The use of microtracers in food-effect trials: An alternative study design for toxic drugs with long half-lives exemplified by the case for alectinib

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Abstract
The traditional design of food-effect studies has a high patient burden for toxic drugs with long half-lives (e.g., anticancer agents). Microtracers could be used to assess food-effect in patients without influencing their ongoing treatment. The feasibility of a microtracer food-effect study during steady-state of the therapeutic drug was investigated in an in silico simulation study with alectinib as an example for a relative toxic drug with a long half-life. Microtracer pharmacokinetics were simulated based on a previously published population pharmacokinetic model and used for estimation of a model with and a model without food as a covariate on oral bioavailability of alectinib (assuming a 40% food-effect). Power was defined as the fraction of clinical trials where a significant ($p < 0.01$) food-effect was identified. The proposed study design of 10 patients on steady-state treatment, 10 blood samples collected within 24 h after administration and an assumed food-effect of 40% had a power of 99.9%. The mean estimated food-effect was 39.8% (80% confidence interval: 31.0%–48.6%). The feasibility of microtracer food-effect studies was demonstrated. The design of the microtracer food-effect study allowed estimation of the food-effect with minimal influence on therapeutic treatment and reducing patient burden compared to the traditional study design for toxic drugs with long half-lives.

Study Highlights
WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
The traditional food-effect design is a randomized, balanced, two-treatment, crossover design with single dose administration. This study design can be undesirable for food-effect studies in patients with toxic drugs due to a high patient burden. Microtracers (stable isotopically labeled drugs) can be co-administered with a therapeutic dose of an unlabeled drug, allowing simultaneous determination of pharmacokinetics of both compounds, as demonstrated with absolute...
bioavailability. Microtracers could be used to determine the food-effect without influencing therapeutic treatment.

**WHAT QUESTION DID THIS STUDY ADDRESS?**
This study investigates the feasibility of microtracer food-effect studies during steady-state exemplified with alectinib.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**
This study demonstrated the feasibility of microtracer food-effect studies and identified critical aspects of the study design.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**
Microtracer food-effect studies could be used to investigate food-effect without influencing therapeutic treatment, thereby potentially reducing patient burden associated with food-effect studies in patients.

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**INTRODUCTION**

Co-administration of food can alter oral drug pharmacokinetics in various ways, by influencing the physiological processes, such as gastric emptying time, bile flow, and activity of transporters or enzymes. Furthermore, it can interact with the drug itself (e.g., by influencing solubility). In general, food can have four possible outcomes on the bioavailability of a drug: (1) delayed absorption, (2) decreased absorption, (3) increased absorption, and (4) no effect on absorption.

Due to its possible influence on pharmacokinetics, it is essential to study the effect of food intake on drug absorption during drug development. The traditional study design is a randomized, balanced, two-treatment (fed vs. fasting) and two-sequence crossover design with single dose administration in healthy volunteers. It is recommended to use a high-fat, high-caloric meal (800 to 1000 calories of which 50% is fat) because this meal type will have the largest effect on gastrointestinal physiology and therefore will have maximal effect on the oral bioavailability.

The above-described study can give rise to safety concerns when the acceptable risk differentiates between healthy volunteers and patients (e.g., anticancer drugs) or when exposure could rise higher than expected (e.g., food-drug interaction). In these situations, conducting food-effect studies in healthy volunteers might be undesirable. Continuing the study burden of the traditional study design is increased for investigational drugs with a long half-life. An example is alectinib, a tyrosine kinase inhibitor selectively targeting anaplastic lymphoma kinase (ALK) with a half-life of ~32h of which a microtracer is available. A single dose food-effect study for alectinib would consist of two single-dose administrations at day 1 and day 8 of the study with a washout period of 7 days after the single dose administrations. Alectinib treatment will then be able to start on day 15, effectively delaying treatment with at least 15 days (Figure 1a). Another consequence of the long half-life is the long period of sample collection to capture the complete pharmacokinetics of the drug with noncompartmental analysis (NCA; e.g., in a food-effect study of alectinib, blood samples were collected up to 96 hours after administration).

An alternative to the single dose administration would be to study the food-effect at steady-state treatment conditions. In this situation for alectinib, patients have to administer the drug under fasting conditions until steady-state pharmacokinetics is achieved (~7 days), followed by a study day (day 8). The same procedure is to be repeated but under fed conditions where patients have to administer their twice daily alectinib dose with a standardized meal for days 9 up to 15 and a study day (day 16). This study design requires considerable time investment and commitment of the included patients (Figure 1b).

