.2.2.1 Glibenclamide Glibenclamide is predominantly metabolized by CYP2C9 into its two main metabolites: 4′-hydroxy glibenclamide and 3′-hydroxy glibenclamide [44]. Therapeutic pharmacokinetics of glibenclamide (0.5–3.5 mg) are reported to be significantly different between CYP2C9 genotypes [45, 46], indicating that glibenclamide could be used as a probe for CYP2C9 activity. There is no information available about the sensitivity of phenotyping metrics to inhibition or induction of CYP2C9. However, a genotyping study reported the difference in AUC_{∞} and $t_{1/2}$ of a glibenclamide microdose (10 µg) between genotypes, indicating a possible use as a microdose phenotyping probe [33]. However, no statistical analyses were performed. The pharmacokinetics of glibenclamide were moderate to poorly scalable from 10 µg to 2.5 mg [33, 44, 47]. Taken together, glibenclamide shows potential as a microdose probe for CYP2C9 activity, but the sensitivity of AUC_{∞} at the microdose level during enzyme inhibition and induction needs to be established before clinical application (Table 1).

Table 1 Overview of probes and metrics available for in vivo phenotyping of Cytochrome P450 enzymes and the evidence level for their application

Enzyme	Probe	Dose (µg)	Route of administration	Metric	Evidence level		Comment	Recommendation	References
					Sens.	PK			
CYP1A2	Caffeine	25	PO	AUC_{∞}	A	B	Only evidence for inhibition	Data is needed about performance of AUC_{∞} during enzyme induction	[21, 41-43]
CYP2C9	Glibenclamide	10	PO	AUC_{∞}	NA	C/D	No information about sensitivity	Sensitivity studies at microdose level need to be performed	[33, 44, 47]
	Losartan	100	PO	AUC_{∞}	NA	B	No information about sensitivity	Sensitivity studies at microdose level need to be performed	[49]
	Tolbutamide	25	PO	AUC_{∞}	C	B	Only evidence for inhibition	Sensitivity studies at microdose level need to be performed	[21, 51]
	Warfarin	10-100	PO	AUC_{∞}	NA	C	No information about sensitivity	Sensitivity studies at microdose level need to be performed	[31, 33, 52, 53]
CYP2C19	Lansoprazole	50-70	PO	AUC_{∞} Cl/F AUC 5-OH/LSP	NA NA NA	D D D	Difference in enzyme activity between genotypes	Sensitivity studies at microdose level need to be performed	[33, 55]
	Omeprazole	100	PO	$AUC_{0\text{-}24}$ AUC_{∞} Cl/F	A/B	C/D		Suitable for *in vivo* phenotyping at microdose level	[26]
CYP2D6	Yohimbine	50	PO	AUC_{∞} AUC YH/11-OH YH Cl/F	B B B	A/B A/B A/B		Sensitivity studies at microdose level need to be performed	[56, 57]
CYP2E1	Chlorzoxazone	2.5-50	PO	AUC_{∞} Cl/F	NA NA	A/B A/B		Sensitivity studies at microdose level need to be performed	[60, 61]
CYP3A4	Midazolam	75	PO	AUC 1-OH/MDZ	B/C	A/B	Only evidence for inhibition	Not suitable for *in vivo* phenotyping	[15, 20, 27-32, 34-39, 66, 68, 78-85]
		33	PO	$AUC_{0\text{-}10}$	A	A/B	Only evidence for inhibition	Suitable for *in vivo* phenotyping at microdose level	
		10	PO	$AUC_{2\text{-}4}$	A	A/B	Only evidence for inhibition	Suitable for *in vivo* phenotyping at microdose level	
		0.1-75	PO	AUC_{∞}	A	A/B	Dependent on perpetrator dose and rout of administration	Suitable for *in vivo* phenotyping at microdose level	
		0.1-75	PO	Cl/F	A	A/B A/B		Suitable for *in vivo* phenotyping at microdose level	
		3-10	PO	Cl_{met}	A				
							No effect with cyclosporine with or without fluconazole		
	Quinidine	100	PO	AUC_{∞}	NA	B		Sensitivity studies at microdose level need to be performed	
				AUC 3-OH/QND	NA	B		Sensitivity studies at microdose level need to be performed	
				AUC N-OX/QND	NA	B		Sensitivity studies at microdose level need to be performed	[66]
	Verapamil	100	PO	AUC_{∞}	NA	A/B		Sensitivity studies at microdose level need to be performed	[66]
				AUC NVP/VPN	NA	A/B		Sensitivity studies at microdose level need to be performed	

AUC_{∞} area-under-the-concentration-time-curve extrapolated to infinity, AUC_{0-24} AUC from 0 to 24 h, AUC_{0-10} AUC from 0 to 10 h, AUC_{2-4} AUC from 2 to 4 h, *AUC N-OX/QND* AUC of N-oxide quinidine divided by quinidine, *AUC NVP/VPN* AUC of norverapamil divided by verapamil, *AUC YH/11-OH YH* AUC of yohimbine divided by 11-hydroxy yohimbine, *AUC 1-OH/MDZ* AUC of 1′-hydroxy midazolam divided by midazolam, *AUC 3-OH/QND* AUC of 3-hydroxy quinidine divided by quinidine, *AUC 5-OH/LSP* AUC of 5-hydroxy lansoprazole divided by lansoprazole, *Cl/F* apparent clearance, *PK* pharmacokinetics, *PO* oral administration, *Sens.* sensitivity

3.2.2.2 Losartan The major metabolic route for losartan is its metabolism by CYP2C9 into the E3174 metabolite [48]. There is no information available about the sensitivity of phenotyping metrics (AUC, Cl, or metabolic ratio) at the microdose level. The scalability of losartan pharmacokinetics over a dosing range of 100 µg to 50 mg was considered good in both healthy volunteers and hypertensive patients (See Supplementary Table 2) [49]. Before losartan can be used for in vivo phenotyping at the microdose level the sensitivity of the phenotyping metric to changes in CYP2C9 needs to be investigated at the microdose level.

