Citation: Clin Transl Sci (2017) 10, 337–350; doi:10.1111/cts.12464

REVIEW

Intra-Target Microdosing (ITM): A Novel Drug Development Approach Aimed at Enabling Safer and Earlier Translation of Biological Insights Into Human Testing

T Burt[1,*], RJ Noveck[2], DB MacLeod[3], AT Layton[4], M Rowland[5] and G Lappin[6]

INTRODUCTION

The safe and efficient translation of biological insights into human applications is a major public health challenge. Safety concerns are associated with substantial preclinical developmental costs, delaying entry into first-in-human testing. We introduce a novel drug development tool, Intra-Target Microdosing (ITM), combining features of "microdosing," the systemic subpharmacological testing of drugs, and intra-target drug delivery. The approach could enable early, safe, and inexpensive detection of safety- and efficacy-relevant biomarkers and pharmacokinetic data with minimal risk.

BACKGROUND

Intra-target microdosing (ITM) is a novel concept that amalgamates two disparate techniques: "microdosing" and intra-target (e.g., intra-arterial, intra-muscular, intra-thecal, topical, subcutaneous) drug delivery whereby a drug under development is administered in its first-in-human (FIH) study locally leading to therapeutic-level exposure only in a small proportion (about 1/100th) of the total body mass. When the drug enters the systemic circulation it is diluted (about 100-fold) such that the resulting systemic concentration is subpharmacological, meeting the definition of a microdose, with the implied safety profile and associated regulatory leniency. The initial exposure of the target organ to pharmacological concentrations, albeit for a short period of time, may be sufficient to generate responses of biomarkers indicative of the drug's local efficacy and/or toxicity. Such local pharmacodynamic (PD) data could be collected either in the vein draining the target, through imaging or other physiological testing of the target, which could be relevant to systemic effects and actions in other organs/tissues. Such knowledge on pharmacological exposure of human tissue *in vivo* to test articles is the critical "missing link" of preclinical drug experimentation and would be invaluable for developmental decision making. In a recent publication we proposed the term "in-humano" to describe this type of testing, conducted in the living human with no therapeutic intent, with minimal systemic exposure and associated toxicity risks.[1] ITM thus offers to be a novel drug development approach, complementing and augmenting the existing drug development "tool-kit" by allowing the safer, earlier, quicker, and relatively inexpensive safety, efficacy, and pharmacokinetic (PK) testing of new drugs in targeted human organs or tissues of interest. In this concept article we cover the regulatory background, theoretical and conceptual aspects, discuss the proof of concept and feasibility studies and related mathematical modeling used to extrapolate the data to the full, pharmacological-level exposure, outline some of the applications, and finally discuss the limitations of the approach.

Relevance to drug development

Most drugs fail during clinical development with consequent wasteful human and animal testing and associated resources, unnecessary exposure to risks, and costly delays in delivery of healthcare benefits of successful drugs.[2–6] The translational stage, moving from animal models to humans, represents a major bottleneck and attrition source in drug development, with considerable improvements in the science, technologies, and strategic implementation identified by regulators, academic entities, and patient advocacy groups as potential improvements over traditional approaches.[4,7] The conventional Investigational New Drug (IND) application process necessitates a substantial package of preclinical safety data, genotoxicology, and manufacture of sometimes kilogram amounts of the test articles to Good Manufacturing Practice (GMP) standards that can take 12–18 months to complete (**Figure 1**). Prior to undertaking this expensive and lengthy process limited, "exploratory," entry into human testing has been offered by the regulators,[8] approaches sometimes called "phase 0," or "exploratory clinical trials," among which is microdosing.[1,9] Microdosing and other phase 0 approaches can increase the efficiency and reduce the costs of drug development by arriving

[1]Burt Consultancy, LLC, Durham, North Carolina, USA; [2]Medical Director, Duke Clinical Research Unit, Durham, North Carolina, USA; [3]Department of Anesthesiology, Duke University, Durham, North Carolina, USA; [4]Robert R. and Katherine B. Penn Professor of Mathematics Arts and Sciences Council Chair Professor of Biomedical Engineering, Duke University, Durham, North Carolina, USA; [5]School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, UK; [6]Reader in Pharmaceutical Science, Lincoln School of Pharmacy, University of Lincoln, Lincoln, Lincolnshire, UK. *Correspondence: T Burt (talburtmd@gmail.com)
Received 4 January 2017; accepted 1 March 2017; published online on 18 April 2017. doi:10.1111/cts.12464

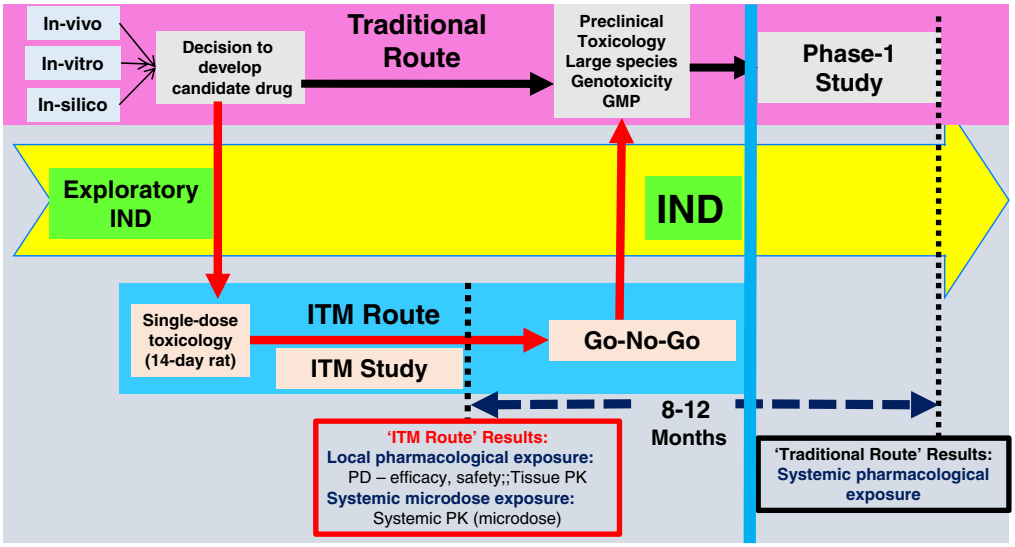

Figure 1 Intra-target microdosing (ITM) in drug development. ITM may result in 8–12-month quicker arrival at human-based "go-no-go" decisions. The figure illustrates the traditional (black) and ITM (red) pathways for entry into human testing: IND (Investigational New Drug) or Exploratory IND (eIND), respectively. GMP, Good Manufacturing Practices; PK, pharmacokinetics; PD, pharmacodynamics.

at human-based developmental decisions prior to the expensive and time-consuming full phase I programs.[1,9,10] In addition, these approaches could lead to savings estimated to be greater than USD 300 million per "no-go" decision, allowing successful back-up compounds to proceed in clinical development 8–12 months earlier, thus adding to the value of their patent-life.[9]

The 2006 US Food and Drug Administration (FDA) eIND guidance includes the following as justification for the establishment of this category of clinical trials: "Existing regulations allow a great deal of flexibility in the amount of data that need to be submitted with an IND application, depending on the goals of the proposed investigation, the specific human testing proposed, and the expected risks. The Agency believes that sponsors have not taken full advantage of that flexibility and often provide more **Supplementary Information** in INDs than is required by regulations."[11] Considering the main goal of IND applications is to ensure safety of human testing, it is remarkable that a regulator would advise its constituents against overdiligence in safety preparations. However, it is also a testament to the regulator's responsibility to promote the efficiency of new drug development through implementation of just-sufficient regulations to ensure human safety.[4] As with many ideas, initially intended for one situation, in this case human testing prior to full phase I investigation, some phase 0 ideas, in particular microdosing, have found wider application in drug development such as studies in vulnerable populations.

Intra-arterial drug delivery

A convenient and common intra-target drug administration is intra-arterial (IA) drug delivery. We will refer to this method as the default intra-target approach throughout this article, even though other intra-target approaches are available, such as intra-muscular, intra-thecal, and topical. There is decades-long experience with intra-arterial therapeutic and diagnostic target delivery that administers high-potency, often toxic agents into local arteries supplying target organs, thereby attaining high exposure at the target organ while minimizing systemic exposure.[12–16] Intra-arterial drug delivery has seen an increase in popularity since the advent of more advanced microcatheters and other endovascular devices.[17] The goal is to reduce systemic exposure to toxic agents and thereby spare the body adverse effects of high potency interventions but at the same time maintain high concentrations at the target organs and tissues. Examples include transarterial chemoembolization delivered into the hepatic artery for treatment of hepatocellular carcinoma,[18] intra-arterial chemotherapy for pancreatic cancer,[19] and retinoblastoma,[20] tissue plasminogen activator (TPA) delivered into the carotid arteries for the treatment of stroke,[21] verapamil to treat cerebral vasospasm,[22] and intra-carotid injection of sodium amytal for diagnosis of speech dominance lateralization.[15] Related approaches using intravenous (i.v.) and intra-nasal administration, can be described as "targeted investigational medicine" have been reported,[23–25] albeit without measurement of test article concentrations in the tissues or vessels of interest, and were never used, to the authors' knowledge, in clinical drug development.

