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PERSPECTIVES

OPINION

Developing drug prototypes: pharmacology replaces safety and tolerability?

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Abstract | New medicines are designed to bind to receptors or enzymes and are tested in animal cells, tissues and whole organisms in a highly scientific process. Subsequently they are often administered to human subjects with tolerability as the primary objective. The process of development is considered to be linear and consecutive and passes through the famous four phases of development (Phase I–Phase IV). This is efficient for those projects for which the uncertainty about the development is low. There is, however, an increasing number of new prototypical compounds resulting from the increased biological knowledge with a high level of uncertainty. For these prototypical drugs development has to proceed in a much more adaptive manner, using tailor-made objectives, the development of special methodology and a cyclical rather than a linear type of project management.

In 1785 a physician from the county of Shropshire in England, UK, discovered that extracts from the leaves of the foxglove (*Digitalis purpurea*) had an impressive effect on oedema. He did not know that the oedema was caused by heart failure and described foxglove leaves as a diuretic from his observations of the mobilization of the extracellular fluid as increased urine output. William Withering wrote a masterpiece, describing the clinical effects and side effects of digitalis leaves in great detail. His careful clinical observations followed by clinical experiments with dose and administration forms were done in a systematic manner and interpreted with great skill. *The Account of the Foxglove and its Medical Uses* is a tribute to the power of logical scientific reasoning in an age of limited technology[1] (FIG. 1).

Withering not only performed brilliant clinical science, using the limited tools available to him, but he was also years ahead of his time by indicating the need to study drugs by other modes of analysis than just the observation of clinical signs. Lacking these, "their virtues therefore must be learnt, either from observing their effects upon insects and quadrupeds; from analogy, deduced from the already known powers of some of their congenera, or from the empirical usages and experience of the populace." Note that this country doctor predicted biomarkers, 'me too' medicines (that is, drugs that largely duplicate the action of existing drugs) and clinical trials, as well as animal toxicology being applied to new medicines.

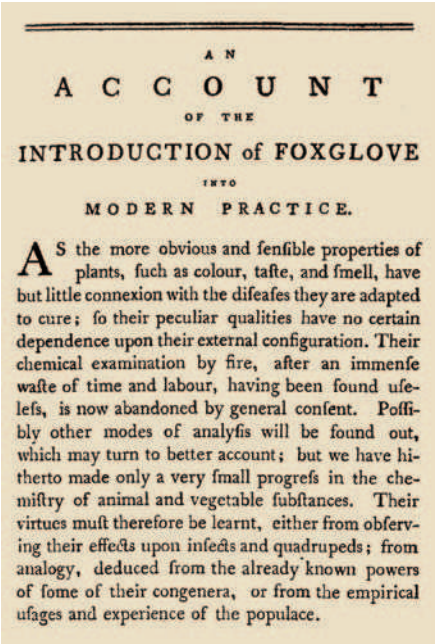
Today many of the technologies that Withering hoped for are in the toolkit of the clinical drug developer. Unfortunately many of these are not always used in the early development of new medicines and Withering, if faced with a standard clinical trial today, would see a surprising similarity to his own drug research. This Perspective will cover the historical reasons for this and provide some new concepts for drug development on a pharmacological (knowledge about the biological effects of the drug) base rather than the pure process (time) base that is currently still predominant. Not all new drugs require such a novel approach and it will also be attempted to indicate a system to differentiate between new drugs with low uncertainty and new drugs with high uncertainty, projects which I term prototypical.

Drug development as a linear process

Standard textbooks of drug development describe the development of a new drug in different phases[2,3]. This neatly consecutive discovery process starts with a therapeutic concept, which is generally based on existing knowledge of the aetiology of a disease. This leads to the well-known steps of target selection, target validation, lead identification and lead optimization, which may result in a drug candidate. This candidate undergoes preclinical development and when sufficient data are acquired to determine suitability for administration to humans, a new sequence of chronological events ensues, which can be divided into four clinical phases. This whole sequence can be described in a linear manner — with compounds progressing forwards through each step until the compound either reaches the market or its development is terminated.

However, in practice this process is not appropriate in all cases, as there are instances in which it does not lead to the drug being appropriate for the indication or the correct predication of dose. A large proportion of registered drugs require dosage adjustments[4] or display pharmacological[5] or toxicological effects that subsequently lead to discontinuation of the product. For example, only the lipid-lowering effects of the cholesteryl ester transfer protein inhibitor torcetrapib were pharmacologically studied in detail[6]. Effects on aldosterone and blood pressure, which may have led to its demise in the clinic when the compound did not produce the expected effect on mortality, were only studied much later[7].

The question is therefore whether the traditional four clinical phases of drug development are still the right approach for all projects. Indeed, the US Food and Drug Administration (FDA) abolished this concept as a basis for classifying clinical trials — most emphatically in a guideline in 1997 (REF. 8) — replacing the classification by much more appropriate study types



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AS the more obvious and ſenſible properties of plants, ſuch as colour, taſte, and ſmell, have but little connexion with the diſeaſes they are adapted to cure; ſo their peculiar qualities have no certain dependence upon their external configuration. Their chemical examination by fire, after an immenſe waſte of time and labour, having been found uſeleſs, is now abandoned by general conſent. Poſſibly other modes of analyſis will be found out, which may turn to better account; but we have hitherto made only a very ſmall progreſs in the chemiſtry of animal and vegetable ſubſtances. Their virtues muſt therefore be learnt, either from obſerving their effects upon inſects and quadrupeds; from analogy, deduced from the already known powers of ſome of their congenera, or from the empirical uſages and experience of the populace.

Figure 1 | **The account of the foxglove and its medical uses.** William Withering laments the lack of chemical methods available to study drugs and accepts that the second-best method is the study in whole animals and in humans. Figure is reproduced, with permission, from REF. 45 © (1985) Oxford University Press.

(TABLE 1). It is of interest why this concept of the four phases of drug development is being perpetuated virtually unchallenged, even by the authority that issued the new guidelines. Why does the traditional model still exist and are there possible alternatives?

The heyday of the linear model

It is debatable whether the linear clinical research process, which may have been historically appropriate, is suitable for all the current compounds in development. At the beginning of the 1990s an analysis by the stock analysts Lehman Brothers sent ripples of concern through the pharmaceutical industry. To fund the level of investment in research and development of the pharmaceutical industry of that year, sales of future products had to increase to an unsustainable level, unless each company produced approximately two products a year with a turnover of at least a billion dollars each.

This was the era of the angiotensin-converting enzyme inhibitors and the cholesterol-lowering agents that were reasonably safe, could be used widely for many indications and were marketed at a relatively high price. So the feasibility of marketing several large turnover products frequently and, above all, rapidly seemed realistic. Speed and progression of the clinical trials were therefore essential in the development of these products.

This required strong attention to efficiency and a linear development model with consecutive phases, as is appropriate in such cases, when the unforeseen uncertainty about the project is low[9]. At the start of a project there is always uncertainty but in some cases this uncertainty can be fairly easily and conclusively resolved. In the course of this article uncertainty in a project is defined as a high level of foreseeable uncertainty (that can be resolved) and also a high level of residual uncertainty (that cannot be resolved). The residual uncertainty category is in other disciplines often termed the unknown unknowns (or unk unks). I refer the reader to the excellent book by Christoph Loch about risk management in projects for further theoretical background[10].

