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ARTICLE

A naïve pooled data approach for extrapolation of Phase 0 microdose trials to therapeutic dosing regimens

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Abstract

Microdosing is a strategy to obtain knowledge of human pharmacokinetics prior to Phase I clinical trials. The most frequently used method to extrapolate microdose (≤100 μg) pharmacokinetics to therapeutic doses is based on linear extrapolation from a noncompartmental analysis (NCA) with a two-fold acceptance criterion between pharmacokinetic metrics of the extrapolated microdose and the therapeutic dose. The major disadvantage of NCA is the assumption of linear extrapolation of NCA metrics. In this study, we used a naïve pooled data (NPD) modeling approach to extrapolate microdose pharmacokinetics to therapeutic pharmacokinetics. Gemcitabine and anastrozole were used as examples of intravenous and oral drugs, respectively. Data from microdose studies were used to build a parent-metabolite model for gemcitabine and its metabolite 2′,2′-difluorodeoxyuridine (dFdU) and a model for anastrozole. The pharmacokinetic microdose models were extrapolated to therapeutic doses. Extrapolation of the microdose showed differences in pharmacokinetic shape for gemcitabine and dFdU between the simulated and observed therapeutic concentrations, whereas the observed therapeutic concentrations for anastrozole were captured by the extrapolation. This study demonstrated the possible use and feasibility of an NPD modeling approach for the evaluation and application of microdose studies in early drug development. Last, physiologically-based pharmacokinetic modeling might be an alternative for microdose extrapolation of drugs with complex pharmacokinetics such as gemcitabine.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The current golden standard for the evaluation of microdose Phase 0 trials is noncompartmental analysis (NCA). The main limitation of NCA is its unsuitability for extrapolations to therapeutic doses.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study describes an alternative method for the evaluation and extrapolation of microdose Phase 0 trials: a naïve pooled data (NPD) approach. Pharmacokinetic models were developed based on microdose data and extrapolated to therapeutic doses.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
This study demonstrated the feasibility of an NPD approach for the evaluation and extrapolation of microdose Phase 0 trials as an alternative to NCA.
HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
The NPD approach could add available information to inform first-in-human doses for Phase I clinical studies.

INTRODUCTION

The concept of microdosing was introduced in the 1990s as a method to obtain early in vivo human pharmacokinetic data.[1] The first study using microdosing was published in 2003.[1] Subsequently, the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) first addressed microdosing trials in 2004 and 2006, respectively.[2,3] This resulted in the current international International Conference on Harmonization (ICH) M3[R2] guideline on experimental investigational new drug studies by the EMA.[4] During this period, a microdose was defined as 1/100th of the anticipated therapeutic dose with a maximum of 100 μg[3].

The original idea of microdosing was assessment of the pharmacokinetics of a new drug in an early phase prior to Phase I clinical studies.[5] Because microdosing trials precede Phase I clinical trials, they are often referred to as Phase 0 studies. In these Phase 0 studies, a small population of healthy volunteers (and sometimes patients)[6] is administered a microdose of the drug candidate. The aim of these studies is to quickly establish whether a novel drug has an appropriate pharmacokinetic profile in the human body without therapeutic or diagnostic purpose.[6] Consequently, Phase 0 microdose studies enhance early selection of promising drug candidates and help in selecting the starting dose, thereby reducing costs and time, and improving efficiency of drug development.[7]

The concept of microdosing is based on the assumption that pharmacokinetics of a microdose can be extrapolated to therapeutic doses. The most frequently used method to evaluate the pharmacokinetic extrapolation of microdose to therapeutic dose is by performing a noncompartmental analysis (NCA) and determine the fold-difference in dose-normalized pharmacokinetic metrics between the microdose and therapeutic doses.[8,9] The acceptance criterion is typically a two-fold difference, which is commonly used in allometry.[8,9] Based on this criterion, the predictability of microdose to therapeutic pharmacokinetics has been summarized previously, where the predictability of orally administered drugs was 62% (n = 25)[10] and 68% (n = 41),[11] whereas the predictability of intravenously (i.v.) administered drugs was 100% (n = 12)[10] and 94% (n = 16).[11] Phase 0 microdose studies appear to be more successful in predicting therapeutic human pharmacokinetics compared to the traditionally used techniques for in vitro to in vivo extrapolation (IVIVE) such as allometry or physiologically-based pharmacokinetics (PBPKs). A predictability of 51–79% for IVIVE is reported in literature for both oral and i.v. administered drugs and with a two-fold error.[12–15]

Assessing microdose predictability using NCA with a two-fold criterion, however, has several disadvantages. Drugs with pharmacokinetic metrics just outside the two-fold criterion are classified as not predictive. However, it is debatable whether drugs with metrics just outside this interval are relevantly different from drugs with metrics just inside the interval. Additionally, the two-fold criterion is difficult to interpret for pharmacokinetic parameters or metrics that have boundaries. For example, bioavailability cannot be larger than 100% and organ clearance is bound by blood. Furthermore, the current evaluation method ignores the overall shape of the pharmacokinetic curve when only summary measures for exposure are used for evaluation (e.g., a similar area under the concentration time curve [AUC] value could represent two different pharmacokinetic curves).

