The Changing Landscape for Human Absorption, Metabolism, and Excretion: Practical Experiences From a Data Analysis of 500 Studies

John S. Kendrick[1,*], Claudine Oelke[2], Catherine Laing[3], Lee Crossman[1], Ruth Stow[1] and Colin Webber[4]

Coincidental with the intensified regulatory and industry focus on the design and conduct of human absorption, metabolism, and excretion (hAME) studies in the past 12 months, we have recently completed our 500th cohort involving radiolabeled test item administration to humans. Here, we build upon a recent industry white paper in this journal[1] and share some of our own experiences as a Contract Research Organization based upon collaborations with numerous pharma companies and their differing approaches to design and timing, to add further context to the discussion regarding hAME studies and the pivotal role that drug metabolism and pharmacokinetics plays. In this article, we explore how both changing relationships within the industry and shifting regulatory guidelines are impacting strategies, and compare EU and US pre-study approval requirements, before evaluating the trends from over 500 studies conducted at our global facilities conducted over more than 30 years. We conclude with a review of how improved technical capabilities and strategies are influencing the design and conduct of hAME studies, before speculating on some of the driving factors which may shape the direction they take in the future.

Drug metabolism and pharmacokinetics (DMPKs) is the pivotal discipline in drug development[2] that aids valuable go/no-go decision making from early discovery to lead optimization through to regulatory approval (and beyond). **Figure 1** illustrates the touch points where *in vitro*, *in vivo*, formulation development, and more recently *in silico* modeling are typically applied in the drug development life cycle, culminating in the radiolabeled nonclinical and human absorption, metabolism, and excretion (hAME) studies which typically occur between phase II and phase III of clinical development.

As a Contract Research Organization (CRO), we are uniquely positioned to observe and advise on differing strategies that pharma companies take to obtain their hAME data. Timing of the conduct of the hAME study can significantly differ, with many larger pharma companies opting for the "as late as possible" approach, based upon regulatory guidance; compared with conducting hAME as part of a single ascending dose (SAD) study, early in phase I. Both smaller and larger companies tend to utilize CRO partners for the hAME study given the expertise and experiences that have been established in CROs, that run numerous hAME projects annually. Different companies also have differing approaches to the amount of radioactivity applied during an hAME study. The vast majority still consider a macro-tracer dose of radioactivity (typically ca 100 µCi) as the optimal approach, but ca 10% of companies we work with opt for a micro-tracer (< 1 µCi) use. Then, there are others that utilize both a micro- and a macro-tracer 2 phase study design, incorporating assessment of absolute bioavailability and systemic clearance using an i.v. micro-tracer, followed by an appropriate macro-tracer dose of radioactivity in the second study phase to confirm mass balance and aid metabolite identification and quantification. The pros and cons of these varying approaches are described in this paper.

CHANGING RELATIONSHIPS AND INTERDEPENDENCIES

The drug development landscape has certainly changed over the past 10 years. Scientific knowledge and data have provided insight into the mechanism of action of compounds, and biology has continued to expand exponentially as genomics and metabolomics have driven decision making. As pharma companies have focused effort on the key biology of the therapeutic targets, the conduct of DMPK studies has been considered more of a routine element, and as such has continued the outsourcing flow of such studies to CROs.

The developing and continuing trust and reliance in DMPK expertise to help inform decision making and help de-risk drug development has continued with inclusion, participation, and leadership by CRO members in scientific workshops, discussion groups, and influence in both regulatory guidance and industry leading welfare groups. Furthermore, with the flow and consolidation of DMPK expertise within CROs, there is now an increasing onus upon them to hire, train, and nurture the next generation of DMPK scientists. This expertise encompasses radiolabeled nonclinical and clinical hAME studies. Synthesis and utilization of radiolabeled molecules is expensive, not only in production of a molecule, but the

[1]Labcorp Early Development Laboratories Inc., Harrogate, UK; [2]Fortrea Clinical Research Unit Inc., Madison, Wisconsin, USA; [3]Fortrea Clinical Research Unit Inc., Leeds, UK; [4]Labcorp Early Development Laboratories Inc., Huntingdon, UK. *Correspondence: John S. Kendrick (john.kendrick@labcorp.com)

Received June 8, 2023; accepted August 21, 2023. doi:10.1002/cpt.3040

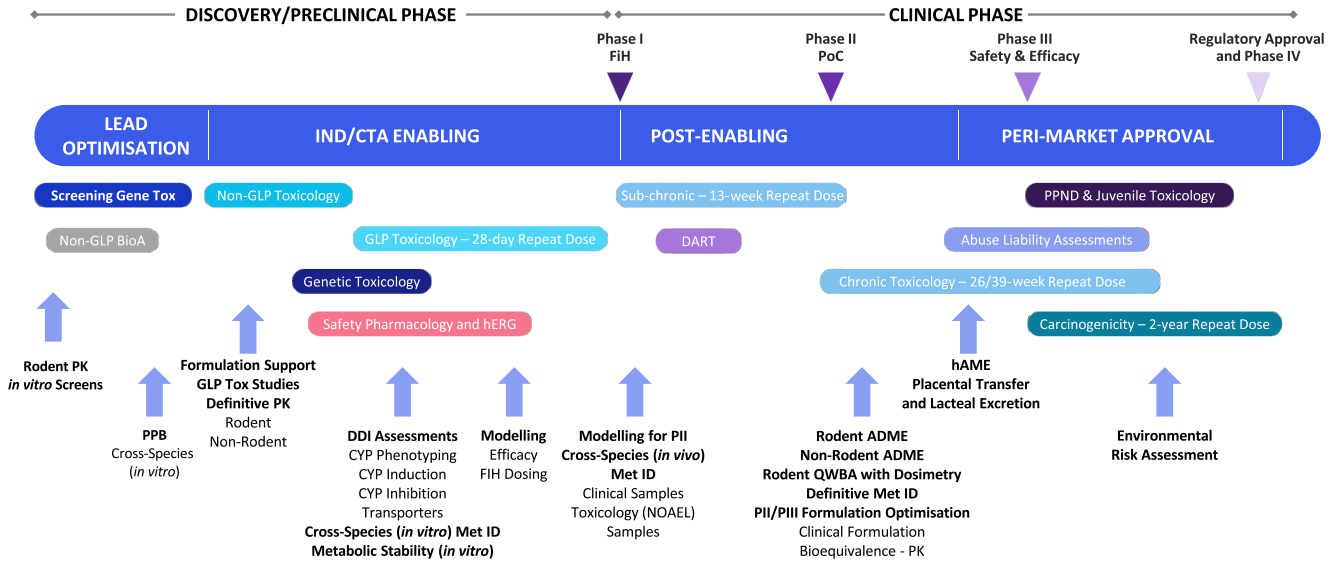

Figure 1 The continued importance of DMPK inputs during the drug development cycle. ADME, absorption, distribution, metabolism, and excretion; CTA, Clinical Trial Authorization; DDI, drug-drug interaction; DMPK, drug metabolism and pharmacokinetics; FIH, first-in-human; GLP, good laboratory practice; hAME, human absorption, metabolism, and excretion; IND, investigational new drug; NOAEL, no observed adverse effect level; PK, pharmacokinetic; PoC, proof of concept; PPB, Plasma Protein binding; PPND, Pre and post-natal development.

regulatory environment for receipt, transport, and storage through to the analysis of samples generated from radiolabeled studies, and eventually the downstream disposal of radioactivity. Therefore, it is unsurprising that CROs offering these services across a multitude of clients, can do so cost-effectively, compared with the pharma companies that operate alone on a project-to-project basis. With this service offering comes the significant value that CROs offer outside of the conduct and analysis of the studies. Experience in the pitfalls and challenges of how radiolabeled studies are designed and conducted, from the specific activity of the drug administered to the limits of detection for metabolite quantification, become hugely important to the success of a project, and this experience and knowledge is becoming increasingly concentrated within a few select CROs.

It was evident throughout the last 2 decades that the number of laboratories conducting radiolabeled studies declined. This was in part due to consolidation of the CRO industry, but also as a consequence of recognition by pharma companies that to conduct preclinical requirements of hAME studies (i.e., quantitative whole-body autoradiography (QWBA) studies) in house, required a large amount of outlay on cryo-microtomes, imaging equipment, and software, as well as specialized expertise to evaluate the data from these distribution studies. This is certainly one area that is almost now exclusively conducted within CROs, where industry relies upon partners, such as Labcorp, to not only conduct these studies on their behalf, but also to provide training and education to their study monitors on the pitfalls and challenges of undertaking these studies.

