Citation: Clin Transl Sci (2017) 10, 351–359; doi:10.1111/cts.12477

ARTICLE

Intra-Target Microdosing – A Novel Drug Development Approach: Proof of Concept, Safety, and Feasibility Study in Humans

T Burt[1,*], D MacLeod[2], K Lee[3], A Santoro[2], DK DeMasi[4], T Hawk[5], M Feinglos[6], M Rowland[7] and RJ Noveck[8]

Intra-Target Microdosing (ITM) is a novel drug development approach aimed at increasing the efficiency of first-in-human (FIH) testing of new molecular entities (NMEs). ITM combines intra-target drug delivery and "microdosing," the subpharmacological systemic exposure. We hypothesized that when the target tissue is small (about 1/100th of total body mass), ITM can lead to target therapeutic-level exposure with minimal (microdose) systemic exposure. Each of five healthy male volunteers received insulin microdose into the radial artery or full therapeutic dose intravenously in separate visits. Insulin and glucose levels were similar between systemic administration and ITM administration in the ipsilateral hand, and glucose levels demonstrated a reduction in the ipsilateral hand but not in the contralateral hand. Positron emission tomography (PET) imaging of ^{18}F-fluorodeoxyglucose (FDG) uptake demonstrated differences between the ipsilateral and contralateral arms. The procedures were safe and well-tolerated. Results are consistent with ITM proof-of-concept (POC) and demonstrate the ethical, regulatory, and logistical feasibility of the approach.
Clin Transl Sci (2017) **10**, 351–359; doi:10.1111/cts.12477; published online on 8 July 2017.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ ITM enables the study of PD and PK characteristics of drugs with minimal systemic exposure. An animal POC study was conducted using the same ITM methodology.[23]

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ The study aimed to demonstrate the POC and feasibility of the ITM approach in humans.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ This is the first reported ITM study in humans. The ITM, in its multimodality, adaptive design is feasible from scientific, ethical, and practical considerations. PK and PD information can be obtained in humans with minimal systemic risk.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ ITM could help address limitations of traditional microdosing approaches and the challenges of FIH studies in general to accelerate development of new drugs in humans.

Drug development is a risky, expensive, and error-prone process and becoming more so.[1–3] Increasing productivity of basic science discoveries inspired by decoding of the human genome and greater understanding of disease processes, together with decreasing productivity of clinical development, have led to a virtual bottle-neck at the translational interface, from discovery into human testing.[4–9] These dynamics have made the safe and efficient translation of biological insights into human applications a major public health priority and challenge. Safety concerns associated with new molecular entities (NMEs) lead to substantial preclinical developmental costs, delaying entry into first-in-human (FIH) testing. To help address these challenges we introduce a novel drug development approach, Intra-Target Microdosing (ITM), combining features of "microdosing," the systemic subpharmacological testing of drugs, and intra-target drug delivery.[10]

Microdosing studies, which are an exploratory investigational new drug approach, also described as "exploratory clinical trials," are a regulatory framework aimed at improving the efficiency of drug development and specifically of the entry of NMEs into human testing.[11–14] The microdosing approach exposes human research participants to only a fraction of the phase I doses (1/100th of the anticipated minimal effective dose or 100 μg, whichever is lower)[15–18] and the inherent safety of the approach leads to significant reduction in expense and duration of preclinical requirements. This allows meaningful developmental

[1]Burt Consultancy, LLC, Durham, North Carolina, USA; [2]Department of Anesthesiology, Duke University, Durham, North Carolina, USA; [3]MI, CCC-TDI, OPS, SV, Siemens Medical Solutions USA, Inc., Knoxville, Tennessee, USA; [4]Wake Forest School of Medicine, Winston Salem, North Carolina, USA; [5]Department of Radiology, Duke University, Durham, North Carolina, USA; [6]Department of Endocrinology, Duke University, Durham, North Carolina, USA; [7]Manchester Pharmacy School, University of Manchester, Manchester, UK; [8]Duke Clinical Research Institute, Duke University, Durham, North Carolina, USA. *Correspondence: T Burt (talburtmd@gmail.com)
Received 13 March 2017; accepted 25 April 2017; published online on 8 July 2017. doi:10.1111/cts.12477

Table 1 Intra-Target Microdosing proof-of-concept program

Primary hypothesis: ITM ≅ SF (glucose)			
		ITM ≅ SF (insulin; ^{18}F-FDG uptake) ITM >> SM; SF >> SM	
		Observations	
Secondary hypotheses:		**Ipsilateral**	**Contralateral**
Interventions	ITM	ITM	SM
	Systemic full-dose	SF	SF

FDG, fluorodeoxyglucose; ITM, Intra-Target Microdosing local PK/PD during ITM intervention; PK/PD, pharmacokinetic/pharmacodynamic; SF, systemic PK/PD during systemic full-dose administration; SM, systemic PK/PD after microdose exposure (e.g., contralateral effects post-ITM).
The primary hypothesis is that the effect of insulin on glucose levels in the anatomic target (i.e., the ipsilateral hand) after ITM intervention is similar to that after systemic full-dose administration (ITM ≅ SF [glucose]). The secondary hypotheses are: (1) insulin levels and ^{18}F-FDG uptake in the ipsilateral hand are similar after ITM and systemic full-dose administration (ITM ≅ SF [insulin; ^{18}F-FDG uptake]); (2) effects in the ipsilateral hand after ITM are much larger than those elsewhere in the body (e.g., contralateral hand; ITM >> SM); and (3) effects in areas other than the anatomic target after systemic full-dose administration are much larger than those after ITM (e.g., in ITM contralateral; SF >> SM). Interventions in the rows are matched with observations in the columns in testing the primary and secondary hypotheses.