Microdosing

Microdosing is a technique that emerged in the late 1990s whereby subpharmacological doses of a drug are administered systemically to human subjects to obtain preliminary PK information.[26] The regulatory guidelines were internationally harmonized through the ICH (International Conference on Harmonization) M3 guidelines in 2009.[8]

The test article in these exploratory clinical trials (also known as phase 0 studies, or exploratory IND) is administered at 1/100th of the NOAEL (no observed adverse effect

level, practically meaning the pharmacological effect threshold, as estimated by the results of the single-dose toxicity rodent preclinical study) or 100 μg, or 30 nmol for biologics, whichever is lower.[1,8,9,11] The very low doses involved are considered to have no significant toxicological concerns and therefore can be administered to humans based upon a much reduced safety package compared with that required for a full phase I clinical study.[2,27,28] The microdose can be administered to human subjects by any route, although oral and i.v. are currently the most common.[29] Sensitive bioanalytical techniques (Accelerator Mass Spectrometer (AMS), Positron Emission Tomography (PET), and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)) are required to measure the very low concentrations generated by the approach. Each analytical technique provides advantages that have been comprehensively reviewed elsewhere.[1,9,30] AMS is the most sensitive technique but requires labeling the compound with a radioisotope that is rare in body fluids and tissues. The most common isotope employed is ^{14}C. With PET microdosing,[31] drugs labeled with positron emitting radioisotopes (e.g., ^{18}F, ^{11}C, ^{124}I) can be visualized in real time in humans using PET imaging, their target-binding identified, and concentrations in compartments of interest measured over time.[32–34] Recent advances with LC-MS/MS allow the technique to detect many drugs following microdose administration, including characterization of their metabolites, and with the advantage of the absence of exposure to radioactivity.[35] AMS and PET microdosing have been used to select new drugs in development.[27,32,36–38] Microdosing has been primarily used to provide exploratory PK data for the selection of drugs, both small molecules and proteins, entering full development[37–39] but the technique has also been used to detect PD effects,[40] including binding to regions of interest,[32,37] and conversion of drug to its active form.[41] Microdosing has also been applied to the investigation of potential drug–drug interactions (DDI) and pharmacogenomics.[1,42–44]

Further advantages of microdosing include: i) potential for more than 50% savings in developmental costs over conventional IND development strategies[10]; ii) additional savings, potentially worth $300 million in patent-life commercial sales, due to earlier development of successful back-up compounds[9]; iii) the safe study of drugs in vulnerable populations (e.g., children, pregnant women, severely hepatically/renally impaired, the frail elderly) who are routinely excluded from clinical trials due to safety concerns.[41,45–47] In pediatric drug development, for example, it has been suggested that extrapolation from adult data is reliable in less than 20% of cases,[48] and off-label use is extensive and associated with increased incidence of adverse drug reactions (ADRs), emphasizing the need for accelerated and informed drug development[45,49]; iv) potentially better prediction of human PK than alternative preclinical methods. As indicated in our recent analysis of microdosing PK data, 79% of orally administered and 100% of i.v. administered microdoses were directly proportional (within a factor of 2) to full pharmacological exposure PK.[27] On the other hand, traditional preclinical development has been estimated to predict human PK within twofold in only 45% of cases[50]; v) microdose can be administered by any route, including those bypassing first-pass effect (e.g., IA, i.v.), due to its inherent safety; vi) by enabling rejection of candidate drugs before full animal testing, microdosing can help reduce the use of animals in drug development.[5]

Role of microdosing in drug development. The FDA and National Institutes of Health (NIH) have identified microdosing as a key innovative approach.[2,8,11] The industry, however, has not been quick to adapt it as a drug development tool and a recent search of "clinicaltrials.gov" identified a total of only 97 registered microdose studies compared with a total of 35,289 registered phase 0 and phase I studies, or 0.27% (search conducted on 7 September 2016 for the search term "microdosing OR microdose" and a search checking the "phase 0" and "phase I" checkboxes[51]). Even though public reporting is required only for phase II and later studies, the percentage of microdosing studies relative to total phase 0 / phase I studies is probably still a reflection of the limited utilization of microdosing. There could be many reasons for this, including the traditional culture of drug developers,[1,11] but there are methodological challenges as well.

Methodological challenges facing the application of microdosing in early-phase clinical development. Microdosing, in its current format, is designed primarily to provide PK data. This approach, however, does not address the main challenge facing human drug development—namely, obtaining pharmacodynamic (PD) information, including safety, efficacy, and mechanistic data. Microdosing in its current format also faces the challenge of extrapolation from the subpharmacological doses being administered to the full, therapeutic-level exposure in the absence of a human full-dose reference.[1,52] We propose that the ITM approach addresses these challenges by establishing local, in-target full pharmacological exposure, thereby providing previously unobtainable PD data together with contemporaneous systemic microdose data, at the earliest possible stage of human testing without the risks of systemic exposure that would otherwise be needed to generate the same magnitude of response.

Intra-Target Microdosing (ITM)

ITM combines the methodologies and techniques of "microdosing" and "intra-target drug delivery" to test new drugs in target organs and tissues of interest. It produces a full pharmacological exposure in the organ/tissue of interest and a microdose in the rest of the body. Since a microdose is defined as 1/100th of the anticipated pharmacological dose calculated on a total body weight basis, when such a dose is administered into 1/100th of the body mass or less, pharmacological concentrations are briefly (seconds to minutes) generated in the target tissue before entering the general circulation as a subpharmacological dose (microdose) (**Figure 2**). The duration of such exposure can be increased by the use of a tourniquet or other methods, as we have demonstrated (and see below under POC studies).[53,54] Drugs could be administered at constant or varied infusion rates with different durations and concentrations to reflect desired tissue exposure–response time profiles and modeling requirements (see below under "ITM measurement, computational, and modeling considerations").

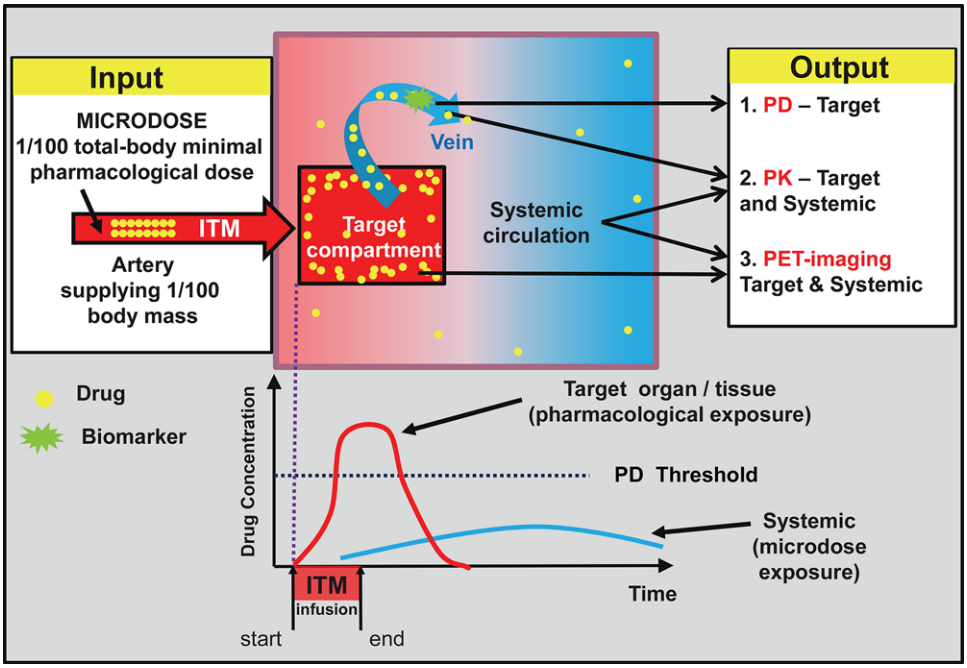

Figure 2 Intra-target microdosing (ITM): Schematic of input and output. By generating concentrations higher than the pharmacodynamic (PD) threshold, ITM allows the capture of local PD data relevant to full (pharmacological, therapeutic-level) exposure, in addition to systemic PK data. Multiple infusion profiles are possible depending on desired exposure–response profiles.

Local tolerance should not be of concern since the organ/tissue of interest receives pharmacological-level exposure corresponding to the (eventual) minimal effective oral (or i.v.) doses. In other words, since anticipated local concentrations with ITM are calculated so that local exposure is around the NOAEL or minimal effective threshold, and since only a microdose is administered, local concentrations are not expected to exceed the range of local exposure and tolerance of a usual phase I study, and definitely not expected to enter the maximal tolerated or toxic range. In addition, the doses given into the target (e.g., intra-arterially) can start very low (subpharmacologically) and be upwardly titrated gradually until the first PD indications in the organ/tissue of interest are observed. This would ensure no excessive exposure in the tissue of interest. Nevertheless, the possibility of pockets of high concentration in the artery prior to complete dissolution in blood are possible, especially with rapidly infused substances (e.g., bolus). This may be addressed during the animal toxicity studies by administering the drug using the same route and infusion parameters and test protocol, including the use of tourniquets, as the intended human study. The effects of the tourniquet could further be tested in the human study by comparing one arm with the other, and the same arm with or without a tourniquet during an ITM administration.