An alternative approach to assess microdose predictability is a naïve pooled data (NPD) modeling approach. In this approach, a population pharmacokinetic model is developed by fitting the combined data of all individuals while ignoring individual differences. The aim of the current study was to demonstrate the feasibility of an NPD modeling approach for extrapolation of a microdose to therapeutic dosing. The NPD approach will be demonstrated with data from two microdose studies[16–18] using gemcitabine, and its metabolite 2′,2′-difluorodeoxyuridine (dFdU) as an example of an i.v. administered drug and anastrozole as an example of an orally administered drug. Pharmacokinetic models were developed using the microdose data and used for the extrapolation to therapeutic dosing. The observed therapeutic data were used to evaluate the predictive value of microdose-based extrapolations.

METHODS

Study design, subjects, and data

Data were used from three studies: a gemcitabine Phase 0 microdose study,[16] an anastrozole microdose study,[17] and an anastrozole clinical dose finding study.[18] Demographic characteristics of the subjects included in the studies are depicted in Table S1.

Gemcitabine study

The gemcitabine Phase 0 microdose study was a prospective, sequential, open-label, single arm microdose study performed at the Antoni van Leeuwenhoek Hospital, The Netherlands. In this study, patients received gemcitabine as a microdose and as a therapeutic dose. The trial received institutional ethical approval, was conducted in accordance with the principles of the Declaration of Helsinki, and was registered in the Netherlands Trial Register (NTR6183). The study design and results of the NCA have been described in detail previously.[16]

Ten patients (>18 years old) with solid tumors and an indication for treatment with gemcitabine according to standard of care were included in the study. Patient characteristics are described in Table S1. Patients received 100 μg gemcitabine and 1250 mg/m^2 gemcitabine within a 24-h interval.[16] Both the microdose and therapeutic dose were administered as a 30-min i.v. infusion. Blood samples were taken at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 4, and 8 h after the start of the infusion. Plasma concentrations of gemcitabine and dFdU after administration of the microdose were quantified using a validated liquid-chromatography-tandem mass spectrometry (LC–MS/MS) method with a lower limit of quantification (LLOQ) of 5 and 500 pg/ml, respectively.[19] Furthermore, gemcitabine and dFdU concentrations after administration of the therapeutic dose were quantified using a validated LC–MS/MS method with an LLOQ of 0.5 and 50 ng/ml, respectively.[20] The accuracy and precision of both assays were within 15%.[19,20]

Anastrozole studies

Anastrozole data was derived from two studies[17,18] using Plot Digitizer (https://sourceforge.net/projects/plotdigitizer/, version 2.6.8). The microdose study included six healthy Japanese men (Table 1). Anastrozole was dosed orally at 1.98 μg simultaneously with cetrozole and TDM-322.[17] Blood samples were taken predose and over a 72-h period after dosing. Anastrozole plasma concentrations were quantified using a validated LC–MS/MS method with an LLOQ of 0.2 pg/ml.[17] The accuracy and precision of this assay were not reported.

In the clinical dose finding study, six postmenopausal women with advanced breast cancer were included in the study (Table S1).[18] Patients received a single oral dose of 1 mg anastrozole. Blood samples were taken predose and over a 100-h period after dosing. Anastrozole plasma concentration were quantified with gas chromatography.[18] The performance of the bioanalytical method was not reported. The study designs and results have been described in detail elsewhere.[17,18]

TABLE 1 Final model parameter estimates for the gemcitabine-dFdU population pharmacokinetics model and naive pooled data approach

	Final model estimate	
Parameter	**NPD**	**95% CI (SIR)**
Fixed effects		
$V_{c,dFdC}$ (L)	32.4	26.3–39.5
$V_{p,dFdC}$ (L)	128	67.7–218
Q_{dFdC} (L/min)	0.294	0.202–0.422
Cl_{trans} (L/min)	2.87	2.42–3.38
$V_{c,dFdU}$ (L)	38.1	33.7–43.3
Cl_{dFdU} (L/min)	0.0307	0.0113–0.0499
Residual variability (σ^2)		
σ^2 proportional$_{dFdC}$	0.569	0.481–0.715
σ^2 additive$_{dFdC}$	0.00125[a]	–
σ^2 proportional$_{dFdU}$	0.461	0.394–0.573
σ^2 additive$_{dFdU}$	0.25[a]	–

Abbreviations: 95% CI, 95% confidence interval; BSV, between-subject variability; Cl_{trans}, conversion clearance from gemcitabine to 2′,2′-difluorodeoxyuridine; dFdU, 2′,2′-difluorodeoxyuridine; NPD, naïve pooled data; SIR, sampling importance resampling; Q, intercompartmental clearance; V_c, volume of distribution of the central compartment; V_p, volume of distribution of the peripheral compartment.

[a]Fixed parameter.

Software

Plasma concentrations of gemcitabine, dFdU, and anastrozole were analyzed using nonlinear mixed-effects modeling software NONMEM (version 7.3; ICON Development Solutions, Ellicott City, MD). Pirana (version 2.9.2), R (version 3.4.3), and Perl-speaks-NONMEM (PsN, version 5.22.4) were used for the numerical and visual evaluation of the model output.

