Foundation review: Progressing preclinical drug candidates: strategies on preclinical safety studies and the quest for adequate exposure

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Drug discovery lead optimization teams face many diverse challenges in the search for drug development candidates. This includes understanding the toxicology profile of a candidate, and some strategies call for *in vivo* preclinical safety studies to be moved increasingly earlier in the discovery phase to increase the likelihood of success in development. One of the final hurdles in these pursuits is achieving adequate exposure to support safety margins for human clinical trials. In this article, we describe several strategies on early toxicology studies along with various enabling formulation methods that can be employed to achieve optimal oral absorption. These two elements of research together can significantly increase the speed preclinical drug candidates can move through development, and the overall probability of success in identifying viable new drugs.

Introduction

Drug discovery lead optimization (LO) teams must optimize a myriad of *in vitro* and *in vivo* biological parameters during the search for a good preclinical candidate, and similar strategies are practiced throughout the pharmaceutical industry [1]. The balancing act of optimizing *in vitro* potency, pharmacokinetics (PKs) behavior and pharmacodynamics (PDs) is universally daunting. To add to the challenge, a final hurdle faced by most potential preclinical candidates is demonstration of an acceptable toxicology profile in several preclinical species. As an LO project nears completion, it is particularly disheartening when all the LO hurdles are met, but the lead compound fails to meet the high exposures often (and associated margins) required in today's preclinical safety programs. Difficulties in achieving adequate oral exposure in preclinical species are often rooted in the suboptimal physicochemical properties of a compound (i.e. solubility); it is a multidisciplinary dilemma. While safety assessment colleagues demonstrate the exposure troubles in their various toxicology studies, pharmaceutical (Pharma R&D) scientists embedded in discovery are usually the unenviable messengers on any root cause physicochemical inadequacies. We surmised that a useful article would describe some key aspects to consider in the early stages of preclinical safety assessment, in the context of helping promising discovery leads progress into development. Towards this end, in this article we describe topics ranging from the increasingly large roster of desirable and required preclinical toxicology studies, the associated

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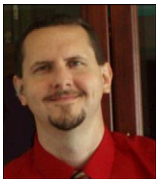

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exposure requirements and some tox-friendly enabling formulation strategies to enhance oral absorption of poorly soluble compounds. Ultimately, we hope to demonstrate that a solid understanding of the interplay between these elements at the drug discovery development interface can make the difference between progressing and killing a compound.

Safety assessment LO strategies

Termination of a late LO discovery candidate is disappointing, but the value of making quick, clear termination decisions during LO is beneficial. By making this decision at an early stage, the organization benefits by saving time and resources that would have been required to move this compound into development for good laboratory practice (GLP) toxicology studies. In many instances, the time the LO team loses on the wrong candidate molecule can cause a delay to the entire development program of several months while the active pharmaceutical ingredient (API) is manufactured and the GLP toxicology studies are conducted. To avoid this costly scenario, an organization that is willing to expend limited resources on exploratory LO preclinical safety assessment studies during the discovery phase can mitigate the risk of failure in early development.

The benefits offered by exploratory LO drug safety assessment studies are (i) to provide confidence and reduce attrition when moving a new molecule into development, (ii) to de-risk for specific preclinical safety issues that might have been identified with a lead candidate, (iii) to understand potential for toxicity related to the mechanism of action or a known pharmacologic mechanism, and (iv) to evaluate the consequences of possible off-target effects based on the counter-screen assays or observations from other studies [2]. Early in the discovery phase around the time of target identification, exploratory preclinical safety also can serve to evaluate the effects of engaging a novel target for which the biology is poorly understood. Although phenotypic data from knock-out or knock-in models might provide some insights, these artificial gene-altered animals often have compensatory homeostatic changes such that the gene that has been knocked in or out are not the only genes and/or pharmacology affected. Validating this target in carefully constructed preclinical safety studies with a prototype molecule capable of activating, blocking or modulating the target site can inform the discovery team of the desirability and risks associated with the efforts to identify new molecules targeted at this site [2].

Safety assessment LO studies

Safety pharmacology

Preclinical safety pharmacology studies conducted in LO have the same objectives and goals as the regulatory safety pharmacology conducted during early development and required by regulation for initiation of Phase I clinical studies [2]. These are studies that evaluate the PD properties of the test molecule on major organ system function in an attempt to understand those which might pose a human risk [3]. Most often, the primary objective of these studies is an assessment of cardiovascular or central (peripheral) nervous system function [4]. However, some institutions might also test for effects on gastrointestinal, renal and respiratory function before candidate nomination depending on the therapeutic class and clinical indication [5]. Overall, this practice is becoming less frequent [4] and is often conducted on a 'case-by-case' basis based on a need to prioritize and focus resources on those systems of greatest concern [2,4,6].

The differences between safety pharmacology conducted in discovery *versus* development might be that development studies include a more robust experimental design, adhere to GLP procedures, and evaluate doses that produce moderate adverse effects [2]. The development studies also might involve the use of different species than those employed in the discovery studies; however, it is preferable to use the same species in all of the toxicology studies. The use of the same species in discovery and early development aids in continuity of design, gains familiarity with species pharmacologic effects, and ensures that the formulation and systemic exposure are adequate for the regulatory toxicology studies. Species continuity also might aid in dose selection refinement for the GLP toxicology studies [2]. Lastly, the amount of preclinical toxicology and pharmacology data is more limited during discovery, and there might be instances when the active pharmaceutical final development physical form and formulation might differ between those studies conducted in discovery and early development.

