PERSPECTIVE

feature

The impact of early human data on clinical development: there is time to win

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Modern accelerator mass spectrometry (AMS) methods enable the routine application of this technology in drug development. By the administration of a ^{14}C-labelled microdose or microtrace, pharmacokinetic (PK) data, such as mass balance, metabolite profiling, and absolute bioavailability (AB) data, can be generated easier, faster, and at lower costs. Here, we emphasize the advances and impact of this technology for pharmaceutical companies. The availability of accurate intravenous (iv) PK and human absorption, distribution, metabolism, and excretion (ADME) information, even before or during Phase I trials, can improve the clinical development plan. Moreover, applying the microtrace approach during early clinical development might impact the number of clinical pharmacology and preclinical safety pharmacology studies required, and shorten the overall drug discovery program.

Introduction

Drug development is a challenging business. Over the years, the window for new opportunities has narrowed, drug targets have become more complicated, and the number of newly approved medicines has diminished. Pharmaceutical companies spend an enormous part of their overall budget on drug discovery; thus, counteracting the falling success rates of drug development is essential [1]. Many compounds fail sooner or later because of undesirable PK, insufficient efficacy, and/or safety concerns that were not foreseen even after having a plethora of data available from animal studies [2]. In a perfect world, information on human metabolism, PK, and AB would be available early during drug development. This ideal situation would

arise if novel candidate drugs could be evaluated directly in humans and, as a result of innovative analytical approaches, such an ideal is now within reach. Microdosing and/or microtracing allows the generation of human data early during development, resulting in a more cost-effective drug discovery pipeline [3–5]. Here, we offer a perspective on the impact of microdosing and/or microtracing research on the clinical development plans of pharmaceutical companies.

A modernized development design minimizes unpleasant late-stage surprises, with the consequent discontinuation of the lead compound. However, this requires general acceptance of advanced methodologies that bring new opportunities to the field. By strategically

selecting the right studies at the right time, experiments can be removed from the critical path. For example, the early availability of human metabolite data enables researchers to plan toxicological studies, and only useful drug–drug interaction studies would need to be performed. Moreover, the number of clinical pharmacology studies during development could be decreased, because a mass balance study can easily be incorporated into a standard Phase I study. Additionally, the formulation of a compound could be optimized if its bioavailability is limiting, and piggy-backing one or more back-up compounds as a microdose onto a standard Phase I study would derisk any program.

Even with these suggested changes, the overall process from drug discovery to market

release would remain lengthy and expensive. In the competitive field of the pharmaceutical industry, the period to profit from a temporary monopoly is essential for a return on investment, and such revenues support research and development (R&D) activities in new fields. Increasing the effective length of the patent of a compound positively affects the financial picture. Earlier release of the new therapeutic would also be in the interest of patients, because their medical needs would be met sooner.

Studies including a ^{14}C label

In many clinical studies, the compound levels in plasma whole blood, urine, and faeces are analyzed. In traditional mass balance studies, radioactive dose levels are typically between 50 and 200 μCi, although studies have been published administering doses of up to 200 mCi [6]. To dose these amounts of radioactivity, human dosimetry calculations are required, for which several models can be applied [7]. The models are based on International Commission on Radiological Protection (ICRP) recommendations [8–11], and these are only possible when at least rat ADME and rat whole-body autoradiography (QWBA) data are available. The Novartis models also include human PK [12]. Moreover, the radiolabelled drug product for clinical use must be produced under good manufacturing practices (GMP). The advantage of a traditional mass balance study is that the analysis can be performed using straightforward and generally accepted liquid scintillation counters (LSC). However, because of the relative complexity of the human ADME study and the post-study labour-intense biotransformation laboratory work, these studies are often performed relatively late during development, at the end of Phase II or later.

The advantages of microtrace and/or microdose studies have widely been addressed in the literature [3,13–15]. The ability to dose at extremely low levels has substantial benefits. A typical microdose administered to human adult volunteers contains less than 1% of the therapeutically active dose or ≤100 μg (30 nmoles for biologicals) [16] and, in general, only 100 nCi ^{14}C radioactivity. Given that the resulting drug concentrations in the body are extremely low, safety issues (toxicological and radiological) are omitted. Quantitative whole-body autoradiography (QWBA) is not required, and a 14-day rodent toxicology study is sufficient to allow dosing of humans [16].

