4 Effective Early Drug Development

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4.1 INTRODUCTION

The process by which a new chemical entity (NCE) results in a new medical entity (NME) that, when administered to patients, results in a successful therapeutic intervention that improves the patients' quality of life is a long and costly process. This has remained so in spite of advances in all of the sciences that are contributors to and constitute the process. Even with the result of a greatly increased number of NMEs with promising pharmacological properties being routinely identified, the challenge remains: which molecule will be predictive of efficacy and safety in humans and what is the process that will support these outcomes?

In drug development, each pharmaceutical company has its own system, which (probably) is a reflection of previous successes. Although when "all is said and done," drug candidates become drugs by going through similar stepwise regulatory-driven process that demonstrates safety and efficacy. In this context, a faster and less costly process as a definition of "efficient drug development" is highly unlikely. This does not preclude that within the process, the attainment of conclusive Go/No-Go milestones can be attained effectively (efficiently) and thus result in an efficient process overall. In

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this context, the addition of effective drug development should also be used in defining drug development.

Considering the number and diversity of molecules for potential development, some with multiple activities with active indication profiles, an all encompassing treatment of drug development is beyond the scope of this chapter. Thus, we have not attempted to be all inclusive or exhaustive in terms of a drug class or indication or to compile a drug development study checklist with the intent of "one size fits all." Instead, we have attempted to present in outline concepts that define aspects/landmarks of early nonclinical and clinical development, as these concepts contribute to the traditional path to facilitate the first developmental outcome desired: an open investigative new drug (IND).

In one respect, drug development can be considered as a single path with a single outcome. However, it is not a line-driven process. Once started, the path forward quickly branches into the primary disciplines that will support safety and efficacy, which in turn develop subbranches to form a mosaic(s) to constitute total development as defined in the new drug application (NDA). Every path has to have a beginning: the path forward for a formal drug development is the IND. It should be stated that the IND, once opened, precedes the conduct of the multidisciplinary studies that continue mostly by paths that validate drug safety and efficacy. Once the initial studies are supportive of further development, continuation of full panels of studies are conducted on the way to the NDA. All of the studies that will define the content of the NDA are not addressed here in detail; however, for continuity of the development process, descriptions of IND-targeted studies that are germane to further development are presented. This theme will be defined and expanded further in some detail and is introduced here.

In early drug development, nonclinical studies are conducted in two to three species to determine a dose–toxicological profile in relationship to the proposed human dose (animal to human safety margins) in the first clinical trial(s), that is, phase 1.

It is important to state that phase 1 (and phase 2a) studies are the *learning phases* of drug development and, if appropriately coordinated and conducted, provide information that increases the efficiency/effectiveness for the rest of the drug development process [1]. In addition, phase 1 provides decision-making data and information for labeling. Some examples of such coordination and impacts on decisions will be presented later. In this context, the pivotal phase 3 studies (and phase 2) of drug development are *confirmatory studies* that define the safe and effective use of the drug in the targeted patient population. Careful consideration for the selection, design, and timing of phase 1 trials provide a solid foundation for the conduct of one or two proof of safety and efficacy studies in phase 3. The final approval process as entailed in phase 3 is outside the scope of the present report.

In addition, recent use of techniques such as biomarkers and surrogate markers [2–4], pharmacokinetic/pharmacodynamic (PK/PD) correlations [5], and microdosing [6], all of which can increase the understanding of the drug PK/PD properties in both early and later stages of development, are outside of the scope of this present report. These are mentioned as they are utilized in current drug development. The use of biomarkers and surrogate markers to define/predict clinical outcomes is an important consideration to an efficient drug development process [7]. As biomarkers and surrogate markers are developed, it must be kept in mind that the time to think about their selection should be early in the drug discovery and development process. Thus, a linkage(s) to clinical outcomes will transition the marker into a surrogate end point

and have a prospective value as the surrogate will predict an outcome. An essential component in the selection of biomarkers and surrogate markers is their validation. In this context, information must not be confused with knowledge and reliability with validity. The criteria for validity should be concurrent in that supporting evidence is obtained at the same time, predictive in that the evidence is also obtained at a later time, and prescriptive by providing the appropriate treatment.

More recently, the use of genotyping/phenotyping to provide information for predicting drug response and variation that are currently used with increasing regularity are also mentioned only in passing. These ultimately do contribute to the overall aspects of development, especially as polytherapy is the norm in today's aging population to provide effective patient management and contain the promise of individual therapy [8–12], but at present, this remains in the future.

To provide an adequate bibliography is prohibitive; thus, an abbreviated list of reference citations is provided for additional and in more depth insights for the process of drug development. Considering that drug development is essentially a regulatory process, FDA guidance documents are also included where applicable to provide a regulatory framework for the concepts presented.

4.2 OVERVIEW OF DRUG DEVELOPMENT

The basis for the marketing approval of a NME is safety and efficacy. To this end, the *road map* to develop a drug is both defined and regulated by FDA and ICH (International Committee for Harmonization) guidances. However, a road map primarily gives us the destination. To efficiently navigate, the road map requires both a plan and *road markers* that can ascertain progress as one moves forward. These road markers are studies and, even more importantly, data that are *needed* (not what can be generated) for Go/No-Go decisions. Even with these guides, there are few specifics provided that are "Not to Do". However, the specifics of what to do, how to do it, and how to evaluate the validity of the results obtained during the drug development process remains time consuming and costly. This is true in spite of the very large number (e.g., 100,000) of new molecules that are synthesized yearly, the availability of high throughput screening for determining pharmacological activity, validated *in vitro* and *in vivo* disease models, the use of genomics and proteonomics, and other new and innovative technologies to expedite drug discovery and development. The expectation for new and numerous drugs available for therapeutic use have not been met, and in fact, there is a decline in the new drug discovery/development pipeline.

When all is said and done, only 1–2 of the 100,000 molecules actually reach the market for therapeutic use after a development time of 7–12 years (or more) and costs that can exceed $700 million before an NDA is submitted to the FDA and filed for their review and evaluation. The review of the NDA adds additional time of 10–18 months until marketing approval. A goal during development is to obtain Go/No-Go milestones as quickly as possible to maintain the entry of drug dossiers for review by the regulatory agency. An outline of the development process (road map) is shown in Fig. 4.1 in which drug development is outlined in distinct phases. In addition, guidance documents issued by the FDA are available, which address specific studies/aspects of development, provide a wealth of specific information [13], and provide efficiency to a development process by resulting in an effective drug development.

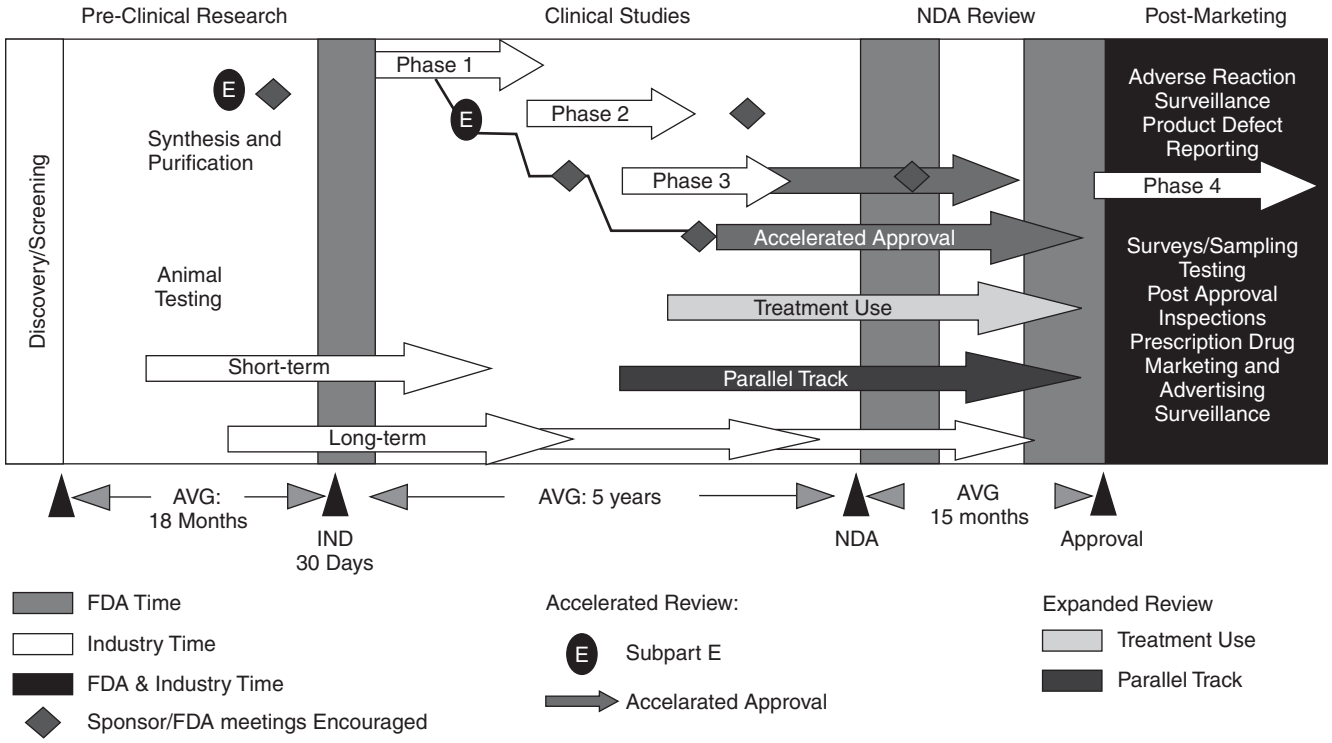

Figure 4.1 New drug development process.

Figure 4.1 outlines the distinct phases of drug development: discovery, preclinical studies (pharmacology and toxicology/safety, which lead to an open IND, enabling the first study(ies) in humans), the phase 1 studies (clinical pharmacology), followed by the phases 2 and 3 clinical studies that demonstrate and confirm safety and efficacy and thus provide the basis for marketing approval.