The temporary pharmacological concentration generated in the tissue or organ of interest may be sufficient to detect changes in local biomarkers and collect PD data relevant to the drug's safety, efficacy, and/or mechanistic information. In addition, PK data, in the tissue of interest (via PET imaging) or systemically (via sampling of a vein that is independent from the one associated with the ITM sampling), can be obtained as in conventional microdose studies (**Figure 2**), thereby maximizing the body of informative data from a single clinical study. In fact, the systemic microdose and local exposure are two distinct but contemporaneous studies. When the dose is administered by a vascular route, estimation of disposition kinetics, such as volume of distribution (V) and clearance (CL), can be obtained. These parameters can be very important for drug developers, particularly where there is uncertainty in the prediction of clearance from nonhuman-based methods such as allometry.[39] Moreover, data to date suggest that prediction of V and CL for microdose studies is highly reliable.[39] We have previously reported POC studies in rats and humans (described below).[54,55] To our knowledge this is the first conceptual description of ITM in drug development.

Organs of interest, including limiting organs (i.e., with toxicity liability) can be identified based on the single-dose toxicity study, other preclinical *in vivo* studies, and known targets of similar drugs and the disease state. In addition, in ITM studies where PET imaging of the labeled test article and/or a relevant biomarker/s is undertaken, a full-body PET imaging may provide an unbiased, exploratory, and comprehensive investigation of potential targets in the living human that may have not been identified by the earlier preclinical studies and theoretical considerations.

ITM allows the study of key properties that are relevant to drug development "go-no-go" decision making (**Figure 3**). Even though there is no therapeutic intent with ITM, it effectively combines a regular microdosing study with a simultaneous study of local therapeutic-level exposure, allowing the study of the characteristics that have been called "the pillars of pharmacology": i) plasma PK, ii) target/tissue PK (relevant

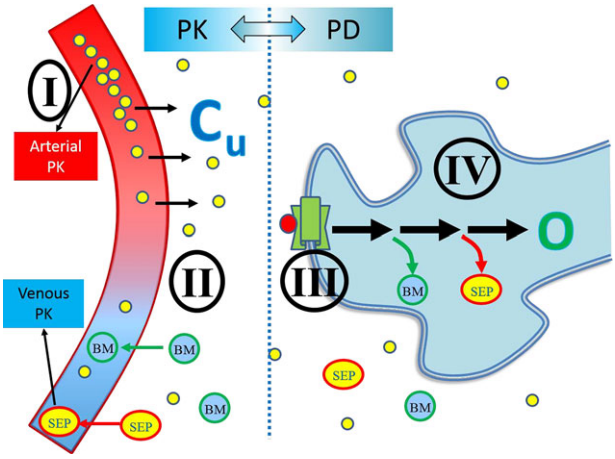

Figure 3 PKPD continuum. ITM allows study of drug effects in the following domains: (I) plasma PK; (II) target/tissue PK (for efficacy and toxicity targets); (III) receptor binding and displacement; (IV) pharmacological effects; biomarkers and/or clinical outcomes. PD, pharmacodynamics; PK, pharmacokinetics; Cu, concentration unbound in tissue; O, clinical outcome; BM, biomarkers/metabolites; SEP, surrogate end points. (Adapted from Burt *et al.* 2016).

to efficacy and toxicity targets), iii) target receptor binding and displacement, and (i.v.) PD (biomarkers, surrogate end points, and clinical outcomes).[56] Of these, it is recognized that outcomes that require medium to long-term exposure cannot be identified using ITM, but even then, intermediate biomarkers and surrogate end points, if they exist and are known and validated, may be studied in the ITM timeframe (see below under "ITM applications").

Historical precursors to ITM: Comparison with other investigational, diagnostic, and therapeutic interventions

We are not aware of previous reports of investigational medicine, diagnostic, or therapeutic approaches that are fully consistent with the ITM definition. We restrict the definition of ITM to the following criteria: i) the study is FIH; ii) there is no therapeutic intent (i.e., this is an exploratory clinical trial meeting the ICH M3 definition[8]); iii) the test article is administered as a microdose (i.e., 1/100th of the NOAEL or 100 μg, or 30 nmol for biologics, whichever is lower[8,11]); iv) the microdose is administered into an area about 1/100th of the total body mass; v) local and systemic measurements of the test article and/or biomarkers relevant to test article effects are undertaken. There are, however, reports of methods using similar principles and here we describe a few.

To begin with, the aforementioned therapeutic intra-arterial interventions (e.g., intra-arterial chemotherapy, intra-carotid TPA) are examples of localized administration that may well fit the definition of 1/100th of the body mass. If only one carotid artery is infused in a TPA intervention, for example, the exposure of one-half of the brain, at around 670 g, is a close approximation of 1/100th of the body mass. However, these approaches deliver usually doses much higher than a microdose, as the aim is not to have subpharmacological systemic exposure but rather to avoid systemic toxicity. Cardiac angiography is an interesting example, in that it involves an intra-target administration (into the coronary arteries) of a diagnostic contrast agent that provides its effects only locally without the interference of systemic background contrast material. In other words, here it is the principle of visual signal entropy, rather than safety, that motivates the ITM-like approach. Since the heart also is about 1/100th of the body mass, the presence of effects only locally would meet the definition of a microdose. These are obviously not FIH investigations, and there is therapeutic intent, but if they were, in the case of development of new contrast agents and systemic PK measurements would be undertaken, it would meet the definition of ITM. An important implication of carotid TPAs and cardiac angiography is the procedural familiarity with direct intra-arterial administration of substances into these highly sensitive organs to the point where they could be considered routine. If there is confidence with routine therapeutic administration of substances into these organs, would it not be justified to administer microdoses of test articles to small groups[4–8] of research volunteers to investigate novel therapeutic or diagnostic agents?

There are several reports of investigational medicine and drug development that utilize common principles to ITM. In 2005, Dhaun *et al.* evaluated local responses to the novel endothelin ET_A receptor selective antagonist BMS-193884 in the human forearm vasculature by infusing low doses of the test article.[57] However, the lowest dose administered (300 nmol = 5 nmol min^{-1} x 60 min) of the new biologic is 10 times the upper limit of the microdose definition for biologics,[11] which would make it consistent with the starting dose for regular phase I studies. Importantly, full pharmacological doses were given orally in two of the three study groups, something that will not be possible in an ITM study under the exploratory IND framework.

ITM can be applied to the development of marker or challenge agents. In their 2006 report Hesse *et al.* described a modified Aellig dorsal hand vein compliance technique used to study neurokinin-1 (NK-1) receptor antagonist SLV317.[58] As part of the technique, substance P, a natural agonist of the NK-1 system, was administered locally (dorsal hand veins) into an area consistent with 1/100th of the total body mass. However, veins were studied only ipsilaterally, while with ITM the contralateral side is studied as well, where possible (as is the case of symmetric/bilateral organs/tissues such as the hand), as well as systemic exposure. In addition, by virtue of administering substance P until maximal venodilatation is achieved (as opposed to awaiting only minimal venodilatation effect) the technique is, by definition, inconsistent with ITM. Indeed, markers used for this purpose are usually already approved for human use in full dose at the time they are used as validated markers in the development of investigational drugs, and therefore do not need to be administered as a microdose. However, if the marker itself is being developed for human use then the use of ITM to study its effects may be an appropriate choice for the FIH study.

ITM advantages, disadvantages, and limitations over existing FIH approaches

ITM offers the following advantages over existing phase 0 / microdosing, and phase I FIH approaches (**Table 1**): i)

Table 1 Advantages of ITM over existing FIH approaches (phase 0 / microdosing and phase I studies)

		Advantage/disadvantages over existing FIH approaches		
	ITM Advantage	**Microdosing**	**Nonmicrodosing phase 0**	**Phase I**
1.	Human-based data to triage preclinical candidates	++ (Microdosing provides only human PK)	++ (Non-microdosing phase 0 approaches can provide limited human PK, PD and therapeutic data but with the disadvantage of systemic exposure)	++ (phase I provides human-based data but with the risk of systemic exposure)
2.	Only limited body mass is exposed	++	++	++
3.	Short (seconds-minutes) exposure to full-dose levels	++	++	++
4.	Low systemic exposure	N/A	+ (some therapeutic-range systemic exposure, but less than MTD)	++
5.	Detection of PD effects	++	+	N/A
6.	Dual study using bilateral / symmetric organs / tissues allowing contemporaneous own control	++	++	++
7.	Tissue exposure to therapeutic-level doses can be stopped immediately	N/A (tissue exposure is already subtherapeutic)	++	++
8.	Guides selection of phase I end points	N/A	N/A	++
9.	Not limited to healthy volunteers	N/A	N/A	++ (except in high-risk populations, e.g., oncology)
	ITM Disadvantages			
1.	Limited exposure to the test article	N/A (	–	—
2.	Risk of intra-target administration	—	—	—
3.	Safety risk due to full exposure at the target	—	–	N/A
4.	Complexity of intra-target perfusion parameters	—	—	—
5.	Practical challenges of intra-target procedure	—	—	—
6.	Modeling requirements	–	–	—

++ considerable advantage; + partial or limited advantage; — considerable disadvantage; – partial or limited disadvantage; N/A, no advantage/disadvantage; MTD, maximal tolerated dose.