Table 2 Participant characteristics and insulin administration schedule

Subject	Age, years	BMI (kg/m^2)	Insulin Dose		5-min tourniquet
			Systemic	ITM	
A	24	25.4	2 IU	0.02 IU	–
B	23	22.4	2 IU	0.02 IU	–
C	21	26.2	2 IU	0.2 IU	+
D	34	28.4	2 IU	0.2 IU	+
E	30	26.1	2.5 IU	0.03 IU	+

BMI, body mass index; ITM, Intra-Target Microdosing.
All research participants were young healthy men. After the lowest insulin effective thresholds were determined in visit 1, the table indicates the doses administered during visit 2 ("systemic," intravenously) and visit 3 ("ITM," intra-arterially). Plus (+) or minus (-) signs indicate the application, or not, respectively, of a 5-min tourniquet to the ipsilateral arm (i.e., the arm where insulin was administered intra-arterially) immediately after administration of insulin. In those subjects (C, D, and E) where the tourniquet was applied, it was applied also during the systemic visit as well (visit 2) to establish comparable conditions for the ipsilateral hand.

decisions to be made prior to the more expensive full-dose human testing stages by providing human data, mostly systemic pharmacokinetic (PK) data, earlier and cheaper in the development process.[19–21] Nevertheless, concerns about extrapolation of data from microdose to full dose levels of exposure and the absence of pharmacodynamic (PD) data have limited the application of microdosing in drug development.[15,22]

To address these microdosing limitations, the objective of the ITM development program was to demonstrate that ITM is associated with local full-dose exposure but only microdose-level systemic exposure, and, hence, that local PD and systemic PK data may be obtained, simultaneously, with minimal or no systemic risk. Thus, it allows simultaneous comparison of microdose exposure and therapeutic-level exposure in the same individuals, and provides insight into the PD effects of the test article (both efficacy and toxicity related) at therapeutic-level exposures, addressing the limitations of traditional microdosing approaches. **Table 1** outlines the study objectives and expected outcomes. In addition to the scientific objectives, we aimed to demonstrate the operational, ethical, and regulatory feasibility of the approach as well as its safety. We previously reported the results of a rodent ITM study using similar methodology.[23] Here, we present, to our knowledge, the results of the first feasibility and proof-of-concept (POC) human study of the ITM approach.

METHODS

Study objectives and hypotheses

The primary objective of this pilot, feasibility, and safety POC study was to demonstrate that local effects after ITM are similar to those after systemic full-dose, therapeutic-level administration (**Table 1**). The secondary objective was to demonstrate that local exposure after ITM is higher locally than systemically in the same individuals at the same time. The corresponding hypotheses are (**Table 1**): the primary hypothesis is that the effect of insulin on glucose levels in the anatomic target (i.e., the ipsilateral hand) after ITM intervention is similar to that after systemic full-dose administration (ITM ≅ SF [glucose]). The secondary hypotheses are: (i) insulin levels and ^{18}F-fluorodeoxyglucose (FDG) uptake in the ipsilateral hand are similar after ITM and systemic full-dose administration (ITM ≅ SF [insulin; ^{18}F-FDG uptake]); (ii) effects in the ipsilateral hand after ITM are much larger than those elsewhere in the body (e.g., contralateral hand; ITM >> SM); and (iii) effects in areas other than the anatomic target after systemic full-dose administration are much larger than those after ITM (e.g., in ITM contralateral; SF >> SM). Insulin was chosen as the test article for PK measurements, and glucose plasma levels (the primary outcome) and ^{18}F-FDG uptake – the biomarkers for PD measurements.

Ethics and regulatory

The study was performed at the Duke Clinical Research Unit, at Duke University Medical Center, was approved by the Duke University Medical Center Institutional Review Board, and was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guideline for Good Clinical Practice (E6). Volunteers underwent an informed consent process and signed the institutional review board-approved informed consent prior to initiation of study procedures. The US Food and Drug Administration (FDA) provided an investigational new drug exemption for the intra-arterial administration of insulin in this study. The study was registered with the clinicaltrials.gov database (NCT02304211).

Volunteers. Inclusion/exclusion criteria

Eleven male volunteers were screened, six passed the inclusion/exclusion criteria, and five (subjects A–E, mean age 26.4 ± 4.8 years; body mass index 25.7 ± 1.9 kg/m^2; **Table 2**) completed the three study visits. One eligible participant did not complete the study due to incompatibility with the study schedule. Participants were healthy, nonsmoking male subjects, and free of medication during the prior 14 days. A modified Allen's test was performed to confirm ulnar artery patency and safety of radial artery cannulation.[24]