A second example is in the arena of accelerator mass spectrometry (AMS). Since the application of AMS toward drug development by companies, such as CBAMS and Vitalia in the early 2000's, industry, regulators, and larger CROs have all shown an interest in the technology, and how its use could either accelerate drug development or bridge the preclinical to clinical translational gap earlier. Phase 0 clinical trials involving a micro-tracer amount of radiolabeled drug together with AMS detection has ebbed and flowed in popularity. Most commonly i.v. micro-tracer administration enables a determination of the absolute bioavailability of an oral therapeutic dose in the clinic and is the study approach involving AMS we see most at Labcorp. As with QWBA, the expense and expertise to conduct AMS studies in-house within large pharma companies has declined or been shelved, with the CROs taking on this mantle and opportunity to provide these as out-sourced activities. Thereby the reliance on CROs has grown, and partnerships between CROs and industry pharma companies established to conduct hAME with AMS as an end point.

The evolving regulatory landscape

All global agencies involved in drug development have moved toward a greater understanding of the metabolic disposition of a drug before large-scale patient studies, and this continues to be driven earlier in development than ever before. This has focused understandably on the scientific and safety considerations of unique or disproportionate metabolites, and the rates and routes of elimination of the drug and its metabolites in humans. However, extensive understanding of the metabolism of a drug and potential for drug–drug interaction (DDI) studies is now required prior to any first-in-human (FIH) study conducted, in agreement with the US Food and Drug Administration (FDA) guidance.

The regulatory bodies and the frequency of the issuance of the regulatory updates from these agencies has increased particularly over the past 5 years (**Figure 2**), and little by little the major agencies across the globe (the FDA, International Council for Harmonization (ICH), and the European Medicines Agency (EMA)) have become more closely aligned in terms of the prerequisites they are asking pharmaceutical companies to consider as

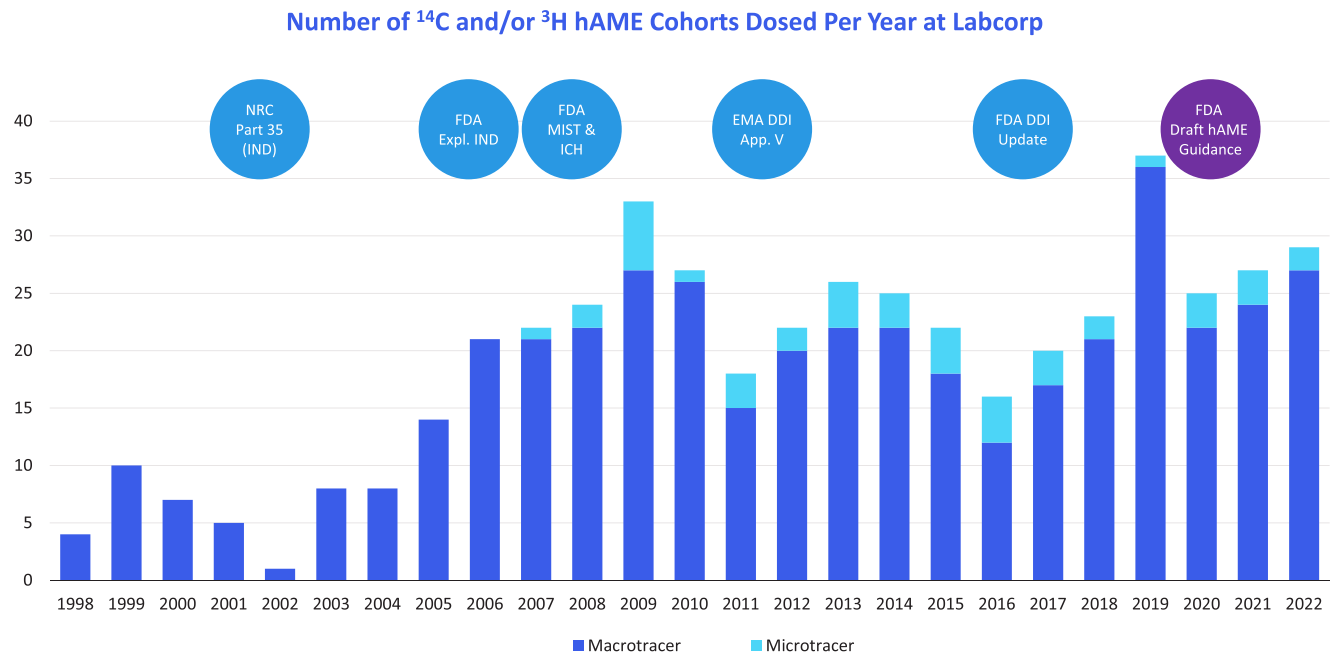

Figure 2 The number of macro- and micro-tracer hAME studies conducted at Labcorp on an annual basis overlaid with regulatory guidance release updates. DDI, drug-drug interaction; EMA, European Medicines Agency; FDA, US Food and Drug Administration; hAME, human absorption, metabolism, and excretion; ICH, International Council for Harmonization; IND, investigational new drug; MIST, Metabolites in Safety Testing; NRC, Nuclear Regulatory Commission.

development of a new chemical entity progresses into clinical trials. Through adoption and alignment of these guidance documents within Labcorp, we now typically conduct 20 or more hAME studies annually in our clinical research units (CRUs), supported by analysis within our co-located DMPK laboratories.

Pre-2008, an hAME study would simply be conducted to fulfill the requirements of a regulatory new drug application (NDA) at the FDA. The purpose was primarily seen as confirming the rates and routes of elimination of a drug and its metabolites (i.e., gaining a mass balance). This exercise was largely conducted very late in the development of a compound (during phase III typically) with the onus on parent drug safety the primary consideration. Then, the FDA Metabolites in Safety Testing (MIST) guidance was issued[3] and this had a profound impact on the design, planning, and conduct of a clinical hAME study, but importantly on the safety associated with metabolites in humans. The emphasis was on the systemic exposure of these metabolites, which may be either unique to humans, disproportionately present compared with the metabolites in preclinical species, or potentially pharmacologically active or reactive.[4] A cutoff for further investigations, and those that were of regulatory safety concern was included for the first time (concerning metabolites that were present at > 10% of circulating parent concentrations), and a timeframe indicated that these data should be available "as early as possible."

Further definition of the timing that metabolite data should be available from an hAME study was shared in the ICH M3(R2) guidance in 2009[5] where a need to understand as completely as possible the metabolism of a drug was highlighted, with pharmacokinetic (PK) data relevant to potential drug interactions required before phase III, being noted. As with the FDA MIST guidance, the need to compare human and animal metabolites and determine if additional testing was warranted was highlighted.

It was only in 2012, with publication of the EMA Drug–Drug Interaction Guidance[6] that several of the standard aims of an hAME study we recognize today was described – "Total recovery of radioactivity in urine and feces should exceed 90% of dose in a human mass balance study, where > 80% of the recovered radioactivity is then identified", (i.e., the high-level characterization of clinically relevant metabolites was prescribed).

Further updates to many of the guidances followed, but it is only recently that the FDA finally released draft guidance on the clinical pharmacology considerations for human radiolabeled mass balance studies.[7] This draft guidance summarizes the objectives of the hAME study in terms of the overall pathways of metabolism and excretion of an investigational drug, and the identification of those circulating human metabolites. The guidance further defines study design, conduct, participant enrolment, radioactive dose, as well as sample collection, bioanalysis, and reporting of data. All these aspects have been defined over many years and through experiences of our phase I Clinical Pharmacology Research Units in collaboration with our DMPK laboratories at Labcorp, and we have shared these in more detail throughout this paper.

The Clinical Trials Directive. It has not only been the regulatory authorities that have driven the evolution of the conduct of hAME studies. In 2001, the European Union issued Directive 2001/20/EC, commonly known as the Clinical Trials Directive,[8] governing the approval of clinical trials involving medicinal products in the European Union. It introduced the norm that investigational drugs should be manufactured to Good Manufacturing Practice

(GMP), and this included those radiolabeled molecules used in hAME trials. Although the GMP manufacture of investigational drugs is generally conducted, where a micro-tracer study is used (i.e., with < 1 µCi of radioactivity) or where the radiolabel represents a minor proportion of the total drug-related chemical dose (i.e., < 10%), GMP of the radiolabel is not always required by qualified persons releasing the dose for human administration, or by the radiosynthesis providers generating the material. This of course remains a contentious point, with many CROs, including Labcorp, preferring a robust and cautionary approach of requesting the availability of GMP material for all hAME studies.

Examples of the differences in front-end approval for hAME studies from two global sites

The regulations preceding the approval to conduct hAME studies vary around the world. **Figure 3** shows examples of the approval requirements from both the United Kingdom and the United States[9].