3.2.2.3 Tolbutamide Tolbutamide is a recommended probe by the EMA owing to its predominant metabolism by CYP2C9 to 4'-hydroxy tolbutamide [13, 50]. The AUC_{∞} of tolbutamide appears to be sensitive to CYP2C9 inhibition at the microdose level (25 µg) since inhibition resulted in an increase in AUC_{∞} of 1.8-fold, but the change from baseline was not significant, possibly owing to the large variability in tolbutamide pharmacokinetics following the administration of the perpetrators compared to baseline [21]. This was attributed to use of moderate CYP2C9 inhibitors (ketoconazole and fluvoxamine) [21]. There is no information avail-

able about the sensitivity of AUC_{∞} to CYP2C9 induction at the microdose level. Continuing, the pharmacokinetics were good scalable over a dosing range of 25 µg to 125 mg [51]. Summarizing, there is currently insufficient evidence to recommend a microdose tolbutamide as a phenotyping probe for CYP2C9.

3.2.2.4 Warfarin S-warfarin is recommended as a probe for CYP2C9 activity at therapeutic level by the EMA [13]. Currently, there is no evidence that the pharmacokinetics of S-warfarin are sensitive to changes in CYP2C9 activity at the microdose level. A microdose genotyping study did not find any significant differences in warfarin AUC_{0-2} of a 10 µg microdose between different CYP2C9 genotypes, indicating that warfarin might not be a sensitive probe for CYP2C9 activity at the microdose level [33]. However, the authors reported the quantification of racemic warfarin instead of (S)-warfarin and an incomplete capture of the pharmacokinetics as possible causes of the nonsignificant findings [33]. Furthermore, the pharmacokinetics of warfarin were moderately scalable from the microdose level (10–100 µg) to therapeutic level (5–7.5 mg; Supplementary Table 3) [31, 33, 52, 53]. In conclusion, S-warfarin cannot be currently recommended as a microdose probe for CYP2C9 activity (Table 1).

3.2.3 CYP2C19

3.2.3.1 Lansoprazole The predominant metabolic pathway for lansoprazole is its 5-hydroxylation by CYP2C19 to 5'-hydroxy lansoprazole [54]. Currently, there is no sensitivity information available at the microdose level but results from a pharmacogenetic-pharmacokinetic study suggests the possible sensitivity of lansoprazole AUC_{∞} (50–70 µg) to changes in CYP2C19 activity, but no statistical analyses were performed [33]. The pharmacokinetics of lansoprazole demonstrated nonlinearity over a dosing range of 50 µg to 30 mg (evidence level D) [33, 55]. Concluding, sensitivity studies at microdose level are needed before the application of lansoprazole as a microdose probe (Table 1).

3.2.3.2 Omeprazole Owing to omeprazole's good selectivity for CYP2C19 and its tolerability, it is recommended as a CYP2C19 probe by the EMA at therapeutic level [13]. At the microdose level the AUC_{0-24} is sensitive to inhibition and induction of CYP2C19 [26]. The effect size at the microdose level on AUC_{0-24} during both inhibition and induction of the enzyme was similar to the therapeutic level [26]. Furthermore, the pharmacokinetics of omeprazole were nonlinear over a dosing range of 100 µg to 20 mg (evidence level D) [26]. Pharmacokinetics of omeprazole have been reported to be nonlinear over a dose range of 20–40 mg, which has been mostly attributed to the metabolic saturation of the S-enantiomer of omeprazole. Moreover, S-omeprazole also inhibits CYP2C19 after multiple dosing, complicating the exploration of phenotyping results at the therapeutic dose level [26]. Taken together, a microdose of 100 µg omeprazole is suitable as an in vivo phenotyping trope of CYP2C19 (Table 1) and might even be more predictive for CYP2C9 activity compared with a therapeutic dose.

3.2.4 CYP2D6

3.2.4.1 Yohimbine Yohimbine has been investigated as a probe for CYP2D6. At microdose level yohimbine AUC_{∞}. The Cl/F and metabolic ratio of yohimbine to 11-hydroxy yohimbine seemed to be sensitive to perpetrators of CYP2D6 (see Supplementary Table S1) [56, 57]. However, the fold-difference in pharmacokinetic parameters at microdose was smaller compared with the therapeutic dose and no statistical tests were conducted. The pharmacokinetic parameters of yohimbine were not well scalable from microdose to therapeutic dose, while the metabolic ratio of yohimbine to 11-hydroxy yohimbine was good scalable (Supplementary Table S2) [57]. This was credited to the high variability in yohimbine pharmacokinetics [57]. Concluding, yohimbine seems to have potential as a CYP2D6 probe at the microdose level, but sensitivity to changes in CYP2D6 activity needs to be established at the microdose level before yohimbine could be considered as a microdose probe.

3.2.5 CYP2E1

3.2.5.1 Chlorzoxazone The metabolic ratio of 6'-hydroxy chlorzoxazone and chlorzoxazone is a commonly used metric for CYP2E1 activity [58, 59]. At this moment, there is no data available regarding the sensitivity of the metabolic ratio to perpetrators of CYP2E1 at the microdose level. Chlorzoxazone pharmacokinetics were linear over a dose range from 25 µg to 5 mg [60, 61]. Currently, chlorzoxazone cannot be recommended as a microdose probe for CYP2E1 activity.