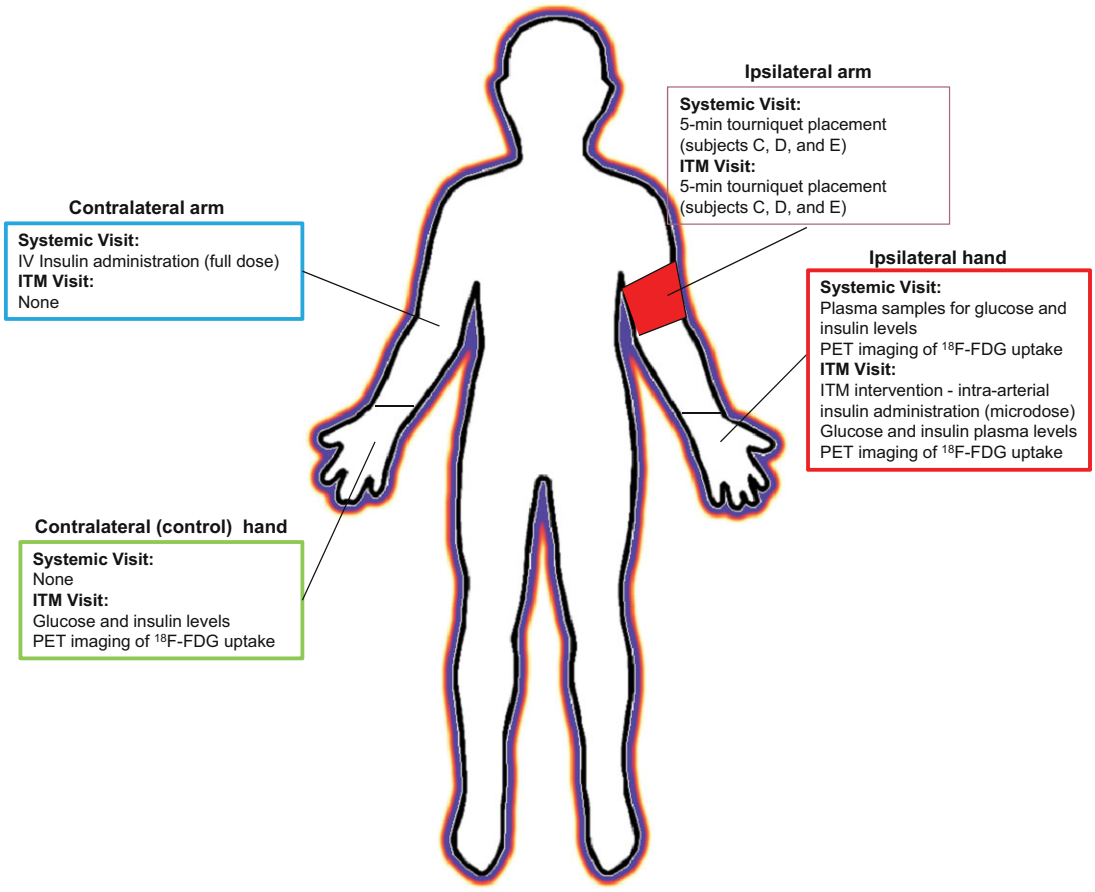

Figure 1 Intra-Target Microdosing (ITM) study: schematic of procedures. The "systemic" and "ITM" visits were separated by 1 week. During the "systemic" visit, insulin full-dose was administered intravenously into the arm (median cubital) vein, glucose and insulin plasma levels obtained from the ipsilateral superficial (cephalic) hand veins, and simultaneous positron emission tomography (PET) imaging performed of ^{18}F-fluorodeoxyglucose (FDG) uptake into ipsilateral hand muscles. During the "ITM" visit, insulin microdose was administered into the ipsilateral radial artery, glucose and insulin plasma levels were obtained from the ipsilateral and contralateral superficial (cephalic) hand veins, and simultaneous PET imaging performed of ^{18}F-FDG uptake into ipsilateral and contralateral hand muscles. A tourniquet was placed for 5 min immediately after insulin administration on the ipsilateral arm during both "systemic" and "ITM" visits to increase local exposure to insulin (only in subjects C, D, and E). Ipsilateral is the side of ITM intervention. IV, intravenous.

Evaluations included medical history, physical examination, and routine blood and urine laboratory testing, including toxicology screen to determine health status. The small sample size and concerns about variability of the results precluded recruitment of female participants.

Study design

The study followed an open-label, sequential group, adaptive design protocol (**Figure 1**). Eligible participants entered a 3-visit protocol over 3 weeks. During visit 1, the minimal insulin dose necessary to generate a measurable systemic reduction in plasma glucose levels was determined. In visit 2, the minimum insulin dose was administered systemically, and in visit 3, the calculated ITM dose was administered intra-arterially into the nondominant, ipsilateral, radial artery. Insulin administration in visits 2 and 3 was followed by positron emission tomography (PET) imaging of ^{18}F-FDG uptake and simultaneous blood sampling for glucose and insulin plasma levels from ipsilateral and contralateral superficial hand veins.

Adaptive design

We used an adaptive design that allowed two interventions to be modified in sequential groups according to the results of the earlier groups. Groups varied by amount of intra-arterial insulin administered and whether a tourniquet was applied on the ipsilateral arm (the arm of the intra-arterial administration).

Primary and secondary outcomes

Glucose concentrations in the vein draining the ipsilateral (i.e., side of the intra-arterial insulin administration) during

ITM vs. systemic administrations was the primary outcome, and vs. contralateral administration was the secondary outcome (**Table 1**). A positive change consistent with insulin effect was predefined as 20% or greater reduction in glucose plasma levels vs. baseline. Respective insulin plasma levels were secondary outcomes. ^{18}F-FDG-uptake in ipsilateral, contralateral, and systemic regions of interest (ROI) was also a secondary outcome.

The hand was chosen as a model of peripheral drug action, being less than 1/100th total body mass (about 0.6%), familiarity with radial artery cannulation, accessibility, mobility, and minimal invasiveness. Insulin was chosen for its known physiological actions, quick responding biomarkers (glucose and ^{18}F-FDG uptake), abundance of reference information, relevance to the hand, and known antidote, glucose. Additionally, insulin has been previously administered intra-arterially in the forearm.[25–27] ^{18}F-FDG was chosen due to its safety, familiarity, and quick visualization of glucose uptake.[23] These design characteristics were used in our animal POC study.[23]

Tourniquet placement

In subjects C, D, and E, a tourniquet was placed for 5 min on the ipsilateral arm to increase duration of local exposure to insulin after intra-arterial administration (**Table 2**). The tourniquet was applied as control during the corresponding "systemic" visit and to ensure comparable conditions.

Dose formulation and administration

The Duke Investigational Research Pharmacy prepared insulin syringes for intra-arterial or intravenous administration using regular insulin (Humulin R; Eli Lilly). For each subject a "stock syringe" was prepared and then transferred the respective volumes to dosing syringes. To prepare the dose, a volume of 0.3 mL of Humulin-R (100 units/mL) was mixed with a volume of 29.7 mL 0.9% sodium chloride preparation. This prepared a 30 mL "stock syringe" of 1 unit/mL. The volumes corresponding with the desired dose were then drawn into appropriate-sized syringes with the desired concentrations and corrected for residual doses in the administering apparatus, including syringes. Insulin was administered either intra-arterially into the radial artery (ITM intervention) or into the arm vein (systemic intervention) over $\sim$20 s.

Intra-arterial and venous catheters

During visit 3 (ITM visit), the arterial catheter was placed into the nondominant radial artery (in the left side for all volunteers) after performing the modified Allen's test. For serial blood collections, an indwelling catheter was placed into the forearm. Venous drainage of the hand is predominantly on the dorsum of the hand so the sampling catheter was placed in the cephalic vein. Distal forearm catheters were used for the collection of biomarker (glucose) and test article (insulin) samples in the ipsilateral arm, whereas systemic samples were obtained from the contralateral arm.

