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ARTICLE

Quantitative methods and modeling to assess COVID-19-interrupted in vivo pharmacokinetic bioequivalence studies with two reference batches

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Abstract

The coronavirus disease 2019 (COVID-19) has presented unprecedented challenges to the generic drug development, including interruptions in bioequivalence (BE) studies. Per guidance published by the US Food and Drug Administration (FDA) during the COVID-19 public health emergency, any protocol changes or alternative statistical analysis plan for COVID-19-interrupted BE study should be accompanied with adequate justifications and not lead to biased equivalence determination. In this study, we used a modeling and simulation approach to assess the potential impact of study outcomes when two different batches of a Reference Standard (RS) were to be used in an in vivo pharmacokinetic BE study due to the RS expiration during the COVID-19 pandemic. Simulations were performed with hypothetical drugs under two scenarios: (1) uninterrupted study using a single batch of an RS, and (2) interrupted study using two batches of an RS. The acceptability of BE outcomes was evaluated by comparing the results obtained from interrupted studies with those from uninterrupted studies. The simulation results demonstrated that using a conventional statistical approach to evaluate BE for COVID-19-interrupted studies may be acceptable based on the pooled data from two batches. An alternative statistical method which includes a "batch" effect to the mixed effects model may be used when a significant "batch" effect was found in interrupted four-way crossover studies. However, such alternative method is not applicable for interrupted two-way crossover studies. Overall, the simulated scenarios are only for demonstration purpose, the acceptability of BE outcomes for the COVID19-interrupted studies could be case-specific.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Pharmacokinetic (PK) bioequivalence (BE) studies usually compare single manufacturing batches of test and reference products.

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WHAT QUESTION DID THIS STUDY ADDRESS?

This study evaluated the impact of using two batches of a reference product due to product expiration in PK BE studies that are interrupted by the coronavirus disease 2019 (COVID-19) pandemic, via a quantitative methods and modeling approach with hypothetical interruption scenarios and batch-to-batch variations.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

A "batch" effect could be considered in the statistical model when two batches of a reference product are used in a single study. The selection of statistical approaches could be case-specific based on study types, degree of batch-to-batch variations, interruption types, etc., and should be accompanied with adequate justifications and not lead to biased equivalence determination.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

Quantitative methods and modeling approaches may provide scientific justifications for alternative analysis approaches to be used for COVID-19 related study interruptions, such as the usage of two batches of a reference product in a single PK BE study.

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a large outbreak of a global public health emergency not seen since the 1918 flu pandemic.[1] Since its initial outbreak in late 2019, COVID-19 has continued to spread in 2021 across the world.[2] To combat the COVID-19 pandemic, the US Food and Drug Administration (FDA) has approved two vaccines, marketed as Comirnaty and Spikevax, for the prevention of COVID-19 disease, and issued emergency use authorization for several drug and biological therapeutic products and vaccines.[3–6] Although more treatment and preventive options are available, the COVID-19 public health emergency has presented unprecedented challenges to drug development. Many ongoing clinical trials have been interrupted due to national guidelines and restrictive measures, including site closures, quarantines, and travel restrictions.[7,8] A recent report revealed that around 80% of non-COVID-19 related clinical trials were suspended or interrupted as a result of the COVID-19 public health emergency with thousands of trials.[9] In March 2020, the FDA issued guidance for industry investigators, and institutional review boards *Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency* (March 2020, updated on August 2021).[10] The guidance noted the impact of COVID-19 on ongoing clinical trials and indicated that protocol modifications might be required to assure the safety of trial participants, maintain compliance with good clinical practice, and minimize risks to trial integrity. Later, the FDA issued guidance for industry *Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency* (June 2020).[11] The latter guidance outlined considerations on the statistical analysis to ensure that affected trials would provide interpretable findings with appropriate statistical quantification of uncertainty.

The COVID-19 public health emergency may make it difficult to meet protocol-specified procedures for clinical trial execution, including those for abbreviated new drug applications (ANDAs). The FDA recognized the impact of COVID-19 on conduct of bioequivalence (BE) studies for ANDAs on its website[12] and published guidance for industry *Protecting Participants in Bioequivalence Studies for Abbreviated New Drug Applications During the COVID-19 Public Health Emergency* (January 2021; COVID-19 BE Guidance).[13] The website and the COVID-19 BE Guidance are intended to provide recommendations toward the safe conduct of BE studies for those involving human subjects. The COVID-19 BE Guidance recommends alternative approaches for sampling, such as using alternative pharmacokinetic (PK) modeling approaches, spaced dosing and sampling approaches, or off-site sampling. Moreover, study protocol modifications and considerations may be required to protect the study participants' rights, welfare, and assure the quality and integrity of the data.