Model development

Gemcitabine and 2′,2′-difluorodeoxyuridine model

For the structural model, one and two compartment models with linear elimination were tested for gemcitabine and dFdU based on previously published models with therapeutic dose data.[21–23] Model development of gemcitabine and dFdU was performed sequentially. The Laplacian estimation method and subroutine ADVAN13 (with TOL equals 9) were used. Full conversion of gemcitabine to dFdU was assumed and described with a first-order conversion rate constant. This assumption was made to account for the missing plasma concentrations of other metabolites so the renal clearance of gemcitabine would be reliably estimated. Residual errors were described by a proportional error model for gemcitabine observations above LLOQ (Equation 1), a combined error model for gemcitabine observations lower than LLOQ but above the limit of detection (LOD; Equation 2) and a combined error model for dFdU observations (Equation 2).

$$C_{obs,ij} = C_{pred,ij} * \left(1 + \varepsilon_{prop,ij}\right) \quad (1)$$

$$C_{obs,ij} = C_{pred,ij} * \left(1 + \varepsilon_{prop,ij}\right) + \varepsilon_{add,ij} \quad (2)$$

where $C_{obs,ij}$ is the jth observed concentration of the ith subject, $C_{pred,ij}$ the predicted concentration for the jth observed concentration of the ith subject, and $\varepsilon_{prop,ij}$ the proportional residual error and $\varepsilon_{add,ij}$ the additive error both assumed to be distributed following $N(0, \sigma^2)$.

The additive errors for gemcitabine and dFdU were fixed to half of their respective LLOQ to reduce overparameterization of the model. Beal's M3 method was used to handle observations below the limit of detection (<LOD).[24] Bayesian estimation was used to obtained conditional weighted residuals (CWRES).

Anastrozole model

For the structural model of anastrozole, one and two compartment models with linear elimination were evaluated. First order absorption and zero order absorption were tested to describe anastrozole absorption. Residual errors were described by a proportional error model (Equation 1). The first-order conditional estimation method with the interaction option and subroutine ADVAN4 TRANS4 were used.

Model selection and evaluation

Model evaluation was performed throughout model building by consideration of the physiological and scientific plausibility, general goodness-of-fit (GOF), individual fit, precision of parameter estimates, and change in objective function value (OFV). A decrease in OFV greater than or equal to 7.879 ($p < 0.005$ based on χ^2 distribution with one degree of freedom) was considered statistically significant for hierarchical models. Sampling importance resampling was performed on the final models to obtain 95% confidence intervals of the parameter estimates. Furthermore, the predictability of the model was evaluated with visual predictive checks.

Extrapolation to therapeutic dosing

The final models were used for extrapolation to therapeutic pharmacokinetics with the therapeutic dosing regimens of gemcitabine (1250 mg/m^2) and anastrozole (1 mg).[17] Plasma concentrations after therapeutic dosing were simulated 1000 times using the final microdose model. The geometric mean was calculated from these simulated concentrations at each timepoint. The geometric mean concentrations over time were plotted using a two-fold error margin around the geometric mean. A two-fold error was deemed acceptable due to its frequent use in allometry,[8,9] and its use in the NCA evaluation of microdose studies. This two-fold error margin represented the acceptable discrepancy between the extrapolated therapeutic pharmacokinetics from a microdose and the expected therapeutic pharmacokinetics. The width of the error margin can be adjusted according to the expected or desired therapeutic window of a new drug entity. Because, in this study, therapeutic pharmacokinetics were known, the observed therapeutic plasma concentrations were visually compared to the simulated therapeutic plasma concentrations. The fraction of observed therapeutic plasma concentrations within the two-fold error margin were calculated. Because extrapolation rather than accurate prediction is the aim of microdose Phase 0 studies, extrapolation to therapeutic dosing was considered adequate if 70% of the observed therapeutic plasma concentrations fell within the two-fold error margin. For anastrozole, the mean plasma concentrations were compared instead of the geometric means because the original publication only reported mean plasma concentrations.

RESULTS

Gemcitabine and 2′,2′-difluorodeoxyuridine model

A total of 99 gemcitabine plasma concentrations from nine patients and 88 dFdU plasma concentrations from

eight patients were included in the data analysis, of which seven (7.1%) were less than the LOD. Two patients were excluded from the original dataset. One patient was excluded due to dosing errors, receiving 1 mg instead of 100 µg. Another patient was excluded from the dFdU analysis due to exhibiting highly different dFdU pharmacokinetics. This individual caused CWRES to fall outside the ±4 range.

The data were best described by a two-compartment model for gemcitabine and a one-compartment model for dFdU with a first-order conversion rate constant from gemcitabine into dFdU and a first order elimination for dFdU. The final model estimates are depicted in Table 1 and GOF plots are depicted in Figures 1 and 2. Overall, the model adequately described the data. CWRES versus time and versus observations are depicted in Figure 1b,c for gemcitabine and Figure 2b,c for dFdU respectively. CWRES showed a bias toward high concentrations which could not be improved with further model development. The final model adequately predicted the gemcitabine and dFdU concentrations over time (see Figures S1 and S2).

Anastrozole model

A total of 66 anastrozole plasma concentrations from six patients were included in the data analysis. A two-compartment model with linear elimination best described the data. The absorption phase could not be captured by first order absorption. Therefore, zero order absorption was modeled as zero order absorption with a duration of 1 h. Estimation of the duration of the zero order absorption resulted in instability of the model. The additive error was omitted because it was estimated to be close to zero.

The final model estimates are depicted in Table 2. The GOF plots are depicted in Figure 3. Predictions versus observations were evenly distributed around the line of unity (Figure 3a). CWRES distribution demonstrated a small bias at low and higher concentrations which could not be improved with further model development. However, the final model predicted the data well (Figure S3).

Extrapolation to therapeutic dosing

Therapeutic plasma concentrations of gemcitabine, dFdU, and anastrozole were extrapolated with the models described above. The geometric mean of the extrapolated therapeutic plasma concentrations is depicted with the observed therapeutic plasma concentrations in Figure 4.

