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Snapshot PK: a rapid rodent *in vivo* preclinical screening approach

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Described in this article are strategies implemented to increase the throughput of *in vivo* rodent pharmacokinetic (PK) studies using the snapshot PK study design and automated methods for compound submission, sample processing, data analysis and reporting. Applying snapshot PK studies to categorize the oral exposure of >1300 discovery compounds as low, moderate or high resulted in an attrition rate of 86%. The follow up full PK studies on the remaining compounds found that 98% of the compounds were predicted in the correct (69%) or adjacent (29%) oral exposure category by the snapshot PK studies. These results demonstrate that the snapshot PK screen in rodents can serve as an effective and efficient *in vivo* tool in the compound selection process in drug discovery.

Introduction

Lack of efficacy, serious toxicity and unacceptable pharmacokinetics (PKs) are the three main reasons for clinical failure of drug candidates. According to a survey by Food and Drug Administration (FDA) in 1991, approximately 40% of clinical failure was attributable to poor PK properties [1]. By 2000 the PK attrition rate was down to 10% [2]. This significant improvement was due to a change in drug discovery strategies, as pharmaceutical companies began assessing PK properties of new chemical entities at the very early stages of drug discovery [3,4]. *In vitro* absorption, distribution, metabolism and elimination (ADME) assays facilitate early elimination of compounds with poor drug-like properties and selection of potential candidates for *in vivo* PK profiling. *In vivo* animal PK studies provide a reality check which guides the medicinal chemists to optimize the chemical structure of compounds. *In vivo* animal PK information also assists pharmacologists to design effectively *in vivo* efficacy studies and accurately interpret pharmacodynamic (PD) observations.

In recent years, the throughput of the drug discovery process has improved because of the implementation of high-throughput *in vitro* ADME assays. Hundreds of compounds can be screened *in vitro* per week, providing scientists with a wealth of data [5]. By contrast, *in vivo* PK studies are still conducted in a traditional low-throughput manner in most pharmaceutical companies. Therefore, there is a need to bring *in vivo* PK studies into a higher throughput arena. Multiple steps are involved in *in vivo* PK studies, including compound submission, study protocol preparation, formulation, animal dosing and sampling (in-life portion), sample processing and analysis (analytical portion), PK regression and data reporting. In the past, many efforts to increase PK throughput have focused on the 'in-life' [6–9] or analytical portions of PK studies [10,11]. In this paper, we report the snapshot PK approach which attempts to address every step in the PK study process from the study request stage to the final data reporting.

Snapshot PK study design: dosing and sampling strategy

Animal dosing and sampling is one of the major time-consuming steps in *in vivo* PK screening. Conventional (full) PK studies use the dosing strategy of a single compound per animal to avoid potential drug–drug interaction problems. A full PK study typically includes two study arms (i.v. and p.o.) and takes 6–12 serial blood samples

Abbreviations: AUC, area under the plasma concentration time curve; CL, clearance; V_{ss}, volume of distribution at steady-state; $T_{1/2}$, half-life of elimination; C_{max}, maximum plasma concentration; T_{max}, time at which C_{max} occurs; C_{last}, the last observed plasma concentration; T_{last}, time at which C_{max} occurs; BA, bioavailability.

