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Research paper

In vivo models and decision trees for formulation development in early drug development: A review of current practices and recommendations for biopharmaceutical development

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A B S T R A C T

The ability to predict new chemical entity performance using *in vivo* animal models has been under investigation for more than two decades. Pharmaceutical companies use their own strategies to make decisions on the most appropriate formulation starting early in development. In this paper the biopharmaceutical decision trees available in four EFPIA partners (Bayer, Boehringer Ingelheim, Bristol Meyers Squibb and Janssen) were discussed by 7 companies of which 4 had no decision tree currently defined. The strengths, weaknesses and opportunities for improvement are discussed for each decision tree. Both pharmacokineticists and preformulation scientists at the drug discovery & development interface responsible for lead optimization and candidate selection contributed to an overall picture of how formulation decisions are progressed. A small data set containing compound information from the database designed for the IMI funded OrBiTo project is examined for interrelationships between measured physicochemical, dissolution and relative bioavailability parameters.

In vivo behavior of the drug substance and its formulation in First in human (FIH) studies cannot always be well predicted from *in vitro* and/or *in silico* tools alone at the time of selection of a new chemical entity (NCE). Early identification of the risks, challenges and strategies to prepare for formulations that provide sufficient preclinical exposure in animal toxicology studies and in FIH clinical trials is needed and represents an essential part of the IMI funded OrBiTo project. This article offers a perspective on the use of *in vivo* models and biopharmaceutical decision trees in the development of new oral drug products.

1. Introduction

The ability to predict *in vivo* dosage form performance has been under investigation for more than two decades [1]. Nevertheless, preformulation scientists at the drug discovery interface responsible for lead optimization and candidate selection have a difficult task. Following the selection of a new chemical entity (NCE), information beyond drug dissolution from the formulation under biorelevant conditions is needed but today cannot always be well obtained from *in vitro* and/or *in silico* tools alone. Preparation for formulations that provide sufficient preclinical exposure in toxicology studies in animals and in the clinical trials in patients requires early assessment of the associated risks, challenges and development strategies. Approaches that are followed may be different [2–5,29] but a stepwise approach that considers a combination of crucial elements such as the dose to be administered orally in relation to the API solubility in biorelevant media, i.e. a biorelevant dose number (DN) is frequently included. In addition, *in vivo*, *in vitro* and *in silico* information about the API characteristics are necessary to complement the single parameter concepts such as the volume to dissolve the applied dose (VDAD), which is closely linked to the DN concept.

In an unpublished survey held across European Pharmaceutical Industry Association (EFPIA) partners involved in the Public-Private-Partnership OrBiTo funded from the IMI initiative, it was evaluated when, how and which *in vivo* tools are currently being applied during the oral formulation development process. It turned out that all 12

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responders applied animal models for their formulation development, especially in the drug discovery or early development phase. Dogs and rats are by far the most popular species. Next to animal studies, more than half of the responders indicated to often or always apply human studies in formulation development. Overall, the survey clearly indicated that companies heavily rely on animal but also human models for their formulation development.

In line with the survey, several publications prior to the initiation of OrBiTo have indicated that rats, dogs and nonhuman primates are the most commonly used *in vivo* models for pharmaceutical API and formulation development. Therefore, in the present manuscript, the current decision trees with respect to animal use are examined, keeping in mind that the 3Rs (Replacement, Reduction and Refinement) is a fundamental factor in all decisions to use animals in research. In addition, some guidance on study design, techniques, *in vivo* and *in vitro* parameters which have important roles in defining suitable cut-offs for decision making and how *in silico* modelling in early development can assist in making decisions is provided. Previously proposed decision trees exist [6–8]. Uppsala EUFEPS 5th World Congress on Drug Absorption, Transport and Delivery [9], indicated that many are mainly based on absolute and relative bioavailability data of solution and suspension formulations, respectively, and have been implemented e.g. at Janssen Pharmaceutica and Bayer.

The decision trees (DT) presented as a consensus of the OrBiTo project in the present manuscript also have connections to new methods for *in vitro* analysis of both API and formulation products which have been developed by the other work packages within OrBiTo (WP1- *in vitro* characterization of drug substances, WP2 – *in vitro* characterization methods for formulated products and WP4 – *in silico* models). The contents draw from new information gained in human gastrointestinal physiology and its comparison to those of the most common animals used.

In a recent review article it has been pointed out that the characteristics of the canine gastro-intestinal tract (GIT), especially the luminal characteristics of large dogs (body weight of about 30 kg), show various similarities with man. It was concluded that the most important distinctions from the human GIT relate to the higher bile concentrations and higher solubility/dissolution of BCS Class II drugs, and to the higher absorption of BCS class III drugs, and to differences in colonic characteristics [12].

In comparison of the dog with the rat as screening tools, a recent retrospective analysis of a compound database containing data in both, the rat and the dog, has shown that in dog the very poorly soluble compounds in FaSSIF (< 0.01 mg/mL) mostly corresponded to a relative bioavailability of a suspension versus a solution ($F_{rel,susp/sol}$) of less than 20%. For most APIs with a FeSSIF solubility higher than 0.1 mg/mL, the exposure in the dog after dosing a suspension was comparable to the exposure after dosing of the API in a solution ($F_{rel,susp/sol}$ close to 1). Unlike in the dog, the trend in the rat was less clear: higher relative bioavailability or even complete oral absorption was observed in the rat for very poorly soluble APIs, pointing out that the dog may in fact be more discriminating than the rat in case of low soluble compounds. Differences in permeability in the database evaluated appeared to be less critical as an explanation for the observations in both the rat and the dog [14].

The $F_{rel,susp/sol}$ in rat was higher compared to the $F_{rel,susp/sol}$ in the dog for most of the APIs for which both data in rat and dog were available. Neither the prandial state, nor the % ionized in stomach or duodenum appeared to be an explanation for the difference between the rat and the dog [14].

Other issues that have not been discussed in the literature up to date involve the effects of critical excipients as formulation constituents and the ability of preclinical species to reflect their effects on absorption. For example, when looking for a formulation for either oral solution or intravenous administration, the use of solubilizing agents is often employed. For example, PEG400 administered per os tends to increase the water volume in the intestine *in vivo* and may impact the permeability in the gut. For intravenous administration, dosage forms that contain Tween 80 (or Solutol) are known to cause histamine release in the dog where no such effects can be observed in the rat. DMSO is another commonly used solubilizing agent for both routes of administration and even though the percentage by volume is low, it can produce unwanted permeability effects in both species. Efflux transporter inhibition by PEG400 was observed *in vitro*; not proven *in vivo* by now [12,13].