In a linear model many functions, such as the trial design, the execution of an experiment, the reporting and the publication, can be standardized and separated into distinct specialized organizational units, which increases the efficiency of the drug development process. The separation of scientific departments from operational departments in pharmaceutical companies — that were still integrated in the 1980s — was a logical consequence of this. Furthermore, the outsourcing of the operational performance of a clinical trial in its entirety was made possible by the growth of the process-oriented contract research organizations[11].

The demise of the large turnover product rofecoxib (Vioxx; Merck)[12], after having been marketed, confirmed mounting fears that this model (sometimes now called the blockbuster model) was unsustainable[13]. This led to a realization that there was much more uncertainty in the development of new drugs than perhaps previously considered, even for compounds such as rofecoxib, which could be considered an incremental innovation of the traditional non-steroidal anti-inflammatory drugs.

The observed drop in the number of innovative products reaching the patient led to much reflection and publications about the potential causes. Problems were ascribed to a wide range of culprits, which included the hypothesis that all the easy targets were being covered ('low hanging fruit') and issues related to managerial and organizational concerns[14]. There is no doubt that the number of medicines being marketed each year is not increasing, despite an increase in the research and development budgets of the pharmaceutical industry[15]. Conversely, there is also much evidence that the rapidly increasing knowledge about disease mechanisms will produce a large number of highly innovative drugs to be evaluated, but that the evaluation process will take longer than expected or hoped[15,16].

Adaptation of the model

The development of innovative compounds is rife with unknown unknowns. This requires a more innovative approach to development[10,17] than consecutive, phased project management, which is intended to deal with low uncertainty projects. Not all drugs to be developed are at the same level of innovation. A compound that is chemically innovative may not affect a new biological mechanism or be a therapeutic innovation. These compounds have less uncertainties in development and require a different project management style compared with an entirely (that is, chemically, mechanistically and therapeutically) novel molecule. Such novel compounds are termed prototypical in this paper; in contrast to less innovative standard compounds (BOX 1). In fact such a differentiated approach to prototypical versus standard product development is quite common in the electronics and software industry and has a solid underlying theoretical basis[9,18–20].

Development of standard products that are directly intended for the market (as project uncertainty is deemed to be low) is appropriate for projects that can have the remaining uncertainties removed in confirmatory research. Any loss of time in getting the product to market is loss of sales and the priority of the project is high speed and low cost.

Industrialization of the processes needed to perform the research, through the differentiation of functions in different organizations, is possible in these cases and the transfer of technology between these organizations is easy. For example, in such cases the writing of a standard study protocol can be done by medical writers, the regulatory submission and discussions by a consultant, the performance of the study in many different countries by a contract research organization and the reporting by a statistical consultancy group.

By contrast, a prototypical project is not directly intended for the market. This is because uncertainties (unknown unknowns) need to be identified and subsequently removed by redesigning of the molecule or by returning to an earlier stage of the development plan or even by the performance of new animal or laboratory tests (that is, back to the drawing board). The priority

Table 1 | **Classification of study objectives according to FDA clinical trial guidelines**

Objective of study	Study examples
Human pharmacology study	
• Assess tolerance • Define and/or describe pharmacokinetics and pharmacodynamics • Explore drug metabolism and drug interactions • Estimate drug activity	• Dose tolerance studies • Single and multiple dose pharmacokinetic and/or pharmacodynamic studies • Drug interaction studies
Therapeutic exploratory study	
• Explore use for the targeted indication • Estimate dosage for subsequent studies • Provide basis for confirmatory study designs, end points and methodologies	• Earliest trials of relatively short duration in well-defined narrow patient populations, using surrogate or pharmacological end points or clinical measures • Dose–response exploration studies
Therapeutic confirmatory study	
• Demonstrate and/or confirm efficacy • Establish safety profile • Provide an adequate basis for assessing the benefit–risk relationship to support licensing • Establish dose–response relationship	• Adequate and well-controlled studies to establish efficacy • Randomized parallel dose–response studies • Clinical safety studies • Studies of mortality and morbidity outcomes • Large simple trials • Comparative studies
Therapeutic use study	
• Refine understanding of benefit–risk relationship in general or special populations and/or environments • Identify less common adverse reactions • Refine dosing recommendations	• Comparative effectiveness studies • Studies of mortality and morbidity outcomes • Studies of additional end points • Large simple trials • Pharmacoeconomic studies

FDA, US Food and Drug Administration. This table is adapted from REF. 8.

in these projects is on the knowledge and the integration of information from many sources involving learning research rather than confirmation. It is best done by groups of specialists in one location, as technology transfer of part of the process to a specialized organization is generally impossible as a result of the many remaining uncertainties.

The concept of concurrent engineering (in which stages are not consecutive but parallel) and prototype learning cycles has been rarely applied in drug research, although it is used regularly in engineering and software development. In line with this, it was suggested some 13 years ago that drug development should proceed in so-called 'learn and confirm' cycles and that a change in the style of project management is necessary[21]. Unfortunately this view was not widely accepted or practiced. This is perhaps not surprising because most of the previous generation of marketed products with big turnover did not require this approach. It may therefore be useful to make a differentiation between standard and prototypical projects and to tailor the development process and knowledge management to the project, rather than adapting the project to a rigid process.

Experiments with prototypes

The proposed method of differentiation between standard and prototypical drug development is shown in FIG. 2 as a matrix with two dimensions. The first dimension of the matrix describes the strength of the knowledge about the linkage between the biological mechanism of a drug and the clinical effect (defined as an effect on 'feelings, function or survival'). For example, the link between the inhibition of the enzyme involved in inflammation, mitogen-activated protein kinase, (that is, the biological mechanism) and an effect on joint destruction in rheumatoid arthritis (that is, the clinical effect) is not established for an inhibitor with a novel mechanism of action. A standard drug has by definition a 100% linkage between its biological mechanism and the clinical effect, as it has already been established for the original compound.

The second dimension of the matrix is the availability of methods that allow this link to be established, which I call a linkage marker. This could be a biomarker or another measure, such as the plasma concentration of the drug. A generic drug has a near perfect measure in the plasma concentration and bioequivalence to an existing marketed compound. On the other side of the spectrum is the entirely prototypical compound, which affects a new mechanism that cannot be evaluated with existing methodology. The inhibition of mitogen-activated protein kinase and its effects on cytokine release in relation to the concentration of a drug may require specialized development of the assay. Depending on the project, the marker (or group of markers) could be various measures, such as a questionnaire, a sophisticated brain imaging technique or the measurement of a cytokine in an unusual body fluid[22].

The task of a drug developer can then be simply described by moving the prototypical project from the lower left hand of the graph (FIG. 2) to the upper right hand through an understanding of the link between molecular mechanism and disease using innovative trials with a toolkit of markers. This approach suggests that the linkage marker toolkit cannot be developed and validated at the same time as the link between the biology and clinical effect is tested. Generally, the markers have to be developed ahead of the evaluation of the link. This in itself requires a different approach to project planning. Some of the activities to develop these markers have little to do with the development of the compound as a medicine, but much more to do with the methodology. If the fact that markers need to be developed ahead of the compound is not recognized in time serious delays in projects will occur as the technology is not ready when the project is. For non-prototypical projects this is less likely to happen.