In the United Kingdom, as with all clinical trials, Clinical Trial Authorization (CTA) submission needs to be made to the Medicine and Healthcare Products Regulatory Agency (MHRA) for a clinical trial of an investigational medicinal product. The main difference for submission for an hAME study compared with other clinical studies is that an Investigational Medicinal Product Dossier for the radiolabeled material needs to be submitted, including the necessary supporting data for radiolabeled Active Pharmaceutical Ingredient (API) and non-radiolabeled API.

The MHRA and Ethics Committee (EC) submission and review process is conducted in parallel via the Integrated Research Application System (IRAS) system. This can take ~4–6 weeks from submission, depending on whether comments are raised by the MHRA/EC after their initial review. The Administration of Radioactive Substances Advisory Committee (ARSAC) submission is completed at the same time as the MHRA and EC submission via a separate online portal. The role of ARSAC is to check that the radiation exposure of trial subjects is within acceptable limits. In addition to the documentation that the EC receive, the ARSAC review also includes a form containing the following:

> An overview of the research; the purpose of the research; details of the radioactive material (extracted from the dosimetry report); a radiation risk assessment.

The ARSAC review can take up to 8 weeks, depending on queries raised, but approval is only required for dosing, therefore screening can be initiated ahead of ARSAC approval, taking this off the critical path for the hAME study. Recruitment of participants can take place in parallel to all three submissions (the CTA, EC, and ARSAC submissions) so that screening can commence once the MHRA and EC approvals are received.

In the United States, the Radiation Safety Committee (RSC) at the Labcorp CRU will review the proposed radioactive dose for each study to confirm that it conforms to FDA regulations and the RSC Chair will provide an official memo of the approval of the radiation dose to the institutional review board (IRB) with the study submission. After IRB review, which can take up to 1 week, recruitment can begin up to 4 weeks prior to screening depending on the investigational new drug status of the test compound (**Figure 3**). Following this, a 28-day screening period takes place before dosing.

STUDY AIMS AND END POINTS

In a typical hAME study, the main aims are to determine absorption (following an extravascular dose), PKs (total radioactivity,

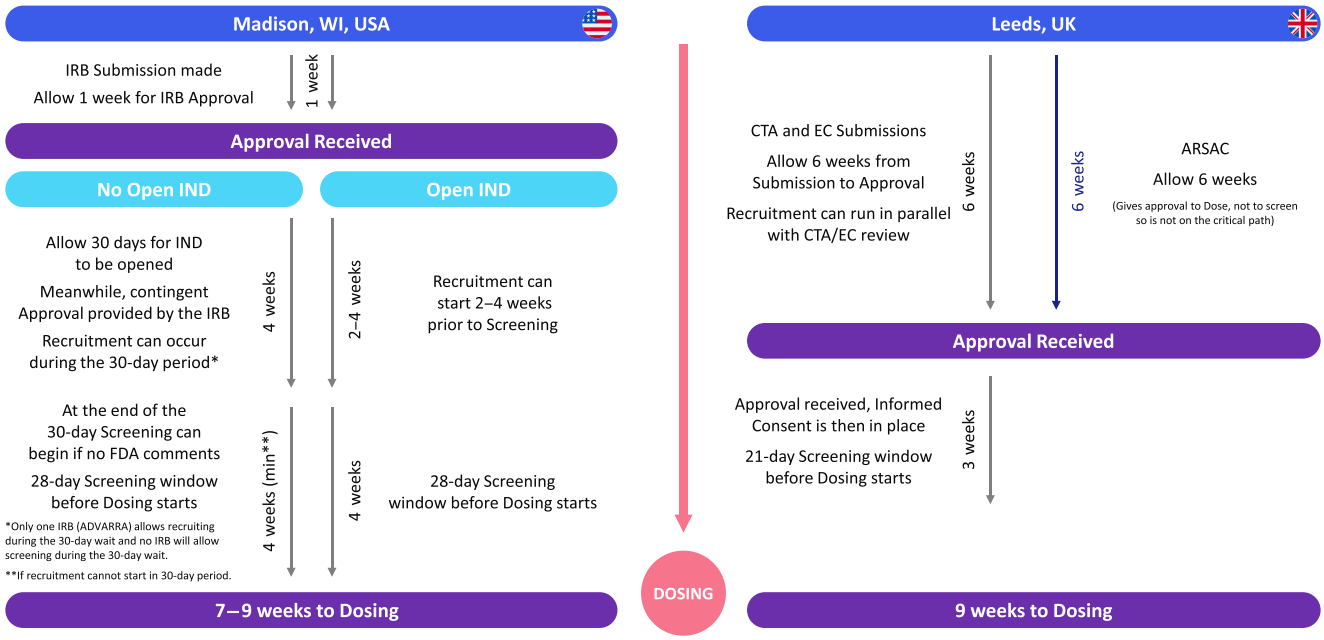

Figure 3 Comparison of the typical approval process flow with timelines for initiation of hAME studies in the United Kingdom and the United States. ARSAC, Administration of Radioactive Substances Advisory Committee; CTA, Clinical Trial Authorization; EC, Ethics Committee; FDA, US Food and Drug Administration; IND, investigational new drug; IRB, institutional review board.

and parent and selected metabolites), rates, and routes of excretion of drug-related radioactivity and a mass balance of radioactivity administered. The precious samples are always used for subsequent metabolite profiling, enabling identification of metabolites of radioactive drug-related material in urine, feces, blood, plasma, tissue biopsies, cerebrospinal fluid, and possibly expired air and bile, if collected.

As part of the mass balance end point, exit criteria are typically achieved when radioactivity in two consecutive plasma collections are below the limit of quantification, and at least 90% of the radioactivity administered in the dose has been recovered in the excreta (urine and feces) and < 1% of the total radioactive dose has been voided in 2 consecutive 24-hour periods. Several studies of drugs with potentially protracted excretion have involved in-clinic stays routinely up to 336 hours, but occasionally 672 hours or more. Furthermore, in the past, occasional outpatient visits were required for additional urine and feces collections and recovery data interpolated to provide the best estimate of overall excretion. However, our experiences have demonstrated that subjects are excellent at following study requirements and compliant to the collections of urine and feces during 24-hour periods at home if extended spot collections are needed once the clinical in-house time period has concluded. Safety is a secondary assessment, with the dose level selected being the planned clinical dose, and already established to be well-tolerated in healthy subjects. However, the dose can be reduced, especially in consideration of enabling a higher specific activity of drug to be administered, which will improve analytical sensitivity downstream. Although the stability of the radiolabel within the drug moiety is often confirmed by collecting and analyzing expired CO_2 during preclinical absorption, distribution, metabolism, and excretion (ADME) studies, several study designs include sparse collection of expired air to preclude expiration of unique volatile human metabolites. Human bile may also be collected and analyzed with minimally invasive devices, such as the EnteroTracker or other duodenal fluid sampling approaches.[10–12]

Radiolabeled studies are not statistically powered so the panel size typically requested is small and has ranged from 4 to 12 over the last 27 years for which we have data. With the new FDA guidance issued (May 2022) a recommendation was made for at least six evaluable subjects and as a result most studies now are planned with a recruitment panel of eight subjects, allowing for any unexpected subject dropouts, with at least six subjects fulfilling the study requirements. Attrition (postdose subject withdrawal) has been rare (< 2%) for Labcorp studies and successful recruitment was maintained throughout the recent pandemic, despite being much more difficult.

Predominantly, mass balance studies are conducted in healthy adult subjects of 30–65 years old. Where safety concerns preclude the enrollment of healthy subjects, or where the administration of a single dose to healthy subjects cannot be justified, such of the studies can be conducted in the patient population of interest. Labcorp has the ability to conduct radiolabeled studies in oncology patients where this is necessary, with relationships established at various National Health System hospitals in the United Kingdom to facilitate conduct of these studies.

Studies have typically been conducted in male subjects (> 95%), in alignment with the regulatory guidance. The predominantly male study design is due to female reproductive toxicity concerns from ingesting a radiolabel. Where female subjects have been included (< 5%) they have either been postmenopausal/surgically sterilized or the drug indication has been female-gender specific.

The benchmark for a starting radiolabeled dose for an hAME trial has historically been 100 μCi per subject (a macro-tracer dose). Typically sampling regimens for blood collections, together with urine and feces collected at typically 24-hour intervals, explores the distribution, metabolism, and elimination of radioactivity over 7 days, and has regularly been sufficient to ensure complete determination of the study end points using radioactivity detection via Liquid Scintillation Counting (LSC) for the mass balance, and radio-high-performance liquid chromatography with flow detection for systemic and excretory metabolite investigations. With LSC radio-analysis can generate real time recovery data and allow subjects to be released from the clinical stay as soon as the exit criteria are met, and this could be earlier than the typically designed minimum stay period of 7 days for instance. Nonetheless, it is occasionally not possible to dose enough radioactivity to conduct these hAME studies using traditional radioanalytical methods to achieve the study end points. Such studies are termed micro-tracer studies and will administer a much lower radioactive dose in the range of 1 μCi or less. Samples from these studies are analyzed by AMS (same process as carbon dating) and due to the extended analytical processing times for this technique, "real-time" analysis of samples is precluded. Therefore, the clinical trial protocol must include a specific strategy that is the worst-case scenario for allowing subjects to stay in the clinic for as long as possible, with outpatient visits also included routinely in case of protracted elimination (**Table 1**).

