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Improved early clinical development through human microdosing studies

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Since the early 1990s, the productivity of pharmaceutical R&D has been the subject of growing attention, both from the industry itself and external commentators. Rising R&D costs and falling numbers of marketing approvals have led to what has been described as a 'productivity crisis' [1–4].

Pharmaceutical R&D is a complex business. Its management requires clear strategies and decision-making processes to tackle the trade-offs in cost, time, product value and success probability that occur within individual projects and across product portfolios. The importance of this endeavour is underlined by the stark reality that 75% of the cost of drug development is on failures concentrated in the early stages [5] and reducing the cost of failure – either by failing candidates sooner or by improving the overall probability of success – is the most powerful solution to improving R&D productivity [6].

There is no simple recipe for 'fail early, fail cheaply'. However, consideration of a basic principle in pharmaceutical R&D provides some pointers. To a large extent, the outcome of drug development – success or failure against the target product profile – is predetermined at the point when a candidate new medicine is selected. Essentially, outcomes of efficacy and safety are a function of the interaction between the chemical or biological entity and the body. In other words, whether a drug will have the appropriate balance of desired and undesired effects is inherent in its physicochemical properties and physiological or biochemical processes. The same is true regarding the fate of the drug within the body – its absorption, distribution, metabolism and excretion (ADME) and hence pharmacokinetics (PK). Because neither the physicochemical properties nor biological processes are changed during drug development, pharmaceutical R&D can be viewed as a process by which the properties of the drug candidate are 'unveiled'. Therefore, strategies for 'fail early, fail cheaply' should provide information that differentiates successes from failures at the earliest opportunity and at the lowest possible cost.

Microdosing studies: human preclinical investigations?

Although toxicity is a growing cause of drug attrition, inappropriate PK properties remain the most cited reason for early development failure and account for 40% of exits from Phase I [7–9]. Consequently, confidence that a candidate medicine will have a PK profile consistent with the overall target product profile significantly enhances the probability of development success. Conversely, identification at an early stage of those compounds with inappropriate PK parameters enables them to be 'failed' before the high costs of later development are incurred.

In the past decade, several *in vitro* and *in silico* approaches have been developed that aim to predict probable PK properties ahead of drug candidate selection. However, although considerable progress has been made in building new tools and 'modernizing' the drug discovery mindset, a truly predictive ADME approach still eludes the pharmaceutical industry [10–12]. Therefore, although *in silico* and *in vitro* screens will improve the quality of drug candidate selection, there remains, for the foreseeable future, a need to confirm PK in the target species – humans.

Human microdosing (HMD) studies elucidate the PK profile, for example, rate (C_{max}) and extent of absorption (area under curve), plus half-life ($t_{1/2}$), at an earlier stage and at lower cost than can be achieved in 'traditional' first-in-human studies. From this information, compounds with inappropriate PK can be de-selected without additional development costs and those that meet the target product profile can be progressed with greater probability of success and hence confidence for further investment. These pre-Phase I or 'Phase 0' studies are performed using a fraction (usually μg quantities) of the proposed therapeutic dose and as such require a reduced preclinical safety package. The benefits of HMD studies can be measured in terms of time and cost (Table 1).

A HMD ADME study, therefore, comprises the administration of a sub-pharmacological, sub-therapeutic dose of novel drug candidate(s) to obtain essential PK information [13–16]. However, it should be noted that no efficacy or safety data are obtained from a HMD ADME study.

Before HMD, researchers might typically identify one or more drug candidates that

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TABLE 1

Summary of the requirements and benefits of microdosing versus traditional first-in-human studies

Requirement	Human microdosing	'Traditional' first-in-human studies	Benefits of human microdosing
Materials	Gram quantities	Kilogram quantities	Significantly lower quantities at a time when compound supply is often rate-limiting
Preclinical toxicology package	Reduced package	Standard	Significant reduction in cost and time
Time to completion	4–6 months	12–18 months	8–12 months

have demonstrated pharmacological activity *in vitro* and in animal models: some limited animal safety testing also needs to be conducted. After administration of doses (oral and/or intravenous) to human volunteers, relevant body-fluid samples are collected for subsequent analysis. This new method contrasts with the traditional approach in that early *in vivo* human metabolism data can make a key contribution to candidate selection. The ADME data from microdose studies can be fed into *in silico* PK models to obtain a much better estimate of the probable pharmacological dose in future efficacy studies (Figure 1).