The FDA acknowledged the possibilities of drug expirations during the COVID-19 public health emergency in COVID-19 BE Guidance and included drug expiration-related questions in its guidance for industry *Development of Abbreviated New Drug Applications During the COVID-19 Pandemic – Questions and Answers*

(April 2021, updated September 2021).[13,14] The COVID-19 BE Guidance states that the use of expired Reference (R) or Test (T) product is not recommended in general. Prospective applicants that intend to use a different batch of reference product for such purpose may submit specific questions through controlled correspondences or pre-ANDA meeting request pathways, including discussing alternatives approaches to demonstrate BE.[13,14] In general, only a single manufacturing batch of a reference standard (RS) is used in a PK BE study, such as for the two-way and fully replicated four-way crossover design.[15] The reason is that using two batches of an RS may potentially introduce the risk of passing bio-inequivalent (BIE) products due to between batch variations. If more than one batch of an RS is used in a PK BE study due to RS expiration during the COVID-19 public health emergency, the impact of this un-prespecified study modification needs to be evaluated.

Quantitative methods, such as modeling and simulation approaches, are useful tools to assess the outcome of BE studies, such us evaluating unexpected protocol changes.[16] Those quantitative methods may be utilized to test assumptions and find better study designs in a more efficient way.[17] Per COVID-19 BE Guidance recommendations, the FDA encourages prospective applicants to find and perform alternative analysis approaches for COVID-19-interrupted BE studies, accompanied with adequate justifications that would not lead to biased equivalence determination.[13] The aim of this work is to demonstrate how scientific justifications could be provided using modeling and simulation approaches for unexpected changes in study execution during the COVID-19 public health emergency, such as to conduct a single PK BE study with two batches of an RS. Based on real case scenarios submitted under controlled correspondences by the applicants, this work evaluated two-way and fully replicated four-way crossover study designs as case examples. Simulations were performed with a hypothetical orally administered product by a two-compartment model. Fully replicated four-way crossover studies were simulated with a hypothetical narrow therapeutic index (NTI) drug as an example which requires use of the same lots of the T and R formulations for the replicated administration.[15] The acceptability of the BE results was evaluated by comparing interrupted BE studies that were conducted with two batches of an RS with uninterrupted BE studies that were conducted with one batch of an RS. Overall, the results obtained from this study are case examples to demonstrate the acceptability of BE results, which could be varied and case-specific depending on study design, statistical analysis methods, and generic products.

METHODS

Model-based simulation

Both two-way and fully replicated four-way crossover studies were simulated with and without interruptions for hypothetical drug products. The uninterrupted two-way crossover studies were simulated as two periods, two treatments, two sequences with RT/TR designs, and conducted with the same batch of the RS across all periods (Figure 1a). The COVID-19-interruption was designed to occur after the completion of period one in the two-way crossover study (Figure 1b). More specifically, in the interrupted study design, the first batch of the RS (i.e., R1) was used before the interruption. The second batch of the RS (i.e., R2) was used after the interruption due to the expiration of the first batch because of unexpected study pause. In this study, we assume no product expiration for the T product, and only one batch of the T product was used across all periods. Similarly, a fully replicated four-way crossover study was simulated for four periods, two treatments, and two sequences with TRTR/RTRT design for NTI drug (Figure 1c). Two types of interruptions were

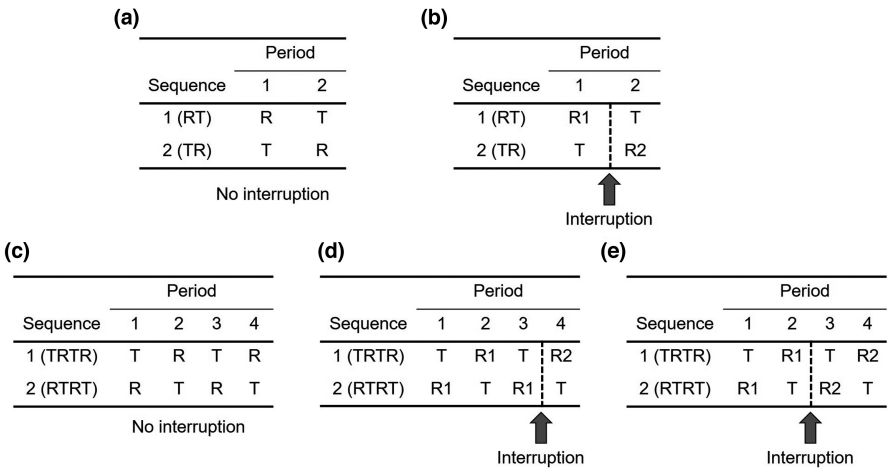

FIGURE 1 Simulated uninterrupted and interrupted study designs. (a) A two-way crossover design without interruption. (b) A two-way crossover design with interruption after the completion of period 2. (c) A four-way crossover design without interruption. (d) A four-way crossover design with interruption after the completion of period 3. (e) A four-way crossover design with interruption after the completion of period 2

simulated that were interrupted after the completion of either period two or period three (Figure 1d,e).

