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The use of preclinical pharmacokinetic and pharmacodynamic data to predict clinical doses: current and future perspectives

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Abstract

The obtainment of appropriate pharmacokinetics and pharmacodynamics is critical to achieving an efficacious and safe clinical dose range. Therefore, the combination of pharmacokinetic and pharmacodynamic considerations at the preclinical discovery stage should lead to drugs with optimum performance characteristics in the clinic. Indeed, the pharmacokinetic phase, incorporating the absorption, distribution and clearance of the compound can have a profound impact on the in vivo potency, duration and selectivity of the compound being tested. Human pharmacokinetic parameters can be predicted from preclinical pharmacokinetic and metabolism data, and thus, human oral exposure of pharmacologically active free drug can be estimated. The potential variability in human oral exposure, due to factors such as variable absorption and polymorphic enzymes, can also be predicted. The potency of a compound against the target receptor can be determined from values such as K_i, pA_2, EC_{50}, etc. However, in order to turn such a value into an appropriate target efficacy concentration, it is necessary to know the degree of receptor occupancy required to exert an effect. It is known that the required receptor occupancy can vary considerably from as little as 20% for agonists to close to 100% for enzyme inhibitors. If these issues can be resolved dose projections for efficacy can be made, from anticipated oral exposure and knowledge of the occupation of the target receptor, to guide dose selection for initial clinical dose escalation studies. In a similar manner, safety and toleration can be predicted from the potency of the compound against other receptors demonstrated in broad ligand binding studies.

Keywords: Free drug theory; Extrapolation of pharmacokinetics; Clearance; Pharmacological potency; Receptor occupancy

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1. Major factors in dose predictions from preclinical data

The ultimate goal of pharmaceutical companies is to develop novel therapeutic agents for the treatment of diseases. The drug discovery and development process is scientifically complex and full of risk, and is, therefore, expensive and time-consuming. Typically, the cost of discovery and development of a new chemical entity (NCE) is hundreds of millions of dollars and requires a decade or longer to reach the market place. A significant factor in the cost and success of bringing NCEs onto the market is their high attrition rates in preclinical and clinical development. In a study by DiMasi [1] the reasons for termination of 1099 investigational new drug (IND) candidates between 1964 and 1989 were explored. The main reasons for failure were unacceptable efficacy, which accounted for 46% of candidates, and safety issues, which were responsible for termination of 23% of INDs. In another study [2], a similar trend was observed with 39% and 30% of 198 NCEs in clinical development being dropped due to inappropriate pharmacokinetics and lack of efficacy, respectively. Indeed, inappropriate pharmacokinetics was also a major factor in the discontinuation of 39% of NCEs in clinical development in an earlier analysis by Prentis et al. [3]. These studies show the need for candidate and dose optimisation to start early in the drug discovery process and, in particular, the need to target pharmacokinetic and pharmacodynamic issues.

Therefore, the main role of preclinical pharmacokinetics and pharmacodynamics in drug discovery and development is to optimize candidate selection for the target therapeutic area, taking into consideration the type of agent required, and to predict the dose and dosing regimen for initial clinical trials with due concern to the requirements for effective treatment in the target therapeutic area. In order for this approach to be successful, a clear understanding is required for both the pharmacological target and drug disposition (absorption, clearance and distribution) of NCEs [4,5]. A fundamental tenet in linking the pharmacokinetic and pharmacodynamic phases is that free drug in the systemic circulation is in equilibrium with the receptors. In the pharmacokinetic phase, only free drug can be cleared, and drug is reversibly bound to tissues and blood [6]. The pharmacodynamic phase is further subdivided into the interaction with the drug receptor triggering post-receptor events, eventually leading to actual drug effect. In this phase, only free drug can exert pharmacological effect [7] and the free concentration of drug in plasma is in direct equilibrium with the intestitial fluid bathing most cells, since the capillary wall contains sufficient aqueous pores to allow the rapid passage of relatively small molecules, regardless of physicochemistry. Most receptor targets are accessed extracellularly. We can, therefore, expect that all drugs regardless of physicochemistry will be in direct equilibrium, at these targets, with free drug in plasma.