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from 6 to 8 animals per compound, which therefore requires a minimum of 36 samples per study. Because of the lower blood sampling volume allowed in the mouse, a full PK study in mice often requires twice the number of animals over that in a rat PK study. The high animal usage and labor intensive dosing and sampling place a hurdle to the throughput of the assay. Cassette dosing, that is, simultaneous administration of several compounds per animal, affords significant savings in both animal usage and dosing resources [6,7]. However, its application has been limited by several considerations: first, there are no safeguards against drug–drug interactions even when using low dosage and eliminating known inhibitors [12]; second, inaccurate PK information generated from cassette dosing studies was reported to be as large as sixfold [13]; third, difficulties in preparing a usable dosing formulation from a mixture of multiple compounds frequently occur in cassette dosing. A survey conducted in 2003 from 31 pharmaceutical companies indicated a decline in the frequency of use of cassette dosing in drug discovery [12]. To overcome the limitations of cassette dosing, a rapid rat PK screen was first reported and later modified as the cassette accelerated rapid rat screen (CAARS) by scientists at Schering-Plough [8,14,15]. Furthermore, scientists at Pfizer reported using a three-point sampling rapid rat PK method on 123 compounds to quickly overcome problems of poor correlation of *in vitro* ADME data and the *in vivo* clearance [9]. In the meantime, GNF has developed and validated a similar idea described herein as snapshot PK studies (Fig. 1). Snapshot PK studies are predominantly performed in mice (90%) because the large majority of rodent efficacy models at GNF are conducted in mice. The snapshot PK study is conducted using standardized protocols that are different from the individualized protocols typically used in full PK studies. The test compound is prepared using a standardized formulation of 75% polyethylene glycol 300 (PEG300) in 5% dextrose (D5W) solution at 2.5 mg/ml. Each animal receives a 20 mg/kg (mouse) or 10 mg/kg (rat) dose of a single compound by oral gavage, which requires only a small amount of test compound, that is, 3–5 mg per mouse study and 12–15 mg per rat study. The dosing and sampling strategy in the snapshot PK protocol resembles that used in rapid rat PK screening, but with smaller blood sampling volumes (50 μl versus 100 μl) and fewer sampling points (4 versus 6) over a shorter time frame (five hours versus six hours). Blood samples (50 μl) are drawn via the retro orbital sinus in mice and from a surgically implanted jugular vein catheter in rats. Alternatively, serial blood samples in mice can be collected by saphenous vein or tail vein bleeds. The total blood volume of 200 μl collected per study equals approximately 13% of the circulating blood volume (~1.6 ml) in a 25 g mouse. Compared to full PK studies, the snapshot PK design reduces animal usage from the typical six to eight animals to two animals per compound. The sampling period is shortened from 24 to 5 hours with fewer sampling points. By standardizing the dosing formulation, reducing animal usage and limiting sampling points over a shorter sampling period, significant savings are achieved in the in-life portion of the PK studies. The animal staff resources required to conduct a snapshot PK study is reduced three- to fivefold compared to those in a full PK study.

Snapshot PK study design: analytical sample pooling strategy

Sample analysis is another major time-consuming step for *in vivo* PK studies. Several approaches have focused on increasing the throughput of sample analysis by using technologies such as automated online sample extraction [16] or robotic liquid handlers [17] to reduce the time required for sample preparation. Other solutions to increase capacity include shortening the analytical procedures by using faster LC/MS/MS methods [18] or by reducing the numbers of samples by sample pooling and running cassette

