Human ADME and Studies with Radiolabeled Compounds: Phase I-IIa

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Executive Summary: Assessing Absolute Bioavailability and Human ADME

Radiolabeled medication is widely used in the assessment of human ADME and also increasingly in assessing absolute bioavailability (BA). Conducting an ADME study early in the clinical development program — generally before or in parallel with Phase Ila — is prudent since the outcome may generate the need for additional toxicology studies. An established approach used to define the absolute bioavailability of almost any drug without the need for an i.v. toxicology program is in designing the clinical study to administer an intravenous $^{14}$C-labeled microdose at the same time as an oral pharmacological dose. The PK of the intravenous $^{14}$C-microdose is then assessed by Accelerator Mass Spectrometry (AMS) and the PK of the oral dose is assessed by LC-MS/MS or other bioanalytical method.

During clinical drug development there can be several situations where the use of radiolabeled medication or other radiolabeled compounds is required or favorable to accomplish the study objectives. This whitepaper is mainly focusing on human ADME studies and “microdose” studies to assess absolute bioavailability (BA). The major part of the information in this overview is also relevant for other clinical studies with a radiolabeled compound.

Background

A human ADME study with radiolabeled medication (usually $^{14}$C-label) is generally conducted as part of the clinical development of a drug. The objectives of such a human ADME study are:

- To assess absorption and distribution.
- To elucidate the routes and rates of excretion (see Figures 1 and 2 for examples).
- To assess the mass balance: recovery of administered $^{14}$C-label from all collected urine and feces (and expired air, if applicable).
- To assess the metabolite profile and metabolite identification.

It is prudent to have this study relatively early in clinical development, since the outcome may generate the need for additional toxicology studies in the same or additional animal species. This is the case especially if the human metabolite profile appears to be significantly different from the metabolite profile in the species used thus far for the toxicology program, as addressed in applicable guidance documents (ICH M3 R2 and FDA’s Guidance for Industry: Safety Testing of Drug Metabolites). Such differences may occur in the form of human-specific metabolites or metabolites that are formed in much higher quantities in humans compared to animal species used in the toxicology program. The timing of the ADME study should therefore be as early as possible in the clinical development program, generally before, but ultimately in parallel with, Phase Ila (i.e., after the multiple dose Phase I study and before Phase Ilb).

Figure 1: Cumulative Excretion
Design of a Human ADME Study

The most common design for an ADME study is a single dose administration of the radiolabeled medication in 4 to 8 healthy male subjects. If needed based on the mode of action, ADME studies for oncology compounds can be conducted directly in (oncology) patients. Within PRA Early Development Services, 14C-ADME studies in oncology patients are conducted through our Patient Phase I facilities in Central and Eastern Europe. The route of administration should be the same as the intended route of administration for the product. If the intended route of administration is non-parenteral, it is sometimes compared with intravenous administration in a two-way crossover design or in two parallel groups. The administered dose should be in the therapeutic dose range.

Usually, the radioactive dose is in the range of 0.75 to 3.7 MBq (20 to 100 microCurie; see section on dosimetry below). Obviously, the in-clinic period for an ADME study is variable, and discharge depends on radioactivity levels in the excreta, which are measured on a daily basis using Liquid Scintillation Counting (LSC). Usually, the collection of urine and feces is terminated when less than 1% of the dose is excreted per 24 hours during two consecutive 24-hour intervals.

For compounds with a very long half-life, we have successfully applied an alternative approach. Thus, the subjects collect their excreta quantitatively over a fixed in-clinic period (e.g., approximately 2-3 half-lives). Thereafter, they can be discharged and return at weekly or two-weekly intervals for 24-hour or 48-hour in-clinic collections. From the excretion data, excretion rates in urine and feces versus time will be plotted; the cumulative excreted amounts are calculated from the area under the curve.

Additionally there are “microtracer” human ADME studies where the radioactive dose is < 0.1 MBq (= 2.5 microCurie), and where Accelerator Mass-Spectrometry (AMS) is used for bioanalysis. This has been applied for compounds with an extremely long elimination half-life, based on the expectation that the radiation burden for the subjects would become too high when using a conventional radioactivity dose.

Designing a Microdose Study to Assess Absolute Bioavailability

As shown in the graph below, in a microdose absolute BA study, a pharmacologically relevant, unlabeled (“cold”) oral dose is combined with an intravenous radiolabeled microdose. The intravenous microdose (e.g., 100 microgram) is usually given as a short infusion (e.g., 15 min) that is started shortly after the oral dose (preferably around Tmax). The number of subjects may range from 4 to 8.

