Adaptive-Optimal Design in PET Occupancy Studies

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Positron emission tomography (PET) is an imaging technique that is used to investigate ligand–receptor binding in the living brain and to determine the time course of plasma concentration/receptor occupancy (RO). The purpose of this work was to demonstrate the added value of an adaptive-optimal design for PET scan timings and dose selection over traditional study designs involving fixed or educated selections of timings and doses. A k_{on}–k_{off} model relating plasma concentration to PET data was applied to generate the simulated data. Optimization was performed on scanning timings and doses using the D-optimality criterion. Optimal designs as applied to scanning timings provided unbiased estimates and improved the accuracy of results relative to those of fixed and educated designs. Optimization of both timings and dose provided improvements in accuracy and precision when the initial dose selection was noninformative regarding the time course of RO. These results indicate that adaptive-optimal designs can provide an efficient experimental design for RO studies using PET, by minimizing the number of subjects required and maximizing information related to the plasma concentration–RO relationship.

The use of imaging in early drug development, mainly as related to neurosciences, has contributed greatly to the increased efficiency and optimization of the development process of new molecular entities.[1] Of the various *in vivo* imaging techniques, positron emission tomography (PET) in conjunction with a suitable radioligand appears to have the best ability to measure receptor occupancy (RO) in humans.[2] The integration of PET occupancy studies into clinical drug development plans provides a valuable tool for dose selection and optimization in humans by accurately characterizing the relationship between the time course of plasma drug concentration and target RO.[3,4] In fact, a rational approach to selecting dosing regimens should be based on a knowledge of the onset and duration of RO (which is expected to be more closely related to drug effect) rather than the time course of drug concentration in plasma.[5] Clearly, the choice of dosing regimens should also account for the time course of downstream transduction, in case these are rate-limiting effects; therefore, an appropriate integrated approach that takes into account both phenomena should be considered.[6]

The methods proposed for the analysis and quantification of these exposure–time–occupancy relationships are based on preclinical (animal model) characterization of the time course of RO.[6,7] These methods have been extended to investigate the relationship between dose and exposure in human studies in which very sparse data relating to PET measurements were available (typically only at the time of peak plasma concentration).[8–10] However, recent publications, mainly involving dopamine D2 receptor antagonists, demonstrate the importance of accounting for the time course of RO in humans in the dose-selection strategy for chronic treatment.[11–13]

RO time-course studies in humans are not without their constraints. In a PET study, both the total number of subjects and the number of scans per subject are limited either by cost or for ethical reasons (e.g., concerns regarding overall radiation exposure may limit the number of scans per subject, typically to a maximum of three). It is within these constraints that an appropriate experimental design should be adopted in order to best characterize the exposure–time–occupancy relationship. In a recent study involving a novel antipsychotic drug (D2 antagonist), Lim *et al.*[14] used serial PET scans in healthy volunteers to assess the time course of RO. An indirect model that included an effect compartment was successfully identified using population approaches. In this study, fixed time points were used for PET assessment. In view of the constraints encountered in these studies, the application of adaptive-optimal design methods

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should be considered in order to arrive at a proper allocation of drug doses and PET scan timings.

Optimization of experimental designs has been successfully used to increase the efficiency and minimize the cost of clinical trials[15,16] by optimizing dose allocation and sampling schedules. D-optimal designs have been applied to population approach studies and have demonstrated more precise population parameter estimates.[17–19] In recent work,[20] a series of theoretical designs was considered for exploring the influence of optimizing the PET scan timing allocation, determining the number of subjects to be assigned to elementary ("fixed") designs, and determining the number of dose levels; the allocation of appropriate scan timings appeared to be the most critical factor for improving efficiency rather than the number of groups/doses.

The major limitation in the application of optimal design in early phase I PET studies is the need for detailed information about model structure and parameter values. In fact, although information can be derived from preclinical experiments regarding distribution of receptors in the target organ and the associated affinity of the labeled compound to the receptor (k_{on}/k_{off}), a conspicuous degree of uncertainty remains when translating this information from animal studies to application in humans.[21]

In general practice, PET studies are conducted according to a sequential adaptive design. An initial cohort of subjects is treated with an initial dose (selected on the basis of preclinical pharmacokinetic (PK) RO information and early human PK data), with scan timings targeting the maximal plasma concentration and/or a lower concentration (usually 24 h after dose).[8] The decisions regarding sample size, doses, and scan timings for the second cohort of subjects are derived from the analysis of the initial data. This procedure is repeated for all the cohorts of subjects planned in the study protocol. The number of subjects treated, the selection of informative doses, and the scan timings remain critical issues for a precise and accurate characterization of the PK–RO relationship. This is what is referred to here as an "educated" approach. When PET studies are performed with the same scanning timings for all subjects, we refer to this as a "fixed" approach.

The purpose of this work is to present methodology that enables optimal sequential PET occupancy experiments. The algorithm presented aims to provide an optimal strategy for the selection of doses and allocation of scan timings for each subject, leading to a more accurate estimation of the PK–RO relationship. The method is developed in a framework that combines the benefit of a "population–optimal design" methodology[22,23] and a "sequential adaptive approach"[24] leading to more informative experimental designs compared to more traditional fixed or educated approaches.

Adaptive-optimal design has recently been proposed as a method of improving the assessment of RO time courses in PET experiments.[25] In the present work, we have expanded this concept by including the optimization of dose and by improving the adaptation/optimization algorithm. An indirect model (k_{on}–k_{off}), using the binding potential (BP) data typically estimated from PET studies,[26] was developed to account for baseline intersubject variability. A comparison was performed for adaptive-optimal designs vs. traditional ("fixed" or "educated") designs of PET scan allocations, when optimizing either the sampling schedule alone or both sampling schedule and dose.

Two different scenarios were explored. Scenario 1: the initial dose was considered to be informative (i.e., a dose providing RO >50%) based on prior information (i.e., preclinical data). Scenario 2: the initial dose is chosen to be noninformative (i.e., a very low dose in the dose–occupancy relationship), in order to test the performance of the adaptive-optimal design in experiments in which initial dose selection based on prior information is misleading (for instance, when preclinical data are not predictive of the behavior of the drug in humans).