Sample collection

Blood samples were collected at baseline, prior to dosing, and at 5, 10, 15, 30, and 60 min after dosing. The time points for sample collection and PET imaging were based on the known half-life of insulin (4–6 min). This allowed observation duration to cover five times the insulin half-life, an accepted standard for the measurements of drug PK. The blood samples were centrifuged soon after collection at 15,000 g to produce plasma, which was then transferred into duplicate tubes and stored at -70°C.

Insulin plasma levels

Plasma insulin was analyzed in duplicate by the enzyme-linked immunosorbent assay method with kits purchased from the American Laboratory Products Company (ALPCO, Salem, NH; catalog number 80-INSHU-E01.1). All tests were performed in accordance with the manufacturer's instructions.

PET procedures

PET imaging was performed on a Discovery IQ PET/CT (GE Healthcare, Milwaukee, WI). This system has a large (26 cm) axial field of view. Subjects were positioned with their hands gently resting on hemispherical protrusions from a rigid foam plastic board resting on the thighs, isolating the hands from background activity on other parts of the body to the degree possible. Low-dose computed tomography (CT) imaging was done for purposes of PET attenuation correction and used for subsequent ROI identification. PET data acquisition commenced at the time of FDG injection (185.52 $\pm$ 7.51 MBq; $n = 10$ [systemic $= 5$; ITM $= 5$]) and continued for 1 h using the list mode. Data were subsequently played back into 12 5-min frames (12 frames $\times$ 300 s) and reconstructed. Images were reconstructed into a 128 $\times$ 128 $\times$ 79 volume with 3.9 mm $\times$ 3.9 mm $\times$ 3.29 mm voxels using the ordered subsets expectation maximization algorithm. Corrections for attenuation and scatter (both using the CT images), random events, and dead-time were applied. Injected dose (corrected for residual activity in the injection device) and patient weight were recorded in the image sets for subsequent calculation of standardized uptake values (SUV-body weight).

PET image processing was performed using the Inveon Research Workplace 4.2 package (Siemens Medical Solutions). The ROIs were drawn on the CT images with a soft tissue threshold applied (Hounsfield units -300 to 300). Right and left hand ROIs were drawn to cover the area of the metacarpals (wrist to the first knuckle), and circular ROIs in the adductor pollicis and the hamstring muscles. ROIs were applied to the dynamic PET data set and time activity curves were generated with a unit of SUV-body weight.

PET image analysis

PET FDG SUV time-activity curves, reflecting ^{18}F-FDG uptake into tissues in ROIs were established according to previously reported methodology.[23] Time-activity curves were corrected for baseline values for each subject. To calculate the difference between systemic, ITM ipsilateral (ITM[IL]), and ITM contralateral (ITM[CL]) for each subject, paired *t*-test was used ($P < 0.05$). The systemic observations were taken from the same side of the ITM observations (e.g., if ITM[IL] was the comparison arm, it was compared with the ipsilateral side during the systemic imaging).

Safety considerations and monitoring

Key safety considerations were related to the intra-arterial procedure and potential hypoglycemia postinsulin administration. The intra-arterial procedure was performed only in subjects with a normal Allen's test, confirming patency of the ulnar artery. The procedure was performed using ultrasound guidance with a high-resolution ultrasound transducer probe by an experienced anesthesiologist using a local anesthetic to increase the accuracy of the procedure and reduce subject discomfort and the likelihood of multiple puncture wounds and/or extravasation. Even though the concentration of insulin infused into the artery was within physiological range, for added safety, an infusion of dextrose-in-water was maintained on standby throughout the experiment to counter potential symptoms of hypoglycemia. Blood glucose levels, heart rate, and blood pressure were repeatedly measured and recorded. Adverse events were recorded.

RESULTS

Safety

Study participants experienced no major adverse events. Minor events included local bruising associated with intra-arterial and intravenous catheters and mild tiredness and diaphoresis possibly associated with hypoglycemic effects postsystemic insulin administration.

Subjects A, B, C, and D each received 2 IU and subject E received 2.5 IU of insulin systemically during the second visit (based on results of visit 1). Subjects A and B received 0.02 IU intra-arterially during the third visit. Because no changes were observed in insulin or glucose plasma levels in these subjects (**Supplemental Information Table A**), a decision was made to increase the dose and apply tourniquets in the next group. Subjects C and D received 0.2 IU intra-arterially during the third visit and had a tourniquet inflated for 5 min over the ipsilateral arm (the arm receiving the intra-arterial insulin) to prolong tissue exposure to insulin.

In subjects C and D, there was an increase in ipsilateral insulin levels and a corresponding drop in glucose levels (**Supplemental Information Table A; Figures 2 and 3**) after intra-arterial administration in the ipsilateral arm but not the contralateral arm. Based on these results, subject E received 0.03 IU intra-arterially (corresponding with a microdose calculated on a total body basis) during the third visit and had a tourniquet inflated for 5 min over the ipsilateral arm. As with subject Cs and D, subject E experienced a reduction in the ipsilateral glucose levels after ITM corresponding to an increase in ipsilateral insulin levels (**Supplemental Information Table A; Figure 4**). Compared with baseline, maximal reductions in glucose plasma levels in subjects C, D, and E, were 29%, 24%, and 33% with systemic intervention, 39%, 18%, and 19% with ITM(IL), and 6%, 5%, and 13% with ITM(CL), respectively.

Notably, both insulin and glucose level changes post-ITM were brief and limited to 5–10 min after insulin microdose administration. Insulin plasma levels after systemic administration were also brief but glucose plasma level changes were more protracted (and in subjects A and E led to brief hypoglycemic symptoms that necessitated administration of dextrose-in-water after 30 min). There were no meaningful changes in glucose or insulin plasma levels in the contralateral arm after ITM, consistent with the hypothesized systemic microdose exposure to insulin.