The clinical stay may also be partly influenced by the nature of the therapy under investigation, or the choice of dose route. Oncology directed therapies may have a greater tendency to warrant additional safety monitoring as part of a single dose hAME study; or development of psychoactive compounds that have transient and exaggerated pharmacology, may also require specific sample collection considerations.

The more unusual dose routes or durations, such as longer-term i.v. infusions, long-acting dermal patches, and slow-release enteric coated capsules for gastrointestinal tract delivery, will also require careful monitoring and planning during the development of a clinical hAME study, and these are becoming more common in our clinics as approved compounds are being redesigned, repurposed for differing targets, or reformulated to alter PK and pharmacodynamic (PD) characteristics.

The choice of radio-isotope is key when considering the hAME study. The vast majority (> 95%) of the studies we have conducted at Labcorp have utilized ^{14}C isotopes, but occasionally ^{3}H has been the choice of the sponsor. This is mainly driven by the ease and cost of synthesis of the radiolabeled molecule. It must not be forgotten that although standard radio-analytical LSC techniques can measure the scintillation resulting from multiple isotopes, such as ^{14}C,

Table 1 Advantages and disadvantages of AMS use in hAME studies

Traditional macro-tracer	AMS based micro-tracer
+ Well-established and accepted practice	+ Does not require QWBA study
+ Real time recoveries	+ Minimal requirement for ^{14}C synthesis
+ Simultaneous generation of radio-chromatographic profile and metabolite identification data	+ AMS sensitivity
+ Metabolite identification generally within 4–8 weeks	+/– Nonclinical studies generally not conducted by AMS
+ Ability to evaluate more samples in a cost-effective way	– Unable to achieve real time recoveries routinely
– Requires QWBA and dosimetry ahead of a hAME trial	– Can lengthen overall reporting timelines
– Higher ^{14}C requirement	– Metabolite profiling and metabolite identification are separate and sequential
	– Chromatographic resolution can be limited

AMS, accelerator mass spectrometry; hAME, human absorption, metabolism, and excretion; QWBA, quantitative whole-body autoradiography.

^{3}H, ^{35}S, and ^{125}I, that are used in preclinical animal studies, AMS is more limited, and typically are set up for the analysis of ^{12}C, ^{13}C, and ^{14}C only.

STUDY PLANNING AND PREPARATION

The hAME studies require substantial resources, investment, and a multidisciplinary multisite collaboration; therefore, these studies require thorough planning to ensure that all objectives are met and to save time and resources.

A comprehensive planning process for integrated preclinical and clinical stages should generate an optimized timeline of between 12 and 18 months for end-to-end preclinical and clinical radiolabeled studies.

The rate limiting step is often the radiosynthesis stage, which can take as long as 10–12 months from initiation to the nonclinical batch of non-GMP material, through to the GMP radiolabeled drug being available for the clinic. The availability of the radiolabeled drug and its radiochemical stability determines when one can start dosing in the hAME study. With the ongoing conflict between Russia and the Ukraine, which has resulted in sanctions against Russia, the export of the ^{14}C radiolabeled starting materials, which form the precursor to the vast majority of organic syntheses of radiolabeled small molecules, has been subject to bottlenecks and uncertainty. Consequently, CROs offering radiosynthesis have been reluctant to take on projects with complex syntheses or low product yields, because this can minimize the number of projects they can initiate.

The radiosynthesis stage is usually conducted in two parts: first, production of the non-GMP material, which is used in preclinical studies, and second, the GMP synthesis that produces the material for human use. The number of GMP synthesis stages is determined on a case-by-case basis and is dependent on three factors: the radiochemical dose, the isotopic dilution, and the formulation to be administered. Combining radiosynthesis of an ADME radiolabeled drug and a GMP hAME radiolabeled drug as one project within one radiosynthesis laboratory can significantly reduce timelines and costs. If the drug development strategy includes conducting the hAME earlier in the clinical phases, then creating additional stock of the key intermediates for the radiosynthesis during the non-GMP manufacture of the ADME radiolabeled drug has only a small incremental additional cost, but can pay dividends in saved time and cost for the clinical GMP manufacture, often cutting many months from the timeline.

As a minimum for the preclinical studies, rodent tissue distribution investigation (typically by QWBA) and, for the United Kingdom in particular, a rodent excretion balance study are required to support the radiolabeled hAME study. These preclinical investigations help establish the safe radiolabeled dose that is targeted during an hAME trial, with the generation of a dosimetry calculation. The dosimetry calculation itself is determined with reference to differing International Commission on Radiological Protection (ICRP) guidelines. In the United States, the calculations are derived in reference to ICRP 30, whereas, in the United Kingdom, the ICRP 60 and ICRP 62 guidelines are used to derive the values. This provides a slightly different perspective on the final dosimetry interpretation, with the United Kingdom taking the value and assessing risk based upon classification according to category I, II, or II from the World Health Organization (WHO), or category I, IIa, IIb, or III from the ICRP bodies; whereas our US teams take the derived dosimetry value and establish the level of risk and exposure of the radioactivity in the planned hAME trial as an overall annual exposure limit for approval by the IRB (**Table 2**). Neither approach is more appropriate or advantageous, and our experiences have enabled us to appreciate and understand if the categorization of any defined dosimetry value will provide undue risk, or cause challenges to meeting the end points of a study should that level be fixed in the hAME trial.

Therefore, determining the dosimetry value and assessing options for both the dose levels set (radiolabeled and chemical dose) and the analytical approaches that are best suited to provide the highest levels of success in attaining the scientific end points is the default action of any hAME trial planning.

It is important to remember that although the hAME study is not required until large scale clinical trials are initiated (phase III), there is no reason that these studies cannot be conducted sooner. After all, the unique deliverable of an hAME study is to definitively identify and provide quantitative data on the nature of the compound-related material and its metabolites that may have an implication on the safety of the drug. We have seen hAME studies occasionally used in ranking of candidates prior to full SAD and multiple ascending dose (MAD) phases much like

Table 2 Dosimetry risk categories by regulatory guidance and effective dose administered within each category

Risk category	Regulator	Effective dose (E_B; mSv)	Level of risk
Category I	WHO[13]	<0.5	Within variations of natural background
Category II	WHO	0.5–5	Within dose limits for members of the public
Category III	WHO	<5–50	Within dose limits for persons occupationally exposed to radiation
Category I	ICRP[14]	<0.1	Trivial
Category II			
IIa	ICRP	0.1–1	Minor to intermediate
IIb		1–10	
Category III	ICRP	>10	Moderate

ICRP, International Commission on Radiological Protection; WHO, World Health Organization.

a preclinical PK screen, through to incorporation into an arm of the SAD study, included in a fed/fasting study, a DDI study, or, as described above, a more commonly used standalone clinical pharmacology radiolabeled trial. The cost of generating radiolabeled material, as well as that of a clinical trial itself, often pushes the point of initiation down the critical path, only to be initiated once a proof of concept (PoC) point has been reached on efficacy and greater confidence on the progression of the drug through the clinic has been established. Therefore, the timing of when to conduct an hAME study becomes as much a risk-based consideration on whether unique or disproportionate human metabolites are detected at the first opportunity, and then addressed through preclinical evaluations, or whether the project team/company have confidence that the situation of unique or disproportionate human metabolites will not occur. Although we do regularly observe disproportionate human metabolites when we conduct the quantitative metabolite evaluations on human samples compared with preclinical species, the twofold threshold for further safety evaluation directed by MIST are not often exceeded. It is even less common to observe unique human metabolites that have not been observed in any *in vitro* or *in vivo* preclinical investigations, but we have seen this on 3 occasions in the last 10 years, which has ultimately led to immediate hold on the clinical development of those compounds. To add context, this represents ca 0.6% of the hAME trials that we have conducted (**Figure 2**).

RESULTS AND DISCUSSION

Labcorp undergo a thorough IRB and EC review of all study protocols, and informed consent was in place for all data generated and presented here.