ITM provides human-based data to triage preclinical drug candidates (**Figure 1**), which could substantially supplement and strengthen existing methods such as allometry and extrapolation from *in vitro* and *in vivo* data[50,59]; ii) only *a-priori* determined, small, and limited body mass is exposed to therapeutic-level concentrations of the test article. Tissues/organs that meet the approximate threshold of 1/100th of the body mass include the hand, kidney, heart (e.g., during cardiac angiography), one-half of the brain (e.g., during intra-carotid TPA[60]); iii) with ITM, target exposure to the pharmacological level of test articles is shorter, hence safer; iv) systemic exposure with ITM is to no more than very low (microdose) concentrations; v) pharmacological-level exposure to the test article in tissues of interest enables detection of changes in biomarkers indicative of drug efficacy or toxicity, such as displacement of PET ligands at pharmacologically active concentrations (an advantage vs. traditional microdosing and preclinical testing); vi) ITM can be stopped and tissue exposure terminated almost immediately at any indication of adverse reaction; vii) in testing symmetric or bilateral organ/tissues (e.g., hands, kidneys, brain) ITM provides the ability to use individuals as their own control in real time. For example, if a drug is given into the radial artery such that the ipsilateral hand will receive a threshold pharmacological exposure, then the contralateral hand will receive a microdose. It is rare in medical research to have a subject as their own control in real time. This holds the potential for considerable reduction in variability of the data and sample size requirements; viii) ITM data can guide the selection of phase I study doses and end points.[1,9,61] Otherwise, choice of doses and end points in phase I clinical trials of novel compounds may need to rely on human *in vitro* and/or preclinical *in vivo*

data[8]; ix) rather than being limited mostly to healthy volunteers because of safety concerns and uncertainty about PK, FIH studies using ITM could be conducted in patients and vulnerable populations as well.

We anticipate that the ITM approach will be well received by study participants, patients, and patient and animal advocacy groups due to the reduced risk in exposure to new drugs and anticipated acceleration of their development.

ITM has several methodological limitations and practical challenges when compared with existing FIH approaches

1. The main practical limitation is the invasiveness and corresponding risk of the intra-target procedure (e.g., when done intra-arterially using an internal organ artery). However, such procedures would be performed only in a limited number (4–12 research participants) for the life of the drug and executed in specialized clinical research facilities with access to tertiary care services and procedural expertise. The risk/benefit ratio will depend on the target organ/tissue, the route of administration, the complexity of the procedure, expertise of the research team, and the expected benefit of the drug. The risk of the procedure would be reduced in individuals who are already cannulated with intra-arterial catheters for diagnostic and/or therapeutic purposes (e.g., chemotherapy) or during elective surgery. In such cases it is proposed that the ITM procedure take place shortly prior to the therapeutic intervention to minimize delay of any anticipated therapeutic benefits but also to reduce the potential confounding impact of the therapeutic intervention on ITM results.
2. The main methodological challenge to ITM is identifying biomarkers relevant to drug efficacy or safety within the short window of seconds to minutes of local exposure that ITM would provide. The reality of interactions of pharmacology with pathophysiology is that they can change over a time-scale of days/months/years, while ITM allows study of seconds/minutes and hours at most. Indeed, if perfusion, membrane permeability, and receptor engagement are not (almost) instantaneous, the ITM results may not reflect those after the intended route of therapeutic administration. It may be particularly challenging with drugs acting by novel mechanisms of action, where a drug's metabolism and chemical reactions with safety and efficacy targets are not sufficiently characterized or validated. Binding to target receptors, however, is likely to occur within the short timeframe of ITM. Such binding, to identifiable, tissue-specific targets, detected and measured by PET imaging of appropriately labeled drugs, might function as the mechanistic biomarker of the drug under development (**Figure 3**). For test compounds acting extracellularly, the distribution to, and engagement with, the target site are likely to be rapid and observable with ITM, as the vascular endothelium is highly permeable, at least for small molecules, except for some organs, such as brain and testes. However, for large molecules, such as monoclonal antibodies, distribution may be too slow to observe measurable changes at the target site or downfield changes in biomarkers. The same may apply with intracellular targets, as the cell membrane permeation may be slow, although for small lipophilic compounds, tissue distribution, including cells, is likely to be perfusion rate limited.
3. There is a safety risk due to the full pharmacological exposure at the target tissue. This will be minimized/mitigated in the following ways:
   a. A toxicity study in rodents using the intra-arterial route prior to human testing (part of the limited toxicity study required for the eIND application);
   b. The ability to start at low tissue exposure and titrate gradually to therapeutic-level tissue exposure while maintaining systemic exposure at a microdose level;
   c. Limited exposure in time (second to minutes) and limited amount of tissue (1/100th of the body mass);
   d. Ability to terminate ITM immediately upon any signs of toxicity (unlike systemic administration).

As an exploratory (phase 0) approach, ITM lies somewhere on the risk spectrum between traditional microdosing and traditional phase I approaches.[8] This is since the traditional phase I approach generates the ITM-type exposure in the entire body (i.e., ~100-fold by mass) and for a longer periods of time (i.e., for at least five half-lives with traditional phase I, vs. local exposure of seconds to minutes with ITM). Similarly, in the local target, exposure with traditional microdosing is less than ITM, i.e., ~1/100th of the ITM exposure.

4. The perfusion profile during ITM should be similar to the target/tissue perfusion profiles postoral or i.v. administration of pharmacological concentrations in order to permit generalization to the latter routes of administration. This may be challenging at a phase in development when no human full pharmacological exposure has yet been produced orally or i.v., although simulations using such highly mechanistic approaches as physiologically based pharmacokinetics (PBPK), which incorporate *in vitro* and physicochemical data, should help to define the likely concentration–time profiles anticipated with such administrations.[62]
5. The amount of tissue perfused: The challenge of finding an artery that supplies 1/100th of body mass.
6. The microdose definition. If the microdose represents less than 1/100th of the pharmacological dose (i.e., when the upper limit of a microdose (100 μg) is less than 1/100th of the lowest anticipated pharmacological dose), then a correspondingly smaller tissue would have to be exposed to the ITM application in order to ensure that the tissue exposure is in the therapeutic range. This may represent technical challenges in arterial access and biomarker capture. However, this scenario is less likely to be a problem with the usual high potency achieved with many modern drugs.

ITM POC in animals

We conducted a POC study in rats to test the hypotheses that after administering a microdose of insulin into the femoral artery, the effects on ipsilateral leg muscles are

similar to those after systemic therapeutic dose administration
with minimal or no systemic effects.[55] The primary outcome was ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake into tissues. Insulin affects FDG similarly to glucose and leads to its uptake into tissues within minutes. PET imaging allows dynamic, continuous visualization and quantification of this uptake. ^{18}F-FDG uptake was measured by the slope of the standard uptake value (SUV) plot. Secondary outcomes were glucose and insulin plasma concentrations. ^{18}F-FDG uptake into ipsilateral leg muscles after insulin administration into the femoral artery was similar to that after systemic (tail vein) full-dose administration, but was statistically significantly greater than systemic microdose and sham intervention. Similarly, femoral vein glucose levels were reduced after insulin administration into the ipsilateral femoral artery. At the same time, contralateral and systemic effects remained at the microdose, subpharmacological, baseline levels, statistically significantly less than the ipsilateral effects. The results demonstrate the feasibility of the approach and support the two study hypotheses: first, that local, intra-target administration can produce pharmacological effects similar to those after systemic full-dose administration; and second, that such local administration can lead to subpharmacological, microdose-level, systemic exposure.

ITM POC in humans

We have just completed, to our knowledge, the first human POC study of an ITM-type methodology.[54] The study, conducted at the Duke University Medical Center (DUMC), followed a similar methodology as the animal study in five healthy human volunteers. The nondominant radial artery was the ITM delivery conduit, with a tourniquet used in three of the subjects to reduce venous return and increase exposure at the ipsilateral side. Insulin and glucose plasma levels were obtained in the arm veins of both arms, and ^{18}F-FDG uptake measurements into hand and leg muscles, bilaterally, were obtained. A local increase in insulin levels associated with reduction in local glucose levels were observed in the ipsilateral side, with minimal changes systemically. The results were consistent with the ITM concept but require further validation in larger controlled studies in multiple therapeutic models, target organs or tissues, and administration routes. The results also demonstrated the ethical, regulatory, and procedural feasibility, and specifically the contemporaneous intra-arterial administration, PET imaging, and blood sampling. The approach was well tolerated by the study volunteers.

These ITM POC studies demonstrated the practicality and feasibility of ITM from regulatory, ethical, logistic, and modeling considerations, in addition to demonstrating the scientific value of using the hand as an ITM target for generic systemic drug effects (glucose uptake) and a biomarker (FDG) as the labeled article. While these findings can be generalized to many other developmental circumstances, additional POC studies are required for demonstration of scientific and practical applicability in other therapeutic areas and anatomical targets.