PET imaging analysis

Results of the PET analyses are shown in **Table 3**. Subject C was excluded because the CT-based attenuation correction was not established. Differences between systemic and ITM(IL) interventions were statistically significant for three

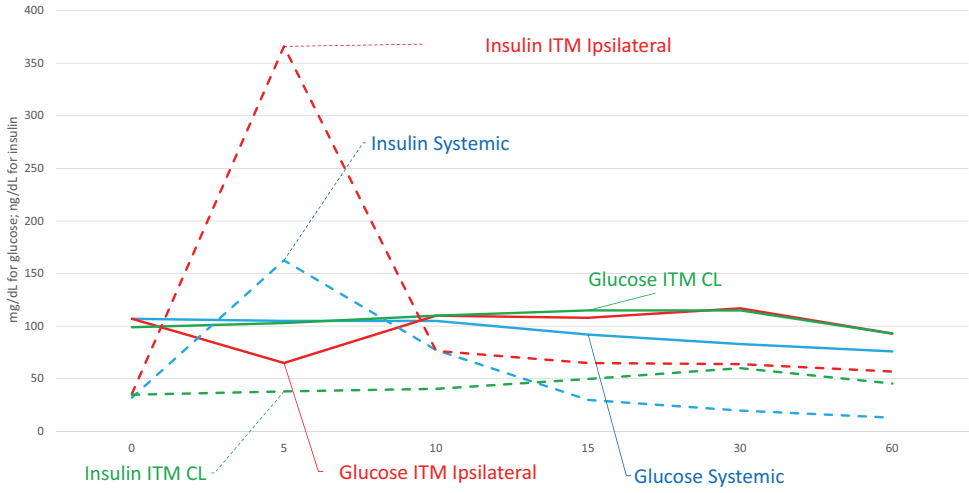

Figure 2 Subject C. Insulin and reciprocating glucose plasma level changes post-Intra-Target Microdosing ipsilateral (ITM[IL]) and postsystemic insulin administration. As with postsystemic administration, glucose levels drop post-ITM with corresponding insulin level changes. A positive change consistent with insulin effect was predefined as 20% or greater reduction in glucose plasma levels vs. baseline and was the primary outcome. Glucose levels were reduced 29% with systemic intervention (from 107 to 76 mg/dL) and 39% with ITM(IL) (from 107 to 65 mg/dL). No meaningful reduction was observed with ITM contralateral (ITM[CL]; 6%; from 99 to 93 mg/dL). Ipsilateral = side of ITM intervention; plasma levels obtained from the ipsilateral arm vein; Contralateral = plasma levels from the contralateral arm vein during the ITM intervention.

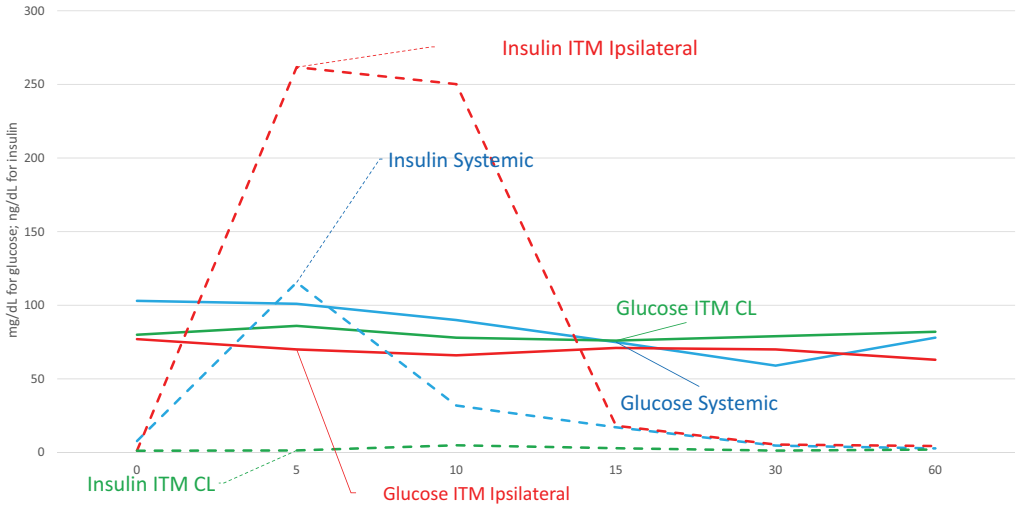

Figure 3 Subject D. Insulin and reciprocating glucose plasma level changes post-Intra-Target Microdosing ipsilateral (ITM[IL]) and post-systemic insulin administration. As with postsystemic administration, glucose levels drop post-ITM (albeit briefly) with corresponding insulin level changes. A positive change consistent with insulin effect was predefined as 20% or greater reduction in glucose plasma levels vs. baseline and was the primary outcome. Glucose levels were reduced 24% with systemic intervention (from 103 to 78 mg/dL) and 18% with ITM(IL) (from 77 to 63 mg/dL). No meaningful reduction was observed with ITM contralateral (5%, from 80 to 76 mg/dL). Ipsilateral = side of ITM intervention; plasma levels obtained from the ipsilateral arm vein; Contralateral = plasma levels from the contralateral arm vein during the ITM intervention.

