

REVIEW

Strategic, feasibility, economic, and cultural aspects of phase 0 approaches

Is it time to change the drug development process in order to increase productivity?

Tal Burt^{1,2}  | Ad F. Roffel³ | Oliver Langer^{4,5} | Kirsten Anderson⁶ | Joseph DiMasi⁷ 

¹Phase-0/Microdosing Network, New York, New York, USA

²Burt Consultancy, LLC, New York, New York, USA

³ICON plc, Groningen, The Netherlands

⁴Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria

⁵Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Vienna, Austria

⁶Presage Bioscience, Seattle, Washington, USA

⁷Tufts Center for the Study of Drug Development, Tufts University, Boston, Massachusetts, USA

Correspondence

Tal Burt, Phase-0/Microdosing Network, 1214 5th Avenue, 32G, New York, NY 10029, USA.
Email: tal@talburmd.com

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Abstract

Research conducted over the past 2 decades has enhanced the validity and expanded the applications of microdosing and other phase 0 approaches in drug development. Phase 0 approaches can accelerate drug development timelines and reduce attrition in clinical development by increasing the quality of candidates entering clinical development and by reducing the time to “go-no-go” decisions. This can be done by adding clinical trial data (both healthy volunteers and patients) to preclinical candidate selection, and by applying methodological and operational advantages that phase 0 have over traditional approaches. The main feature of phase 0 approaches is the limited, subtherapeutic exposure to the test article. This means a reduced risk to research volunteers, and reduced regulatory requirements, timelines, and costs of first-in-human (FIH) testing. Whereas many operational aspects of phase 0 approaches are similar to those of other early phase clinical development programs, they have some unique strategic, regulatory, ethical, feasibility, economic, and cultural aspects. Here, we provide a guidance to these operational aspects and include case studies to highlight their potential impact in a range of clinical development scenarios.

INTRODUCTION

Drug development is a complex, lengthy, expensive, and error-prone process.^{1–3} The past few decades have witnessed an exponential increase in the complexity and costs of clinical development without the commensurate increase in success rates. Perhaps most impactful is the poor translation of progress in basic science, such as that

driven by genomic discoveries, into clinical applications. Poor translation means that drugs that enter clinical development without sufficient supportive evidence have increased chance of failing, and often do so after costly human testing.

There are two main methodological approaches that can result in increases in the success rates and productivity of clinical development. One is the reduction of “false

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negatives” (e.g., by increasing the power and quality of the studies) and the second, and potentially more impactful, is to have better candidates enter the clinical development process.^{2,4-6} Data from phase 0 studies can contribute to both of these factors. The addition of *in vivo* human data (or “*in humano*,”⁷ in addition to the *in vitro* human data) to preclinical candidate selection can reduce the likelihood of good candidates being falsely eliminated (i.e., false negatives) and can also increase the quality of candidates entering clinical development, with both effects potentially resulting in reduced attrition rates.

Phase 0 studies have several methodological and strategic advantages over traditional approaches. Methodological advantages include inpatient testing for first-in-human (FIH) studies, intravenous microdose administration of oral drugs, cassette microdosing, and intra-target microdosing (ITM). Strategic advantages include the use of *in vivo* human data to select preclinical candidates, resolve conflict or inadequacy of the preclinical data, accelerate drug development in vulnerable populations, and facilitate entry into human testing with limited availability of resources. The biggest potential contribution of phase 0 to the efficiency and effectiveness of drug development is in obtaining pharmacodynamic (PD) and mechanism of action (MOA) data. This is because at this early stage of development such mechanistic data are typically unavailable for decision making.⁸⁻¹²

REGULATORY FRAMEWORK

After establishing formal frameworks for phase 0 approaches in the three main international regulatory jurisdictions, in Europe by the European Medicines Agency (EMA) in 2003,¹³ in the United States by the US Food and Drug Administration (FDA) in 2006,¹⁴ and in Japan by the Ministry of Health, Labour, and Welfare (MHLW) in 2008,¹⁵ the frameworks were integrated, harmonized, and ratified in 2009 under the International Conference on Harmonization (ICH) M3 “Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.”¹⁶ Section 7 of the ICH M3 guidance is devoted to Exploratory Clinical Trials, which include microdosing and nonmicrodosing approaches. Table 1 summarizes the five approaches outlined in the ICH M3 guidance. Approaches 1 and 2 are single and multiple microdose approaches, respectively, and approaches 3, 4, and 5 describe the nonmicrodosing phase 0 approaches. The range can be described on continuums of dosages and duration of exposure from the most restrictive (single microdose) with increasing exposures up to the no observed adverse effect level (NOAEL) area under the curve (AUC) of the non-rodent

species for up to 14 days. In addition, phase 0 studies should not have therapeutic intent and are not meant to study tolerance.¹⁶

The continuum of exposure also generally corresponds with the extent of the preclinical studies that need to be undertaken and the rigor of the standards that need to be met before entry into human testing. This, in turn, corresponds with the time and resources required to prepare the regulatory submission package and follows the general principle that the more limited the exposure, the safer is the anticipated exposure in humans, and less efforts need to be undertaken to mitigate any associated risk. The most restricted exposure, a single microdose, is comparable to routine exposures to environmental toxins,¹⁴ and is the safest of the phase 0 approaches and the one associated with the least amount of submission material and extent of preclinical testing.^{14,16}

ADVANTAGES OF PHASE 0

The benefits of phase 0 approaches have been demonstrated in numerous developmental applications. An extensive discussion of the topic is available elsewhere,^{6,7,18} however, a brief discussion is provided here in relation to strategic decisions. Favorable developmental scenarios for use of phase 0 approaches include multiple undifferentiable preclinical candidates or nonoptimized formulations or conjugate candidates. Additional scenarios include conflicting preclinical data, poor preclinical models, target populations consisting of vulnerable populations, and the prospect of studying PD and MOA.⁶ Whether phase 0 should be applied more extensively or universally in drug development should await detailed economic analysis and demonstration of applicability in a wider range of developmental scenarios, including drug classes, drug targets, and therapeutic areas. In addition, it should be appreciated that the ultimate demonstration of benefit is contingent on controlled comparisons with traditional approaches and is discussed in greater detail under “Future directions” below. Several potential disadvantages of phase 0 approaches also need to be considered.

Phase 0 approaches could be used in both patients and healthy volunteers to anticipate and potentially obviate the need for phase I or phase II studies. Therefore, a substantial developmental benefit of phase 0 approaches is the potential to terminate inappropriate drug candidates earlier and without the costs of expensive phase I or phase II programs. This has been demonstrated in numerous studies in actual drug development.¹⁹⁻²⁷ For example, Okour et al. reported in 2017 on the termination of development of a GlaxoSmithKline (GSK) anti-malarial drug using a microdosing study. The elimination half-life of the

TABLE 1 Phase 0, including microdosing approaches from the ICH M3 guidance¹⁶

	Approach 1	Approach 2	Approach 3	Approach 4	Approach 5
	Microdosing				
Dose definition	≤1/100 th NOAEL and ≤1/100 th of pharmacologically active dose (scaled on mg/kg for i.v. and mg/m ² for oral)	Same as approach 1	Starting at subtherapeutic dose and moving into the anticipated therapeutic range but <1/2 NOAEL	Starting dose:<1/50 th of NOAEL in the species with the lowest AUC at NOAEL. Into the anticipated therapeutic range. Highest dose:<1/10 th of preclinical AUC if no toxicity in both species, or < NOAEL if toxicity identified in one species, or <1/2 the AUC at the highest dose in the species not showing toxicity, whichever is lower	Starting dose:<1/50 th NOAEL in the species with the lowest AUC at NOAEL. Into the anticipated therapeutic range. Highest dose:< non-rodent NOAEL AUC, or < 1/2 rodent NOAEL AUC, whichever is lower
Cumulative dose	100 µg	500 µg			
Limit per dose	100 µg	100 µg			
Maximal daily dose	100 µg	100 µg			
Number/duration of dosing	1 (could be divided to multiple doses with a total of 100 µg)	5	1	Multiple <14 days	Multiple <14 days
Washout	N/A	6 or more half-lives between doses	N/A	N/A	N/A
Pharmacology	In vitro and receptor profiling PD model supporting human dose selection	Same as approach 1	Same as approach 1 + core battery of safety pharmacology	Same as approach 1 + core battery of safety pharmacology	Same as approach 1 + core battery of safety pharmacology
General toxicity studies	14-day extended single dose toxicity	7-day repeated-dose toxicity	Extended single-dose toxicity; in rodent and non-rodent	14-day repeated-dose toxicity in rodent and non-rodent	14-day repeated-dose toxicity in rodent and non-rodent
GLP	Yes	Yes	Yes	Yes	Yes
Genotoxicity Studies	Not recommended SAR included if available	Same as approach 1	Ames assay	Ames assay + chromosomal damage test	Ames assay + chromosomal damage test
Dosimetry estimates	For highly radioactive agents	Same as approach 1	Same as approach 1	Same as approach 1	Same as approach 1

Intermediate forms are possible, and the optimal approach should be arrived at based on discussions with local regulators. Adapted from Ref. [17].

Abbreviations: AUC, area under the curve; GLP, good laboratory practice; ICH, International Conference on Harmonization; N/A, not applicable; NOAEL, no observed adverse effect level; PD, pharmacodynamic; SAR, structure-activity relationship; NOAEL, no observed adverse effect level; AUC, area under the curve; GLP, good laboratory practice; SAR, structure-activity relationship; N/A, not applicable.

drug, at 17h was determined to be too short to be consistent with developmental objectives.¹⁹

When there are multiple undifferentiable preclinical candidates (Figure 1), phase 0 approaches are superior to traditional preclinical approaches because they add the potentially differentiating “in humano” data to the animal and in vitro data to help select the lead candidate. The alternative to conducting a phase 0 study, potentially in a cassette design with all candidates administered simultaneously and studied concurrently, is to conduct multiple expensive phase I studies or select a lead candidate based on more limited data, risking the false negatives of the candidates being eliminated based on preclinical data alone. For example, Madan et al. published in 2009 a microdosing study by Neurocrine Biosciences of five histamine H₁ receptor antagonists, diphenhydramine, and four analogues, with the goal of obtaining pharmacokinetic (PK) data for candidate selection.²² Historical data of diphenhydramine were used to demonstrate linearity with the microdosing data, and the results were deemed useful for compound selection.

