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Human Absorption, Metabolism, and Excretion (hAME) Mass Balance Studies in Patients With Cancer

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ABSTRACT

Radiolabeled mass balance human studies are a critical component of clinical pharmacology programs supporting the development of new investigational drugs. These studies provide information about absorption, metabolism, and excretion (AME) of the parent drug and metabolite(s) in the human body and are normally conducted in healthy volunteers (HVs). However, in some instances, based on the characteristics and toxicity of the molecule, conducting the study in HVs may not be a viable option and therefore cancer patients (CPs) need to be enrolled. Successful navigation of aspects such as pharmacologic characteristics, dose administration, patient clinical status and comorbidities, confinement period, study extension, team training, and requirements of the hospitals/units are essential to successfully deliver radiolabeled mass balance studies in CPs. This article provides an overview of the main aspects that must be taken into consideration to conduct AME studies in CPs.

1 | Introduction

Human mass balance studies, also called human radiolabeled mass balance studies or human absorption, metabolism, and excretion (hAME) trials, represent a critical component of clinical pharmacology studies supporting the development of new investigational drugs in line with the regulatory guidance. Mass balance studies should be conducted early during the drug development program and together with radiolabeled toxicology studies, provide essential information on the exposure of the parent compound and metabolites.

The objectives of hAME are to elucidate the pharmacokinetic (PK) characteristics of the drug, including (1) identifying and quantifying circulating parent and metabolites and determining the exposure to metabolites which may be either unique to humans or disproportionately represented, compared to the metabolites in preclinical trials and (2) demonstrating the elimination pathways of the product. Therefore, information obtained from hAME studies is crucial to understand (1) if additional nonclinical studies are required to assess the safety of human metabolites, (2) the potential for drug–drug interactions (DDI) and consequently if specific DDI studies are needed and (3) whether renal and/or hepatic impairment studies are required [1–3].

hAME studies with non-mutagenic targeted molecules in oncology may be conducted in healthy volunteers (HVs). However, for some mechanisms of action, drugs with genotoxic effect or in case of other safety concerns, it may be necessary to conduct these trials in CPs which constitute around 10% of all hAME studies [2, 4].

A typical hAME study starts with the administration of a single dose of the candidate drug linked to a radiolabeled isotope. Different isotopes have been used, including ^{3}H, ^{14}C, ^{32}P, and ^{35}S, but the most commonly used radiotracer is ^{14}C which has a long half-life. Unlike hydrogen, which readily forms bonds and

Study Highlights

- **What is the current knowledge on the topic?**

Radiolabeled mass balance human studies are an essential study of the clinical pharmacology package needed for the development of new investigational drugs. These studies are normally conducted in HVs. However, in the case of drugs with epigenetic, genotoxic, carcinogenic, or clastogenic effects, and when adverse effects are irreversible and difficult to monitor, or toxicological data from animal models show poor relevance to humans, CPs need to be enrolled.

- **What question did this study address?**

This article provides an overview of the main aspects that must be taken into consideration to conduct hAME studies in CPs, from protocol design, including patient population, study extension and confinement periods, bioanalytic aspects, and site selection.

- **What does this study add to our knowledge?**

A thorough evaluation is necessary to decide if a specific hAME study should incorporate CPs or HVs, considering the features of the investigational agent and the specific requirements of the protocol. It will be crucial to have a good knowledge of the ability of the investigational site and pharmacy to manage such complex studies.

- **How might this change clinical pharmacology or translational science?**

It provided insights into how CPs metabolize drugs, leading to a better understanding of pharmacokinetics and pharmacodynamics aspects of the molecule. Data from these studies can lead to better dosing strategies and reduced toxicity and may help refine predictive models for drug behavior in CPs, improving the translatability of preclinical data. Consequently, the insights obtained can strengthen collaborations among pharmacology, oncology, and toxicology, fostering innovative approaches to drug development.

TABLE 1 | Summary of the main regulatory guidance.

Year-regulatory body	Guidance
2008-**FDA**	**MIST**. Describe the importance of understanding the safety associated with metabolites in humans, particularly ones that represent more than 10% total drug exposure and are disproportionally present compared to the metabolites in pre-clinical species [1, 8].
2009-**ICH**	**ICH M3 (R2)**. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals, emphasizing the need to understand drug metabolism as comprehensively as possible [7, 9, 10]
2012-**EMA**	**DDI guideline** that, for the first time, indicated the requirement of greater than 90% recovery in urine and feces [7, 9, 10].
2024-**FDA**	First guidance that fully described the design and requirements for a **human radiolabelled mass balance study** [2, 7]
2025-**EMA**	**Question-and-answer document** related to the 2012 guidance "Guideline on the investigation of drug interactions" [2, 7]
2019-**FDA** 2024-**EMA**	Guideline focused on **oncology therapeutic radiopharmaceuticals** to assist sponsors in the design of appropriate nonclinical programs for the development of radiopharmaceuticals compounds to treat cancer and to provide recommendations in key aspects of the marketing authorization and product labeling [11, 12].