RESULTS

A total of 12 subjects were considered for all the designs, with groups of 2, 3, and 4 subjects being allocated for a given elementary design. **Table 1** reports details of sampling timings and doses for the fixed and educated designs.

Table 1 Selection of doses and sampling timings for fixed and educated designs

Scenario	Setup	Design	Method	Doses (mg)	Sampling timings (h)
1. Informative initial dose	1	4 subj/3 groups	Fixed	6, 1.5, 4	{0, 6, 24}; {0, 6, 24}; {0, 6, 24}
	1	4 subj/3 groups	Educated	6, 1.5, 4	{0, 6, 24}; {0, 3, 12}; {0, 8, 36}
	2	3 subj/4 groups	Fixed	6, 1.5, 4, 3	{0, 6, 24}; {0, 6, 24}; {0, 6, 24}; {0, 6, 24}
	2	3 subj/4 groups	Educated	6, 1.5, 4, 3	{0, 6, 24}; {0, 3, 12}; {0, 8, 36}; {0, 12, 48}
	3	2 subj/6 groups	Fixed	6, 6, 1.5, 1.5, 4, 4	{0, 6, 24}; {0, 6, 24}; {0, 6, 24}; {0, 6, 24}; {0, 6, 24}; {0, 6, 24}
	3	2 subj/6 groups	Educated	6, 6, 1.5, 1.5, 4, 4	{0, 6, 24}; {0, 6, 24}; {0, 3, 12}; {0, 3, 12}; {0, 8, 36}; {0, 8, 36}
2. Noninformative initial dose	4	4 subj/3 groups	Educated	0.5, 1.5, 6	{0, 6, 24}; {0, 3, 12}; {0, 8, 36}
	5	3 subj/4 groups	Educated	0.5, 1.5, 4, 6	{0, 6, 24}; {0, 3, 12}; {0, 8, 36}; {0, 12, 48}
	6	2 subj/6 groups	Educated	0.5, 6, 1.5, 3, 4, 8	{0, 6, 24}; {0, 6, 24}; {0, 3, 12}; {0, 3, 12}; {0, 8, 36}; {0, 8, 36}

Initial sampling timings for the adaptive-optimal designs were selected to be the same as in standard designs {0, 6, 24}. In the design that optimized for sampling timings alone, the dose selected was equal to the doses in the fixed and educated designs, with the same number of subjects and groups. Only the initial dose (6 mg in scenario 1 and 0.5 mg in scenario 2) was selected in the design that optimized both sampling timings and doses.

subj, subject.

Table 2 Performances of fixed, educated, and adaptive-optimal designs

				Fixed		Educated		Optimal (timing only)		Optimal (timing + dose)	
Scenario	Setup	Design	Performance	k_{on}	k_{off}	k_{on}	k_{off}	k_{on}	k_{off}	k_{on}	k_{off}
1	1	4 subj/3 groups	Bias (SME)	0.87	1	0.13	0.12	−0.0095	−0.029	0.0086	−0.0088
			Precision (RSE)	2.56	2.82	0.383	0.437	0.185	0.205	0.195	0.196
			Accuracy (RMSE)	4.86	5.74	0.449	0.506	0.183	0.201	0.196	0.194
	2	3 subj/4 groups	Bias (SME)	0.83	0.93	0.096	0.076	−0.0010	−0.012	−0.0078	−0.02
			Precision (RSE)	3	3.19	0.434	0.487	0.199	0.238	0.201	0.214
			Accuracy (RMSE)	5.56	6.21	0.485	0.53	0.199	0.235	0.2	0.21
	3	2 subj/6 groups	Bias (SME)	0.87	1	0.13	0.12	0.013	0.0022	0.011	0.0004
			Precision (RSE)	2.56	2.82	0.383	0.437	0.196	0.227	0.182	0.2
			Accuracy (RMSE)	4.86	5.74	0.449	0.506	0.199	0.228	0.185	0.2
2	1	4 subj/3 groups	Bias SME			0.164	0.161	0.019	0.014	−0.023	−0.040
			Precision (RSE)			0.435	0.532	0.376	0.427	0.332	0.335
			Accuracy (RMSE)			0.532	0.638	0.383	0.433	0.325	0.324
	2	3 subj/4 groups	Bias (SME)			0.171	0.161	0.036	0.027	0.014	−0.0057
			Precision (RSE)			0.535	0.687	0.292	0.342	0.229	0.257
			Accuracy (RMSE)			0.649	0.814	0.304	0.352	0.233	0.256
	3	2 subj/6 groups	Bias (SME)			0.101	0.095	0.037	0.022	0.022	−0.0001
			Precision (RSE)			0.306	0.359	0.244	0.295	0.216	0.251
			Accuracy (RMSE)			0.352	0.405	0.256	0.302	0.222	0.251

RMSE, root mean square error; RSE, relative standard error; SME, scaled mean error; subj, subject.

Scenario 1 simulation studies: informative dose selection

Optimization of sample timings results in a clear improvement with respect to bias (scaled mean error), precision (relative standard error), and accuracy (root mean square error) of the population estimates (k_{on} and k_{off}) when compared to traditional (fixed and educated) designs (scenario 1, Table 2). Scaled mean error, relative standard error, and root mean square error are defined in the Methods section. Bias (with 95% confidence intervals) and boxplots of relative error are illustrated in Figure 1 for the adaptive-optimal and nonoptimal study designs. These results indicate that the two traditional designs occasionally provided severely misspecified parameter estimates (outliers), resulting in relatively high bias values. The optimal designs do not suffer from this problem. Unbiased mean estimates were found for the optimal designs; a great improvement in accuracy (25- to 30-fold) was found when using optimal designs as compared to fixed designs, and a significant improvement was found when comparing optimal designs with educated designs (two- to threefold). Overall, both educated and optimal designs provide plausible parameter estimates in the majority of simulated studies, whereas, as expected, the fixed designs based on three fixed time points typically provided estimates that were highly biased and imprecise. Figure 2 shows the distribution of optimal sampling timings for the four-step design (scenario 1, setup 2 in Table 2) when optimizing only sample timings.