Cardiovascular and/or neurological toxicity de-risking

Evaluation of cardiovascular function in the discovery phase includes an assessment of test substance effects on cardiovascular hemodynamics in addition to cardiac ventricular repolarization, which is one of several risk factors for the drug-induced polymorphic ventricular tachy-arrhythmia, torsades de pointes (TdP) [3]. Excluding TdP risk is a crucial step in selecting a compound that can be safely advanced through the clinical phases without concerns of TdP-related cardiac toxicity [5,7]. Employed at an early stage of discovery are 'moderate' throughput *in vitro* assays (e.g. dofetilide binding, Rb efflux, automated patch clamp) and at a later stage, a more comprehensive electrophysiology assay [2] (e.g. conventional whole cell patch clamp, Purkinje fiber, isolated Langendorf heart), with confirmation of batch concentrations of the test substance.

In vivo cardiovascular function evaluation can include an assessment of cardiac electrophysiology (e.g. surface electrocardiogram) and systemic hemodynamic properties of a test article [2]. Hemodynamic function is usually assessed by measuring changes in heart rate and arterial blood pressure (systolic, diastolic and mean arterial pressures); however, under special circumstances, other hemodynamic measures, such as cardiac output, peripheral vascular resistance, and systemic and regional blood flow might also be included in the early testing [2,5].

Careful selection of species for these tests is important to assure that the preclinical model emulates the human response to the test agent and requires close collaboration with the experimental biologists and/or pharmacologists from the LO team [2,3,8]. For example, evaluation of cardiac ventricular repolarization in rodents should be avoided since the prominent ion channel responsible for repolarization in rodents differs from that of humans [9]. Furthermore, if the compound plasma protein binding is different in the selected species *versus* human, the free fraction exposure multiple achieved in the animal study compared with that projected for clinical efficacy should be determined [10]. Radio-telemetry technology provides an easily adapted method for

assessing electrocardiographic and hemodynamic function in freely moving conscious animal species (e.g. rats, guinea pigs, dogs, mini-swine, and/or monkeys), keeping in mind the caution of electrocardiographic measures in rodents [2]. At a minimum, safety parameters evaluated include arterial blood pressure, heart rate and interrogation of the electrocardiogram for PR, QRS, QT, and heart rate corrected QTc, intervals, changes in morphology and the presence of arrhythmias [2,5].

Evaluation of abnormal drug-induced neuro-behavior following the scheme originally described by Samuel Irwin and modified by Virginia Moser in rodents provides an early sign of potentially adverse PD properties of a new molecule [2,11,12]. This is particularly important in the selection of discovery candidates possessing neuropharmacodynamic properties; but will also be important for those compounds that demonstrate affinity for off-target sites in the central or peripheral nervous system, those backup candidates that have been previously shown to possess a class-related propensity for adverse neuro-behavior, or to generally exclude possible concerns of the central (peripheral) nervous system before advancing the lead candidate into development [2]. A more detailed evaluation of neuro-toxicities might be pursued before candidate recommendation based on known target or off-target activity for the therapeutic class or compound.

Evaluation of drug effects on the gastrointestinal, renal and respiratory systems is often limited during LO and these preclinical pharmacology studies have been described in other excellent publications [3,5,13].

Genetic toxicology

For most therapeutic classes, early screening of potential chemical lead series for mutagenicity and possibly clastogenicity is crucial [2]. Discovery candidates with potential or known genotoxic liability are routinely excluded during LO. It is important when testing a LO candidate to consider possible hydrolysis products, degradants, and potential metabolites that might adversely influence the genotoxicity of the LO candidate. Exceptions to this strategy include those small molecules that are known to be genotoxic owing to their PD activity [e.g. fluoroquinolones, nucleoside analogs, cytotoxics (e.g. mustards, alkylating agents)], in those cases wherein the therapeutic benefit of the molecule outweighs the potential risk of producing cancer (e.g. treatment of certain types of tumors) [2].

The first line of testing for potential genetic toxicity is to conduct an evaluation of the chemical structure for potential alerts for genetic toxicity. *In silico* tools, such as MCASE and DEREK, in addition to other electronic databases and experience with particular structures or metabolic pathways, are available to provide support for the LO chemist and team. In addition, 96 or 384 well high-throughput assays of clastogenicity and mutagenicity are commercially available to screen a large number of compounds for prioritization early in the discovery phase (e.g. Vitotox, AMESII, Radarscreen Assays) [2]. At a more advanced stage of identifying the development lead, assays, such as the mini-Ames, using three bacterial strains (i.e. TA100, TA102, TA97a) and *in vitro* micronucleus assays in V79 cultured Chinese hamster cells will provide a relatively more definitive indication of mutagenic and clastogenic potential before a compound is advanced into development [2,14].

The purity of the active pharmaceutical ingredient used in LO genetic toxicology studies can be important too. In development, the GLP studies evaluating the genetic toxicity of a molecule not only evaluates the molecule, but also any impurities, degredation products, residual solvents and other process residuals that remain in the batch of material are intended for the preclinical safety and early clinical studies. In this way, the safety of the API is assured before the use of the material in human clinical study. At the time of the discovery studies, however, the final drug substance form and manufacturing process might not be known. As a result, at this stage the importance of testing for genetic toxicology potential lies with the active chemical and any major metabolites that are formed [2]. To achieve this goal, the active pharmaceutical ingredient is purified for the purpose of genetic toxicology testing. With regards to the other tests described in the following sections, the purification of the drug substance batch is not essential, but the purity of the material to be tested should be known. The purity of the test API and proactively saving small portions of the LO batch tested can be helpful when trying to understand why a LO candidate is positive for genotoxicity [2].