Discovery/screening studies are not included herein, as these are drug/indication specific and as such beyond the scope of the present report. Needless to say, those type of studies are carried out in order to provide a rationale for the development of the lead candidate(s). Usually, a relatively small number of such studies are conducted; the positive results from which support a rationale for further development. It is not uncommon that four to five animal pharmacology studies suffice, two or three of which hit the desired end point. Such an approach is alluded to in the *Exploratory IND Studies* guidance document [14] issued by the FDA as one of the steps to facilitate the entry into the ultimate model: humans. The phase 2 and pivotal phase 3 studies are also not addressed; the emphasis is on the traditional segment of drug development that is representative of what constitutes learning to understand the drug and thus allows development to move forward into the confirmatory aspects of drug development (phase 2b and phase 3) on which marketing approval is ultimately based.

The concepts that will be described are for the development of an oral drug, as this is the most desired commercially, and perhaps the most prevalent, route of administration from several perspectives. However, the development of a drug with vascular (or topical) administration would follow the same path (type of studies) to establish the safety profile of the intended route of administration for the management of patients.

4.3 REGULATORY CONSIDERATIONS

Drug development is subject to numerous the FDA and ICH guidances and regulations [Good Laboratory Practices (GLPs), Good Clinical Practices (GCPs), etc.]. These provide a wealth of information on both specific and general issues that contribute to any drug development process.

The need for more *better and cheaper* drugs is a widely used phrase. At a relatively recent national meeting, the FDA shared their concerns to this end by presenting data showing that new compounds entering phase 1 today have an 8% chance of reaching the market (vs. 14% chance 15 years ago); the failure rate in phase 3 is 50% (vs. 20% 10 years ago). In this context, the FDA is actively pursuing a critical path initiative (CPI) [15] in the attempt of modernizing the techniques and methods used to evaluate safety, efficacy, and quality of drugs as they move through the development and approval processes. The three areas of the CPI are (i) a new approach to clinical trials in terms of early proof of concept (POC) studies, computer modeling, microdose studies, and new and innovative methods of data analysis; (ii) the FDA will issue a guidance on the validation of biomarkers and surrogate markers; and (iii) the use of modeling and simulations. It is hoped that these will expedite the entry of drugs into the marketplace.

4.4 IND-ENABLING DEVELOPMENT

Drug development has its beginnings in pre-IND as the following factors are considered:

- understanding the disease process and therapeutic approach;
- determining the mechanism of action and establishing appropriate models;
- identifying and validating clinical safety and, if possible, efficacy markers for decision making;
- conducting *in vitro* metabolism screens using toxicology species and human liver microsomes to identify the cytochrome P450 (CYP450) isozymes involved;
- developing the appropriate toxicology–safety program (see ICH guidance).

At some time during the above development, it is advantageous to write a draft label for the indication sought, as this will provide focus to all aspects of drug development as it moves forward. It is also important to address the manufacturing of the drug substance and set the impurity limits/specifications and prepare the clinical supplies for the first human studies. At this stage, it is not necessary to have a final galenic formulation for the initial clinical studies, as these first such studies can easily be conducted with simple hand-packed capsules. It is given that the galenic formulation is ongoing for formal drug development. Also, in parallel, the impurity profile—qualitative and quantitative—is determined. This is important to ascertain that the drug substance used in the animal toxicity/safety studies that were conducted to open the IND did not have an impurity profile that exceeds that of the drug substance that is to be used in the initial clinical trials.

Using the FDA-recommended Exploratory IND, as mentioned previously, is an efficient and effective roadmap to an acceptable IND, in that a Go/No-Go decision can be reached relatively quickly and may include a pivotal first study in the ultimate model: humans. It is worth repeating that 15% of drug development programs fail at phase 1. In general, the Exploratory IND consists of a panel of general *in vitro* and *in vivo* safety pharmacology, toxicology/safety studies in two animal species, with the duration of such studies linked to the duration of the proposed post-IND trial. Safety pharmacology/toxicology (cardiac safety including a QTc prolongation in the telemetry dog), genotoxicity, and ADME (absorption, distribution, metabolism, and excretion) studies are normally required for the Exploratory IND before dosing in humans.

In this context, the current regulatory environment encourages INDs with minimum content, that is, pharmacology to support a rationale for drug development and appropriate animal toxicity–safety studies that allow development to move forward into the first studies in humans (exploratory INDs). Thus minimum as describing the requirement for the IND are a misnomer; the studies provide key nonclinical information for conduct of a human PK study. However, there are no shortcuts, and if development is to move forward, additional toxicology studies will be conducted at higher doses and with increased duration.

There is always value in stating (or restating) what has been (and will remain) the foundation of drug development: the IND. The IND consists of the following sections:

- Form FDA 1571
- Table of contents

- Introductory statement
  - List of references
- General investigational plan
  - Clinical development plan
  - Planned exposure
- Investigator's brochure (IB)
- Protocols
- Chemistry, manufacturing, and controls (CMC)
- Nonclinical pharmacology and toxicology
- Previous human experience
- Additional information.

It is important to have a clear objective(s) for the first human studies (phase 1) as this will have a direct impact on the nonclinical pharmacology and toxicology studies that will precede it. In this context, it is of value to utilize the FDA guidance document titled Exploratory IND Studies, which provides concepts that facilitate an early entry into clinical studies by focusing on the core nonclinical studies required. It should be stated, however, that should development move beyond the first phase 1 study and into formal development, it will be necessary to go back and conduct the studies that constitute a traditional IND/NDA.

4.5 ANIMALS TO HUMANS

Once a candidate for development has been chosen, the first goal is to obtain an open IND, thus allowing the first study in humans. As was outlined above, the dossier for the IND is started by conducting a series of nonclinical toxicology/safety studies that includes at least two animal species, usually rat and dog, that emerge as the primary species for the determination of a toxicologic and safety profile projected for human exposure.

Throughout the whole process (IND to NDA), measuring drug concentrations to determine the extent and duration of systemic exposure to the drug provides windows of understanding of both safety and efficacy (or the lack thereof). This is true of nonclinical toxicity/safety [toxicokinetics (TK)] and clinical studies (PK). The fundamental difference in animal studies and human studies can be outlined as follows:

Animals	Humans
Once/day dosing; defined regimen	qd, bid, tid, qid; widely varying regimens
Wide range of doses/dose strengths	Single, two to three strengths with narrow range
CMC suspensions, dietary admix (rats); neat drug in capsules (dog)	Elegant galenic formulation
Species differences	Ethnic and gender and/or age differences

4.5.1 Bioanalytical Considerations

As was stated above, the systemic exposure to the administered drug and the elucidation of its dispositional parameters is of importance. To this end, it is mandatory to develop and validate the analytical methods that would support both nonclinical and clinical studies. Usually, the method utilized for determining different aspects of the drug substance, foe example, dose verification, purity, and stability is high performance liquid chromatography (HPLC). For determining drug concentrations in animal and human biofluids (blood, plasma, urine, tissues), the usual method(s) is LC/MS-MS (liquid chromatography/mass spectrometry/mass spectrometry) assay(s) for its sensitivity [low limit of quantitation (LOQ)] and specificity for drug (and metabolite). Validated bioanalytical methods for animal studies (TK, ADME) and humans studies characterize the biopharmaceutics (BP) and PK for phase 1/phase 2a clinical pharmacology studies. Thus, such bioanalytical methods are conducted under GLP regulations. The validation of such assays results in stand-alone reports that demonstrate selectivity, accuracy, precision, reproducibility, and recovery [16]. As most biological samples (animal/human) are stored frozen and bundled prior to assay, short- and long-term stabilities are required. The inclusion of BP and PK principles in the conduct and evaluations of animal toxicity/safety studies and GLP toxicity studies is virtually mandated. Using full or sparse sampling profiles over selected time points over the course of toxicology studies, TK provide drug systemic drug concentration data that aids in understanding the findings.

As development moves forward, a validated LC/MS-MS assay for drug and primary metabolite in human plasma (and urine) with a low LOQ should also be developed, the report(s) of which should be included in the IND in support of the conduct of the first phase 1 PK study.

4.5.2 Early Nonclinical Pharmacology and Toxicology

An IND-targeted program will start with nonclinical pharmacology and toxicology to provide the data from which safety assessment can be projected for safe use in the first human studies. The early nonclinical toxicology program to support an open IND is outlined below, although details of study design are not addressed. It should be stated that the success/failure of drug development program can depend on the early nonclinical toxicology/safety studies that provide a rationale/guidance for effective evaluation that provides rational safety margins [17]. Only selected studies are addressed, and specific guidance documents are referenced for additional information. As development continues, the nonclinical toxicology dossier ends up being one of the major portions of the NDA; a full in-depth review is not feasible within this report. However, in the guidance for the format and outline of the toxicology section of the NDA, one can obtain specific design information from the table of contents [18].

Acute toxicity studies [19] are the initial nonclinical studies conducted. These are usually conducted in rats, although a second species may be used. There are two basic study designs: escalating doses in the same animal with a 14-day observation period with incremental doses to ascertain any latent toxicity or escalating doses in parallel groups with a 14-day observation period. Doses are escalated until a clear toxicological outcome is reached. There is no requirement for conducting acute studies under the GLP regulations. However, most Sponsors do conduct these studies under GLP, in that

if a global marketing strategy is contemplated and proceeds forward, some overseas regulatory agencies require that acute toxicity be conducted under GLP regulations. As was stated above, acute toxicity studies are dose escalation studies, the primary objective is not to cause mortality per se but to have a clear concept of the dose–toxicity relationship (food consumption, body weight loss, clinical pathology findings, etc.) as an acute response and/or a delayed response after dose administration. In addition, the acute toxicity response may provide a window for investigating potential human overdose. It should also be mentioned that gross pathology, histopathology, or clinical pathology are not requirements; however, body weight loss, gross pathology, and the addition of key clinical chemistry indicators can be used in establishing a dose at which toxicity begins.