The radioactive dose is less than 0.1 MBq (“tracer label”) with AMS being used to assess the pharmacokinetics of the intravenous microdose. The PK of the oral dose is assessed by LC-MS/MS or another “cold” bioanalytical method. The absolute bioavailability is derived from the dose-corrected ratio of AUC oral/AUC i.v. The graph below shows the mean results from an absolute BA microdose assessment for an investigational drug which showed approximately 100% bioavailability (note the long half-life). In this study, the intravenous microdose was given as a 15-min infusion starting at 4 hours after the oral dose.

An absolute BA microdose study and human ADME study can be efficiently combined in one study protocol, using the same subjects and the same batch of 14C-labeled active compound. However, an absolute BA microdose study can also be conducted as a stand-alone protocol or as part of any Phase I study protocol.
Composition of Radiolabeled Medication

The $^{14}$C-label should be incorporated in the drug molecule at a metabolically site, so that it will be found in most metabolic degradation products of the drug.

Generally the dose consists of labeled drug substance corresponding with the required radioactive dose and supplementary cold drug substance to achieve the proper total dose. PRA can assist with outsourcing radiosynthesis activities to qualified vendors; but we can also accept material sourced by our clients.

Regulatory Aspects

Calculation of radiation burden (dosimetry)

Usually, the radioactive dose for a human ADME study is in the range of 0.75 to 3.7 MBq (20 to 100 microCurie), but will be chosen such that the radiation burden for healthy volunteers remains in principle below 1 mSv (category IIa according to ICRP Publication 62). The radiation burden for the volunteers should be estimated on the basis of non-clinical and clinical pharmacokinetic data. Usually, these should include at least one mass balance study and an organ distribution study with (Q)WBA in pigmented rats.

Calculation of the expected radiation burden will be done by one of our radiation experts, and the dosimetry calculation is reviewed by the IEC as part of the standard CTA review process.

No dosimetry calculations are required for absolute BA microdose studies and other “microtracer” studies where the radioactive dose is < 0.1 MBq ($\approx$ 2.5 microCurie).

Toxicity Data to Support an Intravenous Microdose

It is important to note that no intravenous toxicity data will be required to support an intravenous microdose, assuming that there is a normal toxicity program to support oral dosing in humans and human safety/tolerability data from at least a single ascending dose study.

Regulatory Approval

In The Netherlands, no national regulatory authority on radiation safety is involved in the review process. As with any other Phase I study, the CTA is submitted in parallel to the Independent Ethics Committee (IEC) and Competent Authority (CA). Both CA approval and IEC approval can effectively be obtained by 2-3 weeks after CTA submission.

ADME studies in oncology patients are performed in the Patient Pharmacology Service Unit in Budapest. The European Directive for Phase I clinical trials is followed here, and approval from EC and CA is expected within 60 days (in parallel).
Summary

For many drugs, a human ADME study is part of the development program. For drugs with an extremely long half-life, the conventional approach with 20-100 microCurie (0.75-3.7 MBq) radioactivity doses and analysis by LSC can cause unacceptable radiation exposure, and microtracer labeling combined with AMS for analysis can be preferable. Cumulative excretion can be derived from the area under the curve of excretion rate versus time.

For almost any drug, absolute bioavailability can be assessed by simultaneous administration of an oral cold dose and an i.v. microdose with tracer labeling. AMS is then used for assessment of the i.v. pharmacokinetics. Thus, an assessment of absolute bioavailability and a conventional human ADME study can be efficiently combined in one study protocol.

About PRA Health Sciences

PRA’s Early Development Services group provides comprehensive services for Phase I and Phase IIa clinical research, bioanalytical research and data support. Our facilities in the US and The Netherlands include over 440 beds, bioanalytical laboratories and a GMP-licensed pharmacy.

At PRA, human studies with radiolabeled medication are a regular part of our Phase I study program. We have an extensive track record for conduct of Phase I isotope studies according to specific requirements, including:

- Use and maintenance of dedicated and licensed isotope facilities in our Clinical Research Units (CRU) and laboratory:
  - Obligatory cleaning, swab testing and release of isotope unit in CRU after completion of in-house part of clinical study
  - Separate ventilation procedures and maintenance program for isotope units
- Deployment of specialized and dedicated PRA employees:
  - Licensed Radiation Safety Officers (continuous obligatory training)
  - Experienced Isotope Project Teams

- Compliance to Law on Nuclear Energy (Kernenergiewet):
  - Monitoring and renewal of licenses
  - Authority notification process for import of radiolabeled drug substance(s)
  - Separate sample storage (limited capacity) in dedicated isotope facility
  - Radioactivity calculation and check for radioactivity limit compliance on all outgoing (external) shipments by Laboratory Radiation Safety Officer
- Separate destruction of all contaminated (radioactive) waste
  - Materials from isotope unit in CRU
  - Materials from isotope laboratory facility
  - Remaining drug product (including Statement of Destruction)

Manufacturing of Radiolabeled Medication

We offer you manufacturing of infusion solutions and oral formulations in our GMP-licensed state-of-the-art pharmacy with QP release by our pharmacists.