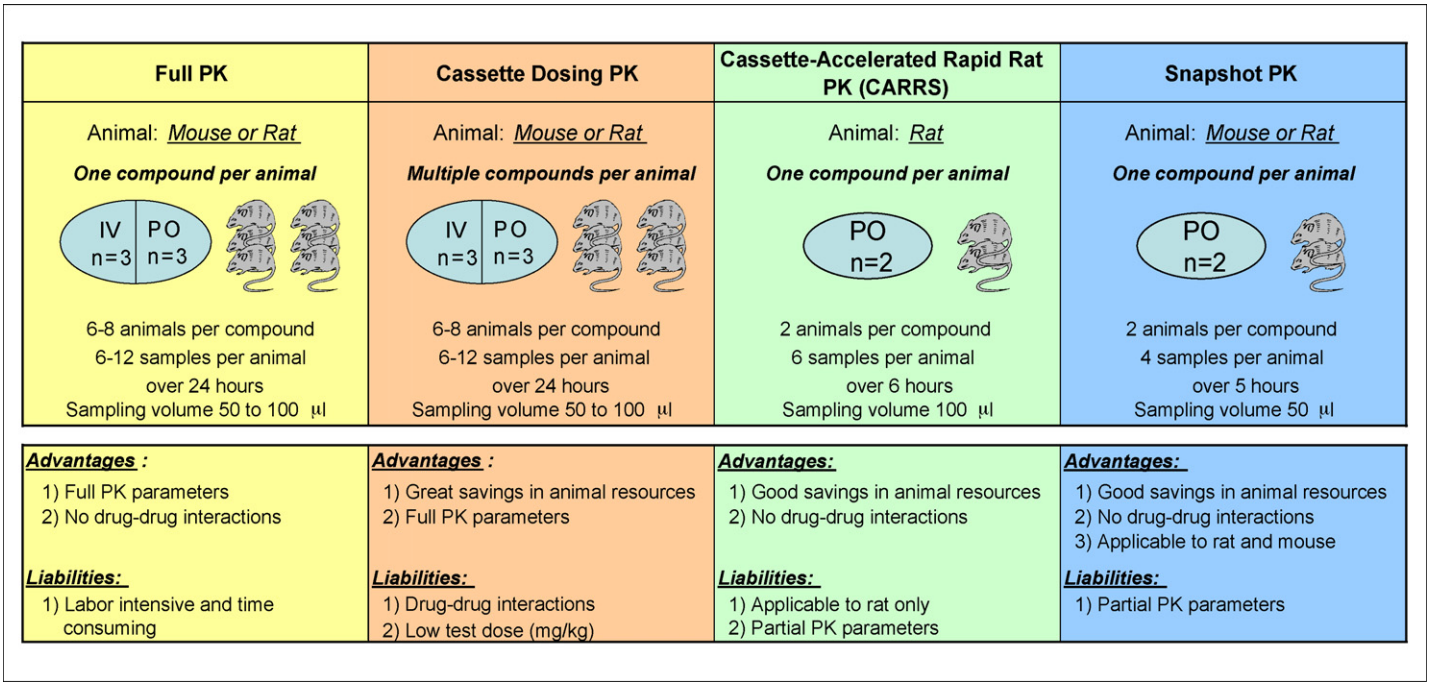

FIGURE 1

In-life portion strategies of snapshot PK: comparison against other *in vivo* PK study designs.

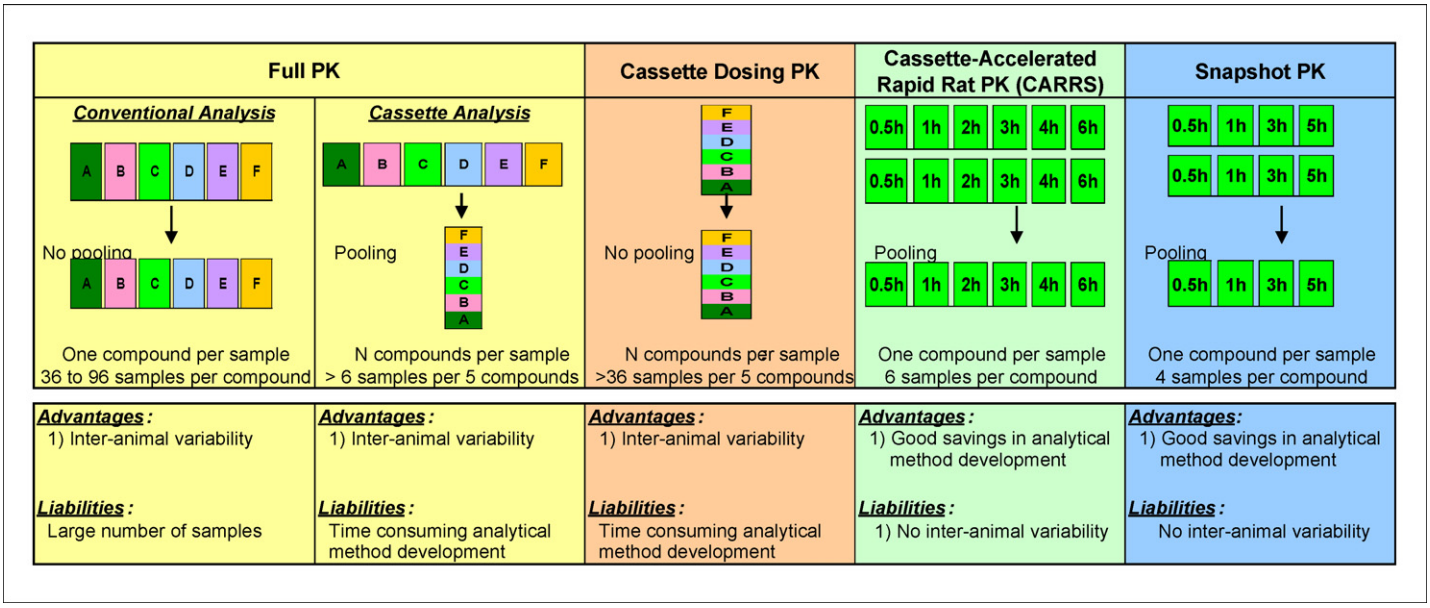

FIGURE 2

Sample analysis strategies of snapshot PK: comparison against other *in vivo* PK study designs.