A review of Labcorp historical data (over 500 studies since 1989) reveals that radiocarbon (^{14}C) is by far the most prevalent isotope used on hAME studies (>98%), with only nine studies using tritiated (^{3}H) material and one study using a dual (^{3}H and ^{14}C) labeled approach. Following evolving regulatory requests and the regulatory shift to better understand metabolites, the setting of radioactive doses is first based upon the dosimetry report that is generated, but then this is evaluated following extrapolation of the human plasma PK data (unlabeled test material) and preclinical plasma and excreta metabolite data (radiolabeled material) in order to predict the minimum radiolabeled dose required to fulfill these guidelines. This has resulted in a trend of administering a more targeted radioactive dose to ensure that we administer a sufficient amount of radioactivity to fully analyze and understand the circulating metabolites.

The guidelines prescribed by the FDA and EMA require the radioactive dose to be "as low as (is) reasonably achievable" (ALARA) and total exposure to radioactivity is targeted to ICRP 62 category IIa (< 1 mSv) and the WHO[13] guidelines category 2 (< 5 mSv). Where sufficient radioactivity cannot be dosed to meet the study objectives and fulfill the ICRP category IIa requirements, approval to expose the volunteers to up to 5 mSv of radioactivity has previously been obtained (i.e., ICRP category IIb). Even though we may be administering most of our studies at or above 100 µCi, the mSv exposure data for almost 80% of the studies conducted are still at or below the 1.0 mSv threshold. This is a consequence of dosimetry being a cumulative calculation of radiation risk associated with exposure to many tissues of the body, that have differing weighting factors that contribute to the overall assessment.[15] For instance, the gonads and intestines (for oral doses) have particular emphasis within the calculation, therefore exposure measured during preclinical studies can have a disproportionate impact on the overall radioactive dose that is safely permissible to humans.

A notable increase in oncology products coming through the drug development pipeline with a mechanism of action and safety profile which enables them to be given to healthy volunteers, has resulted in oncology products accounting for 25% of the drugs administered in our healthy volunteer hAME studies in the last 15 years (**Figure 4a**). New chemical entities (NCEs) developed to address clinical conditions related to infectious diseases and cardiovascular diseases are two additional areas we have seen a higher demand for hAME trials. This is probably unsurprising given these areas are the most common therapeutic areas for NDAs and drug approvals in the past 2 decades.[16]

Doses are prepared following GMP regulations. Oral formulations are most common (accounting for 86% of dosing in Labcorp hAME studies) due to the increased patient compliance and favorable target product profile of orally administered drugs; however, different parenteral formulations and dermal creams have been evaluated in hAME trials (**Figure 4b**). It is also evident to note that the powder in capsules, as well as various formulations in capsules, are often a bridge between a true clinical tablet dose, the FIH SAD/MAD trials and the oral suspensions often used in the preclinical safety and PK studies.

Most studies conducted at Labcorp have been in healthy normal volunteers with those subjects being mainly men (94%; **Figure 5a**). Female subjects are typically excluded to align with regulatory guidance 21CFR361.1. The ALARA principle prescribed by the FDA recommends that radiation exposure to subjects should be kept ALARA; therefore, if no specific reason exists to include female subjects (i.e., no available data suggest metabolism of the investigational product is different in female vs. male subjects),

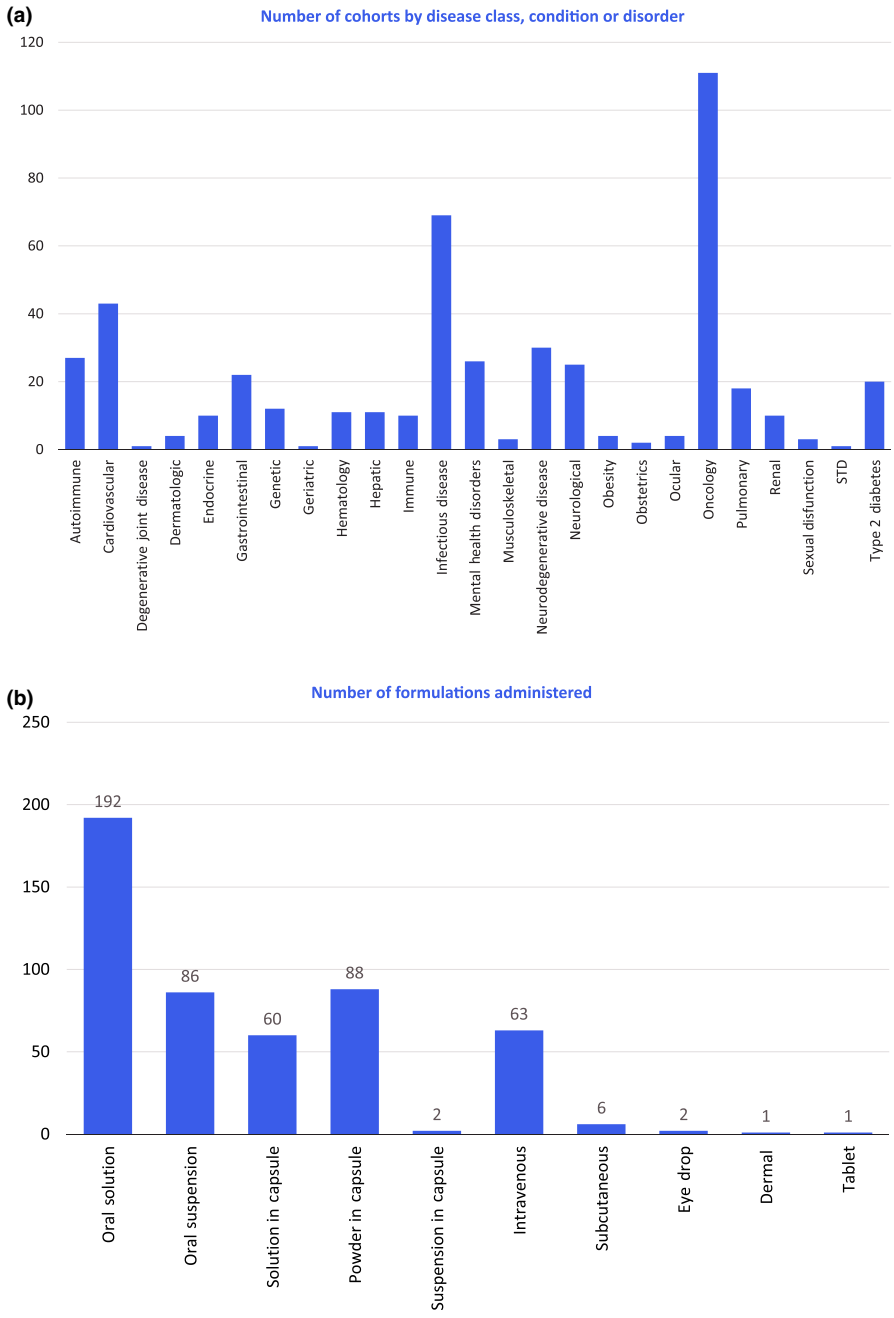

Figure 4 (**a**) Therapeutic areas involving hAME studies that have been conducted at Labcorp. (**b**) Dose routes and administration regimens in hAME trials at Labcorp. hAME, human absorption, metabolism, and excretion.

then the radiation exposure to female subjects is kept at zero by not including female subjects and only enrolling and dosing male subjects. About 65% ($n = 81$) of female subjects included in Labcorp studies have been for oncology products.

The number of subjects included in Labcorp hAME studies has ranged from 4–12 with the most common panel size being 6 or 8 subjects (42% and 41%, respectively; **Figure 5b**). Within the FDA draft guidance issued May 2022, a recommendation was made to have at least six evaluable subjects. It is therefore advisable to dose approximately eight subjects to ensure completion of at least six evaluable subjects.

About half (46%) of the hAME studies conducted at Labcorp targeted a radioactivity dose of 100 µCi (3.7 MBq) and this remained constant either side of issue of the MIST guidelines. However, the proportion of studies using a higher radioactive dose increased from 17% to 25% and in our last 100 studies (2019–2022), about 40% have administered > 100 µCi (**Figure 6a**).

The choice of the dose level, as described earlier, is influenced by the known disposition and PK properties of the drug, the potency, and therefore overall chemical dose that can be administered, as well as the analytical strategy that is being used.