One of the major advantages provided by the UK regulatory environment for microdosing studies has been recognition by the Medicines and Healthcare Products Regulatory Agency (MHRA) that Good Laboratory Practice (GLP) active pharmaceutical ingredient (API) can be used for these Phase 0 investigations. This constitutes a significant saving in time and cost for moving into early human studies because it reduces significantly the validation and documentation of the synthesis process. In addition, it also indicates that the regulators recognize the argument of 'fitness for purpose' in the differing stages of drug development.

Preclinical requirements for human microdosing studies

Obtaining efficacy and safety data is not the essential aim of microdosing studies, rather they gather PK data for new drug candidates. This contrasts with conventional Phase I studies, which try to demonstrate tolerability and safety in a small number of subjects starting with a relatively low drug exposure. A recent position paper from the European Agency for the Evaluation of Medicinal Products (EMEA) proposed that a human microdose should be a dose one-hundredth of the pharmacological dose derived from animal and *in vitro* models or a dose in the low µg range with a maximum to be administered of 100 µg (www.emea.eu.int/pdfs/human/swp/259902en.pdf).

The EMEA's encouraging stance is to be welcomed, because microdosing helps to move the focus of early drug development away from laboratory animals to safe and ethical studies in humans via a reduced preclinical safety package for low-dose clinical studies. The main aim of conducting animal studies before HMD is to ensure no harm will result in the volunteers when a microdose is administered. Such preclinical tests should be designed to: use as few animals as possible; save time and cost; use minimal amounts of drug substance; and permit several compounds to be put through the safety program in parallel. The growing public demand for a reduction in the use of animals for pharmaceutical development highlights a key potential advantage provided by the microdosing concept. Current statistics show that over 750 drugs entered Phase I development in 2004, with the overwhelming majority following the traditional paradigm that requires significant preclinical evaluation before first human dose (Pharmaprojects 2004). A large number of these molecules will be subsequently dropped from development in Phase II (or worse still Phase III) for ADME and/or bioavailability reasons. If these candidates could be de-selected at Phase 0 with limited preclinical testing, then a marked reduction in animal studies would be achievable.

The non-clinical program should be able to support a maximum human clinical dose of 100 µg (i.e. 2 µg/kg for a human weighing 50 kg). Based on allometric scaling (ratio of 5 for rats) and a safety factor of 1000, this equates to a maximum dose of 10 mg/kg in the rat. Clearly, the exact preclinical toxicology program must be based on the chemical structure and nature of the test molecule, its pharmacological class and other known chemical and biological information. As a starting point in designing an appropriate non-clinical program of work, it is perhaps judicious to assume that there are basically three types of candidate that could be evaluated by a preclinical program. These are summarized below, along with the suggested preclinical studies:

(i) A novel molecule with little preliminary supportive information:
- *In vitro* metabolism study.
- Extended single dose toxicity study in one species.
- Standard *in vitro* mutagenicity battery.

(ii) A novel molecule within a well-known pharmacological class of similar chemical structure to other members in that class:
- *In vitro* metabolism study.
- Extended single dose toxicity study in one species.
- Abridged (screening-type) *in vitro* mutagenicity battery.

(iii) A novel molecule within a well-known pharmacological class with known potential safety issues:
- *In vitro* metabolism study.
- Extended single dose toxicity study in one species.
- Standard mutagenicity battery.
- Specific safety pharmacology and/or toxicity studies as deemed appropriate to address the potential safety issue of concern, for example, assessment of QT interval prolongation.

As well as the proposed studies, additional background data, such as therapeutic class regarding target organs and safety pharmacology screening, should be available.

A detailed summary of the probable extended single dose toxicity study has been provided in a recent practical guide to microdosing [16]. The mutagenicity package includes full or abridged *in vitro* bacterial reverse mutation test (Ames) to detect point mutations and an *in vitro* cytogenetic evaluation of chromosomal damage in