No translational development in phases

Virtually all new compounds are of small molecular mass, chemically synthesized or large biologically-made molecules and most, if not all, are developed with the intention of having some molecular interaction with a receptor or an enzyme. These are normally evaluated preclinically in accordance with the scheme shown in FIG. 3. This logical progression of the acquisition of knowledge is disturbed when the compound is given to human subjects at the start of Phase I trials. At this point, according to standard teaching, the drug is first evaluated for its tolerability and safety. This is logical for a standard compound, as the only function of the first administration to humans is to confirm that the drug is indeed well tolerated.

For a prototypical compound a stepwise approach that confirms the intended pharmacological effect of the compound in human receptors, isolated cells and tissues is more appropriate, and is analogous to the studies done in the animal models.

Box 1 | **Standard versus prototypical drug development**

This box gives the usual properties of the 'standard' development programmes with regard to preclinical and clnical research. By contrast the rquirements for a solid prototypical drug are given.

Standard drug development

- Pharmacological experiments in animals done using dose–response relationships
- No concentration measurements in preclinical pharmacology
- No strategy for linking results from animal experiments to human pharmacology or physiology
- Link between animals and humans through the no observed adverse effects level (NOAEL) dose in toxicology studies in animal models
- No measurements of pharmacological or physiological drug effects in toxicological experiments
- First dose in humans determined by a fraction of NOAEL
- Only clinical observation of adverse effects in the first human experiments
- Dosing in humans until the tolerability level is exceeded
- Development is linear and as rapidly and as cheaply as possible

Prototypical drug development

- Concentration–effect relationship established in pharmacological experiments
- Clear predefined strategy that is based on the relationship between the scientific questions that lead to animal testing and the questions to be answered in experimental testing in humans
- Link between the effect of the drug in animals and in humans based on quantitative experimental results from animal and human receptors, cells, tissues and whole organisms
- Measurement of drug effects rather than side effects or tolerability in animal toxicology experiments
- First dose in humans based on pharmacological effects
- In addition to observation of side effects, quantitative measurements of drug effects from first dosing in humans onwards
- Extensive use is made of the fact that regulatory agencies allow the possibilities of exploratory trials, microdosing and other opportunities for obtaining information about the usefulness of a compound rather than its adverse effects
- Determination of tolerability of the drug is not a primary aim
- Development is cyclical, with maximization of information as a priority over speed and cost

In this way, the transition to humans should repeat some of the steps that were systematically taken during the preclinical phases of development. The FDA guidelines for exploratory investigative new drug studies may be of value for this[23]. The 'straight into human' approach might be considered too risky for prototypical compounds[24] as it involves no testing of the actions of the compound in human cells, tissues or organs before progressing to human subjects. By contrast, the animal experiments that are performed with the molecule follow a logical progression from molecular interactions to cellular effects, tissue physiology and finally effects on the whole organism. The quantitative aspects of this, using plasma concentrations and effect measurements, can be integrated using pharmacokinetic–pharmacodynamic models[22,25]. From this data, a dose that is likely to produce a pharmacological effect in humans can be estimated. This is still rarely done. For example, because there is generally no information regarding the plasma concentrations of drugs that produce pharmacological activity in animal models[25], it makes it impossible to predict the concentration–effect relationships in humans.

The 'straight into the organism' approach is also used when determining the potential preclinical toxicology in animal studies. The drug is generally given to two animal species in doses that are sufficiently high to induce toxic effects. A lower dose at which these effects do not occur is then established and this no observed adverse effect level (NOAEL) dose, diminished by a rather arbitrary safety factor, determines the human dose[26]. Moreover, the requirements to determine the pharmacological effects of the drug in the toxicological species are limited to the general requirement for the species to be responsive to the primary pharmacodynamic effect of the substance (see the document from the European Medicines Agency: *Note for Guidance on Repeated Toxicity* (CPMP/SWP/1042/99)).

For biological substances the relevant receptor or epitope has to be expressed in the species. It is nowhere stated in this guidance that this has to be experimentally confirmed in the repeated dose toxicity tests by ascertaining that this also leads to functional or physiological effects that are similar to those in humans. This theoretically allows the use of an animal species for toxicological tests in which the drug functionally lacks its primary biological actions, when for example, the receptor is expressed but has different functional effects in this species than in humans and hence will tell us little about its toxic effects induced by excess pharmacology. At this point it should be recalled that the research of Withering in 1785 was similar.

Tolerability and safety. Unattainable?

An ethical committee should reject protocols for studies in humans for which the methodology does not allow the objectives to be reached. I studied the Phase I studies submitted to the competent authority for clinical trials in the Netherlands in 2009 (the Central Committee on Research Involving Human Subjects). For this assessment the full protocols and investigative medicinal product dossiers of these studies were examined. The system in the Netherlands guarantees completeness of the data set as submission in the national database is required by law.

There were 26 Phase I studies in patients, of which 27% were first administrations of the drug to humans. Most of these studies (65%) were in cancer and virtually all protocols involved highly innovative therapeutic concepts and products. Yet, 85% of all these studies had as their primary objectives the safety and tolerability of the compound and 50% attempted to reach a maximally tolerated dose as its end point. There were 81 studies carried out in 4,754 healthy volunteers, of which 40% were first-in-human studies. All of these studies were performed using only safety and tolerability as the primary end points.

Only in 42% of the studies were biomarkers used at all, although generally they were used as a secondary end point and never as linkage markers. In 60% of the studies in which biomarkers were used at all, considerably more human pharmacology could have been done, either by more frequent assessment of these markers or by more pharmacologically appropriate ones to allow the measurements to be used in decisions about the potential activity of the compound. For example, in a protocol of a highly innovative analgesic compound with a new mechanism of action, no experimental pain model was involved. At high doses serious adverse effects developed in humans but it was unknown how these related to analgesic dosage, producing serious delays in the development of the

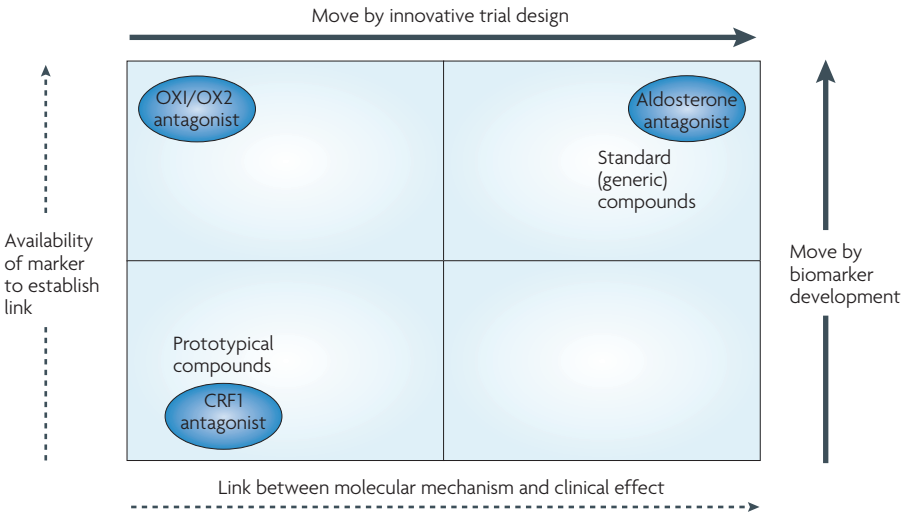

Figure 2 | **Determining the prototypical nature of a project.** An aldosterone antagonist, such as eplerenone, could undergo standard development because the link between the inhibition of the receptor and the clinical effects in heart failure are well known and the methodology to study this is mature. By contrast, a corticotropin-releasing factor 1 (CRF1) antagonist for social anxiety is prototypical because the link between this receptor antagonism and the beneficial effects on the clinical symptoms, as well as the methodology to study it, is not yet validated. The orexin 1 (OX1) or OX2 antagonist described in REF. 39 is an example of a prototype for which the methodology (sleep studies) is well established to show linkage between molecular action and clinical effects.

compound. It was remarkable that even for first-in-class biologicals — for which pharmacodynamic measures are generally an integral part of the first-in-human clinical trial protocol — there was limited evaluation of pharmacodynamic measures.