To ascertain, no potential for genotoxicity is a benchmark in early development. The core genotoxicity studies are the Ames test with and without S9 activation, chromosomal aberration (CHO), and *in vitro* mouse micronucleus test [20,21]. If there are no findings indicative of genotoxicity or mutagenicity, no further genotoxicity testing is required. If the genotoxicity results are positive in identifying a signal for mutagenicity, the relationship to dose relative to the first human dose should be carefully examined. It is possible that the program should be stopped as the genotoxic dose is too close to the proposed human dose.

The safety pharmacology studies in Refs 22,23 represent the *in vitro* and *in vivo* panel of nonclinical studies that support cardiac safety during an open IND. These are acute administrations at doses that result in systemic concentrations that are at least 100× the human plasma concentration of the projected clinical dose. These studies are conducted in animal models to identify undesirable PD properties of the NCE that may have a relevance to human safety. The core battery of safety pharmacology trials [22] addresses organs or systems toxicities and includes the following.

1. Effects on the central nervous system are assessed in terms of motor activity, behavior changes, coordination and sensory/moor reflex responses, and learning and memory.
2. Cardiovascular system studies assess cardiac output, ventricular contractility, blood pressure, heart rate, and the ECG (electrocardiography) *in vivo* (telemetry dog) and *in vitro* repolarization and conductance [23]. The effects of *in vitro* hERG potassium channels expressed in human embryonic kidney cells on cardiac ionic currents are assessed. Also, isolated rabbit Purkinje fibers assess the effects on a potential changes in the action potential duration (ADP). These *in vitro* studies are routinely conducted to be complementary to the human study.
3. Respiratory system assessed by measurements of the respiratory rate and function.

The duration of the proposed clinical studies are directly linked to the duration of the animal toxicity studies: two-week studies in two animal species (usually rat and dog) allow two-week studies in humans. It is usually more efficient to conduct four-week toxicity studies at this time, which will allow more flexibility for the first human study(ies). As early nonclinical trials indicate a signal in regard to the relationship to safety and an advantageous PK profile of the drug candidate, animal studies of longer duration (e.g., 4 and 13 weeks in rats and dogs, 6 month in rat, 9 month in dogs, 2-year carcinogenicity in mice and rats, the full panel of reproductive toxicity in rodents) will be conducted in sequence. These tabulated studies are needed to obtain an open IND

and thus allow the conduct of the first human studies (PK/tolerance) to initiate formal drug development [14,18].

In this context, the challenge in the early development program is to set the doses for the first animal toxicity study. It is not recommended to guess at the dose level because of either underdose or overdose and therefore, not allowing one to obtain an accurate picture of the dose–toxicity relationship. The early conduct of the acute toxicity studies (rats and dogs) can provide some information for setting the doses for the two-week toxicity studies, although conducting a 7-day dose range finding study with initial and terminal TK is more informative. The inclusion of TK into the acute toxicity studies is not a requirement; however, TK can focus on the drug plasma concentration relationship and the onset of toxicity.

Today, the inclusion of TK in toxicology studies is assumed [24]. In addition to ascertaining systemic absorption and a level of exposure of the drug in animals, it is also important to understand the systemic disposition of the drug in animals relative to human studies. Thus, the conduct of animal ADME studies is important in this transition. Animal studies with IV and oral dosing can be readily conducted to obtain a metric of bioavailability (BA), area under the curve (AUC), half-life, clearance, and volume of distribution. All of these parameters provide an understanding to the TK determinations that are routinely obtained from the toxicology studies performed.

It is prudent in early development to conduct studies that determine the metabolic profile of the drug using standard protocols and that utilize rat, dog, and human hepatocytes and microsomes (i.e., the metabolic stability of the drug). Drug metabolism studies are required for marketing approval, and metabolites must be identified and their pharmacological activity as well as their toxicological potential must be assessed [25]. In addition, the CYP450 isozymes involved will be identified, including whether the drug induces or inhibits their activity, and based on the collective information obtained, whether there is a potential for a drug–drug interaction in the therapeutic setting of the targeted population. Thus, the early elucidation of the metabolic pathway can be an important consideration to the overall development program.

The determination of the extent of plasma protein binding in rats, dogs, and humans is also important in these early drug dispositional studies. Only the free fraction of a drug is available for metabolism and excretion, pharmacological activity, and binding to tissues. The determination of the extent of protein binding (i.e., the free fraction of drug) over a concentration range that encompasses and exceeds the TK and clinical PK range is conducted in animal and human plasma. Whether binding is concentration dependent/independent can provide insights as to the activity and/or toxicity seen; determining the main carrier proteins of the drug [albumin, alpha-1 acid glycoprotein (AGP)] in the systemic circulation may have a relevance in certain disease states.

A tabulation of a nonclinical program in early development that supports continuing development is provided in the next two tables. The GLP status, the amount of drug needed, and a usual time from start to final report are presented. As development continues, an NDA-targeted toxicology program would include subchronic, reproductive, chronic toxicity studies including *in vivo* carcinogenic studies in two species.

Study	GLP status	Drug needed (g)	Time of start to final report
Analytical/Bioanalytical			
HPLC assay for dose verification	Y	10	One week for development and validation; Four weeks for report
LC/MS-MS for drug concentrations in rat plasma (for TK and PK)	Y	1	Four to six weeks for development and validation
LC/MS-MS for drug concentrations in dog plasma (for TK and PK)	Y	1	Four to six weeks for development and validation
LC/MS-MS for drug concentrations in human plasma	Y	5	Four to six weeks for development and validation
• Acute oral toxicity in rats	Y	50	Three to four months
• Acute oral toxicity in rats with necropsy and clinical path	Y	50	Three to four months
• Acute oral toxicity in dogs	Y	100	Three to four months
• Ames test with/without S9 activation	Y	2	Six weeks
• Chromosomal aberration (CHO test)	Y	5	One month
• *In vivo* mouse micronucleus	Y	10–15	One month

Study	GLP status	Drug needed	Time of start to final report
QT interval prolongation in conscious dog	Y	50 g	Four weeks in life, four weeks for report
In vitro hERG assay	Y	100 mg	Three to four weeks to QA'd draft report
Behavior in rats (Irwin test)	Y	1 g	One week in life, three to four weeks for QA'd draft report
Respiratory effects in rats	Y	1 g	One week in life, three to four weeks for QA'd draft report
Two-week oral toxicity/TK in rats	Y	300 g	Two weeks in life, six to seven weeks for QA'd draft report
2-Week oral toxicity/TK in dogs	Y	1 kg	Two weeks in life, eight week for QA'd draft report
Four-week oral toxicity/TK in rats	Y	500 g	4 weeks in life, 12 weeks for QA'd draft report
Four-week oral toxicity/TK in dogs	Y	2.2 kg	4 weeks in life, 12 weeks for QA'd draft report

(*continued overleaf*)

(*continued*)

Study	GLP status	Drug needed	Time of start to final report
IV/PO single-dose PK in rats	N	2 g	Five to six weeks
IV/PO single-dose PK in dogs	N	15 g	Five to six weeks
Protein binding in rat, dog, and human plasma	N	250 mg	Four weeks
Metabolic profile, rat, dog, and human liver microsomes	N	100 mg	Five to six weeks

4.5.3 Biopharmaceutical Considerations

BP, the use of PK to evaluate dosage forms, is an integral part of clinical development. In preclinical toxicology/safety studies, the dosage forms are not as complicated as they are for clinical use. However, the issue of dose selection is of importance, in that a dose–toxicological profile that, will hopefully, reflects what will be found in humans will be obtained. Thus, a good prospective projection regarding safety in humans based on preclinical systemic exposure would use both BA and BP principles in animal studies to ensure that the dosage/dose administered results in the maximum drug release profile [26].

Biopharmaceutical and BA studies are more complex for animal studies, and a maximal drug release profile should be determined in order to define the line between pharmacology and toxicology. To this end, the BA of the dosage form should be determined to confirm that the presence or absence of a toxic or pharmacologic effect is real and not a result of a low or no drug absorption (brick dust). In this context, ADME studies (including IV administration) and the use of *BP screens* in dogs and primates to evaluate the dosage forms for toxicology studies are very useful and have been presented earlier.

4.5.4 Go/No-Go

Usually, Go/No-Go decisions are used in the context of clinical studies. In the context of nonclinical development, toxicity in the animal safety studies in early development is the main reason for failure. Thus, it is important to ascertain a maximum drug release profile (TK) in early development. This is especially true if the toxicity found is organ specific or by an unknown mechanism. When a well-defined dosage form administered at $1\times$, $3\times$, and $10\times$ of the projected human dose does not result in safety margins of $\geq 3\times$, the development should normally not go forward.

In early development, Go/No-Go decisions are not based on testing/evaluations of a single compound. In practice, depending on the resources, multiple NCEs are evaluated and the most advantageous are selected and brought forward for further development. It is usual that development proceeds for two to three candidates in parallel to expedite further development into the pre-IND stage. A low initial BA is not a *show stopper*, and BP considerations are evaluated to determine if the low BA is an intrinsic property or the physicochemical property of the drug substance. Thus, early development evaluates absorption as a function of dosage forms with micronized drug (reduced particle size),

various salts, or esters and routes of administration to obtain maximum absorption. Although pharmacological activity may not be uncovered and if shown not to be correlated with an extent of systemic exposure, further development could proceed at an accelerated pace.

Other factors that result in failure at the preclinical stage of development are summarized in the following table.