analysis [11,19]. Increased throughput of the sample analysis in snapshot PK studies was achieved using a sample pooling strategy to reduce the number of plasma samples. Instead of pooling multiple compounds into one sample as reported in the cassette pooling strategy, snapshot PK relies on a pooling strategy which results in a single test compound per sample. Korfmacher *et al.* first reported using this technique in the CARRS assay [14], which was an improvement of their earlier pooling strategy used in the rapid rat PK assay, where samples from multiple time points were mixed and time-course data were lost [8]. In snapshot PK studies, a set of eight plasma samples are generated per compound (i.e. four time points and $n = 2$ animals). The eight plasma samples, each 20 μl, are pooled across the two animals at each time point to provide four pooled samples of 40 μl (Fig. 2). Although the sample reduction with the snapshot PK pooling strategy is not significant as the cassette pooling strategy, the ease of analytical method development and determination is maintained. The pooling strategy applied in snapshot PK studies allows pooling of samples taken at the same time point from two individual animals, hence obtaining an average plasma concentration at each time point. Information on the concentration changes over the time is thus retained for an accurate determination of $AUC_{(0–5\ hours)}$.

Automation of the snapshot PK studies

PK study requests and compound submission

Implementation of snapshot PK resulted in a large increase in compound submission, which made it impractical for PK scientists to conduct manually the sample weighing, tracking and solution preparation. A key change in the PK sample processing workflow at GNF was to apply existing technologies within the compound management group (CMG) for PK compound submission. CMG relies on an electronic compound requesting system to track the hundreds of compounds that enter and leave the department each day [20]. Project team members enter the compound information into an online PK study tracking system. Compounds are submitted in barcoded vials to CMG for weighing, tracking and applying of labels that contain information on project, structure, salt form and amount. The barcoded vials allow PK scientists to track electronically each compound through the formulation and dosing stages. By automating the study request and compound submission process, significant increases in PK study throughput was accommodated without hiring additional scientists or negatively impacting the quality of the data generated.

Sample preparation, analytical method development and analysis

Robotic liquid handling systems are commonly used to increase the throughput of sample preparation of *in vivo* PK studies [17,21] and sample automation technology was implemented at GNF to process samples from snapshot PK studies. Pooled plasma samples (40 μl) from the *in vivo* studies, typically eight compounds per preparation, are transferred into 96-well plates and sample processing and protein precipitation carried out on programmable Tecan and Tomtec liquid handling workstations. The automation of sample preparation cut the five hours manual preparation into one hour for every eight compounds, reducing the preparation time by 80%.

With increasing number of compounds submitted for PK studies, it became necessary to automate the analytical component in order to increase the throughput of PK sample analysis [22,23]. Commercially available automated software, such as Automaton®, allows automated compound tuning, development of analytical LC/MS/MS methods, analysis of mass spectrometry data and generation of quantitative results. These advances in automated software for mass spectrometry significantly reduced the time needed for routine method development, without sacrificing the success rate of methods developed, thereby allowing the scientist to focus on those problems that merit extra attention. Pharmaceutical companies have reported using these software tools for *in vitro* assays [24] and we validated the use of Automaton® for analysis of *in vivo* snapshot PK samples [25]. The automated tuning had a success rate

