Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

July 2024
Clinical Pharmacology

Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies

Guidance for Industry

Additional copies are available from:
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353
Email: druginfo@fda.hhs.gov
https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

July 2024
Clinical Pharmacology

TABLE OF CONTENTS

Contains Nonbinding Recommendations

Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance describes the FDA's recommendations regarding clinical pharmacology considerations for conducting human radiolabeled mass balance studies of investigational drugs, including: (1) deciding whether and when to conduct the study, (2) designing the study, and (3) reporting results.[2] The recommendations in this guidance are, in part, based on the FDA's experience reviewing human radiolabeled mass balance studies submitted to the Agency in recent new drug applications (NDAs).[3] This guidance does not cover animal mass balance studies, safety testing of drug metabolites, or recommendations for selecting the radioactivity dose.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A human radiolabeled (most commonly ^{14}C or ^{3}H) mass balance study is the single most direct method to obtain quantitative and comprehensive information on the absorption, distribution,

[1] This guidance has been prepared by the Office of Clinical Pharmacology in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] 21 CFR 201.57.

[3] Ramamoorthy A, G Bende, ECY Chow, H Dimova, N Hartman, D Jean, S Pahwa, Y Ren, C Shukla, Y Yang, S Doddapaneni, and ZY Danielsen, 2022, Human Radiolabeled Mass Balance Studies Supporting the FDA Approval of New Drugs, Clin Trans Sci, 15(11):2567–2575.

metabolism, and excretion (ADME) of the drug in the human body. The mass balance study can provide information to:

- Determine the overall pathways of metabolism and excretion of an investigational drug
- Identify circulating metabolites
- Determine the abundance of metabolites relative to the parent drug or total drug-related exposure

The results from mass balance studies help to:

- Provide information on which metabolites should be structurally characterized and which metabolites should undergo nonclinical safety assessment or drug-drug interaction (DDI) evaluation[4,5]
- Assess whether renal or hepatic impairment studies or certain DDI studies are recommended for the investigational drug
- Assess the extent of absorption of the investigational drug

III. RECOMMENDATIONS FOR HUMAN RADIOLABELED MASS BALANCE STUDIES

In general, mass balance studies should be conducted for all new molecular entities, as information obtained from the mass balance study helps inform the subsequent drug development program. When a human radiolabeled mass balance study is not conducted, the sponsor should provide adequate justification. Unless clinical concerns suggest otherwise, a mass balance study might not be recommended in some circumstances, for example:

- Drugs for which mass balance study results can be obtained from acceptable literature sources or FDA-approved product labeling
- Drugs (e.g., monoclonal antibodies, oligonucleotide therapeutics, and endogenous substances such as peptides and hormones) with known metabolism and excretion pathways based on basic pharmacology and nonclinical ADME information, unless structural modifications are expected to change its ADME properties.

[4] See the FDA guidance *Safety Testing of Drug Metabolites* (March 2020). We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[5] See the FDA guidance *In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions* (January 2020).

- Drugs with the majority of the dose (i.e., greater than or equal to 90 percent) recovered in the urine as the unchanged parent drug with minimum metabolism.

- Drugs with no or negligible systemic exposures.

When a human radiolabeled mass balance study is not feasible (e.g., antibody drug conjugates, safety concerns because of the potential for radiolabeled moieties distributing extensively to critical organs), the sponsor should use alternative approaches, such as animal mass balance studies, non-radiolabeled clinical sample analysis via nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS), or in vitro assessments to characterize the ADME of the investigational drug.

Sponsors considering alternative approaches should consult with the appropriate FDA review division.

IV. TIMING OF MASS BALANCE STUDIES

Mass balance studies should generally be conducted early in drug development, at the latest before initiating any late-phase clinical trials. This timing allows information from the mass balance studies to be incorporated into the overall development program by:

- Providing information on metabolism and excretion pathways. This information, together with other in vitro and in vivo data, can inform the recommendation for and the design of DDI studies specific to the pathways involved in metabolism and excretion. For additional information on DDI studies, refer to the FDA guidances on drug interaction studies.

- Identifying metabolites for which nonclinical safety assessments should be performed.

- Guiding decisions for conducting renal and/or hepatic impairment studies. For additional information on organ impairment studies, refer to the FDA guidances on renal and hepatic impairment.[6,7]

- Avoiding unnecessary exclusions of patients with varying renal and/or hepatic function or informing dosing for such patients in the safety and efficacy clinical trials that support product approval.

V. CONSIDERATIONS FOR DESIGNING MASS BALANCE STUDIES

[6] See the FDA guidance *Pharmacokinetics in Patients with Impaired Renal Function — Study Design, Data Analysis, and Impact on Dosing* (March 2024) for more information.