Individual PK profiles were simulated for a hypothetical orally administered product using a two-compartment model with first-order absorption and elimination. The simulation codes used to simulate BE studies are included as Table S1. The selected Population Pharmacokinetics model would not significantly impact the generalizability of the results. All PK parameters were assumed to be log-normally distributed, and a combined proportional and additive error model was used to describe the residual variability. Although different batches of an RS are expected to be comparable, batch-to-batch variations of the RS were assumed to be ranged from 5% to 30% for illustration purpose to cover the extreme cases. The batch-to-batch variations were simulated from the PK model by changing the relative bioavailability factor (Frel) between R1 and R2. Frel was also used to create different magnitude of exposure for T versus R product by assuming different Frel between T and R1 or R2. Simulations accounted for a wide range of geometric means of T/R ratios for each RS batch-to-batch variations with scenarios, including BE, borderline BIE, and clear BIE studies. A range of geometric means of T/R ratios were simulated, which were equally divided into 50 increasing values, with 200 replicates for each value, for a total of 10,000 simulated studies for each selected batch-to-batch variation. In two-way crossover studies, the median value of within subject coefficient of variation (CV) of the log-transformed PK metrics (e.g., area under the curve [AUC] or maximum concentration [C_{max}]) estimated from simulated studies is ~20%. In four-way crossover studies that used NTI drugs as an example, the median value of within subject standard deviation of the R-to-R ratio (S_{WR}) that estimated from simulated studies is 0.15 and are similar to within subject standard deviation of the T-to-T ratio (S_{WT}). Uninterrupted studies were simulated with one batch of the RS (i.e., either R1 or R2). Interrupted studies were simulated by a combination of datasets from R1 and R2 following the designed interruption pattern which used R1 and R2 representing data collected before and after the interruption, respectively. It should be noted that the simulated scenarios are only considered as case examples, which cannot be extrapolated to all interrupted two-way and four-way crossover studies, the acceptability of the BE results could be case-specific. A summary of simulated scenarios is shown in Table 1. All the graphs were created using R version 3.6.3.

BE evaluation and statistical analysis

Two-way crossover studies

Pharmacokinetic end points (e.g., AUC or C_{max}) follow a general linear/linear mixed effect statistic model. The following model (Equation 1) was considered for the two-way crossover design:

$$Y_{ijk} = \mu + F_{(j,k)} + P_j + \pi_k + S_{ik} + e_{ijk} \quad (1)$$

where Y_{ijk} is the response observed log-transformed PK parameters (e.g., AUC or C_{max}) in the ith subject ($i = 1, 2, ..., n_k$) in period j ($j = 1, 2$) of sequence k ($k = 1, 2$), and μ is the overall mean; $F_{(j,k)}$ is the direct fixed effect of the formulation administrated in sequence k at period j; P_j is the fixed effect of period j; π_k is the fixed effect of sequence k; S_{ik} is the random effect of the ith subject in sequence k; and e_{ijk} is the within-subject random error associated with Y_{ijk}. It is assumed that the error terms S_{ik} and e_{ijk} are normally distributed with zero mean and constant variance, that is, $S_{ik} \sim N(0, \sigma_S^2)$ and $e_{ijk} \sim N(0, \sigma_e^2)$.[18]

For each simulated two-way crossover study, a conventional average BE (ABE) was calculated based on log-transformed C_{max}. To establish BE, a 90% confidence

TABLE 1 Summary of simulated scenarios for two-way and four-way crossover studies

Variables	Tested values	
	2-way crossover studies	4-way crossover studies
Number of subjects	24	24
Log-transformed T/R ratio	0.67–1.5; equally divided 50 steps with 200 replicates on each step	
Batch-to-batch variation	5%–30%	5%–30%
Within subject variability estimated from simulated studies	Median CV% ≈ 20%	Median $S_{WR} = S_{WT} \approx 0.15$
Interruption	After completion of period 1	a. After completion of period 2 b. After completion of period 3

Abbreviation: CV%, coefficient of variation percentage.

interval for the ratio of the averages for the measures of the T and R was calculated using SAS 9.4 (SAS Institute Inc.) PROC GLM or PROC MIXED procedure with α of 0.05. Studies were considered as BE if the 90% confidence interval of T/R ratio was within the 80.00%–125.00% limits. Significant level of effect for each variable was estimated based on the type III analysis of variance (ANOVA) table.