ITM measurement, computational, and modeling considerations

ITM data can be used to parameterize physiologically based computational PK/PD (PBPK/PD) models. Unlike statistical models, PBPK/PD models can be used to simulate what-if scenarios and generate predictions.[63] The structure of a PBPK/PD model should be chosen based on the specific ITM application with the advantage of using human-based data to inform the models. As an example, we describe a simple compartmental PBPK/PD model applied to the intra-arterial version of ITM (**Supplementary Information**).[55,64]

An open question is how best to translate the ITM data to a likely therapeutic dose. Much depends on the level of detailed information gathered following ITM administration, but a few guiding principles apply. First is the need to translate administered amount(s) to relevant concentration(s). Following ITM, the local arterial concentration during administration is R(t)/Qa, ignoring the minute recirculating concentration, where R(t) is the input rate time profile, which if precision is required may be delivered via a computer-controlled infusion pump, and Qa is the local arterial blood flow rate, which can either be assumed, based on reference physiological data, or directly measured.[62,65] In the hand, for example, arterial supply may be challenging and difficult to predict from reference values, and could require dedicated preintervention measurements to obtain accurate baseline values.[66] Additionally, local venous concentration measurements can be obtained during and for some time after ITM administration. When tissue distribution is perfusion rate limited, as might be expected to apply to lipophilic compounds that readily permeate cell membranes, or even to small more polar compounds with access limited to the interstitial space (owing to the porous nature of many vascular endothelial membranes), venous output concentration better reflects tissue concentration than arterial measurements. Moreover, if steady-state conditions prevail across the local tissue, as might occur during a period of constant rate input, or approximated for drugs that rapidly reach distribution equilibrium during most of the duration of the arterial input, venous concentration (which does not require an estimate of tissue blood flow rate) equals arterial input and approximates to that needed to be generated following systemic therapeutic dose administration. Furthermore, when both local arterial and venous data are available, these can be modeled to provide greater insight into the tissue concentration–time profile, and hence to the tissue concentration–response relationship, as illustrated with the ITM insulin example (**Supplementary Information**).[53]

Second, is to recognize that ITM provides systemic PK disposition kinetics considering that ITM is a parenteral administration that leads to a systemic microdosing exposure. However, if the drug is intended for oral administration, oral bioavailability will either need to be predicted, using for example PBPK, or determined by administering a microdose orally, preferably to the same subjects receiving ITM on a separate occasion, recognizing that microdose PK is a reasonable predictor of therapeutic dose PK, or an amalgam of such data.[50,62,67]

Third, a limitation to the arterial concentration that can be generated following ITM needs to be considered. In the

simplest case of a constant rate input, R_0, of duration θ, the arterial concentration, $Ca = R_0/Qa = 0.1$ mg/($Qa \bullet \theta$), given that the maximum dose is fixed at 0.1 mg for an adult. Clearly, the maximum arterial concentration is heavily dependent on local blood flow rate, which for a radial artery is relatively low but is high for the renal artery. While in many cases high enough arterial concentrations can be produced to generate measurable pharmacological responses, in some cases this may not possible. Careful consideration of the above aspects are important in the design, execution, and interpretation of ITM studies.

Lastly, drugs with meaningful target-mediated drug disposition (TMDD) may complicate measurements of volumes of distribution and disposition with ITM, as with traditional phase I studies, possibly making extrapolation to full-dose exposure unreliable at times. However, as we discussed in a previous publication,[1] it is not the linearity *per se* that is required for effective extrapolation, but an accurate predictive model that may well be nonlinear but predictive nevertheless. Such a model may be deducted from the same single toxicity preclinical study where a microdose can be given followed by a full dose in the same animals. In addition, ITM offers the opportunity to study the target directly, especially with PET imaging, to provide direct information on target disposition. In the final analysis, however, ITM will never provide the same quality information as a full-dose, full-duration phase I study. As drug development is a continuous process of reduction in uncertainty regarding the test article, an assessment will have to be made on a case-by-case basis whether the ITM study can provide the amount and quality of information necessary to make meaningful developmental decisions.

ITM applications

We propose that, for certain drug classes and physiological/therapeutic targets, capturing data on biomarkers in the short ITM timeframe of seconds to minutes is feasible and capable of generating biomarkers relevant to drug effects (**Table 2**). The timeframe would typically be in the seconds to minutes range, unless venous drainage is reduced (e.g., by applying a tourniquet), in which case target exposure could be increased to about an hour. Drug action can be demonstrated by obtaining chemical biomarkers in target tissue venous drainage indicative of reactions in the chemical pathway relevant to efficacy or toxicity (e.g., substrate phosphorylation and activation/deactivation of parent compound), increased or decreased production of endogenous metabolites, or physiological changes in the tissues/organs of interest (e.g., vasodilation/vasoconstriction, analgesia, muscle contraction/paralysis). In addition, demonstration of target receptor binding through PET imaging generally occurs rapidly, is relevant to virtually all drugs, and is a powerful mechanistic support of drug action.[33]

ITM approaches

The following ITM approaches could utilized in select developmental scenarios. Considering the small numbers required for such exploratory clinical trials (mostly 4–12 research subjects) and that patients rather than mostly healthy volunteers can be recruited, both feasibility and risk/benefit ratios may be favorable even with the more extreme of these approaches (e.g., cannulation of an internal organ). Availability and consideration of such ITM approaches should contribute to the strategic versatility of development programs (**Table 3**):

1. **Cannulation of a peripheral artery.** A peripheral artery that is easily accessible and known to supply about 1/100th of the body mass is used. The peripheral tissue could be used to test systemic drug effects, i.e., those that are not organ- or tissue-specific, such as the peripheral actions of insulin, thyroid hormones, immune modulators or vasodilators/-constrictors. A suitable local artery could be the radial artery supplying the hand, as the hand represents about 1/100th of the body mass, and a vein draining the area could be cannulated for collection of plasma biomarkers.
2. **Cannulation of the artery of an internal organ.** An artery supplying an internal organ is cannulated together with the respective vein draining the organ. This may be preceded by angiography to determine arterial and venous distributions to ensure accessed area is within 1/100th of the body mass.
3. **In the course of a diagnostic or therapeutic intra-arterial procedure.** ITM could make use of existing arterial and or venous access during the course of diagnostic and/or therapeutic procedures (e.g., in the course of intra-arterial chemotherapy, or cardiac angiography). This would obviate the excess risk associated with the intra-arterial cannulation procedure.
4. **Arterial access in the course of surgery.** Access obtained in the course of elective surgery could facilitate the study of organs or tissues that have challenging access such as tumors or small internal organs such as the pituitary. Biomarkers obtained when ITM is performed during surgery could include direct tissue visualization, physiological manipulation and testing, and histological examination of samples of healthy and/or pathological tissues exposed to the ITM intervention.
5. **Subcutaneous biologic administration.** Some biologic drugs are intended to be administered via the subcutaneous route. Normally in such administrations aimed at systemic doses, the local exposure is much higher than the anticipated therapeutic exposure. Using ITM, however, allows administration that causes only local therapeutic-level exposure while exposing the rest of the body to microdoses.

ITM application examples

The following are examples of potential application scenarios of ITM in drug development (**Table 3**):

1. **Neuromuscular blocking drugs (NMBD) and muscle strength.** Localized administration of an NMBD into the radial artery would have effects on hand muscles within seconds to minutes.[78,79] Reduction in muscle strength, measured as percent "twitch response," serves as the biomarker associated with drug efficacy. Electrical stimulus is applied to the proximal ulnar nerve to induce a depolarization with the subsequent release of

Table 2 Potential drug categories, physiological and therapeutic targets for ITM applications

Drug categories	Organ/tissue	Biomarker
CNS stimulants and depressants (e.g., hypnotics, sedatives, anxiolytics), NMDA antagonists[68]	CNS	Neuronal activity (e.g., Wada test[15])
Chemotherapy	Liver, kidney, brain, breast	Receptor binding (with PET imaging of radiolabeled drug)[37]
Nitrates, inotropes, adrenergic, muscarinic, PDE5 inhibitors, NEP inhibitors, natriuretic peptides[69–72]	Peripheral vascular	Vasodilation, vasoconstriction, cGMP spillover measurement[72]
Anesthetics, analgesics (e.g., $Na_v1.7$ inhibitors[73,74])	Peripheral organ / tissue	Anesthesia, analgesia
Triptans	Blood vessels	Analgesia, substance P and CGRP levels[75]
Neuromuscular blocking agents	Skeletal muscles	Muscle relaxation/paralysis
Anticoagulants, antiplatelet[76]	Blood	Coagulation parameters, platelet aggregation
Immune modulators, antihistamines	Blood	Cytokines, allergic symptoms[77]
Hypoglycemics, SGLT-2 inhibitors, diuretics	Kidney	Glucose levels, reabsorption in proximal tubule (by ^{18}F-FDG)

CNS, central nervous system; NMDA, N-methyl-D-aspartate; PET, positron emission tomography; PDE5, phosphodiesterase type 5; NEP, neutral endopeptidase; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; SGLT, sodium glucose cotransporter; FDG, fluorodeoxyglucose.
The table is not comprehensive in the sense that there may be drugs with well-characterized targets, receptor binding, or biomarkers from other classes not mentioned in the table that would make the application of ITM feasible. The table also is not representative of the frequency of use and applicability in drug development, but clearly, CNS and oncology (chemotherapy) are large categories in terms of drug development with an appeal for the application of ITM due to the well characterized anatomopathological target, and the difficulty of studying it directly otherwise.