Toxicity	In one or more species Insufficient safety margins
Pharmacokinetics	Long or short half-life Nonlinear kinetics
Low bioavailability	Insufficient systemic exposure
Metabolism	High first pass CYP induction or inhibition
Instability	Short shelf life of product Instability of substance in biomatrices
Complex/costly synthesis	Low yield Difficult scale-up Unacceptable impurities in final product

It should be pointed out that there are caveats linked to the therapeutic target where some of the above factors are not pivotal. A long or short terminal elimination half-life may not be a show stopper: a long half-life would not be appropriate for a centrally acting drug but could be advantageous for an antiarrhythmic drug. Even if there is a high first-pass effect, depending on the targeted therapeutic area, this may not be problematic providing the drug does not have a very narrow therapeutic index (TI) and is not highly variable. However, if the first-pass effect is concomitant with a high systemic metabolism, it may be prudent not to go forward with development. Thus, detrimental results have to be evaluated and understood, and rather than a "No Go" point, it may be a "Slow Down" point, as in some cases, the detrimental results can be overcome with a good therapeutic agent brought ultimately to market. In this context, a phased approach has been proposed to facilitate a smart approach to development [27].

4.6 CLINICAL PHARMACOLOGY

It was stated earlier that basic research has used today's technology to advance the understanding of disease(es) to new levels, which has resulted in a vast array of new NCEs to be translated into new drugs that improve patient health and management. However, in spite of all the advances on all fronts of the "medicinal sciences", the successful transition/translation of discovery to a new therapeutic entity on the market is still not a routine process. It is our proposition that clinical pharmacology may be a foundation for the effective translation/transition of research and development to effective and safe medicines [28].

It goes without saying that drug development of a NME is not for animals but for humans; however, drug development starts with animal studies. Up to this point, an overview of animal nonclinical studies that have been outlined were presented in the context of determining a safety profile in animals that would support a first study in humans and thus continue development by conducting studies that collectively constitute clinical pharmacology essential for an NDA-targeted development.

In the simplest terms, clinical pharmacology can be defined as a qualitative and quantitative assessment of drug action once it enters the systemic circulation: an understanding of what the drug does to the body and what the body does to the drug. In the drug development process, these are studies in humans, single-dose and/or repeated-dose studies of short duration from which come the quantitative measurements of pivotal drug disposition. These first human studies are designated as phase 1 and are the learning phase for effective patient management, that is, safe and effective therapeutic use. These are usually conducted in normal subjects, although the use of patients may not be excluded. In general, exposure is limited to small numbers of subjects and safety/tolerance is always a looked-for outcome, whereas efficacy is not.

Clinical pharmacology has been a part of drug development for the past 30 years. In the recent past, there has been a trend to reduce the number of PK studies contained in an NDA submission to those considered to be essential, that is, BA, bioequivalence (BE), and dose proportionality, thus reducing unnecessary exposure to drug in healthy human volunteers. It was considered by some that these phase-1-type studies are not needed in depth as the NDA is approved based on the pivotal phase 3 safety and efficacy trials. In addition, PK studies not included in the NDA would/could be conducted as phase 4 commitments. More recently, the FDA has placed a new emphasis on PK/phase 1, as such studies are learning studies that provide understanding of drug disposition and thus being important to patient management. This can be attributed to the emergence of factors, such as race, gender, age, disease state, and administration with food, that can alter the PK–drug exposure profile and thus have an important impact on dosing regimens. This emphasis on clinical pharmacology/PK/BP and their importance to patient management is shown by the clinical pharmacology guidance documents issued by the FDA with recommendation that the results of clinical pharmacology studies should be included in the NDA and thus in the label. PK/BP studies are an integral part of the NDA and enhance an understanding of the drug in the therapeutic setting in addition to providing basic drug disposition information even those that may be conducted postmarketing (phase 4) to fill a gap.

It should be kept in mind that approval/approvability does not necessarily remove phase 4 commitments. Having some data that retrospectively addresses factors that affect drug disposition is not the same as formal studies designed to elucidate aspects that, today, are part and parcel of clinical pharmacology. Approval can be obtained, but what usually suffers is the label. In this context, it is worth remembering that a strong/complete label at approval will have a positive impact on marketing. The successful completion of phase 4 studies three to four years after approval has little impact other that fulfilling a regulatory requirement. In addition, at that time the argument may arise as to "why pay a $200,000–$300,000 fee for a labeling supplement in addition to the study cost."

Often the value of phase-1-type studies is overlooked as emphasis is placed on phase 2 and phase 3 studies. It is certainly true that the data obtained from phase 1 studies does not approve an NDA. The issue falls back on *approvability* relative to having approval with a comprehensive label that provides important information for patient management. In other words, having a label that can be used by marketing and that differentiates the product from the competition. It should be mentioned that most of the negotiations/issues with FDA on the final label deal with the pivotal phase 3

studies, not with phase 1 data. This is not to say that a focused package of phase-1-type studies in early post-IND will not provide information for a better phase 2 program; additional phase 1 studies can, and should, be conducted on the way to the NDA.

4.6.1 First in Humans

The duration and safety margins obtained in the early animal toxicity/safety studies determine the entry of drug into human clinical pharmacology studies. However, before such evaluations, it is of value to estimate a maximum safe starting dose (MSSD) based on the safety profile obtained from the early animal toxicity studies. In this context, although not presented in detail, the algorithm to estimate the MSSD for first in human clinical trial has been issued by the FDA [29].

Such determinations are primarily dependent on observed preclinical toxicities and administered doses. In general, toxicity should be avoided at the initial doses. The doses chosen should allow a demonstration of the tolerability and the PK profile, which are the goals of early phase 1. The major elements in the animal species are the no observable adverse effect level (NOAEL); the level for each species tested should be converted to the human equivalent dose (HED) and converted by appropriate body surface area conversion factor (BSA-CF). This conversion factor converts animal dose in milligram per kilogram to the appropriate human dose in milligram per kilogram. In general, the resultant safety factor should be at least 10-fold. The guidance document presents tables of the factors to convert animal doses to HEDs based on the body surface area, which are to be used in the estimation of the first maximum human dose.

Usually, the first human study conducted is a single oral dose (SD) tolerance study that can also provide the first window on the clinical PK disposition of the drug: parameters such as C_{max}, T_{max}, AUC, $t_{1/2}$, Cl/F, and Vd/F. If an intravenous form is available, conducting this study as a PO/IV crossover would yield additional information such as absolute BA and a true estimate of Cl and Vd. In practice, a parenteral dosage form is not usually available; even if available, an IV toxicology study of at least one week duration has to be performed in at least one species (rat) to support the IV human study. There may also be resistance from management for using an IV dosage under the rationale that "we are not going to market an IV dosage form." It should be pointed out that the rationale is not to develop an IV dosage form but to provide an important PK information, for example, BA to the overall development process. Therefore, drug is typically administered orally in hand-packed capsules.

Validated bioanalytical methods for measurements of drug concentrations are essential for the elucidation of drug disposition, and their importance has been presented earlier. The early validation of a very sensitive and robust bioanalytical method specific for drug substance can present an opportunity to conduct a microdose study in early development. Microdose studies administer doses resulting in suboptimal exposure and thus do not determine the safety or efficacy; the primary objective is to determine the drugs' PK profile. As such, they are used solely as a screen for ranking the ADME properties of drug candidates [14]. Such information early in development can contribute to better candidate selection for development. The advantage of microdose studies is thus readily discerned, obtaining a window on drug disposition before implementing

formal/traditional phase 1 clinical pharmacology studies. Every drug development program is not appropriate for microdose studies. However, if and when implemented, the quality of development decisions can be improved and thus decrease the drug failure rate.

Accelerated mass spectroscopy (AMS) is a new analytical technique that has much promise in the future [30]. AMS is really not mass spectroscopy as it is generally known. In short, ions are accelerated to extremely high kinetic energies before mass analysis. The special strength of AMS is that for techniques that measure decay, it separates a rare isotope from its abundant neighboring mass, for example, ^{14}C from ^{12}C and ^{14}N from ^{14}C. To a large extent, this is still experimental and not readily in use and thus primarily for macrodose studies, but its cost prohibits larger use. Considering the advances in MS/MS technology and highly improved detection limits, AMS may not be an added value as an analytical technique for drug disposition.

Multiple dosing (MD) studies for 7 days with dose escalation can follow until a maximal tolerated dose (MTD) is achieved, that is, an observed undesirable side effect or in rare instances, a measurable therapeutic effect is observed. In addition, a dynamic response may sometimes be measured, for example, diuresis; but a PD response has to be treated with some caution as normal volunteers (NVs), not patients are used.

4.6.2 Overview of Clinical Pharmacology

The text that outline studies that are examples of clinical pharmacology programs that give guidance for phase 2/3 dosing regimens. The conduct of these studies defines a comprehensive presentation of clinical pharmacology and, ultimately, become part of the product label as the guide for general therapeutic use. The figures show examples of clinical pharmacology studies that are conducted as regulatory requirements; the sequence of conduct is neither absolute nor important and is dependent on the individual drug, the route of administration, the targeted indication, and the information obtained to that date. It is a *menu* of studies required, the order of conduct can be *choose and pick* and thus provide a rational clinical pharmacology program with impact on the design of phases 2b and 3 that results in verifiable safety and efficacy leading to marketing approval. It should be stated that the content of the figures is not new or original to the authors, although they are arranged/grouped in an order that has been found to be advantageous in past experience.

In drug development, there always is a time-line-driven push to do more with less and faster. It was stated previously that such an approach may result in an undesired outcome and ultimately, in failure of the program. The research and development staff should thus focus on their goal and the desired outcome of the development program: a favorable package insert for filing. They need to decide how to get from where they are now to where they need to be (marketing approval) in the most the efficient, effective, and shortest time as possible.

In certain instances or places a Go/No-Go study may be suggested. These studies are conducted in NVs, although studies in patients in the targeted patient population are not excluded and used where/when warranted. Details of study designs are not given in detail; but some elements of design and the specific guidance to conduct the studies are provided.