Four-way fully replicated crossover studies

The BE of four-way fully replicated crossover studies was evaluated using the reference-scaled ABE (RSABE) following the draft product-specific guidance on *Warfarin Sodium* (recommended December 2012)[19] with a modification that allows the batch effect to be estimated in scaled BE. All simulated studies are balanced cases which contain no missing values. A scaled BE is determined by calculating the 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta S_{WR}^2$. The implied BE limits on the T/R ratio are scaled by S_{WR} but are capped at 80.00%–125.00%. In the draft product-specific guidance on *Warfarin Sodium*, $\mu_T - \mu_R$ is calculated based on the observed difference between the average of the log-transformed replicated T and R measures for each subject using Equation 2 below:

$$I_{ik} = \frac{T_{ik1} + T_{ik2}}{2} - \frac{R_{ik1} + R_{ik2}}{2} \quad (2)$$

where $T_{ijk} = j^{th}$ observation ($j = 1$ or 2) on T for subject i within sequence k; $R_{ijk} = j^{th}$ observation ($j = 1$ or 2) on R for subject i within sequence k.

To estimate and add the "batch" term to the calculation, the estimate of $\mu_T - \mu_R$ are calculated following a linear mixed effect statistic model, as shown in Equation 1. As simulation creates balanced cases which contain no missing values, the unbiased estimate of $\mu_T - \mu_R$ underlying the linear mixed effects statistic model should be comparable with the estimated difference calculated based on observed value as recommended in the current guidance.[19]

When more than one batch of an RS are used, the treatment group R is divided into two subgroups (i.e., R1 and R2). Unlike other effects (i.e., period or sequence effects) in the mixed effect model, batch R1 and R2 can only be found in treatment group R, and batch T can only be found in treatment group T. Therefore, the batch effect was considered as a nested effect under the treatment effect, denoted as batch(trt), in the mixed effects statistical model. For each simulated four-way fully replicated crossover study, a preliminary test for batch(trt) was investigated by a type III ANOVA table from a PROC MIXED procedure. The statistic model would include or exclude the batch(trt) term in both scaled and unscaled BE upon evidence of a significant batch(trt) effect ($p \leq 0.1$), or lack of significance ($p > 0.1$), respectively. The estimated mean and variance for the ratio of T and R were calculated using PROC MIXED, followed by mathematical calculations for S_{WR} and critical bound using R. Uninterrupted studies were analyzed with a model that excluded batch(trt) effect with modified model. When using the RSABE, every study should pass the scaled BE and also regular unscaled BE limits of 80.00%–125.00%. In addition, the upper limit of the 90% confidence interval for the ratio of S_{WT} and S_{WR} (S_{WT}/S_{WR}) should be ≤2.5.

Comparison of uninterrupted and interrupted studies

Simulations were conducted for both two-way and four-way fully replicated crossover studies with and without interruptions. As we aimed to evaluate the possibility of a false BE conclusion from interrupted studies, all interrupted studies which passed BE were collected to assess the chance of being BIE products if conducted without interruption. The BE outcomes from uninterrupted studies were used as a reference to evaluate the BE outcomes from the interrupted studies. We would assess whether a T that can pass the BE in an interrupted BE study can pass BE using a conventional BE approach to at least one RS batch (R1 or R2, or both) at a given batch-to-batch variation.

RESULTS

Analyses of interrupted two-way crossover studies

Interrupted two-way crossover studies which passed BE with the conventional ABE approach were selected, and their results were compared with those obtained from the same products but with an uninterrupted design to assess the risk of passing BIE products. Figure 2 lists the outcome distributions in terms of the percentage falling into one of four scenarios when the study was conducted as uninterrupted designs (i.e., T vs. R1 or T vs. R2). In scenario 1, T would pass BE for both T versus R1 and T versus R2; and, in scenario 2, T would pass BE for either T versus R1 or T versus R2, but not both. In both scenarios 3 and 4, T would not pass BE for either T versus R1 or T versus R2 but, in scenario 3, the PK exposure of T falls within the PK exposures of the two R batches, whereas in scenario 4, the PK exposure of T falls outside those of the two R batches. The percentages of studies that fell into each scenario were used to inform the acceptability of the statistical method