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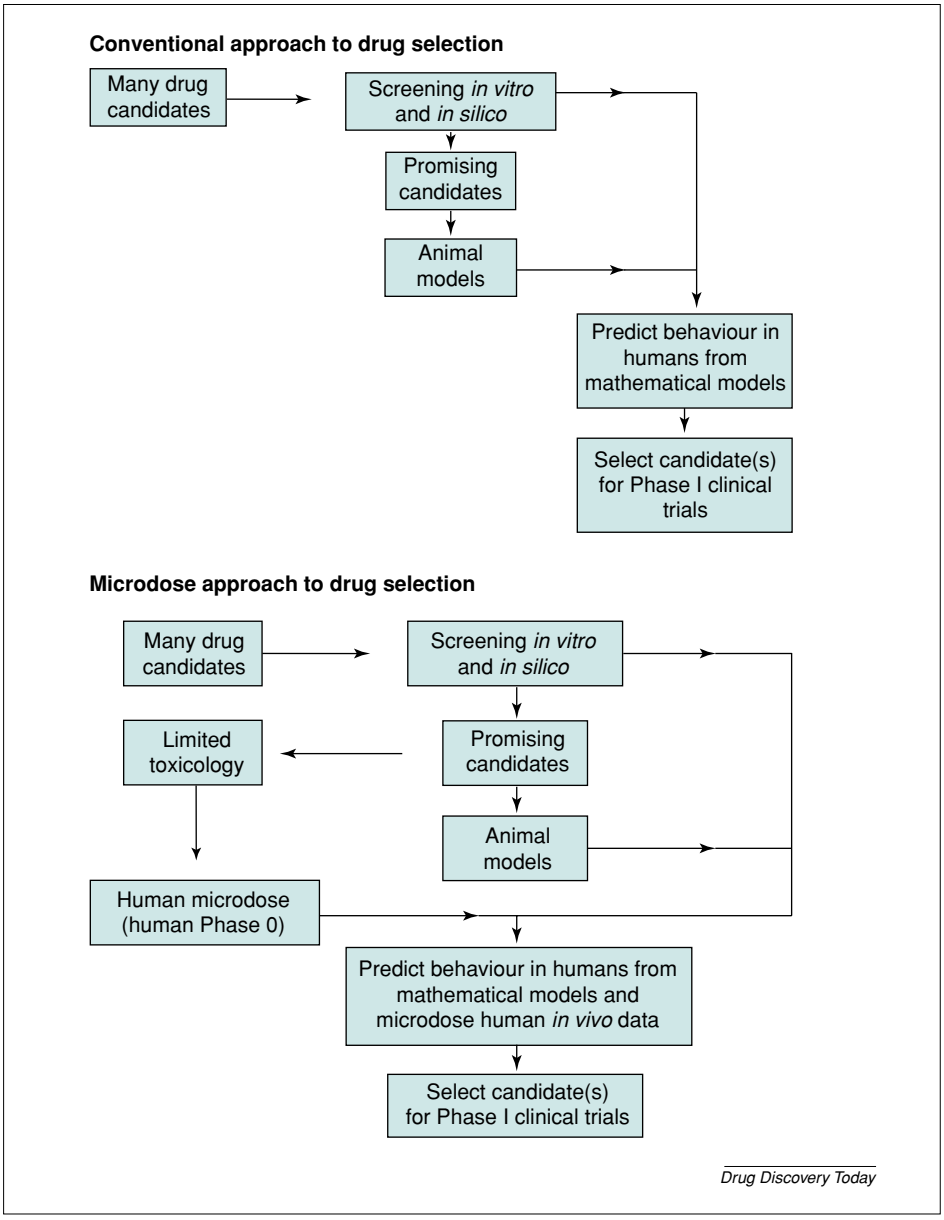

FIGURE 1

Comparison of the conventional and microdose approach to drug candidate selection.

mammalian cells (human peripheral lymphocytes or Chinese hamster ovary cells) or full or abridged *in vitro* gene mutation and clastogenic test (mouse lymphoma thymidine kinase assay).

Bioanalytical challenges for microdose studies

Microdosing studies for ADME investigations are dependent on ultrasensitive analytical techniques, such as accelerator mass spectroscopy (AMS) and ultrasensitive LC–MS/MS [17,18]. In contrast to LC–MS/MS, AMS requires isotopically labelled candidate drugs; however, LC–MS/MS might not currently have the necessary sensitivity to measure drug concentrations at microdoses. The current perspective focuses more on AMS as the bioanalytical technique of choice but great strides are being made with LC–MS/MS, and therefore perhaps year on year the technical gap will shorten.