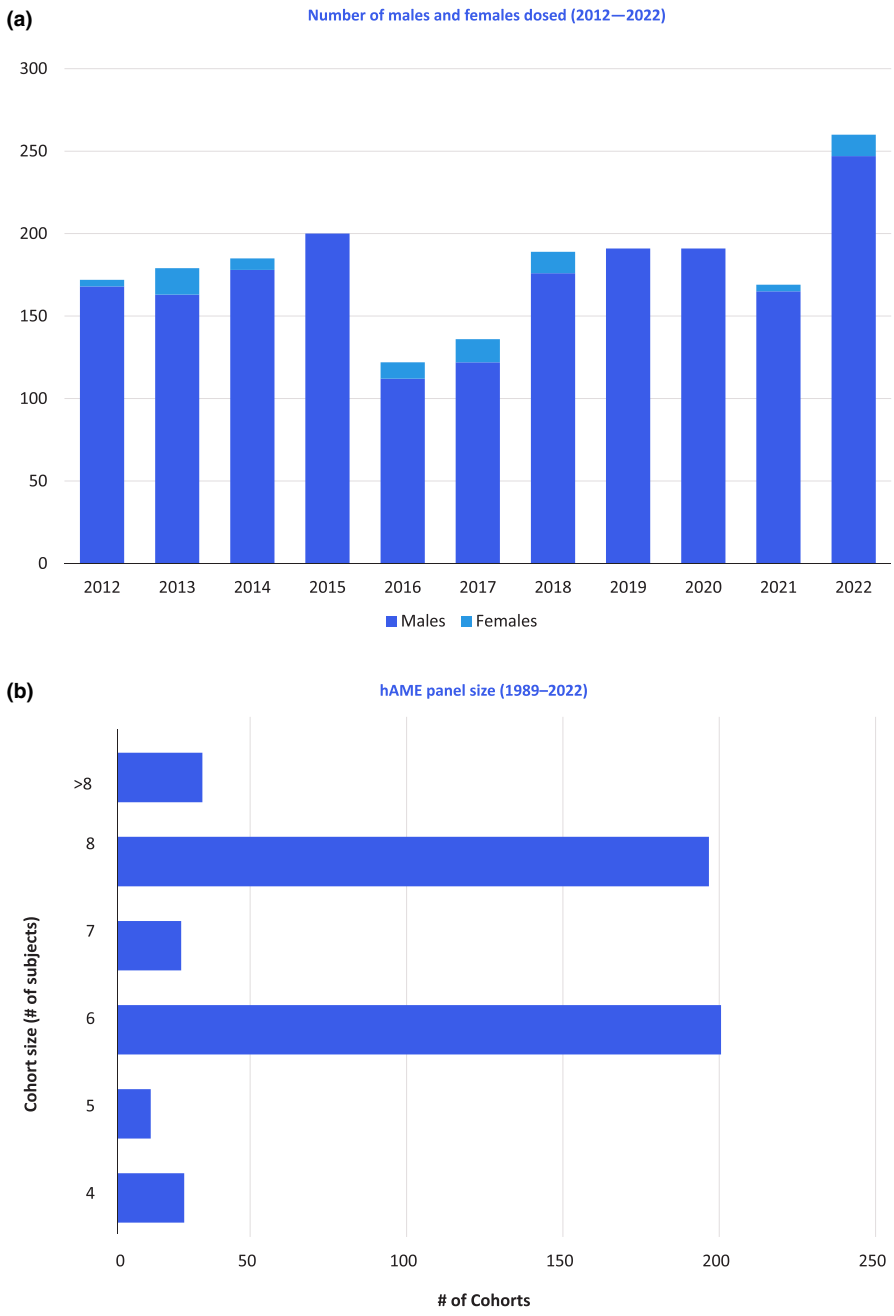

Figure 5 (**a**) The number of male and female subjects enrolled in hAME trials annually at Labcorp. (**b**) Comparison of the cohort size recruited for hAME trials at Labcorp. hAME, human absorption, metabolism, and excretion.

Our hAME studies have been conducted almost exclusively with small molecules with relatively short half-lives. Therefore, the duration of the in-house study period for most studies is up to 14 days, however, 3% have had study in-house confinement for > 30 days. With some molecules of longer half-life, additional visits consisting of a 24 or 48-hour stay to collect excreta samples may be required of subjects to more completely assess excretion (**Figure 6b**).

Regulatory bodies and scientific needs unite on the intent that an hAME study should achieve at least a 90% recovery of administered radioactivity. As a result, subjects are typically discharged from the clinic when they have achieved that milestone and/or when they are consistently excreting < 1% of the administered radioactivity in excreta samples. There may also be an additional requirement that radioactivity in blood and/or plasma samples must be below the limit of quantitation before subjects complete the study. Using this process, we have been able to obtain recoveries that routinely meet these criteria (**Figure 6c**).

Lower than optimal recovery of radioactivity can have multiple causes. Non-compliance of subjects is not usually an issue during hAME studies, but accidental oversight (e.g., spilling the sample or forgetting to collect the sample) is the likely reason for

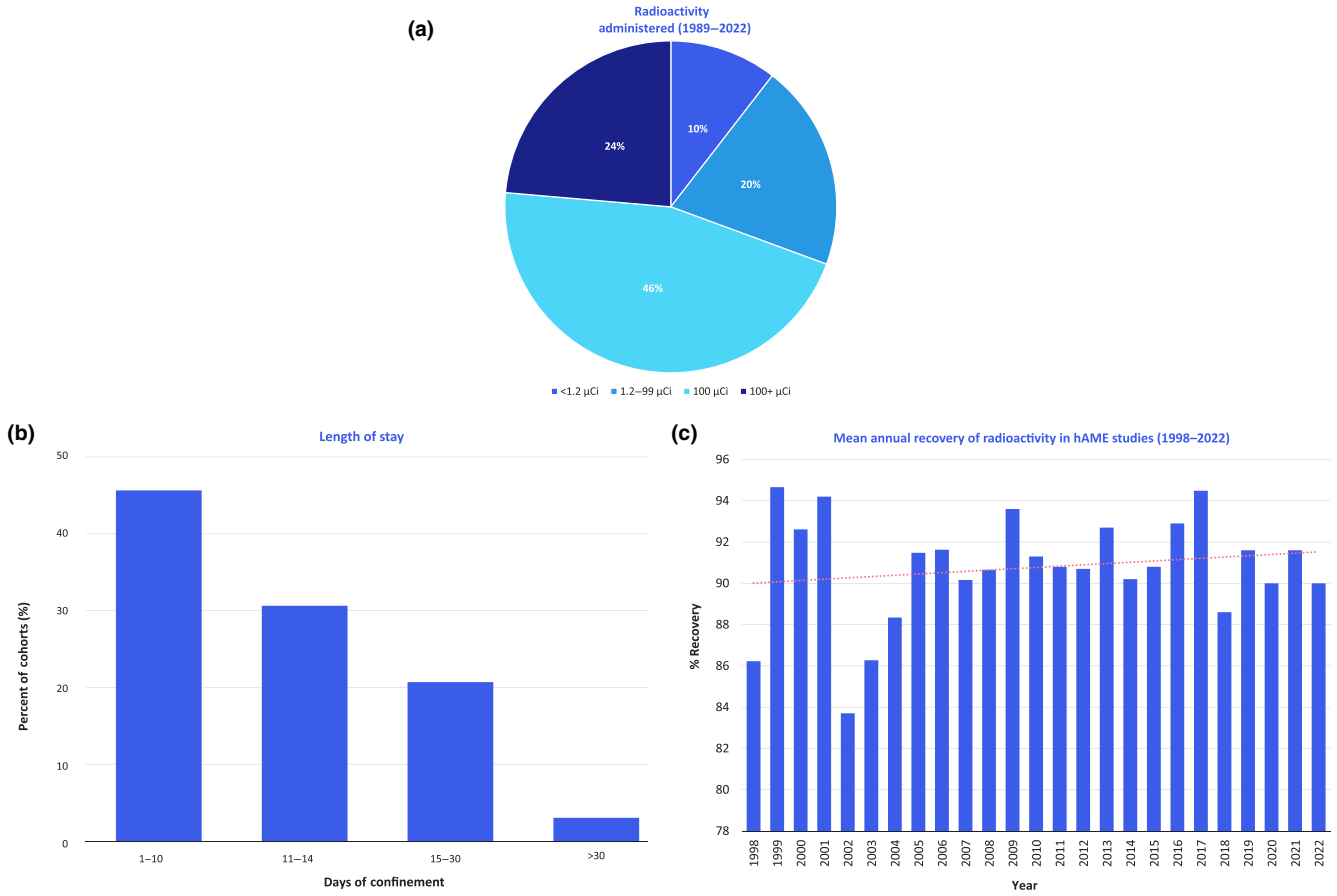

Figure 6 (**a**) Proportion of studies conducted administering various amounts of radioactivity at Labcorp. (**b**) Comparison of the protocolled clinical in-house period for hAME studies at Labcorp. (**c**) Mean recovery of radioactivity in hAME studies over the last 5 years at Labcorp. hAME, human absorption, metabolism, and excretion.

noncompliance when observed, and could result in low recovery of radioactivity for a specific subject and study. More commonly it is the nature of the test material administered that is the causal variable in low recovery, such as prolonged elimination half-life, with radioactivity recovered at levels around the limit of quantification for many days post dosing (e.g., ca 1% of the administered dose recovered daily). Utilizing an inappropriate solvent in fecal homogenization is also a common cause of variable recovery, especially for those compounds that have pronounced insolubility profiles, and can be more common after capsule dosing.

