

**REVIEW**

# Human radiolabeled mass balance studies supporting the FDA approval of new drugs

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**Abstract**

Human radiolabeled mass balance studies are an important component of the clinical pharmacology programs supporting the development of new investigational drugs. These studies allow for understanding of the absorption, distribution, metabolism, and excretion of the parent drug and metabolite(s) in the human body. Understanding the drug's disposition as well as metabolite profiling and abundance via mass balance studies can help inform the overall drug development program. A survey of the US Food and Drug Administration (FDA)-approved new drug applications (NDAs) indicated that about 66% of the drugs had relied on findings from the mass balance studies to help understand the pharmacokinetic characteristics of the drug and to inform the overall drug development program. When such studies were not available in the original NDA, adequate justifications were routinely provided. Of the 104 mass balance studies included in this survey, most of the studies were conducted in healthy volunteers (90%) who were mostly men (>86%). The studies had at least six evaluable participants (66%) and were performed using the final route(s) of administration (98%). Eighty-five percent of the studies utilized a dose within the pharmacokinetic linearity range with 54% of the studies using a dose the same as the approved dose. Nearly all studies were performed as a single-dose (97%) study using a fit-for-purpose radiolabeled formulation. In this analysis, we summarized the current practices for conducting mass balance studies and highlighted the importance of conducting appropriately designed human radiolabeled mass balance studies and the challenges associated with inadequately designed or untimely studies.

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## INTRODUCTION

Human mass balance studies, often referred to as the absorption, distribution, metabolism, and excretion (ADME) studies, are an integral part of the clinical pharmacology programs supporting the development of new investigational drugs. A radiolabeled mass balance study (from here on referred to as mass balance study) is the most common method used to characterize the disposition of the parent drug and its metabolite(s) in the human body, although a few other methods may be available. The main objectives of mass balance studies are to: (1) elucidate the overall pathways of metabolism and excretion of an investigational drug, (2) identify and quantify circulating metabolites, and (3) determine the abundance of metabolites relative to the parent drug and/or total drug-related exposure. Information obtained from these studies, along with other *in vitro*, *in vivo*, and *in silico* data, can then inform the overall clinical and nonclinical development program of the investigational drug. For example, a human mass balance study provides information on which metabolite(s) should be structurally characterized and which metabolite(s) would be subject to nonclinical safety assessment.<sup>1,2</sup> In addition, metabolism and excretion information obtained from the human mass balance studies can inform the need to further evaluate the impact of renal and/or hepatic impairment as well as drug–drug interaction (DDI) studies during drug development.

Several publications have discussed the importance of mass balance studies in the drug development and regulatory submissions.<sup>3–9</sup> Of note, on May 5, 2022, the US Food and Drug Administration (FDA) published a draft guidance for the industry titled “Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies.”<sup>10</sup> Additionally, the European Medicines Agency (EMA) has provided regulatory guidelines for conducting and interpreting the *in vivo* mass balance studies.<sup>11</sup> Further, the mass balance study was identified as one of the most frequent areas of regulatory concern during the EMA’s evaluation of marketing authorization applications for new chemical entities.<sup>4</sup> In fact, the paucity of information from these studies for some drugs restricted the benefit/risk assessment in all populations leading to precautions and restrictions included in the product labeling.<sup>4</sup>

Herein, we collected and analyzed information from new molecular entity (NME) new drug applications (NDAs) that were approved by the FDA over a period of 5 years (2014–2018). The goal was to evaluate the conduct and design of mass balance studies that were submitted as a part of the NDAs. The results from this regulatory research also informed the development of the FDA draft guidance on mass balance studies.<sup>10</sup> Here, we summarize

the current practices as well as outline some best practices for conducting radiolabeled mass balance studies.