Another general developmental category is where preclinical data produce conflicting data or where the animal models are inappropriate as predictors of drug effects in humans. Pfizer used a microdosing study to select the lead candidate among four Nav1.7 channel blockers that demonstrated species differences in in vivo preclinical PK studies.²⁶ The differences were identified to be related to the organic anion-transporting polypeptide (OATP)-mediated hepatic uptake, a mechanism for which few validated human modeling approaches were available. Bauer et al. used a positron emission tomography (PET)-microdosing study with a potential anti-amyloid drug in patients with Alzheimer's disease to assess passage across the blood–brain barrier (BBB) and binding to anatomic targets associated with beta amyloid rich regions.²⁰ Both the passage of the human BBB and the pathophysiology of beta amyloid are poorly predictable from animal models.

Phase 0 approaches have demonstrated their advantages in the development of drugs in vulnerable populations who may otherwise be typically excluded from drug development studies and especially of novel compounds and in early stage development. In the first demonstration of the utility of microdosing in vulnerable populations, ursodiol PK was studied in preterm infants using accelerator mass spectrometry (AMS) technology.²⁸ No previous study of ursodiol in infants was done leaving treatment decisions vulnerable to the typical erratic response patterns in infants. The AMS-microdosing study enabled the safe study of ursodiol in infants and generated PK data previously only available in adults to guide pharmacotherapy of parenteral nutrition-associated cholestasis. Recently, a meeting of drug development experts with the

FDA identified microdosing as an attractive approach to provide evidence-based data to support pharmacotherapy in pregnancy.²⁹

Perhaps the most attractive and potentially meaningful knowledge that phase 0 approaches can generate is of novel drugs' PD and MOA. Subtherapeutic exposures may still lead to pharmacological effects at efficacy and toxicity targets, although this will typically require the use of highly sensitive analytical tools and/or surrogate biomarkers, or specialized applications (e.g., ITM^{30–34}) and study designs in order to be detected. Although, currently, such applications depend on favorable developmental circumstances, a growing number of applications can be found in the literature and in industry projects,^{30–36} and, in principle, with the availability of appropriate and sufficiently sensitive analytical tools, detection of biomarkers should be possible in most if not all drug development scenarios. An example of biomarker detection with systemic microdose exposures is that of DNA adducts, biomarkers of chemotherapeutic action, in peripheral blood mononuclear cells (PBMCs).^{35,36} In these studies, two important elements of chemotherapeutic MOA, drug entry into PBMCs, and formation of DNA adducts inside the PBMCs, were demonstrated using AMS-microdosing through the localization of ¹⁴C-labeled DNA adducts in PBMCs after microdose administration of ¹⁴C-labeled drugs. Additional case studies are included in Appendix S1.

Intra-Target Microdosing (ITM)

An important emerging advantage of phase 0 applications is Intra-Target Microdosing (ITM) (Figure 2). Since the emergence of the concept in 2015, ITM has been adopted by industry as a developmental approach for the introduction of novel pharmaceuticals into clinical development.^{30,31,34,37,38} Presage has applied ITM in oncology drug development, using a cassette microdosing approach to administer multiple drugs into localized tumor environments, enabling the simultaneous study of therapeutic-level exposures and drug-drug interactions in a controlled microenvironment while ensuring no more than the microdose systemic exposure.³⁴ Presage has partnered with Celgene, Bristol Myers Squibb (BMS), Takeda, and Merck around this technology.^{34,39,40} Sjögren et al. reported on an AstraZeneca study that used the concept to study localized skin exposure to therapeutic levels of transient receptor potential vanilloid receptor 1 (TRPV1) antagonists for analgesia and obtain pain-related biomarkers while ensuring no more than microdose systemic exposures.³² A group affiliated with the Massachusetts Institute of Technology and Harvard Medical School has applied ITM and cassette

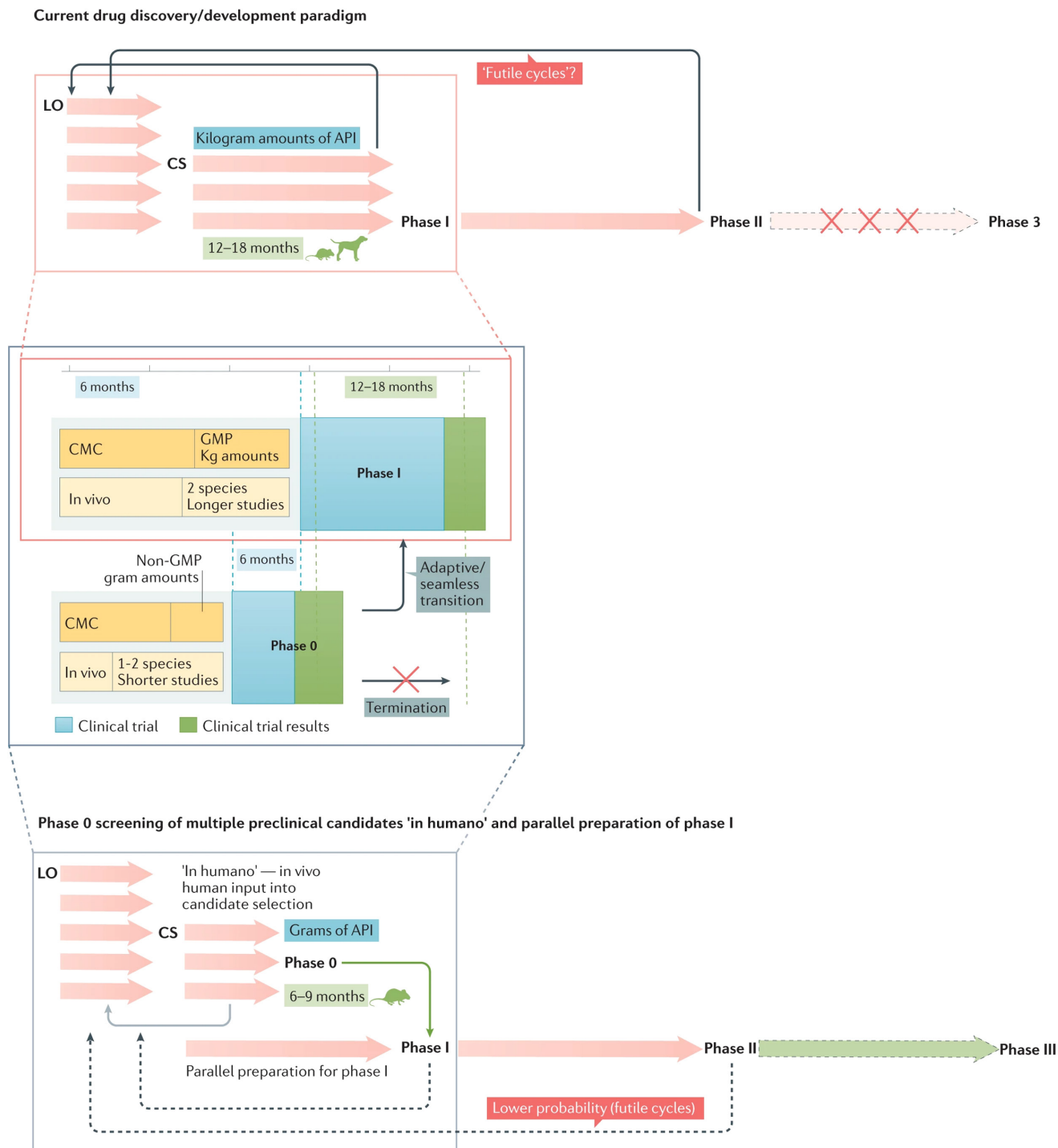


FIGURE 1 Phase 0 and phase I timelines. It is recommended that preparations for phase I continue in parallel to the conduct of the phase 0 study. This will allow for seamless transition to phase I or, alternatively, early termination of phase I based on the phase 0 results. The costs of additional preclinical preparations, that might be lost in the event of termination of development, are minimal compared with the savings in time that such parallel development allows (see text). The contribution of in vivo human testing (i.e., in humano⁷) to the selection of preclinical candidates is highlighted. This has the potential to reduce false negatives (i.e., the good drugs wrongly deselected based on inappropriate animal or in vitro data), and also discover earlier, and therefore in a less expensive manner, those false positives that may be discovered only at the end of expensive phase I in healthy volunteers, or phase II in patients. Phase 0 approaches can provide human data for developmental decision 8–12 months prior to traditional phase I. In the case of adaptive phase 0/phase I design the phase 0 application must be withdrawn prior to initiation of the phase I study (required by the FDA but not in the EU). The traditional drug development approach uses phase I as the first-in-human (FIH) approach. The parallel phase 0/phase I approach is an adaptive design. Abbreviations: API, active pharmaceutical ingredients; CMC, chemistry; manufacturing, and controls; CS, candidate selection; EU, European Union; FDA, US Food and Drug Administration; GMP, Good Manufacturing Practices; LO, lead optimization. Reprinted with permission from Burt et al.⁶