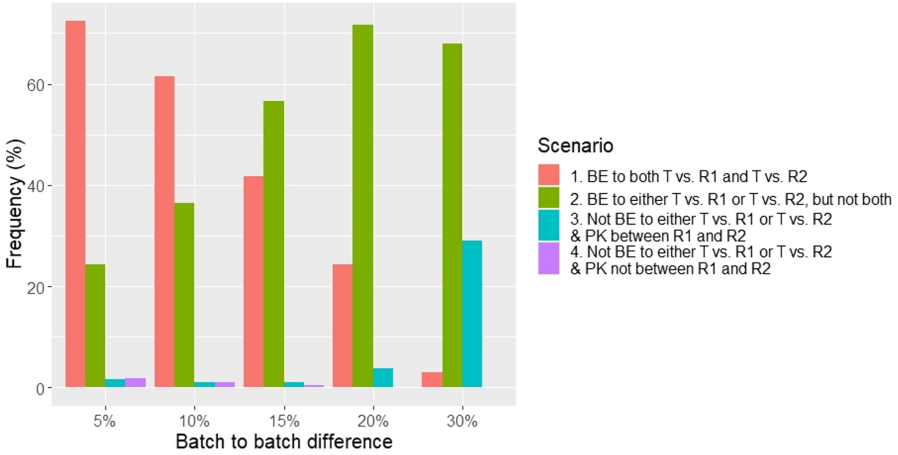

FIGURE 2 Analyses of interrupted BE studies with two-way crossover design that passed the conventional average BE (ABE) evaluation. The figure represents the frequency observed in each scenario when compare with uninterrupted studies. Scenario 4 is considered as a BE failure scenario. BE, bioequivalence; PK, pharmacokinetic

TABLE 2 Percentage of studies showing significant fixed effect

	Categories		Batch-to-batch variations, %				
			5	10	15	20	30
% Studies showed significant period effect	Uninterrupted studies	R1 only	6%	6%	6%	6%	6%
		R2 only	6%	6%	6%	6%	6%
	Interrupted studies	R1 + R2	7%	13%	21%	35%	64%
% Studies showed significant sequence effect	Uninterrupted studies	R1 only	4%	5%	4%	4%	4%
		R2 only	4%	5%	4%	4%	4%
	Interrupted studies	R1 + R2	5%	10%	17%	29%	57%
% Studies showed significant effect for both period and sequence	Uninterrupted studies	R1 only	<1%	<1%	<1%	<1%	<1%
		R2 only	<1%	<1%	<1%	<1%	<1%
	Interrupted studies	R1 + R2	<1%	2%	4%	11%	38%

that was used for analyses. The fourth scenario was considered as the failure scenario for not being BE.

When the batch-to-batch variation is low (e.g., 5%), 72% of studies that can pass the BE with interruption fell into scenario 1 (T can pass the BE to both R1 and R2), and 24% fell into scenario 2 (pass either T vs. R1 or T vs. R2, but not both). The percentage of studies in scenarios 3 and 4 (not BE for either T vs. R1 or T vs. R2) were close or equal to 0. Conversely, when the batch-to-batch variation is as high as 30%, the chance of passed interrupted studies falling into scenarios 1 and 3 decreased to 3% and increased to 29%, respectively. Among studied batch-to-batch variations, the chance of studies that fell into the BE failure scenario (scenario 4) was low (respectively, 2% and 1% for batch-to-batch variations of 5% and 10%) or less than 1% (for batch-to-batch variations of 15%, 20%, and 30%). The simulation results indicated that using a conventional ABE method for interrupted two-way crossover studies might be acceptable as the chance of studies falling into the BE failure scenario was close or equal to 0. If a T can pass the BE assessment in an interrupted study, the same T can pass the BE assessment if the study is conducted without interruption.

Additionally, to evaluate the impact of using two different batches of an RS on period and/or sequence effects in the mixed effect model, type III ANOVA table results were collected from both uninterrupted and interrupted studies (Table 2). The batch effect cannot be identified separately in the statistical model for interrupted studies with a two-way crossover design. As shown in Figure 1a, in an interrupted two-way crossover study, R1 only appeared in period 1, and R2 only appeared in period 2, same for the sequence. Thus, using two batches may lead to inflated uncertainties on period and sequence effects. Table 2 shows that period and sequence effects are not significant when studies are conducted without interruption. If studies are conducted without interruption with one batch of the R, we observe close to or less than 5% of studies with significant effects ($p \leq 0.05$) for period, sequence, or both. However, the percentage of studies with significant period or sequence effects, or both, increased when studies were conducted with interruption with two batches of the R. The chance of having significant period or sequence effects seems to increase with increasing batch-to-batch variation and can be as high as 60% when the batch-to-batch variation is 30%.