Regarding the above-described study designs, there is a need to reduce the patient burden of food-effect studies for toxic drugs with long half-lives. A strategy would be to use microtracers for the determination of food-effect at steady-state. Microtracers are stable isotopically labeled drugs that are dosed at a microgram level (maximum 100μg). These microtracers have originally been used for the determination of the absolute oral bioavailability. Pharmacokinetics of the microtracer could be used for the determination of food-effect, whereas the therapeutic treatment remains unchanged. To reduce the period of sample collection necessary for the complete capture of alectinib pharmacokinetics with NCA, a population pharmacokinetic approach was used for data analysis. The aim of the current study was to demonstrate the feasibility
of a microtracer food-effect study exemplified for the anticancer drug with a relative long half-life. Secondary objectives were to decrease the patient burden of the study and to identify critical aspects of the study design with a sensitivity analysis.

**METHODS**

**Criteria and proposed study design**

Several criteria were defined to reduce the patient burden of the proposed study design for microtracer food-effect studies. The criteria were as follows: (1) the study should consist of maximally 2 study days where the patient is hospitalized and available for the collection of blood samples, (2) the duration of the hospitalization should not exceed 24 h, (3) the microtracer should be administrated as a single dose, and (4) the blood sampling schedule should be optimized to reduce the number of samples collected.

The study design was deemed feasible when the following criteria were met: (1) a sample size of ~10 patients (maximum 15), and (2) power greater than or equal to 80% to detect a minimal clinically relevant food-effect of 40%. A food-effect of 40% was considered the minimal clinically relevant food-effect due to the previously reported intra-individual variability of 27% in alecatinib trough levels.12

The proposed study design is depicted in **Figure 1c**. Patients who have been treated with alecatinib for more than or equal to 7 days (steady-state) will co-ingest the microtracer with their therapeutic dose and a standardized meal on study day 1. After a washout period of the microtracer of five times the half-life of alecatinib (~7 days), patients will receive the microtracer under fasted conditions (day 9). For drugs with a relative long half-life, population pharmacokinetic analysis is recommended over NCA.11 With NCA, the area under the plasma concentration-time curve (AUC) is extrapolated to infinity by dividing the last observed concentration by the elimination rate constant. For drugs with long half-lives, such as alecatinib (32 h8), the extrapolation to infinity can contribute significantly to AUC calculation relative to the AUC from zero to the last observed concentration. In these cases, population pharmacokinetic analysis is recommended because pharmacokinetic parameter estimates of population pharmacokinetic models are independent of a calculation of the AUC extrapolated to infinity.11 Blood samples will be collected before administration of the microtracer (predose), and at 0.5, 1, 1.5, 2, 4, and 6 h to capture the absorption of alecatinib (time to maximum concentration is 4–6 h).8 To capture the elimination of alecatinib, blood samples will be collected at 8, 10, 12, and 24 h after administration.

**Population pharmacokinetic model**

Because it is assumed the microtracer (alecatinib-d6) has equal pharmacokinetics to alecatinib, the population pharmacokinetic model in the assessment report from the European Medicines Agency (EMA) was used.13 This model was built on pharmacokinetic data from 138 patients with locally advanced or metastatic ALK+ non-small cell lung cancer (NSCLC). All patients received 600 mg of alecatinib bidaily with a meal.14 The model was a one-compartment model with first-order elimination and sequential absorption.13 Allometric scaling was included for clearance and volume of distribution with power coefficients of 0.75 for
clearedance and 1.0 for volume of distribution. The residual error model account for unexplained variability (e.g., bioanalytical variability and model misspecification) consisted of a proportional error and an additive error. The additional error was excluded from the residual error model due to the difference in concentration range between therapeutic alectinib dose and the microtracer (ng/nL vs. pg/mL).