Therapeutic pharmacokinetics of gemcitabine are depicted in Figure 4a. Visual comparison indicated that the extrapolated therapeutic pharmacokinetics from a microdose underestimated the initial distribution phase and largely overestimated the terminal elimination phase. This is confirmed by the numerical comparison: the fractions of the therapeutic observations within the two-fold error margin of the geometric mean were 75–100% between 9.39 and 50.5 min after infusion and 0–50% between 50.5 minutes and 10 h after infusion (see Table S2). The simulated therapeutic pharmacokinetics of gemcitabine would possibly underestimate a recommended dose for Phase I studies due to overestimation of the terminal elimination phase. However, the range of concentrations were similar between extrapolated and observed pharmacokinetics.

The simulated and observed therapeutic pharmacokinetics of dFdU are depicted in Figure 4b. Comparing the extrapolated concentrations and the observed concentrations visually revealed a major difference in pharmacokinetic profile. Therefore, a microdose of gemcitabine is not predictable of therapeutic dFdU pharmacokinetics and would potentially result in misguided dose recommendations for Phase I studies. However, the numerical comparison indicated good predictability with 70–100% of the observed concentrations within two-fold of the geometric mean between 9.39 min and 3 h after infusions (see Table S2). This discrepancy between visual and numerical comparison demonstrates the importance of visual comparison.

The therapeutic data of anastrozole is depicted as a mean with an SD (Figure 4c). Extrapolated therapeutic pharmacokinetics showed a similar pharmacokinetic profile compared to observed therapeutic pharmacokinetics indicating good predictability. This was supported with numerical comparison: all mean observed concentrations were within two-fold of the extrapolated mean concentration. Continuing, the SDs of 10 out of 11 concentration timepoints also fell within two-fold of the extrapolated mean concentration. Whereas the last observation (C_{last}) differentiated most from the extrapolated mean concentration, the numerical differences were relatively small between the extrapolated and observed therapeutic plasma concentrations for C_{last} (2.0 vs. 4.1 µg/L, respectively). From Figure 4c it was concluded that extrapolated therapeutic anastrozole concentrations based on microdose pharmacokinetic model would be informative for dose recommendations for Phase I clinical trials.

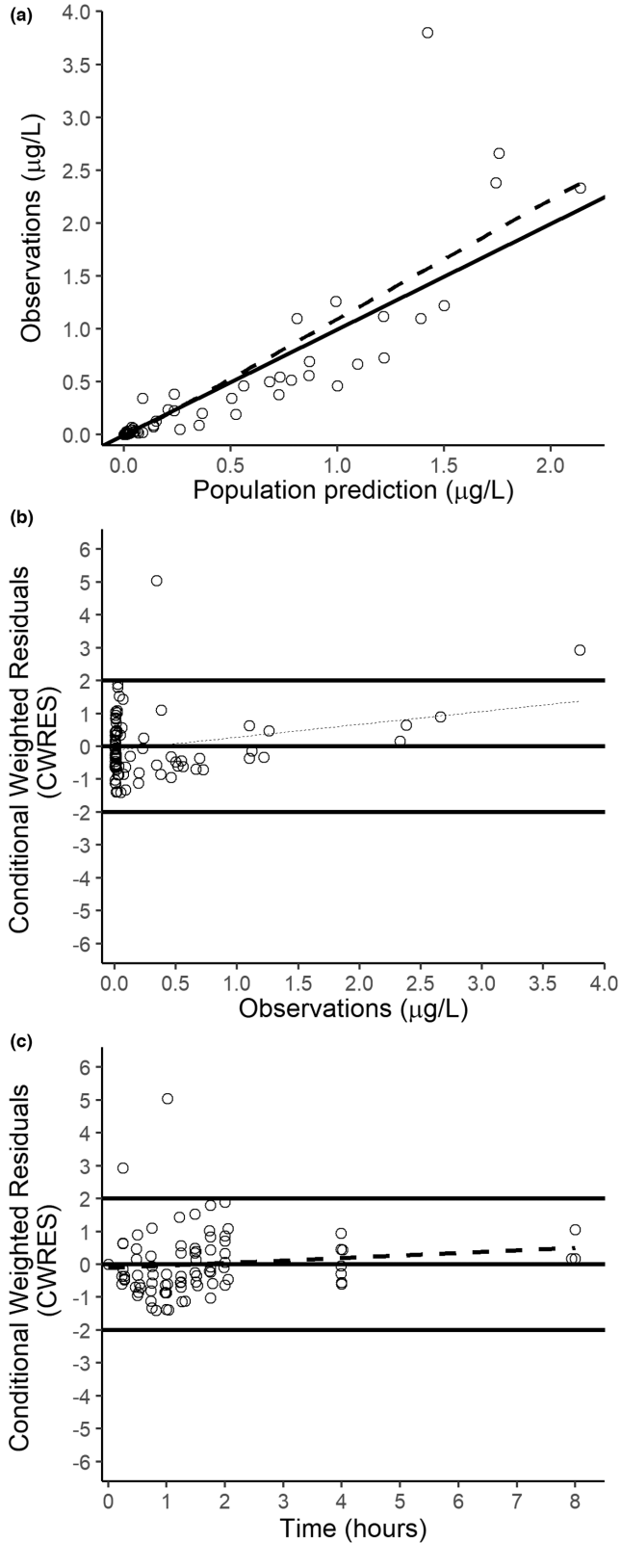

FIGURE 1 Diagnostic plots for the final naïve pooled data modeling approach of gemcitabine. (a) Observations versus populations predictions. (b, c) Conditional weighted residuals (CWRES) versus observations and time after dose, respectively. The black dashed line depicts the trend in the data