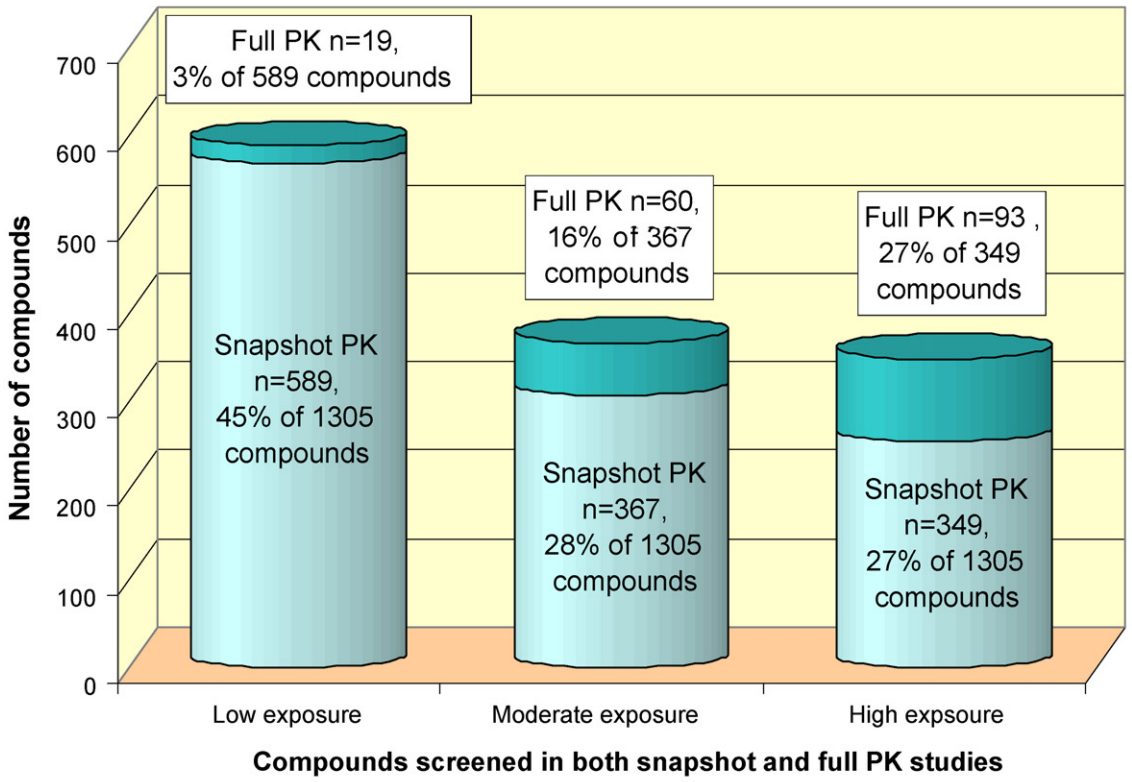

FIGURE 3

Distribution of 1305 compounds in oral exposure categories in snapshot PK studies and selection of compounds for full PK studies.

of 95% and the incorporation of automated LC/MS/MS tuning and method development in the snapshot PK analysis resulted in a sixfold increase in throughput relative to manual LC/MS/MS method development. The plasma drug concentrations from snapshot PK studies are determined with a generic LC/MS/MS system using a five-point standard curve. Raw data reports from the analysis of snapshot samples are generated automatically using a linked result template to integrate all LC/MS/MS information onto one platform. Overall, it takes less than three hours to complete the automated assay for eight compounds in snapshot PK studies, including compound tuning optimization, sample preparation and raw data reporting (excluding the over-night LC/MS/MS analysis). In comparison with the previous manual bioanalytical assay run, the automated assay has comparable accuracy, precision and levels of quantitation. In addition, it led to a four- to fivefold improvement in throughput of sample processing and data reporting while reducing potential errors associated with manual pipetting and raw data reporting.

PK regression analysis and data reporting

The majority of commercially available PK software (e.g. WinNonlin®, Nonman®, ADAPTII® and Gastroplus®) is designed to conduct PK regression analysis of a variety of study designs, including complicated multiple dose PK and PK/PD studies. Their wide range of features provide great flexibility; however, this also implies more manual parameter specifications, including settings for fitting model (noncompartment/compartment), input dosing route, dosing amounts and concentration units, which is time-consuming and low throughput. At GNF there was a need for a more automated and customized solution to meet the throughput requirements of PK data analysis. Drug discovery PK profiling is most efficiently accomplished using noncompartmental PK analysis as described in the literature [26]. A PK fitting program capable of noncompartmental PK regression analysis using standardized units of the input parameters (dose, concentration and time) and output parameters (AUC, CL, V_{ss}, $T_{1/2}$, C_{max}, T_{max}, C_{last}, T_{last} and BA) was developed at GNF. This custom program enabled further development of an informatics system that not only calculates all key PK parameters for multiple time series in a batch mode but also generates dynamic PKs plots for visual analyses which were published to the GNF database and made accessible to authorized scientists. Web publishing tools rapidly create PK report pages as data become available in the database. A typical PK report page contains all historical PK data in both tabular and graphical formats, as well as all ADME measurements for the ease of correlation comparison.