A further field of investigation relates to the strain of a particular animal as *in vivo* absorption/bioavailability model and the pretreatment conditions. In general Beagle dogs are used most commonly for early phase formulation development [19,20].

Formulation studies are seen with and without pretreatment of the dog with pH decreasing agents (e.g. per os 1 N HCl or 0.6 mg/kg pentagastrin intramuscularly to better predict human physiology [18]. A discussion on aspects of species choice and physiologic variables will be presented in the article.

The timing of animal studies and the design of the studies from 3 of 7 top Pharmaceutical companies will be explored in this article. Based on best practices from these scientific communities and the drawing of experiences within their own companies, a consensus-driven decision tree or strategy will be presented. The first will review the decisions and data which are required to select an NCE or active pharmaceutical ingredient (API) with properties that allow future development activities to move forward with the best formulation early in preclinical development [25].

The current paper will summarize some of the current practices used in several of the major pharmaceutical companies and put forth some common rules or standards for best practices for *in vivo* studies during early formulation development.

2. Current decision trees for early formulation selection of the participating EFPIA companies

2.1. Bayer

2.1.1. Bayer current decision tree

Decision making on the type of formulation development of drug candidates for clinical phase 1 studies at Bayer is based on (i) the volume needed to dissolve the applied dose (VDAD) in human, and (ii) the relative oral bioavailability ($F_{rel,susp/sol}$) of the micronized crystalline compound material administered perorally in rat as suspension versus solution [37]. This procedure was recently published [4,14].

The calculation of the VDAD is based on the measured thermodynamic solubility of the crystalline, solvent free, micronized API in aqueous solution at pH1 (0.1 M HCl) and pH7 (phosphate buffer) and the predicted human therapeutic dose.

The decision for development of a conventional immediate release (IR) tablet is suggested for neutral compounds if the VDAD is < 3 L, for acids if VDAD at pH7 is < 6 mL, and for bases if VDAD at pH1 is < 175 mL. For all other compounds a rat $F_{rel,susp/sol}$ study is performed and used for the decision making as follows: Briefly, the test compound is administered orally to catheterized male Wistar rats at the estimated therapeutic human dose on a mg/kg basis in two different formulations: (i) e.g. ethanol/PEG400/water, 10/50/40 or ethanol/Solutol/water, 10/20/70, with the optimal vehicle being selected based on solubility data to ensure that the compound material is applied as solution, and (ii) suspension vehicles consisting of a 0.5% Tylose solution, in which the respective compound is suspended as micronized material. Plasma samples are collected over a period of 24 h post-dose and analyzed by LC/MS/MS. Pharmacokinetic parameters are estimated from plasma concentration-time profiles with standard PK software. $F_{rel,susp/sol}$ is calculated as the AUC ratio obtained from the suspension vs the solution arm. Based on a retrospective comparison with $F_{rel,susp/sol}$ data obtained in human, APIs with $F_{rel,susp/sol}$ values greater than 50% in rat qualify for conventional IR tablet formulation

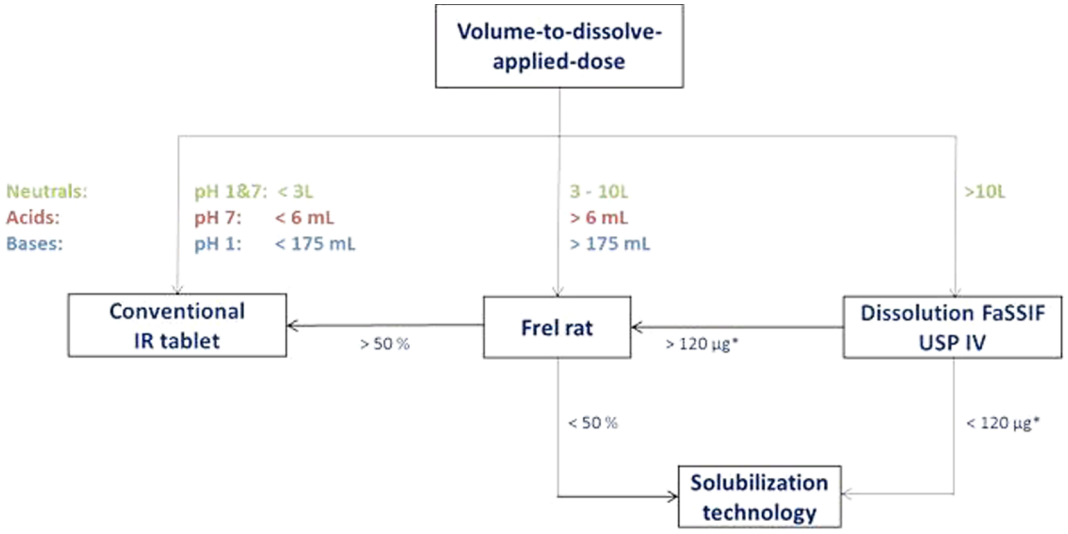

Fig. 1. Bayer Decision Tree.

development, whereas the development of enabling formulations (EF) is indicated for APIs with $F_{rel,susp/sol}$ values less than 50% (Fig. 1).

2.1.2. Bayer SWOT analysis

API formulation selection for clinical phase 1 studies is first performed at the stage of development candidate selection for which a comprehensive *in vitro* and *in vivo* data set is being generated. The profiling encompasses an in-depth physicochemistry, pharmacology, DMPK and toxicology characterization that is used as basis for the decision which candidate is going to enter preclinical development [25].

The decision on whether the candidate drug is suitable for a conventional IR tablet formulation or whether an EF development is needed, is based on a decision tree (DT) that combines *in vitro* as well as *in vivo* data such as (i) the aqueous solubility of the well characterized micronized compound material, (ii) the predicted human efficacious dose of the compound, and (iii) the $F_{rel,susp/sol}$ of well characterized micronized compound material given as suspension vs solution in rat (male, fasted) at the human equivalent therapeutic dose on a mg/kg basis. The data is used for decision making as described above under Section 2.1.1.