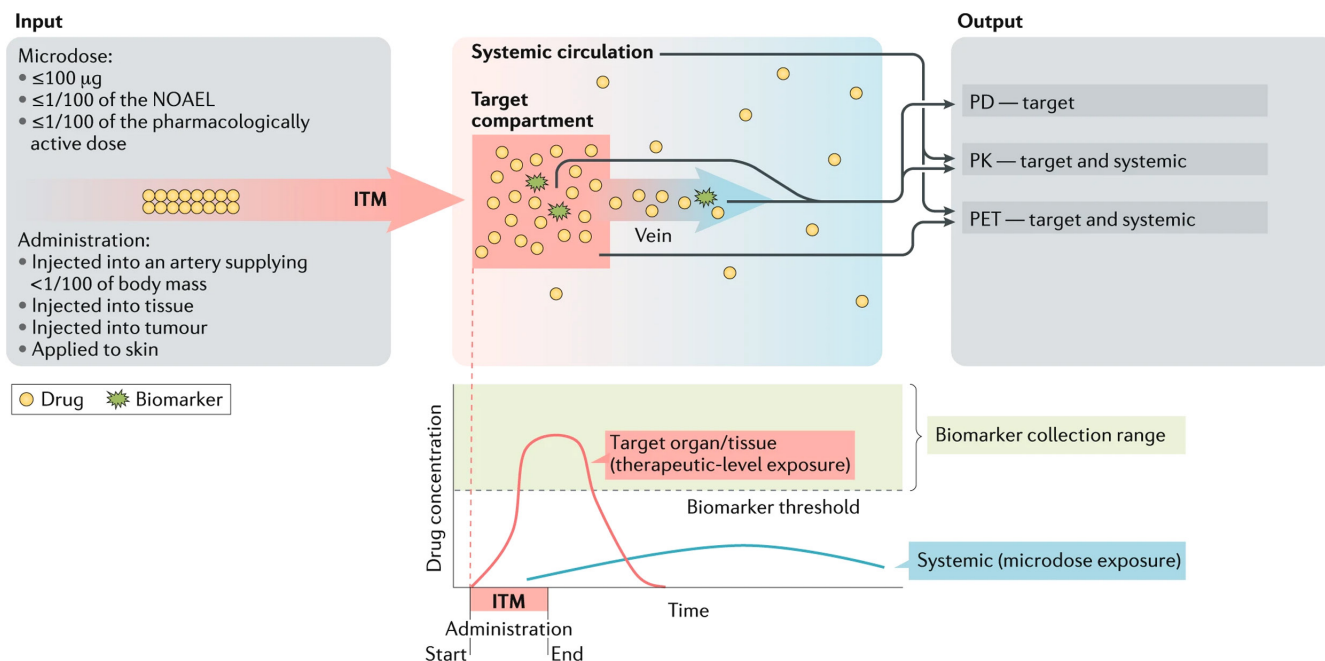


FIGURE 2 Intra-Target Microdosing (ITM).^{18,33,37} In this illustration, using an intra-arterial administration method, administering a microdose ($\leq 100 \mu\text{g}$, or pharmacologically active dose, or $1/100^{\text{th}}$ the NOAEL) temporarily surpasses the therapeutic-level exposure in a small target ($\leq 1/100^{\text{th}}$ of the body mass) and may allow collection of PD, biomarker, and MOA data, as well as systemic microdose PK. Reprinted with permission from Burt et al.⁶ Abbreviations: MOA, mechanism of action; NOAEL, no observed adverse effect level; PD, pharmacodynamic; PET, positron emission tomography; PK, pharmacokinetic

microdosing principles using implantable microdevice to deliver multiple (up to 20) different drugs and drug combinations into tumors to study tumor microenvironment's response to drugs.^{31,41–43} Detailed case studies of ITM applications in a range of therapeutic areas and targets are included in a review of the topic¹⁸ and in Appendix S1.

DISADVANTAGES OF PHASE 0

The main argument against application of phase 0 approaches in any specific developmental program is the lack of an a priori identifiable potential to generate information that could be useful for meaningful developmental decisions. In such cases, phase 0 studies would likely constitute superfluous expenditure in developmental resources and time. A detailed benefit/risk assessment of phase 0, considering costs and timelines, should be conducted well in advance, preferably 1.5–2 years prior to anticipated entry into clinical development.^{6,7,17}

As an example, phase 0 studies may not serve a clear purpose/deserve a place in early clinical development in cases where one studies a single, well-characterized test article, with few toxicity concerns, and no inconsistencies between the preclinical sources of information (e.g., between different animal models or between in vivo and in vitro data). Other disadvantageous scenarios include scenarios in which extrapolation from microdose to

therapeutic-level exposure is unreliable, where radiolabeling proves challenging or impossible, where the exposure to radioactivity may be considered unacceptably high (e.g., for drugs with long elimination half-lives that are labeled with radionuclides with long radioactive half-life), or when delay to timelines is expected in a manner not justified by the data obtained. Thus, in summary, when there are no significant uncertainties about PK and/or PD properties of the compound being developed and no need to choose from multiple, equivocal candidates, the advantages of phase 0 may be limited.⁶

TOP-LEVEL STRATEGY

When considering incorporating a phase 0 approach in a clinical development strategy, understanding the developmental needs of the drug candidate, platform, or pipeline should guide decision making. These needs can then be matched with the developmental advantages and capabilities that phase 0 approaches can provide. The top strategic decisions can be made at the pipeline level to ensure that the drug candidates with the highest probability of success will move forward. At the platform level, phase 0 approaches can screen multiple preclinical candidates for best PK properties, on-target activity, or biomarker engagement (Figure 1).

Phase 0 strategy should focus on unique advantages that these approaches provide in generating information

TABLE 2 Strategic elements that should be prioritized in phase 0 approaches

<p>Matching test article developmental needs with phase 0 design options by identifying</p> <ul style="list-style-type: none"> Drug targets at molecular and organ/tissue levels as they may be relevant for potential efficacy and toxicity effects Drug's MOA in potential efficacy and toxicity effects Drug arrival at the target tissues (PK) <p>And validating indicators of drug action at the target (MOA, PD)</p> <ul style="list-style-type: none"> Binding to receptors at efficacy and toxicity targets Identification of biomarkers or surrogate biomarkers generated as a result of post-receptor modulation.
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Logistics

Study preparation timelines – initiate phase 0 programs at least 6–8 months prior to anticipated phase I program, so that data are available prior to initiation of phase I. Use of decision tree to anticipate key outcomes and contingencies

Multistakeholder engagement and coordination (e.g., regulators, preclinical and clinical pharmacology, toxicology, chemistry, biomarkers, statistics, modeling, analytics, economics, and patient advocacy)

Study design and execution – optimally, use of adaptive phase 0/phase I with preparation for phase I taking place in parallel to phase 0

Abbreviations: MOA, mechanism of action; PD, pharmacodynamic; PK, pharmacokinetic.

earlier than traditional approaches or information that is not otherwise available using traditional approaches (Table 2). The strategy should be guided by the pharmacological properties of the test compound, its pathophysiological targets, and any anticipated therapeutic or toxic effects. It is recommended that discussions whether to use phase 0 approaches or not be initiated at least 1.5–2 years prior to anticipated initiation of phase I in any drug development programs in consideration.⁶ The developmental strategy should then focus on the timelines necessary to produce the preclinical data, engage the appropriate stakeholders, and initiate the phase 0 clinical trials, preferably 6–8 months prior to anticipated initiation of the phase I programs (Table 2). This is so that phase 0 data are available prior to initiation of the phase I program and therefore would either lead to termination of development prior to initiation of phase I or will not delay the phase I should a “go” decision be undertaken. In the latter case, phase 0 data could also be used to enhance the phase I (and/or phase II, in case patient data are obtained) program design.

PREPARATION FOR PHASE 0

Phase 0 programs are typically smaller and shorter than the equivalent phase I and/or phase II programs of the same compound, requiring less nonclinical study data and preparations (e.g., safety pharmacology) and as a result cost about a third of phase I program (Table 3).

Regulatory

The internationally harmonized ICH M3 guidance governs the application of phase 0 approaches, or, as they are referred to in the guidance, “Exploratory Clinical

Trials” (also see Appendix S2). Some variations exist among the regulatory jurisdictions that ratified the guidance, and therefore a pre IND (investigational new drug or pre-clinical trial application [CTA] meeting in Europe) early consultation with the local regulatory authorities is recommended at least 6 months prior to initiation of the phase 0 study for consideration of the proposed data package and phase 0 design. These meetings will not have impact on the subsequent review and timelines for the traditional IND.

There may be flexibility in the regulatory package requirements (e.g., toxicology, pharmacology testing, and manufacturing standards) and the amount of data included in the investigator's brochure. These should be negotiated and clearly outlined and agreed upon with the regulators at the first meeting. Other documents, such as clinical study protocol, informed consent forms, medication labels, laboratory, and pharmacy certifications, will be similar to other clinical developmental phases and may take up to 4 months or as little as 5 weeks to prepare. Drafts of these documents should be included in pre-meeting packages for regulatory review.

In the United States, the timeframe for response to the exploratory IND application is 30 days. In the European Union, the maximal turnaround for the CTA that includes approval by both the country authority (competent authority) and an accredited ethics committee (EC) ranges from as early as 14 days in the Netherlands to up to 4 months in other countries. In the United Kingdom the turnaround is 60 days for Medicines and Healthcare Products Regulatory Agency (MHRA) approval and 35 days for ECs. In the United States the exploratory IND application is intended to be withdrawn at study completion. A traditional IND must be filed to allow initiation of the phase I study, but these activities can be done in parallel.

TABLE 3 Comparison of phase 0/microdosing with traditional phase I approaches

	Phase 0/microdosing (exploratory IND)	Traditional phase I (IND)
Program		
Preclinical package	Limited, variable; depends on extent of exposure to the test article and experimental goals	Full requirements
In vitro models	Full requirement	Full requirements
Toxicology	Limited, variable	Full requirements
Genotoxicology	None or limited	Full requirements
GMP	Flexible, depending on available preclinical information and route of administration (e.g., sterility ensured for i.v. route)	Full requirements
Regulatory review	30-day (US)	30-day (US)
Usual duration of program	4–12 months	12–24 months
Cost of program	\$0.7–1.25 M	\$1.75–3.5 M
Clinical trial		
Therapeutic intent	None	Possible
Study of systemic tolerability	None	Yes
Study of PD/MoA	Possible (e.g., PET receptor binding and displacement, ITM)	Possible
Size (typical)	4–10 participants	6–30 participants
Duration (per participant)	1–14 days*	6–60 days*
Number of study sites	Single	Single/multiple
Maximal dose	<<MTD (\leq NOAEL/MABEL)	MTD
Exposure	Limited (see Table 1)	Multiple doses allowed
Population	Healthy volunteers or patients Vulnerable populations	Usually health volunteers (unless toxicity risk is high, e.g., in oncology trials)
Sensitive analytical tools	Typically required	Typically not required

Adapted from Burt et al.¹⁷

Abbreviations: *, on average, could be longer with longer half-life drugs; GMP, Good Manufacturing Practice; IND, investigational new drug; ITM, intra-target microdosing; MOA, mechanism of action; MTD, maximum tolerated dose; NOAEL, no observed adverse effects level; MABEL, minimal anticipated biological effect level; PD, pharmacodynamic; PET, positron emission tomography.

In Japan, there is an option to request consultation with the Pharmaceuticals and Medical Devices Agency (PMDA) prior to starting clinical development, and phase 0 approaches can be discussed then. Japan has issued a national guidance on the conduct of microdosing studies in 2008¹⁵ and currently also adheres to ICH M3 guidance. Trial submission (clinical trial notification (CTN)) is as for phase I trials, with 30-day initial protocol review. In South Korea, the concept of phase 0 was incorporated into law in 2016, and the regulatory mechanism offers advice on phase 0 if requested.