3.2.6 CYP3A

3.2.6.1 Midazolam Midazolam is a highly selective probe for CYP3A activity owing to its selective metabolism by CYP3A to its main metabolite 1′-hydroxy midazolam [62]. Furthermore, midazolam is a recommended CYP3A probe by the EMA [13]. Of the available phenotyping metrics, AUC_{∞} and Cl/F had the best evidence for sensitivity (Supplementary Table 1) [5, 7–9]. The metabolic ratio of 1′-hydroxy midazolam/midazolam is thought to give a more accurate estimation of CYP3A activity because it correlates well with hepatic CYP3A4 content [63, 64] and is, therefore, the metric of preference [13]. Surprisingly, the meta-

bolic ratio was not sensitive to CYP3A induction [28]. The authors did not comment on this result, but concluded that the metabolic ratio determined 30 min after administration correlated well with midazolam Cl (Spearman correlation; $p < 0.005$) [13]. Moreover, two metrics that are determined with limited sampling strategies, $AUC_{0–10}$ and $AUC_{2–4}$ were investigated. Both of these metrics were found to be sensitive to changes in CYP3A activity at the microdose level. Overall the pharmacokinetics of midazolam can be considered as good scalable over a wide range of doses (0.003–7.5 mg). Concluding, midazolam AUC_{∞} and Cl/*F* are both suitable for in vivo phenotyping of CYP3A at the microdose level (Table 1).

3.2.6.2 Quinidine Quinidine is predominantly metabolized by CYP3A4 and CYP2D6 [65]. There is currently no information about the sensitivity of microdose phenotyping metrics for quinidine (AUC_{∞}, Cl/*F*, or metabolic ratio). Quinidine pharmacokinetics were considered to have good scalability (0.1–10 mg), while its metabolite quinidine N-oxide was not well scalable (Supplementary Table 2) [66]. Before its application as an in vivo phenotyping probe, sensitivity to changes to CYP3A activity at the microdose level should be established (Table 1).

3.2.6.3 Verapmil Verapamil is a substrate for both CYP3A and CYP2C8 enzymes [67]. It is currently unclear if any of the phenotyping metrics of verapamil (AUC_{∞}, Cl/*F* or metabolic ratio) are sensitive to changes in CYP3A activity. Verapamil had highly scalable pharmacokinetics from a dose of 100 µg to 16 mg [66]. Continuing, the metabolic ratio for the main metabolite norverapamil and verapamil was good scalable over the same dosing range [66]. Sensivity studies at the microdose level are essential to establish whether verapamil should be used as a probe for CYP3A owing its multiple metabolic pathways (Table 1)

4 Discussion

The suitability of microdosing for in vivo phenotyping of CYP enzymes was reviewed. Several recommendations can be made regarding the above described literature regarding the use of microdosing for the in vivo phenotyping for CYP enzymes (Table 1). The use of microdosing for in vivo phenotyping of CYP1A2, CYP2C9, CYP2D6, and CYP2E1 cannot be currently recommended since there is lacking evidence of the sensitivity of the phenotyping metrics of the respective probes. However, there is potential for caffeine, glibenclamide, and yohimbine as microdose probes for CYP1A2, CYP2C9, and CYP2E1, respectively. The AUC_{∞} of a 25 µg dose of caffeine was sensitive to CYP1A2 inhibition, but no evidence has been reported for CYP1A2 induction yet [21]. Moreover, both glibenclamide and yohimbine might be sensitive probes at the microdose level since microdose pharmacokinetics differed between different CYP2C9 and CYP2E1 genotypes, respectively [33, 56]. Microdosing could be used for the in vivo phenotyping of CYP2C19 and CYP3A4. For CYP2C19, the recommended microdose phenotyping test is the omeprazole $AUC_{0–24}$ after a 100 µg. CYP3A activity could be best determined with an oral 0.1–75 µg dose of midazolam and the midazolam AUC_{∞} or Cl. Moreover, there are two metrics available that are determined with a limited sampling strategy: $AUC_{0–10}$ and $AUC_{2–4}$.

Three conclusion could be drawn based on the currently presented literature. Firstly, dose-proportional pharmacokinetics from (sub)therapeutic to microdose is not necessarily predictive of the sensitivity of a probe at the microdose level. This is illustrated by omeprazole and tolbutamide. For omeprazole, AUC_{0-24} is sensitive to changes in CYP2C19 but its pharmacokinetics are not linear, while for tolbutamide the pharmacokinetics are linear but the phenotyping metric at a microdose is not sensitive to changes in enzyme activity. Dose-nonlinear pharmacokinetics might be irrelevant at the microdose level since dose-nonlinear pharmacokinetics are often caused by saturable processes, such as metabolism, and a microdose will result in exposure below the threshold of saturation. For omeprazole, specifically, dose-nonlinear pharmacokinetics over a dosing range of 20–40 mg is thought to be caused by the metabolic saturation of the S-enantiomer and its auto-inhibition of CYP2C19 [26]. Secondly, microdoses might be more predictive of the in vivo enzyme activity. Owing to the low administered dose of microdoses, microdose pharmacokinetics are not influenced by saturable processes or auto-inhibition/induction that can occur at (sub)therapeutic doses, such as for omeprazole. Lastly, there is a need for recommendations regarding the design of studies investigating sensitivity of potential probes. Some sensitivity studies had suboptimal results owing to the choice of perpetrator [21], the choice of probe [33], or high variability in pharmacokinetics [21]. There are currently no guidelines on in vivo phenotyping of CYP enzymes. However, recommended probes and metrics in the drug–drug interaction guidelines from the EMA and FDA could be used [13, 14]. Characteristics of a validated probe are listed in these guidelines, but the use of microdosing for in vivo phenotyping is not discussed. However, the following recommendations can be made. A probe should be a selective substrate of the respective enzyme and in the case of a racemic drug, the isomer with the highest selectivity should be chosen as the probe (e.g., S-warfarin [33]). Perpetrators should be chosen on the basis of their strength of inhibition or induction of the enzyme in question [13, 14]. Moreover, the dose [68], duration of exposure [14, 68, 69], and time of administration of the perpetrator [14, 68] should

be chosen in such a way that the inhibition or induction of the enzyme is maximized. Lastly, if the probe is meant to be used in a phenotyping cocktail, drug–drug interaction studies at the microdose level should be conducted making sure that the individual probes in the cocktail do not influence each others metrics.