The DT draws its strength from a clinical validation that is based on more than 20 in-house compounds for which both human $F_{rel,solid/sol}$ data (IR tablet vs liquid formulation) and $F_{rel,susp/sol}$ data in rat have been generated. Based on the data set used, the cut-off $F_{rel,susp/sol}$ value in rat for the recommendation of conventional IR tablet or EF development has been defined as 50% [5]. An additional strength of the DT is the use of the predicted human therapeutic dose of the actual drug development candidate, i.e. not a standard dose, which is used for the $F_{rel,susp/sol}$ study in the rat and the calculation of the volume needed to dissolve this dose in human [21]. The solubility is determined in aqueous buffer at different pH values and for a compound state that resembles at this stage of development as closely as possible that of the API in an IR tablet, i.e. defined crystalline material that is micronized with a mean particle size of d50 $<$ 10 µm. The DT also accommodates the charge state of the compounds, i.e. to what degree they will be neutral, basic or acidic in the different parts of the GIT. Together with the use of the predicted human therapeutic dose this information is used to estimate any potential solubility-limited and/or dissolution limited absorption issues for a given drug development candidate. In addition, the oral absorption behavior in human is also assessed by the absolute oral bioavailability in preclinical species of the compound administered as solution, and the permeation behavior in the *in vitro* Caco-2 assay in order to identify any permeability-limited absorption issues.

The DT has been successfully applied in many drug discovery and development projects from multiple indications, but acknowledgment of its limitations is important for the reliability of the formulation recommendation for every new drug development candidate. One of these aspects concerns the solution arm in the rat $F_{rel,susp/sol}$ study, where it is essential to assure that the compound completely remains in solution, i.e. does not precipitate in the GIT *in vivo* thereby leading to an overestimate of the $F_{rel,susp/sol}$ value. The selection of the formulation for the rat $F_{rel,susp/sol}$ solution arm thus needs to be made with great care prior to the performance of the *in vivo* study. First, formulations for which the highest solubility of the compound can be achieved have to be identified. However, as supersaturated solutions tend to precipitate in the GIT *in vivo*, the formulation has also to be tested in an *in vitro* precipitation assay as second step in order to qualify for its suitability for the solubility arm in the *in vivo* study. Another limitation of the DT concerns the human dose range of the compound set used in the publications by Muenster and co-workers [4]. Most compounds in the data set used are applied at human doses of below 200 mg. This needs to be taken into account when using the DT for compounds with projected human doses exceeding this dose range. For such compounds, *in vitro* dissolution tests in biorelevant media at increasing doses are of particular importance in order to be able to predict the absorption behavior at the different dose steps of the human phase 1 dose escalation [21]. While in the past decisions have been based predominantly on solubility estimates in aqueous buffers, they are now increasingly complemented by estimates that are derived from biorelevant media [22]. Furthermore, project support and decision making is also being increasingly supported by physiologically-based oral absorption modelling [23–26].

2.2. Boehringer Ingelheim

2.2.1. Boehringer Ingelheim current decision tree

At Boehringer Ingelheim, the developability assessment is formally done based on solubility and estimated human dose and the respective dose number (DN; i.e. (dose/250 mL) * (solubility)). Other parameters like permeability, bioavailability and dissolution rate are considered as well but not reflected in the BI decision tree (DT).

There are three different formulation scenarios (A, B and C). Formulation scenario A is used for compounds with intrinsic DN (DN in water) $<$ 10. Scenario A compounds are developed as conventional formulations only and no problematic bioavailability is expected. Compounds with an intrinsic DN $>$ 10 are differentiated in two categories. The compounds with an intrinsic DN $>$ 10 and a DN in

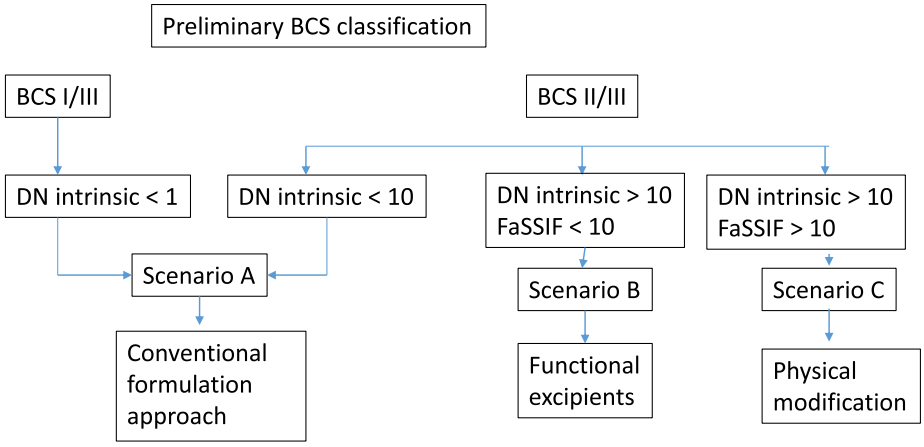

Fig. 2. Boehringer Ingelheim Decision Tree; DN = [(dose/250 mL)]/compound solubility.

FaSSIF < 10 are regarded as scenario B and the compounds with DNs > 10 in water and FaSSIF are scenario C compounds. In case of scenario B compounds, a more complex formulation development is performed; in addition to the conventional formulation several modified conventional formulations containing surfactants or organic acids are developed. Biorelevant dissolution and *in vivo* studies are utilized to choose the best formulation for phase 1 studies. The most complex scenario is scenario C. Non–conventional formulations or EFs containing amorphous or nanocrystalline API are being developed in parallel to the other two types. During phase 1 of scenario C compounds, several formulations are expected to be tested in the clinic (Fig. 2).

2.2.2. Boehringer Ingelheim SWOT analysis (before phase I)

For example, the DN is calculated based on the estimated therapeutic human dose. The decision of the formulation principle applies for the intended commercial formulation. During Phase 1 studies, higher doses are administered and the DN needs to be calculated with these doses to assess whether an EF is needed for these kinds of studies. The threshold of the DN is based on experience with former compounds and not determined by a correlation of DN and absolute/relative bioavailability. An internal validation of this threshold is needed.

The decision tree is based on solubility only and F_{rel} studies are not performed as part of this decision process. If the DN is low, this is an advantage because it leads to fast decision-making process in favor of conventional formulation development and animal studies can be avoided. However, it might be a disadvantage because animal studies for the above mentioned DN > 10 might show that a conventional formulation approach is still good enough and costly non-conventional formulation development can be avoided. In the BI decision tree, the kinetic solubility after 2 h of mixing is regarded as biorelevant solubility and no thermodynamic solubility is determined. This makes it difficult to compare with the other decision trees.

2.3. Bristol Myer-Squibb (BMS)

2.3.1. BMS current decision tree

At the Discovery-Development interface, animal PK studies are typically conducted in Sprague Dawley rats and Beagle dogs to assess biopharmaceutical risks of APIs and early clinical formulations. The dog model is more routinely used for formulation and dosage form decisions and assessing the biopharmaceutics risks to the early clinical trials.