Regulatory aspects of ITM

The regulatory approach to ITM is guided by the following considerations^{18,30,31,33,43}:

1. The flexibility in the regulations is commensurate with the risks of the proposed study.¹⁴
2. Exposure to the drug is at the lowest end of therapeutic-level exposure range (or NOAEL).
3. No more than 1/100th of the body mass is exposed to the new drug.
4. The therapeutic-level exposure to the new drug at the anatomical target is typically brief. Exposure of a mass equivalent to 1/100th of the body mass will last no more than seconds until the drug is cleared from the tissue. If target size smaller than 1/100th of the body mass is used^{30,31,43} then longer exposures are possible, effectively compensating for the increased duration of present exposure by reducing the amount of tissue that is exposed to the risk.
5. Systemic concentrations are never more than those after systemic administration of a microdose.

Toxicology

Toxicology requirements are outlined in the ICH M3 guidance for each of the five example phase 0 approaches that are described in Table 3 of the guidance.¹⁶ For example, Approach 1, a single microdose, the approach that involves the least exposure to the test article, requires only one, extended single dose toxicity study in one animal species, typically rodents, with follow-up at day 14. Approach 5 which enters into the low therapeutic dose range but with limited duration (<14 days), with no therapeutic intent, and not intended to evaluate tolerance, uses a 2-week repeated dose toxicity study in rodents followed by a confirmatory study in a non-rodent species for the duration of the intended exposure in the phase 0 study and at least for 3 days. Intermediate approaches 2, 3, and 4 are available as well as others that may be specifically tailored to the needs of the test article and should be negotiated directly with the regulators.

Chemistry, manufacturing, and controls

When the active pharmaceutical ingredient is available, drug formulation needs to be developed and tested. Initial steps can be done on technical grade material. Data will then be included in the exploratory IND application in the United States, the investigational medicinal product dossier in the European Union, the CTN in Japan, or their equivalent in other jurisdictions. The chemistry, manufacturing, and control (CMC) could take about 3 months, depending on the drug properties and required formulation and may be done in parallel to other steps in the phase 0 preparation process.

Manufacture of the drug requires Good Manufacturing Practice (GMP) standards and procedures in some regulatory jurisdictions but not in others. Some may require GMP-like standards and procedures depending on the expected extent of exposure to the drug compound. Expectations should be clarified with the regulators at the first meeting as it may significantly impact costs and timelines and therefore also affect the main potential benefits of phase 0 approaches. Previous publications have expressed the opinion that manufacturing standards of phase 0 studies should be more lenient when compared with phase I,^{14,44} because exposures in microdosing studies are low and comparable to routine exposures to environmental toxins. In addition, the level of impurities in such doses is negligible, suggesting that impurity test processes can be abbreviated.

As per EU legislation,⁴⁵ investigational medicinal products, as used in clinical trials, are to be manufactured under GMP; this also applies to phase 0 studies. Similarly, the FDA guidance on current GMP (cGMP) for investigational drugs⁴⁶ states that cGMP must be in effect for the manufacture of each batch of IMP used during

phase I clinical trials. However, the FDA guidance also states that manufacturers may implement manufacturing controls that are “appropriate” for the clinical trial stage of development. The overarching objective of the guidance (as also shown in the FDA exploratory IND guidance¹⁴) is to allow to determine whether the investigational drug to be used in the clinical trial is sufficiently safe to permit the trial to proceed. The guidance acknowledges that some manufacturing controls for product quality and their extent differ between the various phases of clinical trials corresponding with the expected risks and the goals of the investigation. If pre-negotiated with the agency, this approach could be used efficiently in phase 0 programs, in which low doses with reduced risk to human subject safety in terms of quality or purity related issues are used.

The FDA has issued separate guidance on Investigational New Drug Applications for Positron Emission Tomography (PET) drugs,⁴⁷ that supplements an earlier guidance on cGMP standards for PET.⁴⁸ It specifies selected CMC quality criteria in order to ensure that the drug meets “appropriate” chemical, pharmaceutical, radiochemical, and radionuclide standards of identity, strength, quality, and purity, as needed for the safety and scientific significance of the research conducted. Similar guidelines have been prepared by the European Association of Nuclear Medicine.⁴⁹

EXAMPLES OF NONCLINICAL PACKAGES SUPPORTING PHASE 0 APPLICATIONS

Example 1: Nonclinical package and GMP requirements for a phase 0 candidate selection study

A phase 0 candidate selection study was conducted to investigate the PK properties of three chemically related prodrugs of an approved small molecule medication in humans. Intravenous (i.v.) dosing was chosen to assess the primary PK parameters clearance and volume of distribution, as well as derived PK parameters (especially the plasma elimination half-life), without interference of absorption processes. The ¹⁴C-labeling (10 kBq, 270 nCi per dose) and AMS were used to measure concentrations of prodrug and active moiety in plasma. The nonclinical package supporting this phase 0 study consisted of:

1. An in vitro receptor screen and in silico genotoxicity assessment for the prodrugs.
2. A PK study of each candidate drug in two animal species (one rodent and one non-rodent), to assess the release of the active moiety and to model the expected human PKs.

3. An extended single dose toxicity study at three dose levels in rats, i.v. dosing only, with toxicology assessments as per ICH M3R2 Approach 1. Three (3) dose levels were tested in order to have flexibility as to the choice of the microdose to be used in humans; thus, if the highest dose tested in rats (allowing for a 90- μ g dose in humans) would not be safe and tolerated, the medium or low dose levels could still potentially allow for the microdose study to be conducted, at a lower dose, in humans.
4. No other nonclinical studies were conducted (e.g., hERG channel inhibition or pharmacology in an animal model), because these studies had already been done for the active moiety.

The above approach was seen as sufficient based on the available knowledge for the active moiety, and theoretical considerations as to the potential pharmacological activity of the chemical structures defining the prodrugs. This was accepted without any objection by the regulatory authorities. Synthesis of the radiolabeled drug material was done under GMP, as per the Sponsor's strict quality standards; manufacturing of the drug formulation was done under GMP as per EU legislation.

Example 2: Nonclinical package and GMP requirements for a phase 0 early human PK study

A phase 0 study was conducted to investigate the PK properties of a follow-up oncology drug in humans without full nonclinical characterization (early PKs in humans). The lead compound (with same mode of action) had already entered clinical testing, based on a regular nonclinical package, and had shown suboptimal PKs in humans. Therefore, the follow-up compound was tested in a phase 0 microdose study, with i.v. dosing in order to assess primary and derived PK parameters, and the lead compound was administered as an i.v. microdose as well, for comparative purposes. The investigational drugs were not labeled with ^{14}C , and bioanalysis was through high-sensitivity liquid-chromatography tandem mass spectrometry (LC-MS/MS). The nonclinical package supporting the administration of the follow-up compound in this phase 0 study consisted of:

1. An in vitro receptor screen and in vitro as well as in vivo target pharmacology testing to assess the MOA and the potential for clinical efficacy in the therapeutic indication (as required for ICH M3 R2 Approach 1).
2. A single dose i.v. PK study in rats, to assess basic PK parameters and predict the PK in humans.
3. An extended single dose toxicity study at one dose level in rats, i.v. dosing only, with toxicology assessments as per ICH M3R2 Approach 1. The one dose level tested

would allow up to 100 μ g as a microdose to be used in humans; the sponsor was confident that assessing one dose level would pose no risk for failure based on other, preliminary toxicity testing.

4. In vitro hERG assay and genotoxicity testing were conducted as per local preference, based on the notion that one would want to know about these potential safety issues prior to any administration of novel investigational drugs to humans.
5. Plasma protein binding, transporter assays, and metabolism in hepatocytes in vitro, to compare the suitability of the follow-up compound versus the lead compound.

Synthesis of the drug material and manufacturing of the drug formulation were done under GMP as per the sponsor's and EU standards. This example involved more nonclinical testing than required per ICH M3 R2; this was done because the sponsor had taken the lead compound into humans as per regular nonclinical and clinical testing, found the PKs to be suboptimal, and developed a follow-up compound (with the same MOA) that was then tested in a microdose PK study. Therefore, the sponsor had and wanted more than the minimum, comparing the follow-up to the lead compound.

Analytics

The low concentrations of the test compound present in tissues or in the systemic circulation in phase 0 studies, and especially in microdosing studies, often necessitate the use of sensitive analytical tools to detect and measure them. The three approaches most commonly used are LC-MS/MS, AMS, and PET, each having their advantages and disadvantages toward accomplishing study objectives, with combination approaches possible, and are described below and in [Table 5](#).

Radiochemistry

For approaches that require radiolabeling, such as those using AMS and PET, elucidation of radiochemistry procedures should be given at least 6 months unless known in advance. This is required to synthesize suitable radiolabeling precursor molecules and to develop fast and efficient radiolabeling approaches under GMP-like conditions, which are compatible with the short radioactive half-lives of PET radioisotopes (e.g., ^{11}C , half-life 20.4 min).⁵⁰ Labeling with ^{11}C for PET microdosing or ^{14}C for AMS microdosing is attractive because of the almost universal presence of carbon atoms in test compounds. However, longer half-life PET isotopes (e.g., ^{18}F , ^{89}Zr , and ^{124}I) may be required in certain developmental scenarios (e.g., long PK half-life of the test compound).

Dosimetry

AMS and PET microdosing studies involve the administration of radiolabeled drugs to healthy volunteers or patients. The use of ionizing radiation in medical research has to be justified in the study protocol submitted to the institutional review board (IRB)/ethics committee (EC). In study protocols, the risk associated with radiation exposure is typically categorized according to International Commission on Radiological Protection (ICRP) classification,⁵¹ and is based on estimates of the radiation exposure of study participants (i.e., the effective dose in millisievert [mSv]). In addition, critical organs relevant to the absorbed dose need to be identified and local radiation exposure needs to be considered with respect to the administration route of the radiotracer. Dosimetry estimates are usually based on whole-body biodistribution data in preclinical species (rodents or non-human primates). The effective radiation dose of a radiolabeled drug depends both on the properties of the drug and the used radionuclide, including the type of radiation being emitted and the corresponding radioactive half-life.