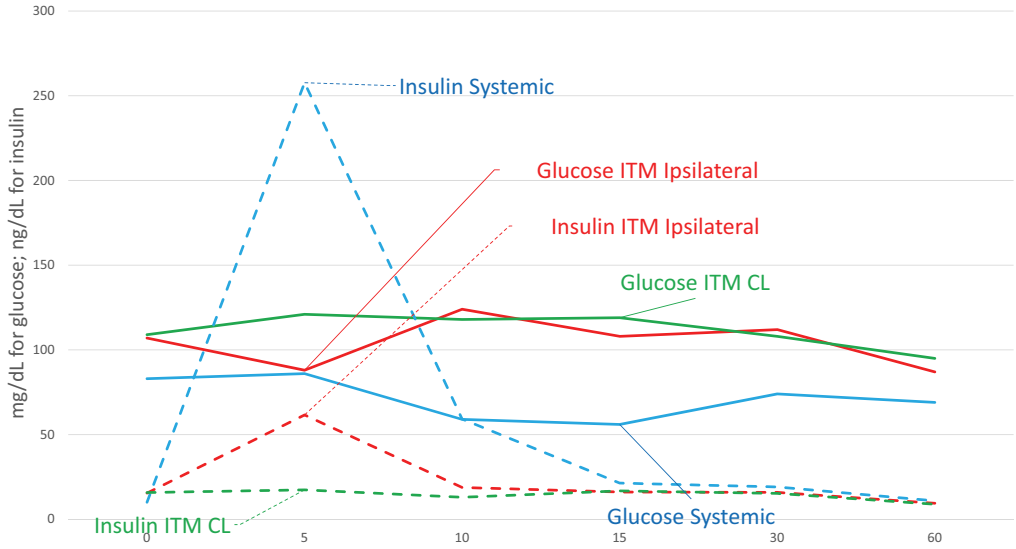

Figure 4 Subject E. Insulin and reciprocating glucose plasma level changes post-Intra-Target Microdosing ipsilateral (ITM[IL]) and post-systemic insulin administration. As with postsystemic administration, glucose levels drop post-ITM (albeit briefly) with corresponding insulin level changes. A positive change consistent with insulin effect was predefined as 20% or greater reduction in glucose plasma levels vs. baseline and was the primary outcome. Glucose levels were reduced 33% with systemic intervention (from 83 to 56 mg/dL) and 19% with ITM(IL) (from 107 to 87 mg/dL). No meaningful reduction was observed with ITM contralateral (ITM[CL]; 13%, from 109 to 95 mg/dL). Ipsilateral = side of ITM intervention; plasma levels obtained from the ipsilateral arm vein; Contralateral = plasma levels from the contralateral arm vein during the ITM intervention.

subjects (A, B, and E). Differences between systemic and ITM(CL) were significant for subjects A, D, and E, consistent with the secondary hypotheses. Differences between ITM(IL) and ITM(CL) observations were significant for subject B, and trend significant for subjects A and E, also consistent with secondary hypotheses.

DISCUSSION

Drug development is a risky, lengthy, expensive, and error-prone process, especially in clinical development where >90% of new drug candidates fail.[28–34] Much of the expense and duration of drug development is spent on ensuring safety

Table 3 PET imaging analyses

	Paired t- test (p-value)		
Subject	**Sys vs. ITM(IL)**	**Sys vs. ITM(CL)**	**ITM(IL) vs. ITM(CL)**
A	0.001	0.002	0.092
B	0.012	0.768	0.003
D	0.079	0.021	0.312
E	0.035	0.006	0.067

CL, contralateral; FDG, fluorodeoxyglucose; ITM(IL), Intra-Target Microdosing (ipsilateral); PET, positron emission tomography; Sys., systemic.
Results of PET imaging analysis of ^{18}F-FDG uptake. The systemic measurements were taken from the same side as the ITM comparison (i.e., ITM[IL] compared with systemic ipsilateral). Subject C was excluded because computed tomography-based attenuation correction was not established. The cell shades represent the different intervention groups as per Table **2** (A and B, C and D, and E).

in humans, with many vulnerable populations (frail elderly, pediatric, women, renally impaired, and hepatically impaired) routinely excluded from testing of new drugs due to safety concerns. For example, only a minority of drugs approved in young adults have been fully tested in frail elderly populations; even though elderly, major consumers of prescription drugs require age-related pharmacotherapy adaptations.[35,36] Off-label use is extensive and associated with increased incidence of potentially inappropriate medication use and adverse drug reactions.[37–39] Traditional development necessitates a substantial package of preclinical safety data, genotoxicology, and compliance with Good Manufacturing Practice standards that can take between 12 and 18 months to complete. It is in this context of risk, expense, and duration of development of traditional approaches that the need for alternative approaches is appreciated. Regulators now allow FIH testing with limited amounts and/or duration of exposure to the new drug under an exploratory mechanism sometimes called "phase 0."[12] This mechanism has a few critical limitations that our ITM approach proposes to resolve.

We propose ITM as a novel methodology complementing and enhancing existing drug development approaches to accelerate development of NMEs and of existing drugs in vulnerable populations.[10,15,22,23] The approach is uniquely positioned to address current translational research limitations. Our ITM concept manuscript discusses the many applications of the approach with detailed examples and applicable developmental scenarios.[10]

Microdosing

Among "phase 0" approaches, microdosing allows testing of subpharmacological doses in humans prior to traditional phase I trials to safely gain initial insights into drug response. The very low doses involved are considered to have no significant toxicological concerns and, therefore, can be administered to humans based on a much-reduced safety package compared to that required for a full phase I trial.[40–42] This means quicker and cheaper human testing, informed selection among multiple preclinical analogues, and the ability to safely study old drugs in vulnerable populations. Sensitive bioanalytical techniques (e.g., PET of radiolabeled drugs) are required to measure the very low concentrations generated by this approach.[18,43,44] Microdosing has been strongly endorsed by both the FDA and the National Institutes of Health.[11,41]

Current limitations of microdosing addressed by ITM

Microdosing in its current format provides only PK data because of the subpharmacological exposures, by definition, do not generate any measurable biomarkers. Although this is the basis for microdosing's safety attributes, the inability to learn about drug actions with microdosing limits its application and utility in drug development. Another limitation of microdosing is the concern about predictability of the full dose from microdose data. Indeed, the presence of any nonlinear pharmacological mechanism in the absorption, distribution, metabolism, and elimination of the test article would weaken the predictability of full-dose data from microdosing data. Using ITM (where "target" indicates an anatomopathological, not molecular, entity) addresses these major microdosing limitations by allowing testing of drug effects only in a very small part of the living human body and for a very brief duration, thus reducing the toxicity risks. The ITM approach, to which we coined the term "in-humano" in a recent publication to indicate preclinical testing in humans,[22] also allows the local full-dose effects to be compared simultaneously with systemic subpharmacological measurements, thus addressing concerns about linearity, extrapolation, and predictability of microdosing observations.

In our study, consistent with the primary hypothesis of glucose plasma levels in subjects C, D, and E demonstrated a hypoglycemic-like reduction in the ipsilateral side after ITM insulin administration and after systemic insulin administration (**Figures 2, 3, and 4**). The magnitude of the reduction was similar in both interventions; however, the duration of the reduction seemed shorter after ITM than after systemic administration. Subjects A and B did not demonstrate a discernible reduction in glucose plasma levels. Subjects C, D, and E differed from subjects A and B by having a tourniquet placed on the ipsilateral arm for 5 min after insulin administration into the radial artery of the same side to increase the exposure to insulin in the ipsilateral hand. Subjects C and D also had a 10-fold increase in the ITM dose (from 0.02 IU to 0.2 IU) reduced again to microdose levels in subject E.