Formulation development plans hinge on the BCS class of the clinical drug candidate. A preliminary assignment is made based on the permeability (Caco-2 and PAMPA) and solubility of the highest dose used in early animal studies (e.g. F_{rel} rat or toxicology). Early preclinical studies include absolute bioavailability assessment from oral dosing of a drug solution formulation in rats, followed by relative BA of a suspension formulation. If the BA of crystalline suspension is > 50% relative to a solution formulation, conventional formulation approaches for tablet or capsule development are considered regardless of the BCS class. However, if the BA is < 50% for the suspension especially for BCS-II or IV compounds, an enabled API (e.g. high solubility salt form, co-crystal, or size reduced API) or enabled formulation (EF) approach (e.g. amorphous dispersion, or solubilized system) is likely to be adopted. Based on the physicochemical properties and preformulation studies, specialized biorelevant dissolution *in vitro* studies and dog PK studies may be used to further understand biopharmaceutical risks such as food-effect, sensitivity to pH-modifying agents, and risk of low BA (e.g. gastric instability, poor wetting, or poor initial dissolution) at a clinically relevant dose. The use of these biorelevant tools and their link to preclinical and clinical PK has been published previously [28,29] (Fig. 3).

2.3.2. BMS SWOT analysis

The decision process is grounded on the biopharmaceutical risks of the API, taking into consideration the clinical dose, physicochemical data, and a range of biorelevant (SGF, FaSSIF and FeSSIF) *in vitro* dissolution data, *in vivo* pharmacokinetics, and *in silico* modelling activities. The strength of the formulation decision process relies on the universal, health agency accepted, designation of the BCS class and the relatively simple requirement of hitting 50% bioavailability threshold relative to a solution formulation. This has been applied to every clinical development candidate over several years and continually verified through retrospective analysis after FIH clinical PK data is available and additional mechanistic understanding of the controlling risk factors.

In early clinical formulation development, dog studies are primarily used to test the API and formulation options for the same reasons described previously. Pre-treatments with pentagastrin and famotidine are used to control the inherently variable dog gastric pH and replicate conditions more like the human experience. However, at this early phase several unknowns exist when adopting such a simplistic approach. For instance, the API crystal properties (final form) may not be finalized and the effect of particle size not fully known; the dose range can change significantly; and certain patient-centric biopharmaceutical risks such as susceptibility to pH-effect and/or food-effect can emerge to influence the formulation options. To navigate this uncertainty, biorelevant dissolution models and *in silico* PBPK modelling are used to gauge the severity of the risks and perhaps justify the need for additional animal PK testing.

2.4. Janssen

2.4.1. Janssen current decision tree

In early development, formulation selection is guided via a

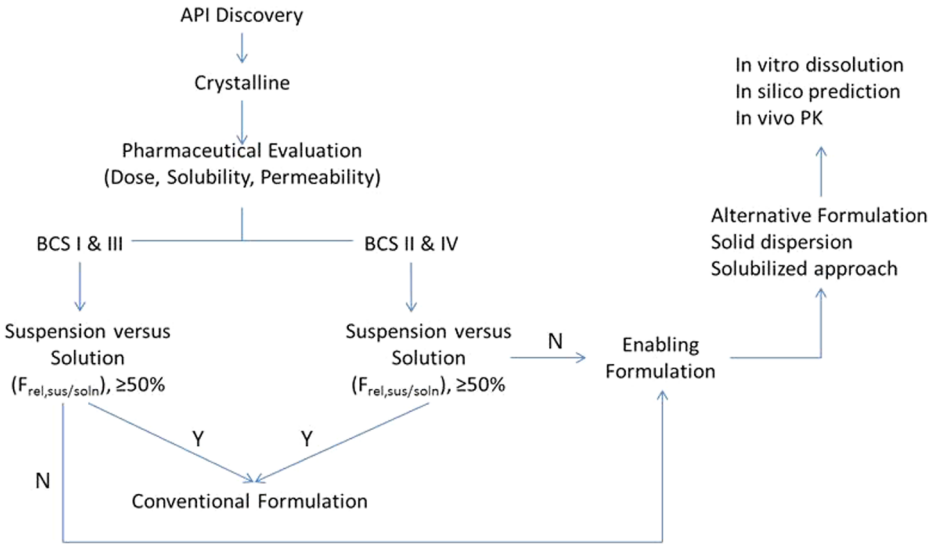

Fig. 3. Bristol Myers Squib Decision Tree.

preclinical study, determining the absolute bioavailability of the drug in solution ($F_{abs,sol}$) and the $F_{rel,susp/sol}$ in Sprague Dawley rats or Beagle dogs [14]. PEG400 and cyclodextrins are typically used as solubilizers in the oral solution formulations, while methylcellulose is often used as a suspending agent. Commonly administered doses in this preclinical suspension/solution bioavailability study are standard doses, e.g. 5 mg/kg for rat and 10 mg/kg for dogs.

The outcome of the study can provide an indication as to which formulation type has to be developed for clinical studies. A distinction is made between conventional, enhancing or EF technologies, because they drive the development cost and timelines. Conventional formulations are formulations without deliberate efforts to modify drug solubility, release/dissolution rate, permeability and/or stability (e.g. direct compression, dry/wet granulation, encapsulation technologies). Solubility or permeability enhancing formulations use conventional technologies to enhance respectively API solubility/dissolution (e.g. salt forms, inclusion of solubility or dissolution enhancing excipients) or permeability (e.g. addition of permeation enhancers). The term solubility EFs is used when API solubility/dissolution enhancement is targeted using complex manufacturing technologies (e.g. solid dispersions, nanosuspensions, lipid-based systems).

For compounds with a high $F_{rel,susp/sol}$ (≥ 0.8) a conventional formulation can be developed when $F_{abs,sol}$ is high (≥ 0.7) or when $F_{abs,sol}$ is low due to a high first pass effect. In case $F_{abs,sol}$ is low due to a low P_{eff} or precipitation of solubilized API, respectively, permeability enhancing or solubility enhancing/EF are proposed. For compounds with a low $F_{rel,susp/sol}$, solubility EFs should be considered when $F_{abs,sol}$ is low, while solubility enhancing formulations may be appropriate when $F_{abs,sol}$ is high. If the clinical DN is known or estimated to be low, conventional formulations may be developed in cases where solubility enhancing or enabling technologies are proposed (Fig. 4).

2.4.2. Janssen SWOT analysis

The DT has been validated using an extensive database of Janssen compounds (mainly weak bases with log P > 2 and intestinal human effective permeability (P_{eff}) > $2 * 10^4$ cm/s), indicating that preclinical data in the dog corresponded well with the BCS classification of the compounds and the developed clinical formulation type, while the alignment was less clear for preclinical data in the rat [14]. In the validation dataset, most rat data were generated in fed state, whereas most dog data were generated in fasted state. The average higher bile concentration in the intestine in the rat compared to the dog due to a permanent biliary drainage in the rat and a food-induced secretion of bile in the dog, may have led at least partially to these observations.