Abbreviations: DDI, Drug–drug interaction; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; MIST, Metabolites in Safety Testing.

can be exchanged with environmental elements, carbon-14 (^{14}C) forms stable bonds that are unlikely to exchange with the environment, thereby minimizing loss of radiolabel activity [5, 6]. After the dose administration with the radiolabeled tracer, blood samples and excreta (urine and feces) are collected during a period long enough to achieve the objectives of the study.

Based on United Sates Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) guidance, most Phase 1 hAME protocols the excrete collections continue until at least 90% of the administered radioactivity has been recovered in the urine and feces and the subjects are excreting $<1\%$ of the administered radioactivity for two consecutive days [2, 7]. Table 1 provides a summary of the main regulatory guidance's.

hAME studies are required for regulatory filing. Ramamoorthy et al. reviewed regulatory packages submitted between 2014 and 2018, especially if hAME studies were used to support the development and approval of new drug applications (NDAs) for new molecular entities (NMEs) [13]. Interestingly, and despite regulatory requirement to submit the hAME study as a part of the submission package, out of 154 drugs analyzed, only 101 had information from hAME studies conducted during the development program. For all antiviral drugs and most oncology drugs, hAME studies were conducted as a part of the drug development programs; however, for ophthalmological indications, no hAME studies were included in the regulatory submission.

This paper provides an overview of the main aspects to take into consideration when CPs need to be enrolled in hAME studies.

2 | Method

An electronic search of the US FDA and EMA websites, the International Conference on Harmonization (ICH), and the PubMed database were reviewed to obtain the most important

regulatory guidance documents and main literature to discuss the relevant aspects of this topic. Search terms included: healthy volunteers, oncology, cancer, mass balance, administration, metabolism, and excretion (AME). Also, a comprehensive manual review of ClinicalTrials.gov was done; the filters included: (1) period: from January 2009 to September 2025, (2) target population: oncology and cancer, and (3) other terms: mass balance, administration, metabolism, excretion, or AME. All studies with these filters, independently of the type/mechanism of action of the molecule and the sponsor, were reviewed and are included in Table 2.

3 | Results

3.1 | Selection of Subjects: Healthy Volunteers (HVs) vs. Cancer Patients (CPs) and Differences in the Design

The selection of HVs vs. CPs depends on the characteristics of the drug under development, mechanism of action, preclinical package, toxicology findings, and clinical safety data.

While oncology hAME studies conducted in HVs may be justifiable in some cases, CPs may be selected in case of drugs with potential epigenetic, genotoxic, carcinogenic, and/or clastogenic effects, with non-reversible and non-readily monitored adverse effects, and poor translatability of the animal model toxicological data [14–17].

A review of clinicaltrials.gov data covering the period from January 2009 to September 2025 found 44 hAME studies conducted in CPs [4]. The patient population enrolled was mainly "all-comer" or solid tumors, and a few in CPs with hematological malignancies. The majority of the candidate drugs were epigenetic targeted agents with a variety of mechanisms of action, and in all, ^{14}C was the radioisotope used (see Table 2).

With respect to the design of a hAME study, there is very little difference in whether the study is conducted in CPs or in HVs (see Table 3). A hAME study conducted on CPs usually has more focus on minimizing, as much as possible, the burden to the patients. The minimum duration of the study is determined by the plasma elimination half-life ($t_{1/2}$) of the compound, and typically the subjects would have excreta and blood collections for at least 5.5 times the longest $t_{1/2}$ for the parent or any known metabolites. For HVs, the study duration may be extended further, on an individual subject basis, if insufficient recovery of total radioactivity has been obtained. However, for CPs it is preferable to have a fixed duration for the minimum time expected to be necessary to meet the study objectives, typically 5.5 times the $t_{1/2}$.

In terms of the sample size, US FDA guidance [2] recommends that a mass balance study should include data from at least six evaluable HVs and therefore, for HVs studies typically eight subjects are dosed to ensure at least six provide evaluable data. However, in CPs, the data can be reviewed after each patient to determine if they are considered evaluable to ensure a minimum number of patients are dosed. In addition, if the results are clear and the variability is low, it may be possible to meet the study objectives with fewer than 6 evaluable CPs.