Table 3 Examples of translational applications and respective ITM approaches

			ITM approaches				
Translational application	**Biomarkers**	**PET imaging**	**Cannulation of a Peripheral Artery**	**Cannulation of an Internal Artery**	**With Diagnostic / Therapeutic Intervention**	**During Elective Surgery**	**Subcutaneous Biologic**
Neuromuscular blocking drugs (NMBD)	Muscle strength Receptor binding	+	+	-	+	+	-
SGLT-2 inhibitors	Urine glucose Venous glucose Receptor binding	+	-	+	+	+	-
Natriuretic peptides	Vasodilation Venous cGMP Receptor binding	+	+	+	+	+	-
Tumor chemotherapy	Venous markers Receptor binding	+	+	+	+	+	+

PET, positron emission tomography; SGLT, sodium glucose transporter; cGMP, cyclic guanosine monophosphate.

acetylcholine (Ach) at the neuromuscular junction. The resulting contraction of the adductor pollicis muscle is the "twitch response." This response is calibrated as a full twitch (100%). When the NMBD is administered, the reduction of the twitch can be graded (0–a100%) where 0% represents full neuromuscular blockade. The pharmacodynamic action of the drug (onset, duration of blockade and offset) can then be recorded in terms of % twitch. This represents a considerable advantage over the current NMBD development process whereby a dose is administered systemically, with resultant complete muscle paralysis requiring full ventilatory support. In addition, systemic NMBD studies are conducted under general anesthesia to avoid the extreme anxiety-provoking experience of complete paralysis. The utility of ITM would be the ability to administer NMBD peripherally to a circumscribed target for the purpose of studying its effects while sparing the rest of the body unnecessary exposure and expensive procedures. Furthermore, reversal of NMBD residual muscular block is of clinical importance and could also be studied in a safe and controlled manner using ITM in the early clinical development of NMBDs.[80,81] This could prove of particular value in vulnerable populations such as children and the elderly.[82,83]

2. **SGLT-2 inhibitors and glucose excretion in the kidney.** An SGLT-2 (sodium/glucose transporter type 2) inhibitor is administered into the renal artery. Glucose reabsorption into the proximal tubule is thus inhibited and glucose is excreted in the urine.[84] The biomarkers would be urine glucose, obtained by catheter in real time, and ^{18}F-FDG excretion and reabsorption (a proxy of glucose excretion and reabsorption), or ^{11}C-labeled drug as measured by dynamic PET imaging.
3. **Natriuretic peptides, vasodilation, and cGMP as biomarkers.** Natriuretic peptide (analog of the naturally occurring atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)), a proposed vascular modulator with cardioprotective properties, is administered into the radial artery.[85] Vasodilatation and cGMP concentrations serve as physical and chemical biomarkers, respectively, of drug action, and have been shown to take place within minutes of infusion.[72] Vasodilatation, conveniently studied in peripherally accessed vessels,

is measured using venous occlusion plethysmography. Plasma cGMP concentrations are measured using an enzyme immunoassay.[72]

4. **Tumor chemotherapy.** A chemotherapeutic agent for hepatocellular carcinoma is administered into the hepatic artery.[17] The drug or a biomarker (e.g., FDG) is radiolabeled. Demonstration of binding to tumor tissue through PET imaging will be proof-of-concept that the chemotherapeutic agent reaches, penetrates, and binds to cellular targets.[86] Displacement studies could confirm binding to a target of interest by administering competitive agonists or antagonists.[87] Chemical biomarkers could be obtained in the downstream vein. If done during surgery, blood samples could be more easily obtained, and tissue samples of the tumor could be obtained for histological markers of therapeutic or toxic effects. Since the concentration returning to the systemic circulation becomes a microdose, the traditional microdose study where systemic PK and binding to extrahepatic tissues can be studied as well. Dynamic PET imaging obtained to show concentrations over time could help study intra-tumor PK, systemic microdose PK, and together with the chemical data could be used to scale results to the whole-body full pharmacological exposure.[37]

The regulatory position and safety

Regulations of first entry into human experimentation focus primarily on safety and are governed by the internationally harmonized ICH M3 guidelines.[8] These guidelines cover "microdosing" under "Exploratory Clinical Trials" but do not specify or limit the routes of administration, except to note that the preclinical study should use the intended route of administration of the clinical study.[8] For example, in the case of ITM using the intra-arterial route, discussions with the FDA confirmed that the limited preclinical toxicity study from one species (usually rodent) is to be conducted using the intra-arterial route as well, and special attention should be paid to pathology in the target organ and in the arterial blood vessels delivering the test article.[1] No genotoxicology studies are required (**Figure 1**).[9] Other forms of "Exploratory Clinical Trials" are covered in the guidelines that allow limited capture of PD data with greater than microdose exposure, but these require additional preclinical studies and genotoxicology studies to be conducted prior to human testing.[1] Under this framework, ITM should be viewed as a microdose study since the systemic drug concentrations would be no higher than that from a microdose administered i.v. (**Figure 2**). It is nevertheless recognized that local drug concentrations are at levels that would elicit a pharmacodynamic response (albeit over a short period of time) and so, with development drugs, it may be necessary to include some additional tissue exposure studies (e.g., histological evaluation of those tissues that experienced a pharmacologic-level exposure) in the safety package to ensure safety during the human phase 0 study.

Examples of drug development scenarios that favor use of ITM

The following examples of hypothetical developmental scenarios illustrate situations that favor the use of ITM in drug development. In addition to advantages provided by general microdosing,[9] ITM would be particularly useful in situations in which concerns about extrapolation from animal models and traditional microdosing exist and where early availability of PD data is desired, thus benefitting from these two key and unique features of ITM, as described earlier. The features of these examples can be generalized to other drug classes and other therapeutic scenarios as outlined in **Table 2**.

1. Monoclonal antibody for the treatment of breast cancer

Pre-IND information: Preclinical studies suggest potential for adrenal toxicity. The drug is associated with target-mediated disposition (TMDD). Labeling with ^{11}C proved quick and inexpensive. Biomarkers are available within seconds to minutes.

Reasons for choosing ITM: the presence of a clearly defined target, 1/100th or less of total body mass (the breast tumor) with accessible arterial supply favors ITM, as does the likelihood of systemic toxicity with full-dose phase I studies. TMDD underlines the importance of studying the target directly with full-dose exposure levels and having the ability to compare full pharmacological exposure (ipsilateral breast) with microdose exposure (contralateral breast and systemically) using PET imaging. Straightforward labeling and quickly available biomarkers are important for ITM feasibility.

Study design: ^{11}C-labeled drug administered intra-arterially through the mammary arteries or during elective surgery followed by total-body PET imaging used to detect tumor drug disposition and any extra-tumor targets. Plasma samples are collected for biomarkers. If done during surgery, tumor samples may be obtained for histopathological examination.

2. Antiepileptic drug for temporal lobe epilepsy

Pre-IND information: The drug affects neuronal excitability within seconds. Animal models suggest clear EEG signature plus unique plasma biomarkers. Production of the drug is very expensive. ^{11}C is not straightforward or cheap, but possible. There is uncertainty regarding extrapolating blood–brain barrier (BBB) findings in animal models.

Reasons for choosing ITM: A clearly defined target within 1/100th of body mass (brain hemisphere). Expensive production is a general reason to choose microdosing studies[9] because of the difficulty in scaling up to the doses required for a full phase I study. EEG and ^{11}C labeling are attractive due to the noninvasive nature of measurement in an otherwise organ/tissue with difficult access, as well as confirmation of BBB passage.

Study design: The drug is administered into the carotid artery associated with the side of the epileptic focus followed by PET imaging of the labeled drug, EEG measurement of signature effects, and plasma biomarkers.

3. Vasodilator for the treatment of Raynaud syndrome

Pre-IND information: Systemic hypotension is a concern from theoretical considerations and preclinical study results.

Physical effect (vasodilatation) and release of biomarkers to plasma occur within seconds to minutes.

Reasons for choosing ITM: Clear target about 1/100th of the body mass (hand), and quick biomarker effects.

Study design: Drug administered into radial artery followed by ipsilateral and contralateral drug and biomarker measurements. Tourniquet used to increase local exposure. LC-MS/MS used to measure drug and biomarker concentrations.

4. Diuretic

Pre-IND information: Binding to renal tissue occurred in human *in vitro* but not rodent models. Drug has fluorine and labeling with ^{18}F is feasible. Biomarkers are expected to be available in urine.

Reasons for choosing ITM: Need for quick and relatively inexpensive proof-of-mechanisms demonstration. Presence of target about 1/100th of the body mass (kidney). Ability of labeling to demonstrate binding to specialized renal tissue. Non-invasive ability to measure biomarkers (in urine).

Study design: Drug administered into the renal artery followed by PET imaging of ipsilateral and contralateral kidneys to detect and compare drug binding to regions of interest. Urine collected for biomarkers.

5. Antiarrhythmic drug for the treatment of ventricular fibrillation

Pre-IND information: Drug may have potentially toxic antithyroid effect that is not yet characterized well. There is a need to obtain information for developmental decisions quickly before further investment. Results of human testing will significantly increase the value of this single-drug biotechnology company.

Reasons for choosing ITM: Clear target about 1/100th of the body mass (heart). Desire to avoid systemic toxicity while increasing product value as soon as practicable. Effects (antiarrhythmic) occur within seconds to minutes and can be captured noninvasively (ECG).

Study design: Nonlabeled drug is administered into the coronary arteries followed by ECG measurements and collection of plasma samples. Systemic drug concentrations determined by LC-MS/MS.

CONCLUSIONS

Intra-target microdosing (ITM) is a novel translational tool whereby the test article is administered directly into a small target at locally therapeutic levels but leading to subpharmacological systemic exposure. ITM allows exploratory PK and PD data to be obtained safely in humans ahead of the riskier, more expensive and time-consuming phase I clinical trials. This potentially constitutes significant improvement over traditional drug development (in which full therapeutic-level exposure is induced systemically) and microdosing (in which no biomarker data are expected to be generated). ITM could accelerate the translation of novel biomedical discoveries into therapeutic applications and also allow the safe study of old drugs in vulnerable populations (e.g., children, pregnant women, hepatically impaired, renally impaired, and frail elderly). ITM is limited by the short duration of exposure to the test article and the potentially invasive nature of the procedure and will not be applicable to all developmental scenarios. ITM would be optimized when validated biomarkers or surrogate end points can be obtained within minutes of administration and when benefit/risk considerations are favorable.