## METHODOLOGY

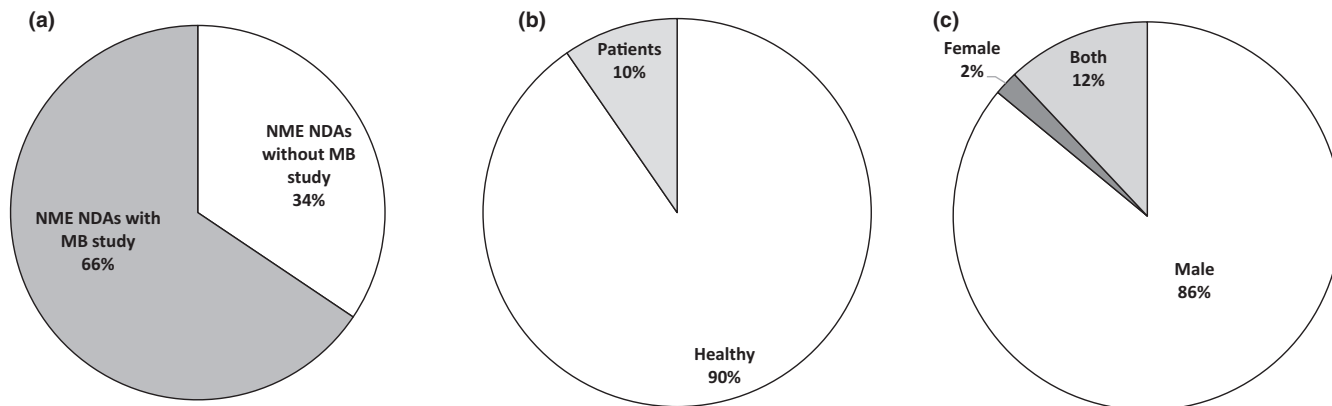
New molecular entity NDAs approved by the FDA between January 2014 and December 2018 are included in this analysis. Data were extracted from applicant (i.e., biopharma companies) submitted final clinical study reports as well as from the FDA clinical pharmacology reviews and original approved drug labeling obtained from Drugs@FDA,<sup>12</sup> to gain insights into current practice on the conduct of human mass balance studies. The assessment includes determination of whether a human mass balance study was conducted and what, if any, justification was provided for the lack of such studies. Specific information on study population (sex, number, and healthy subjects vs. patients), dosage (including single dose vs. multiple doses), administered radioactivity dose, drug (and relevant metabolite) half-life, percentage recovered in different matrices (e.g., urine and feces), route of administration of the approved drug and route of administration used in the mass balance study, and other study objectives like absolute bioavailability was collected.

## CONDUCT OF MASS BALANCE STUDIES

In this analysis, we evaluated if mass balance studies were leveraged to support the development and approval of NME NDAs between 2014 and 2018. Overall, 154 NME NDAs were approved during this period, including 15 drugs approved as combinations of two or more chemical entities. Of the 154 drugs, 101 had information from mass balance studies that were conducted as a part of the drug development program (Figure 1a).

For the 101 drugs, a total of 104 radiolabeled mass balance study reports were submitted as three combination drugs had multiple mass balance studies. Of note, the proportion of drug development programs that conducted mass balance studies was similar between drugs with (63%) and without (68%) orphan designation. When analyzing by therapeutic area, drugs approved for imaging or ophthalmology indications did not have mass balance studies, whereas all antiviral drugs and most oncology drugs (92%; the other 8% included a radiopharmaceutical and two drugs with postmarketing studies) had mass balance studies conducted as a part of the drug development programs.

Fifty-three drugs did not have mass balance studies as a part of their development program. When



**FIGURE 1** Overview of human radiolabeled mass balance studies performed to support the initial approval of new small molecule drugs approved between 2014 and 2018. (a) Human radiolabeled mass balance (MB) study supporting the new molecular entity new drug application (NME NDA;  $n = 154$ ). (b) Study population enrolled in the MB studies ( $n = 104$ ). (c) Sex composition based on the inclusion/exclusion criteria listed in the protocol of the MB study ( $n = 104$ )

evaluating the NDAs without a mass balance study, the following justifications were often provided: (1) not ethical to be conducted in humans (e.g., safety concerns), (2) the drug was a radiopharmaceutical or diagnostic agent, (3) the drug was an endogenous substance or analog (peptide, oligonucleotide, hormone, fatty acid, etc.), (4) the drug had negligible systemic exposure, (5) ADME information was available from literature or approved drug labeling, or (6) the drug was mainly excreted as an unchanged form (e.g.,  $\geq 90\%$ ) in urine from non-radiolabeled phase I studies. Additionally, for two drugs, postmarketing studies were established for conducting human mass balance study at the time of initial approval.

When human radiolabeled mass balance studies cannot be conducted (e.g., safety concerns), alternative approaches, such as *in vitro* assessments and animal radiolabeled mass balances studies, urine sample collections in clinical trials have been used to characterize the ADME of the investigational drug. However, extrapolation from animals to humans can be challenging due to the potential species differences in drug metabolism and excretion. Information from nonclinical and clinical studies are often integrated to elucidate the elimination pathways of the drug. For a few drugs included in this analysis, based on animal studies, it was concluded that a human mass balance study was not ethical due to safety concerns in humans. For example, a human mass balance study was not possible for cariprazine as an animal mass balance study indicated that there is a potential of the radioactivity of the drug to be accumulated in tissues such as the eyes for a prolonged period. In this case, both mass balance and metabolite profiling were conducted in patients with schizophrenia following multiple-dose administration of the non-radiolabeled drug.<sup>13</sup>