Also, it is relevant to consider CPs' perspective to accept their participation in this study, as their direct benefit from the study may be low, except for a potential benefit with continued access/extension phase study.

3.2 | Study Extension

As during the hAME assessment period, no benefit is expected in CPs for a single dose administration; therefore, it would not be ethical to enroll CPs with no other options of treatment and in the late stage of the disease without any further option to receive the study drug. The study should therefore offer the opportunity to CPs to continue the treatment if the principal investigator considers that it is in the best interest of the patient and the patient consents to it [18, 19].

After the completion of the mass balance assessment part, the IMP will continue to be administered in an extension phase, open label access, or roll over protocol (note, a roll over protocol allows continued access to the drug for patients from different protocols but who are no longer eligible to receive the drug under the original protocol) until disease progression and/or unacceptable toxicity, initiation of new anticancer therapy, withdrawal of consent, discontinuation at the discretion of the investigator, non-compliance with protocol, death, or discontinuation for any other reason [18–21].

The protocol and the informed consent form (ICF) must clearly detail the expected duration of the continued access, the dose to be administered, and the required follow-up assessments.

3.3 | Patient Population

Based on the search done in Clinicaltrial.gov, and as previously mentioned in section "Selection of subject", in most cases, CPs enrolled are "all comer" patients with solid tumors or even hematological malignancies. Cancer patients with advanced solid tumors or hematological malignancies who progressed on standard therapy and for whom no other options of treatment are available constitute a fragile population [4, 20, 21]. However, patients selected for hAME studies should be clinically stable with adequate hepatic, renal, biliary, and gastrointestinal function, few comorbidities, and concomitant medications which could lead to potential drug interactions.

Many oncology drugs are developed to target a specific tumor biomarker, and inclusion of CPs who express that marker is burdensome, with potential to lengthen recruitment period of the hAME study. Therefore, patient eligibility criteria need to be carefully considered. As an example, the hAME study of niraparib, a PARPi designed for patients with BRCA-mutated and non-mutated ovarian patients included six female patients (three with ovarian, two with breast and one with colorectal with metastatic or locally advanced tumor) and did not require a positive BRCA mutation status [4, 22–24].

Figure 1 shows the journey of a CP during their participation in a hAME study.

TABLE 2 | Examples of Recruiting/Active/Completed/Terminated hAME clinical Trials in CPs from ClinicalTrials.gov.

NCT ID	Drug	Mechanism of action	hAME Population
NCT02031055 (completed)	TAS-102 (Lonsurf)	Thymidine-based nucleoside analog acting in the synthesis of DNA	Advanced solid tumors, excluding previously treated breast cancer
NCT01583777 (completed)	Belinostat	Histone deacetylase (HDAC) inhibitor	Advanced cancer
NCT04013048 (completed)	Fluzoparib	Poly (ADP-ribose) polymerase (PARP) inhibitor	Chinese patients with advanced solid tumors
NCT05246111 (completed)	Berzosertib	Inhibitor of the enzyme ataxia telangiectasia and Rad3 related (ATR)	Advanced solid tumors
NCT03804515 (terminated-COVID)	Poziotinib	Pan-HER inhibitor	Solid tumors suitable for treatment with poziotinib with either EGFR or HER 2 mutations or EGFR/HER2 overexpression/amplification
NCT02076230 (completed)	TH-302 (Evofosfamide)	Hypoxia-activated prodrug of the cytotoxin bromo-isophosphoramide	Locally advanced or metastatic solid tumor
NCT03057366 (completed)	Pevonedistat	NEDD8-activating enzyme (NAE) inhibitor	Advanced Solid tumors
NCT02390154 (completed)	Roniciclib	Pan CDK inhibitor	Advanced solid Tumors
NCT05371899 (recruiting)	Chiauranib	Multi-target inhibitor against tumor angiogenesis	Small Cell lung carcinoma (SCLC)
NCT01386866 (completed)	AMG 706 (Motesanib)	Multi-target tyrosine kinase inhibitor	Advanced solid tumors
NCT01714947 (completed)	Alisertib	Inhibitor of Aurora A kinase (AAK)	Advanced solid tumors or lymphomas
NCT05976334 (recruiting)	Subasumstat	Inhibitor of small ubiquitin-like modifier (SUMO)-activating enzyme	Advanced or metastatic solid tumors
NCT01953783 (completed)	Ixazomib	Proteasome inhibitor	Advanced or metastatic solid tumor or lymphoma
NCT01387204 (completed)	GSK1120212 (Trametinib)	Inhibitor of the MEK1 and MEK2 (MEK1/2) enzymes	Advanced solid tumors
NCT03070548 (completed)	Talazoparib	Poly (ADP-ribose) polymerase (PARP) inhibitor	Advanced solid tumors, limited to platinum-resistant ovarian carcinoma, cervical adenocarcinoma, small cell lung carcinoma or triple-negative breast cancer
NCT02986100 (completed)	Rucaparib	Poly (ADP-ribose) polymerase (PARP) inhibitor	-Advanced solid tumor -Part II only: known deleterious BRCA1/2 mutation (germline or somatic)
NCT01296568 (completed)	LY2603618 (Rabusertib)	Inhibitor of checkpoint kinase 1 (CHK1) protein kinase	Advanced and/or metastatic solid tumors
NCT01019161 (completed)	AZD1152 (Baracertib)	Aurora B kinase inhibitor	Relapsed, refractory or acute myeloid leukemia (AML), or new diagnosed not suitable for standard induction and consolidation chemotherapy