In experiments with informative initial doses, no clear advantages were found when optimizing both timing and dose (scenario 1, Table 2) as compared to optimization of timing alone. The number of adaptive steps had less influence on design performance than did the method of designing the next step. A slight improvement was obtained in intersubject variability estimates when using optimal designs as compared to nonoptimal designs (data not shown). In general, the estimates of k_{on} and k_{off} intersubject variability were underestimated, with typical variances ranging between 0.04 and 0.07 (against a true variance of 0.1 for both k_{on} and k_{off}). These results are not unexpected, given the low sample size.

Scenario 2 simulation studies: noninformative dose selection

Scenario 2 in Table 2 compares results obtained from educated and adaptive-optimal designs in a scenario in which the initial dose was noninformative, with optimization of sampling timings alone, and also of both sampling timings and dose. As in scenario 1, the optimal-adaptive designs in scenario 2 display a clear improvement in parameter bias precision and accuracy. In addition, the adaptive-optimal design, as applied to both timing and dose, provided better performance than the adaptive-optimal design for timing alone: on average, the improvement in accuracy and precision ranged from 10 to 40%. In this scenario, the number of adaptive steps appears to be more relevant than in the previous scenario (in which initial doses were more informative); designs with four and six adaptive steps showed better performance than the three-step design.

DISCUSSION

Traditional approaches to establishing a PK–RO model are generally based on the assumption of a direct link between

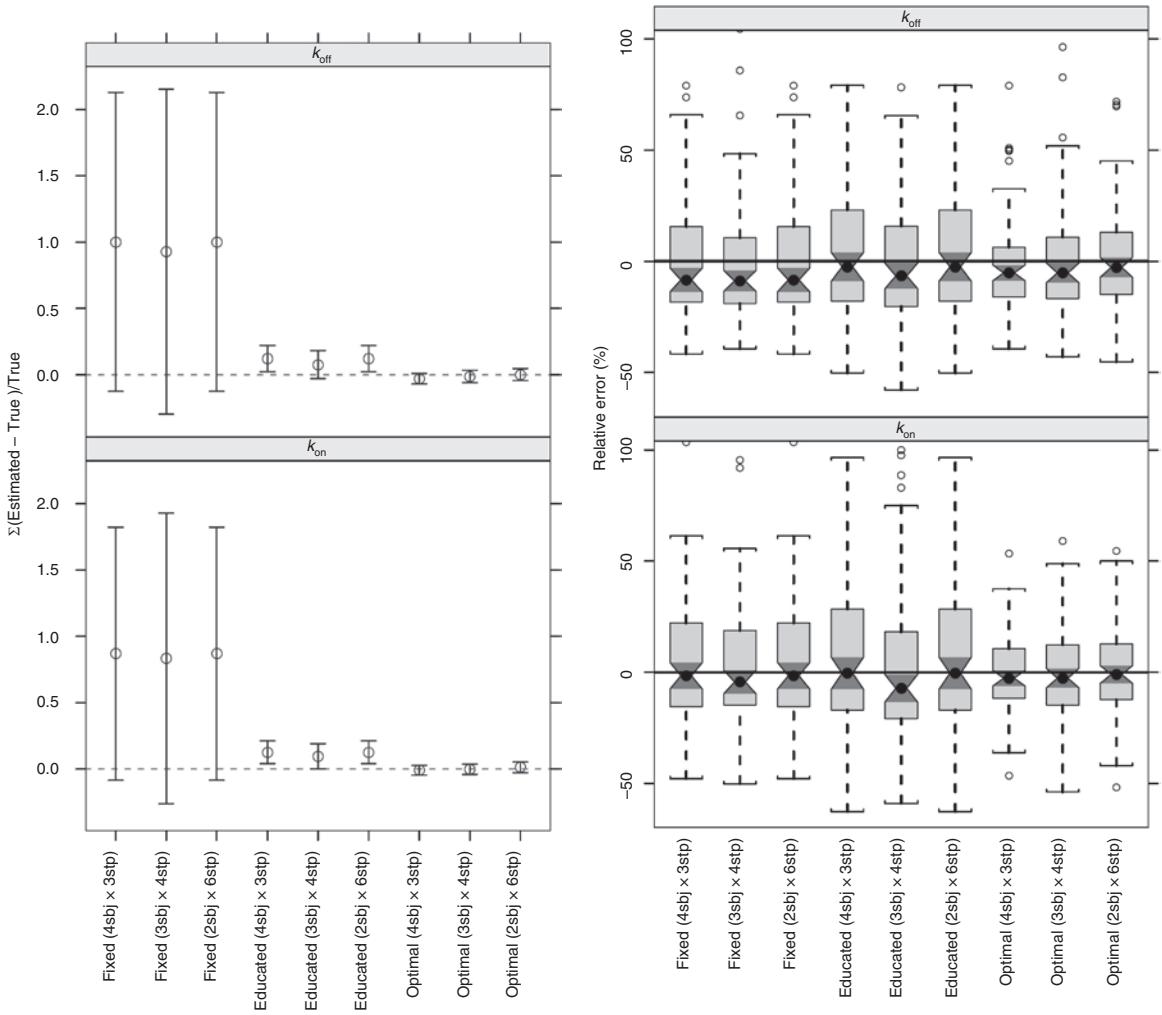

Figure 1 Comparison of performances (bias and relative error) of adaptive-optimal and nonoptimal approaches to various designs (scenario 1). Parametric confidence intervals are shown in the bias plots. sbj, subject; stp, step.

plasma concentration and RO. The direct model assumes both rapid exchanges across the blood–brain barrier and a rapid equilibration at the receptor sites. However, in many cases these assumptions are not appropriate, and alternative models such as effect compartment or association–dissociation models should be used.[27] Clearly, in this context, the application of an optimal study design for the selection of PET scan timings is more relevant than in the case of a direct relationship. In this work, we have characterized the relationship between plasma concentration and occupancy using an indirect model. In practice, this has been implemented using BP as the dependent variable, which offers advantages over the use of occupancy. In fact, BP is the primary variable (measured in the PET scan), whereas RO is a derived variable using BPs at baseline (BP_0) and after dosing (BP). Normalization of all observations per baseline value has previously been shown to lead to significant bias in parameter estimates, especially in studies with small numbers of subjects (as is the case here).[28] In addition, the use of BP allows for modeling (i.e., understanding) of baseline intersubject variability.