- IV/PO single-dose PK in NVs provides the intrinsic PK following the administration of dosage forms (tablets, capsules, liquids) used in the pivotal phase 1 trial

to determine the relative BA and BE [31]. Usually, these studies are conducted as an open label; single-dose crossover design with a small number of subjects (usually, 18–32) determined statistically to meet the "80–125% rule." However, if drug variability is high and requires very large subject numbers (60–80 or more) to meet the BA/BE criteria, use of a single-dose, four-period, four-sequence, randomized, crossover, replicate study design can be used to obtain BE [32].

- Effect of single doses of high fat food on the rate and/or extent of absorption relative to the fasted state studies are a requirement that should be conducted in early development [33,34]. Although the mechanism of the effects of food is not clear, food can also influence factors such as bile flow, gastric emptying, luminal metabolism, the first-pass, and complexity with excipients, all of which can affect drug absorption. If the effect of food is demonstrated, a study on the time of drug administration relative to food administration should be ascertained [34].
- If the drug is targeted for pediatric use, the development program includes the BP of oral solutions or suspensions, dosage forms targeted for the therapeutic use by children. However, if drug development is primarily for use in adults and is/can be used by children, oral dosage forms such as capsules and tablets may present children with difficulty in swallowing. In such case, studies should be designed to determine if the tablet can be crushed and administered with applesauce. These studies are considered *food studies* and are evaluated as described above.

The guidance documents mentioned earlier address the study design, the statistical evaluation for acceptance (90 CI, the 80–125% "rule"), and the number of subjects to be used. The main objective is the respective BA and/or BE study as directly applicable to clinical use. However, the study design generates the intrinsic PK properties of the drug (C_{max}, T_{max}, AUC, $t_{1/2}$), and these properties are included in the data presentation. For repeated-dose studies to steady state, escalating dose MTD/tolerance studies and dose linearity studies to ascertain if PK properties are linear are used. Although there are no specific guidance documents, those mentioned above provide the rationale, study design and statistical evaluation (if needed/appropriate) as above.

An understanding of the metabolism of the drug under development is a requirement for marketing approval. It is required to identify all metabolites formed and their relative concentration (percentage of total mass). The metabolic reaction types such as phase 1 transformations can be either by the introduction of a functional group (oxidation) or by the unmasking of functional groups (hydrolysis) and are the main steps of the metabolic disposition of drug. Phase 2 transformations are primarily the conjugation reactions that result in highly polar derivatives for excretion in urine, bile, or feces. Taken collectively, metabolism studies determine the mass balance of the drug (and drug-related material), characterize the routes of elimination and drug clearance, and identify and quantify the drug remaining in systemic circulation. An additional and more detailed treatment of drug metabolism is presented below in the role of drug interaction studies.

Single-dose metabolism studies using ^{14}C-radiolabeled drug(s) are conducted in early development to ascertain the information on the presystemic metabolism, the mass balance, and the routes of elimination. The use of ^{14}C-radiolabeled drug is a proven tool for drug metabolism; however, the radiosynthesis of the drug substance is required and may, depending on the synthetic route, be costly to obtain the specific

activity required. Replacing ^{14}C with stable isotopes for the use in drug metabolism may be more advantageous [35], and this approach has been used to determine an extensive metabolic disposition of drug in the rat [36].

An important *in vitro* study considered to be clinical pharmacology is the determination of the extent of plasma protein binding to determine the free drug concentration (not to be equated to the free fraction). Included is determining whether plasma protein binding is drug concentration dependent or not and the major carrier proteins, for example, albumin and AGPs.

Clinical pharmacology studies are regulatory requirements to determine drug disposition as affected by intrinsic factors (renal and hepatic insufficiency) that compare the PK of subjects with normal organ function to matched groups of the respective impaired organ [37,38]. This evaluation is important since the liver is the main organ of drug metabolism, whereas excretion of drug and metabolites are mediated by the kidneys.

It is generally recognized that numerous drugs are metabolized by the CYP450 enzymes located primarily in the liver. Most of the drug-metabolizing enzymes are in the CYP1, CYP2, and CYP3 families. The activation and metabolism of many drugs are dependent on the enzymes of the CYP450 system and can lead to wide interindividual differences in the clearance of drugs. These differences in metabolism can alter the dose–response relationship, leading to an increase in adverse events and toxicity as well as therapeutic failure [1,2].

Of the 50 CYP450 enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 are responsible for the metabolism of $\sim 90\%$ of currently marketed drugs [39]. These enzymes are predominantly located within the liver but may also occur in the small intestine, lungs, placenta, and kidneys [39]. The CYP3A4 isozyme is very abundant and important in the metabolism of many drugs. Its presence in the gastrointestinal (GI) tract is responsible for the low oral BA of many drugs. It should be noted that nondrug substances such as grapefruit juice and herbal supplements such as St John's wort can cause significant inhibitory and inducing effects, respectively, on CYP3A4 substrates.

Of the major isozymes identified above, three have shown genetic variance in their alleles (CYPC9, CYP2C19, and CYP2D6), which can be expressed phenotypically in patients as poor, intermediate, extensive, or ultrarapid metabolizers. Therefore, drug metabolism via CYP450 isozymes exhibits variability (polymorphism) that can influence an individual patients response to a drugs.

Genotyping for patients who receive mono- or polytherapy medication that are metabolized by isozymes that show variable individual metabolism (CYP2C9, CYP2C19, and CYP2D6) may assist clinicians in avoiding adverse drug–drug interactions and to individualize treatment with new pharmaceutical agents better. In the future, the use of genotyping to determine metabolic status of polymorphic drug-metabolizing enzymes will be integral to efficient patient care and management.

Although not all inclusive, the above outline of clinical pharmacology reiterates and confirms the role of clinical pharmacology/phase 1 and represents the learning stage of development during which an understanding of the drug and how to use it is obtained, whereas, as was stated previously, phase 2/3 uses what has been learned and confirms safety and efficacy.

4.6.3 Decision-Making Studies

As mentioned above, for some indications, certain studies can (and should) be conducted in patients rather than in NVs. Studies on drug interactions and hepatic and renal dysfunction must be conducted according to their respective guidance's. Once a drug substance is targeted for development, the expeditious conduct of a core toxicology package allows the initiation of the early clinical studies: an effective development program. It should also be mentioned that, as indicated/suggested in the figures below, many of the studies can be conducted in parallel, resulting in the overall development time line on the way to the NDA.

It has been stated previously that today PK/BP are well-defined components of drug development. There has been a progressive and increased emphasis by the FDA on the understanding of the PK and PD of drugs not only in healthy subjects but also in special populations. In this context, there is a great emphasis to evaluate and understand the potential for *in vivo* drug interactions, especially as polytherapy is the norm with an aging population. Thus, the "Clinical Pharmacology" and "Dosing Information" sections that correspond to these sections of an NDA dossier can comprise the major part of the label as the information therein is targeted to guide patient management (as an example, the labels of the two marketed cyclooxygenase 2 (COX-2) inhibitors contain the results of 17–22 drug interaction studies). On the basis of the regulatory guidance documents, pharmacology studies will provide data that are decision making as the development program moves forward. In the following sections, examples of several scenarios that illustrate effective drug development in phase 1 with inclusion of the current emphasis on drug interactions are presented in some detail.

4.6.3.1 Decision Based on Early Phase 2. This development outline is based on an early understanding of the drug as to its therapeutic value and thus continuing development.

Figure 4.2 introduces a concept that is an important contributory to the Go or No-Go decision: POC. In its simplest definition, a POC is a demonstration that a certain method (or idea) is supported by data of its feasibility and whose purpose has the potential of being used. In drug development, POC is not synonymous to proof of principle as

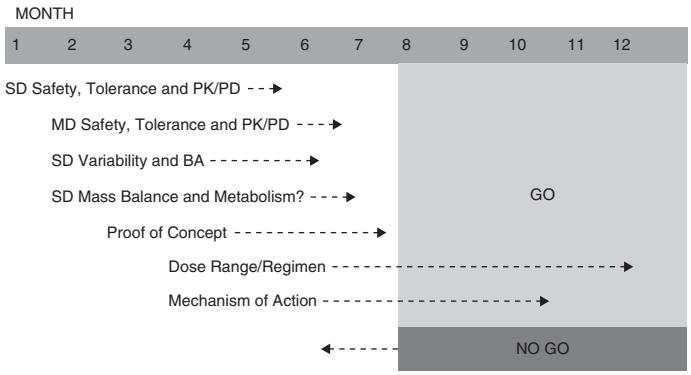

Figure 4.2 Phase 2a for Go/No-Go decision.

they lack clear definitions and they vary in usage between authors, investigators, and institutions and in usage over time.

In these early studies, it is usual that the preliminary dosage forms are used, for example, hand-packed capsules rather than galenic formulations. In many, perhaps, in most cases, the defined PK profile is the main objective. As/when development continues and a final dosage form is developed (for phase 1b or phase 2), a bridging BP study would be conducted relative to the initial form. The absorption PK would be compared as to the validity of the early study using a study design to determine whether the PK is linear over a projected dose range.

POC refers to early development, and the convention is to divide it into *phase 1* and *phase 2a* studies. Phase 1 studies are usually small ($n = 10–20$) single-dose studies in healthy volunteers; a short course of repeated doses (one to two weeks) can be conducted. A single-dose study determines whether the desired PK profile is achieved and the tolerance of the administered dose; with repeated doses, a signal for the desired clinical activity may be an outcome. At this stage, a Go decision implements the ADME studies required for complete development. Phase 2a is usually conducted in up to 100 patients to ascertain tolerance/safety and pharmacological activity and select a dose for the NDA-targeted studies.

The above panel outlines a series of studies that would allow the conduct of a POC study, the results of which determine the Go/No-Go decision.