Potential for on-target and/or off-target activity

On-target activity refers to the site of action of the test substance (e.g. target receptor or enzyme) that results in the desired PD effect. Off-target activity refers to all other targets for which the molecule has affinity; with the outcome of activation, blockade, or modulation resulting in a functional effect. In many cases, the off-target activity of the molecule might be subclinical and of limited concern. However, the off-target activity might result in side effects of the active agent that range from a minor nuisance to a severe adverse event in the preclinical studies and/or clinical trials [2].

An early assessment of the potential for a molecule to have off-target activity is based on understanding the molecular structure of the target site and how well conserved the on-target protein is when compared with off-target protein homology. For example, selectivity of agents that affect kinases belonging to a specific family as their target, might also affect other members of that family based on the conservation of the protein structure within the family and the potential similarity in the specific target site within the kinase where the test agent acts to produce the desired effect [15]. In the same way, off-target activity at one site might predict the probability for off-target activity at related sites within the family of proteins. Typically in an early LO program, the team will engage in a significant effort to demonstrate the affinity and/or activity of the molecule for related proteins to understand the selectivity of the molecule for the desired target in addition to the liability for the off-target protein activity [2]. The chemistry effort would be focused to identify unique chemical or structural features within the target protein to enhance selectivity and limit possible off-target activity.

To account for those off-target sites that might not be predicted based on genotypic profiling during lead optimization, 'counter-screen' assays will be conducted to identify those targets that could potentially be of concern. Typically, the counter-screen assay evaluates affinity for a variety of target receptors, enzymes, and ion channels with a binding assay (including screens for many of the 500 known kinases) [16–19]. It is important to realize that these are high-throughput affinity assays and a positive result

suggests a concern would be followed by a more robust evaluation of the concentration-related displacement of the ligand, enabling a determination of a 50% inhibitory concentration (IC50) for that binding site. This work would be followed by a functional assay to demonstrate whether binding to the protein leads to a functional result (i.e. functional agonist or antagonist). In some cases, affinity for a protein target does not necessarily translate into a functional outcome [2].

Counter-screening is an important component during LO of new molecules and determines whether there is a potential concern of progressing the compound into development. In evaluating the data from affinity assays, the questions that might be posed are (i) is the efficacy of the test agent at the off-target site sufficient to result in a biologic effect (e.g. IC10, IC50, IC90); (ii) is the exposure multiple between the concentration projected to achieve therapeutic efficacy (on-target site) and that required for activity at the off-target site sufficiently large (e.g. 100–1000-fold exposure multiple) such that the off-target effect is unlikely at clinically relevant concentrations; (iii) was the assay protein an expressed human target or a target from another animal species and is the degree of homology between the targets in the different species sufficient to feel that the data accurately estimate the true risk to humans; and (iv) what is known regarding the PD properties of an agonist, antagonist, inverse agonist at that target site and are those measurable endpoints in planned animal safety studies [2]. After careful evaluation, if the data suggest that the functional relevance of binding to an off-target site would be evident in one of the *in vivo* safety studies, the team might decide to progress the compound through these assays and if no unwanted effects are observed, the compound is progressed into development.

LO safety assessment studies

The goal of LO safety assessment studies, also referred to as exploratory and/or investigative discovery toxicology, is to provide the toxicology data to support the selection of the 'strongest' discovery candidate for development with the best overall opportunity of achieving marketing authorization [20,21]. Up to this point, most of the preclinical safety studies reviewed did not include detailed antemortem properties (e.g. clinical observations, food consumption, and body weight) or anatomic and clinical pathology at doses extending into a toxic range. The value of adding safety assessment studies before a candidate is nominated for early development is to identify the potential safety liabilities, on and off-target toxicities, and target organs of toxicity that might be evident in either single- or multiple-dose toxicology studies. In addition, the LO team might be able to identify or develop safety biomarkers that can help monitor the safety liability in development [21,22]. Furthermore, the multiple-dose toxicology studies often aid and help justify the selection of doses for the GLP toxicology studies. In addition, identification of the toxicity profile of a lead compound can be useful for the backup program where the goal is often an improved safety margin. The design shall also provide possible valuable data about potential enzyme induction, test article exposure over 7–14 days of dosing, and toxicogenomics of selected organs (e.g. liver and kidney) [2]. Although the strategy is intended to mitigate the risk of failure in development, it is important to note that toxicology studies of 7–14 days duration cannot completely exclude those toxicities which appear following longer periods of exposure to the test substance (e.g. slow developing chronic degenerative conditions, hyperplastic responses and carcinogenic effects). A typical roster of routine LO safety assessment studies is tabulated in Box 1.

BOX 1

Routine LO safety assessment studies.

Genetic toxicology
- Ancillary and/or safety pharmacology
- Exploratory mutagenicity assay (Ames test)
- Exploratory clastogenicity assay (*in vitro* or *in vivo* evaluation)

Ancillary and/or safety pharmacology
- *In vitro* dofetilide binding, Rb efflux, or automated patch clamp
- *In vitro* whole cell patch clamp
- *In vivo* cardiovascular study (hemodynamic and electrophysiology)

Exploratory general toxicology
- 5–14-day rodent toxicology study
- 1–14-day nonrodent toxicology study

Nonroutine LO safety assessment studies

Depending on the therapeutic target and the perceived safety liabilities, LO teams might want to modify the design of the routine safety assessment studies to include specialized biomarkers or assays to help de-risk their LO candidate. In some instances, nonroutine LO toxicology studies are added to better define the specific safety issues or better understand the safety implications. Furthermore, LO teams would hope that *in vitro* assays are available to address these special concerns because of the limited API available and the number of candidates available for screening. These investigative assays can be varied and might involve any specialized field of safety assessment (e.g. immunotoxicology and developmental whole embryo cultures). However, LO teams might need to consider complex, multiple-dose toxicology studies that usually vary from two to four weeks.