As described in the introduction, Lim *et al.*[14] recently proposed the use of serial PET scans in healthy volunteers to assess the RO time course, using an indirect model. However, scans at fixed timings in all subjects were used in this study, which may have had an impact on both bias and uncertainty, especially around effect parameters (k_{eo}, k_{on}, or k_{off}). Two possible methods of improving the results in the study by Lim *et al.* are to increase the study size and to change other aspects of the design of the study. Clearly, given that PET studies are expensive, an increase in sample size may not be a practical suggestion. However, by optimizing and adapting other aspects of the design (e.g., scan timings and doses) benefits with regard to reduction in bias and improvement in accuracy and precision may be achieved at no extra cost.

In both the study by Lim *et al.* and our current work, the use of the population modeling approach is proposed. One of the many advantages of this approach is the ability to "borrow" information between individuals, leading to accurate and precise parameter estimates when data from individual modeling approaches might be too sparse for parameter estimation. These results indicate that adaptive-optimal design for PET occupancy studies can provide more accurate information on the PK–RO relationship.

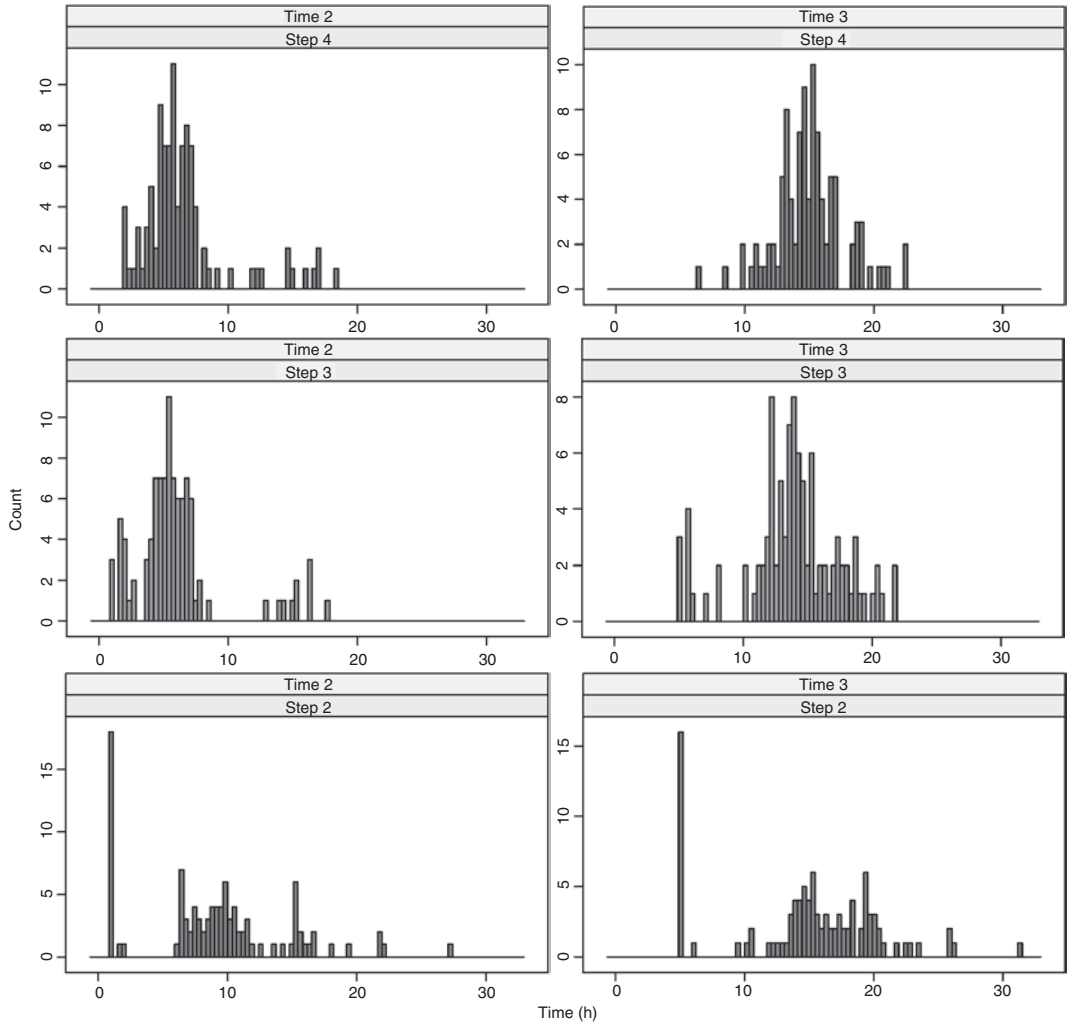

Figure 2 Distribution of optimal sampling timings for scenario 1, setup 1 (100 simulated studies). Note that the time point for the first sampling is always zero (baseline) and is not shown here.

In our first example (scenario 1), informative initial dosing, based on previous knowledge of the system, allowed for a spread of doses in the cohorts, resulting in high, medium, and low occupancy levels. Consequently, optimization of dose was found to have no significant influence on the results. In scenario 2, where an initial misleading dose was selected, the optimization of both timing and dose showed a clear advantage over the optimization of timing alone. In addition, in these experiments, all designs that did not optimize doses in each cohort relied on "educated" dose adjustment. Optimization of dose makes the successful outcome of the experiment less dependent on the experience of the people running it, leading, in general, to a less error-prone experimental design.

Increasing the number of steps of the adaptation in this work appeared to improve precision and accuracy, especially when the initial dose selection was not informative. In a real (nonsimulated) experiment, the selection of a nonoptimal initial dose can easily occur and should be considered the norm. These results suggest that, given a certain sample size, increasing the number of groups—and therefore reducing the number of subjects per group—should be considered. This is especially true in the initial steps, when uncertainty in dose selection is much greater than in the subsequent steps.

Further refinements of this adaptive-optimal approach can be envisaged. For example, in this work we have assumed a model structure and parameter values for this model at each step in which an optimal design is computed. However, this information can be very uncertain, especially in the first step of the adaptive sequence, when the model and parameter values might be based on estimates from preclinical information that may not be directly applicable to human subjects. It would therefore make sense to include this uncertainty in the calculations involved in the optimal design. This can be done in two ways: (i) uncertainty about the parameter values for the model can be incorporated using ED-optimal design[29] and (ii) multiple model structures can be used to find the optimal designs.[30]

We expect that the optimal characterization of the PK–RO relationship after a single dose should allow for the prediction of the occupancy that will be achieved after a repeat dosing trial.