The ultimate aim of in vivo phenotyping is contribution to individualized dosing by explaining (in part) the variability in drug clearance. There are indications of the clinical relevance of in vivo phenotyping. Simvastatin dosing could be improved when CYP3A activity was taken into account [8]; accounting for the CYP3A phenotype in the dose calculation of irinotecan improved the predictability of the pharmacokinetic and toxicity profile [9], and midazolam Cl was found to be highly correlated with sunitinib exposure and explained a large proportion in the observed interpatient variability in pharmacokinetics [10]. A second study reported a significant correlation between the metabolic ratio of midazolam and sunitinib, but concluded that it did not predict variability in sunitinib clearance sufficiently to be useful in clinical dosing strategy [70]. Of these four studies, only the first study used a microdose for phenotyping [8]. Another study used a microdose cocktail to investigate the influence of renal impairment on the pharmacokinetics of the probes [71]. Lastly, microdosing could also be used to investigate time course of indication or inhibition [72, 73]. Taken together, in vivo phenotyping has the potential to beneficially influence pharmacological treatment.

The clinical application of microdose in vivo phenotyping for clinical dosing strategies is enabled by recent advances within the field of bioanalysis. Innovations made it possible to use relatively simple equipment, such as liquid chromatography tandem mass spectrometry (LC–MS/MS), for the quantification of extreme low plasma concentrations (Table 2) instead of accelerated mass spectrometry (AMS; Table 2). The advantages of LC–MS/MS over AMS are

Table 2 Overview of the bioanalytical methods used for the quantification of drug concentrations in the support of in vivo phenotyping of cytochrome P450 enzymes at the microdose level

Compound	Matrix	Sample volume (µL)	Internal standard	Sample preparation	Quantification method	Run time (min)	LLOQ (pg/mL)	EMA/FDA[c]	Refs.
^{14}C-caffeine	Plasma	190	Caffeine	PP	HPLC-AMS	30	5.21	No	[21]
Chlorzoxazone[a]	Plasma	500	$^{3}H_2$-chlorzoxazone	LLE	LC-MS/MS	5.5	2.5	Yes	[60]
Dextromethorphan[a]	Urine	100	n.r.	LLE	LC-MS/MS	n.r.	10	No	[33]
Glibenclamide	Plasma	200	n.r.	SPE	LC-MS/MS	n.r.	1	No	[33]
Lansoprazole[a]	Plasma	100	n.r.	LLE	LC-MS/MS	n.r.	10	No	[33]
Losartan	Plasma	1000	Candesartan	LLE	LC-MS/MS	n.r.	50	No	[74]
^{14}C-midazolam	Plasma	190	Midazolam	PP	HPLC-AMS	30	5.75	No	[21]
^{14}C-midazolam	Plasma	500	Midazolam	LLE	HPLC-AMS	NA	0.1[b]	No	[31]
Midazolam[a]	Plasma	250	$^{2}H_5$-midazolam	SPE	UHPLC-MS/MS	2.5	0.05	Yes	[82]
Midazolam[a]	Plasma	1000	N-ethyloxazepam	LLE	Gas chromatography	NA	10	No	[78]
Midazolam	Plasma	750	Rosuvastatin	SPE	LC-MS/MS	6.5	5–200	No	[29]
Omeprazole	Plasma	300	Lansoprazole	LLE	LC-MS/MS	2.5	34	No	[26, 86]
Omeprazole	Plasma	100	$^{2}H_3$-omeprazole	PPE	LC-MS/MS	NA	10	Yes	[87]
Quinidine[a]	Plasma	500	$^{3}H_2$-Quinidine	PP	LC-MS/MS	4.5	5	No	[66]
^{14}C-Tolbutamide	Plasma	190	Tolbutamide	PP	HPLC-AMS	30	5.84	No	[21]
Yohimbine	Plasma	25	$^{13}C^{2}H_3$-yohimbine	LLE	LC-MS/MS	3	5	Yes	[57]
^{14}C-warfarin	Plasma	500	Warfarin	LLE	HPLC-AMS	20	10[b]	No	[31]
Warfarin	Plasma	200	n.r.	SPE	LC-MS/MS	n.r.	50,000	No	[33]
Verapamil[a]	Plasma	500	$^{2}H_6$-verapmil hydroxide	PP	LC-MS/MS	4.5	1	No	[66]

LLE liquid–liquid extraction, *LLOQ* lower limit of quantification, *n.r.* not reported, *PP* protein precipitation, *SPE* solid phase extraction

[a]Simultaneous quantification of metabolite

[b]Lowest measured concentration

[c]Validated according the guidelines of the European Medicines Agency (EMA) and/or US Food and Drug Administration (FDA)

the fast data acquisition, the independency of radioactive isotopes, and the higher accessibility in laboratories [74]. Another crucial aspect for clinical application is a suitable pharmaceutical formulation for microdose phenotyping test. At present, therapeutic formulations are adjusted, such as the dilution of intravenous infusions or dissolving and dilution or oral formulations. However, commercially available microdose formulations are desired to increase the reliability of the administrated dose and to increase the accessibility of microdose phenotyping. Furthermore, patient burden of in vivo microdose phenotyping tests should be minimized. Limited sampling strategies could reduce the number of blood samples necessary for phenotyping as well as reducing the time spent at the clinic. Single-time concentrations or parent over metabolite concentrations could be investigated [75], or maximum posteriori Bayesian estimation using population pharmacokinetic models could be investigated [76, 77]. Moreover, oral administration would be preferable as well as less invasive sample collection methods, such as volumetric absorptive microsampling (VAMS) from a finger prick instead of venepuncture.