Mass balance studies are typically conducted sometime between phase I and phase III of the investigational drug development program.<sup>6</sup> The EMA recommends that the results of the mass balance studies should generally be available before starting phase III.<sup>11</sup> As the information from mass balance studies is leveraged to inform the overall drug development program, it is a good practice to conduct mass balance studies early in the drug development program, or at least prior to any large late-phase clinical trials such that there is sufficient information to justify or expand the eligibility criteria in these trials. Delaying the conduct of the mass balance study until later stages of drug development (i.e., after confirmatory trials are initiated or completed) can cause significant delays in the approval of the drugs if there are any residual uncertainties related to safety or can limit the usage of the drug in certain patient populations if there is residual uncertainty related to the drug's metabolism or excretion pathways. For example, one common issue stemming from inappropriate study design is inadequate understanding of the metabolite profile. Metabolite profiling is generally performed in plasma, urine, and feces samples to assess and identify potential significant metabolites. The ratio of plasma metabolite to the parent drug and/or total drug-related exposure can provide information on whether some metabolites and which should be considered for further nonclinical safety evaluation or DDI evaluation.<sup>1,2,14,15</sup> Generally, if a metabolite accounts for more than 10% of the total drug-related exposure in plasma, the metabolite should be structurally characterized.<sup>1</sup>

The FDA can issue refuse-to-file (RTF) letters or complete response letters (CRLs) for drugs that have significant clinical pharmacology deficiencies due to a lack of understanding of the pharmacokinetic characteristics of the drug. In some instances, this could be avoided if

appropriately designed mass balance studies were conducted to inform the subsequent drug development program. For example, inadequate characterization of a major active metabolite, as in the case of ozanimod, led to the RTF letter being issued, with subsequent approval of the drug after the stated deficiencies were addressed during the NDA resubmission.<sup>16,17</sup> Similarly, the initial application for the approval of deutetrabenazine received a CRL because of clinical pharmacology and non-clinical deficiencies.<sup>18</sup> It was concluded that the clinical pharmacology studies failed to determine all the major metabolites of deutetrabenazine. A subsequent successful resubmission of the NDA included re-analysis of the retained samples from the mass balance study for specific metabolites to provide definitive human plasma exposure data.

In other instances, when insufficient information of the pharmacokinetics of the drug was provided during the NDA submission, postmarketing studies were established for a mass balance study at the time of drug approval. This was because some information gleaned from the mass balance study conducted after the original approval of the drug can still be important to promote therapeutic optimization of the drug in certain patient populations. For example, mass balance studies were established as postmarketing studies for belinostat and rucaparib at the time of approval to obtain critical clinical pharmacology information of the drug that is absent in the original NDA.<sup>19,20</sup> In the case of belinostat, animal mass balance data was only available at the time of initial NDA submission, and a human mass balance study was ongoing. Clinical data were available for patients with mild renal or hepatic impairment. At the time of approval, due to insufficient data to evaluate the potential risk of toxicity in patients with moderate and severe organ dysfunction, a postmarketing requirement was established to identify the excretion route in humans. In the case of rucaparib, a human mass balance study was also ongoing at the time of initial NDA submission. Clinical data were available for patients with mild to moderate renal or hepatic impairment. In addition, *in vitro* studies identified several rucaparib metabolites, but there is a lack of information on the relative abundance of these metabolites *in vivo*. As such, a postmarketing commitment was established to submit the final report of an ongoing mass balance trial to determine elimination pathways of rucaparib and identify major metabolites of rucaparib in humans. These data would determine the need for additional studies to evaluate the pharmacokinetic and pharmacodynamic characteristics of any potential major metabolite that was disproportionately identified in humans.<sup>21</sup>

For an orally administered drug, it is difficult to ascertain the route of elimination without knowing the

absolute bioavailability of the drug, especially for drugs that are excreted extensively in feces as unchanged parent drugs. Of the 101 drugs with radiolabeled mass balance studies, 13% ( $n = 13$ , including seven oncology drugs) had determination of absolute bioavailability as one of the objectives. When oral formulation of the drug is being developed, it is possible to combine an absolute bioavailability study with a radiolabeled mass balance study in a two-part study design within a single protocol. All of these 13 drugs used the two-part study design in the mass balance studies. Specifically, part A is a radiolabeled mass balance study for the orally administered investigational drug; and part B determines the absolute bioavailability of the investigational drug administered as an oral non-radiolabeled dose and an intravenous radiolabeled microdose. The absolute bioavailability study can use a microdose without the need for an intravenous toxicology program if the existing oral toxicity studies provide adequate exposure margins.<sup>2</sup> If part A and part B are conducted sequentially, an adequate washout period is needed in the study.

## STUDY POPULATION

Most mass balance studies are conducted in healthy, male volunteers.<sup>4,22</sup> In this analysis, 90% ( $n = 94$ ) of the studies were conducted in healthy subjects (Figure 1b). About 10% ( $n = 10$ ) of the studies were conducted in the target patient population due to the mechanism of action and/or benefit–risk related considerations (i.e., anticipated undesired effects in healthy subjects). Of these 10 studies conducted in patients, nine were in patients with cancer and one was in patients with endocrine-related disorder (secondary hyperparathyroidism in adult patients with chronic kidney disease on hemodialysis).