Food-effect was included in the model as a covariate on oral bioavailability. The oral bioavailability was fixed to 1 for microtracer administration with food. As described in 2.1 Criteria and proposed study design, a food-effect of 40% was defined as the minimal clinically relevant food-effect. Therefore, the oral bioavailability was fixed to 0.714 for microtracer administration under fasting conditions. Interoccasion variability was included in the model on oral bioavailability to account for the variability in oral availability between the 2 study days that is not explained by the food-effect. Intra-individual variability was described in the model by the food-effect, interoccasion variability on oral bioavailability, and unexplained residual variability. Because the intra-individual variability in alectinib trough levels was reported to be 27.0% and it was assumed the food-effect will be the main source of variability on oral bioavailability between the two administrations, a relatively small inter-occasion variability of 8.0% was assumed. Demographic data, model parameter estimates, and the model code are included in the Data S1.

**Clinical trial simulation**

Stochastic simulation and estimation (SSE) was used to simulate microtracer pharmacokinetics with a food-effect of 40% on bioavailability and perform parameter estimation using two alternative models: (1) a model without food as a covariate on bioavailability, and (2) a model with food as a covariate on bioavailability. The SSE was repeated 1000 times for each trial design. For each SSE, bodyweight was (re)sampled from bodyweights from a previous study with patients with NCSLC treated with alectinib (median: 77.5 kg, range: 49–123 kg). For each SSE, the difference in objective function value (dOFV) was calculated between the two models. The food-effect was calculated with the following equation:

\[
FE = \left( \frac{F - \theta}{\theta} \right) \times 100\%
\]

where FE is the food-effect and \( \theta \) the estimated oral bioavailability for administration under fasting conditions. The oral bioavailability \( F \) of the microtracer after co-administration with food was fixed to 1. A schematic overview of the clinical trial simulations is visualized in Figure 2.

**Feasibility study**

Power was defined as the fraction of clinical trials with significant food effect that was found with a \( p \) value of less than 0.01 (dOFV < -6.63). The 80% confidence interval (80% CI) of the estimated food-effect was calculated based on the 1000 study replicates.

**Sensitivity analysis**

A sensitivity analysis was performed to identify the critical aspects in the design of a microtracer food-effect study. The following aspects of the study design were investigated: Samples size, duration of blood sample collection (8, 10, 12, and 24 h), and number of blood samples collected. For the later samples, the following sample schedules were investigated: predose, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, and 24 h (11 samples), predose, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 h (10 samples), and predose, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 h (9 samples). Moreover, the effect of an included patient with an outlier bodyweight (40 and 200 kg), the effect size of the food-effect, the effect of the proportional error, and the effect of interoccasion variability on oral bioavailability were investigated.

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**FIGURE 2** Flowchart representing the steps of the SSE method used. dOFV, difference in objective function; R, programming language R; SSE, stochastic simulation and estimation; WT, bodyweight.
**Software**

R (version 4.1.2) was used for data preparation, body-weight resampling, and data analysis. SSE was performed using NONMEM (version 7.5; ICON Development Solutions) and Perl-speaks-NONMEM (PsN, version 5.2.6).

**RESULTS**

**Feasibility study**

Figures 3 and 4 provide an overview of the power and estimated food-effect of the study design and the sensitivity study. The proposed study design had a power to reject the null hypothesis that there is no food-effect of 99.9%. The mean estimated food-effect was 39.8% (80% CI: 31.0%–48.6%).

**Sensitivity analysis**

**Study design**

Changes in the study design demonstrated the robustness of the study in terms of power (see Figure 3) and accurate estimation of the food-effect (see Figure 4). There was no relevant impact of the sample size of included patients on the power and outcome of the study. For a sample size between 5 and 15 patients, the power ranged between 89.3% and 99.9% indicating that sample size was not a critical aspect of the proposed study design (see Figure S3). As anticipated, the variability in estimated food-effect decreased with increasing sample size with a sample size of 15 patients having the narrowest CI (80% CI: 32.3%–46.5%; see Figure 4).

The power was not influenced by the reduction of the duration of blood samples collection (Figure 3). Moving the collection time of the last blood sample from 24 to 8 h after administration of microtracer slightly influenced the estimated food-effect with an estimated food-effect of 39.8% (80% CI: 31.0%–48.6%) for 24 h sampling and 39.1% (80% CI: 29.9%–48.3%) for 8 h sampling (Figure 4). A similar effect on the estimated food-effect was observed for the removal of samples in the elimination phase (Figure 4). The sample’s schedule of 10 samples and nine samples had an estimated food-effect of 39.5% (80% CI: 30.2%–48.8%) and 39.2% (80% CI: 29.5%–48.8%), respectively, compared to the full blood collection schedule (39.8%, 80% CI: 31.0%–48.6%). These results indicate that the hospitalization time could be reduced to 8 h after administration of the microtracer.