In general, the LO safety strategy is contingent upon and varies depending on the availability of resources and the level of risk accepted by an organization. Furthermore, the resource and risk burden might vary depending on other related factors such as the therapeutic class and clinical indication, level and timing of competition, and whether or not the target is a novel entity or back up candidate. In many pharmaceutical companies, development candidates are selected based on data from studies in rodents, primarily rats. Subsequent to candidate nomination, testing in nonrodents proceeds as soon as sufficient test substance is available. However, in those cases where there is a cause for concern about advancing a molecule into development, resources are mobilized to prepare sufficient amounts of the active pharmaceutical ingredient to conduct the large animal exploratory safety studies before candidate recommendation for early development. Given the commitment of resources and manufacturing time needed to prepare test substance for early development, the great effort to synthesize drug substance is justified to have all of the data necessary to make an informed decision of whether to progress a potential development candidate.

It is important to stress the close collaboration and coordination required across various functional areas of the discovery team to ensure optimal timing and quality of the data generated from the safety assessment studies. Pharmaceutical sciences will identify

and evaluate API solid state forms and formulations that are acceptable for safety studies and provide data supporting a reasonable period of stability. As suggested earlier, both these parameters can change as a compound progresses.

A common LO failure that highlights the importance of LO collaboration includes resolution of poor systemic exposure following oral administration in the GLP toxicology studies. Poor systemic exposure can be the result of species differences, metabolism issues, poor oral absorption in addition to changes in the final form of the drug substance or the formulation. All too often, LO teams use an unoptimized amorphous drug phase and/or preclinical formulation. This early phase and formulation often gives good oral exposure that cannot be duplicated with the optimized phase and formulation used for GLP regulatory toxicology studies downstream [23]. Close collaboration between chemists, pharmcokineticists, safety colleagues, and pharmaceutical formulators is required to ensure a suitable phase and is available for the regulatory GLP toxicology studies. This example highlights the importance of building robust exposure margins and the regulatory implications discussed below.

Exposure margins generated in safety assessment and current ICH guidelines

During LO it is important to confirm that the compound and the associated formulation will provide adequate systemic exposure to support the Phase I clinical trials. Often the most crucial element of this evaluation is support and selection of the high doses for the early GLP regulatory toxicology studies. To this end, LO teams frequently attempt to define the high-dose limit for the toxicology species (rodent and nonrodent) if there is adequate PK profiling, formulation development and API available. According to the M3(R2) International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals, limit doses and/or exposure are defined by (i) a maximum tolerated dose (MTD), (ii) large exposure multiples, (iii) saturation of exposure, or (iv) use of a maximum feasible dose (MFD) (ICH Topic M3 (R2) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals [24].

Based on the M3 (R2) guidance, the upper limit dose for acute, subchronic, and chronic toxicity studies can be 1000 mg/kg for both rodents and nonrodents except when a dose of 1000 mg/kg does not achieve a mean exposure multiple (exposure generally refers to mean AUC) of tenfold the anticipated clinical exposure and the clinical dose exceeds 1 g per day. If the clinical dose exceeds 1 g per day or the mean exposure is less than tenfold, the upper limit dose for the toxicology studies might be limited to a tenfold exposure margin, or a dose of 2000 mg/kg, or the MFD, whichever is lower. In the rare cases when a limit dose of 2000 mg/kg results in an exposure that is less than the anticipated clinical exposure, a higher dose up the MFD should be considered. A workflow that links formulation activities in relation to desired exposure margins is discussed below.

Given the early LO timing, it is often difficult to predict with certainty the anticipated clinical dose in addition to a tenfold exposure multiple. As such, LO nonclinical toxicity studies frequently try to achieve a MTD, dose-limiting target organ toxicity or dose animals to achieve a 50-fold margin of exposure for the pharmacologically active compound. When calculating the 50-fold exposure margin, the high dose in the toxicity studies should be selected to produce a 50-fold exposure margin (exposure margins usually calculated using the group and/or cohort mean AUC values) over the anticipated clinical exposure at the highest dose proposed for Phase II and III studies [25]. The 50-fold exposure multiple is important because Phase III clinical trials in the USA requires that dose-limiting toxicity should be identified in at least one of the toxicology species when using the 50-fold exposure margin as the limit dose. Exploring the limit dose during LO enables the discovery team to avoid timely exposure and formulation delays and/or issues during early development.

A survey of simple and enabled preclinical formulations

The continuum of preclinical formulation options

Before we delve into the realm of preclinical formulations, we should note that we have observed that it is preferable that a suitable API phase is identified and its fundamental solubility and intrinsic physicochemical properties understood before formulations are pursued. This strategy is recommended since the state properties of a compound frequently impact the formulation, which will not be robust if those properties are a moving target. We have previously reviewed this topic in part [23], and it will not be discussed further here except for occasional highlights in relation to formulation performance and robustness.