Virtually all of these first-in-human studies were done with highly innovative prototypical compounds. This situation is consistent with the apparent current state of affairs in early drug development. Even in cases when the human pharmacology of a new compound can be tested it is either not done or postponed to a later stage of development. In the data set that was studied, the objectives of the studies were generally standardized and did not seem to be dependent on whether or not the compound was innovative or to be dependent on the nature of its pharmacological intended activity. Further study of these phenomena in larger data sets is of interest to detect whether this is an international generalization.

Some compounds with excellent tolerability and stated safety profiles are neither safe nor well tolerated in the patient population as a whole and this is only identified late in development or after marketing of the drug. With most of these compounds a safety problem is identified that is relatively infrequent but serious. The probability of detecting this in a typical Phase I study with perhaps 10 to 20 subjects per dose level is too low and the studies are therefore seriously underpowered to detect safety issues[27] of importance when used in the whole patient population taking the drug. Small studies can detect frequently occurring tolerability issues and are useful for this, although such effects are generally pharmacological and are much more easily detected by quantitative measurements, rather than by event rates.

Tolerability can be determined in early drug-development studies but it is questionable whether this is useful (FIG. 4). The assumption is that the therapeutic dose of a medicine is more plausibly related to its pharmacological actions than to its toxic effects. The method of determining the therapeutic dose as a certain fraction of a toxic dose (the maximally tolerated dose) originates from a time when most drugs were relatively toxic (when these measures were probably closer together) and its value has been based on old literature[28,29] relating to classical cytotoxic drugs. Therefore this method only works for drugs with a narrow therapeutic margin for which the toxicity of the compound is linked to the clinically wanted effect. This is the case for the classical cytotoxic drugs because of bone marrow depression (the usual tolerability problem for these drugs) being a marker for an effect of the drug on rapidly dividing cells, but it is not applicable to any other modern medicines. Even the current generation of anticancer drugs is becoming increasingly selective.

The current methods of drug discovery tend to select compounds with a wide therapeutic margin and this renders the maximally tolerated dose method uninformative for such compounds. Although it is important to determine the clinical tolerability of a drug or its maximally tolerated dose, this measure may not be the first or the most important uncertainty that needs to be resolved for a prototypical drug. The relevance of the human toxicology rather than the human pharmacology of a compound is dependent on its perceived therapeutic margin. For example, there would be no disputing that attempting to determine the tolerability of healthy subjects to a penicillin derivative

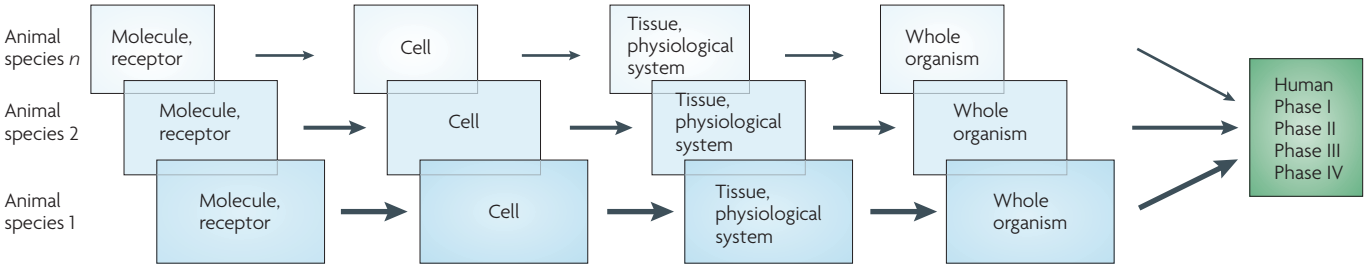

Figure 3 | **Linear non-translational development.** Data are collected for each species but are not connected in a meaningful way. The first introduction of the compound into humans is directly into the whole organism, using tolerability and safety as primary end points, rather than pharmacological or physiological markers. Once the development reaches its intended target species, humans, the experiments become increasingly empirical.

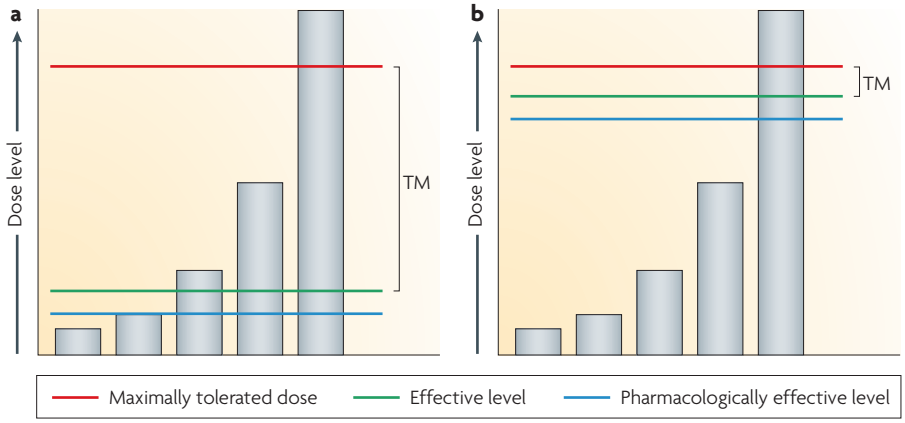

Figure 4 | **Representation of ascending dose study design.** A constant relationship between the pharmacologically active dose and the therapeutic dose is assumed in both situations. **a** | A maximally tolerated dose design is used for a drug with a large therapeutic margin. If a dose for further trials is chosen just under the maximally tolerated dose then overdosing will occur. **b** | The situation for a drug with a narrow therapeutic margin (that is, a classical cytostatic) is shown. The maximally tolerated dose is close to the effective dose level and in such a situation a reasonable estimate of dose is obtained. Note that the effective level is unknown at this early stage of development and if no pharmacological effects are measured only the maximally tolerated dose will determine the dose level in both cases. TM, therapeutic margin.

would be futile. In this case, knowledge of the inhibitory concentrations in a sensitive microorganism, in combination with the plasma or tissue concentrations of the drug, would determine the therapeutic dose.