The strength of performing *in vivo* studies is that they are able to evaluate the combined effect of compound solubility and permeability on drug exposure. These studies often serve multiple purposes, including the initial understanding of pharmacokinetics and preparation for toxicology studies in addition to formulation type selection. By generating and evaluating both $F_{rel,susp/sol}$ and $F_{abs,sol}$ early in the discovery process, in combination with some *in vitro* readouts (e.g. permeability, precipitation testing, etc.), opportunities for mechanistic understanding are created. In silico modelling based on *in vitro* data may be applied as well, but model building requires a significant time investment and validation against *in vivo* data is advisable.

Since therapeutic dose estimates made at the discovery and preclinical research stage have uncertainty when only based on *in vitro* and/or *in vivo* animal pharmacology models, the proposed preclinical bioavailability studies are performed with a standard dose. However, this dose may not reflect the intended clinical dose. In case the clinical dose is known to deviate significantly from the proposed standard dose, the preclinical studies may be performed with a more representative dose.

It is recognized that particle size and physical form of the API and excipients of the solutions and suspensions can influence the solubility and/or dissolution behavior of the compounds tested and therefore the outcome of the *in vivo* bioavailability studies. If changes occur for these parameters during the development process, the impact on formulation type has to be assessed.

3. Interactions with API decision tree (*in vitro* characterization of drug substances)

The Drug Development Decision Tree, an outcome of physicochemical tools in the OrBiTo project, is based on several new small-scale biorelevant *in vitro* tools, developed to characterize physicochemical properties of NCEs, and thus to evaluate if an EF approach is needed. In a first step of the DT, it is suggested to determine the solubility of the NCE using a set of simulated intestinal media, developed by applying Design of Experiment (DoE) to ensure that all relevant intestinal fluid compositions, possibly encountered in the GIT, are covered. Using this DoE approach it is possible to identify which factors are driving the solubility of a NCE, e.g. pH, bile salts, phospholipids, monoglycerides etc., which may also give indications of which formulation approach is suitable for the given NCE. The DoE solubility data will result in a range of dose numbers for each selected dose of the

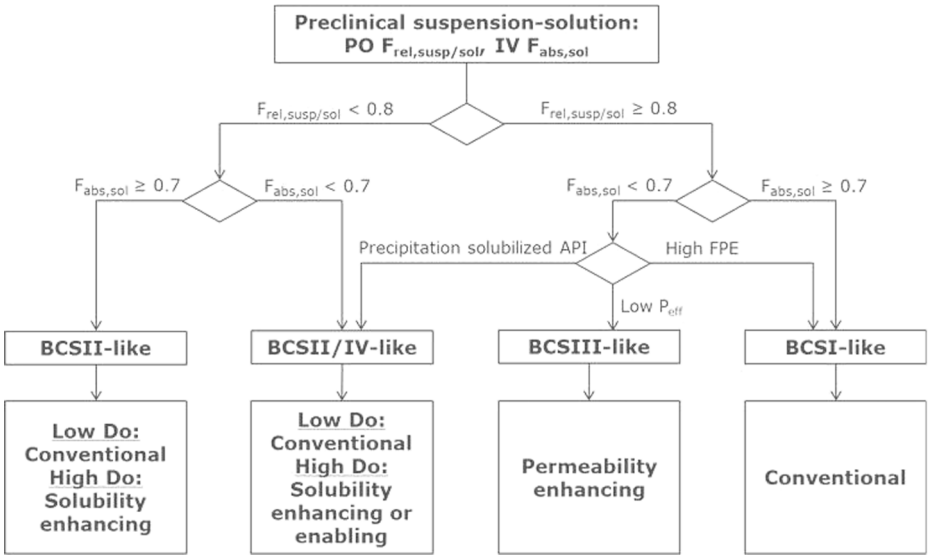

Fig. 4. Janssen Decision Tree.

NCE, which in turn can be used for further evaluation of the development strategy for the NCE.

If one or more of the dose numbers are above 1, it is suggested to carry out a small-scale intrinsic dissolution study (IDR), as described by Andersson et al. [30,31]. Disk, powder or controlled suspensions can be applied depending on the solubility of the NCE. Controlling the size of the NCE particles enables the evaluation of the impact of particle size on dissolving a given dose of NCE. This will inform on whether it will be feasible to reduce the particle size to a level where complete dissolution within the transit time available for absorption is possible [30].

In cases where it is not possible to achieve complete dissolution by reducing particle size, the supersaturation propensity of the NCE can be explored. For this purpose, a small scale standardized *in vitro* supersaturation and precipitation method (SSPM) has been developed [40]. In short, the NCE is dissolved in DMSO and added in excess to a biorelevant medium at four different concentrations. This enables determination of the maximal degree of supersaturation, the induction time prior to precipitation and the rate of precipitation under four standardized conditions. Thereby, the supersaturation propensity can be determined. In addition, the SSPM is also useful for screening the ability of different excipients to delay precipitation or reduce the rate of precipitation.

The physicochemical DT, described for WP1, has several interfaces with the current proposed "decision map/strategy" giving information on the physicochemical properties of the NCE which enables better decisions on which animal studies to select. First of all, the dose numbers obtained based on the DoE approach, could easily be implemented as the dose number applied by the companies for decision making. Further, the IDR studies could add further information as to whether an *in vivo* $F_{rel,susp/sol}$ study is needed. The supersaturation propensity may inform on risk of *in vivo* precipitation in the small intestine and also on the formulation development approach to select.

4. Role of modelling & simulation (M&S) during development (WP4 – In silico modelling tools for *in vivo* data)

In recent years the role of modelling and simulation using physiologically based pharmacokinetic (PBPK) software or other types of M&S tools has become very important to the development of NCEs, especially in late development using the clinical data derived from the studies within any given development program.

The use of PBPK, in particular, can be very useful during early formulation development. Generally, if sufficient *in vitro* parameters and physicochemical information are available, modelling can provide useful information or, at minimum, give insight into which parameters are most important for further formulation optimization. Additionally, these models can improve and/or confirm mechanistic understanding of what is happening in the gastrointestinal tract. Their value in early formulation design is to enhance the validity of the early animal models for parameter sensitivity analysis and to provide the ability to simulate scenarios at a later point in development when more clinical data are available. Refinement during development of these models should continue to be performed as more human clinical information is gathered.