For compounds that are likely to be soluble in the gastrointestinal milieu at the required oral dose, simple methocel solutions and/or suspensions are usually adequate for driving the required oral absorption. For poorly soluble compounds, various enabling formulation options (ranging from simple to heroic) can be engaged for enhancing oral absorption [26] and several early formulation guidance or decision trees have been reported [27,28]. One way to envision some broad formulation options and their relationship with oral absorption as the formulation and/or API moves along the gastrointestinal (GI) tract is shown in Fig. 1. Before one embarks on an enabled formulation quest, a simple expression (Fig. 1) for calculating the 'maximum absorbable dose' (MAD) of a compound can be employed to gain an understanding of the oral formulation challenges with a particular compound. MAD is described elsewhere [29,30], and will not be discussed further here except to note that it is a simple approach enabling one to estimate the amount of an API that can be absorbed from the GI tract, using the solubility and/or permeability of a compound along with GI fluid volume and transit time. As formulations are pursued, MAD is a good parameter to take into consideration to set expectations on oral absorption targets.

When a suspension is used, micronization (i.e. reduced particle size to $\leq$20 (m median range) is a relatively simple and well established means of increasing dissolution rate and reducing absorption variability, and there are a variety of milling options available at mg-scale. As the particle size is further reduced, nanoparticles ('nanos', particle size below 1 μm) come into play for maximizing dissolution rate [31]. Small-scale methods for making nanoparticles, such as media milling and high-pressure homogenization are now common, and formulations can be made in quantities to support discovery LO through GLP toxicology studies. Generally, the addition of a surfactant stabilizer is required to keep the nanoparticles from agglomerating. Regardless of particle size, it is worth noting that suspension formulations should be characterized at least by light microscopy, so as to have some understanding of what was dosed.

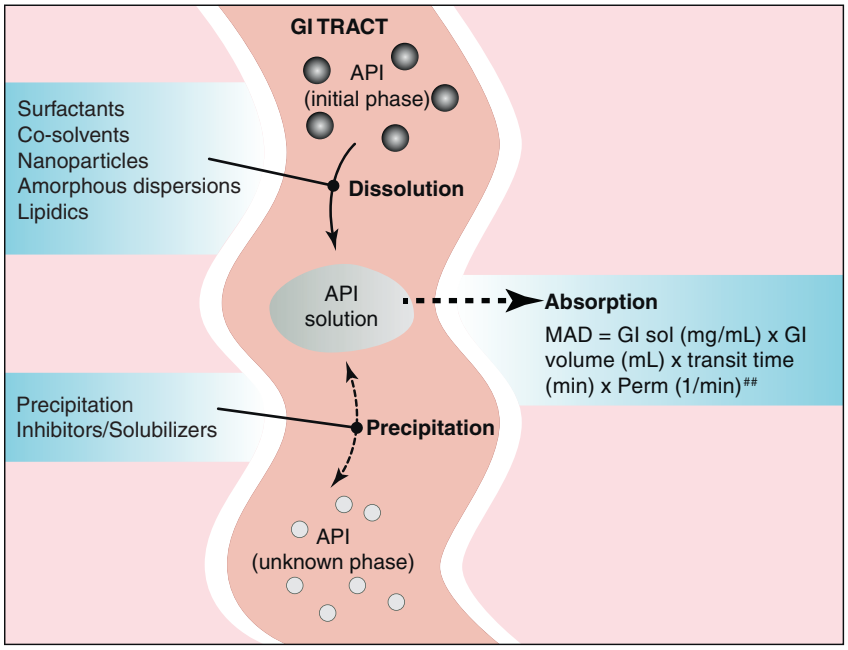

FIGURE 1

API disposition along the GI tract. Some general formulation types and an expression for predicting dose absorbed based on solubility and physiologic parameters. *Abbreviations*: API, active pharmaceutical ingredient; GI, gastrointestinal; MAD, maximum absorbable dose; Sol, solubility in simulated GI fluid; Perm, permeability in $CaCo_2$ cells.

Although a simple solution or suspension formulation is always preferred, poor solubility or the need for increased exposure often requires us to employ more aggressive formulation methods. There is a wealth of information available on the use of various enabling excipients, such as surfactants, co-solvents, or lipid and/or emulsion formulations [32,33], which all can be effective for enhancing the absorption of insoluble compounds. It is worth noting that although significant improvement in oral exposure can be gained through the use of enabled formulations, such measures cannot fix poor intrinsic API properties, such as low cell membrane permeability (often seen initially through low permeability in the *in vitro* $CaCo_2$ cell model) or high *in vivo* clearance rates due to extensive metabolism. An example of the use of a small *in vivo* enabled formulation screen for enhancing exposure of an insoluble compound is shown in Fig. 2. An observation here was that powerful solubilizing excipients, such as surfactants and coslovents and SMEDDS self emulsifying drug delivery systems (SMEDDS) provided little increase in aqueous

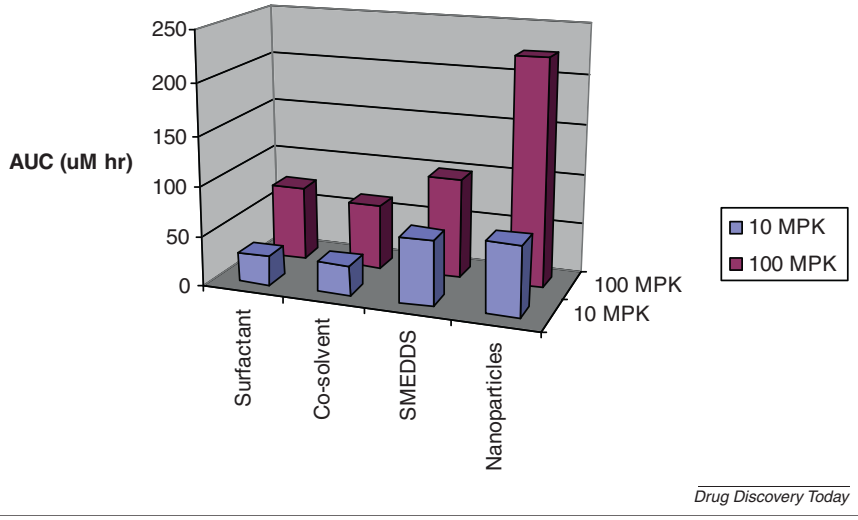

FIGURE 2

Oral exposure in rats for an insoluble compound (solubility <1 mg/mL in all vehicles) using various enabled formulations [male Wistar rats, 10 and 100 mgs/Kg (MPK)]. *Abbreviations*: AUC, area under curve; SMEDDS, self emulsifying drug delivery system.