Due to the very high sensitivity of AMS, the AMS microdosing studies require the administration of only very low ¹⁴C amounts (10–100 kBq). In addition, as the energy of the beta particles emitted in the decay of ¹⁴C is relatively low, AMS microdosing studies mostly fall within risk category I according to the ICRP classification (trivial risk, effective dose <0.1 mSv).⁵¹

Among commonly used PET radioisotopes, the effective radiation dose increases, as a rule of thumb, with the half-life of the radioisotope. A maximum effective radiation dose of 10 mSv, corresponding to risk category IIb (minor to intermediate risk) is usually considered acceptable for studies in healthy volunteers aged less than 50 years. Such a radiation exposure is compatible with the administration of most ¹¹C and ¹⁸F-labeled drugs. In case of ¹¹C-labeled drugs, which have an average effective dose of ~5 μSv/MBq,⁵² and assuming a typical i.v. injected radioactivity amount of 400 MBq, repeated administration (up to 5 times) of ¹¹C-labeled drug to the same subject should be feasible within the effective dose limit of 10 mSv. This is particularly useful for receptor occupancy studies, in which participants undergo PET scans at baseline and after administration of different doses of unlabeled drugs. For other, longer-lived PET radionuclides (e.g., ⁸⁹Zr and ¹²⁴I) effective radiation doses are about two orders of magnitude higher than for ¹¹C, so that their use may fall outside risk category IIb. Studies with these radionuclides usually correspond to risk category III (moderate risk, effective dose >10 mSv)⁵¹ and would require a substantial benefit for the study participant, which is “usually directly related to the saving of life or the prevention or mitigation

of serious disease.” Recently introduced total-body PET scanners with very high sensitivity may permit performing PET microdosing studies with ⁸⁹Zr- and ¹²⁴I-labeled compounds after injection of substantially reduced radioactivity, thus mitigating dosimetry concerns.⁵³

Biomarkers

A common misconception is that biomarkers cannot be studied with phase 0 approaches. With approaches 3, 4, and 5 in the ICH M3 guidance there is entry into therapeutic exposures and collection of PD biomarkers is straightforward. However, collection of biomarkers is possible also with microdosing^{18,30–32,35,54–59} (approaches 1 and 2 in ICH M3) and is more attractive due to the reduced regulatory package associated with such approaches and the ability to initiate human testing earlier than with the other approaches.¹⁶ Whereas microdosing exposures are subtherapeutic, they are not “sub-pharmacological” in the literal sense. Pharmacological and biological effects may take place at “subtherapeutic” exposures but may not be readily observable and require sensitive analytical tools to detect them.

One straightforward microdosing methodology that can be used to generate and measure PD effects is ITM, whereby the microdose is administered directly into the target tissue to generate local therapeutic-level exposures.^{18,30–32} Other methodologies include use of radiolabeled compounds to detect low concentrations of biomarkers, such as imaging of drug binding at the target, presence of drug phosphorylation or DNA adducts in PBMCs as biomarker of chemotherapeutic drug effects.^{24,35,54–56,58,60,61}

Advance preparation may be needed to validate targets and biomarkers and other components of the study design (e.g., PET tracers to determine target occupancy and displacement agents at the drug binding receptors, see below under “Positron emission tomography”). However, one of the advantages of phase 0 approaches is that they can be used to expand the early knowledge base about the investigational molecule by accelerating the validation of target, biomarker, and drug-target interactions for use in future clinical trials.

Clinical site selection and preparation

As with traditional phase I studies, site selection and preparation ensure familiarity with the subject matter, and methodological approaches (including Phase 0 approaches), availability of technological capabilities, FIH study expertise, and access to research subject pool. It is

also important to ensure that the regulatory environment, both local and national, is optimal and receptive to phase 0 approaches. Prior site familiarity with phase 0 is preferred but not essential as long as sufficient training of the site, investigators, IRB/EC, patient, and healthy volunteer groups is possible. Educational activities directed at patients and patient advocacy groups are recommended and could help with recruitment efforts, because these groups are typically appreciative and responsive to approaches, such as phase 0 that enhance patient safety and have the potential to accelerate development of novel therapeutics (Appendix S2).

ETHICS OF PHASE 0 APPROACHES

Phase 0 studies are clinical trials conducted in human volunteers and as such should comply with the Declaration of Helsinki and any relevant local regulations.^{62,63} Like with other human research, fundamental ethical principles of participation in clinical research, such as beneficence, justice, autonomy, dignity, privacy, confidentiality, and animal welfare, should be adhered to. These principles manifest in the application of informed consent, favorable benefit/risk ratio, adequate representation of all relevant groups, and protection of vulnerable populations.⁴⁴ In addition, demonstration of the scientific value, validity, and generalizability of phase 0 study methodologies and results is required.^{64,65} Transparency of study results and reporting in the public domain should also be sought whenever possible, even though not all regulatory jurisdictions require FIH studies to be reported in the

public domain (for example, in the United States reporting in the public domain of phase 0 and phase 1 studies in healthy volunteers is not required,⁶⁶ however, in Europe it is required). Nevertheless, the Declaration of Helsinki, required by many ethics committees as standard of clinical trial ethics, requires registration in a public database prior to recruitment of the first human subject in a trial.⁶²

Phase 0 approaches also have unique features that lead to unique ethical considerations (Table 4). The two ethical principles that are impacted by these unique features are “beneficence” and “justice.” Beneficence is the consideration of benefit/risk ratio. The inherent safety of phase 0 and especially microdosing studies means that human volunteers are exposed to less risk from the test article, requiring less testing in animals, and allowing for earlier entry into human testing. Phase 0 approaches are characterized by their subtherapeutic exposures, which imply increased safety but also nontherapeutic potential and sometimes exposure to radiation emitted by radiolabeled compounds.⁴⁴ Typically, the riskiest element of phase 0 studies is the exposure to the novel drug. The most invasive procedures are usually the obtaining of blood samples, although occasionally biopsies are obtained. To justify their application, the benefits of knowledge obtained from phase 0 studies, corresponding with the concept of “scientific necessity,” should outweigh any associated risks.⁶³ Knowledge of the benefits are to future patients and society at large through the translation and development of therapeutic or diagnostic agents. Because of the lack of therapeutic benefit, ethical considerations go beyond safety of drug exposure, especially when phase 0 studies are conducted in patients. For example, in the case of patient studies, participation

TABLE 4 Ethical considerations in phase 0 studies

1. Beneficence – benefit/risk ratio	
a. Increased benefit	<ul style="list-style-type: none"> i. Less risk to human subjects from exposure to the novel drug ii. Less use of animals for human research iii. Earlier knowledge (“scientific necessity”) with less resources iv. Acceleration of drug development
b. Decreased benefit:	<ul style="list-style-type: none"> i. Exposure to radiation ii. No direct therapeutic benefit to patients
2. Justice – equitable distribution of research risks and benefits	Early development of drugs in vulnerable populations (including patients) – for children “minor increase over minimal risk” ⁶⁸

in the phase 0 trial must not prevent or significantly delay treatment or participation in a therapeutic clinical trial, and study procedures, such as biopsies, should be justified in light of the PD information in the clinical setting.⁶⁷

Other features of phase 0 with relevance to ethical considerations include their applicability to vulnerable populations, including patients, and their potential to save developmental time and resources, reduce attrition due to failed compounds, and therefore accelerate the process of drug development. Patients and other vulnerable populations that are typically excluded from early phase clinical development (e.g., pediatric, women of childbearing potential, frail elderly, the hepatically and renally impaired, polypharmacy, and comorbidity) can participate earlier in clinical research of drugs that may benefit their conditions in the future.⁶⁸ ICH M3 guidance specifically encourages inclusion of patients in microdosing studies¹⁶ (see also under “Study design” below). This is consistent with the ethical concept of justice that postulates equitable distribution of risk and benefit in populations of prospective beneficiaries of drug development, thus anticipating the availability of target-specific information to inform administration of the drug in these populations.^{44,62,63} For example, in the United States, it is acceptable to conduct microdosing studies in children who suffer from the condition under study if participation in the study poses no more than minor increase over minimal risk (i.e., risk expected as a consequence of engagement in daily routines).⁶⁸ Whether microdosing studies can be conducted in healthy children is more controversial.⁶⁸ Similar restrictions may apply to other vulnerable groups.

Animal rights groups have been interested in phase 0 for their potential to advance the 3R's principles “reducing, replacing, and refining” of animal testing.^{69–71} Phase 0 approaches minimize the use of animals in support of human research primarily by reducing and replacing animal testing with human testing.^{44,70,71} Whereas parallel preparation for phase I may, in some cases, lead to completion of the phase I animal testing prior to the completion of the phase 0 program, it is nevertheless possible that data obtained in the phase 0 studies will lead to termination of development prior to the phase I animal testing and obviate the need for such testing.

ANALYTICAL TOOLS

The low systemic exposures associated with phase 0 approaches, and especially those of microdosing studies, typically necessitate the use of sensitive analytical tools such as AMS, PET, and LC-MS/MS to detect the test drug in plasma and other biologic tissues¹⁷ (Table 5). AMS was the analytical tool of choice in the early days of the

phase 0 space (2000–2010) due to its high and unique sensitivity, use of the long half-lived ¹⁴C, the versatility of the labeling options, and the low exposure to radioactivity. However, with the emergence of ultra-sensitive LC-MS/MS instruments that also had the advantage of low cost, wide availability, and no radioactivity exposure, they became the most frequently used in phase 0 studies. Increase in radiolabeling options for PET together with the recognition of the value of minimally invasive dynamic measurement of drug tissue concentration levels, and the potential to measure receptor binding and post-receptor modulation, have led to an increase in the utilization of PET microdosing, although still well below what would be a fitting reflection of its potential value to drug developers. A recent review found that of the 116 published phase 0 studies, 45% used LC-MS/MS, 29% used AMS, and 23% used PET.⁶

Whereas AMS, PET, and LC-MS/MS have been most commonly associated with phase 0 and especially microdosing studies because of their high sensitivity that allows detecting the low exposures after microdose administration, there have been reports of other approaches, mostly in non-microdosing phase 0 and ITM studies (total of 11 studies) using near infrared fluorescence,^{72–74} immunohistochemistry,^{30,31,43,75} single-photon emission computed tomography,⁷⁶ and γ -camera,⁷⁶ that are summarized in Appendix S1 of Burt et al.⁶

ACCELERATOR MASS SPECTROMETRY

With sensitivity in the attomole to zeptomole range (10^{-18} to 10^{-21} mole) AMS is the most sensitive analytical tool available to quantify concentrations of organic compounds in biological tissues⁶⁰ (Table 5). AMS is a tracer technology that uses radiolabeling, typically with ¹⁴C, to discriminate between administered dose and other, non-labeled sources of carbon, such as endogenous compounds or other administered compounds.⁷⁷ After administration of the radiolabeled compound, plasma, or other biological samples, such as biopsies, cerebrospinal fluid, bronchial lavage, and blister fluids, may be collected for AMS bioanalysis at intervals that allow the desired characterization of the compound's disposition. Because the sample is combusted and converted to carbon dioxide in the AMS sample preparation process, AMS analysis cannot discriminate between the various molecular forms carrying the radiolabel, for example, parent compound and metabolites. However, using liquid chromatography (LC) prior to AMS sample preparation in a process called LC+AMS or high performance LC/ultraperformance LC+AMS this methodology can allow characterization of