As a group, we do not recommend any specific software but suggest that scientists observe the validation guidelines for PBPK software proposed by the Health Authorities in general.

Ultimately, the use of M&S or PBPK modelling in early formulation work is now becoming a standard activity. Historically, the major drawbacks have been the complexity of PBPK models and the lack of data to perform a suitable modelling in animal species. It is not unusual for EFPIA companies to perform permeability experiments only in human cell-lines or enzymatic studies with human tissues. Hence, this would make modelling in a rat, for example, very difficult and there has been much debate internally regarding the value of performing such experiments to support modelling in animals. With newer, more user-friendly software and the explosion of pharmacometric expertise, it is the consensus of the authors that the value of modelling cannot be understated even if there is a cost to doing so in early discovery. Many compounds fail because of the inability to achieve F_{rel} or $F_{absolute}$ in animals. This is one small reason for undertaking a course which is not directly focused on human development. During early discovery, high-throughput methods to determine parameters such as permeability or metabolic turnover in animal models are a small price to pay for providing the best information as a basis for developing a new compound and its formulation.

5. *In vivo* and physiochemical considerations

5.1. Rat

The typical strains for formulation development are the Sprague Dawley or Wistar rat. In rats, liquid microsampling (including dried blood spots) is now possible to enable evaluating intra-individual testing and potentially the same animals could be used for intravenous administration to obtain absolute bioavailability as is done in the dog.

The main concerns are washout time based on half-life required to maintain weight and age of rat. Blood volume issues are not usually a confounding factor with DBS or liquid microsampling [32]. *In vivo* rat studies may include animal numbers per treatment group ranging typically from 3 to 6 depending on API availability and statistical power to detect differences for a given API/BCS class.

The studies represented in our comparisons were mixed with respect to feeding state. For example, Bayer used fasted rats in most of their studies versus Janssen which used fed animals for their *in vivo* studies. Generally, the usual fasting period is ~16 h overnight for rats. Another consideration is that the rat has higher bile salt concentration in the intestine (possibly related to the continuous bile flow due to the absence of a gall bladder) than dogs or humans [12]. However, it is clear that there are physiologic differences between rat and dog that may explain some of the differences noted between the 2 species.

5.2. Dogs

In general, Beagles are used as the dog strain for formulation development in early phase studies due to their low body weight (~10 kg) [15]. According to an OrBiTo gap analysis [11,33] the dog tends to over-predict human bioavailability of BCS class III compounds. Based on a comparison between rat and dog $F_{rel,susp/sol}$ bioavailability data, Janssen observed the opposite, but concluded that the dog is better at discriminating between formulation type selection than the rat [14].

In general, the number of dogs used in formulation experiments during the early phase depends on a compound's half-life in order to determine the wash-out period if using the same dogs in a cross-over design. The use of a cross-over design in dog allows minimizing inter-individual variation and allows for intra-individual comparison. The number of dogs to be used will ultimately be determined by the number of compounds tested, the amount of API available for a given compound and the variability between animals (if known from other *in vivo* studies). In addition, it has been shown that the pH of the dog stomach is considerably higher than the human gastric pH [5,16]. Therefore, many authors have used pretreatment with pentagastrin (intramuscularly 6 mg/kg) or 1 N HCL per os. Measurement of the gastric pH has been performed by a number of researchers using simple pH test strip as well as more sophisticated tools such as the Smart Pill® [5,10,11,17]. Additionally, in the literature, the distribution of intestinal transporters in the dog almost resembles the human situation [41,42].

Dogs were generally fasted prior to administration. Some companies administer 50 mL tap water after tablet administration in proportion to the 250 mL administered to humans in a bioequivalence study.

5.3. Other species and information

Non-human primates have also been used in *in vivo* studies in the literature assuming that they are most similar to the human [34]. However, animal treatment guidelines and primate feeding habits are significantly different from the human situation [33]. Also minipigs have been used in *in vivo* experiments. Both species may have applicability for use in relative bioavailability trials but animal housing and 3Rs considerations favor the use of either dog or rat [35].

Which formulation vehicles are feasible for use in each species may be limited by their interactions with the species being used. For example, PEG increases the water volume in the intestine of most animals *in vivo* which may impact the permeability in the intestine. Efflux transporter inhibition by PEG400 was observed *in vitro*; however, this fact has not been proven experimentally *in vivo* yet [36,38].

5.4. Compound information: main properties, solubility and dissolution

If we dive deeper into the specific methods to characterize the drug substance, it becomes clear that some characteristics are inherent features of the compounds like the classification into acidic or basic properties. Thus, these characteristics are method-independent and not the subject of discussion at this point.

On the other hand, other features can be characterized in various manners and the result may depend strongly on the specifically utilized method. With reference to the formulation, many companies start the decision process by getting early information about the solubility and dissolution properties of the compound.

Solubility determination can be performed in two general kinds of media: aqueous and biorelevant. Utilizing aqueous media gives a basic overview about solubility in general at different pH values, within the overall physiological range from about pH 1–8. However, it is a well-known fact that these plain aqueous media only reflect in a limited manner the properties of physiological conditions within the GIT [27]. Biorelevant media like e.g. SGF, FaSSIF and FeSSIF aim to reflect more closely the *in vivo* GI conditions and consist of several other components present *in vivo* like bile salts, phospholipids or more *in vivo* relevant salts. These media were implemented about twenty years ago. The ongoing evaluation and development of further derivatives, not only addressing human conditions, but also those of different animal species, show the crucial importance of this *in vitro*-tool. A more in-depth look into the complex influence and interplay of different components of biorelevant media on the solubility has been developed within work package 1 from OrBiTo [39,40]. Section 3 describes the interaction with work package 1 in more detail.

Within EFPIA partners, aqueous media can serve as a start to get an idea about solubility, but biorelevant media are also wide-spread to determine solubility and, building up on this, to perform DN calculations.

While comparing the approaches of the different EFPIA partners, it was noticed that most of them prefer solubility determination at a steady-state between precipitation of the solution and in parallel ongoing dissolution of precipitate, thus, the thermodynamic solubility. The time at which this steady state is reached after stirring a suspension can be highly compound dependent, but a time frame of 24 or 48 h as a rough estimate is often used to determine the thermodynamic solubility. Some EFPIA partners prefer a shorter time frame for solubility determination like 2 or 4 h which better reflects the GI transit time. In most cases, a steady state of solubility concentration is not reached, so that this procedure will serve to determine more a kinetic solubility. In comparison to the thermodynamic solubility, this procedure relies more on the particle size of the compound, but probably better reflects the relevant *in vivo* concentrations, taking oversaturation and slow dissolution procedures into account and could be better suited for up following PBPK calculations.