solubility [34] and no enhancement in oral exposure, whereas a nanoparticle was effective in driving exposure at the higher dose.

A well known caveat should be addressed when concentrated solution formulations that contain significant amounts of a solubilizing excipient are used. This is the likelihood that the API will 'crash' out of solution once the formulation enters GI tract milieu. This is not always insurmountable, since the compound might precipitate as a reasonably soluble form, such as an amorphous phase or nanoparticles. Regardless, the potential impact of this precipitation process can be evaluated by *in vitro* means, where the formulation is sequentially diluted into simulated gastric fluid followed by a second dilution into simulated intestinal fluid [34]. After removal of any precipitates, assay of the final concentration of the supernatant solution enables for a formulation series to be rank ordered for solubility enhancement (or precipitation resistance) in the GI tract.

When all other methods fail, amorphous drug phases (the most soluble solid state form) can be used for preclinical formulations. Since amorphous drug phases are prone to unexpected conversion to less soluble crystalline phases, it is generally preferred to disperse the amorphous drug into an amorphous polymer (i.e. a 'solid solution' dispersed in a cellulosic polymer) to prevent crystallization [35]. The practical methods preparing amorphous drug-polymer dispersions are spray drying or hot melt extrusion. Although it is fairly common in academic and development settings, the routine use of amorphous drug dispersions in a discovery setting is less mature, partly owing to the relatively large quantities of drug required to make the dispersion. As the preparative methods increase in efficiency (and decrease to mg scale), amorphous dispersions are now finding increased utility well before entry into development.

API stability in formulations

Once a suitable preclinical formulation is identified, the chemical and physical stability of the API in the formulation must be well understood and carefully monitored. Although the API chemical stability requirement is obvious, solid state phase changes often are more subtle but can be just as deleterious to the performance of the formulation. This concern is frequently born out when over time the initial drug phase converts to a new less soluble polymorph over time, resulting in a drop in oral absorption. Ultimately, this scenario supports the strategy of performing at least some initial polymorph screening before any significant preclinical safety studies start, to build confidence that the most stable API phase is in hand and adequate exposure can be maintained.

Unique elements of toxicology formulations

Definition of a robust vehicle that can provide the uniquely high exposure levels required to support a toxicology program often is the most challenging part of the preclinical formulation search. It is important to note that there are fewer formulation options for toxicology studies as compared with efficacy or PK studies, owing to the concern that the presence of some excipients might confound the toxicity profile of a drug. In this regard, there are several useful reports on the excipients that are widely accepted for use in toxicology studies and the associated dose limitations [36].

Finally, it should be recognized that the intended route of administration directly impacts the choice of formulation excipients. While there is significant overlap with some excipients [i.e. PEG is useful for oral, intravenous (IV) and IP routes] the excipient selected for a particular formulation must be tolerated when administered by a given route. The various limitations are fairly well known and formulations for each administration route have been described in several recent reports [37].

Some general principles for a robust API phase and preclinical oral formulation are listed below.

(i) When achievable, a physically and chemically stable crystalline drug form is preferred [23].

(ii) The formulation (including the API phase present) must be physically and chemically stable for the time duration of dosing ($\geq$4 hours).

(iii) API salts should be carefully selected based on acceptability for utilization in toxicology and downstream human clinical studies. Care should also be applied in evaluating the amounts of any counterions used [38]. It is often desirable to administer salts *in situ* as opposed to a solid-state counterion.

(iv) pH adjustment can be used, but low ($\leq$2) or high ($\geq$9) extremes should be avoided so as to not cause any deleterious local *in vivo* effects. pH adjustment with nonstandard acids and/or bases also needs to be scrutinized carefully.

A phased formulation approach to achieving required exposure multiples

As described previously, the demonstration of either a dose-limiting toxicity (DLT) or a 50× exposure multiple *versus* the target efficacious exposure level is becoming a regulatory expectation. However, there is scant evidence in the literature and in practice for correlation of exposure multiples obtained and observed DLT. In other words, one is just as likely to observe a DLT at an exposure multiple of 10× as one is to observe a DLT at 50×. Hence, a phased approach to formulation investment as shown in Fig. 3 is one strategy that could be used to mitigate unnecessary investment in formulation and exploratory PK studies in an effort to obtain 50× exposure multiples when a much lower exposure margin is sufficient.