To examine the validity of this model data from a number of G-protein, coupled receptor antagonists working at non-CNS sites (antimuscarinics, antihistamines, β-adrenoreceptor blockers, etc) were examined [8]. Potency values from in vitro pharmacology studies (K_b, pA_2, etc) were compared with steady-state free drug concentrations at clinically efficacious doses. A 1:1 relationship was observed (Fig. 1), which demonstrates that the free concentration present in plasma is that actually seen at the receptor. Using these principles, the potential impact of the pharmacokinetic phase, incorporating absorption, distribution and clearance, on in vivo potency, duration of action and

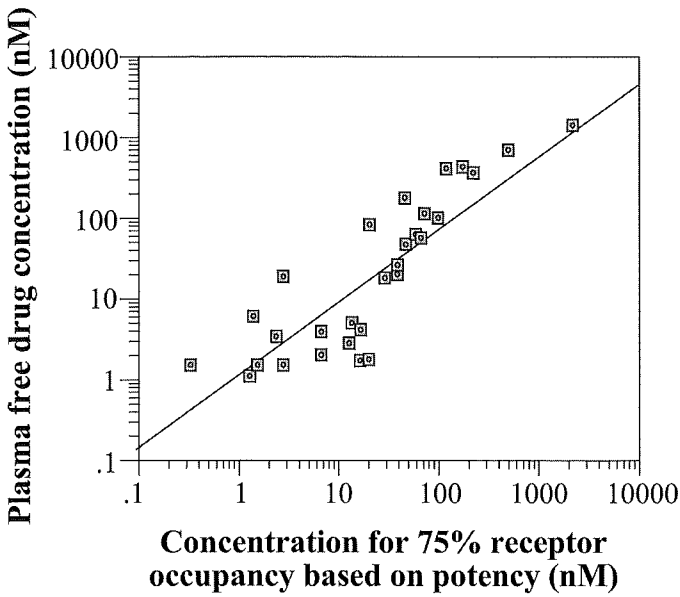

Fig. 1. Correlation of in vitro measure of potency with plasma-free drug concentrations required for efficacy for a number of receptor antagonists.

selectivity can be estimated. In fact, clearance is the key parameter, which governs exposure (AUC, area under the curve) of unbound drug and in the case of hepatic clearance, unbound intrinsic clearance (Cl_{iu}) determines the exposure to free drug following oral dosing assuming complete absorption (Eq. 1):

$$AUC_u = (Dose \times F)/Cl_{iu} \quad (1)$$

Therefore, it follows that unbound intrinsic clearance determines the required dose to provide adequate exposure of free drug when expressed as AUC_u (Eq. 2). AUC_u can be rewritten as C_{ss} (target free concentration) $\times$ T (dosing interval) (Eq. 3):

$$Dose = Cl_{iu} \times AUC_u \quad (2)$$

$$Dose = Cl_{iu} \times C_{ss} \times T \quad (3)$$

It follows that human clearance is a key pharmacokinetic parameter that impacts upon anticipated human dose. Therefore, optimizing human clearance, in order to have the most appropriate clinical dosing regimen, is a key focus in preclinical drug discovery.