5.5. Decision tools: dose number and animal studies

There is broad consensus within most EFPIA partners that the decision between conventional or EF is taken as a two-stage process with calculation of DN and animal studies serving as tools.

The calculation of the DN is basically a result of the solubility of the drug compound in relation to a dose. The solubility was discussed in detail in previous sections and as outlined, it can be subject of discussions which solubility is recommended for this evaluation. It is also a crucial issue which dose to take for calculations. Different ideas exist on this topic within the strategies of EFPIA partners, but basically they revolve around the question whether to take a standard dose, a predicted dose within the estimated therapeutic range or a kind of "worst case" dose which marks the highest dose due to tolerability investigations applied in Phase I. Dose predictions are complex due to the steadily evolving knowledge of the drug candidate, so this is a moving target and likely a matter of change. Adjustment of dose prediction to higher drug amounts could easily end up in a shift from a well suitable to an unfavorable DN. On the other side, working with a standard dose circumvents changes of the DN evaluation. However, a lot of therapeutic areas work with different doses. With a high clinical standard in

Table 1
Physicochemical and *in vivo* parameters of the data set used.

Dose [mg/kg]	Solubility pH 1–2.2 [mg/L]	Solubility water [L]	Solubility pH 7–7.4 [mg/L]	Solubility FaSSIF (mg/L)	Solubility FeSSIF (mg/L)	$F_{rel,\ suspension/solution}$ Rat [%]	DN[b] pH 1	DN pH 7	DN FaSSIF	DN FeSSIF	Type	Acidic pKa	Basic pKa
1	26		22	19.5	39.5	117	10.77	12.7	14.4	7.1	neutral	10.8	
1	0.1		4.4			90	2800	63.6			acid	4.34	2.60
3	0.1	2.4	820	522	491	68	8400	1.02	1.61	1.7	acid	3.4	
5	114	0.5	0.6			66	12.28	2333			base	11.2	4.18
3	18	19	17	15.9	31.8	59	46.67	49.4	52.8	26.4	neutral	10.7	1.17
3	0.2	4.1	4			59	4200	210			acid	10.7	
1	6000		0.1			59	0.05	2800			base		4.66
1	44	1	1	1	1	56	6.36	280	280	280	base		3.44
31	18	120	2	3	100	60	483	4346	2897	86.9	base		
10	11	4	2	6	20	80	255	1400	467	140	base		
12	11	4	2	9	29	84	305	1680	373	116	base		
2.2	5400	5300	600			79	0.11	1.03			base		
10	90		0.1[a]	36	38	78	31.1	28,000	77.8	73.7	base		4.12
10	10[a]		10[a]	5	11	68	280	280	560	255	base		3.21
10	23,000		60	98	226	70	0.12	46.7	28.6	12.4	base		4.87
40	2220		27	53	132	140	5.05	414.81	211	84.8	base		4.91
10	70		10[a]	10	40[a]	23	40.0	280	280	70.0	base		4.56
5	100[a]		100[a]	10	10	35	14.0	14.0	140	140	acid	8.25	
10	10000[a]		70	135	2147	50	0.28	40.0	20.7	1.30	amphoteric	10.9	6.30
5	38000[a]		60	150	332	113	0.04	23.3	9.33	4.22	base		4.44
5	16		2	41	72	84	87.50	700	34.1	19.4	base		0.10
10	3600		460	1490	2590[a]	95	0.78	6.09	1.88	1.08	base		8.52
10	2		10[a]	40	59	77	1400	280	70.0	47.5	base		4.15

[a] Number either > or < number.
[b] DN = Dose number = solubility in media/250; FaSSIF = Fasted Solubility in Simulated Intestinal Fluid; FeSSIF = Fed Solubility in Simulated Intestinal Fluid.

already quite well studied therapeutic fields like cardiovascular diseases, lower doses are more common and can be an important factor for the success of a drug candidate. In comparison, there is a higher tolerance towards a higher dose in more severe and life-threatening diseases with a high demand of new treatments such as oncological therapies. A standard dose should take these differences of various therapeutic standards into account.

Another topic is also the dose escalation within phase I trials to get information about the tolerability of a drug candidate. A conventional IR formulation could work at lower doses, but for higher doses EF may be needed. It is not only double the effort to develop two different formulations, but also the development of EFs could be more complex and time-consuming in comparison to a similar dosed conventional formulation. The statistics for determination of a cut-off value within the sponsor's laboratories will rely on additional variables which can be measured *in vitro* before moving into an *in vivo* animal study.

A dose number is an easy and convenient way to get an idea about the likelihood of sufficient bioavailability of a conventional formulation. Within the OrBiTo data base, many examples for DN calculations of different companies are available (see Table 1). A direct comparison among the EFPIA partners is difficult since every company assessed the DN in their own way with reference to the aforementioned differences in the utilized dose and solubility. Within the shown examples of decision trees, an often utilized and thus, well-established DN cut-off is 10 (see Section 2). With a higher DN, most companies base their decision on a formulation strategy from the results of *in vivo* animal studies.

5.6. Other factors to be considered

The other factors that should be considered when using an *in vivo* model can be described as minimum criteria before any formulation assessment is performed. These factors are *in vitro* permeability using human cell lines, intravenous (IV) pharmacokinetic data in animal species being used for formulation assessment (to calculate absolute bioavailability and the estimated efficacious human dose and/or 'worst case dose for Phase I tolerability') which can be modelled by allometry or PBPK simulations. Ultimately the combination of these factors, species to be used and physicochemical characteristics can give enough information to determine if $F_{rel,susp/sol}$ in animal species should be performed.

After initial PK studies are conducted to enable formulation decision as per the decision trees, oftentimes changes could emerge during progression towards clinical trials. For instance, doses can change, formulation strategy can change (e.g. switch in dosage form, fasted to fed conditions, or inclusion of subjects on pH-modifiers) and API properties can change on scale-up (such as a new more stable polymorph, salt, or a preferred particle size range). PK studies may not be required for all these changes, only for those situations that pose a risk of altered dissolution/absorption. When a PBPK model exists that clearly demonstrates no impact to PK and biorelevant dissolution supports complete dissolution of the dose, animal PK studies become redundant. This would be more likely applicable to BCS-1 and 3 drugs.

6. Dose number evaluation of OrBiTo compounds

As discussed above, an early decision tree or strategy is one where formulation development is conducted before availability of clinical data. The information gathered by 4 of the EFPIA companies is shown in Table 1 and Fig. 5.