In this approach, conventional formulation options could be evaluated in exploratory toxicity studies to understand whether more modest exposure multiples are sufficient to trigger a DLT response. If DLT is achieved with a conventional formulation, irrespective of multiple, no additional formulation work is required. If DLT is not achieved at less than or equal to the maximum feasible dose of a conventional formulation, enabling formulations can then be used to escalate exposure multiples to either drive to a DLT or to obtain as close as possible to the 50× exposure multiple target. Of course, if the latter target is not achieved with enabling formulations, these efforts constitute the requisite due diligence required by regulatory authorities, since additional oral formulation work will be highly unlikely to exceed these enabled formulation approaches. Lack of achieving a DLT or 50× exposure multiple does not necessarily trigger the end of the road for a development compound, but rather might prompt a different strategy to test the molecule in the clinic or

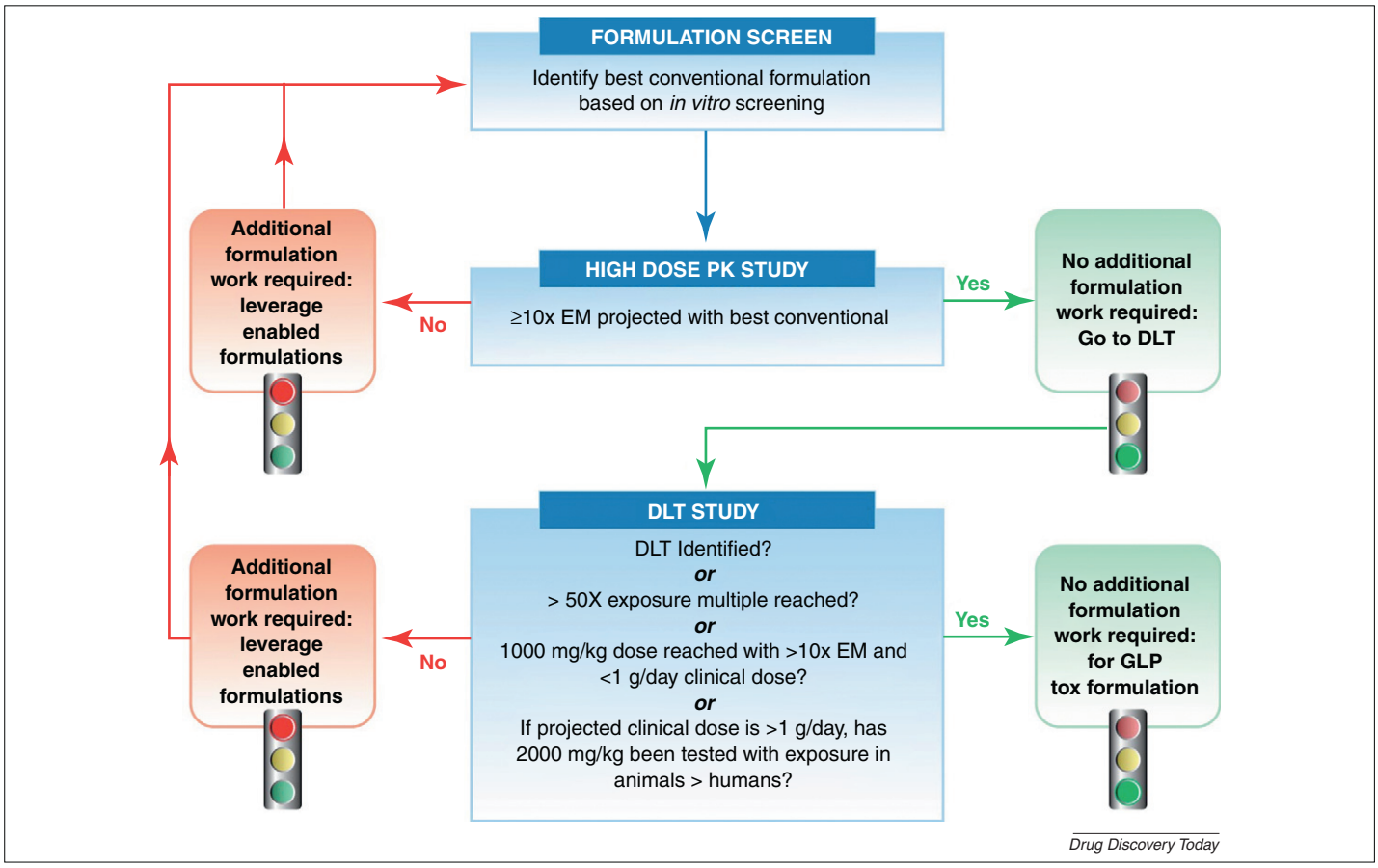

FIGURE 3

A phased approach for *in vitro*/*vivo* studies to identify appropriate toxicology formulations and the associated EM. *Abbreviations*: DLT, dose-limiting toxicity; EM, exposure margins; GLP, good laboratory practices; PK, pharmacokinetics.

discussions with regulatory authorities prior to proceeding with planned studies.

Concluding remarks

In this article, we have highlighted how early attention to preclinical safety assessment and associated formulations to drive exposure can benefit drug research programs in both the discovery and development stages. The strategies discussed represent some of the many different approaches and studies that can be applied, starting upon the identification of a preclinical drug and enabling rapid and confident progression into human clinical trials. Although early metrics on reducing drug candidate attrition support this approach, it is worth noting that this strategy comes at cost, because significant time and resources from several areas spanning the discovery-development continuum must be engaged to execute it.