When looking at the inclusion/exclusion criteria of the mass balance study protocol, 86% ( $n = 89$ ) stated that only male subjects were eligible to be enrolled, whereas 12% stated that both sexes were eligible to be enrolled and 2% stated that female subjects were eligible to be enrolled (Figure 1c). The male-only study design appears to be more prevalent, possibly because of the potential reproductive toxicity concerns in women of childbearing potential and the convenience of sample collection. Sex-specific indication and sex-specific toxicity profiles were some possible reasons for female-only studies. For example, in the case of olaparib, which was initially indicated for the treatment of ovarian cancer, the mass balance study enrolled female patients. In the case of venetoclax, only female healthy subjects were enrolled in the mass balance study because of testicular toxicity identified from preclinical studies. Of the 12% ( $n = 13$ ) of studies that stated that both sexes were eligible to be



enrolled, eight studies were conducted in patients for drugs indicated to treat cancer and one study was for a drug indicated for endocrine-related disorder (secondary hyperparathyroidism in adult patients with chronic kidney disease on hemodialysis).

Typically, formal sample size estimations are not performed in mass balance studies. Sample size of six subjects is commonly adopted in a mass balance study. This analysis confirmed that the mean, median, and mode values of the sample size from the 104 mass balance studies were all six. The range of sample size in these studies was from 3 to 16. Thirteen percent ( $n = 14$ ) of the studies reported results from  $<6$  evaluable subjects. While looking at drugs that enrolled more than eight subjects, most of these studies had multiple objectives, such as evaluation of the mass balance of a drug with different radiolabeling positions, different formulations, or determination of the absolute bioavailability. The sample size of the mass balance study is dependent on the objectives, the study design, and the anticipated pharmacokinetic variability. In general, it is recommended that a mass balance study includes at least six evaluable subjects who have completed the study procedures as required by the protocol, as having fewer evaluable subjects may limit the interpretability of the data, especially if pharmacokinetics of the drug are variable.

Some small molecule drugs (data not included in this analysis) are substantially metabolized by polymorphic drug metabolizing enzymes (e.g., CYP2D6, CYP2C19, UGT1A1, and NAT2). For these drugs, including subjects with different genotypes, may be of scientific value. The results from pharmacogenomic analysis can help interpret the potential pharmacokinetic variability either prospectively or retrospectively.

## RADIOLABELED AND ADMINISTERED RADIOACTIVITY DOSE

This analysis showed that of the 104 studies, 99% ( $n = 103$ ) radiolabeled mass balance studies used carbon-14 labeled drug substance and one used tritium-labeled drug substance.

The administered radioactivity dose in humans should comply to the Regulations (21 Code of Federal Regulations [CFR] 361.1) and guidelines (e.g., the International Commission on Radiological Protection [ICRP], Advisory Committee on Radiological Protection [ACRP]). The administered radioactivity dose is appropriately informed via dosimetry results from animal studies. This analysis shows that the administered radioactivity dose used in the 104 studies ranged from 200

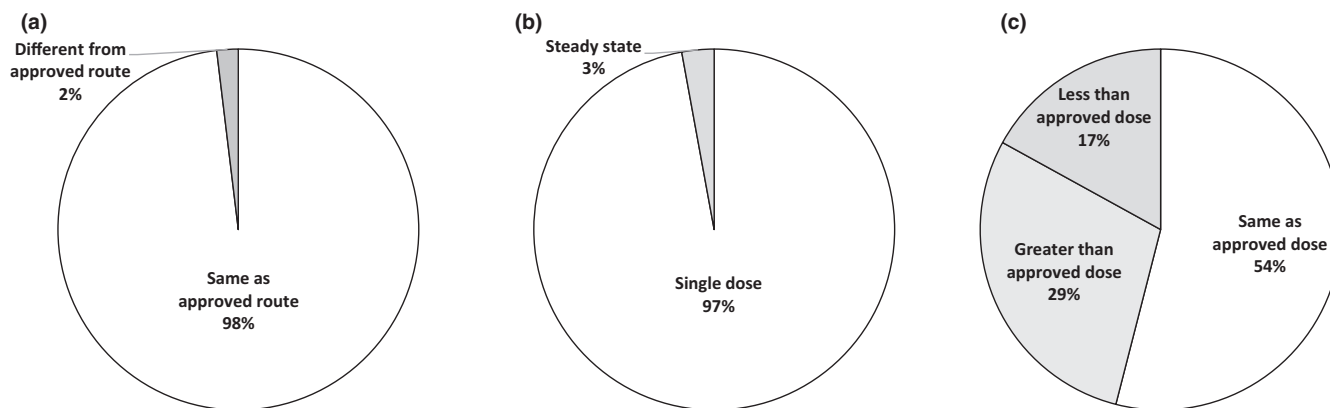
nCi to 500  $\mu$ Ci. Among them, 12% ( $n = 12$ ) performed a study using radioactivity dose ranging from 0.1 to  $<1$   $\mu$ Ci. Additionally, 27%, 45%, and 17% used a radioactivity dose range of 1  $\mu$ Ci to  $<100$   $\mu$ Ci, 100  $\mu$ Ci, and  $>100$   $\mu$ Ci to 500  $\mu$ Ci, respectively.