Analyses of interrupted four-way fully replicated crossover studies

In interrupted four-way fully replicated crossover studies, the batch effect was considered as a nested effect under the treatment effect, denoted as batch(trt), in the mixed effects statistical model, it was considered as the treatment group R is divided into two subgroups (i.e., R1 and R2). The nested effect tests if there is variation between groups, or within nested subgroups of the attribute variable. To determine whether a batch(trt) effect should be included in the statistical model, a preliminary ANOVA test was performed on each interrupted study. The type III ANOVA table results for batch(trt) effect are summarized in Figure 3. The table indicates that the percentage of studies with significant batch(trt) effect increases with increasing batch-to-batch variation. When batch-to-batch variation is higher or equal to 20%, ~50% or more interrupted studies show significant batch(trt) effect regardless of types of interruption.

Following the preliminary ANOVA test results, studies with no evidence of the batch(trt) effect ($p > 0.1$) were analyzed with an RSABE excluding batch(trt) term from the statistical model. Studies with significant batch(trt) effect ($p \leq 0.1$) were analyzed with a statistical model which included batch(trt) term as a fixed effect in the model. Figure 4 shows BE outcomes from the studies that were analyzed with the model that excluded the batch(trt) term. The table lists studies which passed BE and summarizes the frequency observed in each category when compared with uninterrupted study designs. Comparable to what was observed for two-way crossover studies, the chance of falling into the BE failure scenario (scenario 4) was used to inform the risk of passing a BIE product. The chance of studies falling into scenario 4 was close or equal to 0 across all investigated batch-to-batch variations. An RSABE evaluation with batch effect excluded from the model seems to be acceptable to analyze interrupted four-way fully replicated crossover studies with no evidence of batch(trt) effect regardless of the interruption types.

Subsequently, studies with significant batch(trt) effect were analyzed with the statistical model that included "batch(trt)" as a fixed effect. Figure 5 shows the comparison of results between interrupted and uninterrupted studies. In most cases, when batch(trt) effect was included into the model, if a T can pass BE with interrupted design, the same T can pass BE with uninterrupted design and fall into scenarios 1, 2, or 3. The chance of falling into the BE failure scenario is close to 0 for studies with a two-to-two R batch division. However, the chance of studies falling into the BE failure scenario is 5% when batch-to-batch variation is small (i.e., 5%) and interrupted by a three-to-one R batch division. Thus, BE establishment for interrupted studies with significant batch(trt) effect may introduce the risk of falling into the BE failure scenario although a batch(trt) effect term was included in the model for an accurate estimation, depending on the interruption type.

DISCUSSION

In conventional PK BE studies, a single manufacturing batch is usually recommended to represent each of the T

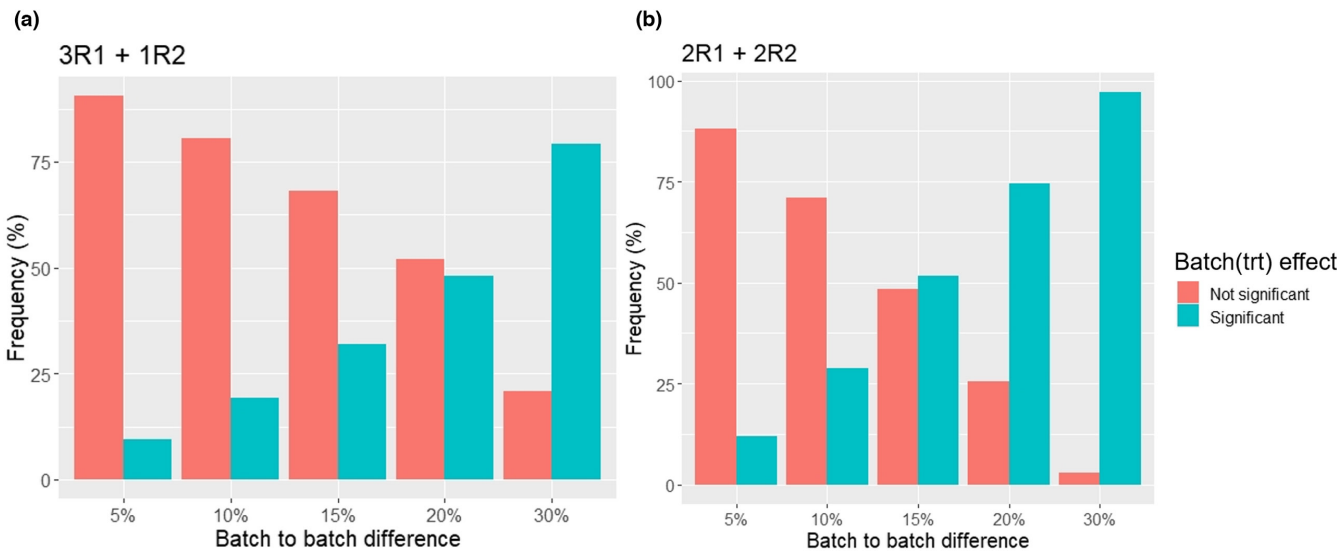

FIGURE 3 Preliminary ANOVA tests of interrupted four-way fully replicated crossover studies for batch(trt) effect. (a) Interruption after the completion of period 3. (b) Interruption after the completion of period 2. Studies with $p \leq 0.1$ are considered with significant batch(trt) effect. ANOVA, analysis of variance