(Continues)

TABLE 2 | (Continued)

NCT ID	Drug	Mechanism of action	hAME Population
NCT01145885 (completed)	BI 6727 (Volasertib)	Inhibitor of the polo-like kinase 1 (PLK1) protein	Advanced solid tumors
NCT01529684 (completed)	OSI-906 (Linsitinib)	Dual inhibitor of insulin-like growth factor-1 receptor (IGF-1R) and insulin receptor (IR)	Advanced solid tumor
NCT05406817 (completed)	Revumenib	Inhibitor of the menin with lysine methyltransferase 2A(KMT2A)	Relapsed or refractory acute leukemia, including MRD-positive participants and participants with isolated extramedullary disease
NCT05008913 (Terminated)	Adavosertib	Inhibitor of the tyrosine kinase WEE1	Advanced solid tumors
NCT01164891 (completed)	RO5185426 (Vemurafenib)	BRAF inhibitor	Metastatic Melanoma
NCT02476552 (completed)	Niraparib	Poly (ADP-ribose) polymerase (PARP) inhibitor	Advanced solid tumors that may benefit from treatment with a PARPi
NCT01262963 (completed)	GSK2118436 (Dabrafenib)	BRAF inhibitor	BRAF Mutant Solid Tumors
NCT01387204 (completed)	GSK1120212	MEK inhibitor	Advanced solid tumors
NCT03010982 (completed)	Tazemetostat (EPZ 6438)	EZH2 inhibitor	B-Cell Lymphomas or Advanced Solid Tumors
NCT01286974 (Terminated)	Linifanib	Inhibitor of vascular endothelial growth factor and platelet-derived growth factor receptor tyrosine kinases	Advanced Solid Tumors
NCT03053219 (completed)	AC0010 (Avitinib)	Pyrrolopyrimidine-based irreversible EGFR inhibitor	Locally advanced or recurrent NSCLC failed to the treatment of EGFR-TKI and harbored T790M mutation
NCT02640755 (completed)	AZD2014	mTOR inhibitor	Advanced solid tumor
NCT03991494 (completed)	Pamiparib	Poly (ADP-ribose) polymerase (PARP) inhibitor	Advanced solid tumors
NCT02828930 (completed)	Idasanutlin	Antagonist of mouse double minute 2 (MDM2)	Advanced solid tumors
NCT02456883 (completed)	ASP2215 (Gilteritinib)	Inhibitor of AXL receptor tyrosine kinase	Advanced Solid Tumors
NCT02578316 (completed)	E7080 (Lenvatinib)	Multitarget tyrosine kinase inhibitor	Advanced Solid Tumors or Lymphomas
NCT00908908 (completed)	Eribulin	Tubulin-based mitotic and antimitotic inhibitor	Advanced Solid Tumors
NCT02622932 (completed)	Anlotinib	Multitarget tyrosine kinase inhibitor	Advanced solid tumors
NCT05613036 (Active, not recruiting)	BI 907,828 (Brigimadlin)	MDM2–p53 antagonist	Advanced solid tumors
NCT01713036 (completed)	Pimasertib	MEK1/2 inhibitor	Advanced solid tumors
NCT06308263 (recruiting)	Tuvusertib	Inhibitor of ataxia telangiectasia and Rad3 related (ATR) kinase	Advanced solid tumors

(Continues)

TABLE 2 | (Continued)

NCT ID	Drug	Mechanism of action	hAME Population
NCT06302140 (terminated)	Nanatinostat	Class I selective HDAC inhibitor	Advanced solid tumors
NCT01164891 (completed)	RO5185426	BRAF inhibitor	Metastatic Melanoma Stage IIIc or IV
NCT01583777 (completed)	Belinostat	HDAC inhibitor	Advanced solid tumors
NCT01023386 (completed)	YM155	Survivin inhibitor	Advanced solid tumors
NCT06713369 (recruiting)	AZD5305 (Saruparib)	PARP inhibitor	Advanced solid tumors