The underlying principle for the POC concept is related to the use of biomarkers and surrogate end points in early clinical trials. This is a very extensive topic and, in fact, is the subject of not only research publications but also entire books; as such, only an introduction of the topic can be accommodated. In simple terms, a biomarker is a substance whose detection has been shown (validated) to correlate with the detection and/or progression and treatment of a disease. In clinical research, a clinical end point is a sign that defines a target outcome of a study. In this context, early drug development programs include investigations to identify and validate biomarkers to ascertain a Go/No-Go decision. In fact, the search for biomarkers is increasing and is now part of drug development in general. The scope and intent of this chapter can only be introductory in nature as a full treatment would warrant its own chapter.

However, it is necessary to distinguish between *disease-related* and *drug-related* biomarkers. Disease-related biomarkers indicate a probable effect of treatment in case(s) if/when the disease already exists. Drug-related biomarkers indicate whether a drug will be effective. At present, there is much effort in the discovery and development of innovative and effective biomarkers; these new biomarkers have become the basis of preventive medicine. However, it should be noted that even though a process can be repeated, it may not mean that it is true; thus, a new biomarker should undergo a rigorous validation.

Even when validated, in a clinical trial, meaningful outcomes based on biomarkers can be expensive or impossible to achieve. In this context, surrogate markers/end points that measure the effectiveness of treatment are used especially when these correlate with a real clinical end point. It is a measurement that is easier to assess than the true end point.

In essence, a surrogate marker (or end point) is a measure of effectiveness, as it is intended to substitute for a clinical end point. Surrogate end points often occur after an intervention in the disease process and can be an effective substitute. As with

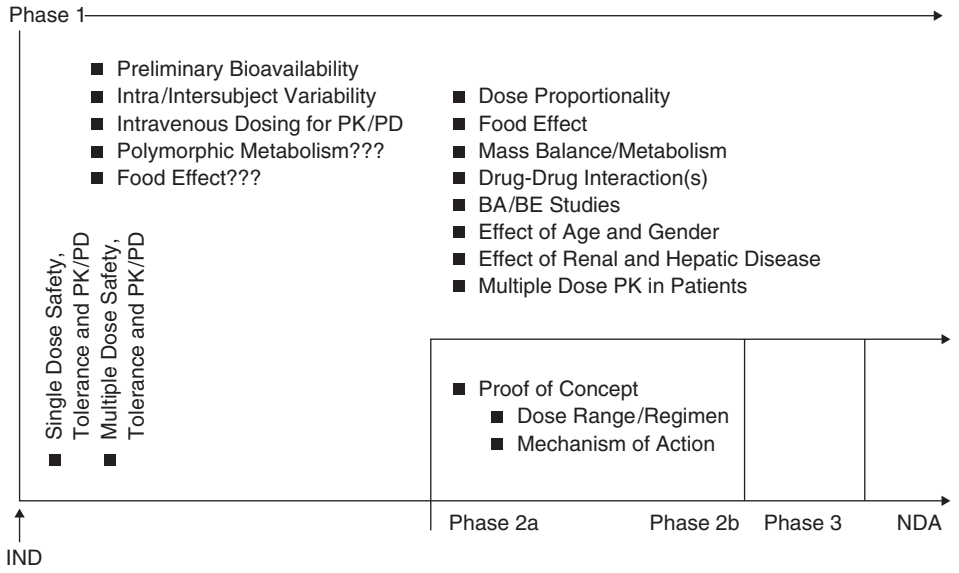

Figure 4.3 Efficient early drug development.

biomarkers, validation is a critical factor. For validation [40] as an end point, these conditions are to be met: (i) evidence that the surrogate predicts the true end point; (ii) end point must be specific, for example, the intervention is mediated via the surrogate end point; and (iii) all information on adverse effects associated with the intervention must be captured.

Thus, it is efficient to conduct a POC study as soon as possible, even though the animal toxicity studies indicate good safety margins for human studies; without efficacy, there is no need for further development.

Assuming that the POC study clearly indicates efficacy, development would enter phases 2a and 2b with studies in the targeted patient population in order to determine the dose(s) for the pivotal phase 3 studies.

Considering that conducting clinical pharmacology studies are not dependent on the final clinical dose, many studies can be conducted in parallel to phase 2. In this case, the sequence of conduct impacts efficiency. This is illustrated in Figs. 4.3 and 4.4, which present the sequence in a somewhat different manner.

There are occasions when the data in early development support a Go decision for further development, but for a variety of reasons, the decision is made to out-license the drug. Again, if there are no safety concerns from the animal toxicity panels conducted, interest to in-license the drug would depend on the clinical studies conducted. A suggested scenario with single-dose human data is shown in Fig. 4.5.

In the development program presented above, the goal was to obtain an open IND and to enter phase 1 clinical development. The above chart presents a program where the objective is to bring the development forward to the stage where out-licensing could be an option.

The above scheme would be greatly facilitated by the *in vitro* determination to determine how metabolism occurs using rat, dog (the toxicity species), and human liver

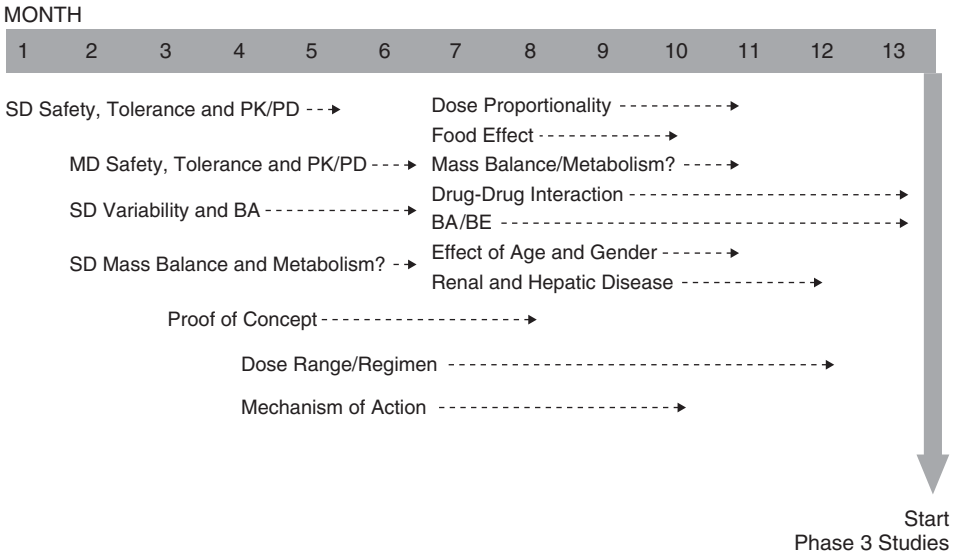

Figure 4.4 Efficient development to NDA.

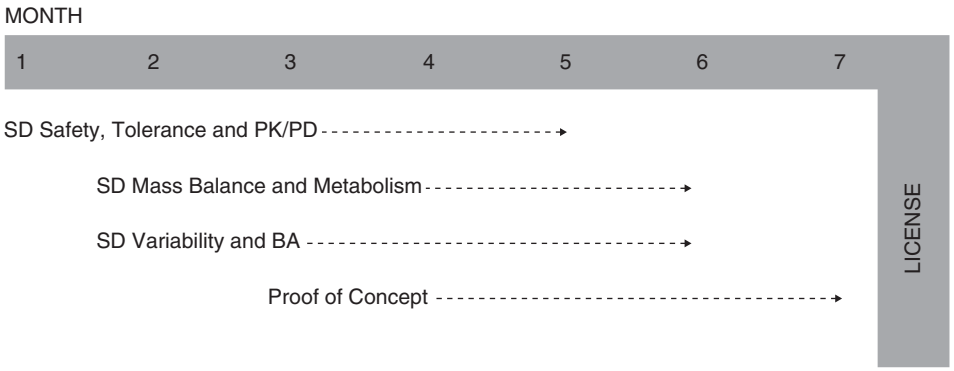

Figure 4.5 Single-dose proof of concept for licensing.

microsomes. This experiment would also identify the CYP450 isozyme(s) involved in metabolism and thus provide information as to a drug interaction potential if development continues. The availability of ^{14}C drug product at this time would greatly facilitate the determination of mass balance (and metabolism); however, using cold assays and collecting urine and feces in the SD study, in addition to plasma, would provide a reasonable preliminary determination of the mass balance without the additional expense of ^{14}C synthesis. However, this would need to be performed once a Go decision is reached after phase 2a.

Out-licensing can also be facilitated by the conduct of repeated-dose clinical pharmacology studies. An outline of such an approach is shown in Fig. 4.6.

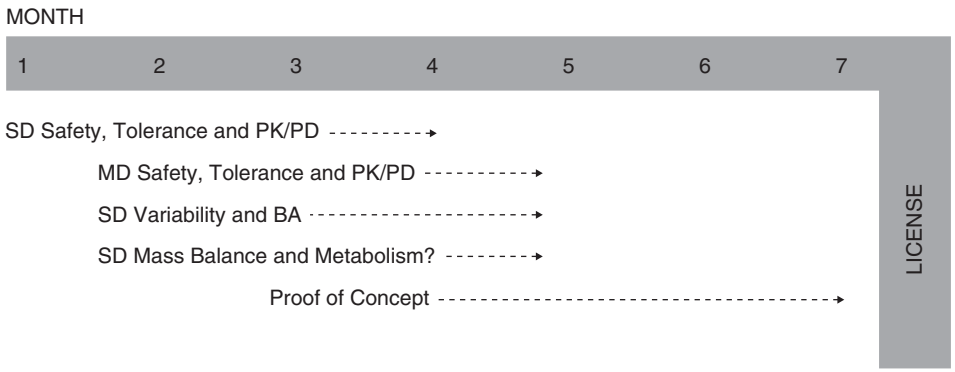

Figure 4.6 Proof of concept for licensing.

The advantage of a positive POC is self-evident. However, the availability of steady-state PK from the multiple-dose study would be an added attraction as such would have an impact on phase 2 studies in the targeted patient population.