## INVESTIGATIONAL DRUG ROUTE, DOSE, AND FORMULATION

Mass balance studies are usually conducted using the same route(s) of administration, as the route that will be eventually approved in the NDA. When administration of the radiolabeled drug through the intended route of administration is not feasible, other routes of administration (e.g., oral or intravenous) can be utilized in the mass balance studies. Of the 104 studies, 98% ( $n = 102$ ) performed the radiolabeled mass balance using the same route of administration as the final approved route of administration (Figure 2a). In our analysis, because of practical considerations, 2% ( $n = 2$ ) of the drugs approved for pulmonary diseases had radiolabeled mass balance studies conducted using a different route of administration (oral and intravenous) in lieu of the approved route of administration (inhalation).

A single dose study is typically considered sufficient for a mass balance study, especially if there are no dose- or time-dependencies observed following the first-pass metabolism or elimination of the drug.<sup>11</sup> Indeed, many studies are done after single-dose administration.<sup>9,22</sup> Results from this analysis indicated that 97% ( $n = 101$ ) of the 104 mass balance studies were single-dose studies (Figure 2b). Three mass balance studies were performed at the steady-state. In such instances, the subjects received a single radiolabeled dose of the drug after reaching steady-state with the non-radiolabeled doses. Of the three drugs,<sup>23–25</sup> two exhibited nonlinear pharmacokinetics: letermovir had greater than dose proportional pharmacokinetics and eliglustat had nonlinear, greater than expected accumulation upon multiple-dose administration. Additionally, one study was conducted in patients with cancer (gilteritinib). Conducting mass balance studies at the steady-state may limit the interpretability of the results.<sup>5</sup> This approach only evaluates the elimination pathway of the radiolabeled drug, which may be altered after multiple doses as compared to a single dose. Bioanalysis of the non-radiolabeled moieties can help understand the accumulation of the parent drug and relevant metabolites at steady-state when interpreting the study results.

The choice of the investigational dose selected for use in the mass balance study may depend on several factors, including the safety and tolerability of the dose in healthy subjects, pharmacokinetic linearity range, expected



**FIGURE 2** Design characteristics of human radiolabeled mass balance studies performed to support the initial approval of new small molecule drugs approved between 2014 and 2018. (a) Route of administration utilized. (b) Mass balance study performed as a single dose study versus at the steady state. (c) Dose of the investigational drug used in the study

effective dose derived from early patient trials, and timing of the study. In our analysis, of the 100 studies (excluding two topical mass balance studies and two mass balance studies with a different route of administration compared to the approved route), 54% ( $n = 54$ ) of the studies selected a dose that was within the range of the final approved dose (assuming a body weight of 70 kg or body surface area of 1.8 m<sup>2</sup> if the dose needed to be adjusted accordingly), 17% ( $n = 17$ ) of the studies were conducted using a dose that was lower than the final approved dose, and 29% ( $n = 29$ ) of the studies were conducted using a dose that was higher than the final approved dose (Figure 2c). Overall, the majority (85%;  $n = 85$ ) of these studies used a dose within the pharmacokinetic linearity range, whereas 12 studies used a dose in the nonlinear range (slightly higher or lower than dose proportional), and three studies used a dose that was below the final approved dose and did not have data on pharmacokinetic linearity assessment. When the dose used in the mass balance study is not the final approved dose and the clearance of the drug is nonlinear, this may limit the interpretability of the mass balance study results. Therefore, it is encouraged that the dose of the investigational drug used in the mass balance study be the dose intended for use in large scale later-phase clinical trials and subsequent inclusion in the drug labeling.

With respect to the drug formulation used in the radiolabeled mass balance studies, typically, it is challenging to manufacture the proposed clinical formulations with radiolabeled drug substance on a small scale. Therefore, the investigational oral drug formulation used in the mass balance study usually tends to be a fit-for-purpose formulation (i.e., a mixture of labeled and non-labeled products in solution or suspension). Although there is a theoretical concern that formulation differences may change drug bioavailability/absorption, the formulation used in the mass balance study is not expected to significantly change

the elimination pathways of the drug, which is sufficient to meet the primary goal of the mass balance study.