3.4 | Pharmacy

Pharmacy considerations for hAME studies in CPs present several challenges. For many hAME studies, a unique formulation may need to be utilized because of the use of the radiolabel. As ^{14}C is the isotope of choice for hAME studies [5, 6], Phase 1 units that perform these studies in HVs are often licensed to use this isotope. However, ^{14}C is not an isotope that is typically used for routine medical procedures; hence, many hospitals may not be adequately licensed to possess and use ^{14}C at their site. This may mean that sites that were previously utilized to conduct oncology non-labeled trials may not be suitable to enroll CPs for the hAME study, which increases the difficulty and complexity to find appropriate sites to conduct such studies.

Most Phase 1 units that conduct hAME trials are experienced with formulating radiolabeled compounds required for these studies. However, a pharmacist in a hospital setting may not have the training and experience for a CP hAME study. Therefore, an investigational medicinal product (IMP) should ideally be provided to a trial site ready to administer with minimally required manipulation. For example, simple reconstitution and/or dissolution in a dosing vehicle, involving only minor stirring or sonication, would be optimal to facilitate ease of administration.

An important aspect to highlight is that in the United Kingdom [25] and the European Union (EU), any formulation beyond a simple reconstitution or dilution would require that the radiolabeled IMP be formulated at a facility under a good manufacture practice (GMP) license [26]. It is not common for a hospital unit to have on such a Radioactive Materials License, and consequently an external pharmacy or contract manufacturing organization (CMO), sometimes in a different country, would be needed to manufacture and ship the radiolabeled IMP unit doses to the study site. In many countries this process may require the identification of a vendor offering Qualified Person (QP) services to batch certify the IMP and an appropriate provider to act as the Importer of Record (IOR). Additionally, utilizing vendors may result in multiple audits, confirming their capabilities including generation of Quality Agreements with these necessary stakeholders before any actual work being performed.

On the other hand, in the US, there is no such requirement that the radiolabeled active pharmaceutical ingredient (API) [27–29] or IMP must be manufactured under GMP conditions, but both must still meet quality standards as set forth by the FDA, United States Pharmacopeia (USP), and/or ICH regulations. However, there may be additional training required at the study site or the possible use of a third-party pharmacy to formulate and ship the radiolabeled IMP to the study site, which should be considered when planning a CP hAME trial.

Finally, the stability and quality of the radiolabeled API and IMP need to be considered as the formulation plan is being determined. The radiolabeled form of an API may not be as stable as the non-labeled form; therefore, stability determination of the API would need to be established. As the recruitment of patients may be a slow process and may be longer than the established stability of the radiolabeled API, repurification of the API may be required by a CMO. A percentage of the material would be expected to be lost during each re-purification; therefore, additional radiolabeled material may need to be manufactured. If the radiolabeled investigational medicinal product (IMP) must be manufactured and transported to the study site, its stability would need to be established over the entire timeframe required for formulation, quality release, shipment, and administration. This process may need to be performed on a per-patient basis.

3.5 | Bioanalytical Aspects

As the main objectives of a hAME trial are to determine the mass balance of total radioactivity administered, which requires data related to absorption, rates and routes of excretion of drug-related radioactivity, PK of total radioactivity, and parent and key metabolites [10], it is necessary to meticulously first collect all the urine and feces that a subject or patient produces during their defined collection period.

Several analytical methods are utilized to meet the study objectives. Liquid Scintillation Counting (LSC), which is a method for determining the content of radioactivity in samples for low energy, beta-emitting isotopes such as ^{14}C, is first used to determine the concentration of radioactivity in all the collected samples and the percentage of radioactivity being excreted in urine and feces samples. Liquid Chromatography (LC) is used to separate the different compounds in a sample, and Mass Spectrometry (MS) is then utilized to enable the identification and quantification of the different compounds based on their mass-to-charge ratio. MS also helps to determine the concentration of parent drug and metabolites in the study samples and in the identification of the different metabolites observed in urine, feces, and plasma samples.