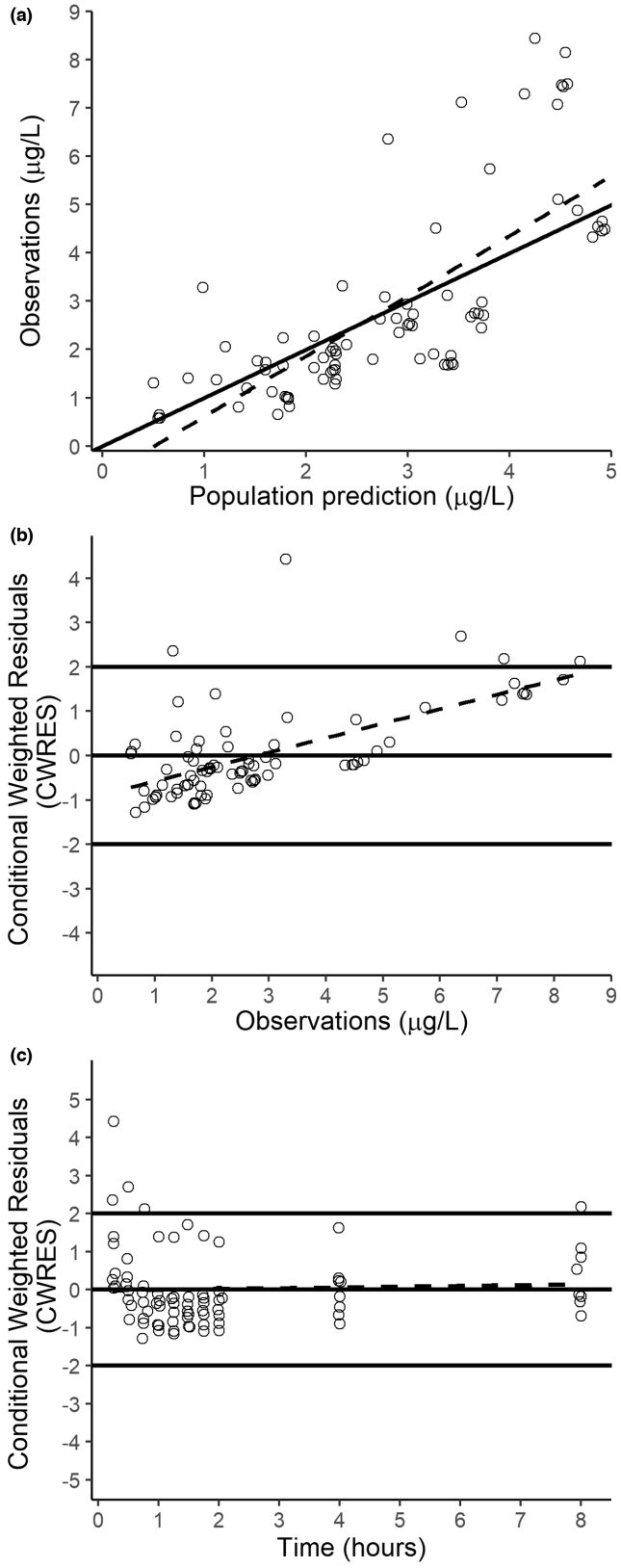

FIGURE 2 Diagnostic plots for the final naïve pooled data modeling approach of 2′,2′-difluorodeoxyuridine. (a) Observations versus populations predictions. (b, c) Conditional weighted residuals (CWRES) versus observations and time after dose, respectively. The black dashed line depicts the trend in the data

TABLE 2 Final model parameter estimates for the anastrozole population pharmacokinetics model and naïve pooled data approach

	Final model estimate (RSE%)	
Parameter	**NPD**	**95% CI SIR**
Fixed effects		
V_c (L)	4.52	0.43–9.62
V_p (L)	79.5	71.6–87.1
Cl (L/h)	1.57	1.44–1.72
Q (L/h)	139	109–177
F	1[a]	–
$D2$ (h)	1[a]	–
Residual variability (σ^2)		
σ^2 proportional	0.0428	0.0309–0.0633
σ^2 additive	0[a]	–

Abbreviations: 95% CI, 95% confidence interval; BSV, between-subject variability; Cl, clearance; $D2$, duration of dosing in the dosing compartment (= compartment 2); F, bioavailability; NPD, naïve pooled data; Q, intercompartmental clearance; SIR, sampling importance resampling; V_c, central volume of distribution; V_p, peripheral volume of distribution.

[a]Fixed parameter.