For quantification of these low drug (related) compound concentrations, an accelerator MS

(AMS) is necessary, which displays exceptional sensitivity [17]. Given this extreme sensitivity, study samples that are expected to contain relatively 'high' levels of activity should be avoided to prevent contamination of the instrument. For example, compounds that exhibit fast urinary excretion are prone to result in relatively large quantities of radiolabel in early samples. Therefore, such samples should be analyzed using conventional LSC. The prerequisite to combining AMS and LSC results into one PK data set is a linear relation between both methods. AMS has been compared with LSC, and has shown a good correlation [18,19]. However, either LSC results were extrapolated to the range of the AMS samples, or the LSC samples were diluted before analysis on the AMS. In both instances, AMS and LSC analysis were not conducted on the exact same sample.

The direction of entrenched drug discovery programs is not easily changed. The ability to make amendments requires trust from pharmaceutical companies, medical and/or ethical committees, and regulatory authorities. Although the added value of microtracer and/or microdosing research has been acknowledged [20–23], it is our view that AMS analysis is still not used to its full potential, which might be partly because of a fear of change. People tend to like things that they are familiar with and, thus, would rather continue using the same old strategies. For us, this brings the challenge of convincing all parties involved in drug discovery of the added value of modern microdosing and/or microtracing strategies. Traditionally, AMS analyses are performed using solid graphite; however, at TNO, we have further developed the direct combustion AMS (cAMS) methodology, initially described by Ruff et al. [24]. cAMS offers several advantages, such as increased sample throughput (^{14}C total count within 15 min/sample) and the requirement for minimal amounts of sample [25]. The limited sample volumes aid the investigation of PK parameters in vulnerable populations, such as neonates and young children [20,21].

Overlapping dynamic ranges for LSC, LLSC, and AMS: a single data set using different technologies

As mentioned for AMS, it is important not to overload the instrument with high levels of radioactivity. Therefore, it would be valuable to be able to select the proper analysis method (LSC or AMS) per sample. One of the challenges is to overcome the suspicion that it is impossible to generate one high-quality data set using multiple techniques. Thus, we performed a direct

head-to-head comparison of cAMS, low-level scintillation counting (LLSC), and LSC.

Figure 1 shows the linear correlation between cAMS, LLSC, and LSC, with an $R^2 = 0.994$. We realize that it is not a common standard to generate a single data set using different technologies; however, this comparison clearly shows that there is absolutely no compromise on data value and quality.

Total count qualifications are independent of the original compound, because single ^{14}C ions are detected. Only the nature of the matrix influences the dynamic range, because the natural carbon levels differ per matrix. Previously, qualification results were published for the ^{14}C total count analysis on plasma using cAMS [25]. Here, we summarize the qualification results for the ^{14}C total count analysis in human whole blood, urine, and faeces (Table 1; for further details, see Tables S1–S12 in the supplementary information online). Out of these four common matrices, faeces was the most challenging. Large sample volumes were processed to guarantee homogeneity, resulting in substantial amounts of ^{14}C and ^{12}C. However, because AMS records the ^{14}C:^{12}C ratios, these were still well within the dynamic range. All qualifications were performed in accordance with the European Bioanalysis Forum recommendation: *Scientific Validation of Quantification by Accelerator Mass Spectrometry* [26].