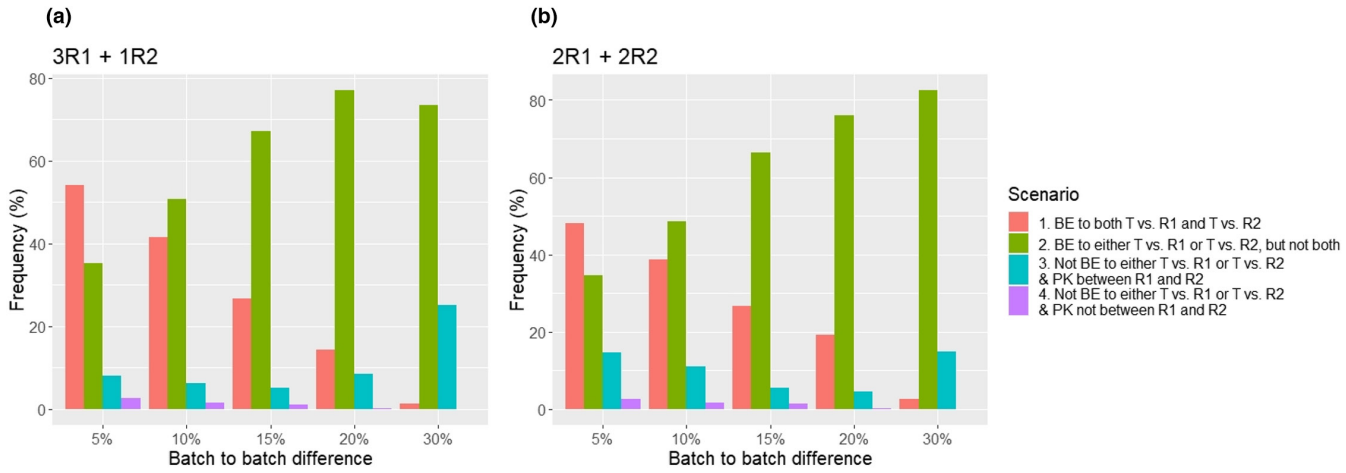

FIGURE 4 Analyses of interrupted four-way fully replicated crossover studies that passed the reference-scaled average BE (RSABE) evaluation with batch effect excluded from the statistical model. (a) Interruption after the completion of period 3. (b) Interruption after the completion of period 2. The figure represents the frequency observed in each scenario when compare with uninterrupted studies. Scenario 4 is considered as a BE failure scenario. BE, bioequivalence; PK, pharmacokinetic