## RECOVERY

Ideally, total recovery of radioactivity in urine and feces should exceed 90% of the administered radioactivity dose to allow for a reliable determination of the contribution of the elimination pathways. The EMA DDI guideline also recommends that preferably total recovery of radioactivity in urine and feces should exceed 90%.<sup>11</sup> Ideally, more than 80% of the administered radioactivity recovered in the excreta should be identified.<sup>11</sup>

Although the patient discharge criteria or sample collection timeline were not quantitatively evaluated in this analysis, many protocols ( $n = 47$ ) specified that urine and feces samples were to be collected until >90% of the radioactivity was recovered and ≤1% of the dose was excreted over a 24-h period on two consecutive sample collection days.

Of 102 mass balance studies (excluding two topical mass balance studies), 64 (63%) had reported ≥90% recovery, 16 (16%) had 85% to <90% recovery, 13 (13%) had 80% to <85% recovery, and nine (9%) had <80% recovery. This appears to be similar to the recovery reported for 22 oncology drugs with ≥90% recovery for 68% of drugs, 85% to <90% recovery for 14% of drugs, 80% to <85% recovery for 9% of drugs, and <80% recovery was reported for 9% of drugs.<sup>22</sup>

There can be a number of potential reasons for incomplete recovery observed in mass balance studies.<sup>8</sup> Some reasons include: (1) inappropriate selection of the radiolabel position on the drug, (2) incomplete sample collection especially for drugs with a long half-life, and (3) an inadequate or insensitive bioanalytical method used.

The position of the radioisotope has to be carefully selected to be chemically and metabolically stable, such that the radionuclide stays with the parent drug and major metabolites and can be readily detected and quantified.<sup>6</sup> Two separate positions on the drug molecule can be used for radiolabeling, if needed.

In our analysis, one notable reason for incomplete mass balance recovery is the long half-life of the parent drug or metabolite(s). Based on the information available in the drug labeling, 20 drugs (either parent drug or metabolite) identified from this analysis have half-lives >72 h: 12 have half-lives between 3 and 7 days, and eight have half-lives >7 days. The majority (80%;  $n = 16$ ) of these drugs have mass balance studies conducted as a part of the drug development program. The recovery or estimated recovery of administered radioactivity ranged from 64% to 95%, including >80% for 13 drugs. Estimation (interpolation and/or extrapolation) of recovery may be needed for drugs with a long half-life where an extended clinic stay is not feasible. However, the estimated recovery results have to be interpreted with caution because these estimations are typically based on the assumption that drug excretion was under a first-order process.

Conducting human mass balance studies for drugs with a long half-life may require some special study design considerations. For drugs with a long half-life, when an extended stay in the clinic becomes impractical to achieve >90% recovery, alternative sample collection strategies can be considered to get an estimate of the final recovery. This may include strategies such as partial collection of samples or collection of urine and feces in an outpatient setting. In addition, sensitive analytical tools, such as accelerator mass spectrometry (AMS), allow for low radioactivity dose (in the range of nCi) to be administered to humans, which alleviates the concern of extended exposure to radioactivity in humans for drugs with a long half-life. For example, the mass balance study was conducted for ixazomib (geometric mean half-life of 9.5 days) with [<sup>14</sup>C]-ixazomib administered at low radioactivity dose (~500 nCi) coupled with utilizing AMS to increase the sensitivity of radioactivity bioanalysis.<sup>26</sup> Given the long half-life, patients were discharged from the clinic on day 8 and returned to the clinic on days 14, 21, 28, and 35 for a 24-h overnight visit. Samples (urine and feces) were collected during each of these 24-h overnight clinic visits. Additionally, patients were instructed to collect feces samples at home for the 24-h period before each overnight clinic visit (days 13, 20, 27, and 34).

## SAMPLE ANALYSIS

Multiple bioanalytical methods are available to identify and determine parent drug and metabolite levels in

biological matrices. The choice of the method depends on the objective of the mass balance study, levels of parent drug and metabolites in the sample matrix, and the limit of detection of the bioanalytical tool. In our analysis, liquid scintillation counting is the most common analytical methods used to measure total radioactivity in biological matrices, whereas high-performance liquid chromatography with radio-detection and AMS (particularly for micro-tracer studies) are also used. Quantification of the parent drug and relevant metabolites with synthesized standards in all applicable biological matrices are performed using a sensitive analytical technique, such as liquid chromatography with tandem mass spectrometry. Given that reliable data from the mass balance study are needed to inform the subsequent development program of the investigational