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Volume linearity

The volume linearity of LSC, LLSC, and cAMS was determined using plasma, urine, and faeces. For LSC and LLSC, the t-SIE value indicates the
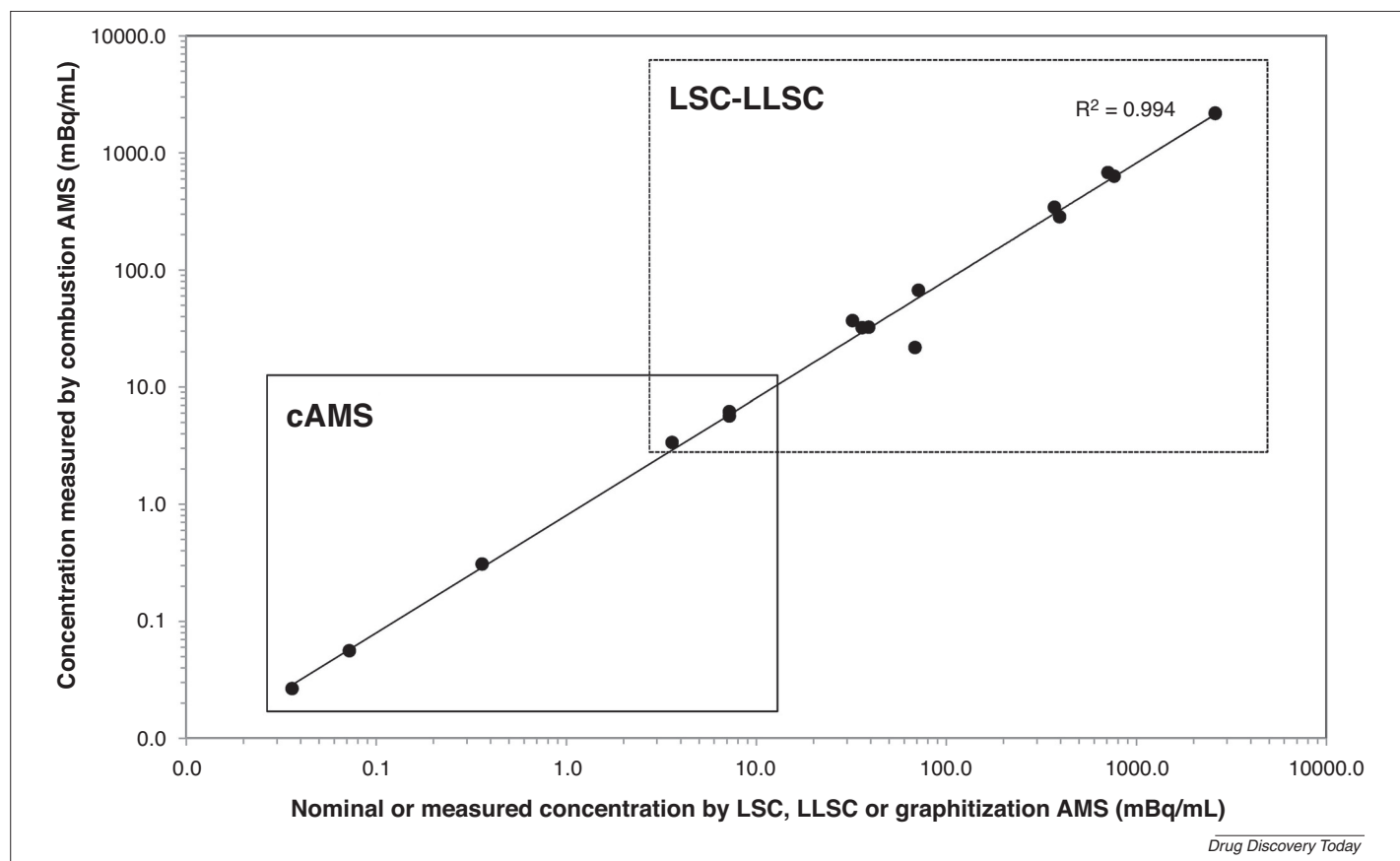
FIGURE 1

A head-to-head comparison of measured ^{14}C concentrations in plasma by liquid scintillation counters (LSC), low-level scintillation counting (LLSC), and graphitization accelerator mass spectrometry (AMS) with combustion AMS. Over the entire concentration range, the various analysing techniques prove to be linear; thus, it is possible to generate a single pharmacokinetic (PK) data set using multiple techniques.

TABLE 1

Total ^{14}C count qualifications performed on human matrices (plasma, whole blood, urine, and faeces)[a]

Matrix	Volume (µl)	Dynamic range (mBq/ml)	Absolute amount of activity (µBq)	Absolute amount of ^{12}C (µg)
Urine	15	0.41–102.5	0.75–1537.5	72.1
Whole blood	5	1.90–950.9	9.50–4754.5	551.9
Whole blood (10× dilution)	8	1.96–147.7[b]	15.68–1181.6	91.7
Faecal homogenate	30	2.64–528.0	79.2–15 840	804.3
Plasma	1.8	0.65–821.2	1.2–1478.2	70.4

[a] All qualifications were performed in accordance with the European Bioanalysis Forum recommendation: *Scientific Validation of Quantification by Accelerator Mass Spectrometry* [26]. This table summarizes the major characteristics per matrix; i.e., processed sample volume, dynamic range, and absolute amounts of ^{12}C and ^{14}C.
[b] Back calculated to undiluted volume.