TABLE 5 Properties of analytical tools used in phase 0 studies

	AMS	PET	LC-MS/MS
Sensitivity	10^{-16} to 10^{-18} g/ml	10^{-11} to 10^{-13} g/ml	10^{-12} g/ml
PK characterization	Very sensitive; limited only by the half-life of the test article	Very sensitive; limited by the half-life of test article and radionuclide	Sensitive; limited by the half-life of the test article and the LLOQ of the instrument
Efficacy demonstration	In special cases (e.g., drug in biopsy of tumor, crucial metabolic conversion is demonstrated in a sequestered compartment ^{55,58})	Possible through demonstration of target organ/tissue penetration, accumulation in regions of interest, receptor occupancy, and biomarker generation	In special cases (e.g., drug in biopsy of tumor, crucial metabolic conversion is demonstrated in a sequestered compartment ^{55,58})
Safety/toxicity demonstration	Unlikely (unless suspected target tissue is known and sampled or biopsied)	Possible, through the demonstration of drug in non-target tissues, or alternatively, the demonstration of non-penetration of suspected non-target organ/tissues (e.g., CNS)	Unlikely (unless suspected target tissue is known and sampled or biopsied)
^a Cassette studies	Not possible to study more than one radiolabeled compound at a time	Not possible to study more than one radiolabeled compound at a time	Simultaneous administration and assessment of multiple compounds is possible
^b Cocktail studies ^{85,116}	Possible	Possible	Possible ¹¹⁶
Discrimination of parent compound and metabolites	Discriminating parent compound from metabolites possible with concomitant HPLC	No discrimination	Discriminating parent compound from metabolites possible
Sample types	Mostly plasma but any samples may be used (e.g., biopsies, bronchial lavage, CSF, urine, feces, blister samples)	Real-time imaging; dynamic, contemporaneous information from multiple tissues/targets	Mostly plasma but any samples may be used (e.g., biopsies, bronchial lavage, CSF, urine, feces, blister samples)
Sample frequency/duration	6–10/h duration unlimited	Continuous/dynamic; duration limited by radioisotope half-life	6–10/h duration unlimited
Plasma sample volume	Typically, 25–50 μ l, but as little as 2 μ l. Samples should contain between 0.2 and 5 mg carbon for ¹⁴ C analysis	N/A; continuous/dynamic “counting” of drug molecules per unit space	Typically, 100 μ l–2 ml, but as little as 25 μ l ^{81,117,118}
Radiolabeling	Mostly ¹⁴ C but ³ H, ³⁶ Cl, ⁴¹ Ca, ²⁶ Al and ¹²⁶ I have also been used	A variety of radionuclides with range of half-lives and chemical properties including: ¹¹ C, ¹⁸ F, ⁶⁴ Cu, ⁸⁹ Zr, and ¹²⁴ I	None
Radiation exposure	Very low	Low-moderate (depending on employed radionuclide)	None
Administration	Mostly p.o. and i.v.	Mostly i.v. but p.o. and intra-nasal administration have been reported ^{119,120}	Mostly p.o. and i.v.
Site of analysis	Can be outsourced	On-site only	Can be outsourced
GMP production standards	Extent of required adherence to GMP standards should be discussed with the regulatory authority in pre-IND meetings	Required in some regulatory jurisdictions ⁸⁷	Extent of required adherence to GMP standards should be discussed with the regulatory authority in pre-IND meetings

TABLE 5 (Continued)

	AMS	PET	LC-MS/MS
Costs per study ^{7,121}	~\$350–700 k	~\$500–800 k	~\$80–140 k
Availability	Limited availability; ~6 facilities dedicated to biomedical research worldwide	Available in specialized centers (e.g., tertiary-care facilities)	Commonly available

Abbreviations: AMS, accelerator mass spectrometry; CNS, central nervous system; CSF, cerebrospinal fluid; GMP, Good Manufacturing Practices; HPLC, high performance liquid chromatography; IND, investigational new drug; LC-MS/MS, liquid chromatography tandem mass spectrometry; LLOQ, lower limit of quantification; N/A, not applicable; PET, positron emission tomography; PK, pharmacokinetic.

Adapted from Burt et al.¹⁷

^aCassette – simultaneous administration of different drugs to assess their PKs.

^bCocktail^{83,116} – simultaneous administration of different probe compounds (“perpetrator” compounds) to assess their effects on the drug under investigation (e.g., through their effects on metabolic enzymes or transporters).

the various analytes including discrimination between the parent compound and its metabolites.^{17,77,78}

The use of ¹⁴C instead of resident ¹²C in drug compounds is convenient because of the ubiquitous presence of carbon atoms in drug molecules. In addition, because ¹⁴C is a very long-lived radioisotope with a half-life of 5730 years, samples can be prepared, shipped, and analyzed without concern about radioactive decay. These advantages mean that most drug compounds can be labeled with ¹⁴C and analyzed using AMS. Another advantage is exposure to very low levels of radioactivity. Typical single dose radioactivity of an AMS microdosing study is 11 kilobecquerels (kBq; equivalent to 300 nanocuries), about half of the natural background radioactivity of ¹⁴C that an adult contains from natural sources.⁶⁰ The exquisite sensitivity of AMS also means that only very small amounts of sample material need to be collected, a considerable advantage when aiming to minimize blood loss in vulnerable populations, such as neonates.^{68,79,80} To enable AMS bioanalysis, samples should contain between 0.2 and 5 mg carbon.¹⁷ Typically, samples are 25–50 µl in volume, but can be as little as 2 µl.¹⁷

Use of AMS is common in absolute bioavailability (AB) studies in which the tracer, typically labeled with ¹⁴C, is administered i.v. at a low dose concomitantly with a higher dose of the non-labeled compound administered orally. The ratio between the doses is at least 100-fold so that the i.v. dose only “traces” but does not “perturb” or meaningfully alter the PK profile originating from the orally administered dose. As an added advantage, using the i.v. microdose can be done without dedicated intravenous toxicity/safety testing in animals/humans. The latter is a major reason why sponsors use this approach. The orally administered dose is usually given in therapeutic-level exposures in AB studies. However, due to the sensitivity of AMS (and, recently, of novel ultra-sensitive LC-MS/MS instruments as well) the entire AB study can be conducted using AMS microdosing, for example, by

giving the i.v. labeled dose as 1 µg or less, concomitantly with 99 µg unlabeled p.o. dose for a total of 100 µg. This is consistent with ICH M3 guidance, approach 1, that allows administration of multiple doses as long as the total is no more than 100 µg (or 1/100th of the NOAEL, or 1/100th of the anticipated minimal therapeutic-level exposure).¹⁶

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

LC-MS/MS combines the high resolution and ability to discriminate and identify multiple compounds offered by liquid chromatography, with the sensitivity of mass spectrometry. Recent increases in sensitivity have made LC-MS/MS instruments adequate to assess the low systemic concentrations associated with single microdose exposures. Already in 2011, an LC-MS/MS study of 31 most commonly used drugs was able to quantify 24 of these drugs (77%) within five half-lives of administration, and 30 (97%) within three half-lives of administration, or about 1/8 of C_{max}, a value usually sufficient to characterize the PK profile of administered organic compounds.⁸¹ Since that publication, the sensitivity of LC-MS/MS instruments has continued to increase, entering in the picogram and even femtogram ranges and allowing analysis of even the most potent modern drugs after microdose administration.^{82–84}

In addition to the advantages of costs, wide availability, lack of radiolabeling requirements, and lack of exposure to radioactivity, LC-MS/MS is well-suited to detect drug metabolites or multiple drugs in the same sample as in cassette microdosing studies⁸⁵ (Table 5). PET, on the other hand, measures total radioactivity in tissue and cannot distinguish different chemical entities to which the radiolabel is bound. AMS analysis, per se, also cannot distinguish different chemical entities in a sample. However, when AMS is combined with chromatographic techniques it offers the

possibility to resolve different compounds just as LC-MS/MS.^{17,77}

POSITRON EMISSION TOMOGRAPHY (PET)

PET is an imaging method which allows the minimally invasive measurement of molecules labeled with positron-emitting radionuclides, or radiotracers.^{61,86,87} PET allows study of drug tissue distribution, tissue PKs, and, in some cases, drug MOA and PD effects as well.^{18,20,37,54,59–61,88} The sensitivity of PET approaches that of AMS (Table 5) and is well-suited to quantify the very low drug concentrations attained in organs and tissues after administration of a microdose.⁸⁹ PET allows the dynamic and simultaneous (depending on the field of view of the used PET scanner) study of multiple target tissues, including both efficacy and toxicity target tissues. There are two approaches for the use of PET in drug development, one that labels and studies the drug, and the other that labels elements in the drug target environment and is used to study target occupancy and post-target modulation induced by an unlabeled drug. Together, these methodologies enable PET to characterize the so-called three pillars of drug development decision making: tissue/target distribution, target occupancy, and post-target modulation.⁹⁰

In all cases in which distribution to tissues relevant for efficacy and/or toxicity is an important question and cannot be reliably predicted from preclinical data, PET microdosing, in particular with total body PET technology (dynamic measurement of the entire body⁹¹), could provide very useful information. Of course, the drug must be amenable to radiolabeling without structural modification (small molecules) and should display metabolic stability over the time course of the PET experiments, in particular when peripheral tissues are studied (less of a problem for the brain, from which metabolites are often excluded due to the BBB).