[7] See the FDA guidance *Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling* (May 2003) for more information.

A. Study Design

Generally, mass balance studies are non-randomized and open-label.

B. Study Participants

Generally, mass balance studies can be conducted in healthy adult volunteers. If safety concerns preclude the enrollment of healthy volunteers, mass balance studies can be conducted in the patient population of interest.

In general, a mass balance study should include at least six evaluable volunteers who have completed the study procedures as detailed by the protocol.[8] Having fewer evaluable subjects may limit the interpretability of the study results. Anticipated or known variability in pharmacokinetics and any relevant polymorphisms in genes coding for drug metabolizing enzymes or transporters should be considered when determining the number of participants for enrollment.

C. Administered Radioactivity Dose and Radiolabel Position

The absorbed dose of radioactivity should be estimated via dosimetry calculations based on data from animal studies. Guidelines of other groups concerned with human safety (e.g., the International Commission on Radiological Protection (ICRP), Advisory Committee on Radiological Protection (ACRP)) should also be considered, as appropriate.

The position of the radioisotope should be chemically and metabolically stable such that the radionuclide is not lost during metabolism, and both the parent drug and metabolites can be detected and quantified. Two separate labeling positions can be used if needed.

D. Investigational Drug Dose

The dose of the non-radiolabeled investigational drug used in the mass balance study should be the final intended dose (if known), or at least in the anticipated therapeutic dose range (taking into account the safety profile of the drug in the study population). If the therapeutic range has not been identified at the time of conducting the mass balance study, the study should use a dose within the pharmacokinetic linearity range.

In general, a single-dose mass balance study is sufficient. A multiple-dose study can be considered in some scenarios; for example, if the investigational drug and/or active metabolite exhibits time-dependent pharmacokinetics, or when a single-dose study is not feasible as the study will be conducted in patients. For a drug that exhibits time-dependent pharmacokinetics, the subjects would receive a single radiolabeled dose of the drug after reaching steady state with non-radiolabeled doses. Because this approach only evaluates the clearance pathway of the

[8] Ramamoorthy A, G Bende, ECY Chow, H Dimova, N Hartman, D Jean, S Pahwa, Y Ren, C Shukla, Y Yang, S Doddapaneni, ZY Danielsen, 2022, Human Radiolabeled Mass Balance Studies Supporting the FDA Approval of New Drugs, Clin Trans Sci, 15(11):2567–2575.

radiolabeled drug, bioanalysis of the non-radiolabeled parent drug and metabolite(s) of interest at steady state can help interpret the results (see section J for more information on bioanalysis).

E. Route of Administration and Formulation of the Investigational Drug

The routes of administration for the mass balance study should include the final intended routes of administration, unless precluded by practical considerations (e.g., inhalation products). If alternative routes of administration are considered, the sponsor should contact the appropriate review division with justification.

The formulation used in the mass balance study contains both radiolabeled and non-radiolabeled drug materials, and this fit-for-purpose formulation is different from the final intended formulation. Although formulation differences may cause some changes in ADME parameters (e.g., absorption), the formulation used in the study should still permit the collection of sufficient information to fulfill the objectives of the mass balance study.

F. Determination of Absolute Bioavailability for Orally Administered Investigational Drugs in a Mass Balance Study

Information on the absolute bioavailability of the investigational drug can help interpret mass balance data and understand the overall drug elimination pathways.

When only the oral formulation is being developed, an absolute bioavailability study can be combined with the mass balance study in a single protocol in a two-part study. One part can be the human radiolabeled mass balance study for the orally administered investigational drug (mixture of non-radiolabeled and radiolabeled doses). The other part can determine the absolute bioavailability of the investigational drug administered as an oral non-radiolabeled dose (see section D for more information on dose) and an intravenous radiolabeled microdose (without the need for an intravenous toxicology program if the existing oral toxicity studies provide adequate exposure margins).[9]

G. Recovery

Preferably, the total recovery of radioactivity in the urine and feces should exceed 90 percent of the administered dose. The potential causes of low recovery or large variability should be provided.

H. Sample Collection and Handling

Plasma, urine, feces, and other matrices as applicable, should be collected for quantitative analysis of total radioactivity and parent drug, and for metabolite profiling.

[9] See the FDA guidance *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (January 2010).

Ideally, sample collection should continue until: (1) the cumulative radioactivity exceeds 90 percent of the administered dose in the urine and feces; and (2) the total radioactivity recovered in the urine and feces is less than 1 percent of the administered dose over a 24-hour period on 2 consecutive sample collection days.