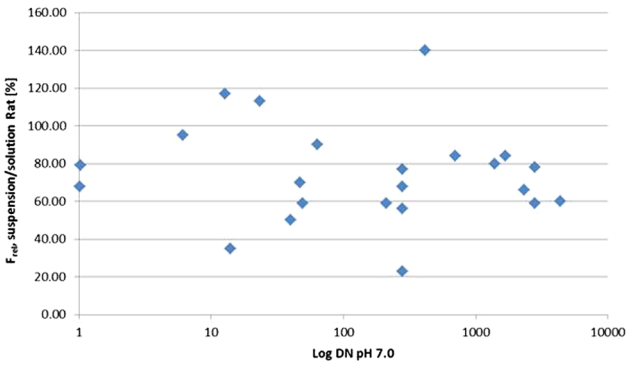

Fig. 5. % F_{rel} in rat versus Dose Number (DN) at pH 7.0–7.4.

Correlations using a linear regression analysis were done in Spotfire and Excel using various parameters against $F_{rel,susp/sol}$ in rat. No discernable or significant correlation was identified with or without log transformation of the DN at any pH (Fig. 5 $R^2 = 0.02$).

7. Conclusion

Within this work, the different strategies for early formulation development approaches in 4 EFPIA companies have been reviewed. It became obvious that there is no conclusion as to which is the superior approach. Whereas these approaches differ in many details like the integration of the dose prediction or the animal species used for *in vivo* studies, each DT is compiled of similar key elements. So, within a broader context, each company derives its formulation decision from similar parameters which can be assessed in a different manner. Every approach has its own rationale making sense in the context of the specific settings of each company, like its laboratory settings and strategy. In this way, an in-house data base is built up serving to compare historical with current data. Instead of aligning the single approaches and thus, losing its unique advantages and chances, this work tries to conclude with an overview about the most important key elements crucial for an early formulation strategy and, therefore, present in the single decision trees (Fig. 6).

Overall, each DT starts with analyzing the physicochemical features of the drug substance like solid state properties and solubility. In a next step, a relation between solubility and dose (DN) and, if deemed necessary, a first exploratory bioavailability study via the targeted application route in animals come into play. These tools help to make formulation decisions regarding whether to develop a conventional or an EF and are successfully applied at the drug development of different sponsors.

Nevertheless, a lack of correlation between F_{rel} to DN was observed (Fig. 5) and several reasons may be proposed. The first possible rationale is that experimental conditions in each laboratory are not performed in a similar manner which has been addressed in Section 2.2.1. A second consideration is that multiple factors may be at play in the determination of such a correlation. This has been observed recently in the dog where many physicochemical parameters are necessary to describe the best fit of F_a data (observed vs predicted) in a PBPK model paradigm [42]. A third possible reason is that a single physicochemical property does not adequately describe the entire chemical space covered. In the preparation of this manuscript, the authors did not look at structure or indication of the space. It would not be unusual for any given sponsor to bring several similar chemical entities to animal studies. Furthermore, there is no reason to believe that the structural series represent either a wide or narrow chemical space.

Therefore, it is difficult in defining an overarching decision tree/strategy that would work across companies and across different chemical classes of compounds. So the recommendation would be that for each project, i.e. chemically diverse set of compounds, there is a need to validate the decision tree and define the most appropriate tools available and relevant for the task.

This review attempted to bring a consistent approach to the decision process for formulation selection based on physicochemical characteristics and/or animal PK studies. It outlines some standards to consider at the Discovery-Development interface where the number of variables is high and uncertainties prevail prior to clinical studies.

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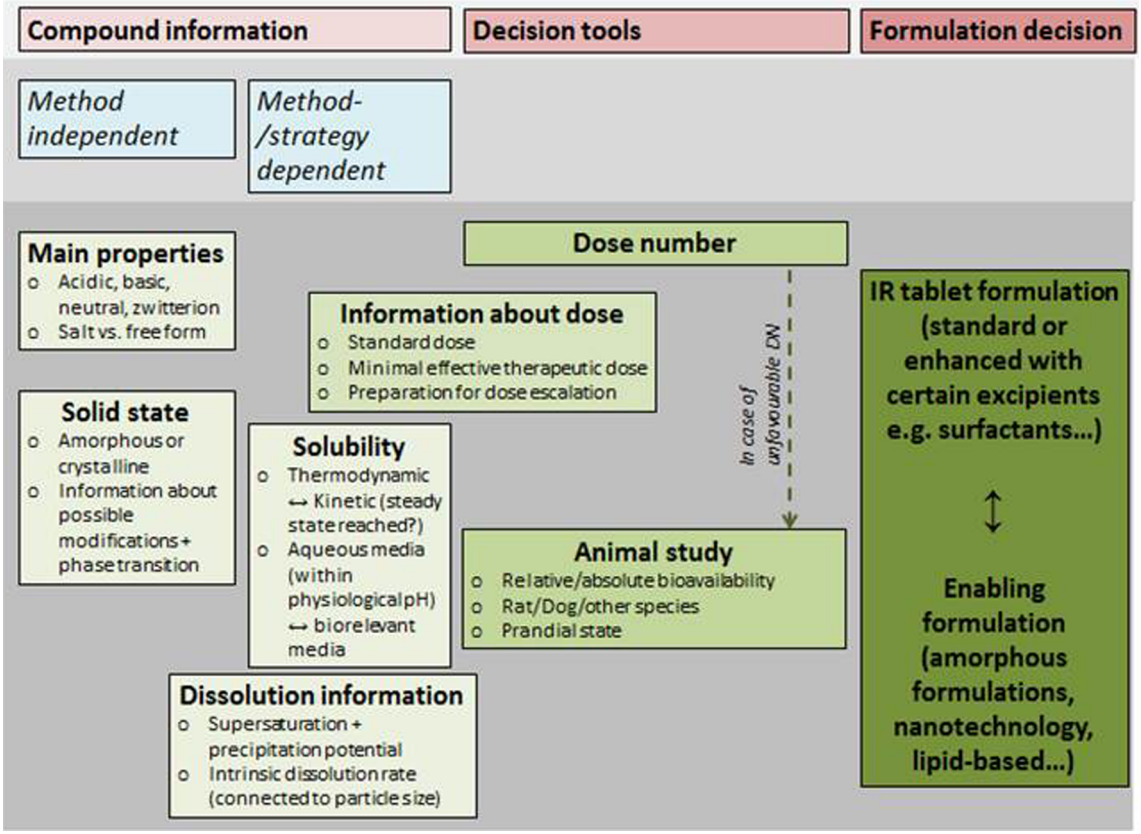

Fig. 6. Physicochemical and *in vivo* tools for formulation Decision making.

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