The current analysis has several strengths and limitations. First, the current narrative review represents a broad and comprehensive overview of the currently published literature regarding the application of microdoses to in vivo phenotyping of CYP enzymes. Moreover, the predefined evidence levels of sensitivity and scalability creates and objective measurement for the comparison of different phenotyping tests. However, owing to the narrative nature of the current analysis relevant citations could have been missed. The risk of missing literature was minimized by the inclusion of the snowballing procedure during literature selection. Lastly, narrative reviews carry a relative higher risk of bias in the selection and interpretation of the data. The risk of bias was minimized by including all the data used for the interpretation of the results presented here in the supplementary tables (Supplementary Tables 1, 2, and 3).

5 Conclusions

In this review we questioned whether microdoses could be used for in vivo phenotyping of CYP enzymes. On the basis of literature, in vivo phenotyping with microdoses is in its infancy. For most CYP enzymes, the use of microdoses for in vivo phenotyping cannot be recommended yet. For most probes, information is lacking regarding its sensitivity to changes in enzyme activity at the microdose level. However, for phenotyping of CYP2C19, a microdose of 100 µg omeprazole can be administered orally and the $AUC_{0–24}$ determined, while for CYP3A4 an oral 0.1–75 µg can be administered and the AUC_{∞} or Cl calculated. Furthermore, a midazolam $AUC_{0–10}$ or midazolam $AUC_{2–4}$ can be considered as metric. For clinical application, more studies are needed regarding the sensitivity of probes at the microdose level, the use of in vivo phenotypes in dosing strategies, limited sampling strategies, and less invasive sampling methods.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13318-024-00896-2.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Author contributions L.H. conceptualized the study and performed data curation and writing—original draft; F.O. performed writing—review and editing; J.H. performed writing—review and editing; A.H.: conceptualized and supervised the study and performed writing—review and editing.

Funding No funds, grants, or other support was received to prepare this review.

Availability of data and materials All data generated or analyzed during this study are included in this published article and its supplementary information files.

Code availability Not applicable.

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References

1. Rendic S, Guengerich FP. Survey of human oxidoreductases and cytochrome P450 enzymes involved in the metabolism of xenobiotic and natural chemicals. Chem Res Toxicol. 2015;28(1):38–42.
2. Guengerich FP, Waterman MR, Egli M. Recent structural insights into cytochrome P450 function. Trends Pharmacol Sci. 2016;37(8):625–40.
3. Ingelman-Sundberg M. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. Naunyn Schmiedebergs Arch Pharmacol. 2004;369(1):89–104.

4. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138(1):103–41.
5. Hohmann N, Haefeli WE, Mikus G. CYP3A activity: towards dose adaptation to the individual. Expert Opin Drug Metab Toxicol. 2016;12(5):479–97.
6. Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. Annu Rev Med. 2006;57:119–37.
7. Song Y, Li C, Liu G, Liu R, Chen Y, Li W, et al. Drug-metabolizing cytochrome P450 enzymes have multifarious influences on treatment outcomes. Clin Pharmacokinet. 2021;60(5):585–601.
8. Stoll F, Burhenne J, Lausecker B, Weiss J, Thomsen T, Haefeli WE, Mikus G. Reduced exposure variability of the CYP3A substrate simvastatin by dose individualization to CYP3A activity. J Clin Pharmacol. 2013;53(11):1199–204.
9. van der Bol JM, Mathijssen RH, Creemers GJ, Planting AS, Loos WJ, Wiemer EA, et al. A CYP3A4 phenotype-based dosing algorithm for individualized treatment of irinotecan. Clin Cancer Res. 2010;16(2):736–42.
10. de Wit D, Gelderblom H, Sparreboom A, den Hartigh J, den Hollander M, König-Quartel JM, et al. Midazolam as a phenotyping probe to predict sunitinib exposure in patients with cancer. Cancer Chemother Pharmacol. 2014;73(1):87–96.
11. Fuhr U, Jetter A, Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. Clin Pharmacol Ther. 2007;81(2):270–83.
12. Streetman DS, Bertino JS Jr, Nafziger AN. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. Pharmacogenetics. 2000;10(3):187–216.
13. European Medicines Agency (EMA). Guideline on the investigation of drug interactions. Guideline on the investigation of drug interactions (europa.eu). Accessed 12 Aug 2023
14. US Food and Drug Administration (FDA). Guidance for the industry, investigators and reviewers: exploratory IND studies. Guidance for Industry (fda.gov). Accessed 12 Aug 2023
15. Donzelli M, Derungs A, Serratore MG, Noppen C, Nezic L, Krahenbuhl S, Haschke M. The basel cocktail for simultaneous phenotyping of human cytochrome P450 isoforms in plasma, saliva and dried blood spots. Clin Pharmacokinet. 2014;53(3):271–82.
16. Chainuvati S, Nafziger AN, Leeder JS, Gaedigk A, Kearns GL, Sellers E, et al. Combined phenotypic assessment of cytochrome p450 1A2, 2C9, 2C19, 2D6, and 3A, *N*-acetyltransferase-2, and xanthine oxidase activities with the "Cooperstown 5+1 cocktail." Clin Pharmacol Ther. 2003;74(5):437–47.
17. Doroshyenko O, Rokitta D, Zadoyan G, Klement S, Schlafke S, Dienel A, et al. Drug cocktail interaction study on the effect of the orally administered lavender oil preparation silexan on cytochrome P450 enzymes in healthy volunteers. Drug Metab Dispos. 2013;41(5):987–93.
18. Marchetti S, Schellens JH. The impact of FDA and EMEA guidelines on drug development in relation to Phase 0 trials. Br J Cancer. 2007;97(5):577–81.
19. Garner RC, Lappin G. The phase 0 microdosing concept. Br J Clin Pharmacol. 2006;61(4):367–70.
20. Hohmann N, Haefeli WE, Mikus G. Use of microdose phenotyping to individualise dosing of patients. Clin Pharmacokinet. 2015;54(9):893–900.
21. Croft M, Keely B, Morris I, Tann L, Lappin G. Predicting drug candidate victims of drug-drug interactions, using microdosing. Clin Pharmacokinet. 2012;51(4):237–46.
22. Lappin G, Noveck R, Burt T. Microdosing and drug development: past, present and future. Expert Opin Drug Metab Toxicol. 2013;9(7):817–34.
23. Lappin G, Garner RC. The utility of microdosing over the past 5 years. Expert Opin Drug Metab Toxicol. 2008;4(12):1499–506.
24. Rowland M. Commentary on ACCP position statement on the use of microdosing in the drug development process. J Clin Pharmacol. 2007;47(12):1595–6 (**author reply 7-8**).
25. van Nuland M, Rosing H, Huitema ADR, Beijnen JH. Predictive value of microdose pharmacokinetics. Clin Pharmacokinet. 2019;58(10):1221–36.
26. Park GJ, Bae SH, Park WS, Han S, Park MH, Shin SH, et al. Drug-drug interaction of microdose and regular-dose omeprazole with a CYP2C19 inhibitor and inducer. Drug Des Devel Ther. 2017;11:1043–53.
27. Mikus G, Foerster KI, Schaumaeker M, Lehmann ML, Burhenne J, Haefeli WE. Application of a microdosed cocktail of 3 oral factor Xa inhibitors to study drug-drug interactions with different perpetrator drugs. Br J Clin Pharmacol. 2020;86:1632–41.
28. Eap CB, Buclin T, Cucchia G, Zullino D, Hustert E, Bleiber G, et al. Oral administration of a low dose of midazolam (75 microg) as an in vivo probe for CYP3A activity. Eur J Clin Pharmacol. 2004;60(4):237–46.
29. Maeda K, Ikeda Y, Fujita T, Yoshida K, Azuma Y, Haruyama Y, et al. Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. Clin Pharmacol Ther. 2011;90(4):575–81.
30. Carls A, Jedamzik J, Witt L, Hohmann N, Burhenne J, Mikus G. Systemic exposure of topical erythromycin in comparison to oral administration and the effect on cytochrome P450 3A4 activity. Br J Clin Pharmacol. 2014;78(6):1433–40.
31. Lappin G, Kuhnz W, Jochemsen R, Kneer J, Chaudhary A, Oosterhuis B, et al. Use of microdosing to predict pharmacokinetics at the therapeutic dose: experience with 5 drugs. Clin Pharmacol Ther. 2006;80(3):203–15.
32. Halama B, Hohmann N, Burhenne J, Weiss J, Mikus G, Haefeli WE. A nanogram dose of the CYP3A probe substrate midazolam to evaluate drug interactions. Clin Pharmacol Ther. 2013;93(6):564–71.
33. Ieiri I, Fukae M, Maeda K, Ando Y, Kimura M, Hirota T, et al. Pharmacogenomic/pharmacokinetic assessment of a four-probe cocktail for CYPs and OATPs following oral microdosing. Int J Clin Pharmacol Ther. 2012;50(10):689–700.
34. Prueksaritanont T, Tatosian DA, Chu X, Railkar R, Evers R, Chavez-Eng C, et al. Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. Clin Pharmacol Ther. 2017;101(4):519–30.
35. Kiene K, Hayasi N, Burhenne J, Uchitomi R, Sunderhauf C, Schmid Y, et al. Microdosed midazolam for the determination of cytochrome P450 3A activity: development and clinical evaluation of a buccal film. Eur J Pharm Sci. 2019;1(135):77–82.
36. Brings A, Lehmann ML, Foerster KI, Burhenne J, Weiss J, Haefeli WE, Czock D. Perpetrator effects of ciclosporin (P-glycoprotein inhibitor) and its combination with fluconazole (CYP3A inhibitor) on the pharmacokinetics of rivaroxaban in healthy volunteers. Br J Clin Pharmacol. 2019;85(7):1528–37.
37. Hohmann N, Mikus G, Haefeli WE, Schwenger V, Gattuso G, Barreca D, Weiss J. A follow-up report on potential drug interactions with clementines: two single case experiments show no effect on CYP3A-dependent midazolam clearance. Eur J Pharm Sci. 2019;15(133):54–8.
38. Hohmann N, Reinhard R, Schnaidt S, Witt L, Carls A, Burhenne J, et al. Treatment with rilpivirine does not alter plasma concentrations of the CYP3A substrates tadalafil and midazolam in humans. J Antimicrob Chemother. 2016;71(8):2241–7.
39. Woolsey SJ, Beaton MD, Choi YH, Dresser GK, Gryn SE, Kim RB, Tirona RG. Relationships between endogenous plasma biomarkers of constitutive cytochrome P450 3A