Expensive lack of pharmacology

The potential danger of the standard approach to drug development can be illustrated using the 5-hydroxytryptamine (serotonin) receptor 4 agonist tegaserod (Zelnorm; Novartis), which was launched in 2001 for the treatment of irritable bowel syndrome and constipation. Early development was done in the standard manner, with attention to safety, tolerability and pharmacokinetics[30,31]. Although the drug was pronounced to be well tolerated and safe following the first studies in humans, it was withdrawn from the market in 2007 because of an increase in cardiovascular events (13 out of 11,614 people taking the drug versus 1 out of 7,013 people on placebo (source: FDA Centre for Drug Evaluation and Research). Although these adverse events might have been the result of some undetected vascular effect of the drug they could also have been the result of chance. The turnover from the drug decreased from US$561 million (growing at 34% per year) in 2006 to $88 million and then to zero. This produced an immediate loss of turnover, only in the first year after discontinuation, of $664 million (data from Astra Zeneca's annual report 2006).

Of the 388 publications about the substance only one dealt with its effect on coronary arteries[32] and no human pharmacological profile was established other than in the target organ, the gut. This was despite the fact that 5-hydroxytryptamine receptor 4 agonists are known to induce the release of vasoactive peptides, such as calcitonin gene-related peptide and substance P. The presence of detailed pharmacological profiling on the cardiovascular system in humans could have played an important part in the evaluation of the safety data and a potential defence against regulatory claims that the increase in cardiovascular events were related to the drug.

It is obviously impossible to say whether the availability of such pharmacological profiling would have made a difference in the final regulatory decisions and there are many other factors, such as the relatively low efficacy of tegaserod, which may have contributed to the final decision about withdrawal of the drug. The fact remains that human pharmacological data were not available and the study on the effect of tegaserod on human coronary arteries (which was negative) only appeared after its discontinuation. The mechanistic aspects of tegaserod in the gut were well studied but the study of collateral pharmacology, in animals and in humans, may have been more important than pharmacokinetics and tolerability. Therefore, such pharmacodynamic data obtained early in the development of a drug may have considerable value in later stages when the benefit–risk ratio of a new compound is finally determined.

When epidemiological data show some sort of increased hazard for drugs on the market, the absence of mechanistic data to show that the drug is not responsible for the adverse event means that the precautionary principles underlying drug regulation allow little else than discontinuation. The presence of mechanistic data may lead to more careful development of the drug in a more select group of patients rather than immediate marketing in large populations. In such a way the pharmacological findings can be studied in detail in the clinic. Such an approach costs time and money but will be more economical in the end when applied to the right (high uncertainty) projects.

Dangerous lack of pharmacology

When a prototypical drug is developed in a standard manner unexpected events may be more likely to occur. The CD28 agonist TGN1412 (developed by TeGenero Immuno Therapeutics) is a good example of a prototypical new drug, resulting from increasing biological knowledge in immunology and the physiology of T cell activation. The link between the molecular effect of the drug on a T cell and the expected clinical effect was not proved and additionally there were no validated biomarkers for this. TGN1412 caused serious damage to six healthy volunteers in a first-in-human trial[33].

TGN1412 was developed in a standard manner with little consideration given to its prototypical nature. Molecular, cellular and whole organism studies were done in several species, but there was little done to connect the findings in a conceptual manner (FIG. 5) in the investigator's brochure of the drug. For example, the amino acid sequence of the receptor that was available in the public databases at the time of the protocol assessment was not homologous between the cynomolgus monkey and humans. This was later shown to be erroneous as the amino acid sequence of the receptor are homologous between the cynomolgus monkey and humans, but the structure of the receptor was only made public after the clinical trial was done and no questions were raised about the lack of homology by either the regulatory agency or the ethics committee overseeing the trial.

Additionally, no *ex vivo* experiments showed similar functional T cell responses in cynomolgus monkeys and humans. In the repeated dose toxicology experiments in monkeys, pro-inflammatory cytokines were

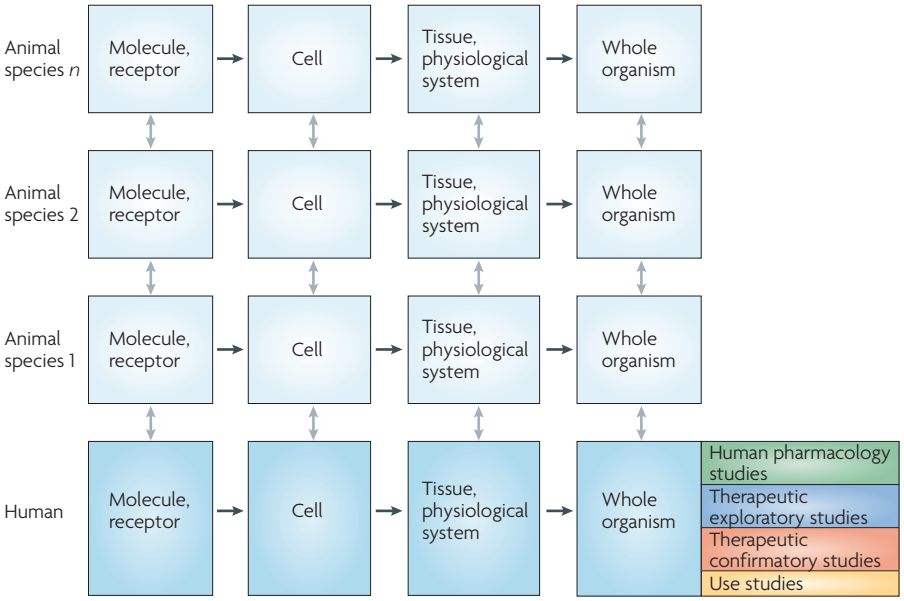

Figure 5 | **Translational drug development.** Black single headed arrows are translational connectors, such as pharmacokinetic–pharmacodynamic models or systems biology models, that allow the quantification of the link between different biological processes. Grey double-ended arrows represent linkage markers. Rather than a linear process a cyclical path can be traced through the process in any direction (in contrast to the usual situation shown in FIG. 3).

measured and, in fact, increased slightly. However, the cytokines interleukin-10 and interferon-γ that are produced by regulatory T cells — the target cell population to be stimulated and increased — were not measured as a primary pharmacodynamic measure. The results from the preclinical tests were presented to the regulatory authority in a simple format without any attempt to integrate the findings obtained in animals, human tissue and cell culture, using concentration–effect modelling (FIG. 5).

The regulatory authority responded with an approval for clinical testing that contained large unchanged sections of the original application. The protocol for this study had just the tolerability and the safety of the compound as its primary objective; T cell and cytokine measurements were considered secondary, unspecified and not included as stopping rules or used as linkage markers. The way the first dose was chosen is exemplified in FIG. 4a.

In toxicological testing, TGN1412 was given to an animal species in which the drug had little effect and as pharmacological effects are not normally included in toxicology experiments the absence of the intended effect remained undetected. Additional data from mice equipped with a human immune system, which showed the human pharmacology of the drug in human tissue and physiology (and incidentally showed the severe depletion of T cells that was seen in the volunteers) was not included in the information submitted to the regulatory authorities[34]. The calculated starting dose was too high as it was based on the NOAEL approach only, disregarding the pharmacological and immunological effects of the protein and its receptor occupancy.