2. Extrapolation of pharmacokinetics across species

Two fundamental challenges in the extrapolation of pharmacokinetics is how to "scale-up" the pharmacokinetic data from animals to humans and how to extrapolate in vitro data

to the in vivo situation. Data from animal and in vitro studies is extrapolated to humans by using appropriate pharmacokinetic principles, but the extrapolation is far from straightforward [9,10]. The difficulty in extrapolation lies in the many intrinsic differences in physiology between animals and humans as well as differences in the disposition of drugs (particularly clearance) between animals and humans. Two main methods are used in extrapolation of pharmacokinetics from preclinical studies to the human situation: allometric scaling and physiological-based pharmacokinetic (PBPK) scaling. As previously discussed, clearance is the key parameter that governs oral exposure, and thus, dose size. It is known that the physiochemical properties of drugs are a key determinant in whether renal or hepatic clearance predominates [4,10], and knowledge of the clearance mechanism is a deciding factor in which method of extrapolation to use.

Allometric scaling is the best described technique to predict human pharmacokinetics from in vivo preclinical pharmacokinetic data [11–13]. It is based upon the premise that many physiological parameters such as hepatic blood flow, glomerular filtration, cardiac output and species organ weights are a function of the size of the animal species (body weight). Therefore, it follows that the major pharmacokinetic parameters such as clearance, volume of distribution and half-life should be related to the size of the animal. Allometric scaling has been shown to be an accurate technique for prediction of human renal clearance from preclinical clearance data [5,14–16], which is based on the fact that glomerular filtration scales allometrically with body weight. Fluconazole is a moderately lipophilic (log P 0.6), neutral compound with minimal metabolic clearance. Consequently, renal clearance accounts for at least 90% of total clearance, and total clearance is less than glomerular filtration rate (GFR) in all species. Therefore, there is a good allometric relationship for renal and total clearance with body weight across the species [17]. An additional benefit of a high degree of renal clearance is that there is low variability in human clearance values ranging from 1.5-fold in healthy volunteers [18] to 4-fold in AIDS patients [19]. In all cases, fluconazole clearance varied in line with variations in GFR. In comparison variations in plasma clearance for metabolically cleared drugs can vary up to 10-fold for CYP3A4, e.g. felodipine [20], triazolam [21], and for polymorphic enzymes such as CYP2D6, clearance can vary 10- to 100-fold, e.g. tolterodine [22] and UK-84,149 [23].

Allometric scaling has also been used to predict hepatic metabolic clearance [13,24,25]. However, the simple allometric approach of correlating metabolic clearance, with body weight across the species, does not adequately predict metabolic clearance in many cases. A major drawback in allometric scaling is its empirical nature. The simple allometric approach for metabolic clearance does not allow for an understanding of species differences in pathways of metabolic clearance and isoenzyme involvement that may have a significant impact on the ability to accurately extrapolate human clearance from preclinical data. However, several publications have proposed novel methods of combining allometric scaling with knowledge of species differences in metabolism derived from in vitro metabolism data to improve utility of allometry for scaling metabolic clearance. Lavé et al. [26] showed that this approach worked well for a range of compounds mainly metabolised by CYP enzymes with a worst case, 2-fold underprediction of human clearance after integration of in vitro metabolism data into allometric scaling from animal pharmacokinetics. In another example, Ward et al. [27] showed that incorporation of a

correction factor for bile flow and microsomal UDP-glucuronosyltransferase activity into the clearance value in each species improved the allometric relationship ($r^2 = 0.96$) for SB-265123 versus the simple allometric approach ($r^2 = 0.71$). Other correction factors to the simple allometric equation, such as maximum life-span potential, brain weight, protein binding, have been used in prediction of human clearance with success for some compounds [24,28].