reliability of the result and is used as a quench-indicating parameter (for details on t-SIE calculations, see the supplementary information online). t-Sie is calculated based on an external standard ratio method using a chemical quench set (Packard Instr). In the lower t-SIE range (<100), the slope of the quench curve (% efficiency versus t-SIE) is too high to allow accurate radioactivity measurements. Therefore, it was considered that results in disintegrations per minute (DPM) of samples showing a t-SIE value <100 were not relevant and should be omitted

(Jock Thomson, Perkin Elmer, Inc., 2014). Depending on the radioactivity level, the counting times may vary to generate accurate results. A summary of the LOD and LLOQ data, including all measurements, are presented in Tables S13–S17 in the supplementary information online. The cAMS analysis showed a high linear correlation over a volume range of 5–100 µl, 3–300 µl, and 10–100 µl for plasma, urine, and faecal homogenate samples, respectively (Table S18 in the supplementary information online).

The volume linearity of LSC, LLSC, and cAMS was determined using plasma, urine, and faeces. For LSC and LLSC, the t-SIE value indicates the reliability of the result and is used as a quench-indicating parameter (for details on t-SIE calculations, see the supplementary information online). t-Sie is calculated based on an external standard ratio method using a chemical quench set (Packard Instr). In the lower t-SIE range (<100), the slope of the quench curve (% efficiency versus t-SIE) is too high to allow accurate radioactivity measurements. Therefore, it was

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Analysing clinical samples from a human mass balance study

So far, the results from the spiked matrices support that cAMS, possibly in combination with LLSC, can be applied to generate high-quality data. As proof of principle, 70 clinical study samples (whole blood, plasma, urine, and faeces) from two finalized human mass balance studies were selected for analysis by cAMS. Previously, these samples had been analyzed by LSC or graphitization AMS [27,28]. The use of these samples for additional research investigations was covered by the clinical protocol and by informed consent.

The cAMS results deviated by less than 15% from the original data, except for two samples. One faecal sample deviated 49%, likely because of sample inhomogeneity, and one plasma sample deviated significantly (140%); however, the particular radioactivity in this sample was at, or very close to, the limit of detection (0.5 mBq/ml). The overall correlation coefficient (R^2), with and without the two outliers, was 0.968 and 0.970, respectively (Fig. 2).

Direct MIST assessment in human volunteers

Finally, we would like to increase the awareness that any microtracer study is a potential metabolite in safety testing (MIST) study. Instead of spending time and resources on animal studies and prediction tools, and still running the risk of being surprised by unexpected human metabolites at a late stage, a 'simple' Phase I study can be used to determine the entire human metabolite profile. Here, a human plasma sample from a mass balance study was analyzed by liquid chromatography (LC)–MS. Directly after chromatographic separation, the flow was split, whereby one fraction was coupled online to a high-resolution MS, and the other fraction was collected simultaneously for off-line total radioactivity analysis by cAMS. A radiochromatogram was reconstructed, showing the major metabolites formed in human volunteers (Fig. 3), which can be identified using the available high-resolution MS data.