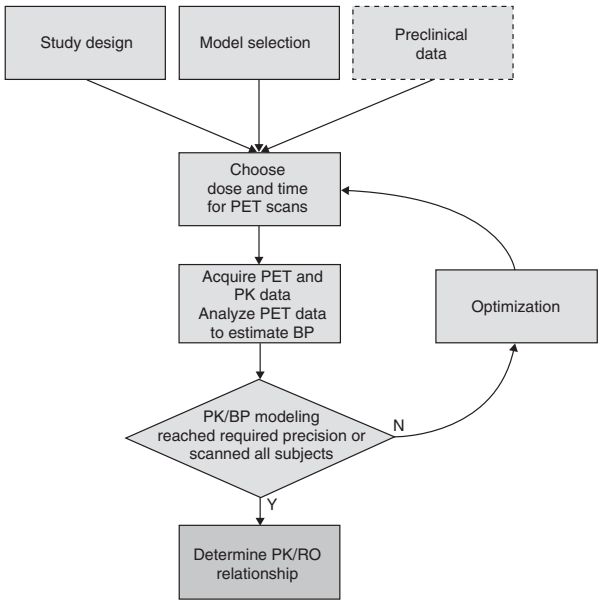

Figure 3 Adaptive-optimal design framework. Initial model, dose, and scanning timings are selected according to prior information. At each adaptive step, parameter estimates from the previous cohorts were determined and used to determine designs for the next cohort. BP, binding potential; PET, positron emission tomography; PK, pharmacokinetic; RO, receptor occupancy.

This expectation is conditional on the assumption of a time–invariant relationship (i.e., association and dissociation parameters remain unaltered after chronic treatment). If this assumption holds, then the results from these single-dose trials can be used to provide additional information earlier in the drug development process.

In a more general context, we have presented a method of adapting and optimizing PET studies of RO such that the results will contain fewer biased parameter estimates and will not rely solely on the judgment of an "educated" clinical team. Optimal-adaptive design is attractive because the methodology can use prior information and also react to data accrued during the study. The idea of adaptive-optimal designs is not a new one[31] but is still relatively rarely used. Previous work has demonstrated the utility of adaptive-optimal designs in phase II dose–response studies from a naive pooled modeling perspective.[24] In this work, we use similar methodology to demonstrate the effectiveness of adaptive-optimal approaches in early phase I studies with a population modeling approach.

METHODS

Adaptation/optimization algorithm. In the proposed adaptive/optimal algorithm, the dose and PET scan time points for the first cohort of subjects are selected on the basis of RO estimated from preclinical studies or from *in vitro* binding studies. In our work, the same initial dose and PET scan time points (pharmacodynamics) were selected for the traditional and optimal designs so that these initial conditions would not influence the comparisons of the various designs. Given the assumption that extensive PK sampling is typically carried out in PET studies, the PK parameters of the PK–BP model are considered to be known and therefore not optimized.

This initial information is then used to estimate model parameter values, and these values are used as priors to select doses and PET scan timings in the next cohort (more details are provided below, under "Methods of simulation, estimation, and optimization"). For each subsequent cohort, the data from all previous cohorts are used as prior information (in the form of updated model parameter estimates and fixed design variables) to select doses and PET scan timings. At each adaptation point, PET scan timings (and, in some scenarios, doses) are chosen on the basis of optimal criteria (see **Figure 3**). In this approach, it is important to emphasize that the selection of initial doses and timings is not based on the true model parameters.

Simulation studies. Simulations were conducted to study the performance of the proposed approach in the context of a real PET study characterized by a limited sample size ($N = 8$–16). Adaptive optimization of doses and sampling timings was performed using the PK–BP model.

A total of 12 subjects, five possible doses (1.5, 3, 4, 6, and 8 mg), and three sample timings per subject were considered. Designs with three, four, and six adaptive steps (cohorts) were investigated. BP time-course data were simulated under the true model from the following designs: (i) empirical fixed designs, in which the sampling schedule was fixed to take place at baseline, RO t_{max}, and trough (24 h) in all subjects, (ii) educated designs, in which doses and sampling schedules were selected to appropriately cover the time–occupancy dynamic range (described below), and (iii) optimal designs, including optimization of sampling schedule alone and of both sampling schedule and dose.

For the educated design, under the assumption of the PK–BP model, the first PET scan was performed at baseline and the second around the RO peak, in order to gather information regarding the association rate; the third scan timing was selected after a sufficient decline in the RO levels, in order to gather information on the dissociation rate (which is dependent on dose). Initial doses were selected to be either informative or noninformative (see above). Doses for the additional cohorts were chosen to cover the dynamic range of RO (high, medium, and low occupancies). When noninformative initial doses are chosen, full coverage of this dynamic range is more difficult to achieve.

Apart from the adaptive-optimal design involving both sampling schedule and dose, the same "educated" doses were used for all the designs (fixed, educated, and adaptive optimal for sampling schedule). Details of sampling timings and doses for the fixed and educated designs are reported in **Table 1**. Because of the poor performance of the fixed design even with informative initial dosing, noninformative dosing was not considered for this design. One hundred studies per design were simulated to test the performance of these various design schemes. A simulated BP, RO, and plasma concentration–time course for a typical individual (scenario 1, setup 1), with optimal PET scan allocation, is illustrated in **Figure 4**.