Disclosures. T.B. holds a patent for Intra-Target Microdosing (ITM). Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR001117 and the National Institutes of Health National Center for Research Resources (NCRR) grant number 1S10RR31792-1 through Duke Small Animal Translational PET/CT Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

1. Burt, T. *et al.* Microdosing and other phase 0 Clinical Trials: Facilitating Translation in Drug Development. *Clin. Transl. Sci.* **9**, 74–88 (2016).
2. Collins, F.S. Reengineering translational science: the time is right. *Sci. Transl. Med.* **10**, 397 (2011).
3. DiMasi, J.A., Hansen, R.W. & Grabowski, H.G. The price of innovation: new estimates of drug development costs. *J. Health Econ.* **22**, 151–185 (2003).
4. FDA. Innovation or stagnation: challenge and opportunity on the critical path to new medical products. http://wwwfdagov/oc/initiatives/criticalpath/whitepaperhtml, (2004).
5. Rowland, M. Microdosing and the 3Rs. In National Centre for the Replacement, Refinement & Reduction of Animals in Research.
6. Paul, S.M. *et al.* How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* **9**, 203–214 (2010).
7. Pammolli, F., Magazzini, L. & Riccaboni, M. The productivity crisis in pharmaceutical R&D. *Nat. Rev. Drug Discov.* **10**, 428–438 (2011).
8. ICH. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals M3(R2). In: *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use* 8–16 (ICH Secretariat, Geneva, Switzerland, 2009).
9. Burt, T., John, C.S., Ruckle, J.L. & Vuong, L.T. phase 0/microdosing studies using PET, AMS, and LC-MS/MS: a range of study methodologies and conduct considerations. Accelerating development of novel pharmaceuticals through safe testing in humans - a practical guide. *Expert Opin. Drug Del.* 1–16 (2016).
10. Yamane, N., Igarashi, A., Kusama, M., Maeda, K., Ikeda, T. & Sugiyama, Y. Cost-effectiveness analysis of microdose clinical trials in drug development. *Drug Metab. Pharmacokinet.* **28**, 187–195 (2013).
11. FDA. Guidance for Industry, Investigators, and Reviewers Exploratory IND Studies. http://wwwfdagov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078933pdf, (2006).
12. Burkhardt, J.K., Riina, H.A., Shin, B.J., Moliterno, J.A., Hofstetter, C.P. & Boockvar, J.A. Intra-arterial chemotherapy for malignant gliomas: a critical analysis. *Intervent. Neuroradiol.* **17**, 286–295 (2011).
13. Joshi, S., Meyers, P.M. & Ornstein, E. Intracarotid delivery of drugs: the potential and the pitfalls. *Anesthesiology* **109**, 543–564 (2008).
14. Sakai, K., Imaizumi, T., Masaki, H. & Takeshita, A. Intra-arterial infusion of insulin attenuates vasoreactivity in human forearm. *Hypertension* **22**, 67–73 (1993).
15. Wada, J. & Rasmussen, T. Intracarotid injection of sodium amytal for the lateralization of cerebral speech dominance. 1960. *J. Neurosurg.* **106**, 1117–1133 (2007).
16. Fikkers, B.G., Wuis, E.W., Wijnen, M.H. & Scheffer, G.J. Intraarterial injection of anesthetic drugs. *Anesth. Analg.* **103**, 792–794 (2006).
17. Yokoyama, J., Ito, S., Ohba, S., Fujimaki, M. & Ikeda, K. A novel approach to translymphatic chemotherapy targeting sentinel lymph nodes of patients with oral cancer using intra-arterial chemotherapy - preliminary study. *Head Neck Oncol.* **3**, 42 (2011).
18. Lau, W.Y., Yu, S.C., Lai, E.C. & Leung, T.W. Transarterial chemoembolization for hepatocellular carcinoma. *J. Am. Coll. Surg.* **202**, 155–168 (2006).
19. Liu, F. *et al.* Regional intra-arterial vs. systemic chemotherapy for advanced pancreatic cancer: a systematic review and meta-analysis of randomized controlled trials. *PLoS One* **7**, e40847, 1–8 (2012).
20. Gobin, Y.P., Dunkel, I.J., Marr, B.P., Brodie, S.E. & Abramson, D.H. Intra-arterial chemotherapy for the management of retinoblastoma: four-year experience. *Arch. Ophthalmol.* **129**, 732–737 (2011).
21. Balucani, C. & Grotta, J.C. Selecting stroke patients for intra-arterial therapy. *Neurology* **78**, 755–761 (2012).