Application of snapshot PK studies

The snapshot PK design was initially validated with 23 compounds which indicated a good correlation ($r^2 = 0.84$) between snapshot PK and full PK studies. Since its introduction at GNF, the snapshot PK approach has been successfully used to guide the drug selection process in drug discovery. During this time, 1305 compounds of diverse structures were screened using the snapshot PK approach (Fig. 3). The compounds were classified as exhibiting low, moderate or high oral exposure based on their dose-normalized $AUC_{0-5\ hours}$ values. The classification criteria

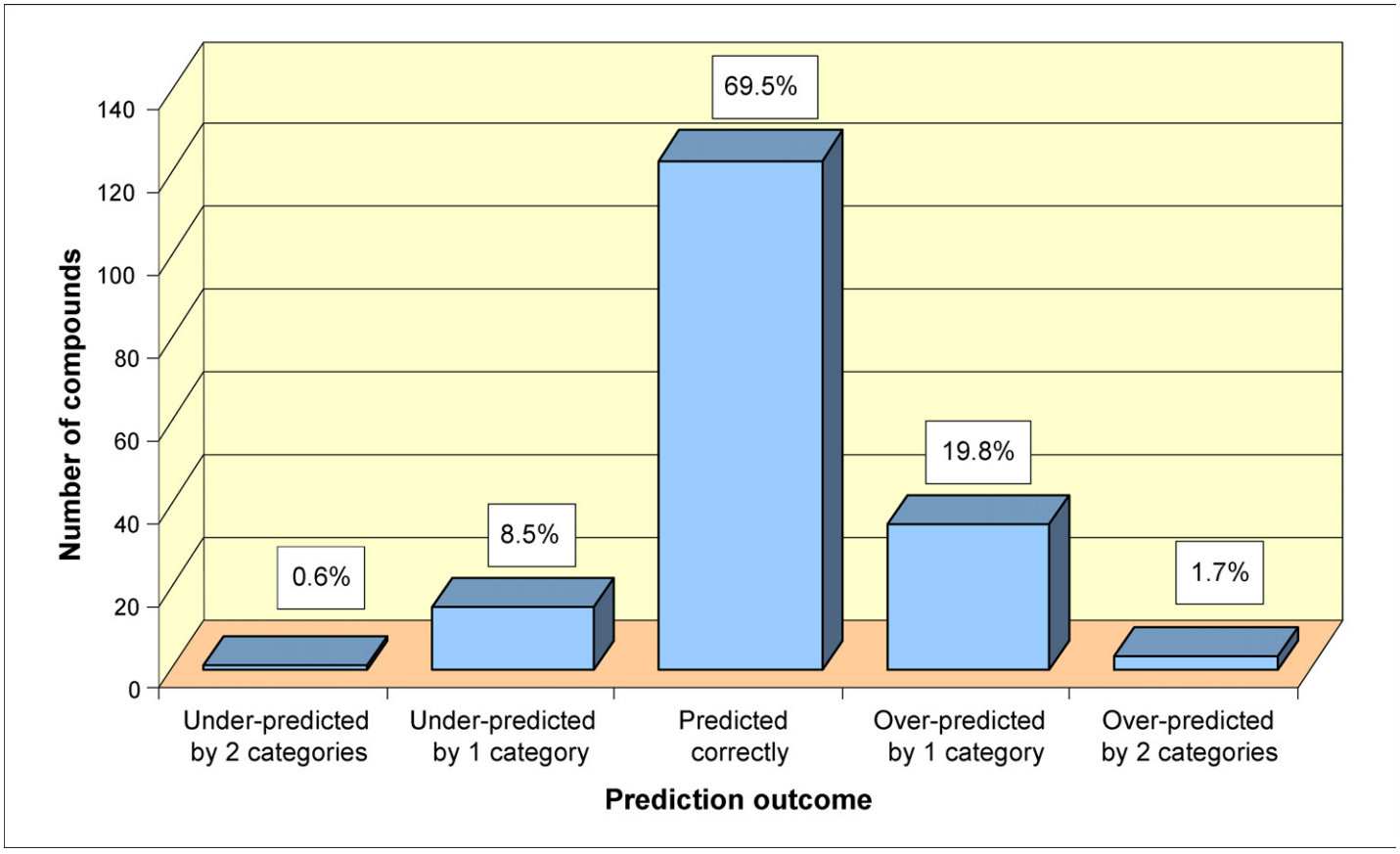

FIGURE 4

Prediction of the oral exposure category of 177 compounds: snapshot PK versus full PK studies.