If a more integrated approach had been chosen for this prototypical compound, the first and most obvious link between concentration and effect on T cells could have been made without performing any additional experiments. A calculation of receptor occupancy of the human receptor, in combination with simple pharmacokinetics, showed that the first dose of the drug resulted in >90% receptor occupancy. This was only done after the adverse events occurred. A logical subsequent experiment would then have been an *in vitro* stimulation test on different populations of human lymphocytes, in comparison with, for example, the lymphocytes of the cynomolgus monkey, but this was not done. The investigating committee[35] that dealt with the clinical trial eventually performed additional experiments in human and primate cells and in primate models, thus providing further linkage markers albeit too late for the subjects who suffered from severe side effects.

Failure to recognize that TGN1412 was not a standard compound may have been the overall cause for the tragic sequel for the subjects in this trial. The trial of TGN1412 is a reminder that standard development of a prototype has the potential to seriously harm humans and destroy a prototypical compound that may still be of important therapeutic value.

Sensible scientific objectives

There is no doubt that knowledge about the tolerability and safety of a drug is paramount. However, I contend that it is impossible to estimate this reliably in early development as the only parameter to determine go/no-go decisions about development. The fact that a compound is well tolerated is of importance, but only if this occurs at a dose or concentration level that is likely to produce pharmacological effects and even then this may not be

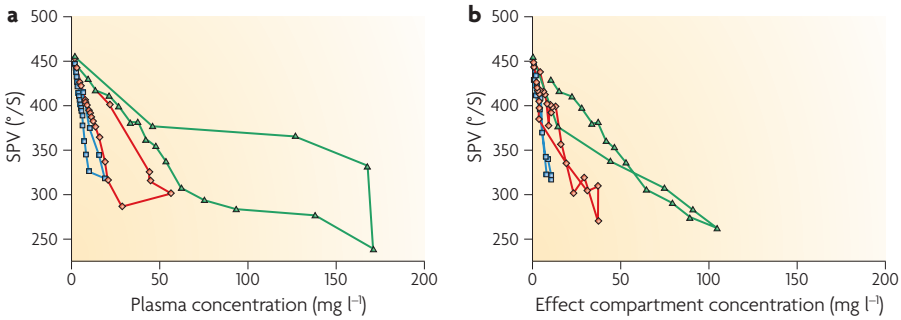

Figure 6 | **A first-in-human pharmacology experiment.** Average effects on a physiological measure of attention, calculated as the saccadic peak velocity (SPV), in response to concentrations of an experimental benzodiazepine Ro 48–8684, measured the plasma concentration (**a**) and the effect compartment concentration (brain) (**b**) in two doses (represented by blue squares and red diamonds) and the positive control midazolam (represented by green triangles). These data produce a dose–response curve for the desired effect in humans and immediately lead to a range of clinically active doses. The data were obtained during the first administration to humans with pharmacological measures as its primary objective. Figure is reproduced, with permission, from REF. 46 © (1997) John Wiley & Sons.

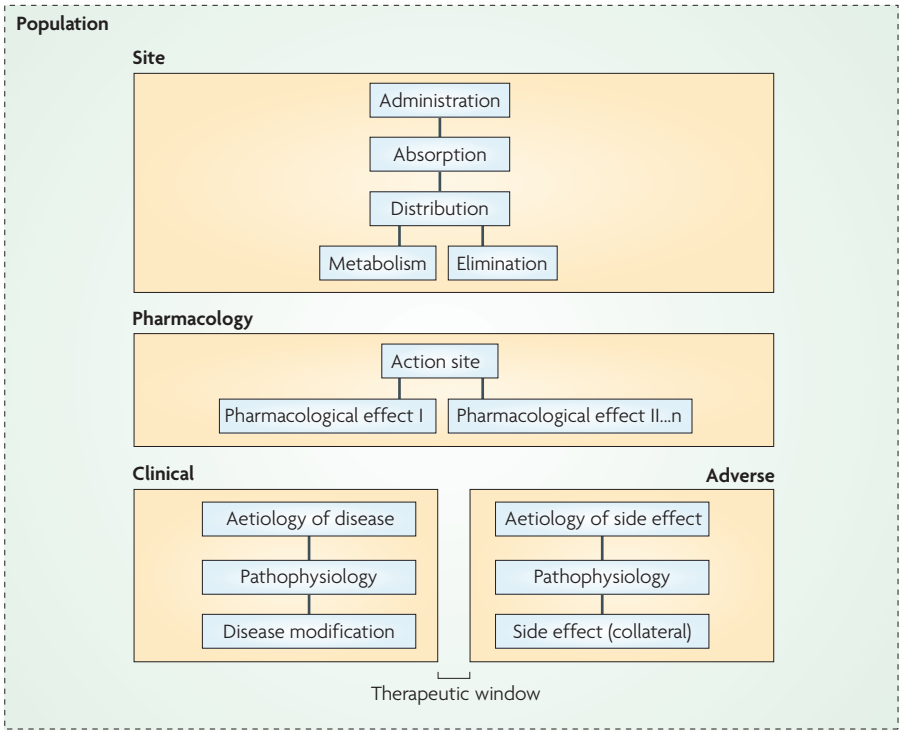

Figure 7 | **A schematic to determine the objectives using the question-based approach.** Six areas of interest can be recognized; population, site, pharmacology, clinical, adverse and therapeutic window. For example, the site group of questions deal with whether the drug can reach its site of action. Biomarkers can be pharmacokinetic measurements in blood or in other body fluids but it can also be positron emission tomography scans of the brain. Pharmacological questions are answered by the most directly related marker of activity on a receptor system or enzyme. Clinical questions attempt to study the relation between modification of pathophysiology and 'feelings, function or survival'.

generally true as the duration of treatment or the condition of the subjects may hide important tolerability problems in other populations. Conversely, non-tolerance at a dose that exceeds the effective dose by far (as was the case for TGN1412) is only relevant for the determination of a safety range or a therapeutic index, but this can only be done with considerable risk for the subject. Especially when the drug has a large safety margin, unrealistically high doses have to be given to reach the tolerability level.

If tolerability and safety in isolation are not sensible primary objectives for an early study, what are? It is plausible that a therapeutic dose of any medicine is in fairly close relation to a dose (or plasma concentration) that causes its intended pharmacological or physiological effect. Therefore, if a good measure of the pharmacological action of a drug can be included early in prototypical development, this will be more useful in determining the range of doses that are likely to be active. The results can then also be used to confirm the minimally anticipated biologically effective level from the animal experiments and to confirm and validate pharmacokinetic–pharmacodynamic models.

In addition, the early discovery of the potentially irrelevant effects of a drug at unrealistically high doses may produce unnecessary concerns about a prototypical drug. Importantly, the safety of the subjects in the clinical trial is much less a concern if there is no need to reach levels at which clinical tolerability is compromised.

The concept of CD28 superagonism — that is produced by TGN1412 — is potentially of great value in immunology and may well be explored again in the future. The TGN1412 trial showed an old principle stated by the sixteenth century Swiss chemist Paracelsus, that everything becomes toxic at a sufficiently high dose.