PBPK scaling is another method which is commonly employed to predict human clearance. Methods for PBPK scaling vary in complexity. Physiological flow models are developed in laboratory animals, and then scaled up to make predictions for human drug disposition. Such models need to take into account blood flow to eliminating organs; tissue and fluid volumes; blood-to-plasma and tissue-to-plasma drug concentration ratios; drug protein binding and enzyme kinetics [24,29,30]. However, given the practical, economic and ethical considerations of performing in vivo pharmacokinetic studies in several animal species to predict human clearance for each compound of interest, a more simplified approach tends to be used in industry. Predictions of human clearance are usually made using intrinsic clearance in one animal species corrected for in vitro metabolism rates in animals and human, or extrapolating directly from in vitro metabolism data to human in vivo clearance [31–33]. One of the simplest approaches is to use the substrate depletion or in vitro $t_{1/2}$ method in human liver microsomes or hepatocytes to calculate in vitro intrinsic clearance (Cl_{int}). This Cl_{int} value is then scaled-up to reflect Cl_{int} in vivo and inserted into a model of hepatic extraction to give a human clearance estimate. Obach [34] demonstrated that this method worked very well for a range of basic compounds, known to be metabolised by various CYP enzymes, in human liver microsomes with an average fold error of 1.37 (predicted Cl_{blood}/actual Cl_{blood}). The human clearance prediction was less accurate when basic, acidic and neutral compounds were considered in total with a fold error of 2.28. Nevertheless, such a method would still be a useful guide to the anticipated human clearance, which would allow subsequent estimates of dose and duration of action to be made. Lavé et al. [35] have showed that a similarly good human clearance prediction could be made from in vitro Cl_{int} using human

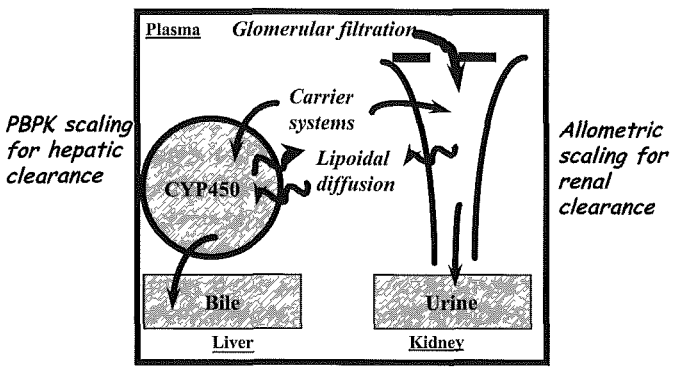

Fig. 2. Schematic representation of the main clearance mechanisms in humans and the preferred methods for extrapolation of pharmacokinetics.

hepatocytes, which provides the added benefit of incorporating phase (I) and (II) metabolism. In addition, hepatocytes can be used to predict non-metabolic clearance as demonstrated in rat hepatocytes for the non-metabolised compound pravastatin. Hepatic clearance of pravastatin had been shown in a number of studies to be mediated by hepatic uptake as the rate-determining step [36,37]. Therefore, Yamazaki et al. [38] demonstrated that extrapolation of in vitro Cl_{int} (from V_{max}/K_m) for active hepatic uptake of pravastatin in rat hepatocytes gave an estimated in vivo clearance (75 ml/min/kg), which is very similar to the actual clearance (83 ml/min/kg).

Therefore, the two main clearance mechanisms, renal and hepatic clearance, that account for the clearance of the vast majority of drugs, can be extrapolated from animals to humans using allometric scaling and PBPK scaling. The allometric approach works very well for renal clearance, whereas, hepatic clearance is more accurately predicted using PBPK scaling (Fig. 2). The traditional approach of extrapolation of pharmacokinetics from in vivo animal pharmacokinetics is gradually being refined and, in some cases, replaced by extrapolation from human in vitro clearance data only.