**Outlier bodyweight**

Furthermore, inclusion of patients with an outlier bodyweight (40 and 200 kg) did neither influence the power of
the study nor the outcome. The respective estimated food-effect was 39.5% (80% CI: 30.8%–48.2%) and 39.7% (80% CI: 30.0%–48.5%).

**Anticipated food-effect**

The proposed study design had sufficient power to detect a food-effect of greater than or equal to 30% (see Figure 3). For an effect-size of 20%, the study design had a power of 78.1% with an estimated food-effect of 19.5% (95% CI: 12.4%–26.6%). To detect a food-effect of 20% with a power 80% greater than or equal to 12 patients have to be included in the study (see Figure S1–S3).

**Unexplained residual variability**

Decreasing the proportional error did not influence the power of the design, but it did influence the precision of the food-effect estimate. An increase of proportional error of 19% to 30% resulted only in a small decrease in power (99.9% vs. 97.7%). However, an increase in the proportional error from 19% to 30% resulted in a decrease in accuracy and precision of the food-effect estimation (39.8%, 80% CI: 31.0%–48.6% vs. 38.6%, 80% CI: 27.9%–49.2%, respectively). However, the differences in the estimated food-effect and the true food-effect were small (<2%) and were therefore deemed clinically irrelevant.

**Interoccasion variability**

To account for variability in oral bioavailability between the two administrations (with and without food) with the addition of the food-effect, a small interoccasion variability of 8% was assumed. Increasing the interoccasion variability from 8% to 19% (half of the reported intra-individual variability in alectinib trough levels) resulted in a drop in power of 99.9% to 75.1%, while an interoccasion variability of 27% resulted in a power of 44.4% (Figure 3). The variability in estimated food-effect increased with increasing interoccasion variability: 39.8 (80% CI: 31.0%–48.6%), 40.0% (80% CI: 24.2%–55.8%), and 40.3% (80% CI: 17.9%–62.7%), respectively.

**DISCUSSION**

The aim of the current study was to determine the feasibility of a microtracer food-effect study during steady-state of the therapeutic drug and to identify critical aspects of the study design by performing a sensitivity analysis. Alectinib served here as an example. The proposed study design of 10 patients with a parallel food-effect arm, and blood collection at predose, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h after oral administration of 100 μg microtracer (alectinib-d6) had a power of 99.9% to detect an anticipated food-effect of 40% with an estimated food-effect of 39.8% (80% CI: 31.0%–48.6%). The sensitivity analysis demonstrated the robustness of the study design. The power of the study was always greater than or equal to 80% except when the anticipated food-effect was 20%. However, a food-effect of 20% can be considered clinically irrelevant given the high intra-individual variability observed in alectinib trough levels. The high power of the study design was thought due to use of a population pharmacokinetic model instead of NCA as a method of analysis and the crossover design of the study. Factors that were critical to the study outcome were anticipated food-effect, sample size, and unexplained residual variability.

The sensitivity analysis revealed several critical aspects of the microtracer food-effect study. First, the anticipated food-effect is an important factor in power calculations and therefore the feasibility of a study. The anticipated food-effect of 20% resulted in the lowest power indicating that more patients have to be included to achieve the desirable power of 80% (see Figure S1). These results demonstrated the importance of defining which food-effect is considered clinically relevant prior to conducting the study. An estimation of a clinically relevant food-effect should be based on (expected) intra-individual variability in pharmacokinetics and the (expected) exposure-response relationship. Food-effect should exceed the intra-individual variability in pharmacokinetics to be clinically relevant for exposure. Continuing, a relatively small food-effect can result in an increase in toxicity for drugs with a small therapeutic window, whereas a relatively large food-effect is needed to establish the same for drugs with a large therapeutic window. Additionally, minimizing variability in the data is critical. Although the parallel study design was robust (e.g., changes in sample collection, inclusion of bodyweight outliers, and sample size minimally influenced the power and the outcome of the study), increasing the unexplained residual variability (proportional error) in the model led to a decrease in accuracy and precision of the estimated food-effect with the 80% CI falling outside a 10% deviation from the true food-effect (80%, 27.9%–49.2%). Moreover, the interoccasion variability on oral bioavailability influenced both the power and estimation of the food-effect. This is a worst-case scenario because the food-effect will be the main source of variability in oral bioavailability between the two occasions of administrations. Continuously, the microtracer food-effect study will be conducted.
in a controlled, structured environment with standardized meals whereas the intra-individual variability was observed in a retrospective observational cohort study. Therefore, it is expected that the interoccasion variability presented in the current study is an overestimation and the simulations with increasing interoccasion variability depict worst-case scenarios. The study design of microtracer food-effect studies, therefore, should be optimized to reduce variability in the data to a minimum (e.g., by standardizing procedures and protocols, and training health staff to record dosing and collection times accurately).