Discussion

Here, we have highlighted how LSC and AMS can be applied interchangeably to determine ^{14}C levels in biomedical samples, such as whole blood, plasma, urine, and faeces, originating from preclinical and clinical ADME studies. The advantage of using multiple techniques to generate a single data set is that samples can be measured in a relatively short timeframe without compromising data quality. LSC counting times of more than 2 h to generate reliable results are
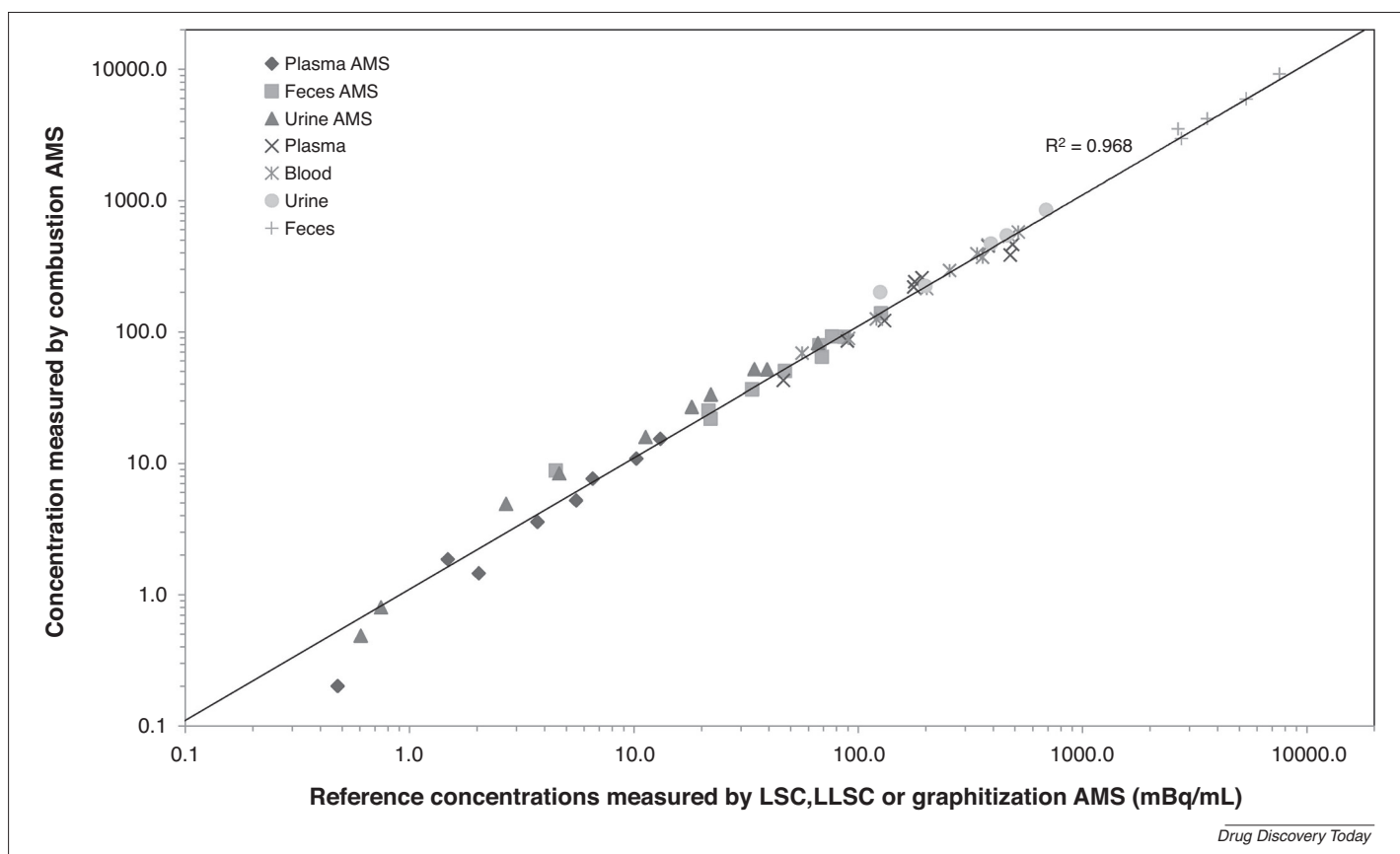