Overcoming the key challenges of hAME studies

There are multiple complexities and parties involved in conducting an hAME study, and one can anticipate many significant challenges that can impact the success of an hAME study. One overarching way of addressing these is by using an integrated development paradigm.

Use an integrated preclinical to clinical approach

The optimal approach for a clinical development program is one integrating early drug development activities to align with the clinical program. Having the resources, expertise, and necessary infrastructure available to design and execute regulatory strategies and early clinical development plans is an effective way of mitigating risk and involves a collaborative and coordinated effort between sponsor and CROs.

An integrated approach allows you to save time on approvals, helps you to understand the molecule as completely as possible, from appreciation of the radio-metabolite profile, metabolite identification challenges, as well as sampling handling, extraction, and stability of the drug and its metabolites in the various matrices, and ensures better planning for optimized analysis and provides consistent results. The hAME studies combine the technical areas of radiosynthesis, chemistry, manufacturing, and control, preclinical ADME with metabolite identification, dosimetry calculations, clinical pharmacology, and pharmacy.

Design preclinical studies that support the clinical studies

As with many facets of drug development, anticipating and designing preclinical studies to predict and bridge the translation to a human setting is paramount within this specific subset of clinical pharmacology. To ensure that preclinical data support the clinical studies, a typical approach involves a robust and comprehensive rodent ADME study, supplemented by other radiolabeled *in vitro* studies and possibly a non-rodent ADME study. These are invaluable in providing a consistently aligned set of parameters in the preclinical study to enable maximum extrapolation and translation to the subjects involved in the hAME study as

well as provide confidence and experience in the handling of the radioactive samples and the evaluation of the compound-derived radioactivity (parent and metabolites) within those samples. To ensure alignment between these studies, the following factors are considered:

- Radioactive isotope – different isotopes have different properties, therefore different risks to tissues in terms of radioactivity exposure.
- Gender specificity related to tissue distribution.
- Route of administration (to ensure ADME properties are fully characterized between the preclinical and clinical expected routes).
- Sufficient sampling times to provide reliable area under the curve (AUC) data, including maximum plasma concentration (C_{max}) and elimination phases.

In addition, the tissue distribution study should cover an appropriate time range, so that the absorption, exposure, and elimination can be evaluated to enable an accurate dosimetry calculation to be performed.

The radioactive dose must be sufficient to allow complete quantification and characterization of metabolites, but within the limits to be safe for humans in clinical trials. Various analytical strategies can be used to optimize recovery in a clinical hAME study. For example, the data obtained from both dosimetry studies with earlier preclinical ADME data must be analyzed thoroughly to allow the establishment of likely concentrations of radiolabeled metabolites that will be obtained in human samples.

In addition, using sample preparation and concentration techniques, such as sample pooling at the point of collection prior to processing; fraction pooling and overlay following radiometabolite profiling with fractionation; or solvent evaporation allowing sample workup to lower the limit of detection where possible.

Technology and analytical strategies

Without an effective analytical strategy, the time and cost of arriving at a mass balance and identification and quantification of the human metabolites, can become grossly exaggerated. We have seen approaches to the analyses of biological matrices change significantly over time. Once, plasma samples from every subject in an hAME trial at 8–10 timepoints were profiled and evaluated. Excreta samples from every timepoint collected in the clinic were also subject to metabolite identification. Samples tend to be pooled to minimize the sample numbers presented for analysis today. With two to four plasma samples and two to three urine and feces samples typically taken for the metabolite identification evaluations. Many companies are content with a scientific strategy that provide the necessary scientific robustness, whereas covering any metabolic liability or risk. Plasma samples are pooled perhaps at C_{max} and an AUC pool prepared per subject to provide indications of those metabolites circulating at the time of highest parent compound exposure and the relative proportions of those metabolites over the time course studies. These are then directly comparable on a subject-to-subject basis.

During excretion balance determinations, LSC is the most common method for determining the amount of radioactivity eliminated in time-defined samples. Although limits of detection are suitable when dosing macro-levels of radioactivity (< 30 μCi per subject) if the dosimetry calculated from the preclinical studies reduce the amount of radioactivity that can be safely administered to a volunteer, then low level counting LSC are required. These offer some additional background reductions to conventional LSC, but to become truly low level, AMS is required. This, however, has the disadvantage of being unable to provide real-time sample analysis, which subsequently allows for the ongoing determination of the excretion balance, or PK evaluation, which allows subjects to be released from the clinic.

PoC in the use of cavity ring-down spectroscopy (CRDS) has been provided in both clinical and preclinical studies,[17,18] and may well offer an alternative, more cost-effective approach to determining low level radioactivity concentrations in biological samples. Mainstream release of these instruments remains on hold, but these benchtop instruments do not require the high technical capabilities as an AMS technician may do and can certainly fit within conventional laboratory spaces without special modification.

Of specific note, however, in the context of the aims and objectives of an hAME study, is the rapid and fundamental development of high-resolution mass spectrometers in the last 10 years, which has revolutionized the detection and quantification of metabolites from these study samples. Common limits of detection are now within the pg and ng ranges, and with the ability to resolve isobaric metabolites based upon their collisional cross-sectional area in ion mobility instruments, there are now only rare instances where we cannot accurately assign biotransformation sites within molecules effectively.

Metabolite identification instruments and the software platforms that enable the low-level detection and determination of metabolites will undoubtedly continue evolving. The use of next generation instruments, which provide greater time of flight paths, or ion mobility improvements via greater collisional cross-sectional areas of the molecular species, is a keenly contested field by the instrument manufacturers. Metabolite identification in the relatively clean matrices obtained during preclinical and clinical studies, such as plasma, urine, and bile, offer the biotransformation scientist the greatest opportunity to evaluation of the diversity of metabolites arising from a small subset of animals or subjects. Extraction of drug-related radioactivity ahead of metabolite profiling in "dirtier" matrices, such as feces, remain commonplace and insightful for orally dosed, or entero-hepatically recycled compounds; but it is with the possible differences in a genetically heterogeneous population, such as humans, together with the overwhelmingly diverse microbiome environment that poses the next barrier to understanding how individuals will respond differently to drugs in terms of their biotransformation, and elimination, and therefore the impact of the personalized medicine approaches we know is the optimal route for drug therapy.

FUTURE PERSPECTIVES

As we have highlighted, the approach to designing and conducting hAME studies, as well as the downstream analyses, data evaluation

and interpretation, and value in conducting hAME studies at various key milestones during the development of a drug, has evolved in complexity over the past 30 years. With the issue of the FDA guidance on hAME study design, we would also expect this trend to continue, with worldwide regulatory authorities complementing the FDA, and outlining expectations on how the studies add insights into the safety of a drug in humans. This would not just broach harmonization across regions, but also build upon the MIST guidance to understand the contributions to safety from circulating metabolites. Within many areas of both clinical and preclinical DMPK, the utilization of modeling and simulation, physiologically-based pharmacokinetic (PBPK) and PK/PD models, continue to add to the predicted efficacy and safety of a molecule. To date, these models play a minor role in the design and conduct of an hAME study, given clinical doses and much of the preclinical safety is well-understood before entering clinical trials with a radiolabeled drug. However, as the mode of action of drugs, especially those that have complex immunomodulatory or widespread biochemical effects are developed, the need for predictive virtual outcomes has become an expectation of regulators. With more intuitive metabolite biotransformation software evolving (add some details) that complement the predictive toxicology platforms, we can see a virtual hAME study being a stepping-stone to the actual conduct of an *in vivo* study in the future.

As technology progresses, then so too will the desire from the drug development industry to build a framework for the consistent utilization of that technology, and standardization of the outputs presented to regulators for approval. The iterative toolbox of analytical instruments that support the generation of data regarding the ADME properties of a drug in human subjects is now more diverse and sensitive than ever before. We would expect to witness the further evolution of more benchtop ready AMS instruments, for instance, that not only allow more standard laboratories to house and utilize them, but also will facilitate the direct injection of samples into the AMS for radioactivity quantitation rather than the current somewhat lengthy and time-bound graphitization process. The emergence of CRDS instruments which take a slightly different approach to elemental carbon analysis in samples, although not commercially available yet, may provide a more cost-effective manner for lower-level radioactivity counting needed when micro-trace amounts of radioactivity are administered in phase 0 type studies. However, it is in the realm of instruments that are perhaps considered niche today, such as various imaging platforms (static cell based and whole-body fluorescence or hand-held 3D body scanners), coupled with real-time biopsies and matrix assisted laser desorption/ionization-mass spectrometry that we may see greater scientific value and addition of multiple end points in the future. This may be particularly important when coupling pre-clinical patient-derived xenograft models and personalized medicine to the understanding of the mode of action of a compound in its tumor micro-environment, whereas minimizing any adverse systemic side effects.