The presented study design has several advantages over traditional food-effect studies for toxic drugs with long half-lives that are unsafe in healthy volunteers. The first advantage of microtracer food-effect studies is the ability to study food-effect without influencing therapeutic treatment (Figure 1c). In comparison, the traditional study design will either result in treatment delay or treatment interruption, which is undesirable and could be unethical (Figure 1a). Studying the food-effect under steady-state conditions (Figure 1b) could be an alternative but would influence treatment by increasing or decreasing therapeutic exposure depending on the food-effect. Furthermore, steady-state conditions also require patients to consume standardized meals for a longer period of time. This not only increases the patient burden of the study, but is also sensitive to errors and could possibly result in an underestimation of the food-effect. Second, the microtracer food-effect study has limited impact of therapeutic exposure because the microtracer is administered at a maximum dose of 100 μg. No additional side effects are expected. Traditional food-effect studies could influence toxicity and/or efficacy during the study period. Last, microtracer food-effect studies are feasible with a reduced hospitalization time and shortened sampling schedule compared to traditional food-effect studies, thereby reducing patient burden.

There are several reasons why the food-effect on the pharmacokinetics of a drug should be investigated. First, food could have a negative effect on efficacy or toxicity. For example, the simultaneous administration of food with nilotinib should be avoided due to the risk of QT prolongation at high peak concentrations. Co-administration with food should also be avoided when exposure is decreased (e.g., capecitabine and afatinib). On the other hand, administration with food could also be an interesting strategy to increase bioavailability of poorly soluble drugs. A previous study demonstrated enhanced efficacy while increasing the trough levels of abiraterone by co-administration with food. In addition, food can also play a role in therapy adherence. Studies have shown that adherence to complex dosing regimens could be improved by coupling dosing with a routine daily activity, such as eating. Food can also reduce the variability in bioavailability and thereby resulting in a more consistent exposure (e.g., rivaroxaban). Furthermore, co-administration with food can be desirable for pharmacodynamic considerations, such as reducing gastrointestinal side effects.

Last, the food-effect on steady-state pharmacokinetics could be different from the food-effect on single dose pharmacokinetics. For example, the food-effect on lapatinib exposure was smaller at steady-state compared to single dose. It is, therefore, not only important to know the maximum magnitude of the food-effect on the pharmacokinetics of a drug but also the food-effect of real-life meals in a real-life context of daily treatment of patients because the food-effect in patients could be different to healthy volunteers due to difference in pharmacokinetics and pharmacodynamics.

CONCLUSION

The feasibility of a microtracer food-effect study for alec tinib during steady-state was demonstrated. The design of the microtracer food-effect study allowed estimation of the food-effect of alec tinib with minimal influence on therapeutic treatment. Continuing the feasibility of the study design was also demonstrated with reduced hospitalization time and a reduced number of blood samples, thereby reducing patient burden compared to the traditional food-effect study design. Future microtracer food-effect studies should account for sample size, anticipated effect-size of food-effect, and (unexplained) variability during study design. Last, the schedule for the collection of blood samples should be reviewed critically because the feasibility study demonstrated robustness of the estimation of food-effect with a reducing number of blood samples collected after peak concentration.

AUTHOR CONTRIBUTIONS

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

FUNDING INFORMATION

No funding was received for this work.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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REFERENCES


SUPPORTING INFORMATION

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How to cite this article: van der Heijden LT, Steeghs N., Beijnen JH, Huijtema ADR, Dorlo TPC. The use of microtracers in food-effect trials: An alternative study design for toxic drugs with long half-lives exemplified by the case for alectinib. *Clin Transl Sci*. 2023;16:2557-2564. doi:10.1111/cts.13647