**TABLE 1** Considerations for designing human radiolabeled mass balance studies

### Key study design considerations

- The study can be non-randomized and open-label
- The study can enroll healthy adult subjects, unless safety concerns exist
- Generally, the study includes at least six evaluable subjects. The final number of subjects to be enrolled depends on drug characteristics and objectives of the study
- Radioactivity dose to be administered can be based on animal studies dosimetry calculations and follow applicable guidelines (e.g., ICRP and ACRP)
- Ideally, the dose of the non-radiolabeled investigational drug used in the mass balance study is the dose intended for drug approval or at least, it is in the pharmacokinetic linearity range
- Typically, single dose studies may be sufficient. In some instances, multiple dose studies may be considered
- To ensure applicability of the findings, the routes of administration for the mass balance study generally include the final intended route(s) of administration (unless precluded by practical considerations)
- The position of the radioisotope is in a chemically and metabolically stable position
- For orally administered drugs, evaluating absolute bioavailability can help understand the overall drug elimination pathways
- Study duration and sample collection aims to maximize the total recovery of radioactivity in relevant biomatrices and support the identification of relevant moieties to ensure interpretability of the findings
- For drugs with a long half-life, alternative sample collection strategies can be considered to get an estimate of the final recovery
- Detection and quantification of radioactivity should be performed in all applicable biological matrices. For non-radiolabeled moieties, validated bioanalytical methods should be used for the matrices that are sampled

Abbreviations: ACRP, Advisory Committee on Radiological Protection; ICRP, International Commission on Radiological Protection.

drug, validated bioanalytical methods should be used for non-radiolabeled moieties, as applicable.<sup>27</sup>

## REPORTING OF THE STUDY RESULTS

The mass balance clinical study report typically include the following: (1) plasma and whole blood concentration versus time profiles of total radioactivity, (2) plasma concentration versus time profiles for the non-radiolabeled moieties including the parent drug, and, if possible, metabolites, (3) descriptive statistics of pharmacokinetic parameters for total radioactivity, the parent drug, and, if possible, metabolites in plasma (area under the concentration time curve [AUC], maximum concentration [ $C_{max}$ ], time to maximum concentration [ $T_{max}$ ], terminal half-life, etc.), (4) the cumulative percentage of the administered radioactive dose recovered in urine, feces, and total excreta (urine and feces combined) versus time profiles, and (5) quantitative information on the radioactivity associated with the parent drug and each identified metabolite in collected matrices (e.g., plasma, urine, and feces). A biotransformation scheme with the structures or descriptions of the metabolites are typically included in the NDA submission. The results from human mass balance studies are generally included in Subsection 12.3 Pharmacokinetics of the approved drug labeling.<sup>28</sup>

## CONCLUSION

In conclusion, a human mass balance study is the single most direct method to obtain quantitative and comprehensive information on the ADME of an investigational drug in the human body. Appropriate attention has to be given to deciding whether and when to conduct the study. Based on the collected experience, some best practice considerations for conducting mass balance studies are provided in Table 1. An adequately designed mass balance study is essential to leverage the mass balance information to appropriately inform the design and conduct of other clinical studies in the entire drug development program.

## AUTHOR CONTRIBUTIONS

Wrote manuscript: A.R., G.B., E.C.Y.C., H.D., N.H., D.J., S.P., Y.R., C.S., Y.Y., S.D., Z.Y.D. Designed research: A.R., G.B., E.C.Y.C., H.D., N.H., D.J., S.P., Y.R., C.S., Y.Y., S.D., Z.Y.D. Performed research: A.R., G.B., E.C.Y.C., H.D., N.H., D.J., S.P., Y.R., C.S., Y.Y., S.D., Z.Y.D. Analyzed data: A.R., G.B., E.C.Y.C., H.D., N.H., D.J., S.P., Y.R., C.S., Y.Y., S.D., Z.Y.D.

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The authors declared no competing interests for this work.

## DISCLAIMER

The article reflects the views of the authors and should not be construed to represent the US Food and Drug Administration's views or policies.

## REFERENCES

1. Food and Drug Administration. Guidance for Industry. Safety Testing of Drug Metabolites. 2016. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/safety-testing-drug-metabolites>. Accessed December 2021.
2. Food and Drug Administration. Guidance for Industry. M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. 2010. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m3r2-nonclinical-safety-studies-conduct-human-clinical-trials-and-marketing-authorization>. Accessed December 2021.
3. Beumer JH, Beijnen JH, Schellens JH. Mass balance studies, with a focus on anticancer drugs. *Clin Pharmacokinet*. 2006;45(1):33-58.
4. Coppola P, Andersson A, Cole S. The importance of the human mass balance study in regulatory submissions. *CPT Pharmacometrics Syst. Pharmacol*. 2019;8(11):792-804.
5. Nijenhuis CM, Schellens JH, Beijnen JH. Regulatory aspects of human radiolabeled mass balance studies in oncology: concise review. *Drug Metab. Rev*. 2016;48(2):266-280.
6. Penner N, Klunk LJ, Prakash C. Human radiolabeled mass balance studies: objectives, utilities and limitations. *Biopharm. Drug Dispos*. 2009;30(4):185-203.
7. Penner N, Xu L, Prakash C. Radiolabeled absorption, distribution, metabolism, and excretion studies in drug development: why, when, and how? *Chem. Res. Toxicol*. 2012;25(3):513-531.
8. Roffey SJ, Obach RS, Gedge JI, Smith DA. What is the objective of the mass balance study? A retrospective analysis of data in animal and human excretion studies employing radiolabeled drugs. *Drug Metab. Rev*. 2007;39(1):17-43.
9. Spracklin DK, Chen D, Bergman AJ, Callegari E, Obach RS. Mini-review: Comprehensive drug disposition knowledge generated in the modern human radiolabeled ADME study. *CPT Pharmacometrics Syst. Pharmacol*. 2020;9(8):428-434.
10. Zhou J, Li F. Potential pharmacokinetic interactions of therapeutic cytokines or cytokine modulators on small-molecule