DISCUSSION

This study demonstrated the feasibility of using an NPD modeling approach for extrapolation of a microdose to therapeutic dosing. Pharmacokinetic models using the NPD approach were developed for gemcitabine, dFdU, and anastrozole based on microdose data. These microdose pharmacokinetic models were used to extrapolate plasma concentrations over time after administration of the therapeutic dose currently used in the clinic. Because therapeutic plasma concentrations were available, the observed therapeutic plasma concentrations were visually compared to the extrapolated therapeutic plasma concentrations. Extrapolation of the microdose models to therapeutic doses demonstrated that the simulated therapeutic plasma concentrations fell within similar ranges to the observed therapeutic plasma concentrations. Although the predictive performance of microdose pharmacokinetics might not be perfect, the similar range of predicted plasma concentrations and observed plasma concentrations demonstrated the suitability of microdose studies to acquire early knowledge about in vivo exposure. However, possible discrepancies between the predicted therapeutic pharmacokinetics and the observed therapeutic pharmacokinetics should be taken into account when microdose studies are used to inform therapeutic doses for Phase I clinical trials. Figure 4 depicts differences in the shape of the pharmacokinetic curve for gemcitabine and dFdU

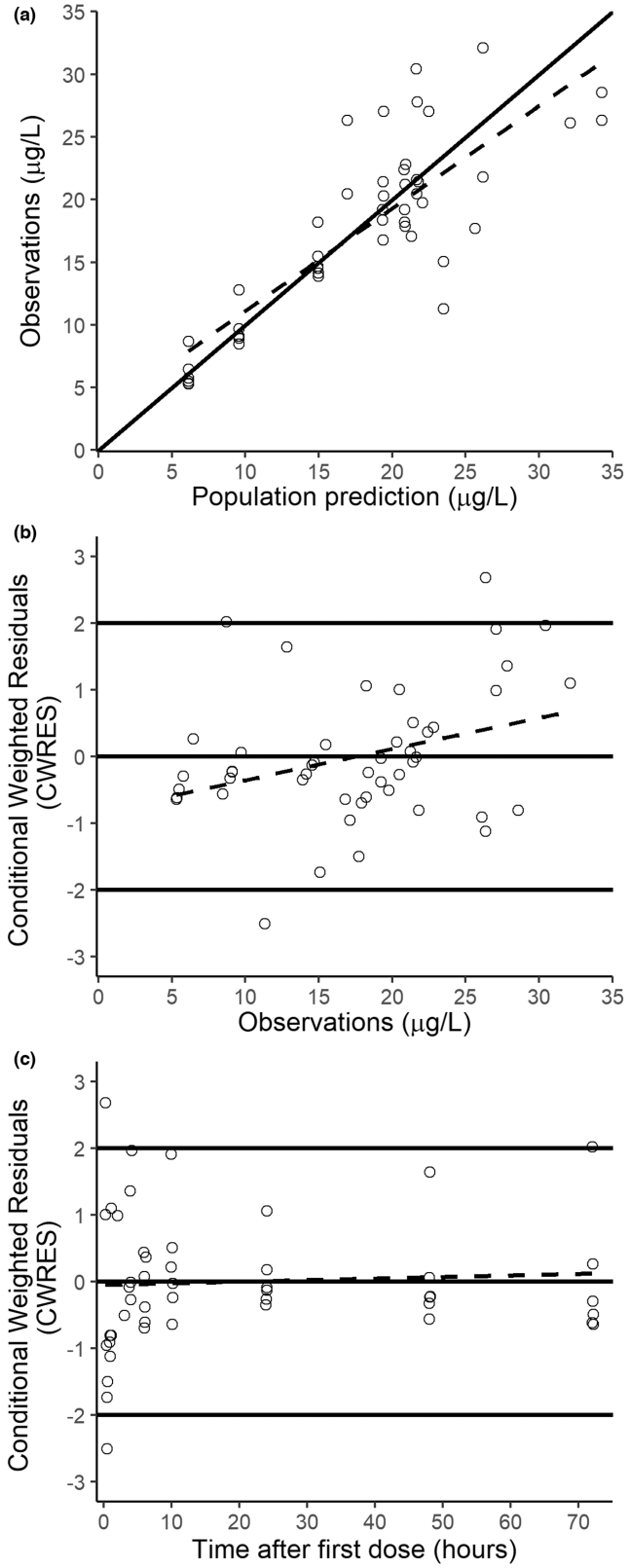

FIGURE 3 Diagnostic plots for the final naïve pooled data modeling approach of anastrozole. (a) Observations versus populations predictions. (b, c) Conditional weighted residuals (CWRES) versus observations and time after first dose, respectively. The black dashed line depicts the trend in the data