3. Relating dose to potency and pharmacokinetics

The importance of clearance for dose predictions results in this parameter, being a key focus of pharmacokinetic predictions from preclinical studies in order to guide dose selection for initial clinical trials. In addition to clearance, the pharmacological potency of the compound is also fundamental in governing dose size. Therefore, from the relationship defined earlier ($\text{Dose} = Cl_u \times C_{ss} \times T$), it can be seen that the pharmacokinetic requirements in a candidate are dependent upon its potency. For a range of 7-TM receptor antagonists, it has been shown that free drug concentrations need to be sufficient for 75% receptor occupancy ($3 \times IC_{50}$) in order to achieve clinical efficacy [4,8]. Therefore, based upon the general approach that pharmacological activity requires an average free drug concentration of $3 \times IC_{50}$ (or equivalent measure of potency), the two fundamental properties of intrinsic clearance and potency can be related to dose requirement. Given that dosing regimens for most therapeutic areas are <200 mg b.i.d., it can be seen that for a compound series with inherent high intrinsic clearance, high potency is needed in order to achieve an acceptable clinical dose size. A good example of high intrinsic clearance compounds are lipophilic, high molecular weight (MW) carboxylic acids. Montelukast, a leukotriene D_4 receptor antagonist, is a lipophilic, high MW carboxylic acid which has a low total human plasma clearance (0.5 ml/min/kg), but because of its likely high plasma protein binding ($\sim 99.99\%$), intrinsic clearance is estimated to be very high (~ 5000 ml/min/kg) [39]. However, montelukast displays very good in vitro potency (K_i 0.18–4 nM) against the leukotriene D_4 receptor [40]. Consequently, the clinical dose of montelukast is only 10 mg, which is driven by the excellent potency of the compound. However, the very high protein binding displayed by lipophilic carboxylic acids (typically $>99.9\%$) is difficult to accurately determine with standard analytical methods. This presents an interesting problem in preclinical studies, making it difficult to correlate free drug concentrations with in vitro potency for such compound series. However, plasma protein binding can be generally determined and free drug concentrations can be correlated with

potency. For example the β-receptor antagonists display a good correlation between in vitro potency (K_b) and unbound steady state plasma concentrations at clinical doses [7]. Indeed, receptor occupancy was constant at ~80% for all the β-blockers regardless of the wide variation in doses used.

4. Conclusion

A key strategy in reducing attrition in clinical development is to target pharmacokinetic and pharmacodynamic issues early in drug discovery. A variety of methods can be used to extrapolate pharmacokinetics, in particular intrinsic clearance, from preclinical studies to human. Thus, the two fundamental properties of intrinsic clearance and potency can be related to predict a human dose for early clinical studies. Optimization of pharmacokinetic extrapolation techniques, in particular in vitro–in vivo scaling would allow more use of in vitro techniques for clearance predictions. A good understanding of the pharmacokinetic and pharmacodynamic issues of drugs is key to confidence in this prediction.

Appendix A. Discussion 1

A. Bye: The task of dose selection in man, in Glaxo Wellcome, falls to the Clinical Pharmacology group in conjunction with its many partners. By the time we are talking about dose, we have gone past the pure research phase and have identified a disease target, some lead molecules and from these one or more drug candidates. Selection of the onward progression of an optimal dose is critical. We need to avoid downstream attrition. Too low a dose range misses an effect, too high can attract inappropriate toxicity, which is difficult to reverse in perception, no matter what future doses may be. Historically, all that was required of Clinical Pharmacology was to show safety of a drug candidate. The actual dose selection was left to the clinical experience in patients. Carl Peck and many of the delegates present in this symposium in the early 1990s, showed that this approach over-estimated the dose and adjustments (usually downwards) came later. Data presented showed that many (>40%) of drugs failed because of pharmacokinetics and toxicity (about 30%), with the balance coming from a variety of commercial issues. The conclusion was that wise use of PK/PD, focussed pre-clinical data and a "learn/confirm" model would drastically improve the situation. It is gratifying to see that from our own company, which invest heavily in PK/PD, we have no PK-based dosing problem in the clinic. What has happened though (post 1992) is that the top slot is taken by Toxicology. Also, with the emphasis on preceded mechanisms, differentiation of products or achieving the expected product profile have become a major issue, accounting for attraction. Why is this?