TABLE 3 | Differences in the design of hAME in CPs vs. HVs.

	hAME in CPs	hAME in HVs
Study Design	Open label, non-randomized, two parts	Open label, non-randomized, single dose
Number of sites	Normally one	One
Dose	^{14}C macrotracer	^{14}C macrotracer[a]/ microtracer[b]
Study Population	Adults CPs with solid tumor and/or hematologic malignancies	Adults Healthiest
Study Duration	Relatively long- precise patients' selection	Short- rapid subjects' accrual
Primary Objectives	- To characterize the primary rates and route(s) of elimination and to estimate the overall recovery in urine and feces of radiolabelled [^{14}C]-IMP -To characterize the PK of total radioactivity following a single oral dose of [^{14}C]-IMP. - To determine the metabolites (if applicable) relative to total [^{14}C]-IMP related exposure, and its identification in the systemic circulation.	
Secondary Objectives	-To assess the safety and tolerability of [^{14}C]-IMP	
Regulatory Requirements- Genotoxic Studies	Not considered essential to support clinical trials	As a minimum a reverse gene mutation test in bacteria (AMES test) is required

Abbreviation: IMP, investigational medicinal product.
[a]Radioactive dose > 1 μCi.
[b]Radioactive dose ≤ 1 μCi.

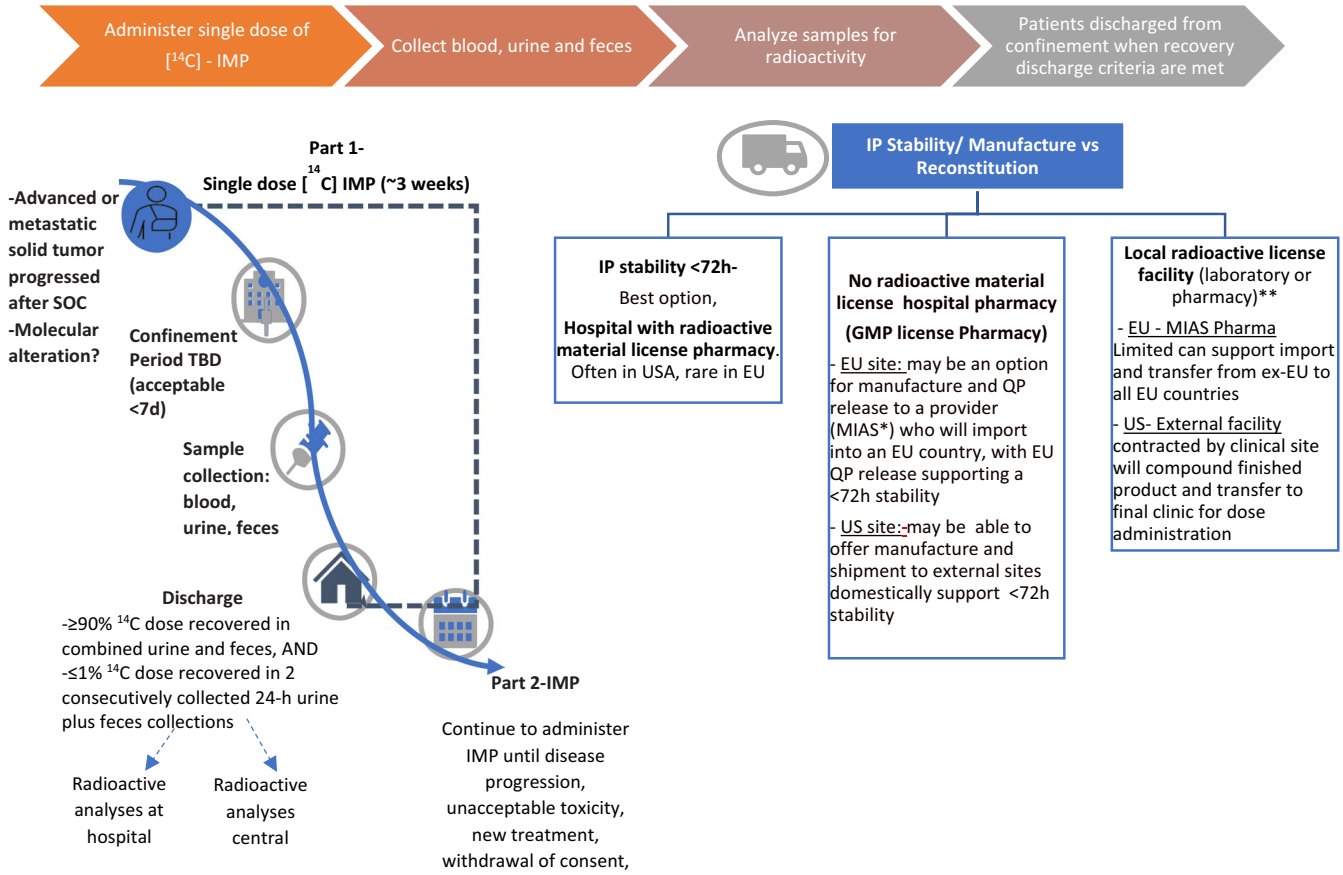

FIGURE 1 | Journey of a cancer patient through a hAME study.