References

1 MacCoss, M. and Baillie, T.A. (2004) Organic chemistry in drug discovery. *Science* 303, 1810–1813

2 Bass, A.S. *et al.* (2009) Exploratory drug safety: a discovery strategy to reduce attrition in development. *J. Phamacol. Toxicol. Methods* 60, 69–78

3 Bass, A. *et al.* (2004) Origins, practices and future of safety pharmacology. *J. Pharmacol. Toxicol. Methods* 49, 145–151

4 Lindgren, S. *et al.* (2008) Benchmarking safety pharmacology regulatory packages and best practice. *J. Pharmacol. Toxicol. Methods* 58, 99–109

5 Bass, A.S. *et al.* (2008) International life sciences institute (health and environmental sciences institute, HESI) initiative on moving towards better predictors of drug-induced torsades de pointes. *Br. J. Pharmacol.* 154, 1491–1501

6 Boyd, R.A. and Lalonde, R.L. (2007) Nontraditional approaches to first-in-human studies to increase efficiency of drug development, will microdose studies make a significant impact? *Clin. Pharmacol. Ther.* 81, 24–26

7 Gintant, G.A. (2008) Preclinical Torsades-de-Pointes screens: advantages and limitations of surrogate and direct approaches in evaluating proarrhythmic risk. *Pharmacol. Ther.* 119, 199–209

8 Hamlin, R.L. (2007) Animal models of ventricular arrhythmias. *Pharmacol. Ther.* 113, 276–295

9 Wymore, R.S. (1997) Tissue and species distribution of mRNA for the Ikr-like K Channel, erg. *Circ. Res.* 80, 261–268

10 Redfern, W.S. *et al.* (2003) Relationship between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsades de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* 58, 32–45

11 Irwin, S. (1968) Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* 13, 222–257

12 Mattson, J.L. *et al.* (1996) A performance standard for clinical and functional observational battery examination of rats. *J. Am. Coll. Toxicol.* 15, 239–250

13 Kinter, L.B. (2003) Safety pharmacology of the renal and gastro-intestinal systems. In *Safety Pharmacology: A Practical Guide* (Williams, P.D. and Bass, A.S., eds), pp. 99–116, TherImmune Research, Gaithersburg, MD

14 Diehl, M.S. (2000) Comparison of the results of a modified miniscreen and the standard bacterial reverse mutation assays. *Environ. Mol. Mutagen* 36, 72–77

15 Chen, M.H. (2008) Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics. *Circulation* 118, 84–95

16 Lu, N.Z. *et al.* (2006) International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacol. Rev.* 58, 782–797

17 Foord, S.M. *et al.* (2011) International Union of Pharmacology. XLVI. G protein-coupled receptor list. *Pharmacol. Rev.* 57, 279–288

18 Clapham, D.E. (2003) International Union of Pharmacology. XLIII. Compendium of voltage-gated ion channels: transient receptor potential channels. *Pharmacol. Rev.* 55, 591–596

19 Hofmann, F. *et al.* (2003) International Union of Pharmacology. XLII. Compendium of voltage-gated ion channels: cyclic nucleotide-modulated channels. *Pharmacol. Rev.* 55, 587–589

20 Fielden, M.R. and Kolaja, K.L. (2008) The role of early *in vivo* toxicity testing in drug discovery toxicology. *Expert Opin. Drug Saf.* 7, 107–110

21 Car, B.D. and Robertson, R.T. (1999) Commentary: discovery toxicology – a nascent science. *Toxicol. Pathol.* 27, 481–483

22 Kola, I. and Hazuda, D. (2005) Innovation and greater probability of success in drug discovery and development – from target to biomarkers. *Curr. Opin. Biotechnol.* 16, 644–646

23 Palucki, M. *et al.* (2010) Strategies at the interface of drug discovery and development: early optimization of the solid state: phase and preclinical toxicology formulation for potential drug candidates. *J. Med. Chem.* 53, 5897–5905

24 ICH Topic M3 (R2) (2009) *Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization of Pharmaceuticals*

25 M3 R2 Implementation Working Group on the M# R2 Guideline (2011) *Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorizationfor Pharmaceuticals; Questions and Answers*

26 Rosen, H. and Abrigat, T. (2005) The rise and rise of drug delivery. *Nat. Rev. Drug Discov.* 4, 381–385

27 Chaubal, M.V. (2004) Application of formulation technologies in lead candidate selection and optimization. *Drug Discov. Today* 9, 603–609

28 Mass, J. *et al.* (2007) An Integrated early formulation strategy – from Hit evaluation to preclinical candidate profiling. *Eur. J. Pharm. Biopham.* 66, 1–10

29 Curatolo, W. (1998) Physical chemical properties of oral drug candidates in the discovery and exploratory development settings. *Pharm. Sci. Technol. Today* 1, 387–393

30 Sun, D. *et al.* (2004) *In vitro* testing of drug absorption for drug 'developability' assessment: forming an interface between *in vitro* preclinical data and clinical outcomes. *Curr. Opin. Drug Discov. Dev.* 7, 75–85

31 Merisko-Liversidge, E. *et al.* (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci.* 18, 113–120

32 Porter, C.J. (2007) Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov.* 6, 231–248

33 Kommuru, T.R. (2001) Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *Int. J. Pharm.* 212, 233–246

34 Dressman, J. and Reppas, C. (2000) *In vitro-in vivo* correlations for lipophilic, poorly water soluble drugs. *Eur. J. Pharm. Sci.* 11, S73–S80

35 Hancock, B. and Zografi, G. (1997) Characteristics and significance of the amorphous state in pharmaceutical systems. *J. Pharm. Sci.* 86, 1–12

36 Neervannan, S. (2006) Preclinical formulations for discovery and toxicology: physicochemical challenges. *Expert Opin. Drug Metab. Toxicol.* 2, 715–731

37 Gad, S.C. (2006) Nonclinical vehicle use in studies by multiple routes in multiple species. *Int. J. Toxicol.* 25, 449–521

38 Stahl, H. and Wermuth, C. (2002) *Handbook of Pharmaceutical Salts*. Wiley