For drugs with a long half-life (parent or metabolites), when an extended stay in the clinic becomes impractical to achieve greater than 90 percent recovery, alternative sample collection strategies should be considered.[10] However, the estimated recovery based on interpolation and/or extrapolation should be interpreted with caution because these estimations are typically based on the assumption that drug excretion is under a first-order process.

Plasma, urine, and feces samples should be properly stored and handled after sample collection and before analysis. The stability of the investigational drug and/or active metabolite in the corresponding matrices should be assessed to avoid misinterpretation of metabolite profiling results due to interference by degradation products.[11]

For quantitative analysis of total radioactivity, parent drug, and metabolites of interest (e.g., active metabolites) in plasma, urine, or feces, samples should be analyzed separately for each subject and pooling is not recommended.

Metabolite profiling is usually conducted after pooling samples in the matrix of interest (plasma, urine, or feces) across timepoints for each subject or across subjects for each timepoint or a combination of both, depending on the purpose of the analysis and practical considerations. Any pooling strategy should be described in detail in the study report.

If scientifically warranted, the sponsor should collect a pre-dose blood sample for prospective/retrospective pharmacogenetic analysis.

I. Parent Drug and Metabolites

In addition to the parent drug, metabolite profiling should be performed in plasma, urine, and feces samples.

The ratio of plasma metabolite to parent drug exposure can provide information on whether and which metabolites should be considered for further DDI evaluation.[12]

---

[10] Ramamoorthy A, G Bende, ECY Chow, H Dimova, N Hartman, D Jean, S Pahwa, Y Ren, C Shukla, Y Yang, S Doddapaneni, ZY Danielsen, 2022, Human Radiolabeled Mass Balance Studies Supporting the FDA Approval of New Drugs, Clin Trans Sci, 15(11):2567–2575.

[11] See the FDA guidance *M10 Bioanalytical Method Validation and Study Sample Analysis* (November 2022).

[12] See the FDA guidances *In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions* (January 2020) and *Clinical Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions* (January 2020).

The ratio of plasma metabolite to total drug-related exposure can provide information on whether and which metabolites should be considered for further nonclinical safety evaluation. Generally, if a metabolite accounts for more than 10 percent of the total drug-related exposure in plasma, the metabolite should be structurally characterized.[13]

Ideally, more than 80 percent of the radioactivity recovered in the excreta should be identified to assess the metabolic pathways of the parent drug. Adequate justification should be provided in instances when less than 80 percent of the recovered radioactivity is identified.

J. Bioanalysis

The choice of bioanalytical quantification techniques and any associated method validation depends on the objective of the mass balance study. Typically, both radiolabeled and non-radiolabeled analytical techniques are used.

- The detection and quantification of radioactivity should be performed in all applicable biological matrices using radioactivity counting techniques (e.g., liquid scintillation counting (LSC), accelerator mass spectrometry (AMS), high-performance liquid chromatography (HPLC) with radio-detection).

- The quantification of the unchanged parent drug and metabolites of interest should be performed in applicable biological matrices using a sensitive analytical technique such as liquid chromatography with tandem mass spectrometry (LC-MS/MS). Validated bioanalytical methods should be used for quantification of parent drug and metabolites of interest.[14]

VI. REPORTING OF HUMAN RADIOLABELED MASS BALANCE STUDY RESULTS

The study report(s) should include the following:

- Plasma and whole blood concentration versus time profiles of total radioactivity

- Plasma concentration versus time profiles for the parent drug and metabolites of interest (refer to section V.J. Bioanalysis)

- Descriptive statistics of pharmacokinetic parameters for total radioactivity, the parent drug, and metabolites of interest in plasma (e.g., the area under the concentration time

[13] See the FDA guidances *Safety Testing of Drug Metabolites* (March 2020) and *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (January 2010).

[14] See the FDA guidance *M10 Bioanalytical Method Validation and Study Sample Analysis* (November 2022).

curve (AUC), the maximum concentration (C_{max}), the time to maximum concentration (T_{max}), terminal half-life)

- The cumulative percentage of the administered radioactivity dose recovered in the urine, feces, and total excreta (urine and feces combined) versus time profiles

- Quantitative information on the radioactivity associated with the parent drug and each identified metabolite in collected matrices (e.g., plasma, urine, feces)

- A biotransformation scheme with the structures or descriptions of the metabolites, if applicable

Results from mass balance studies are generally included in Subsection 12.3 Pharmacokinetics of the approved prescribing information.[15,16]

[15] See the FDA guidance *Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products - Content and Format* (December 2016).

[16] 21 CFR 201.57.