To ensure that enough radioactivity has been recovered in samples to aid in meeting these analytical endpoints, criteria are written into the clinical protocol objectives for subjects to be released from the clinic based on the amount of radioactivity recovered in excreta and sometimes plasma. As a result, and following regulatory guidance [2, 7], most Phase 1 hAME protocols

require that at least 90% of the administered radioactivity be recovered in the urine and feces, and the subjects are excreting < 1% of the total radioactivity before they are discharged from the clinical research unit (CRU). There may also be a criterion that plasma levels of radioactivity are below the limit of quantitation (BLQ) for at least 2 consecutive collection timepoints before they are discharged.

Studies in HVs can be planned for a duration at which the subjects would be expected to meet these criteria. However, for CPs, a prolonged stay in a hospital unit may not be feasible, especially with the flexibility to extend the confinement period if the criteria are not met.

Many hospitals will use a kit system to collect and ship samples to the analytical lab. Collection kits could be provided for the patients to perform at-home collections if possible. Nevertheless, samples may need to be stored at ultra-cold temperatures to preserve the stability of the parent and/or metabolites in the matrix, which could be a limitation of at-home collection, together with the risk that CPs may not collect all samples produced.

Sometimes, there is a need to add a stabilizing agent to the urine or plasma samples to prolong the stability of either the parent drug or a metabolite in the sample or a surfactant may be required in urine samples to prevent the molecule from sticking to the collection containers being used. This may require collections to be performed in a controlled setting where the samples are collected and processed by a trained individual. For this scenario, some additional visits could potentially be added to the study design in which a patient would return to the hospital approximately weekly for a 24-h stay in which to collect blood and excreta samples. An estimate could then be made for the excretion that occurred between visits, based on the known recovery data, which should be done with caution as it assumes drug is excreted under first order metabolism [2].

It should be noted that, when selecting hospital sites to conduct a hAME study, there may be some lack of awareness of procedures for safe handling and disposal of ^{14}C, which, in turn, may cause reluctance by the site to conduct these studies. A hospital site will typically use penetrating radiation for diagnostic tests, imaging or radiopharmaceuticals. There are standard safety measures in place for the handling of the radioactive material, including collection, handling and processing of samples, disposal of remaining materials and even proximity of staff to the patient once dosed. Hospitals may assume that the procedures needed for the use of ^{14}C are similar to those isotopes they frequently use. Accordingly, it should be highlighted that as the sites realize that ^{14}C is a low-level beta emitter, then it can easily be shielded using the same universal procedures used to handle biological samples (i.e., lab coat, gloves, safety goggles). However, it has different limitations on disposal of materials, and no limitations on the proximity to the patient once dosed, which may make a site more amenable to conducting such a study.

Therefore, handling, processing, and storage of the samples are critical for achieving the main objectives of the study, and it will require experienced/trained nursing and local laboratory staff.

3.6 | Dose

There has been a regulatory shift in recent years to stress the importance of having a better understanding of circulating metabolites and the time when such data should be available during the drug development process [17, 30]. Selection of the radioactive dose to be administered is key to ensuring that a sufficient amount of radioactivity is being excreted in samples to provide an adequate analytical sensitivity to detect and quantify circulating metabolites. Dosimetry calculations will inform how much radioactivity would be considered safe to administer for a single study. Once dosimetry has been completed, the laboratory responsible for metabolite profiling and identification can use the available pharmacokinetic (PK) data for the selected chemical dose to estimate the optimal radioactive dose required to support the analytical endpoints.

Historically, about half of the HV hAME studies conducted by our Phase 1 research unit, a global clinical research organization, have administered a radioactive dose of about 100 μCi, but there has been a trend observed in recent years of administering greater than 100 μCi to ensure that sufficient radioactivity is administered to fully analyze and understand the circulating metabolites in plasma. European regulations typically allow radiation exposures from the radioactive dose up to 1 mSv per study, although, in our experience, in the United Kingdom, it has been possible on occasions to justify exceeding this exposure. In the experience of our US clinic in Madison, WI, almost 80% of the radiation exposures were less than 1.0 mSv, even though many of our studies were administered at a radioactive dose at or above 100 μCi [3].

It may not be possible to administer a higher amount of radioactivity due to limitations of dosimetry, molecular limitations for radiolabeling, or a company/sponsor policy of keeping radiolabeled doses as low as possible. This may require administration of the radiolabeled dose at a microtracer level of radioactivity (typically < 1 μCi), with subsequent quantification using accelerator mass spectrometry (AMS). AMS is a highly sensitive technique that uses a particle accelerator to separate ^{14}C ions based on their mass-to-charge ratio, enabling the detection of radioactivity in biological samples.