were established empirically in our laboratory and thereafter used routinely to inform scientists about the relative oral exposures of their compounds [27]. A large fraction of the compounds, 45% ($n = 589$) demonstrated low oral exposure with $AUC_{0–5\ hours}$/dose values <2 (min μg/ml)/(mg/kg), 28% of the compounds ($n = 367$) showed moderate oral exposure with dose-normalized AUC values between 2 and 10, while the remaining 27% ($n = 349$) showed high oral exposure with $AUC_{0–5\ hours}$/dose >10. After completion of the snapshot PK screening, 97% of compounds having low oral exposure were discontinued for further *in vivo* PK profiling. The compounds with moderate and high exposure were prioritized based on their $AUC_{0–5\ hours}$/dose value and plasma concentration profile. Eventually, 86% ($n = 1128$) of all compounds tested in snapshot PK studies were filtered out and only 14% ($n = 177$) of the compounds, predominantly those exhibiting moderate or high exposure, were enrolled in further full PK studies to determine in-depth PKs. Compared to compounds with low exposure in snapshot PK studies, compounds with high exposure were tenfold more probable to be selected for dosing in full *in vivo* PK studies.

Applying the 'right box analysis' concept reported by White and Manitpisitkul [6], the oral exposure of 177 structurally diversified compounds that were studied in both snapshot and full PK studies were categorized (Fig. 4). Based on the dose-normalized AUC values in each study design, compounds were classified into categories of low, moderate or high oral exposure. Retrospective analysis demonstrated that of the 177 compounds studied, 69.5% ($n = 123$) were categorized consistently by both assays and hence placed in 'the right box', 28.2% ($n = 50$) of compounds were off by one category, while only 2.3% ($n = 4$) of compounds were off by two categories.

The majority of the compounds differing by one exposure category were over-predicted by snapshot PK data and showed high rather than moderate oral exposure in snapshot PK studies. The probable reasons for over-predicting the exposure in snapshot PK studies were using soluble salt forms and organic formulation vehicles in snapshot PK studies but less soluble free bases and water-based formulations in full PK studies. Regardless, since 98% of all tested compounds were predicted in the correct or adjacent exposure category, the snapshot PK study design was embraced with confidence by GNF medicinal chemists and biologists as a useful tool to triage medicinal chemistry compounds. The overall high consistency between the two PK assays indicates that the snapshot PK approach can successfully categorize the oral exposure of test compounds, ensuring scientists to correctly prioritize the compounds for further *in vivo* PK and efficacy studies.

Discussion

The strategies used to increase the *in vivo* PK throughput included using the snapshot PK study design with its standardized protocols for dosing and sampling, automated PK request and compound submission system, automated sample extraction and LC/MS/MS bioanalysis, and customized informatics for data processing and