4.6.3.2 Radiolabeled Studies (ADME). The conduct of a single-dose PK drug disposition study (ADME) is a powerful tool in development, especially when conducted early in the development process. A study with ^{14}C drug and the collection of serial plasma samples over a dosing interval can provide a qualitative window on the extent of metabolism from the respective slopes of total radioactivity relative to cold drug. Also, the AUCs of the total radioactivity relative to the cold drug can provide additional information, for example, elimination half-life of both moieties. In addition, from concentration–time data in urine and feces, the major routes of elimination and thus a mass balance can be obtained. Under ideal circumstances, a study in six NVs would be conducted using both IV and oral dosing in a crossover manner; the duration of sampling time would be monitored for radioactivity until the radioactivity is at LOQ. It must be stated that it is a regulation that a rat dosimetry study *must* be conducted to obtain an estimate of the residence time of the radiolabel and the conduct of human study must be approved by government of the state where the study will be conducted.

The challenge of an ADME study is the availability of the ^{14}C drug of sufficient purity and a high enough specific activity for accurate quantitation. There may be resistance to the cost of the synthesis, but it should be pointed out that once available, the radioactive drug can/will be used for required rat tissue distribution and plasma protein binding studies. In addition, in the case where the most appropriate animal species for the pivotal toxicology/safety evaluations is not evident, a radiolabeled study in several species (dog, monkey) would be conducted. By comparing radioactivity concentration in plasma, urine, and feces, it can be estimated which species approximates humans in metabolism and PK.

4.6.3.3 Role of Metabolic Disposition Studies. The role of metabolism in the overall process was mentioned previously. In general, drug metabolism (biotransformation) occurs as two categories: phase 1 and phase 2 reactions. Phase 1 reactions are nonsynthetic reactions and include oxidation, reduction, and hydrolysis. These reactions are

mediated by the CYP450 located primarily in phospholipid bilayer of the smooth endoplasmic reticulum and most of the organs in the body. Phase 2 reactions are synthetic biotransformation reactions that *link*/conjugate the parent drug or a phase 1 metabolite with an endogenous substance. These reactions include glucuronidation, sulfation, acetylation, methylation, and other conjugations. Taken collectively, the purpose of metabolism is to convert the exogenous lipophilic drug to water-soluble metabolites that are readily excreted from the body.

Formal studies to identify the major metabolite(s) in the systemic circulation—the number of metabolites formed *in vivo* , their structure, and whether any of the metabolites have a pharmacological activity; the routes of elimination; and a mass balance—constitute a more comprehensive role for metabolism in drug development. An important outcome of such evaluations is the elucidation of the metabolic pathway of a drug in humans and also in animals.

It is important to the understanding of the drug to determine the site of metabolism (hepatic, presystemic) and the specific member(s) of the CYP450 family of isozymes that mediates the metabolism. The identification of the specific CYP that is responsible for metabolism is virtually mandatory, especially as some isozymes have overlapping activity. The ability of the drug to inhibit or induce a member(s) of the CYP450 family *in vitro* is an important study that has downstream implications on drug–drug interactions potential *in vivo* in the clinical setting; specific protocols for such studies and when such studies need to be conducted can be found in the FDA and ICH guidance documents. It should be noted that these studies should be conducted earlier rather than later, especially if more than one isozyme contributes to the metabolic disposition of the drug. When metabolism occurs via multiple isozymes, clinically significant *in vivo* drug–drug interactions are less likely to occur (also see Section 4.6.3.4). What is more important to understand is the involvement of multiple isozymes rather all possible outcomes.

In Figures 2–6, individual studies are presented as line items that, when taken in the collective, illustrate the mosaic defined as early drug development. As a collection of line item studies, it is natural to see the total, and thus easy to overlook one of the line items in terms of its importance to the whole. Thus, although drug–drug interactions are depicted in one line, their importance greatly exceeds their press in drug development schemes. Today, the understanding of drug interactions with another drug administered concomitantly is an essential part of drug development, especially in polytherapy. Its prohibitive, and not the intention of this report, to recite drug–drug interaction presenting data of specific examples. The intention is to present key elements of drug interaction studies as a process, with study design based on the regulations as they apply to a specific drug and/or therapeutic area.

4.6.3.4 Drug Metabolism–Drug Interaction: In Vitro Studies. Once in systemic circulation, the process of a drug's elimination is initiated either by excretion intact in urine or feces or, primarily, by biotransformation (metabolism) [41]. If/when metabolism is the main process, the metabolic routes(s) are of importance as they can significantly affect the safety and efficacy of the drug. If elimination is via a single pathway, individual differences in the metabolic rate(s) can lead to significant differences in systemic concentrations of drug and/or metabolism as a consequence of activities of the CYP450-mediated metabolism. This metabolic pathway may be relatively simple or may be complicated by the involvement of multiple and

concomitant cytochrome isozymes with additional complexity added if these exhibit bimodal distribution because of a genetic polymorphism. For these reasons, it is important to learn the early stage of development in the metabolic route if the drug is eliminated primarily by metabolism and to ascertain its route of elimination. If metabolism is the primary route, it is important to identify the specific CYP450 isozyme(s) responsible for the drugs biotransformation.

The most prevalent site of drug metabolism is the liver. The most mature technology for the *in vitro* determination of hepatic drug metabolism is the use of human liver microsomes. Using selected commercially available CYP450 inhibitors for specific pathways, the metabolic pathway can be either demonstrated or ruled out. Once a pathway has been determined, the metabolic pathway can be confirmed using recombinant human proteins. Human liver microsomes are also used in assessment of a drug–drug interaction by concomitant incubation(s) using a concentration of the test drug and a standard test probe compound. A complete evaluation in detail is not the purpose here, and such a treatment is found in Ref. 41. It is also important to point out that *in vitro* studies do not adequately define the metabolic pathway and should be subsequently verified in a clinical study.

4.6.3.5 Drug Metabolism–Drug Interaction: In Vivo Studies. Current therapeutics seldom involve monotherapy, as the general population is older and whose treatment utilize multiple drugs on a daily basis [42]. The physiological changes in metabolism associated with aging increases a potential for an occurrence of drug interactions.

Not every drug–drug interaction is metabolism based. Changes in PK due to absorption, plasma protein binding, distribution, and excretion can be a mechanism for drug interaction. Thus, any drug interaction should be adequately assessed in the overall assessment of drug safety and efficacy. In this context, all drug–drug interactions are only relevant when a drugs metabolite(s) have been identified and their pharmacological properties elucidated. And only then, an understanding of the relation of specific drug(s) may emerge as a relationship between *in vitro* and *in vivo* is evaluated.

The potential for a drug–drug interaction is first evaluated *in vitro* by identifying the CYP450 isozyme(s) involved in its metabolism using proper studies designed for this purpose. Once identified, separate studies are conducted to assess the potential for its induction or inhibition and whether the outcome is a linear process. A clinical study is then used to determine the effect on disposition of the drug in question. Whether the uncovered PK changes are clinically significant is determined by the TI of the drug: if the TI is large numerically, changes of even 50% are not significant; however, if the TI is narrow, 10% changes can be significant in the clinical setting.

In general, *in vivo* drug–drug interaction studies compare concentrations of the drug to be evaluated (substrate, S) to determine if its exposure is changed relative to another drug that is interacting (interactant, I). A study can be conducted by several crossover designs and several combinations of single–repeated doses. The above mentioned guidance document provides various study designs, depending on the drug and study objective. One objective of the study design that needs to be kept in mind is that inhibition or induction needs to be evaluated as the end point. In general, induction takes longer to be ascertained, whereas inhibition is attained relatively more rapidly. In addition, the outcome sought may be concentration dependent: the induction of CYP3A4, the most prevalent hepatic isozyme accounting for $\sim$ 40% of hepatic

mass, is both concentration- and time-dependent inductions [43]. In most cases, inhibition/induction drug interaction evaluations are conducted as single-dose studies; if done at steady state, data to document such is needed.

Clinical drug–drug interaction studies are/may be conducted using healthy volunteers. Safety considerations should always be carefully evaluated if the desired outcome may preclude their use because of not knowing if the drug's metabolism is a result of polymorphism. In current drug development, it is not uncommon to determine a subject's phenotype or genotype before study conduct. To characterize a drug as to its potential to be an inducer or inhibitor, the establishment of which CYP isozyme(s) is involved in its metabolism is desired as quickly as possible.

PK end points with studies at maximum or approved doses should be evaluated. Parameters to be assessed include AUC, C_{max}, and time taken to attain C_{max}; PK parameters are clearance, volume of distribution, and half-lives. The exposure parameters are reported as 90% confidence intervals of the geometric mean ratio of the PK measures with and without the interacting drug. The guidance documents [42] is important, as it presents not only specific aspects of study design but also data evaluation.

The importance of understanding drug–drug interactions and its role in therapeutic drug use has resulted in the development of surrogates using *Escherichia coli* for a rapid characterization of CYP450s 1A2, 2 C9, 2 C19, 2D6, and 3A4; these surrogates agree well with corresponding human hepatic isozymes in literature, and thus indicate that such recombinant isozymes may be of value as predictive human metabolism [44].

4.6.4 Drug–Drug Interactions: on the Way to the NDA

The drug–drug interaction studies in early development, by large, can be considered as monotherapy and as such determine the basic foundation of how/when interactions occur: the studies are designed to produce an interaction. A goal in development is and should be to learn the drug disposition, to understand the drug under development, and then to apply this knowledge for successful therapeutic interventions in a large diverse population. Today, monotherapy, although desired, is relatively rare. This is especially true as the general population is elderly and uses multiple therapeutic agents on a daily basis. Thus, as stated above, it is very important to evaluate and understand contraindications of concomitant drug use as the resultant adverse or serious adverse reactions can occur. If the prevalence of these adverse reactions is considered serious with some incidences of death, the drug may be *pulled* from the marketplace.