AMS microdosing

In AMS microdosing, a single dose of trace-enriched ^{14}C-labelled drug is given to a group of ~six human volunteers, preferably in a crossover design in which one dose is given intravenously and the other, after a suitable washout period, by the proposed route of administration (usually oral). In terms of radiolabelling strategy, it is recommended that the location of the ^{14}C moiety should be metabolically stable. Typically, the administered drug range is 1–100 μg. Once the subjects are dosed, blood and urine samples are collected, typically for a period ranging from 24 to 60 h post-dose. The collection period should be sufficient so that three – and preferably five – plasma half-lives will have elapsed for most drug candidates. Urine is often recovered to measure the amount of investigational product excreted by this pathway, whereas blood is collected to establish the amount of total drug and its metabolites that are excreted.

For most drugs, the pharmacologically active moiety is the parent molecule. The proportion of parent drug in either plasma or urine is quantified by extraction followed by chromatographic separation and AMS analysis of the parent drug fraction. From a single chromatography run, the amount of parent drug is measured, as well as the extent of metabolism. If more metabolite information is required, then further sub-fractionation will be necessary. Co-chromatography of radioactive peaks with reference to standard metabolites obtained from *in vitro* incubations or from whole-animal studies provide a strong indication of metabolite structure [19].

Microdosing: predictive of pharmacokinetics at a pharmacological dose?

One of the legitimate anxieties levied at microdosing is the potential for the PK at a microdose to be misleading compared with that achievable at a pharmacological dose. As a consequence, the Consortium for Resourcing and Evaluating AMS Microdosing (CREAM) trial was sponsored by Eli Lilly, Roche, Schering AG and Servier. The results were recently presented by Professor Malcolm Rowland at the *American Society of Clinical Pharmacology and Therapeutics* conference in Orlando, FL, USA, held 2–5 March 2005. The CREAM trial evaluated the microdose PK of five molecules following oral and intravenous dosing compared with PK data obtained at historical

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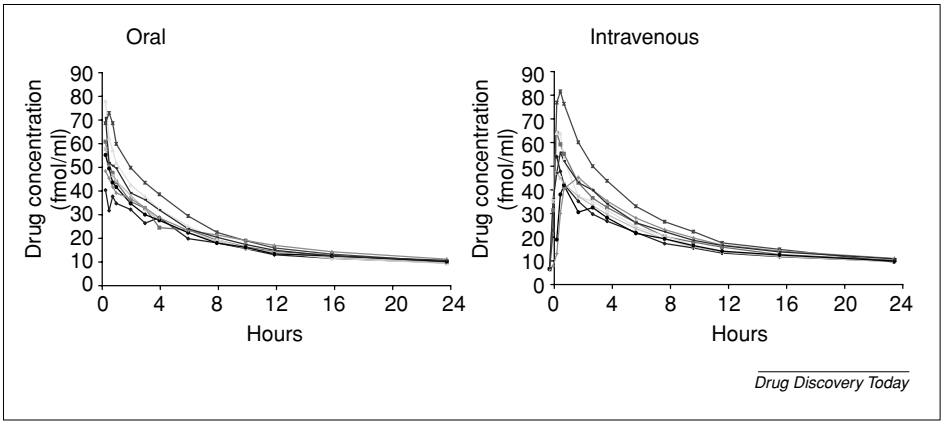

FIGURE 2

Individual pharmacokinetics profiles: oral versus intravenous for α-HGA in a human microdosing crossover study.

pharmacological doses [15]. The compounds were selected on the basis that there would be difficulties in predicting the therapeutic dose PK from a microdose.

For example, midazolam has a high first-pass metabolism mediated by cytochrome P450 (CYP) 3A4 and diazepam is a low clearance molecule eliminated through the activity of CYP2C19. On *a priori* grounds it had been anticipated that the predictive PK capability of a microdose for a pharmacological dose for these types of drugs would be poor as a result of small quantities of drug not saturating the metabolism or clearance mechanisms. However, in both cases, the dose-normalized PK profiles were 'superimposable', providing real evidence of the utility of microdosing even for difficult molecules. Another of the CREAM molecules was a proprietary Schering AG compound, ZK253, for which animal models had failed to predict poor human bioavailability at a pharmacological dose. The drug was dropped after Phase I clinical trials for these bioavailability reasons but not before significant investment. However, would microdosing have provided an equally clear outcome but at a fraction of the cost or would it have generated a false positive because of the low drug dose? After oral microdose administration, the plasma levels of ZK253 were below the limit of quantification (LOQ) for a highly sensitive AMS assay, confirming the poor oral bioavailability of ZK253, thereby building confidence in the decision-making potential of microdosing. Less encouraging was the data for warfarin, which is characterized by extensive, albeit slow, *in vivo* metabolism principally through CYP2C9. The microdose was not truly predictive of a pharmacological dose, although the long $t_{1/2}$ (probably the result of slow metabolism) was manifested in the microdose PK data. Therefore, in general, the trends with microdose PK would have supported decision-making for a pharmacological dose with this molecule. The results with erythromycin were eagerly awaited because the molecule is a substrate for CYP3A4 and P-glycoprotein and, again, saturation of processes with a microdose was likely to be questionable. However, unfortunately, the design of the study led to unprotected oral delivery of erythromycin, which lacks stomach stability, and therefore no result was obtained.