activity and single-time-point oral midazolam microdose phenotype in healthy subjects. Basic Clin Pharmacol Toxicol. 2016;118(4):284–91.
40. Jeppesen U, Loft S, Poulsen HE, Brśen K. A fluvoxamine-caffeine interaction study. Pharmacogenetics. 1996;6(3):213–22.
41. Culm-Merdek KE, von Moltke LL, Harmatz JS, Greenblatt DJ. Fluvoxamine impairs single-dose caffeine clearance without altering caffeine pharmacodynamics. Br J Clin Pharmacol. 2005;60(5):486–93.
42. Perera V, Gross AS, Xu H, McLachlan AJ. Pharmacokinetics of caffeine in plasma and saliva, and the influence of caffeine abstinence on CYP1A2 metrics. J Pharm Pharmacol. 2011;63(9):1161–8.
43. Amchin J, Zarycranski W, Taylor KP, Albano D, Klockowski PM. Effect of venlafaxine on CYP1A2-dependent pharmacokinetics and metabolism of caffeine. J Clin Pharmacol. 1999;39(3):252–9.
44. Lilja JJ, Niemi M, Fredrikson H, Neuvonen PJ. Effects of clarithromycin and grapefruit juice on the pharmacokinetics of glibenclamide. Br J Clin Pharmacol. 2007;63(6):732–40.
45. Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivistö KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. Clin Pharmacol Ther. 2002;72(3):326–32.
46. Kirchheiner J, Brockmöller J, Meineke I, Bauer S, Rohde W, Meisel C, Roots I. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. Clin Pharmacol Ther. 2002;71(4):286–96.
47. Rambiritch V, Naidoo P, Maharaj B, Pillai G. Population pharmacokinetic modeling of glibenclamide in poorly controlled South African type 2 diabetic subjects. Clin Pharmacol. 2016;8:83–92.
48. Sica DA, Gehr TW, Ghosh S. Clinical pharmacokinetics of losartan. Clin Pharmacokinet. 2005;44(8):797–814.
49. Mahajan R, Parvez A, Gupta K. Microdosing vs. therapeutic dosing for evaluation of pharmacokinetic data: a comparative study. J Young Pharmacists. 2009;1(4):4.
50. Bi YA, Mathialagan S, Tylaska L, Fu M, Keefer J, Vildhede A, et al. Organic anion transporter 2 mediates hepatic uptake of tolbutamide, a CYP2C9 probe drug. J Pharmacol Exp Ther. 2018;364(3):390–8.
51. Uchida S, Yamada H, Li XD, Maruyama S, Ohmori Y, Oki T, et al. Effects of Ginkgo biloba extract on pharmacokinetics and pharmacodynamics of tolbutamide and midazolam in healthy volunteers. J Clin Pharmacol. 2006;46(11):1290–8.
52. Frymoyer A, Shugarts S, Browne M, Wu AH, Frassetto L, Benet LZ. Effect of single-dose rifampin on the pharmacokinetics of warfarin in healthy volunteers. Clin Pharmacol Ther. 2010;88(4):540–7.
53. Chan E, McLachlan AJ, Pegg M, MacKay AD, Cole RB, Rowland M. Disposition of warfarin enantiomers and metabolites in patients during multiple dosing with rac-warfarin. Br J Clin Pharmacol. 1994;37(6):563–9.
54. Pearce RE, Rodrigues AD, Goldstein JA, Parkinson A. Identification of the human P450 enzymes involved in lansoprazole metabolism. J Pharmacol Exp Ther. 1996;277(2):805–16.
55. Mainz D, Borner K, Koeppe P, Kotwas J, Lode H. Pharmacokinetics of lansoprazole, amoxicillin and clarithromycin after simultaneous and single administration. J Antimicrob Chemother. 2002;50(5):699–706.
56. Vay M, Meyer MJ, Blank A, Skopp G, Rose P, Tzvetkov MV, Mikus G. Oral yohimbine as a new probe drug to predict CYP2D6 activity: results of a fixed-sequence phase I trial. Clin Pharmacokinet. 2020;59(7):927–39.
57. Vay M, Sauter M, Mikus G, Burhenne J. Quantification of microdosed oral yohimbine and its major metabolite in human plasma in the picogram range. Bioanalysis. 2019;11(16):1459–67.
58. Lucas D, Ferrara R, Gonzalez E, Bodenez P, Albores A, Manno M, Berthou F. Chlorzoxazone, a selective probe for phenotyping CYP2E1 in humans. Pharmacogenetics. 1999;9(3):377–88.
59. Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol. 1991;4(2):168–79.
60. Witt L, Suzuki Y, Hohmann N, Mikus G, Haefeli WE, Burhenne J. Ultrasensitive quantification of the CYP2E1 probe chlorzoxazone and its main metabolite 6-hydroxychlorzoxazone in human plasma using ultra performance liquid chromatography coupled to tandem mass spectrometry after chlorzoxazone microdosing. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1(1027):207–13.
61. Hohmann N, Blank A, Burhenne J, Suzuki Y, Mikus G, Haefeli WE. Simultaneous phenotyping of CYP2E1 and CYP3A using oral chlorzoxazone and midazolam microdoses. Br J Clin Pharmacol. 2019;85(10):2310–20.
62. Arendt RM, Greenblatt DJ, Garland WA. Quantitation by gas chromatography of the 1- and 4-hydroxy metabolites of midazolam in human plasma. Pharmacology. 1984;29(3):158–64.
63. Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Hartwell PS, et al. Use of midazolam as a human cytochrome P450 3A probe: I. In vitro–in vivo correlations in liver transplant patients. J Pharmacol Exp Ther. 1994;271(1):549–56.
64. Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Bacchi CE, et al. Use of midazolam as a human cytochrome P450 3A probe: II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. J Pharmacol Exp Ther. 1994;271(1):557–66.
65. Marcsisin SR, Jin X, Bettger T, McCulley N, Sousa JC, Shanks GD, et al. CYP450 phenotyping and metabolite identification of quinine by accurate mass UPLC-MS analysis: a possible metabolic link to blackwater fever. Malar J. 2013;21(12):214.
66. Maeda K, Takano J, Ikeda Y, Fujita T, Oyama Y, Nozawa K, et al. Nonlinear pharmacokinetics of oral quinidine and verapamil in healthy subjects: a clinical microdosing study. Clin Pharmacol Ther. 2011;90(2):263–70.
67. Tracy TS, Korzekwa KR, Gonzalez FJ, Wainer IW. Cytochrome P450 isoforms involved in metabolism of the enantiomers of verapamil and norverapamil. Br J Clin Pharmacol. 1999;47(5):545–52.
68. Rohr BS, Mikus G. Proposal of a safe and effective study design for CYP3A-mediated drug-drug interactions. J Clin Pharmacol. 2020;60(10):1294–303.
69. Templeton I, Peng CC, Thummel KE, Davis C, Kunze KL, Isoherranen N. Accurate prediction of dose-dependent CYP3A4 inhibition by itraconazole and its metabolites from in vitro inhibition data. Clin Pharmacol Ther. 2010;88(4):499–505.
70. Kloth JS, Klümpen HJ, Yu H, Eechoute K, Samer CF, Kam BL, et al. Predictive value of CYP3A and ABCB1 phenotyping probes for the pharmacokinetics of sunitinib: the ClearSun study. Clin Pharmacokinet. 2014;53(3):261–9.
71. Tatosian DA, Yee KL, Zhang Z, Mostoller K, Paul E, Sutradhar S, et al. A microdose cocktail to evaluate drug interactions in patients with renal impairment. Clin Pharmacol Ther. 2021;109(2):403–15.
72. Nassar YM, Hohmann N, Michelet R, Gottwalt K, Meid AD, Burhenne J, et al. Quantification of the time course of CYP3A inhibition, activation, and induction using a population pharmacokinetic model of microdosed midazolam continuous infusion. Clin Pharmacokinet. 2022;61(11):1595–607.
73. Katzenmaier S, Markert C, Riedel KD, Burhenne J, Haefeli WE, Mikus G. Determining the time course of CYP3A inhibition by