FIGURE 2

In total, 70 clinical samples (plasma, urine, whole blood, and faeces) were selected from two human mass balance studies. These samples had previously been analyzed by graphitization accelerator mass spectrometry (AMS) and liquid scintillation counters (LSC). Reanalysis by combustion AMS (cAMS) resulted in deviations <15% from the original data for 68 samples. Only one faecal sample and one plasma sample showed larger deviations, likely because of sample inhomogeneity and the low levels of activity (at or very close to the limit of detection), respectively.
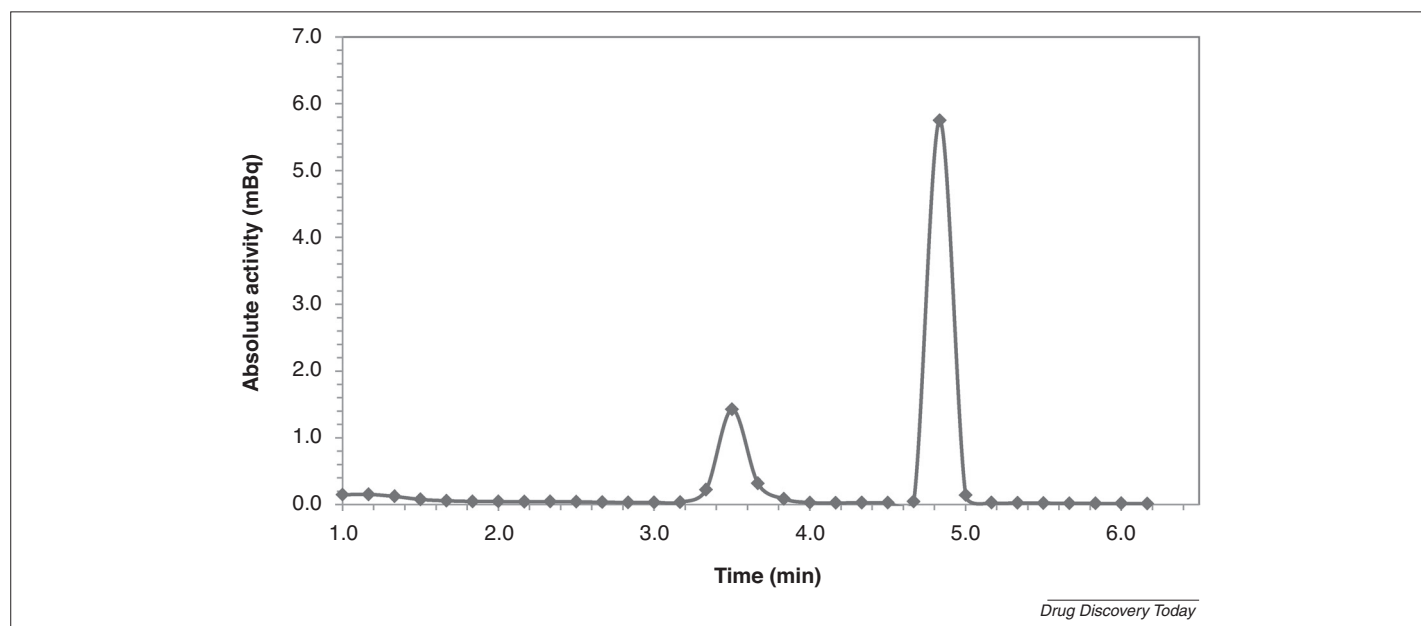
FIGURE 3

A metabolite profile from a human plasma sample analyzed by ultra performance liquid chromatography–combustion accelerator mass spectrometry (UPLC–cAMS). After UPLC separation, the flow was split, allowing simultaneous high-resolution MS analysis and fraction collection. The fractions were quantified offline for ^{14}C levels. This approach enables any Phase I study to become a mass balance study, saving time and resources.

no longer necessary, because, in such cases, cAMS will be faster and more accurate. Vice versa, samples from a mass balance study containing high levels of radioactivity can simply be analyzed using LSC.

Based on current knowledge, we claim that more efficient and effective drug development can be realized when using microdosing and/or microtracer experiments at the right point during compound development [5,14,29,30]. Microdosing studies can easily support the proper drug candidate selection, by providing early PK and AB data for multiple compounds. In addition, microtracer experiments can be used to generate mass balance and metabolite data in plasma and excreta, which are needed to understand the elimination pathways and assess the metabolite plasma exposure against the MIST and ICH M3 guidelines. ADME studies are relatively expensive and, therefore, a significant amount of resources will be saved when only the regulatory required and adequate (on promising compounds) toxicology studies are performed. Moreover, only the parent compound and metabolites comprising 10% of the total drug-related material require toxicology studies. As for microtracer studies, no formal human radioactivity exposure calculations are required [11], and preclinical studies and evaluations needed to determine the human dosimetry of the radioactive dose can be omitted. Taking all the mentioned issues into consideration, the current perception that microdosing and microtracer experiments are rather expensive is no longer valid. Table 2 provides an overview of the important stages during drug development. The

TABLE 2

Comparison of the estimated costs between a microdose and/or microtrace approach and a conventional high-dose study approach for several steps required drug development[a]

Topic	Microdose/microtrace ^{14}C	Approximate cost TNO (k€)	High-dose ^{14}C	Approximate cost (k€)
Production of ^{14}C-labelled compound	Non-GMP often allowed	50–100	GMP material required	100–400
Dosimetry studies	Calculations only	2	QWBA required	50
Clinical trial	Add on to existing trial	–	Separate trial	500
Total radioactivity measurements[b]	AMS	75	LSC	75
Metabolic profiling	UPLC online coupled to high-resolution MS and simultaneous fraction collection for AMS analysis	100 €/sample[c]	UPLC coupled to fraction collection for LSC counting	35 €/sample[c]
Metabolite identification	MS and nuclear magnetic resonance	[d]	MS and NMR	[d]
Data availability	During phase I	–	During phase III	–