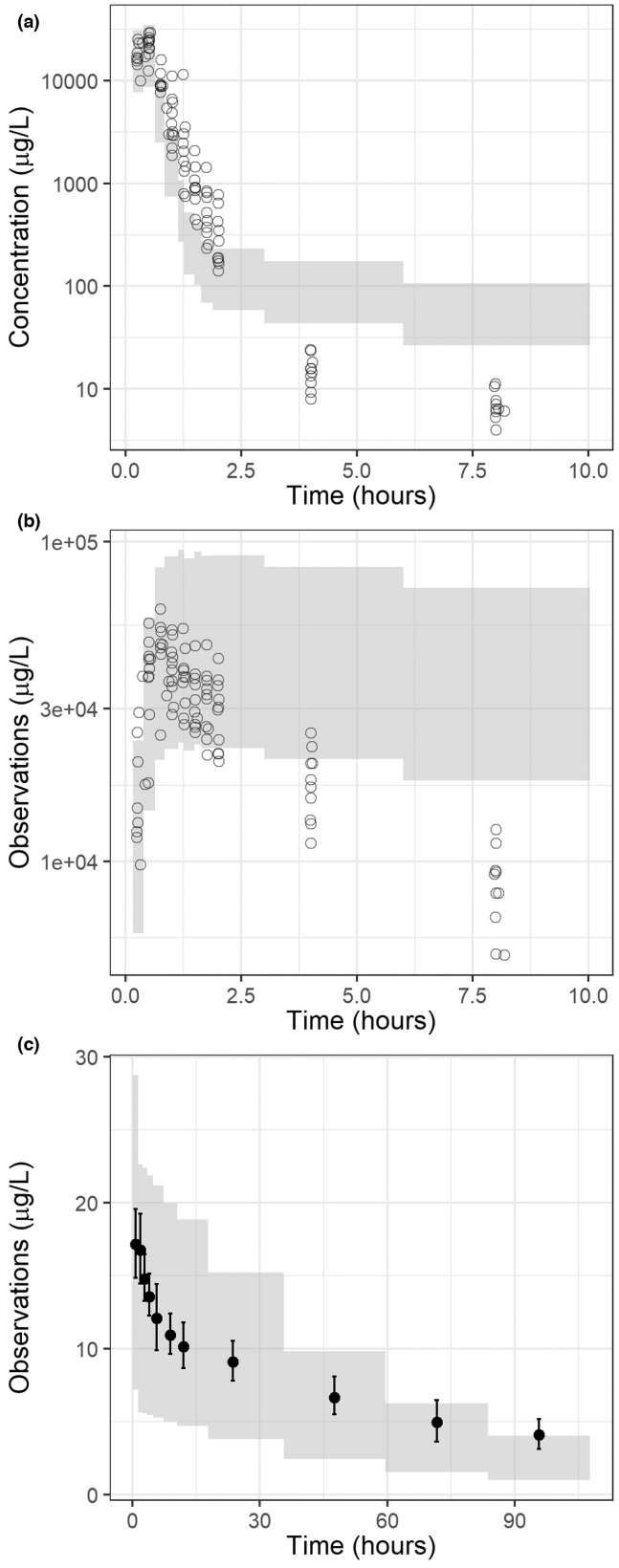

FIGURE 4 The visual predictive checks of the microdose models: (a) gemcitabine, (b) 2′,2′-difluorodeoxyuridine (dFdU), (c) anastrozole. The visual predictive checks were achieved by simulating ($n = 1000$) the therapeutic doses 1250 mg/m^2 (i.v.) for gemcitabine, and 1 mg for anastrozole (oral), using the final naïve pooled data microdose pharmacokinetic models. The gray area is the two-fold range around the geometric mean for gemcitabine and dFdU and the mean for anastrozole of the extrapolated concentrations. The dots represent the observed therapeutic concentrations

between the extrapolated concentrations and observed concentrations. These discrepancies could misinform therapeutic dose recommendations. The possible discrepancies could be taken into account by the error margin, visualizing a predefined acceptable error between the extrapolated therapeutic pharmacokinetics and observed therapeutic pharmacokinetics. Based on the extrapolated therapeutic pharmacokinetics and the predefined error margin, therapeutic dose recommendations for Phase I clinical studies can be defined.

The results from the NPD modeling approach were not fully in accordance with the previously published evaluation of gemcitabine, dFdU, and anastrozole microdose predictability based on NCA (see Table 3).[11,16] The gemcitabine Phase 0 microdose reported predictability for the gemcitabine pharmacokinetic metrics $AUC_{0\text{-}8}$, AUC_{inf}, maximum concentration (C_{max}), and clearance, whereas elimination rate constant, terminal half-life ($t_{1/2}$), and volume of distribution fell outside the two-fold. In addition, the NPD modeling approach demonstrated a difference in shape of the pharmacokinetics profile between the microdose and therapeutic dose despite having similar AUC values. This nonlinearity has been attributed to the saturation of the nucleoside uptake transporter (hENT1), cytidine deaminase (CDA), and deoxycytidine kinase (dCK).[16] CDA is responsible for the rapid and extensive metabolism of gemcitabine to dFdU in plasma, liver, kidneys, and other tissues,[25] whereas dCK is the rate-limiting enzyme for the phosphorylation of gemcitabine to its nucleotide analogs (see Figure S4).[26] Saturation of hENT1 is supported by intracellular data.[16] Saturation of cellular uptake and intracellular phosphorylation of gemcitabine might be an explanation of the difference seen in dFdU elimination between microdose and therapeutic dose (Figure 4b). At therapeutic doses, saturation would result in increased availability of gemcitabine for metabolism to dFdU by CDA in the liver and other tissues, whereas at microdose level the in part reversible phosphorylation could result a balance between gemcitabine phosphorylation and dFdU formation.[26,27] For anastrozole, extrapolation of microdose pharmacokinetics described the therapeutic pharmacokinetics well.[11] The dose-normalized AUC of the microdose and therapeutic dose (16.8 and 10.4 ng*h/ml, respectively) met the two-fold criterion.[17,18] This study showed in addition that the shape of the anastrozole pharmacokinetic curve as well as the individual concentration timepoints were adequately predicted by the NPD modeling approach.

TABLE 3 Direct comparison between the noncompartmental analysis method and the naïve pooled data modeling approach

	Method		
	NCA	NPD	
Criterion	PK metrics ±2-fold[a]	≥70% of observations within 2-fold error margin[b]	Similar trend in PK
Compound			
Gemcitabine	Yes[16,c]	No	No
dFdU	Yes[16]	Yes	No
Anastrozole	Yes[11]	Yes	Yes
Characteristics			
Simplicity	Good	Moderate	
Evaluation of physiologically relevant models	Not possible	Possible	
Visual evaluation	Possible	Possible	
Numerical comparison	Not possible	Possible	
Assumes mono-exponential linear elimination	Yes	No	
Application for clinical trial simulation	Not possible	Possible	

Abbreviations: dFdU, 2′,2′-difluorodeoxyuridine; NCA, noncompartmental analysis; NPD, naïve pooled data; PK, pharmacokinetics.