Studying radiolabeled drugs

The drug is labeled with a radionuclide which is compatible with its PK properties (e.g., ¹¹C, half-life 20.4 min.; ¹⁷F, half-life 109.7 min.; ⁸⁸Zr, half-life 3.3 days; ¹²⁴I half-life 4.2 days¹⁷) and administered i.v. as a microdose. PET is then used to measure the drug's tissue PKs as well as the rate and extent of distribution of the drug from plasma into tissues. Tissues of interest for such studies are most commonly those expected to express the drug's efficacy and toxicity molecular targets, although especially in the case of toxicity targets, one of the attractive capabilities of PET is the ability to detect targets not

previously known. Because an adequate tissue distribution is a prerequisite for the drug to exert its PD effects, PET studies can provide information on the essential link between PKs and PDs.⁹²

Studying target occupancy and post-target modulation

In such studies, the drug is administered unlabeled prior to PET imaging with a radiotracer that is directed toward elements in the target environment, such as receptors or post-receptor biomarkers. This either allows to estimate target occupancy or downstream effects of the drug (post-receptor modulation).^{37,93–97} In order to achieve measurable target occupancy, such studies typically require the administration of therapeutic doses of the unlabeled drug. However, for potent drugs, target occupancy studies may also be feasible within a subtherapeutic dose range allowed for exploratory clinical trials (i.e., approaches 3, 4, and 5 in the ICH M3 guidance, see Table 1). Alternatively, under special circumstances, local administration of a microdose of the unlabeled drug may also achieve a sufficiently high degree of target occupancy measurable with PET (see intra-target microdosing). In target occupancy studies, groups of research volunteers receive single ascending doses of unlabeled drug followed by PET scans to measure target occupancy. Such “dose finding” studies allow identification of appropriate starting doses with respect to target occupancy for subsequent clinical trials.^{98–101} PET tracers to measure target occupancy of investigational drugs should be developed in parallel to drug discovery programs, if possible, to ensure their timely availability when the drug enters clinical testing. The use of PET to assess post-target modulation is particularly common in anticancer drug development using [¹⁷F]FDG to measure tumor metabolic activity or other pathway-specific radiotracers.^{102,103}

PET in the study of target mediated drug disposition

If a drug has a very high binding affinity to its molecular target (e.g., in the nano- to pico-molar range) and achieves a measurable degree of target binding at microdose concentrations, the tissue distribution of the radiolabeled drug may reflect the binding of the drug to its molecular target. This phenomenon has been termed target-mediated drug disposition and has been widely recognized to play a role in the disposition of antibody drugs, but also of some small molecule drugs.⁶ For drug targets with a distinct anatomical distribution pattern, a

PET study with a radiolabeled drug may provide information not only about the drug reaching the target but also whether it binds to a specific molecular target. A classic example are neuroleptics which antagonize dopamine D₂ receptors in the brain. PET studies with radiolabeled neuroleptics (e.g., [¹¹C]raclopride) reveal a distinct uptake pattern in the brain with accumulation in the basal ganglia, a brain region with a high density of dopamine D₂ receptors.¹⁰⁴ Similar use of PET microdosing to detect specific tissue binding as proxy of drug MOA have been used in drug development.^{20,54,59,88} If an appropriate displacement agent is available, which binds to the same target protein as the radiolabeled investigational drug and which can be safely administered at doses which lead to appreciable target occupancy, then target specific binding and MOA of the radiolabeled drug may be confirmed by co-administration of the displacement agent or of a therapeutic-level dose of the unlabeled drug itself (e.g., in phase 0 ICH M3 approaches 3, 4, and 5).¹⁰⁵

Limitations of PET in the study of drug tissue distribution

The following are limitations of PET imaging in the study of drug tissue distribution:

- a. Drug-metabolite distinction. PET measures total radioactivity and therefore is unable to distinguish radiolabeled parent drug from radiolabeled metabolites. If the radiolabel is located in a metabolically stable position of the drug molecule, then both the drug and metabolite may contribute to the PET signal. This is less of an issue with brain PET imaging because typically polar radiolabeled metabolites will be excluded by the BBB.
- b. PET cannot distinguish protein- or lipid-bound drug from the unbound drug that is typically considered the pharmacologically active component. However, the PET data can be supplemented with *in vitro* and *ex vivo* data that can provide estimates of protein- and lipid-binding of the drug. These studies can be done during the preclinical development stage that is part of the Exploratory Clinical Trial regulatory package.¹⁶
- c. Short radioactive half-lives of some radionuclides, especially ¹¹C and ¹⁸F, limit their utilization to imaging durations of approximately 2 and 5 h, respectively. One way to overcome this limitation is to use a combination of PET and AMS microdosing, such as with a mixture of ¹⁴C and ¹¹C-labeled drug, administered simultaneously.¹⁰⁶ The limited duration PET-microdosing tissue PK data can then be supplemented using the long-duration plasma PK from

the AMS-microdosing data to estimate tissue distribution using physiologically-based pharmacokinetic modeling.¹⁰⁷

ECONOMICS OF PHASE 0

Studies of the drug development process have found that pharmaceutical research and development (R&D) costs are high and have increased substantially over time.³ The full cost estimates depend not only on the resource costs of conducting discovery research, preclinical development, clinical trials, and CMC development, but also how long it takes to conduct these activities (time costs for delay in obtaining the returns to successful R&D), and development success rates (accounting for the costs of failure). Increases in the efficiency of drug development, from shortening development cycle times and increasing success rates, therefore reduce R&D costs when the full financial implications are considered. Given that drug development typically occurs over a span of years to a decade or more, and that failures are highly frequent, the potential for substantial financial benefit to drug developers from improvements in cycle times and success rates is high.¹⁰⁸ For example, using data and modeling from a recent study of pharmaceutical R&D costs, [Figure 3](#) indicates that improving overall clinical approval rates (likelihood that a drug that enters clinical development will be approved for marketing) from the study's estimate of one-in-eight to approximately one-in-three would, other things being equal, result in a reduction of clinical period out-of-pocket (resource) costs per approved new drug by nearly one-half.

Framework for assessing phase 0 net benefits

An expected (i.e., risk-adjusted) net present value (eNPV) portfolio approach to evaluating new financial gains to drug developers relative to the standard drug development paradigm from alternative manufacturing contracting practices and from integrating formulation development, real-time manufacturing, and phase I clinical testing that result in shorter drug development times has been applied in several recent studies.^{109,110} The modeling framework used in these studies was parameterized on the development cost side from the results in a study of pharmaceutical R&D costs³ and on the commercial side by net return distribution found in a recent study of rates of return for the pharmaceutical industry.^{108,111} A similar approach could be used to estimate the net financial benefits from phase 0 testing.

Each example of an eNPV approach to estimating the net benefits of an alternative development paradigm will

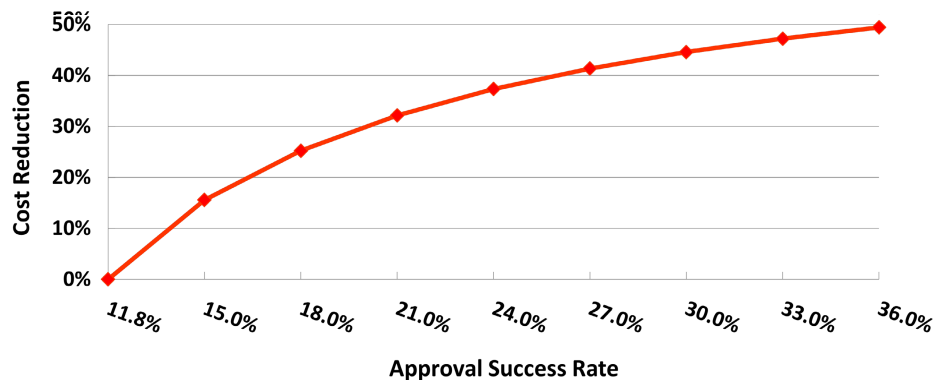


FIGURE 3 Higher clinical approval success rates can reduce developmental costs significantly. Costs defined as “out-of-pocket” clinical period costs. *Source:* DiMasi et al.³

depend on the level of resource costs, the lengths of development phase cycles, phase transition rates under the standard paradigm, and sales distributions for drugs that make it to market. We would also need estimates of the additional resource costs needed to implement the new approach, reductions in resource costs that would otherwise have been made, and of what impacts the alternative approach would have on development phase cycle times and phase success rates.

The industry portfolio approach (i.e., an analysis that presumes that an alternative paradigm is adopted across all projects and across the industry) relies on historical data for the industry. An analysis that is company-specific or asset-specific could still be done in this general manner, but the model would have to be parameterized by subjective estimates of success rates, cycle times, and, to some extent, resource costs. However, the parameterization should be guided by objective historical estimates.

Estimates to consider for an expected net present value analysis

A full economic analysis of the net financial benefit to developers from widespread use of phase 0 approaches must consider the variety of ways that phase 0 testing could help or hinder the return on this type of investment. The following list is a sample of the types of outcomes that can arise from considering phase 0 trials and how they may impact the financial incentives to innovate:

- The early termination (prior to phase I testing) of drugs that will not succeed. This factor should result in the avoidance of clinical testing on some drugs that will not succeed, and so will lower resource costs.
- Optimization of the selection of preclinical candidates for further development, possibly through the use of resource-efficient cassette microdosing design. This will

reduce the likelihood of a preclinical candidate being wrongly eliminated prior to phase I (reducing false negatives).² This factor can also increase the likelihood of finding a drug prior to phase I that will succeed and so can result in higher approval success rates (reducing false positives). This will reduce costs and increase returns on a per drug approved basis.

- Identification of both the nonviability of the lead compound and the viability of a backup compound prior to phase I testing. This factor can result in a compound reaching the market sooner, and, in some cases, extending the period of patent protection. In addition, this can result in the avoidance of some phase I testing costs, thereby increasing the net present value of returns and decreasing resource costs.
- Profits lost from a compound that could have reached the marketplace but was terminated based on phase 0 testing results (increasing false negatives).

The first three factors result in financial benefits to drug developers that must be measured against the added costs of adopting phase 0 testing across the investigational drug portfolio. The last factor is a financial disincentive to drug developers, but the expected value of such losses can be reduced if the threshold for rejecting a lead compound and all backups after phase 0 testing is raised, thereby reducing the likelihood of a false negative. Doing so, however, could also reduce some of the benefits of phase 0 testing (e.g., identifying the compounds that could fail in clinical development, the false positives). These opposing effects would have to be balanced to optimize phase 0 testing decision rules.

A full assessment of the net financial benefit to developers would account for the benefits and costs of all of the factors above simultaneously. To illustrate some of the nuances of the eNPV approach, in the Supplementary Information file, we take one of the cases above and apply some hypothetical, but reasonable, parameter values. It should also be noted that here we are considering only the return on

investment to drug developers from adopting phase 0 testing. The net social benefit from getting safe and effective new drugs to patients sooner or increasing the number of safe and effective drugs available to patients could greatly exceed the private net benefits considered here.

CULTURE OF DRUG DEVELOPMENT

The drug development cultures of industry sponsors may impact the application and effectiveness of phase 0 approaches, with some developmental cultures favoring application of phase 0 and others likely to resist application. Drug development culture could impact the choice of developmental objectives, the strategies used to implement them, study design and execution, interpretation of the data, and, eventually, implementation of study results in developmental decisions.