Case history 1. For a drug like lamivudine, we had novelty, a clear mechanism of action and a good PK/PD relationship. To date, no changes have been made by any authority to the recommendations made by Glaxo Wellcome. Phase IV has added special group dosing and so on. The major item for discussion here was that not all authorities accept PK end-points without clinical confirmation, so instead of submitting on PK/PD,

we submitted conventional data as well. The result was an extra effort, not a saved effort. There is no doubt that understanding mechanism and PK/PD was persuasive but was it really essential? In cost cutting societies it is a debatable point.

Case history 2. Psychiatry is an area where no clear PK/PD exists, and the site of action can only be studied with complex imaging. Do we revert to the tried and tested methods? This is a disease area where nearly all medicines have been reduced in dose since their introduction in the clinic. Certainly in schizophrenia, there is a growing belief that D_2 receptor occupancy is a good predictor of clinical benefit. The mechanistic evidence is compelling. However, filing on a PK/PD argument is still a long way from reality. By building the evidence though, on a learn/confirm basis, the investment is probably worthwhile. The investment in Imaging, quantitative EEG, Psychometric testing and linking via PK/PD is a science which is emerging slowly. On the adverse events side, many CNS penetrating drugs are the same drugs which have Toxicity. Often the Therapeutic window is small and conventional animal exposure cover is non-existent. However, often the toxicity is exaggerated behavioural effects. Is this real toxicity? Most reviewers think it is. The neurosciences represent a huge challenge as often animal does not predict man in either wanted or unwanted effects. By the use of mechanism studies in man (starting at low dose), a putative dose can be selected. We continue to be surprised at how low these doses are compared with conventional doses of drugs clinically used in this class. Dose reduction in neurosciences seems to be at the point of antibiotics some 10 or more years ago. What are we doing to address this? We are almost getting to the point where man dictates the animal toxic doses to be used.

Starting with Paul Morgan's presentation, I think he went through a lot of nice examples where you had a good unbound clearance relationship with doses on and effect, and then you went down to the real world where you showed the 99.99% bound drug. And I must say, from my own experience particularly in the CNS area, they are all 99.99%. So, could you give us some kind of insight as to what you actually would do in that situation?

P. Morgan: On a simplistic level, one could assume that level of protein binding for the worst case scenario. This would allow one to have the most conservative estimate of what free drug exposure is going to be to predict dose. In addition, the analytical issues surrounding measurement of very high protein binding are going to be resolved in the near future. With increased sensitivity from some of the LC/MS methods, we are now finding that we are able to measure high levels of protein binding. So, it is not an unworkable situation even now, to be able to make those types of predictions with no absolute value for protein binding. However, the confidence in that prediction is minimized somewhat because of the assumptions made about unbound concentrations.

N. Holford: A couple of general principles you enunciated. One was that, if it had a short half-life and you wanted it for a once-a-day dose then you would not develop it, in which case you throw away steroids, most beta blockers and most ACE inhibitors, so, that seems like throwing-the-baby-out-with-the-bathwater. And the other one was saying that, if it was an antagonist you needed at least 70% occupancy in order to develop it, and again that would probably throw out digoxin and possibly many others. So, maybe it is the exceptions rather than the rules that you should be developing.

P. Morgan: On the first point, I said that once-a-day agents generally tend to be long half-life. But the key parameter is actually that the duration of action is consistent with once-a-day dosing, and if one is able to look at that in pre-clinical studies, then that would be the parameter which one would follow. In many cases, there are no appropriate models to look at duration preclinically, so half-life is used as a surrogate. And certainly, estimations on required receptor occupancy for receptor antagonists are a guide, to allow us some way to be able to prosecute, looking at a large number of compounds in drug discovery, particularly, if there is no in vivo model to assess dynamics. If the actual receptor occupancy in the clinic is lower, then a lower dose may be efficacious which is always acceptable.

W. Evans: I am curious about the company philosophy. If you encounter a drug in development, that is a substrate for a known enzyme that exhibits functional genetic polymorphism, do you abandon that, or are you incorporating that in your development and going forward?