[a]The extrapolation of the microdose to therapeutic pharmacokinetics was considered good when the dose-normalized pharmacokinetic metrics of the microdose and therapeutic dose fall within two-fold of each other.

[b]The extrapolation to the microdose to therapeutic dosing was considered adequate if 70% of the observed therapeutic plasma concentrations fell within the two-fold error margin.

[c]Area under the concentration time curve (AUC) from zero to 8 h, AUC extrapolated to infinity, maximum concentration, and clearance met the two-fold criterion whereas elimination rate constant, half-life, and volume of distribution did not.[16]

The aim of microdose Phase 0 studies is to obtain knowledge of in vivo human pharmacokinetics prior to Phase I clinical trials. Information obtained from these microdose Phase 0 studies could be used for decision making during further drug development. The NPD modeling approach presented here has several advantages compared to NCA. First, the NPD modeling approach allows the possibility to apply and to evaluate more complex and physiologically relevant models (e.g., multiple compartment models) and thereby improving the extrapolation to therapeutic pharmacokinetics compared to NCA. Second, the visual evaluation allows the simultaneous assessment of the extrapolation of individual concentration timepoints and the shape of the pharmacokinetic curve (comparison between [geometric] mean of the therapeutic concentrations and the extrapolated therapeutic concentrations). Visual comparison is important because two different pharmacokinetic profiles can lead to an equivalent AUC but nevertheless result in differences in target attainment. Furthermore, a numerical comparison can be made by calculating the fraction of therapeutic observations within the prior determined error margin (e.g., 2-fold error margin) which can be dependent on the therapeutic window of the drug. Additionally, numerical evaluation of the NPD modeling approach is also possible by performing a numerical predictive check. Last, NCA assumes mono-exponential linear elimination for the extrapolation of the AUC to infinity and subsequent calculation of clearance.[16] This assumption holds for drugs with biphasic elimination (such as gemcitabine or anastrozole) when there are enough observations in the terminal elimination phase. However, it might be a limitation during early drug development when in vivo human pharmacokinetics is unknown and the terminal elimination phase has not been captured. A direct comparison of the NCA method and the NPD modeling approach is shown in Table 3.

The major advantage of the NPD modeling approach compared to NCA is its ability to extrapolate different therapeutic doses. The different therapeutic doses could be compared in their exposure but also in attainment of a desired target (e.g., time above or below a desired threshold concentration). Visualizations of the extrapolated pharmacokinetics of different therapeutic doses with a relevant error margin (e.g., a two-fold error margin as used here) could be used in decision making to define the start dose of a future Phase I clinical trial. Furthermore, Figure 4 depicts a similar range between the extrapolated concentrations and the observed concentrations. Although the exact pharmacokinetic profile is not always captured by the extrapolation, the extrapolations based on microdose pharmacokinetics were indicative for

the anticipated range of exposure. Therefore, microdose Phase 0 trials have the potential to reduce the number of dose levels in Phase I clinical trials.

In addition to the here presented NPD modeling approach, a full population pharmacokinetic model could also be used to evaluate microdose predictability. An NPD modeling approach might be more appropriate for microdose studies for two reasons. First, the aim of Phase 0 microdose studies is a quick assessment of the human pharmacokinetics of a new drug entity.[5] Estimation of between-subject variability and/or explanation of variability in pharmacokinetics might be more relevant in later stages of drug development, when larger and more heterogeneous populations are being exposed to the drug. Second, microdose Phase 0 studies typically consist of a very small study population (mostly <10 subjects).[28] Due to the small sample size, the final datasets of these microdose Phase 0 studies are modest in size increasing the risk of overparameterization. Furthermore, estimated between-subject variability in these studies might not be an accurate representation of true between-subject variability in a larger patient population. Therefore, it could result in unreliable extrapolation toward therapeutic dosing. A possible next step could be the combination of PBPK modeling and microdosing.[28] Preclinical data (e.g., in vitro enzyme/transporter kinetics studies) could be used to develop a PBPK model for the new drug entity, whereas in vivo human pharmacokinetic data from a microdose study could be used to optimize the model. This method could potentially improve the microdose predictability for drugs with complex pharmacokinetics like gemcitabine and metabolites. However, drugs with pharmacokinetic behavior less dependent on complex enzyme or transporters systems (e.g., anastrozole) may be well extrapolated with the NPD modeling approach.

CONCLUSION

This study demonstrated the use and feasibility of an NPD modeling approach for the evaluation and application of microdose studies in early drug development. The method was shown to adequately describe microdose pharmacokinetics and the extrapolation to therapeutic dosing was informative for the therapeutic exposure. Furthermore, the method allows visual comparison between extrapolated microdose pharmacokinetics and therapeutic pharmacokinetics. This method can further be adjusted to the specific characteristics and requirements of a new drug entity.

AUTHOR CONTRIBUTIONS

L.H., M.N., J.B., A.H., and T.D. wrote the manuscript. M.N., J.B., and A.H. designed the research. L.H. and M.N. performed the research. L.H. analyzed the data.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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