Consistent with the secondary hypotheses, ipsilateral plasma insulin levels post-ITM and postsystemic administration were increased in subjects C, D, and E, reciprocating the reduction in glucose plasma levels (**Figures 2, 3, and 4**). Likewise, plasma glucose and insulin levels remained relatively unchanged in the contralateral side after ITM administration and consequently consistent with a minimal or no PD effects after microdose-level insulin exposure. The PET imaging analyses, consistent with the secondary hypothesis, demonstrated greater effect after systemic administration than contralaterally after ITM administration in subjects A, D, and E, and no statistically significant differences were observed between ^{18}F-FDG uptake in the ITM arm vs. the systemic arm in subject D (**Table 3**). As the insulin plasma levels suggest, exposure in the ITM side was considerably above baseline and yet different from exposure levels after systemic administration. This may account for the statistically significant differences observed in ^{18}F-FDG uptake

between the ITM and systemic interventions in subjects A, B, and E. The small sample size and multiple-group design, however, preclude rigorous hypothesis testing of the PET imaging results. Our animal POC study, which had similar design features but larger sample size, was able to demonstrate differential ^{18}F-FDG uptake in the ITM vs. microdose groups.[23]

The comparison of different test conditions in the same individuals at the same time is a unique feature of ITM. The ability to test full, therapeutic-level exposure in one part of the body while at the same time obtaining safe, systemic microdose data elsewhere greatly reduces the variability between the two conditions. Such information would normally require conducting multiple, crossover experiments over longer periods of time with considerable potential for variability in test conditions even if the same research participants are chosen. Such data could be used to support the extrapolation of microdose to therapeutic level exposure, one of the main limitations of current microdosing studies.[15,22]

This multimodality, FIH-type, pilot study demonstrated the ethical and operational feasibility, and scientific POC of ITM. The approach enabled simultaneous and multimodal target measurement of drug (insulin) and biomarker (glucose) plasma levels as well as imaging another biomarker (^{18}F-FDG uptake). Target exposure (i.e., the hand, in this case) post-ITM was similar to that postsystemic full-dose administration resulting in local effects but with minimal systemic effects. ITM could enable safe, inexpensive, and early testing of novel drugs at the FIH stage. Findings should be validated in larger, controlled studies using a range of targets and classes of drugs.

Limitations and considerations for future ITM studies

The current study has several limitations and should be replicated in larger, controlled studies across multiple therapeutic modalities and drug classes. The small sample size, a requirement of such early POC study, precluded any meaningful statistical analyses of the results, and was compounded by the need to divide the sample into three different interventions. Likewise, the open, nonrandomized, and nonblinded protocol was a requirement of this exploratory pilot study, allowing the adaptive design to progress rapidly through the different permutations, but clearly limits the rigor of the methodology.

When comparing different parts of the body using PET imaging or other measurement modalities (e.g., measurement of blood flow or muscle contraction) efforts should be made to ensure minimal external perturbations if possible. Preferably, direct measurements of blood flow during drug administration and immediately afterward should be performed as they are key to determining accurate tissue exposure. Our use of the hand requires special attention in that regard in that it has dual supply (radial and ulnar arteries) and is highly responsive to environmental and internal stimuli (e.g., temperature, exercise, and sympathetic tone).

CONCLUSIONS

The current study is the first human POC study of the ITM approach. The study involved administration of insulin microdose directly into the radial artery and simultaneous target (hand) measurements of drug (insulin) and biomarker (glucose) plasma levels as well as imaging another biomarker, ^{18}F-FDG uptake. Target exposure post-ITM was similar to that postsystemic full-dose administration resulting in local effects but with minimal systemic effects. The measurement of PK and PD effects with minimal systemic exposure, and simultaneous in-subject study of two (or potentially more) interventions leading to reduction in intersubject and intrasubject variability are valuable for scientific, ethical, and economic reasons, reducing the exposure to potentially toxic novel treatments and the time and expenses required to develop them.

The study represents the culmination of efforts at a drug development paradigm shift on regulatory, ethical, procedural, logistic, scientific, and professional levels, requiring "disruptive" approaches, as well as close multidisciplinary and cross-disciplinary collaborations. ITM could enable safe, inexpensive, and early testing of novel drugs at the FIH stage. Findings should be validated in larger, controlled studies using a range of targets and classes of drugs.

Acknowledgments. The authors would like to thank all the research participants who volunteered to take part in this study and members of the Duke Microdosing Study Group for their continuous support. In addition, the authors would like to acknowledge the role Graham Lappin, Mark Dewhirst, and Andrew Krystal in the conceptualization of this project, and the staff of the Duke Clinical Research Unit and Duke PET imaging facility in the preparation and seamless execution of the protocol.

Author Contributions. T.B. wrote the manuscript. T.B., D.B.M., K.L., M.F., M.R., and R.J.N. designed the research. T.B., D.B.M., A.S., D.K.D., T.H., and R.J.N. performed the research. T.B., K.L., M.R., and R.J.N. analyzed the data.

Disclosure. T.B. holds a patent for Intra-Target Microdosing (ITM). Research reported in this publication was supported by the National Centre for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR001117.

1. Munos, B. Lessons from 60 years of pharmaceutical innovation. *Nat. Rev. Drug Discov.* **8**, 959–968 (2009).
2. 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).
3. Food and Drug Administration. Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. http://wwwfdagov/oc/initiatives/criticalpath/whitepaperhtml (2004).
4. Burt, T. & Dhillon, S. Pharmacogenomics in early-phase clinical development. *Pharmacogenomics* **14**, 1085–1097 (2013).
5. Institute of Medicine (IOM). In *Evolution of Translational Omics: Lessons Learned and the Path Forward* (The National Academies Press, Washington, DC, 2012).
6. DiMasi, J.A., Feldman, L., Seckler, A. & Wilson, A. Trends in risks associated with new drug development: success rates for investigational drugs. *Clin. Pharmacol. Ther.* **87**, 272–277 (2010).
7. Coller, B.S. & Califf, R.M. Traversing the valley of death: a guide to assessing prospects for translational success. *Sci. Transl. Med.* **1**, 10cm9 (2009).
8. Eapen, Z.J., Vavalle, J.P., Granger, C.B., Harrington, R.A., Peterson, E.D. & Califf, R.M. Rescuing clinical trials in the United States and beyond: a call for action. *Am. Heart J.* **165**, 837–847 (2013).
9. Burt, T. & Nandal, S. Pharmacometabolomics in early-phase clinical development. *Clin. Transl. Sci.* **9**, 128–138 (2016).
10. Burt, T., Noveck, R.J., MacLeod, D.B., Layton, A.T., Rowland, M. & Lappin, G. Intra-Target Microdosing (ITM): a novel drug development approach aimed at enabling safer and earlier translation of biological insights into human testing. *Clin. Transl. Sci.* E-pub ahead of print 18 April 2017.