Translational development

Assuming that animal pharmacological data are already available, it would seem plausible to set up the same sequence of molecule to cell to tissue to organism for humans (FIG. 5). In this schematic view, the translational development data are systematically connected in a knowledge management system in which as many links between the different items are established. Using techniques such as pharmacokinetic–pharmacodynamic modelling or systems biology, also allows quantitative links to be established that facilitates the prediction of effects and pharmacokinetics across species. Such a system is not unidirectional anymore and allows a translational path to be traced forwards and backwards to learn and subsequently to confirm findings in another species or situation.

At the Centre for Human Drug Research, Leiden, the Netherlands, such experiments are performed regularly and FIG. 6 shows an example of data obtained from a first-in-human pharmacology experiment in which intensive pharmacodynamic data were obtained in order to get immediate quantitative information about the drug action of a new rapidly acting benzodiazepine. Additionally, its concentration–effect relationship could be determined by comparing it with a relevant positive control. Such data from intensive first-in-human experiments have now been performed at the centre with most classes of central nervous system drugs and also with anti-inflammatory drugs in asthma, diabetes and hypertension[36,37].

Integrated translational development

If the integrative approach presented in FIG. 5 had been undertaken for TGN1412 before the compound was given to human volunteers, the findings from different animal species would have been viewed in the context of the human immune system in a scientifically cohesive manner. This may have led to adequately validated linkage markers; for example, of the effect and binding of TGN1412 to human lymphocytes. This assay could have been safely done using blood samples from the human subjects and would have led to a pharmacological determination of the dose. Moreover the assay could also have been used in different species to investigate their differences. The preclinical development of such assays would have probably directed attention to the necessity of the calculation of receptor occupancy and may have led to a considerably lower choice for the starting dose, as determined by this assay rather than the fraction of the NOAEL, and guidelines for terminating the study or modifying the dose increments would then have been dictated by blood and cell biomarker changes. This is how modern drug development for a prototypical compound should be done.

One of the reasons why this approach is not common practice is that current organizational structures are directed towards standard development; adopting a prototypical approach may require different organizational structures and training of project leaders. Collecting and displaying the research data according to a structure as indicated in FIG. 5 also have several other advantages. It would greatly assist any authority that has to review data from prototypical projects; as indicated earlier, a structured analysis and display of the risks in a first-in-human study will increase the safety of the subjects[24]; and the early data on the intensive development of drugs will show much earlier that the drug works biologically in humans. Even though the measurements of pharmacological effects are not proof of clinical principle, they are proof of pharmacological principle and this by itself may create enthusiasm for further funding and therefore value.

This is illustrated by a project with an orexin antagonist intended as a hypnotic. This antagonist was developed based on the knowledge that deficient orexinergic function leads to narcolepsy in animals and humans. However, there was no previous data proving the link between sedation and blockade of the orexin receptor, but methodology to test this was well validated in earlier studies[38]. This led to a well-coordinated sequence of studies in animal tissues and humans using a range of linkage markers of hypnosis. In the first administration to humans the effects on sleep and sedation could be established in relation to a positive control, zolpidem[39]. This led to immediate identification of the effective dose range.

This sequence of studies indicates well how early addition of information in first-in-human studies can add value by reducing uncertainty beyond the tolerability in humans. This study was also unique in that it immediately faced the comparison with existing therapies and showed potential superiority. The orexin antagonist that was developed in this modern manner was licensed out immediately after the human pharmacology studies for a considerable amount, showing the financial value of the early reduction of uncertainty.

Question-based development

The potential advantage of the traditionally four-phased approach to drug development is that it provides guidance to the planning of the clinical development for any new drug. However, it can be predicted that many of the future potential medicines will be increasingly prototypical and this means no guidance can be obtained from previous experience with similar projects. If a standardized approach will not work, what will?

Any research project or programme starts with the formulation of a set of questions and this approach has been used to design a structured system for the evaluation of new medicines[40]. Question-based drug development makes use of the logical progression of questions as shown in FIG. 7. A new drug has to reach its site of action, will affect a system by its pharmacology, thus affecting (patho)physiology and finally modifying the disease in a certain defined population. A compound may also have unwanted effects (that are also pharmacological[41]) for which the same criteria apply. Such a system can be used to design a set of research questions and to investigate whether the biomarkers and specific linkage markers for answering the questions are validated. This can be done at an early stage of the development and provides insights into what information has to be collected, as well as the methodology that has to be developed.

Essential questions that cannot be answered are a clear representation of the development risk. As stated earlier, a prototypical development project adds much more value to the product by adding information to resolve existing uncertainties rather than by reducing time to market or cost by just performing a standard trial more rapidly. There are still many standard-type drugs in development and so determining how prototypical a project is, is essential to determine the type of project management required. The question-based approach can be combined with advanced decision analysis techniques to determine the added value of obtaining information in the project versus the potential loss of time and the increased costs this may entail[40].

The next chapter of drug development

The increasing knowledge about biology has already led to an explosion of potential drug targets. These may not have led to the explosion in profitable products the pharmaceutical industry promised its shareholders but the signs of a wave of new medicines with entirely new mechanisms of action are already there[42]. Many of these mechanisms will not be in the usual realm of the pharmacologist. For example, the tenth edition of a standard pharmacology textbook[43] devotes 180 pages to the autonomic nervous system (a chief source of 'me too' products) and a meagre 28 pages to the immune system, in which many of the major advances are being made. This means that the uncertainties in many of the projects will increase and dealing with these unknowns requires a radically different approach. Training individuals who can lead these prototypical projects is perhaps the most important step to be taken[44]. Recognizing when the projects are prototypical is the next step, and this will be made considerably easier when done by specifically trained individuals. Development of methodology to deal with the uncertainties will then follow.

Determining whether such an approach would have kept tegaserod on the market is impossible, but the wave of prototypical compounds will probably grow and we need to be prepared for it through the appropriate systems of knowledge management, the investment in alternative techniques beyond counting side effects and the training of researchers to deal with the complicated information. Some of the disasters that have happened when traditional drug development was applied to innovative projects should help us in designing these properly. In doing so we would finally do justice to the simple country doctor from Shropshire who predicted it all.

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1. William Withering. *An Account of the Foxglove and its Medical Uses* (Oxford Univ. Press, London, 1785).
2. Ng, R. *Drugs. From discovery to approval* 2nd edn (John Wiley & Sons, Hoboken, New Jersey, 2009).
3. Rang, H. P. *Drug Discovery and Development*. 1st edn (Churchill Livingstone, Elsevier, Philadelphia, 2007).
4. Cross, J. *et al*. Postmarketing drug dosage changes of 499 FDA-approved new molecular entities, 1980–1999. *Pharmacoepidemiol. Drug Saf.* **11**, 439–446 (2002).
5. Bhogal, N. & Combes, R. TGN1412: time to change the paradigm for the testing of new pharmaceuticals. *Altern. Lab. Anim.* **34**, 225–239 (2006).
6. Clark, R. W. *et al.* Raising high-density lipoprotein in humans through inhibition of cholesteryl ester transfer protein: an initial multidose study of torcetrapib. *Arterioscler. Thromb. Vasc. Biol.* **24**, 490–497 (2004).
7. Zhao, L., Jin, W., Rader, D., Packard, C. & Feuerstein, G. A translational medicine perspective of the development of torcetrapib: does the failure of torcetrapib development cast a shadow on future development of lipid modifying agents, HDL elevation strategies or CETP as a viable molecular target for atherosclerosis? A case study of the use of biomarkers and translational medicine in atherosclerosis drug discovery and development. *Biochem. Pharmacol.* **78**, 315–325 (2009).
8. US Food and Drug Administration. Note for guidance on general considerations for clinical trials. *Fed. Regist.* **62**(242), 66113–66119 (1997).
9. Lenfle, S. & Loch, C. Lost roots. How project management settled on the phased approach (and compromised its ability to lead change in modern enterprises). *Ecole Polytechnique website* [online], http://crg.polytechnique.fr/fichiers/crg/publications/pdf/2010-03-06-1630.pdf (2009).
10. Loch, C., DeMeyer, A. & Pich, M. *Managing the unknown. A new approach to managing high uncertainty and risk in projects*. (John Wiley & Sons, Hoboken, New Jersey, 2006).