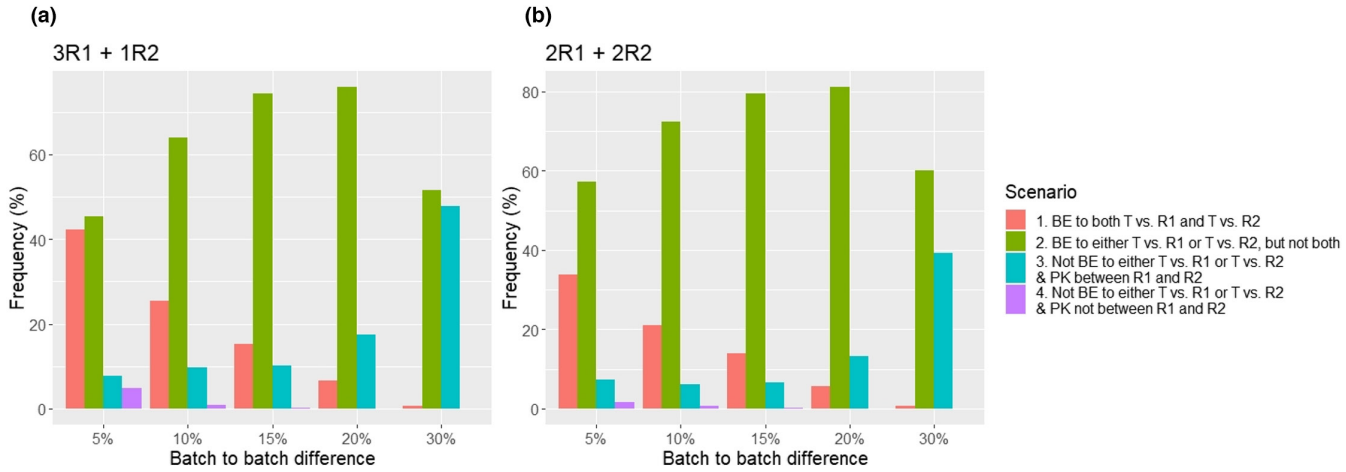

FIGURE 5 Analyses of interrupted four-way fully replicated crossover studies that passed the reference-scaled average BE (RSABE) evaluation with batch effect included from the statistical model. (a) Interruption after the completion of period 3. (b) Interruption after the completion of period 2. The figure represents the frequency observed in each scenario when compare with uninterrupted studies. Scenario 4 is considered as a BE failure scenario. BE, bioequivalence; PK, pharmacokinetic

and R product, especially a four-way fully replicated crossover study design.[15] The active drug content from different batches of an RS is expected to be comparable. When two batches of an RS are used in a single PK BE study, adequate justifications could be used to show two batches are comparable using an additional in vitro test that could provide supportive evidence. In addition, per 21 Code of Federal Regulations (CFR) 320.21(g),[2] for the majority of products, batch-to-batch variation on PK outcomes is not expected to be larger than 25%, simulation conducted in this study is used for the illustration of extreme cases.

For an interrupted two-way crossover study where the interruption happened after completion of period 1, R1 only appears in period 1 and sequence 1, and R2 only appears in period 2 and sequence 2 (Figure 1). In other words, a separate batch effect is unidentifiable because the difference between period 1 and 2 is used to identify either period or sequence effect in a two-way crossover study. In this case, the batch effect can impact both period and sequence effects, as shown in Table 2. The percentage of studies with significant period or sequence effect can be close to 60% when the batch-to-batch variation is high (e.g., 30%). On the other hand, uninterrupted study design only showed about 5% chance of having significant effect, which is at the minimal significance level. In addition, replacing the sequence or period effect with the batch effect in the fixed effects model showed the same results observed without such a replacement (data not shown). Although almost no BE failure study can be found when using a standard ABE to analyze interrupted two-way

crossover studies (Figure 2), an ANOVA test could be done, and further considerations may be needed if significant period and sequence effects are observed.[18,20] The results obtained from interrupted studies may be acceptable if it can be proven that the significant sequence or period effects come from the batch effect, and the significant sequence or period effects would not be biased in the estimation for the formulation difference.

For a four-way fully replicated crossover study that both R and T products would be administered twice to the same individual, the batch effect is identifiable and can be used as a nested effect under the treatment. A preliminary ANOVA test could be performed with the inclusion of the batch(trt) effect which can determine the significant level of the batch(trt) effect. Studies with no evidence of the batch(trt) effect could be analyzed with batch(trt) term excluded from the statistical model, whereas studies with significant batch(trt) effect would be analyzed with batch(trt) term included for a more accurate estimation. The ANOVA results are consistent with our assumption that the batch(trt) term could accurately capture batch changes as the chance of having significant batch(trt) effect increased with higher batch-to-batch variations (Figure 3). From simulation results, BE results obtained from interrupted studies with no significant batch(trt) effect seems to be acceptable when batch(trt) is excluded (Figure 4). However, BE results from studies with significant batch(trt) effect may not be acceptable with the statistical model that included the batch(trt) term, especially for studies with small batch-to-batch variation and three-to-one R batch division (Figure 5). Our simulation showed that, if a four-way fully replicated study is interrupted with a significant batch(trt) effect, the acceptability of BE outcomes is case-specific. In conclusion, the simulated scenarios are only considered as case examples, which cannot be extrapolated to all interrupted two-way and four-way crossover studies, the study results could be case-specific.

CONCLUSION

In this study, we presented a simulation-based method for BIE risk assessment when two batches of an RS were used in a BE study interrupted by COVID-19. Industrial applicants or researchers are encouraged to justify the sufficiency of BIE risk control in a similar approach as presented when the situation arises due to a pandemic-related study interruption.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

All authors contributed to manuscript writing. L.Z., Y.G., K.F., and L.F. designed the research. Y.G., P.Z., and K.F. performed the research. Y.G. analyzed the data.

DISCLAIMER

The opinions expressed in this manuscript are those of the authors and should not be interpreted as the position of the US Food and Drug Administration.

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