11. Food and Drug Administration. Guidance for Industry, Investigators, and Reviewers Exploratory IND Studies. http://wwwfdagov/downloads/Drugs/GuidanceCompliance RegulatoryInformation/Guidances/UCM078933pdf (2006).
12. International Conference on Harmonization. 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).
13. European Medicines Agency. Position Paper on Non-clinical Safety Studies to Support Clinical Trials with a Single Microdose. In: *Position paper CPMP/SWP/2599* (2004).
14. Ministry of Health, Labour and Welfare. Guidance. Microdose clinical studies. (ed. Ministry of Health, L.A.W., Pharmaceutical and Medical Safety Bureau) (Tokyo, Japan, 2008).
15. Burt, T. *et al.* Microdosing and other phase 0 clinical trials: facilitating translation in drug development. *Clin. Transl. Sci.* **9**, 74–88 (2016).
16. 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).
17. Sugiyama, Y. Effective use of microdosing and positron emission tomography (PET) studies on new drug discovery and development. *Drug Metab. Pharmacokinet.* **24**, 127–129 (2009).
18. Bergström, M., Grahnén, A. & Långström, 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).
19. Sugiyama, Y. & Yamashita, S. Impact of microdosing clinical study – why necessary and how useful? *Adv. Drug Deliv. Rev.* **63**, 494–502 (2011).
20. 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).
21. Rowland, M. & Benet, L.Z. Lead PK commentary: predicting human pharmacokinetics. *J. Pharm. Sci.* **100**, 4047–4049 (2011).
22. 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 Deliv.* **14**, 657–672 (2017).
23. 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).
24. Fuhrman, T.M., Reilley, T.E. & Pippin, W.D. Comparison of digital blood pressure, plethysmography, and the modified Allen's test as means of evaluating the collateral circulation to the hand. *Anaesthesia* **47**, 959–961 (1992).
25. Sakai, K., Imaizumi, T., Masaki, H. & Takeshita, A. Intra-arterial infusion of insulin attenuates vasoreactivity in human forearm. *Hypertension* **22**, 67–73 (1993).
26. Imaizumi, T. Effects of intra-arterial infusion of insulin on control of forearm vascular resistance in normotensive and hypertensive subjects. *Hypertens. Res.* **19**Suppl 1, S47–S50 (1996).
27. Zierler, K.L. & Rabinowitz, D. Effect of very small concentrations of insulin on forearm metabolism. Persistence of its action on potassium and free fatty acids without its effect on glucose. *J. Clin. Invest.* **43**, 950–962 (1964).
28. Getz, K.A., Wenger, J., Campo, R.A., Seguine, E.S. & Kaitin, K.I. Assessing the impact of protocol design changes on clinical trial performance. *Am. J. Ther.* **15**, 450–457 (2008).
29. Pammolli, F., Magazzini, L. & Riccaboni, M. The productivity crisis in pharmaceutical R&D. *Nat. Rev. Drug Discov.* **10**, 428–438 (2011).
30. Chalmers, I. *et al.* How to increase value and reduce waste when research priorities are set. *Lancet.* **383**, 156–165 (2014).
31. Button, K.S. *et al.* Power failure: why small sample size undermines the reliability of neuroscience. *Nat. Rev. Neurosci.* **14**, 365–376 (2013).
32. Scannell, J.W., Blanckley, A., Boldon, H. & Warrington, B. Diagnosing the decline in pharmaceutical R&D efficiency. *Nat. Rev. Drug Discov.* **11**, 191–200 (2012).
33. Rowland, M. Microdosing and the 3Rs. In *National Centre for the Replacement, Refinement, & Reduction of animals in Research*. (NC3Rs, London, United Kingdom, 2013)
34. van der Worp, H.B. *et al.* Can animal models of disease reliably inform human studies? *PLoS Med.* **7**, e1000245 (2010).
35. Hajjar, E.R., Cafiero, A.C. & Hanlon, J.T. Polypharmacy in elderly patients. *Am. J. Geriatr. Pharmacother.* **5**, 345–351 (2007).
36. ElDesoky, E.S. Pharmacokinetic-pharmacodynamic crisis in the elderly. *Am. J. Ther.* **14**, 488–498 (2007).
37. Budnitz, D.S., Lovegrove, M.C., Shehab, N. & Richards, C.L. Emergency hospitalizations for adverse drug events in older Americans. *N. Engl. J. Med.* **365**, 2002–2012 (2011).
38. Stevenson, J.M. *et al.* Predicting adverse drug reactions in older adults; a systematic review of the risk prediction models. *Clin. Interv. Aging* **9**, 1581–1593 (2014).
39. Beers, E., Egberts, T.C., Leufkens, H.G. & Jansen, P.A. The views of healthcare professionals, drug developers and regulators on information about older people needed for rational drug prescription. *PLoS One* **8**, e72060 (2013).
40. Lappin, G., Noveck, R. & Burt, T. Microdosing and drug development: past, present and future. *Expert Opin. Drug Metab. Toxicol.* **9**, 817–834 (2013).
41. Collins, F.S. Reengineering translational science: the time is right. *Sci. Transl. Med.* **3**, 90cm17 (2011).
42. Doroshow, J.H. & Kummar, S. Role of phase 0 trials in drug development. *Future Med. Chem.* **1**, 1375–1380 (2009).
43. 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).
44. 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).

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