The identification of which CYP450 isozyme(s) is responsible for the drugs metabolism, whether it can be induced or inhibited, cannot be overemphasized, especially as several CYP isozymes, (e.g., 2D6, 2 C9, and 2 C19) show a bimodal distribution because of genetic polymorphism. Polymorphism can affect the metabolic route of elimination and alter the systemic plasma concentration profile of the drug, with a consequence requiring dose adjustments to maintain safety and/or efficacy.

The common use of pharmacogenetics (genotyping of patients) already influences therapeutics: for Caucasian patients, $\sim 7\%$ cannot metabolize drugs that are primarily metabolized by CYP2D6. Similar information is being uncovered for CYP2C9, CYP2C19, and *N*-acetyltransferase. CYP3A4 activity is involved as the primary isozyme in the metabolic disposition of many drugs, both systemically, as it is the most abundant hepatic enzyme, and pre-systemically (during the absorptive process).

When the role of CYP3A4 is established, a drug interaction with ketoconazole (a strong inhibitor of CYP3A4) is conducted to evaluate the effects on the C_{max} and AUC of the drug; effect–no effect is established by 90% confidence interval, the 80–125% rule.

A current understanding of all factors that impact on metabolism-based drug–drug interactions is available as a guidance document [45], although as a draft. This guidance combines the salient aspects of general factors for the evaluation of clinical drug–drug interaction but of more importance, presents a comprehensive and extensive treatment of all current knowledge of metabolic drug interactions. This information is presented in tabular form and includes current summary of data from recently conducted trials. Such a comprehensive treatment of drug interactions is not in the scope of the present report but is presented to as a continuum and references what can/should be used as the development process continues.

To this end, an overview of some new aspects of development, especially in drug–drug interactions are briefly presented since they are an indication of the direction of effective development being implemented now and refined in the future.

- The pivotal role of CYP3A4 as the most abundant hepatic and intestinal isozyme that metabolizes half of the drugs on the market will be evaluated in development programs of new drugs to determine if drugs are substrates, inhibitors, or inducers of this pathway [46].
- Substrate specificity and regulation of drugs that exhibit polymorphism of their expression, especially of isozymes that are considered to have a low level of their expression will be included in routine drug–drug interaction studies [47].
- The role of transporters will be elucidated to provide advancement to the critical path of drug development [48,49].
- The use of drug cocktails in the conduct of drug interaction trials assist in clearly defining drug interactions in the clinical setting [50–53].

The above represents current advances in technology that have been, and continue to be, incorporated into current drug development, resulting in better therapeutic outcomes.

4.6.5 Past "on the way to the NDA"

Most of the concepts previously presented were targeted for early drug development to support safety and efficacy, especially in drug disposition as the learning process to a successful marketing approval. As such, they are set pieces aimed to open an IND, contribute to the design of the phases 2 and 3 studies, and if/when a successful NDA is achieved, provide specific guidance to the safe and effective management of patients, which is the label. Thus, it is worthwhile to revisit the process as a whole presenting the end of drug development as it is condensed into a primer for patient management.

4.6.6 The Label

A general outline of the studies that would contribute to the final label (Package Insert) has been presented previously. The presentation order of the information in the label

can vary with the drug and the content of the development program. There is, however, a general format that is followed and this is (in part) given below.

Description of the drug product listing the dosage form(s), a brief galenic composition and available strength(s) is usually presented first, followed by the structure, molecular weight, chemical name, the empirical formula, and its appearance and solubility.

Clinical pharmacology: These are the phase 1 single-dose studies in NVs, although repeated-dose studies and using patients are not excluded. Studies dealing with BA; BE; food effects; effects of age, gender, and ethnicity; and the dosing regimen(s) are presented with the appropriate metrics.

Mechanism of Action: The targeted indication is then given with a short summary of the key elements that describe the activity of the drug in the indication.

Pharmacokinetics: The systemic moiety (parent drug and/or metabolite) that affects the activity is then stated. The key PK parameters (C_{max}, T_{max}, AUC, $t_{1/2}$, CL, and V) in NVs and targeted patient population following oral and, if conducted, intravenous administrations are presented. Intra- and intersubject variability is addressed in the context of a dosing regimen. The PK sampling compartment (blood or plasma) is given.

Absorption: The extent of absorption (percentage of relative and/or absolute BA) in the target patient population and NVs is given. If there are different strengths and dosage forms (e.g., 5 mg tablet and 10 mg capsule), the BE of these is determined. If the patient dosing regimen involves several doses, the linearity of the systemic exposure as based on the correlation of the doses to C_{max} and AUC is determined.

Food Effects: The rate and extent of absorption are tested under fasted conditions and compared to the same when the drug is administered in the presence of food. A high fat meal (850 kcal, 46% fat) is the meal tested. If significant, the effect of the high fat meal is compared to a high carbohydrate meal (680 kcal, 85% carbohydrate) relative to the fasted condition. The presented effects are significant relative to a clinical setting are thus described with the addition of the results of the time of food administration relative to drug administration (2 h before, with drug, or 1.5–2 h after the meal). Taken collectively, the food effects are an implicit recommendation for patient management.

Distribution: In very early development, the partitioning of the drug between blood and plasma should be established to ensure that the right compartment for PK is sampled. The extent of plasma protein binding of the parent drug and its major metabolites should be determined *in vitro* by equilibrium dialysis and compared to that in *ex vivo* plasma samples, especially in special populations (renal, hepatic insufficiency). The following should be ascertained: is binding concentration dependent, the contribution of carrier proteins (albumin, AGPs) as their concentration in plasma can change in disease states. If the extent of binding is high (>98%), it should be remembered that even small changes in the free fraction (Ff) can have a large effect on drug disposition.

Metabolism: The extent of metabolism as well as identifying the CYP450 isozymes is presented. The identity of the metabolites, especially the major metabolites, is given with *in vitro* results compared to *in vivo* results. A metabolic pathway is

proposed. The metabolic profile is important to considerations of a potential for drug–drug interactions as was presented above.

Excretion: The routes of elimination (urine, bile/feces) are determined and quantitated, and a mass balance estimation is given. Usually, this is conducted using the ^{14}C drug, although cold assay can be used but may present a challenge in terms of recovery and resolution of drug from noise.

Special Populations: The parameters of exposure (AUC and C_{max}) as well as $t_{1/2}$, V, and CL are determined in patients with renal and hepatic impairment and compared to subjects with the respective normal function.

Issues such as effects of race/ethnicity and gender on drug disposition are addressed as specific stand-alone studies, but such information can also be obtained from the phase 2/3 studies.

Nonclinical studies that impact on safety are also in the label: carcinogenesis, mutagenesis, and reproductive toxicity, which form the basis of the pregnancy category. These studies were stated previously in the outline of the nonclinical studies that are conducted as part of the toxicity/safety evaluations; the study design and resultant evaluations are given in their respective guidance documents.

4.7 SUMMARY AND CONCLUSIONS

When all is said and done, drug development is a costly and time-consuming process; there are no *shortcuts* in spite of a detailed road map and experience. Although the same outcome is desired in principle, each drug and its development process is different in specifics, cost, total development time, and more importantly, regulatory acceptance. If the development is successful, the end product is a dossier of study reports conducted that present a convincing conclusion that based on data, the drug is safe and efficacious and warrants approval to market. In this context, there is always pressure for efficient drug development implying the unspoken caveat meaning "do it quicker" or "less costly" or "be smarter." However, the appropriate description should be effective for this characterization encompasses the process in terms of where it was, where it is, and where it is going, specifically, marketing approval.

It is not the intent of the present report to be all inclusive; in fact, this would not be possible because of pragmatic limitations. We have concentrated on segments in early development that present rationales to move development forward with the caveat that the bulk of development is conducted "on the way to the NDA." Whatever variations in timing and procedures an individual development undergoes, safety and efficacy should never be lost in sight and should be demonstrated by data. Data using rational study designs and flexible study conduct sequences that provide information for Go/No-Go decisions are essential. In other words, an effective development program in which core nonclinical safety studies enable an open IND and is followed by the first human studies (phase 1, clinical pharmacology), which provide the learning tool for information on the drug. As development continues, what has been learned is confirmed in phase 2 and phase 3 and the safety and efficacy of the drug in the context of the dosage and dosing regimen established.

Figures 2–6 that outline several phase 1 scenarios are examples of an effective phase 1 process and are not singular in that these and their variations have been found to be of use by the authors (and others) and are thus given as examples. As shown, it is important to ascertain the drug release profile as an evaluation of the dosage form, the extent of systemic exposure and assess factors that can influence absorption (i.e., food). As a general consideration, mass balance, routes of elimination, major metabolite identification, and the elucidation of the metabolic pathway must be confirmed. Considering that today's polytherapy is the norm in an aging population, the identification of the key metabolites and the CYP450 isozyme(s) mediating such metabolism is essential. Whether these are induced or inhibited is mandated to understand the potential for a clinically meaningful drug–drug interaction(s) and is mandatory for effective patient management with safety and efficacy.

On the basis of today's knowledge, the role of polymorphism in determining drug interaction potential and role of transporters as substrates and/or their induction/inhibition potential are a major part of drug development. It is likely that the ability to understand the overall disposition if drugs, especially metabolism in current terms, will in the future result in individualized therapeutic regimens.

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ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, and Excretion
CMC	Chemistry, Manufacturing, and Controls
CYP450	Cytochrome P450
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
IB	Investigator's Brochure
ICH	International Committee for Harmonization
IND	Investigative New Drug
LC/MS-MS	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
LOQ	Limit of Quantitation
NCE	New Chemical Entity
NDA	New Drug Application
NME	New Medical Entity
PD	Pharmacodynamics
Phase 1	Clinical Pharmacology Studied Primarily in Normal Volunteers
Phase 2	Clinical Studies in Patients; Early Dose Ranging
Phase 3	Pivotal Clinical Studies in Targeted Patient Population to Determine Safety and Efficacy
PK	Pharmacokinetics
POC	Proof of Concept
TK	Toxicokinetics

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