Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies

Guidance for Industry

DRAFT GUIDANCE

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For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology Guidance and Policy Team at CDER_OCP_GPT@fda.hhs.gov.

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

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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] This guidance does not cover animal mass balance studies, safety testing of drug metabolites, or recommendations for selecting the radioactive dose.

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II. BACKGROUND

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

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

[2] 21 CFR 201.57.

- Determine the abundance of metabolites relative to the parent 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.[3,4]

- Assess whether renal or hepatic impairment studies or certain DDI studies are recommended for the investigational drug.

- Assess the absolute bioavailability (see section V.F. Determination of Absolute Bioavailability for Orally Administered Drugs in a Mass Balance Study) and the extent of absorption of the investigational drug with additional data from other studies documenting the investigational drug's stability in the gastrointestinal tract.

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.[5] 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 such as monoclonal antibodies, endogenous substances, and analogs (e.g., peptides, hormones, oligonucleotide therapeutics) with known metabolism and elimination pathways based on basic pharmacology and nonclinical ADME information.

[3] 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.

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

[5] For the purposes of this guidance, except where specifically indicated, references to drugs include drugs subject to approval under section 505(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 355(c)) and biological products subject to licensure under section 351(a) of the Public Health Service (PHS) Act (42 U.S.C. 262(a)) that are regulated as drugs.

- 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 cannot be conducted (e.g., safety concerns because of the potential for radiolabeled moieties accumulating in critical organs), the sponsor should use alternative approaches, such as animal mass balance studies, metabolic profiling using qualitative techniques, urine collection in phase 1 trials, or in vitro assessments to characterize the ADME of the investigational drug. Sponsors should consult with the appropriate FDA review division when alternative approaches are used.

IV. TIMING OF MASS BALANCE STUDIES

Mass balance studies should 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.[6]

- Identifying metabolites for which nonclinical safety assessments should be performed.[7]

- 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.[8]

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

[6] 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).

[7] 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).

[8] See the FDA guidance *Pharmacokinetics in Patients with Impaired Renal Function — Study Design, Data Analysis, and Impact on Dosing and Labeling* (September 2020). When final, this guidance will represent the Agency's current thinking on this topic. See also the FDA guidance *Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling* (March 2003).

V. CONSIDERATIONS FOR DESIGNING MASS BALANCE STUDIES

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

- In general, a mass balance study should include at least six evaluable subjects who have completed the study procedures as detailed by the protocol. 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 subjects 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.

- If the administered radioactivity dose is very low (less than 1,000 nCi), supporting data from dosimetry studies in animals might not be recommended.[9]

- 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, 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

[9] See the *Radioactive Drug Research Committee (RDRC) Program* web page at https://www.fda.gov/drugs/science-and-research-drugs/radioactive-drug-research-committee-rdrc-program.

metabolite exhibits time-dependent pharmacokinetics or when the study will be conducted in patients and a single dose study is not feasible. In such instances, 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 radiolabeled drug, bioanalysis of the non-radiolabeled moieties at steady state can help interpret the results (see section J for 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).

- 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 provide 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. For example, Part A can be the human radiolabeled mass balance study for the orally administered investigational drug. Part B can determine the absolute bioavailability of the investigational drug administered as an oral non-radiolabeled dose (see section D for dose) and an intravenous radiolabeled microdose (without the need for an intravenous toxicology program if the existing oral toxicity studies provide adequate exposure margins).[10] Part A and Part B of the study should be conducted with an adequate washout period. For drugs with long elimination half-lives, a parallel study design might be more practical.

G. Recovery

- Ideally, total recovery of radioactivity in urine and feces should be at least 90 percent. Adequate justification should be provided when recovery is less than 90 percent.

[10] See the FDA guidance *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (March 2013).

H. Sample Collection and Handling

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

- Ideally, sample collection should continue until: (1) the cumulative radioactivity exceeds 90 percent of the administered dose in urine and feces; and (2) the radioactivity 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 to get an estimate of the final recovery.

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

- For quantitative profiling in plasma, urine, and feces, samples should be analyzed separately for each subject and not pooled across subjects.

- Identification of metabolites is usually done after pooling of samples in the matrix of interest (plasma, urine, or feces) within each subject. In certain cases (e.g., low levels of metabolites), it may be helpful to pool samples across subjects.

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

I. Parent 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 exposure can provide information on whether and which metabolites should be considered for further DDI evaluation.[11]

- 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

[11] 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).

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.[12]

- Ideally, more than 80 percent of the radioactivity recovered in the excreta should be identified. Adequate justification should be provided in instances when less than 80 percent of the recovered radioactivity is identified.

J. Bioanalysis

- The choice of bioanalytical 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.

- For the bioanalysis of radioactivity, 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).

- For the bioanalysis of the non-radiolabeled moiety, quantification of the unchanged parent drug and metabolites should be performed in all 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 the matrices that are sampled.[13]

VI. REPORTING OF HUMAN RADIOLABELED MASS BALANCE STUDY RESULTS

- The mass balance clinical study report should include the following:

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

    - Plasma concentration versus time profiles for the non-radiolabeled moieties including the parent drug and, if possible, metabolites (refer to section V.J. Bioanalysis).

    - Descriptive statistics of pharmacokinetic parameters for total radioactivity, the parent drug, and if possible, metabolites in plasma (e.g., the area under the concentration time curve (AUC), the maximum concentration (C_{max}), the time to maximum concentration (T_{max}), terminal half-life).

[12] 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).

[13] See the FDA guidance *Bioanalytical Method Validation* (May 2018).

277 - The cumulative percentage of the administered radioactive dose recovered in
278 urine, feces, and total excreta (urine and feces combined) versus time profiles.
279
280 - Quantitative information on the radioactivity associated with the parent drug and
281 each identified metabolite in collected matrices (e.g., plasma, urine, feces).
282
283 - A biotransformation scheme with the structures or descriptions of the metabolites,
284 if available.
285
286 • Results from mass balance studies are generally included in Subsection 12.3
287 Pharmacokinetics of the approved prescribing information.[14,15]

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

[15] 21 CFR 201.57.