P. Morgan: I think we would very much hope that we would have identified the potential for polymorphism before the compound gets into development, and made a judgement based on the data on progression of the compound. As a general rule of thumb let us say for a CYP2D6 substrate, if it accounted for anything greater than 50% of the metabolism in humans, we would have to think carefully about whether or not we could live with the potential variation in pharmacokinetics that you are likely to observe. Not to say that a CYP2D6 substrate cannot be progressed into clinical development, but there are some very pronounced 2D6 substrates where we have seen a hundred-fold variability in oral exposure in development. That type of compound would be terminated based on that type of variability, to our mind.

A. Bye: You have examples like fluoconazole, a CYP3A4 inhibitor, so why not use it with saquinavir? When you think what that must mean in an early phase setting, it probably is just not worth it, but I would just be interested in some of your views when you are working up these PK/PD responses. How much do you link it with the Phase IV? I was thinking more of how much you would simulate the, kind of, final outcome of some of these almost hypothetical questions. I think the commercial aspects are—particularly for the industry—brought in right from the beginning.

P. Morgan: In many experience, very early on. Commercial opportunities and marketing issues are brought into the discovery cycle as well, as a way of differentiating from competitors.

T. Blaschke: You presented us a lot of very interesting data showing the correlations between the pre-clinical and the clinical dosing. I am just wondering at this point, if you could give us an estimate at least within your own company of how often the kind of data you showed us are actually used as guidelines to the initial dosing in Phase (I) and perhaps into Phase (II) versus how much of it still remains fairly empirical.

P. Morgan: Certainly at Pfizer, we use this approach pretty much 100% of the time, in thinking about free drug concentrations and trying to equate that with efficacy. I think the difficulty is understanding clearly what free drug concentration you actually need to give effects. Then the challenge is: do you understand the dynamic response well enough to be able to know, if it is an agonist for example, what type of receptor occupancy do you really need to give an effect?

T. Blaschke: As a follow-up, then, what kind of lead time do you need in order to be able to develop a dosing guideline for Phase (I)? How long does it take to gather the kind of information that you have shown us in order to be able to use that information, then, in designing your first in man or early phase clinical trials?

P. Morgan: Ideally, this type of basic understanding of the dynamics would come from the exploratory biology, which can take at least 6 months. If there are literature agents out there that can be used to define the dynamic response, then the confidence in using this data for clinical dose setting would be higher. But the experience accrued during the preclinical discovery phase will add to the initial studies to improve the prediction. In short, all preclinical pharmacology and pharmacokinetic data is brought to bear on setting the initial clinical doses. But it is still a prediction to be refined with clinical experience.

A. Bye: I think what Terry's driving at is how do we create this ongoing database. Companies are almost obliged not to talk to one another about the details, because of the competitive element, but if we take the talks that we have heard this morning in isolation of one another, each component is not very valuable. The value comes in joining all the components together. Paul is beginning to say it that if you knew what the answer was in the clinic, I think what we are doing is actually creating those links, particularly in the PK/PD sense. There are a number of examples of where you would make a pretty good prediction of effect, and in fact, in your abstract I see that you are almost giving a general rule for agonism and antagonism on a receptor binding basis, and it is probably not far out, but we do not actually have the same data collected in the patient, because you need something like PET or some other technique to bring it back. That very brief example I showed with the schizophrenia, it does seem to be panning out in general that you need to—in an antagonist sense—be thinking about high occupancy. But even when you look at the data, and it is spread all over the place, it would be very nice now to kind of tighten that up, and see whether it is possible or if there are other factors outside of PK/PD approach, some kind of individualization factors. It is painfully evident from the toxocology that that is true, so why should not it be painfully obvious from the efficacy that it is true? So I think, the general question is, if you started from scratch it will probably take you about 20 years to develop the thing that Terry wants you to say. But if you are following on from something you can learn and probably just take a few months.

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