22. Meng, L., Settecase, F., Xiao, J., Yu, Z., Flexman, A.M. & Higashida, R.T. Initial clinical experience with near-infrared spectroscopy in assessing cerebral tissue oxygen saturation in cerebral vasospasm before and after intra-arterial verapamil injection. *J Clin. Neurosci.* (2016).
23. Aellig, W.H. Venoconstrictor effect of dihydroergotamine in superficial hand veins. *Eur. J. Clin. Pharmacol.* **7**, 137–139 (1974).
24. Fung, M.M. *et al.* Direct vasoactive effects of the chromogranin A (CHGA) peptide catestatin in humans in vivo. *Clin. Exp. Hypertens.* **32**, 278–287 (2010).
25. Gwinn, W.M. *et al.* A comparison of non-toxin vaccine adjuvants for their ability to enhance the immunogenicity of nasally-administered anthrax recombinant protective antigen. *Vaccine* **31**, 1480–1489 (2013).
26. Lappin, G. & Garner, R.C. Big physics, small doses: the use of AMS and PET in human microdosing of development drugs. *Nat. Rev. Drug Discov.* **2**, 233–240 (2003).
27. Lappin, G., Boyce, M.J., Matzow, T., Lociuro, S., Seymour, M. & Warrington, S.J. A microdose study of C-AR-709 in healthy men: pharmacokinetics, absolute bioavailability and concentrations in key compartments of the lung. *Eur. J. Clin. Pharmacol.* **69**, 1673–1682 (2013).
28. Kummar, S. *et al.* Phase 0 Clinical trial of the poly (ADP-ribose) polymerase inhibitor ABT-888 in patients with advanced malignancies. *J. Clin. Oncol.* **27**, 2705–2711 (2009).
29. Lappin, G. Microdosing: current and the future. *Bioanalysis* **2**, 509–517 (2010).
30. Bauer, M., Wagner, C.C. & Langer, O. Microdosing studies in humans: the role of positron emission tomography. *Drugs R&D.* **9**, 73–81 (2008).
31. Wagner, C.C. & Langer, O. Approaches using molecular imaging technology — use of PET in clinical microdose studies. *Adv. Drug Deliv. Rev.* **63**, 539–546 (2011).
32. Bauer, M. *et al.* A positron emission tomography microdosing study with a potential antiamyloid drug in healthy volunteers and patients with Alzheimer's disease. *Clin. Pharmacol. Ther.* **80**, 216–227 (2006).
33. Bergstrom, M., Grahnen, A. & Langstrom, B. Positron emission tomography microdosing: a new concept with application in tracer and early clinical drug development. *Eur. J. Clin. Pharmacol.* **59**, 357–366 (2003).
34. Dunphy, M. *et al.* Progress in first-in-human trial of Hsp90-targeted PET imaging in cancer patients. *J. Nucl. Med. Meet. Abs.* **54**, 279- (2013).
35. Yamane, N., Tozuka, Z., Kusama, M., Maeda, K., Ikeda, T. & Sugiyama, Y. Clinical relevance of liquid chromatography tandem mass spectrometry as an analytical method in microdose clinical studies. *Pharm. Res.* **28**, 1963–1972 (2011).
36. Madan, A. *et al.* A pharmacokinetic evaluation of five H1 antagonists after an oral and intravenous microdose to human subjects. *Br. J. Clin. Pharmacol.* **67**, 288–298 (2008).
37. Heuveling, D.A. *et al.* Phase 0 microdosing PET study using the human mini antibody F16SIP in head and neck cancer patients. *J. Nucl. Med.* **54**, 397–401 (2013).
38. Vlaming, M. *et al.* Microdosing of a carbon-14 labeled protein in healthy volunteers accurately predicts its pharmacokinetics at therapeutic dosages. *Clin. Pharmacol. Ther.* **98**, 196–204 (2015).
39. Rowland, M. Microdosing: A critical assessment of human data. *J. Pharm. Sci.* **101**, 4067–4074 (2012).
40. Henderson, P.T. *et al.* A microdosing approach for characterizing formation and repair of carboplatin-DNA monoadducts and chemoresistance. *Int. J. Cancer* **129**, 1425–1434 (2011).
41. Vuong, L.T. *et al.* Use of accelerator mass spectrometry to measure the pharmacokinetics and peripheral blood mononuclear cell concentrations of zidovudine. *J. Pharm. Sci.* **97**, 2833–2843 (2008).
42. Croft, M., Keely, B., Morris, I., Tann, L. & Lappin, G. Predicting drug candidate victims of drug-drug interactions, using microdosing. *Clin. Pharmacokinet.* **51**, 237–246 (2012).
43. Maeda, K. & Sugiyama, Y. Novel stratergies for microdose studies using non-radiolabeled compounds. *Adv. Drug Deliv. Rev.* **63**, 532–538 (2011).
44. Ieiri, I. *et al.* Pharmacokinetic and pharmacogenomic profiles of telmisartan after the oral microdose and therapeutic dose. *Pharmacogenet. Genomics.* **21**, 495–505 (2011).
45. Kimland, E. & Odlind, V. Off-label drug use in pediatric patients. *Clin. Pharmacol. Ther.* **91**, 796–801 (2012).
46. Roth-Cline, M. & Nelson, R.M. Ethical considerations in conducting pediatric and neonatal research in clinical pharmacology. *Curr. Pharm. Des.* **21**, 5619–5635 (2015).
47. Turner, M.A. *et al.* Pediatric microdose and microtracer studies using (14) C in Europe. *Clin. Pharmacol. Ther.* **98**, 234–237 (2015).
48. Dunne, J. *et al.* Extrapolation of adult data and other data in pediatric drug-development programs. *Pediatrics* **128**, e1242–e1249 (2011).
49. Bellis, J.R. *et al.* Adverse drug reactions and off-label and unlicensed medicines in children: a nested case?control study of inpatients in a pediatric hospital. *BMC Med.* **11**, 238 (2013).
50. Rowland, M. & Benet, L.Z. Lead PK commentary: Predicting human pharmacokinetics. *J. Pharm. Sci.* **100**; 4047–4049 (2011).
51. NIH. *Clinicaltrials.gov.* <http://clinicaltrials.gov/ct2/home> (2016). Accessed February 11th 2014.
52. Lappin, G., Noveck, R. & Burt, T. Microdosing and drug development: past, present and future. *Expert Opin. Drug Metab. Toxicol.* **9**, 817–834 (2013).
53. Burt, T. *et al.* Intraarterial microdosing: a novel drug development approach, proof-of-concept PET study in rats. *J. Nucl. Med.* **56**, 1793–1799 (2015).
54. Burt, T. *et al.* Intra-target microdosing (ITM), a novel drug development approach: proof of concept in humans. In 2016 Annual Meeting of the American College of clinical pharmacology (ACCP).
55. Burt, T. *et al.* Intra-arterial microdosing (IAM), a novel drug development approach, proof of concept in rats. *Clin. Pharmacol. Ther.* **97**, S62 (2015).
56. Morgan, P. *et al.* Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov. Today.* **17**, 419–424 (2012).
57. Dhaun, N. *et al.* Vasodilator effects of the endothelin ET receptor selective antagonist BMS-193884 in healthy men. *Br. J. Clin. Pharmacol.* **60**, 611–622 (2005).
58. Hesse, C. *et al.* Kinetics and dynamics of the peripheral neurokinin-1 receptor antagonist SLV317 in healthy individuals. *Br. J. Clin. Pharmacol.* **61**, 414–419 (2006).
59. Greenblatt, D.J. In vitro prediction of clinical drug interactions with CYP3A substrates: we are not there yet. *Clin. Pharmacol. Ther.* **95**, 133–135 (2014).
60. Kan, P.T., Orion, D., Yashar, P. & Levy, E.I. Intra-arterial thrombolysis and thrombectomy for acute ischemic stroke: technique and results. *J. Neurosurg. Sci.* **55**, 151–160 (2011).
61. Sacks, L.V., Shamsuddin, H.H., Yasinskaya, Y.I., Bouri, K., Lanthier, M.L. & Sherman, R.E. Scientific and regulatory reasons for delay and denial of FDA approval of initial applications for new drugs, 2000-2012. *JAMA.* **311**, 378–384 (2014).
62. Rowland, M., Peck, C. & Tucker, G. Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu. Rev. Pharmacol. Toxicol.* **51**, 45–73 (2011).
63. Zhao, P., Rowland, M. & Huang, S.M. Best practice in the use of physiologically based pharmacokinetic modeling and simulation to address clinical pharmacology regulatory questions. *Clin. Pharmacol. Ther.* **92**, 17–20 (2012).
64. Oliver, R.E., Jones, A.F. & Rowland, M. A whole-body physiologically based pharmacokinetic model incorporating dispersion concepts: short and long time characteristics. *J. Pharmacokinet. Pharmacodynam.* **28**, 27–55 (2001).
65. Tabrizchi, R. & Pugsley, M.K. Methods of blood flow measurement in the arterial circulatory system. *J. Pharmacol. Toxicol. Methods.* **44**, 375–384 (2000).
66. Sabapathy, S.R., Firrell, J.C. & Kleinert, J.M. Evaluation of sympathetic control of digital blood flow. *J. Hand Surg.* **18**, 634–638 (1993).
67. Jones, H.M. *et al.* Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clin. Pharmacol. Ther.* **97**, 247–262 (2015).
68. Zunszain, P.A., Horowitz, M.A., Cattaneo, A., Lupi, M.M. & Pariante, C.M. Ketamine: synaptogenesis, immunomodulation and glycogen synthase kinase-3 as underlying mechanisms of its antidepressant properties. *Mol. Psychiatry.* **18**, 1236–1241 (2013).
69. Sykes, D.A. *et al.* The Influence of receptor kinetics on the onset and duration of action and the therapeutic index of NVA237 and tiotropium. *J. Pharmacol. Exp. Ther.* **343**, 520–528 (2012).
70. Nicholls, D.J. *et al.* Characteristics of AZD9708, a novel, selective beta2-adrenoceptor agonist with rapid onset and long duration of action. *Pulmon. Pharmacol. Ther.* **25**, 293–302 (2012).
71. Debruyne, F.M., Gittelman, M., Sperling, H., Borner, M. & Beneke, M. Time to onset of action of vardenafil: a retrospective analysis of the pivotal trials for the orodispersible and film-coated tablet formulations. *J. Sex. Med.* **8**, 2912–2923 (2011).
72. Minami, H., Yasu, T., Tagawa, T., Yamakawa, K. & Ueda, S. Slower onset of vasodilating action of brain natriuretic peptide (BNP) compared to atrial natriuretic peptide (ANP) in human forearm resistant vessels. *Eur. J. Clin. Pharmacol.* **64**, 859–862 (2008).
73. Krafte, D.S. & Bannon, A.W. Sodium channels and nociception: recent concepts and therapeutic opportunities. *Curr. Opin. Pharmacol.* **8**, 50–56 (2008).
74. Schachtel, B. *et al.* Onset of action of a lozenge containing flurbiprofen 8.75mg: A randomized, double-blind, placebo-controlled trial with a new method for measuring onset of analgesic activity. *Pain.* **155**, 422–428 (2014).
75. Durham, P.L. & Masterson, C.G. Two mechanisms involved in trigeminal CGRP release: implications for migraine treatment. *Headache.* **53**, 67–80 (2013).
76. Alexopoulos, D. *et al.* Double versus standard loading dose of ticagrelor: onset of antiplatelet action in patients with STEMI undergoing primary PCI. *J. Am. Coll. Cardiol.* **62**, 940–941 (2013).
77. Berkowitz, R.B. *et al.* Onset of action of fexofenadine hydrochloride 60 mg/pseudoephedrine hydrochloride 120 mg in subjects aged 12 years with moderate to severe seasonal allergic rhinitis: a pooled analysis of two single-dose, randomized, double-blind, placebo-controlled allergen exposure unit studies. *Clin. Ther.* **28**, 1658–1669 (2006).
78. Foldes, F.F., Nagashima, H., Nguyen, H.D., Schiller, W.S., Mason, M.M. & Ohta, Y. The neuromuscular effects of ORG9426 in patients receiving balanced anesthesia. *Anesthesiology.* **75**, 191–196 (1991).
79. Greenberg, S.B. & Vender, J. The use of neuromuscular blocking agents in the ICU: where are we now? *Crit. Care Med.* **41**, 1332–1344 (2013).
80. Murphy, G.S. *et al.* Postoperative residual neuromuscular blockade is associated with impaired clinical recovery. *Anesth. Analg.* **117**, 133–141 (2013).

81. Schaller, S.J., Fink, H., Ulm, K. & Blobner, M. Sugammadex and neostigmine dose-finding study for reversal of shallow residual neuromuscular block. *Anesthesiology*. **113**, 1054–1060 (2010).
82. McDonagh, D.L. *et al.* Efficacy, safety, and pharmacokinetics of sugammadex for the reversal of rocuronium-induced neuromuscular blockade in elderly patients. *Anesthesiology*. **114**, 318–329 (2011).
83. Yang, C.I., Fine, G.F., Jooste, E.H., Mutich, R., Walczak, S.A. & Motoyama, E.K. The effect of cisatracurium and rocuronium on lung function in anesthetized children. *Anesth. Analg.* **117**, 1393–1400 (2013).
84. Vasilakou, D. *et al.* Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann. Intern. Med.* **159**, 262–274 (2013).
85. Venugopal, J. Pharmacological modulation of the natriuretic peptide system. *Expert Opin. Ther. Patents*. **13**, 1389–1409 (2003).
86. Song, M.J. *et al.* Predictive value of (1)(8)F-fluorodeoxyglucose PET/CT for transarterial chemolipiodolization of hepatocellular carcinoma. *World J. Gastroenterol.* **18**, 3215–3222 (2012).
87. Bench, C.J. *et al.* The time course of binding to striatal dopamine D2 receptors by the neuroleptic ziprasidone (CP-88,059-01) determined by positron emission tomography. *Psychopharmacology*. **124**, 141–147 (1996).

Supplementary information accompanies this paper on the *Clinical and Translational Science* website. (http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-8062)