Intravenous solution for injection or infusion are manufactured in a dedicated clean room class B, while oral formulations will be manufactured in a dedicated clean room class D. The oral formulations include:

- Filling capsules • solutions • suspensions
- Coating tablets with a 14C labeled liquid

All formulations will be QP released. As a release test, PRA can offer the following:

- Total radioactivity
- Radiochemical purity
- Assay (conc. of the compound)
- Related substances
- Identity
- pH /color/clearness/osmolality
- Pyrogen

The radiolabeled compound is considered an excipient for the IMP (CoA and limited other data required). PRA can assist you in writing the IMPD, especially the Drug Product sections.
Clinical Conduct

The healthy volunteer clinical unit in The Netherlands is fully equipped for clinical conduct of radio-isotope studies. Subjects participating in these studies are dosed with the isotope-labeled drug product in a separate, licensed isotope unit, in which sample collection (blood, urine, feces, exhaled air [if required]) is also performed.

The oncology patient unit in Hungary is fully equipped for the clinical conduct of radioisotope studies, and no additional radiation license is required as long as total radioactivity at the site is kept below 10 MBq. Established transport of the IMP from the GMP pharmacy in The Netherlands allows dosing up to 72 hours after manufacturing.

Facility:

• Isotope CRU unit with restricted access
• Fully licensed in accordance with the Dutch Law on Nuclear Energy (Kernenergiewet)
• Located in conjunction to the GMP pharmacy to allow delivery of drug product immediately after manufacturing

Bioanalytical Isotope Laboratory Support

In our bioanalytical laboratory we can give full support for sample processing (including feces homogenization); analysis of concentrations of total radioactivity in plasma; whole blood, red blood cells, urine, feces, exhaled air and saliva samples by ultrasensitive liquid scintillation counting and isotope sample storage. Apart from analysis of radioactivity, we can also offer the "cold" bioanalysis of parent compound and relevant metabolites. In addition, we have a specialized team which is dedicated to metabolite profiling and identification.

For AMS analysis of samples from microdose studies and ADME studies with tracer labeling, we collaborate with industry leaders in AMS analysis in the Netherlands and the US.

Technical information of the most common matrices:

<table>
<thead>
<tr>
<th>Matrix</th>
<th>LLOQ</th>
<th>ULOQ</th>
<th>Vol. Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>30.0</td>
<td>80000</td>
<td>dpm/ml</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>80000</td>
<td>dpm/ml</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>80000</td>
<td>dpm/ml</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>50.0</td>
<td>80000</td>
<td>dpm/ml</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>80000</td>
<td>dpm/ml</td>
</tr>
<tr>
<td>Urine</td>
<td>10.0</td>
<td>100000</td>
<td>dpm/ml</td>
</tr>
<tr>
<td>Feces</td>
<td>40.0</td>
<td>100000</td>
<td>dpm/g</td>
</tr>
<tr>
<td>Exhaled Air</td>
<td>20.0</td>
<td>10000</td>
<td>dpm/2 mmol CO2</td>
</tr>
</tbody>
</table>

* as feces homogenate sample; homogenized with one to two equivalents of water

Figure 5: Most Common Matrices
Facility:

- Isotope laboratory (C-lab) with restricted access
- Separate sample storage (limited capacity) in freezer with temperature registration
- Radioactivity calculation and check for radioactivity limit compliance on all outgoing shipments by Radiation Safety Officer

Equipment:

- Perkin Elmer Sample Oxidizer, Model 307 (for combustion of biological samples)
- 2 Liquid Scintillation Analyzers TriCarb 3100 TR with Low Level Count Mode (LLCM)
- HPLC system equipped with absorbance and radio flow detector (Berthold LB 509)

At PRA, conducting human ADME studies and other studies with radiolabeled medication is executed with the same focus and under the same regulatory requirements as any other Phase I or Phase IIa trial: the same clinical trial regulations apply, there is no separate government body or national authority involved for the radiation safety, and time from protocol submission to approval can be under two weeks. Having over 25 years of experience and close to 50 studies performed over the last 5 years, we know how to do the job and, even better, we will do it for you in the shortest possible timeframe.

Conducting these types of study at PRA means short timelines, dedicated and experienced staff, and having the radiation burden and your 14C-labeled drug product prepared on site.
Contact Information

For further information, or to discuss any aspect of PRA’s services offered in the field of human ADME studies, please contact your Business Development Manager, or the employee listed below:

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