RO time- course model using BP. A general representation of a PK–occupancy–time course model is described in **Figure 5**. However, in PET studies in which only a few PET scans per subject can be acquired, this model cannot be applied, and a simplified version needs to be considered. In our study, a k_{on}–k_{off} model, which characterizes the relationship between the plasma concentration of the drug and the estimates of BP derived from PET data, was considered. This is equivalent to an assumption that the exchange across the blood–brain barrier and the partitioning of the drug in plasma and tissue from free to nonspecifically bound is sufficiently rapid for the association and dissociation of the specifically bound drug to be considered rate limiting. In general, RO is derived from the PET BP measurement, using:

$$\mathrm{RO}(t) = \frac{\mathrm{BP}_0 - \mathrm{BP}(t)}{\mathrm{BP}_0}, \qquad (1)$$

where BP_0 is the baseline binding potential and BP the time-dependent binding potential after dosing. The equation for the model for the time course of RO can be described as:

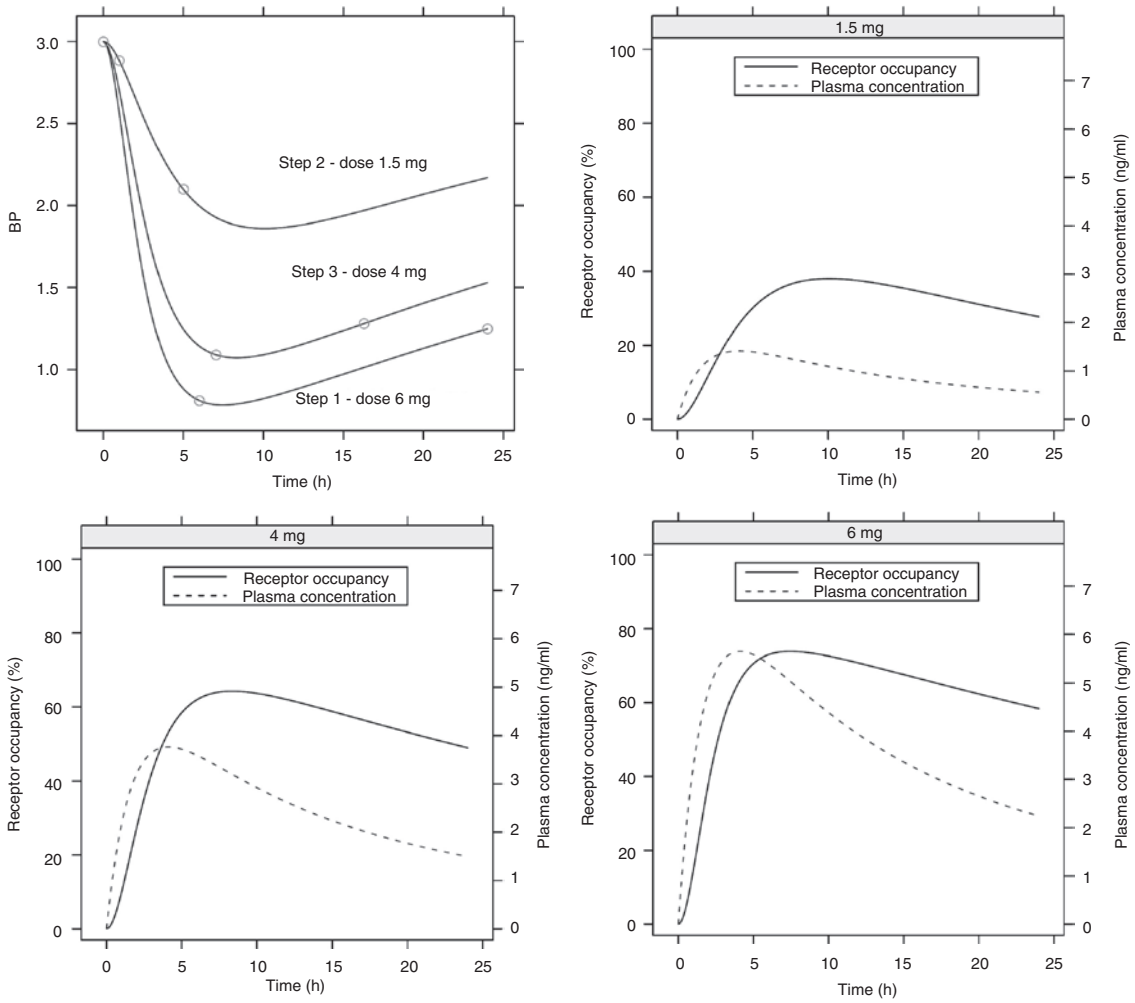

Figure 4 Simulated binding potential–time course for the typical individual at three different dose levels (scenario 1, setup 1). Circles represents the optimal PET scan allocations per group (upper left panel). Time courses of receptor occupancy (continuous line) and of drug concentration in plasma (dotted line) for the typical individual at the three different dose levels (upper right and lower panels). BP, binding potential; PET, positron emission tomography.

$$\mathrm{d}\left(\frac{\mathrm{BP}_0-\mathrm{BP}}{\mathrm{BP}_0}\right)/\mathrm{d}t = k_{\mathrm{on}}\cdot C_{\mathrm{P}}\cdot\left(1-\frac{\mathrm{BP}_0-\mathrm{BP}}{\mathrm{BP}_0}\right)-k_{\mathrm{off}}\cdot\frac{\mathrm{BP}_0-\mathrm{BP}}{\mathrm{BP}_0}, \quad (2)$$

which can be rewritten as:

$$\frac{\mathrm{dBP}}{\mathrm{d}t} = k_{\mathrm{off}}\cdot\mathrm{BP}_0-(C_{\mathrm{P}}\cdot k_{\mathrm{on}}+k_{\mathrm{off}})\mathrm{BP}, \quad (3)$$

where C_P is the concentration of the drug in plasma. In this study, the following parameters were used for the simulation of experimental data: $k_{on} = 0.088\,h^{-1}$, $k_{off} = 0.221\,h^{-1}$, and $BP_0 = 3$. The estimates of parameters were derived from preclinical studies of a $5HT_{1A}$ antagonist (S. Zamuner, unpublished data). An exponential distribution model was assumed for the intersubject variability (coefficient of variance = 30%) of BP_0, k_{on}, and k_{off}, and a proportional error model was assumed for the residual variability (coefficient of variance = 10%).