Computer-assisted learning, or more commonly phrased artificial intelligence (AI), touches our lives in many ways today in both scientific endeavors and other disciplines. AI almost always relies upon robust and extensive databases to aid in machine learning, and to develop confidence that the output directly correlates with a clinical outcome. Database consolidation and sharing confidential intellectual property information on not only those molecules that progress to market, but those that also fail would be needed to assist in developing any AI platform to predict hAME outcomes in terms of excretion balance and biotransformation, which in the future could potentially negate the need for an hAME trial, if those unique human metabolites could be predicted.

One could ask whether the use of radioactivity as a tool will continue fully into the future? Utilizing radioisotopes, such as ^{14}C to enrich NCEs to administer to humans to track a drug and its metabolites is the accepted, and more established, path. However, as global supply of ^{14}C radiolabeled starting materials can be disrupted, particularly as a consequence of territorial conflict, alternative approaches and isotopes may become more prevalent. The use of other naturally abundant isotopes, whether ^{3}H, ^{125}I, ^{13}C, or ^{15}N could become more attractive, because the supply, cost of synthesis and ease of handling, transport, and storage can be lower for a number of these (^{125}I aside). The main barrier to adopting these would likely be the analytical instrumentation, but, as we have noted earlier, elemental analyzers, liquid chromatography mass spectrometry, or even AMS instruments can be adapted to detect alternative isotopes, and if this becomes a focus, the sensitivity and development of these instruments for these alternatives could progress rapidly.

We have focused very much on hAME studies involving small molecule NCEs in the above discussions, and without clear scientific or regulatory need for conducting hAME studies with new biological entities (NBEs) currently, we are unlikely to see this change. What may drive the evaluation of NBEs in hAME trials are the advancements and development of novel therapeutic vehicles, such as lipid nanoparticle scaffolds, cell and gene therapies involving a combination of NBE and NCEs; or NBEs with unique non-naturally occurring components. Establishing the ADME characteristics of these are more likely to lay within the preclinical evaluations prior to clinical trials, but as we have seen regulatory evolution as well as scientific expansion in these new modalities over the past 20 years, we should not be surprised if the need for NBE hAME focused trials occurs to ensure the safety of the development and approval of these therapies in the future. Equally, as the route of administration or medical device development evolves to allow novel application of drugs, the conduct of hAME studies involving microneedle patches, subcutaneous gel deposits, or with orally dispersion tablets and inhalation sprays applied to oral mucosa, or ophthalmic eye administrations, we may well witness more bespoke hAME designs that we already see today.

As a consequence of the regulatory expectations around the need for an hAME study prior to regulatory approval of an NCE, it is not surprising that waivers to perform hAME studies arise infrequently. In our experience, successfully justifying a waiver for an hAME study is rare, but circumstances, such as insignificant systemic exposure to a drug following dermal application of a topically acting drug is one such incidence. Whether it could be argued that the translation of preclinical to clinical radiolabeled evaluations can be made from PBPK modeling in the future when combined with non-labeled clinical data on metabolites and parent compounds alone, remains a bridge too far for many. However, having an open source for hAME and preclinical data from regulatory

approvals (together with any molecules that do not reach that critical milestone) could well enhance the informed decision making for any waivers in the future.

CONCLUSIONS

Well-designed and robust ADME and hAME studies are vital to demonstrating confidence in the safety and efficacy of a potential drug, with knowledge of metabolite profiles of a drug candidate in preclinical species as well as in humans required by regulatory agencies for drug approval. Conducting these studies early in development can have multiple benefits, such as enabling efficient management of metabolites in terms of safety profile, because discovering unique or disproportionate drug metabolites late in drug development can cause significant delays to a clinical program.

Early investment and thorough planning of ADME and hAME studies can mitigate these problems. These studies require the synthesis of radioactive material which is costly, time-consuming, and, in many cases, technically difficult. The data we have presented here from our experiences in over 500 studies demonstrates that there is diversity, yet commonality, in the design and conduct of hAME studies primarily aimed at healthy volunteers to support an NDA. The recent published FDA guidance aligns with the standard approaches to hAME studies we have taken for many years, but it should not be understated that the science behind each molecule is different, and each study needs to be considered on its merit, to ensure the correct study design and downstream analytical approaches are utilized.

Above all, success in conducting hAME studies in a clinical development program requires an integrated early drug development platform involving a collaborative and coordinated effort between different stakeholders across both clinical and nonclinical groups within a CRO. Our experience of the 500 studies we have conducted indicates the need for a high customer centricity, enabling an efficient level of data and information sharing, and an educational experience for many involved, who may be experiencing hAME studies for the first time.

ACKNOWLEDGMENTS

The authors wish to highlight and thank Dr Donald McKenzie and Dr Frederic Thalacker for their continuing support and critical comments on the manuscript.

FUNDING

No funding was received for this work.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

1. Young, G.C. *et al*. Considerations for human ADME strategy and design paradigm shift(s) – an industry white paper. *Clin. Pharmacol. Ther.* **113**, 775–781 (2023).
2. Kendrick, J.S. & Webber, C. One small step in time, one giant leap for DMPK kind – a CRO perspective of the evolving core discipline of drug development. *Xenobiotica* **52**, 797–810 (2022).
3. Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry Safety Testing of Drug Metabolites. U.S. Department of Health and Human Services Revision 2, Pharmacology and Toxicology (2020).
4. Holmberg, A.A., Ekdahl, A. & Weidolf, L. Systemic exposure to the metabolites of lesogaberan in humans and animals: a case study of metabolites in safety testing. *Drug Metab. Dispos.* **42**, 1016–1021 (2014).
5. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals Step 5. EMA/CPMP/ICH/286/1995 (2009).
6. Guideline on the Investigation of Drug Interactions. European Medicines Agency, 21 June 2012, CPMP/EWP/560/95/Rev. 1 Corr. 2**, Committee for Human Medicinal Products (CHMP) – finalized 2013.
7. Food and Drug Administration, Center for Drug Evaluation and Research. Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies Guidance for Industry DRAFT GUIDANCE, U.S. Department of Health and Human Services, Clinical Pharmacology (2022 May).
8. DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
9. ADME/hAME studies optimization to overcome development barriers. Labcorp.com <https://ddblog.labcorp.com/2020/10/optimizing-your-adme-hame-studies-to-overcome-barriers/> (2021). Accessed August 18, 2023.
10. Wang, L. *et al*. Metabolism and disposition of 14C-labeled peliglitazar in humans. *Drug Metab. Dispos.* **39**, 228–238 (2011).
11. Remmerie, B. *et al*. Integrating duodenal sampling in a human mass balance study to quantify the elimination pathways of JNJ-53718678, a respiratory syncytial virus fusion protein inhibitor. *Adv. Ther.* **37**, 578–591 (2020).
12. Wen, B. *et al*. Investigation of clinical absorption, distribution, metabolism, and excretion and pharmacokinetics of the HIV-1 maturation inhibitor GSK3640254 using an intravenous microtracer combined with EnteroTracker for biliary sampling. *Drug Metab. Dispos.* **50**, 1442–1453 (2022).
13. WHO Expert Committee on the Use of Ionizing Radiation and Radionuclides on Human Beings for Medical Research Training, and Nonmedical Purposes & World Health Organization. *Use of ionizing radiation and radionuclides on human beings for medical research, training, and nonmedical purposes: report of a WHO Expert Committee [Meeting Held in Geneva from 1 to 8 March 1977]* (World Health Organization, Geneva, Switzerland, 1977).
14. ICRP. Radiological protection in biomedical research. ICRP Publication 62. Ann. ICRP, Vol. 22. (1992).
15. ICRP. The 2007 recommendations of the International Commission on Radiological Protection. ICRP publication 103. Ann. ICRP 37 (2-4) (2007).
16. Batta, A., Kalra, B.S. & Khirasaria, R. Trends in FDA drug approvals over the last 2 decades: an observational study. *J. Family Med. Prim. Care.* **9**, 105–114 (2020).
17. Kratochwil, N.A. *et al*. Nanotracing and cavity-ring down spectroscopy: a new ultrasensitive approach in large molecule drug disposition studies. *PLoS One* **13**, e0205435 (2018).
18. Zare, R.N. Ultrasensitive radiocarbon detection. *Nature* **482**, 312–313 (2012).