3.7 | Confinement

In accordance with regulatory requirements [2, 5], the collection period for excreta (urine and feces) in the hAME study should be of sufficient duration to ensure adequate recovery of the administered dose. Similarly, the plasma sampling period for metabolite profiling should be sufficiently prolonged to enable comprehensive characterization of circulating radioactivity, including the detection of any late-emerging or persistent metabolites.

Ideally, up to 6 days of hospital stay may be acceptable by sites and CPs. However, it is important to note that CPs enrolled in hAME studies typically share characteristics with those participating in Phase I oncology trials. These patients are generally considered vulnerable, with advanced disease, limited or no remaining treatment options, and a relatively short life expectancy (typically > 12 weeks), and may experience significant emotional and social stress related to the poor prognosis of their disease [18].

Therefore, a study design with low impact on their quality of life related to hospitalization and sample collection should be prioritized. A longer confinement could negatively impact a patient's likelihood to consent, and for sites readiness to allocate scare resources (bed, personnel) to the study.

4 | Discussion

The present overview details the main features, from patient selection to regulatory and hospital/staff requirements, to conduct hAME trial in CPs.

The decision regarding the appropriate study population, i.e., healthy volunteers (HVs) versus cancer patients (CPs), is complex and should take into account the mechanism of action, toxicological findings, and overall safety profile of the investigational agent. In this context, the inclusion of HVs in trials evaluating epigenetic therapies, clastogenic or genotoxic agents, compounds associated with irreversible toxicological effects, and/or agents with adverse effects that cannot be adequately monitored would generally be considered inappropriate [14, 20, 31].

Table 2 illustrates hAME trials with different classes of agents in which CPs were included [4]. Molecules such as histone deacetylase inhibitor, poly (ADP-ribose) polymerase (PARP) inhibitor, inhibitor of the enzyme ataxia telangiectasia and Rad3 related (ATR), tyrosine kinase inhibitors, proteasome inhibitors, BRAF inhibitors, etc. usually require CPs to be included in the hAME trial.

In addition, physiological differences between CPs and HVs may impact trial data. CPs may have gastrointestinal complications, such as nausea and vomiting that can affect drug absorption. The volume of distribution may be altered for hydrophilic and lipophilic drugs based on changes in body composition (e.g., increased adiposity, cachexia) and drug metabolism and excretion could be affected due to liver primary or metastases in CPs or by the presence of comorbidities such as diabetes. It is also important to recognize that CPs are generally an older population who frequently receive multiple concomitant medications, thereby increasing the risk of drug–drug interactions (DDIs) that may complicate the interpretation of hAME study results [32–34]. Therefore, careful selection of study participants is critical to minimize toxicity, reduce dropouts, and limit the need for patient replacement, all of which can impact the timely completion of the trial. For CPs with no remaining standard treatment options, the study design must minimize any impact on their quality of life, particularly with regard to hospitalization and sample collection. Clear communication with patients and their relatives is therefore essential to ensure understanding of the trial requirements and hospitalization needs. Prolonged confinement may negatively affect a patient's willingness to provide informed consent and may also challenge site readiness to allocate scarce resources, such as beds and personnel, to the study.

Additionally, managing the isotopes, handling the samples, and the disposal of the remaining material will require working with experience sites, transversal nurse collaboration, and providing ongoing proper staff training to guarantee trial success.

5 | Conclusion

hAME studies are a critical component of the clinical pharmacology package required to support regulatory submissions for new drugs. Consequently, it is important to develop more efficient strategies for their successful execution. Careful consideration is required to determine whether a particular hAME study should include CPs or HVs, based on the characteristics of the investigational agent, protocol-specific requirements, and the capacity of the investigational site and pharmacy to conduct such complex studies. It is essential that early-phase clinical research units and oncology hospital sites continue to build expertise through experience in conducting hAME studies in CPs, particularly as the oncology drug development pipeline continues to expand.

Author Contributions

B.H., C.O., P.A., H.H., P.N., L.V.B., C.L., J.B., E.C. designed the research, performed the research, and analyzed the data. B.H., C.O., C.L., K.S.S. wrote the manuscript.

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Consent

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Conflicts of Interest

K.S.S. reports consulting fees from the European Commission, and stock and/or other ownership interests in Fortrea Inc. and Quantum Health Analytics (UK) Ltd., outside the submitted work. All other authors declare that they have no conflicts of interest relevant to this manuscript.

Data Availability Statement

All data and references mentioned in this manuscript are from publicly available sources.

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