A two-compartment model with first-order absorption was assumed for the plasma concentration kinetics, and individual PK parameters were assumed as known in the PK–BP model. The population PK model was parameterized as clearance (CL = 34.9 l/h), volume of distribution at steady state (V_{ss} = 1,200 l), intercompartmental clearance (Q = 21.7 l/h), fractional central volume (FVC = 0.671), and rate of absorption (k_a = $0.605\,h^{-1}$). The exponential distribution for intersubject variability was

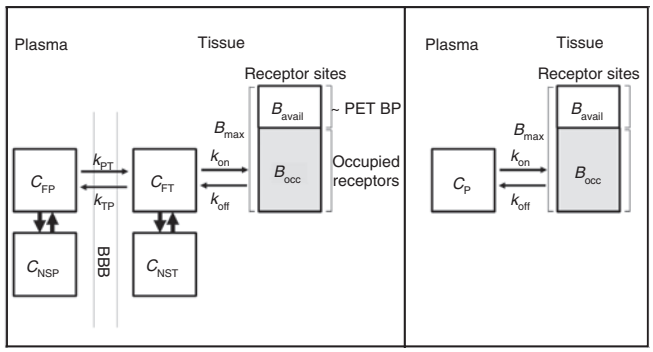

Figure 5 Schematic representations of a PK–receptor binding model (left panel). C_{FP} and C_{NSP} represent the free and the bound concentrations in plasma, respectively, and C_{FT} and C_{NST} represent the free and the bound (nonspecific) concentrations in tissue, respectively. k_{PT} and k_{TP} represent the plasma-to-brain tissue and brain tissue-to-plasma constant rates, respectively. k_{on} and k_{off} represent the association and dissociation rates between free brain concentration and receptor. The simplified model is shown in the right panel, with C_P representing the total concentration in plasma. BBB, blood–brain barrier; BP, binding potential; PET, positron emission tomography.

also used for the PK parameters, employing the following coefficients of variance: CL = 30%, V_{ss} = 40%, Q = 45%, FCV = 30%, and k_a = 45%.

Methods of simulation, estimation, and optimization. Simulation and parameter estimation were performed using NONMEM software, version VI (NONMEM Project Group, University of California, San Francisco, San Francisco, CA).[32] Estimation was performed using the first order with conditional estimation method.

Optimization was performed for scanning timings alone, and also for scanning timings and doses, using a D-optimality criterion as implemented in the PopED software (http://poped.sf.net).[33] A looped linear sequence of random-search, gradient, and line-search algorithms was used to find the optimal design parameters. Optimization of doses and sample timings was carried out using a sequential method; for each of the five dose levels considered, sample timings were optimized, and the most informative dose was chosen. Because we were considering only discrete doses, the simultaneous method was not needed (we could consider all the possible dose levels).[34] For each optimization, the parameter values of the model were updated according to the information from previous cohorts. Because this parameter re-estimation (with all data from all previous cohorts) occurs at each step of the adaptive algorithm, the previous cohorts' designs were included (but fixed) in each subsequent optimal design calculation. That is, information from these cohorts was assumed to contribute to parameter estimation after the next step, but the designs could obviously not be changed because they had already been run. In order to improve run timings, the contribution of the fixed designs of the earlier cohorts was included in the current cohort's design calculations as a fixed addition to the Fisher information matrix. This fixed term was computed once for every optimization, using the new model parameter estimates. With this addition, the D-optimal criterion was

$$\mathrm{Argmax}_{\vec{x}}\left|\mathrm{FIM}\,(\vec{x},\Phi)\right| = \mathrm{Argmax}_{\vec{x}\_n}\left|\begin{pmatrix}\sum_{i=1}^{n-1}\mathrm{FIM}\,(\vec{x}_i,\Phi)\end{pmatrix}+\mathrm{FIM}\,(\vec{x}_n,\Phi)\right|, \quad (4)$$

where $\vec{x}$ is the vector of design parameters (doses, sample timings), Φ is a vector of the updated model parameters, and $\vec{x}_n$ is the nth cohort's design parameters, which can be optimized, whereas the earlier cohort designs (cohorts 1 to $n-1$) are fixed. For the first cohort ($n = 1$), the sum of previous Fisher information matrix values is set to zero.

Comparison of results. The comparison of results was carried out by computing the bias measured as the scaled mean error of the estimated parameter values with respect to the true parameter value; the accuracy was measured in terms of root mean square error of the estimated parameter values with respect to the true parameter value; and the precision was measured in terms of the relative standard error of the estimated parameter values.[35] The definitions of scaled mean error, root mean square error, and relative standard error are as follows:

$$\mathrm{SME} = \frac{1}{A\cdot n}\sum_{j=1}^{n}(E_j - A) \quad (5)$$

$$\mathrm{RSME} = \frac{1}{A}\sqrt{\frac{1}{n}\sum_{j=1}^{n}(E_j - A)^2} \quad (6)$$

$$\mathrm{RSE} = \frac{1}{E}\sqrt{\frac{1}{n}\sum_{j=1}^{n}(E_j - \bar{E})^2} \quad (7)$$

where E_j is the estimated parameter value for the jth simulation, A is the expected value, $\bar{E}$ is mean of the estimated parameters, and n is the number of simulations. In addition, box plots of the relative error pertaining to the estimated parameter values were examined.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