- drugs: mechanistic understanding via studies using in vitro systems. *Drug Metabol. Drug Interact.* 2014;29(1):17-28.
- European Medicines Agency. Drug Interaction Guideline on the Investigation of Drug Interactions. 2013. [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions_en.pdf). Accessed December 2021.
  - Drugs@FDA. <https://www.accessdata.fda.gov/scripts/cder/daf/>. Accessed December 2021.
  - Food and Drug Administration. Clinical Pharmacology Review for NDA 204370 Cariprazine. [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2015/204370Orig1Orig2s000ClinPharmR.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/204370Orig1Orig2s000ClinPharmR.pdf). Accessed December 2021.
  - Food and Drug Administration. Guidance for Industry. In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions. 2020. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions>. Accessed December 2021.
  - Food and Drug Administration. Guidance for Industry. Clinical Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions. 2020. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions>. Accessed December 2021.
  - BioPharma Dive. More Bad News: Celgene Reveals Refuse-To-File Letter for Ozanimod in MS. 2018. <https://www.biopharmadive.com/news/celgene-ozanimod-metabolite-safety-test-approval/522268/>. Accessed December 2021.
  - Food and Drug Administration. Clinical Pharmacology Review for NDA 209899 Ozanimod. [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2020/209899Orig1s000ClinPharmR.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/209899Orig1s000ClinPharmR.pdf). Accessed December 2021.
  - Food and Drug Administration. Clinical Pharmacology Review for NDA 208082 Deutetrabenazine. [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2017/208082Orig1s000ClinPharmR.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/208082Orig1s000ClinPharmR.pdf). Accessed December 2021.
  - Food and Drug Administration. Approval Letter for NDA 206256 Belinostat. [https://www.accessdata.fda.gov/drugsatfda\\_docs/applletter/2014/206256Orig1s000ltr.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/applletter/2014/206256Orig1s000ltr.pdf). Accessed December 2021.
  - Food and Drug Administration. Approval Letter for NDA 209115 Rucaparib. [https://www.accessdata.fda.gov/drugsatfda\\_docs/applletter/2016/209115Orig1s000ltr.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/applletter/2016/209115Orig1s000ltr.pdf). Accessed December 2021.
  - Huang S-M, Temple R, Throckmorton DC, Lesko LJ. Drug interaction studies: study design, data analysis, and implications for dosing and labeling. *Clin. Pharmacol. Ther.* 2007;81(2):298-304.
  - Covance Publication, Kochan R, Leonard L, et al. Advantages and mass balance results dosing [14C]-oncology drugs to normal healthy volunteers: compiled data from 22 AME studies. <https://drugdevelopment.labcorp.com/content/dam/covance/assetLibrary/posters/kochandia13pdf.pdf>. Accessed December 2021.
  - Letermovir labeling. [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2017/209939Orig1s000,209940Orig1s000lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209939Orig1s000,209940Orig1s000lbl.pdf). Accessed July 2022.
  - Eliglustat labeling. [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2014/205494Orig1s000lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205494Orig1s000lbl.pdf). Accessed July 2022
  - Gilteritinib labeling. [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2018/211349s000lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/211349s000lbl.pdf). Accessed July 2022.
  - Gupta N, Zhang S, Pusalkar S, et al. A phase I study to assess the mass balance, excretion, and pharmacokinetics of [(14)C]-ixazomib, an oral proteasome inhibitor, in patients with advanced solid tumors. *Invest New Drugs.* 2018;36(3):407-415.
  - Food and Drug Administration. Guidance for Industry. Bioanalytical Method Validation. 2018. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry>. Accessed December 2021.
  - Food and Drug Administration. Guidance for Industry. Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products - Content and Format. 2016. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-pharmacology-labeling-human-prescription-drug-and-biological-products-content-and-format>. Accessed December 2021.

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