potent reversible and irreversible CYP3A inhibitors using a limited sampling strategy. Clin Pharmacol Ther. 2011;90(5):666–73.
74. Yamane N, Tozuka Z, Kusama M, Maeda K, Ikeda T, Sugiyama Y. Clinical relevance of liquid chromatography tandem mass spectrometry as an analytical method in microdose clinical studies. Pharm Res. 2011;28(8):1963–72.
75. Nguyen TT, Bénech H, Delaforge M, Lenuzza N. Design optimisation for pharmacokinetic modeling of a cocktail of phenotyping drugs. Pharm Stat. 2016;15(2):165–77.
76. Merlé Y, Mentré F. Bayesian design criteria: computation, comparison, and application to a pharmacokinetic and a pharmacodynamic model. J Pharmacokinet Biopharm. 1995;23(1):101–25.
77. Combes FP, Retout S, Frey N, Mentré F. Prediction of shrinkage of individual parameters using the Bayesian information matrix in non-linear mixed effect models with evaluation in pharmacokinetics. Pharm Res. 2013;30(9):2355–67.
78. Eap CB, Bouchoux G, Powell Golay K, Baumann P. Determination of picogram levels of midazolam, and 1- and 4-hydroxymidazolam in human plasma by gas chromatography-negative chemical ionization-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2004;802(2):339–45.
79. Hohmann N, Kocheise F, Carls A, Burhenne J, Weiss J, Haefeli WE, Mikus G. Dose-dependent bioavailability and CYP3A inhibition contribute to non-linear pharmacokinetics of voriconazole. Clin Pharmacokinet. 2016;55(12):1535–45.
80. Saari TI, Laine K, Leino K, Valtonen M, Neuvonen PJ, Olkkola KT. Effect of voriconazole on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. Clin Pharmacol Ther. 2006;79(4):362–70.
81. Chen M, Nafziger AN, Bertino JS Jr. Drug-metabolizing enzyme inhibition by ketoconazole does not reduce interindividual variability of CYP3A activity as measured by oral midazolam. Drug Metab Dispos. 2006;34(12):2079–82.
82. Burhenne J, Halama B, Maurer M, Riedel KD, Hohmann N, Mikus G, Haefeli WE. Quantification of femtomolar concentrations of the CYP3A substrate midazolam and its main metabolite 1'-hydroxymidazolam in human plasma using ultra performance liquid chromatography coupled to tandem mass spectrometry. Anal Bioanal Chem. 2012;402(7):2439–50.
83. Fuchs I, Hafner-Blumenstiel V, Markert C, Burhenne J, Weiss J, Haefeli WE, Mikus G. Effect of the CYP3A inhibitor ketoconazole on the PXR-mediated induction of CYP3A activity. Eur J Clin Pharmacol. 2013;69(3):507–13.
84. Derungs A, Donzelli M, Berger B, Noppen C, Krähenbühl S, Haschke M. Effects of cytochrome P450 inhibition and induction on the phenotyping metrics of the basel cocktail: a randomized crossover study. Clin Pharmacokinet. 2016;55(1):79–91.
85. Chung E, Nafziger AN, Kazierad DJ, Bertino JS Jr. Comparison of midazolam and simvastatin as cytochrome P450 3A probes. Clin Pharmacol Ther. 2006;79(4):350–61.
86. Vittal S, Ganneboina R, Layek B, Trivedi RK, Hotha KK, Bharathi DV, Mullangi R. Highly sensitive method for the determination of omeprazole in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry: application to a clinical pharmacokinetic study. Biomed Chromatogr. 2009;23(4):390–6.
87. Mahmoudi M, Foerster KI, Burhenne J, Weiss J, Mikus G, Haefeli WE. Application of microdosed intravenous omeprazole to determine hepatic CYP2C19 activity. J Clin Pharmacol. 2021;61(6):789–98.