1. Uppoor, R.S. *et al*. The use of imaging in the early development of neuropharmacological drugs: a survey of approved NDAs. *Clin. Pharmacol. Ther.* **84**, 69–74 (2008).
2. Lee, C.M. & Farde, L. Using positron emission tomography to facilitate CNS drug development. *Trends Pharmacol. Sci.* **27**, 310–316 (2006).
3. Gefvert, O., Bergström, M., Långström, B., Lundberg, T., Lindström, L. & Yates, R. Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia. *Psychopharmacology (Berl.)* **135**, 119–126 (1998).
4. Catafau, A.M. *et al*. Pharmacokinetics and time-course of D(2) receptor occupancy induced by atypical antipsychotics in stabilized schizophrenic patients. *J. Psychopharmacol. (Oxford)* **22**, 882–894 (2008).
5. Bergström, M. *et al*. MAO-A inhibition in brain after dosing with esuprone, moclobemide and placebo in healthy volunteers: *in vivo* studies with positron emission tomography. *Eur. J. Clin. Pharmacol.* **52**, 121–128 (1997).
6. Yassen, A., Olofsen, E., Dahan, A. & Danhof, M. Pharmacokinetic-pharmacodynamic modeling of the antinociceptive effect of buprenorphine and fentanyl in rats: role of receptor equilibration kinetics. *J. Pharmacol. Exp. Ther.* **313**, 1136–1149 (2005).
7. Gupta, A., Gillard, M., Christophe, B., Chatelain, P., Massingham, R. & Hammarlund-Udenaes, M. Peripheral and central H1 histamine receptor occupancy by levocetirizine, a non-sedating antihistamine; a time course study in the guinea pig. *Br. J. Pharmacol.* **151**, 1129–1136 (2007).
8. Mamo, D. *et al*. A PET study of dopamine D2 and serotonin 5-HT2 receptor occupancy in patients with schizophrenia treated with therapeutic doses of ziprasidone. *Am. J. Psychiatry* **161**, 818–825 (2004).
9. Meyer, J.H. *et al*. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. *Am. J. Psychiatry* **161**, 826–835 (2004).
10. Bergström, M. *et al*. Human positron emission tomography studies of brain neurokinin 1 receptor occupancy by aprepitant. *Biol. Psychiatry* **55**, 1007–1012 (2004).
11. Takano, A. *et al*. Time course of dopamine D2 receptor occupancy by clozapine with medium and high plasma concentrations. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **30**, 75–81 (2006).
12. Tauscher, J., Jones, C., Remington, G., Zipursky, R.B. & Kapur, S. Significant dissociation of brain and plasma kinetics with antipsychotics. *Mol. Psychiatry* **7**, 317–321 (2002).
13. Bench, C.J. *et al*. The time course of binding to striatal dopamine D2 receptors by the neuroleptic ziprasidone (CP-88,059-01) determined by positron emission tomography. *Psychopharmacology (Berl)*. **124**, 141–147 (1996).
14. Lim, K.S. *et al*. Modeling of brain D2 receptor occupancy-plasma concentration relationships with a novel antipsychotic, YKP1358, using serial PET scans in healthy volunteers. *Clin. Pharmacol. Ther.* **81**, 252–258 (2007).
15. Mentré, F., Mallet, A. & Baccar, D. Optimal design in random-effects regression models. *Biometrika* **84**, 429–442 (1997).
16. Hooker, A. & Vicini, P. Simultaneous population optimal design for pharmacokinetic-pharmacodynamic experiments. *AAPS J.* **7**, E759–E785 (2005).
17. Duffull, S.B., Mentré, F. & Aarons, L. Optimal design of a population pharmacodynamic experiment for ivabradine. *Pharm. Res.* **18**, 83–89 (2001).
18. Retout, S., Duffull, S. & Mentré, F. Development and implementation of the population Fisher information matrix for the evaluation of population pharmacokinetic designs. *Comput. Methods Programs Biomed.* **65**, 141–151 (2001).
19. Hooker, A.C., Foracchia, M., Dodds, M.G. & Vicini, P. An evaluation of population D-optimal designs via pharmacokinetic simulations. *Ann. Biomed. Eng.* **31**, 98–111 (2003).
20. Zamuner, S. & Gomeni, R. Optimal design to estimate the time varying receptor occupancy relationship in a PET experiment. Abstract 1099, Annual

Meeting of the Population Approach Group in Europe, Copenhagen, Demark, 13–14 June 2007 <http://www.page-meeting.org/?abstract=1099>.
21. Kapur, S., VanderSpek, S.C., Brownlee, B.A. & Nobrega, J.N. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on *in vivo* occupancy. *J. Pharmacol. Exp. Ther.* **305**, 625–631 (2003).
22. Zamuner, S., Gomeni, R. & Bye, A. Estimate the time varying brain receptor occupancy in PET imaging experiments using non-linear fixed and mixed effect modeling approach. *Nucl. Med. Biol.* **29**, 115–123 (2002).
23. Liefaard, L.C. *et al*. Population pharmacokinetic analysis for simultaneous determination of B (max) and K (D) *in vivo* by positron emission tomography. *Mol. Imaging Biol.* **7**, 411–421 (2005).
24. Maloney, A., Karlsson, M.O. & Simonsson, U.S. Optimal adaptive design in clinical drug development: a simulation example. *J. Clin. Pharmacol.* **47**, 1231–1243 (2007).
25. Petrone, M., Zamuner, S., Di Iorio, V., Gunn, R., Laruelle, M. & Gomeni, R. Model-based sequential human PET study design for optimal PK/RO assessment. Abstract 1183, Annual Meeting of the Population Approach Group in Europe, Copenhagen, Demark, 13–14 June 2007 <http://www.page-meeting.org/?abstract=1183>.
26. Mintun, M.A., Raichle, M.E., Kilbourn, M.R., Wooten, G.F. & Welch, M.J. A quantitative model for the *in vivo* assessment of drug binding sites with positron emission tomography. *Ann. Neurol.* **15**, 217–227 (1984).
27. Yassen, A. *et al*. Mechanism-based PK/PD modeling of the respiratory depressant effect of buprenorphine and fentanyl in healthy volunteers. *Clin. Pharmacol. Ther.* **81**, 50–58 (2007).
28. Dansirikul, C., Silber, H.E. & Karlsson, M.O. Approaches to handling pharmacodynamic baseline responses. *J. Pharmacokinet. Pharmacodyn.* **35**, 269–283 (2008).
29. Dodds, M.G., Hooker, A.C. & Vicini, P. Robust population pharmacokinetic experiment design. *J. Pharmacokinet. Pharmacodyn.* **32**, 33–64 (2005).
30. Waterhouse, T.H., Redmann, S., Duffull, S.B. & Eccleston, J.A. Optimal design for model discrimination and parameter estimation for itraconazole population pharmacokinetics in cystic fibrosis patients. *J. Pharmacokinet. Pharmacodyn.* **32**, 521–545 (2005).
31. Fedorov, V.V. *Theory of Optimal Designs*. (Academic Press: New York, 1972).
32. Beal, S. & Sheiner L.B. NONMEM User Guides (Nonmem Project Group, 1998).
33. Foracchia, M., Hooker, A., Vicini, P. & Ruggeri, A. POPED, a software for optimal experiment design in population kinetics. *Comput. Methods Programs Biomed.* **74**, 29–46 (2004).
34. Nyberg, J., Karlsson, M.O. & Hooker, A.C. Simultaneous optimal experimental design on dose and sample times. *J. Pharmacokinet. Pharmacodyn.* **36**, 125–145 (2009).
35. Walther, B.A. & Moore, J.L. The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. *Ecography* **28**, 815–829 (2005).