[a] As indicated in the table, study designs are not always equivalent, but both are accepted by the EU and FDA.
[b] This includes the analysis of 15 plasma and 15 whole-blood samples in triplicate, the duplicate analysis of ten urine samples, and the fourfold analysis of ten faecal samples for four individual subjects.
[c] In practice, the study designs differ between the microtrace and high-dose metabolic profiling approaches. Often with AMS, the fractionation intensity is adjusted [i.e., by the analysis of pool samples first at medium resolution (30–60 s fractions), potentially followed by high-resolution fractionation at specific regions of interest (<10 s fractions)].
[d] Independent of the chosen metabolic profiling study design, the time and money spent on metabolite identification are comparable.

estimated costs between a microdose and/or trace approach versus a conventional high-dose study approach are listed.

Human radiolabelled studies will always be required, but there is now a choice of how to design and implement them. When a drug is developed as an oral formulation, producing a radiolabelled dose might be seen as an obstacle; however, the formulation of an IV microdose is relatively straightforward, and even feasible for poorly soluble compounds. Moreover, the material is not required to be produced under GMP [14]. This is somewhat more complicated for oral formulations, but a full GMP-produced clinical batch is often not needed. The radiolabelled investigational medical product (IMP) needs to be manufactured according to the European Union (EU) GMP and US Food and Drug Administration (FDA) guidelines. For most (micro)-trace studies, the radiolabelled IMP is a mixture of nonradiolabelled and radiolabelled drug substance. With the nonradiolabelled drug substance produced under GMP, there is no strict requirement to apply GMP synthesis for the radiolabelled drug substance, in particular when the percent of radiolabelled material is less than 0.15% of the dose. In microtrace formulations, the labelled amount could be considered as an impurity. Still, the facility manufacturing the final radiolabelled IMP should be GMP compliant.

Over the past few years, AMS has mainly been applied to late-stage microtrace human ADME studies [22,31–33]. Many of these studies were successfully completed, even those that were challenging with respect to study duration and complexity [27]. Knowledge of human PK before the selection of drug candidates for full development should preferably be a prerequisite. The long-standing fear of nonlinearity between microdose and therapeutic dose has been shown to be unfounded for most compounds [34]. Recently, a decision tree has become available to predict possible PK nonlinearity risks [30], further reducing the number of negative microdosing studies, either by altering the strategy or simply by skipping the microdosing study.

We have noticed that the increasing number of successful AMS stories in combination with technological developments have triggered more companies to reconsider their development programs. We are only at the start of routine AMS applications. The sample throughput is expected to increase substantially (over 140 samples unattended/day) and the instruments to become more 'user friendly' (i.e., smaller dimensions, less maintenance, and lower purchasing costs). Despite these advancements, we also emphasize that, if sensitivity is not a limiting factor to collecting the data of interest, then LC–MS/MS, possibly in combination with online or offline radioactivity counting, would still be the preferred technology. In addition, the worldwide capacity to perform cAMS is currently far from sufficient.

However, we are convinced that cAMS can help to move innovation in drug discovery on because of the major benefits discussed above. In particular, an understanding of the metabolite exposures and the metabolite pathways will help to set a more targeted and focussed approach for clinical drug–drug interaction studies and in- and exclusion criteria on co-medication Phase II and Phase III trials. The patient population and health care providers will profit from early access to novel oncological compound or drugs under development for other specific diseases for which treatment is still lacking. Under several circumstances, regulatory authorities are already supporting the accelerated release of novel compounds. In such situations, it will also be helpful to have some prior knowledge of the compound before dosing patients.

Concluding remarks

In summary, we have shared our perspective on what attributes could be addressed to enable drug development paradigms to become more efficient. Smart design of the drug development program can substantially save time and costs. In our view, the incorporation of microdosing and/or microtracing research should not be thought of as being high risk. Recent technological improvements in combination with the data presented here and success stories from the past justify the general acceptance of the approach by all parties involved in drug development. Thus, the future for both patients and pharmaceutical companies is positive.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drudis.2016.03.012.

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