11. Shuchman, M. Commercializing clinical trials-risks and benefits of the CRO boom. *N. Engl. J. Med.* **357**, 1365–1368 (2007).
12. Bresalier, R. S. *et al.* Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N. Engl. J. Med.* **352**, 1092–1102 (2005).
13. Garnier, J. P. Rebuilding the R&D engine in big pharma. *Harv. Bus Rev.* **86**, 68–70, 72–76, 128 (2008).
14. Cuatrecasas, P. Drug discovery in jeopardy. *J. Clin. Invest.* **116**, 2837–2842 (2006).
15. Munos, B. Lessons from 60 years of pharmaceutical innovation. *Nature Rev. Drug Discov.* **8**, 959–968 (2009).
16. Lehman Brothers. *The fruits of genomics. Drug pipelines face indigestion until the new biology ripens*. (Lehman Brothers, New York, 2001).
17. US Food and Drug Administration. Challenge and opportunity on the critical path to new medical products. *FDA website* [online], http://www.fda.gov/downloads/Drugs/ScienceResearch/ResearchAreas/ucm079290.pdf (2004).
18. Loch, C., Mihm, J. & Huchzermeier, A. Concurrent engineering and design oscillations in complex engineering projects. *Concurrent Eng.* **11**, 187–199 (2003).
19. Allen, T., Thusman, M. & Lee, D. Technology transfer as a function of position in the spectrum from research through development to technical services. *Acad. Manage. J.* **22**, 694–708 (1979).
20. Roussel P. A., Saad, K. N. & Erickson, T. J. *Third generation R&D: Managing the Link to Corporate Strategy* 1st edn (Harvard Bus School Press, Boston, Massachusetts, USA, 1991).
21. Sheiner, L. B. Learning versus confirming in clinical drug development. *Clin. Pharmacol. Ther.* **61**, 275–291 (1997).
22. Danhof, M., Alvan, G., Dahl, S. G., Kuhlmann, J. & Paintaud, G. Mechanism-based pharmacokinetic–pharmacodynamic modeling-a new classification of biomarkers. *Pharm. Res.* **22**, 1432–1437 (2005).
23. FDA Center for Drug Evaluation and Research. Exploratory I.N.D. Studies. *FDA website* [online], http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078933.pdf (2006).
24. Kenter, M. J. & Cohen, A. F. Establishing risk of human experimentation with drugs: lessons from TGN1412. *Lancet* **368**, 1387–1391 (2006).
25. Cohen, A. Pharmacokinetic and pharmacodynamic data to be derived from early-phase drug development: designing informative human pharmacology studies. *Clin. Pharmacokinet*. **47**, 373–381 (2008).
26. FDA Center for Drug Evaluation and Research. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Guidance for Industry. *FDA website* [online], http://www.fda.gov/do = wnloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf (2005).
27. Cohen, A. Should we tolerate tolerability as an objective in early drug development? *Br. J. Clin. Pharmacol.* **64**, 249–252 (2007).
28. Schein, P. S. *et al*. The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. *Clin. Pharmacol. Ther.* **11**, 3–40 (1970).
29. Freireich, E. J., Gehan, E. A., Rall, D. P., Schmidt, L. H. & Skipper, H. E. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother. Rep.* **50**, 219–244 (1966).
30. Zhou, H. *et al.* Effect of meal timing not critical for the pharmacokinetics of tegaserod (HTF 919). *J. Clin. Pharmacol.* **39**, 911–919 (1999).
31. Appel, S., Kumle, A., Hubert, M. & Duvauchelle, T. First pharmacokinetic–pharmacodynamic study in humans with a selective 5-hydroxytryptamine4 receptor agonist. *J. Clin. Pharmacol.* **37**, 229–237 (1997).
32. Chan K. Y. *et al.* Functional characterization of contractions to tegaserod in human isolated proximal and distal coronary arteries. *Eur. J. Pharmacol.* **619**, 61–67 (2009).
33. Farzaneh, L., Kasahara, N. & Farzaneh, F. The strange case of TGN1412. *Cancer Immunol. Immunother.* **56**, 129–134 (2007).
34. Legrand, N. *et al.* Transient accumulation of human mature thymocytes and regulatory T cells with CD28 superagonist in "human immune system" *Rag2*$^{(-/-)}\gamma_c^{(-/-)}$ mice. *Blood* **108**, 238–245 (2006).
35. Expert Scientific Group on Phase One clinical Trials. *Final Report* (The Stationary Office, Norwich, UK, 2006).
36. de Visser, S. J. *et al*. Concentration-effect relationships of two rilmenidine single-dose infusion rates in hypertensive patients. *Clin. Pharmacol. Ther.* **72**, 419–428 (2002).
37. van der Post, J. P., de Visser, S. J., Schoemaker, R. C., Cohen, A. F. & van Gerven, J. M. Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. *J. Psychopharmacol.* **18**, 221–227 (2004).
38. de Visser, S. J. *et al*. Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br. J. Clin. Pharmacol.* **55**, 39–50 (2003).
39. Brisbare-Roch, C. *et al.* Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nature Med.* **13**, 150–155 (2007).
40. de Visser, S. J. *A question based approach to drug development*. Thesis, Leiden Univ. (2003).
41. Aronson, J. K. & Ferner, R. E. Joining the DoTS: new approach to classifying adverse drug reactions. *BMJ* **327**, 1222–1225 (2003).
42. Franson, K. L. & Cohen, A. F. How it works. *Br. J. Clin. Pharmacol.* **68**, 315–317 (2009).
43. Brunton, L., Blumenthal, D., Buxton, I. & Parker, K. *Goodman and Gilman's Manual of Pharmacology and Therapeutics* (McGraw Hill Medical, USA, 2006).
44. Dollery, C. T. Clinical pharmacology in the molecular era. *Clin. Pharmacol. Ther.* **83**, 220–225 (2008).
45. Aronson, J. *An account of the foxglove and its medical uses, 1785–1985*. (Oxford Univ. Pres, London 1985). *Clin. Pharmacol. Ther.* **83**, 220–225 (2008).
46. van Gerven, J. M. *et al.* Integrated pharmacokinetics and pharmacodynamics of Ro 48–8684, a new benzodiazepine, in comparison with midazolam during first administration to healthy male subjects. *Br. J. Clin. Pharmacol.* **44**, 487–493 (1997).

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Competing interests statement
The author declares no competing financial interests.

FURTHER INFORMATION
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