Drug development culture impacts these developmental elements by creating a hierarchy of values and priorities. For example, the importance of “truth-seeking” versus “progression-seeking” cultures was highlighted recently.^{112,113} Phase 0 approaches, by enabling earlier studies in humans and especially earlier studies in patients, allow earlier identification and validation of drug-related biomarkers and directly address three of the five-dimensional elements of R&D productivity advocated by the truth-seeking model of Morgan et al., identifying the right molecular target, right tissue target, and right patient population.^{12,112} All phases of the drug development process have, of course, truth-seeking objectives, however, progression-seeking incentives may introduce a bias in favor of progression rather than termination in a case where the evidence was in favor of termination but not unequivocal. Incentives can be material and psychological. Progression may be rewarded by specific compensation and career advancements as well as by emotional gratification by the success, or lack of failure, of the product of one's work. Such cultural dynamics may counter one of the most meaningful potential benefits of phase 0, namely, the early termination of nonviable candidates. In a progression-seeking culture, there is no incentive to identify drugs that are less likely to succeed when rewards are attributed to progress and associated with compliance with preset timelines. If an organization's priority is to advance the most promising drug candidates, it must cultivate an ethos that rewards evidence as much as milestones. A top-down perspective evaluating overall pipeline success instills a culture that allows failure as an acceptable, and, in the right scenarios, a desirable outcome. For phase 0 trials to be impactful, teams must understand that short-term loss may drive long-term positive effects.

Adoption of phase 0 may be enhanced by cultures that are receptive to innovation in drug development approaches, methodologies, and applications. However, self-imposed conservatism by sponsors of drug development often discourages exploration of such innovations. In the exploratory IND guidance of 2006, the FDA was keen to emphasize that the regulatory framework has considerable flexibility and that sponsors often do not take advantage of that flexibility and provide more supporting information than is required by the regulations.¹⁴ More information means more resources and more time spent on generating additional data and preparing the regulatory package, often exposing animals and humans to unnecessary testing and risks, instead of using the same time and resources to advance more promising drug candidates. There should be a balance of pragmatism (goal-oriented drug development) and innovation-driven development, whereby novel approaches are progressively tested and applied until validated or refuted.

Another important dichotomy of drug development culture is that between drug-centered development and process-centered development. In the drug-centered approach, the developmental program adapts to and reflects the unique characteristics of the test article, creates the developmental program around it, addresses essential unknowns, and capitalizes on strategic opportunities. In “process-centered” development, the drug is fit, or forced, into an existing process with little flexibility and adaptability to any unique challenges or opportunities presented by the test compound. Process-centered development also misses the opportunity to benefit from process innovations that could be used to extract more data and more knowledge from the drug. Drug-centered development cultures will be those most likely to consider, apply, and benefit from phase 0 approaches.

Modern drug development is increasingly complex and is therefore increasingly dependent on cross-disciplinary and cross-functional collaborations. This is particularly evident in drug-centered development where the unique features of the drug and the unique circumstances of development may call for specialized expertise, such as biomarker development, use of sensitive bioanalysis tools, radiolabeling, and bespoke statistical and study design approaches. Such collaborative and versatile application of biological science knowledge and expertise is likely to extract and exploit the developmental potential of drug compounds more adequately and is especially relevant to application of phase 0 approaches because of their inherent range of application and required specialized expertise.

It is important that phase 0 program management does not “fall between the cracks” of preclinical and clinical development teams' management responsibilities. Who takes ownership, who accepts the risks, and who

pays for phase 0 programs should be decided in advance by the leadership. It would seem sensible that phase 0 would be under the remit of those who oversee the entire pipeline because the benefits of phase 0 approaches will be primarily appreciated on the level of the pipeline. To launch a phase 0 program at the pipeline level, such cross-functional teams must be developed with a shared vision in mind. The organization's own obstacles to both a changed mindset and an adaptive clinical trial design must be identified and included in the advanced planning discussed. Accountability for objectives, timelines, and expenses should be evaluated in this same top-down fashion so progress is not impeded by uncertainty or insufficient authority at the grass roots level. If directives accompany drug development goals, the fear of failure can be removed.

FUTURE DIRECTIONS, BEST PRACTICES, AND POLICY RECOMMENDATIONS

The phase 0 field continues to evolve through research into the science, methodologies, and applications.⁶ These efforts are mostly those of individual research operations, such as those of the drug development industry or academic programs. However, the field could gain considerable benefit from concerted and comprehensive multi-stakeholder efforts including the support of regulators, policy-makers, and industry and academic leadership. For example, regulators could consider minimizing the extent of GMP and good laboratory practice standards required for the preparation of the various phase 0 packages so as to be commensurate with the extent of exposure to the test article and any associated risks. Any progress made by individual research entities could be integrated to drive revised guidelines. It has been more than a decade since the last internationally harmonized regulatory guidance on the topic,¹⁶ or since a study by a consortium of drug development entities collaborated to advance knowledge in this space.^{114,115} The next steps in the evolution of this space, such as comparative phase 0/phase I studies and identification of research and technological priorities, could be undertaken by a consortium of multiple stakeholders, including drug sponsors, operators, regulators, professional organizations, patients, advocacy groups, and other non-profit organizations.

It is important that sponsors of drug development work together to enhance the testing, validity, utility, and applicability of phase 0 approaches. Sponsors could initiate and take part in validation and generalization efforts by sharing data about prior studies and their costs. One straightforward and relatively inexpensive way to

generate valuable controlled data about phase 0 validity is to add a cohort of microdose exposure to otherwise routine phase I studies. Phase 0 data would then be processed by a group blind to the results of the phase I and phase II data in case the phase 0 studies are conducted in patients. The ability of the phase 0 data to predict the results of the phase I and phase II studies and generate the same developmental decisions would then be independently assessed. This will allow expansion of the experience with these approaches to a range of developmental scenarios. Such concerted data generation efforts will expand the phase 0 knowledge base to more therapeutic targets, drug classes, and patient populations, and increase the familiarity and comfort of sponsors with these approaches and the confidence of using them in stand-alone FIH studies.

While the field awaits the more definitive data, especially on applications in a range of developmental scenarios, and on the economic value of routine application, phase 0 should, at a minimum, be considered routinely in all drug development programs as early as feasible in the preclinical stage, preferably 1.5–2 years prior to anticipated entry into phase I testing. This will allow for sufficient preparation for these studies to take place and time to extract their resource-sparing developmental benefits.¹⁷ Finally, professional and advocacy nonprofit organizations should work to dispel common misconceptions about phase 0 among sponsors, operators, patients, policy-makers, and the public at large (Table 6).

CONCLUSIONS AND SUMMARY

Phase 0 approaches are informative and feasible and have the potential to shorten drug development timelines and reduce attrition and expenses in drug development. Phase 0 can be integrated seamlessly into existing drug development strategies and, when initiated sufficiently in advance, can produce valuable information for developmental decisions ahead of traditional approaches, without delaying any eventual progress to mainstream clinical development, while using about a fraction of the resources. Phase 0 programs have several design and logistical features that constitute advantages over a traditional development approach, primarily the ability to enter directly into patient testing in FIH studies, test oral drugs intravenously, simultaneous administration of multiple test compounds (cassette microdosing), and ITM. Logistical and economic advantages include the ability to enter into human testing with a fraction of the risk, time, and resources, and produce data that could considerably increase the value of test compounds earlier, in a safer manner for research subjects, and with less expense.

TABLE 6 Misconceptions about phase 0 approaches

Misconception	Answer
“A phase I will still be required, why add another study?”	Phase 0 may terminate the program, and if not, it may inform phase I design. ^{19,122} Phase 0 data will always reduce the uncertainty about the drug effects in humans and this may reach the threshold for a “go-no-go” developmental decision
“It is risky to accept phase 0 result for developmental decisions. Results could be a “false negative”	This is true. Underpowered, small, and short phase 0 studies have high risk of “false negatives,” and so do phase I studies. The threshold for a “negative” decision should, accordingly, be high
“Delaying the start of phase I is a waste of time”	Delays may be avoided using strategic planning, and/or adaptive phase 0/phase I design. ^{122–125} Obtaining data on the asset and/or its target is not waste and could be used for developmental decisions including termination that would obviate the need for phase I
“The PK data are unreliable because extrapolation is unpredictable”	PK extrapolation is reliable in 70–80% of p.o. and 95–100% of i.v. microdosing ^{64,65}
“You can only obtain PK data”	PD and MOA data can also be obtained
“Prediction of PK no longer critical because of better preclinical models”	~15% of drugs still fail due to erroneous PK predictions. ^{8,9,12} PD and patient PK data also possible with phase 0. ⁶
“The benefits of phase 0 are minimal because they only save on toxicity and genotoxicity, main costs are CMC”	The main savings are due to earlier availability of data to de-risk developmental decisions ¹⁷
“Phase 0 is complex, adds costs, and dilutes resources”	Part of the complexity is due to the unfamiliarity with phase 0. The lack of established phase 0 procedures also means that no dedicated resources are usually available which gives the impression of additional costs and diluted resources. The benefits of phase 0 are appreciated on the pipeline level and should therefore receive endorsement, support, and resources from upper management. Once phase 0 are streamlined into standard development practices they will not be experienced as complex and resource-taxing
“Patients will not accept the radioactivity involved”	Exposure to radioactivity is consistent with regulatory guidance and ethical standards and similar or lower than use of radioisotopes in other medical research and clinical development projects, and in therapeutic and diagnostic applications; Numerous AMS- and PET-microdosing studies have been conducted in healthy volunteers and in patient populations using radioisotopes ^{20,59,80,88,126}
“Parents will not accept it for their children”	Regulators, IRBs, and parents have embraced microdosing for pediatric drug development ^{68,79,80,126}
“Radiolabeling is too difficult”	Radiolabeling is possible with most drug compounds ^{127–129}

Phase 0 approaches are subject to a number of misconceptions about their nature and utility that may interfere with the effectiveness of adoption efforts. Here is a list of those encountered most often and their rebuttal.

Abbreviations: AMS, accelerator mass spectrometry; CMC, chemistry, manufacturing, and controls; IRB, institutional review board; MOA, mechanism of action; PD, pharmacodynamic; PET, positron emission tomography; PK, pharmacokinetic.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

ORCID

Tal Burt  <https://orcid.org/0000-0002-5853-6110>

Joseph DiMasi  <https://orcid.org/0000-0002-3432-6928>

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