The outcome of the study is extremely encouraging for the microdosing concept with truly predictive PK for three 'difficult' candidates and, in all cases, positive trends in the microdose PK compared with pharmacological dose. In general, the CREAM Consortium believed that the study had effectively validated the microdosing concept for decision-making use in early drug development [15].

Microdosing in action: α-HGA study for Tripep

The scientific and commercial context of the microdosing concept can best be recognized from a real 'worked example'. Tripep (Sweden), a growing biotechnology company, was at a crucial go or no-go stage for oral development of a new anti-infective, α-HGA, for HIV. A human microdose study at Pharmaceutical Profiles (PP) was used as a 'gatekeeper' investigation to ensure increased surety of successful development without committing significant funds or delaying the overall clinical plan (www.privataaffarer.se/bors/showpress.asp?intPressid=49443).

The microdose investigation involved a group of eight healthy volunteers undertaking a crossover study to evaluate human ADME following oral and intravenous dosing of a 100 µg drug dose. The study used GLP API and GLP ^{14}C API (100 nCi), which were formulated under Good Manufacturing Practice conditions at PP on the morning of dosing into the oral and intravenous formulations. Regulatory approval for the investigation was sought and obtained under the MHRA Clinical Trials Authorization (CTA) scheme. No formulation stability data were included. Blood and urine samples were collected for 24 h post-dose before AMS analysis at the Lawrence Livermore Institute (CA, USA) in collaboration with Vitalea Sciences.

The human findings were extremely encouraging (Figure 2) and showed that α-HGA was completely orally bioavailable with extremely reproducible PK performance. Oral absorption was rapid with an elimination $t_{1/2}$ longer than envisaged at 9–10 h, making the molecule an ideal candidate for once or twice daily dosing. Review of the combined blood and urine data clearly demonstrated kidney excretion as the predominant elimination route.

From a commercial perspective, the study findings permitted the planned Phase I–IIa program to be commissioned with significantly reduced risk of failure because of knowledge of the excellent oral PK properties. The rapid provision of the human PK data, five months after 'green light' from Tripep, also provided increased confidence in the development program for the external investment community.

Human microdosing: a funding strategy for young biotechnology companies?

The landscape for venture capital (VC) investment in biotechnology companies has changed significantly in the past 20 years. A recent presentation from Marc Ostro

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(TL Ventures) provides a stark message to young embryonic biotechnology companies; human data are an increasing prerequisite for funding [20]. In 1985, VCs were prepared to invest at the basic research stage of the life of a biotechnology company, knowing the public markets would be sympathetic to such a company ~four years later, when the fruits of basic research would first enter humans. However, experience has been a bitter pill for the public market investors and a more cautious approach to Initial Public Offering (IPO) investment has become the norm in 2005. It is now increasingly common that, to achieve a successful IPO at sensible valuations, a biotechnology company should have drugs in late Phase II or preferably Phase III development. Working backwards, this limits VCs to investing in companies that have human data on possible lead molecules. One of the few ways to stretch the VC investment opportunity is via the HMD route; encouraging human ADME data provide an increased chance of commercial success and de-risk the development program.

Interestingly, there is a growing positive perception of HMD studies within the biotechnology community. This was recently highlighted by an interview with Alice Huxley, the entrepreneurial CEO of Speedel based in Basel, Switzerland, who commented: 'We use microdosing as a screening filter for those projects in renin inhibition where we had a few derivatives that showed promising profiles in animal testing in order to pre-screen them before entering the classical, expensive and lengthy human clinical testing' [21].

Conclusions

Microdosing offers enormous opportunities to an industry under pressure from multiple sources. However, to quote Charles Darwin's now famous and seminal work *The Origin of the Species* [22]: 'It is not the strongest of the species that survive, nor the most intelligent but the one most responsive to change.' Is our industry ready to embrace change in the drug development process or will the dinosaurs soon become extinct?

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