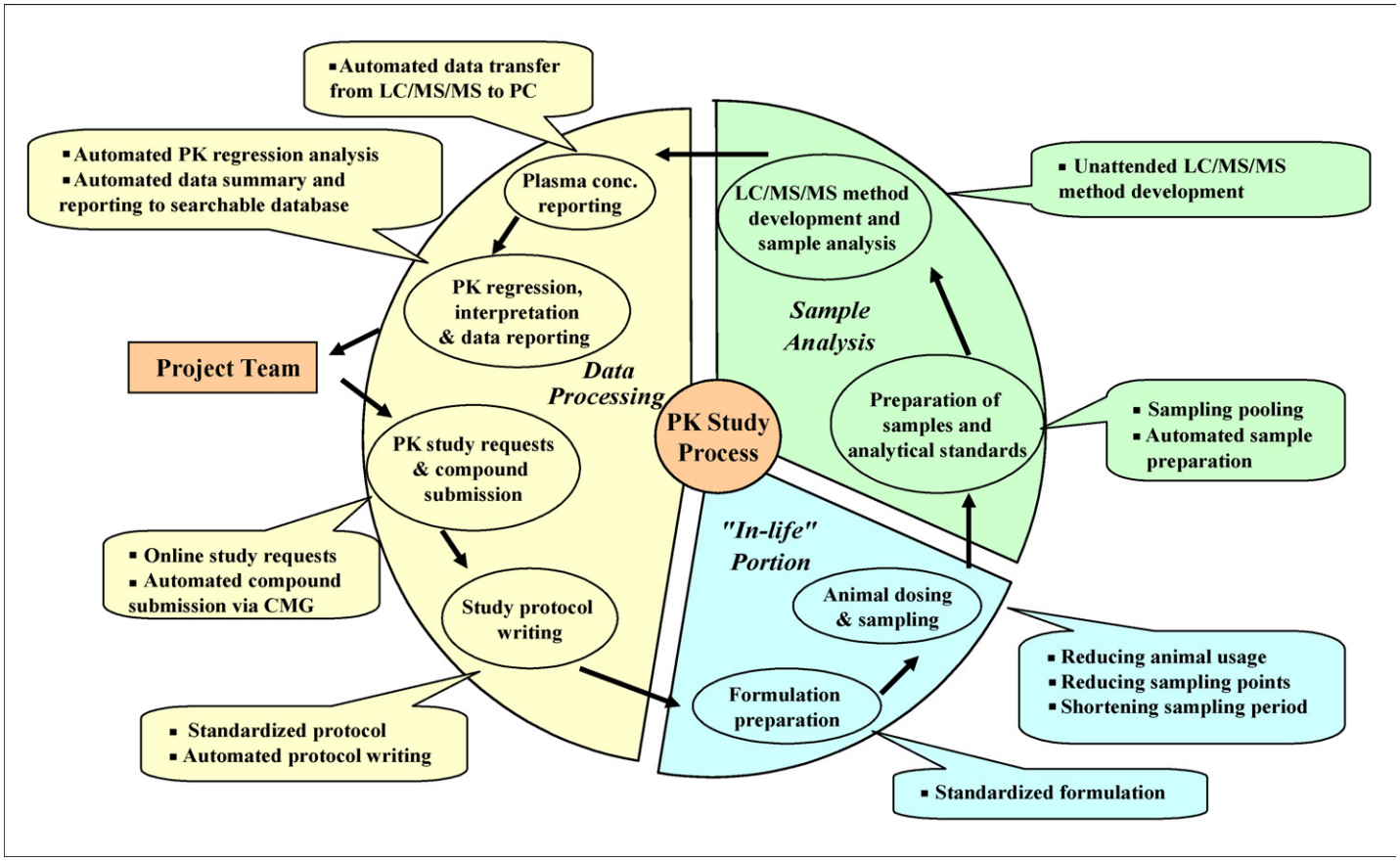

FIGURE 5

Strategies used at GNF to improve the throughput of PK studies.

reporting (Fig. 5). Project team members submit PK requests and compound information via an online PK tracking system. The CMG coordinates weighing and labeling of material for dosing and analytical purposes. Automation accelerates PK sample processing, while automated tuning and method development of the LC/MS/MS instruments minimize the time required to develop and run analytical methods. Successful automation of sample preparation and LC/MS/MS analysis has made the analytical portion of snapshot PK studies 100% 'hands-off'. Through most of these improvements, the PK group worked closely with the Informatics group to streamline all the above components with a customized PK informatics solution. Continuously seeking to improve 'the weakest link' in the chain of events from compound submission to final PK data reporting was crucial to the success in increasing the throughput of *in vivo* PK studies.

Implementation of the snapshot PK design and other high-throughput strategies enabled GNF PK scientists to complete several thousand *in vivo* PK studies over the past few years. A 3.5-fold increase in overall *in vivo* PK throughput was accompanied by a modest 1.4-fold increase in PK personnel. The snapshot PK approach currently is serving as the primary *in vivo* PK screening tool for testing of drug oral exposure in drug discovery. Compounds tested for PK *in vivo* are divided into snapshot PK studies (61%), full PK studies (28%) and PK/PD studies (11%), and combined represent approximately 10% of all newly synthesized compounds by medicinal chemists.

Scientists at Schering-Plough introduced the cassette rapid rat PK screen (CARRS) in 2001 and reported using the *in vivo* approach to successfully triage compounds in drug discovery [14]. The snapshot PK study design was developed at GNF in 2004. It is similar to the CARRS assay, however, the primary animal species is mouse instead of rat. Therefore, the snapshot PK study has fewer samples (4 versus 6) and smaller sample volumes (50 μl versus 100 μl) collected over a shorter time period (five hours versus six hours) than CARRS. The total sample volume (600 μl) collected in CARRS makes the assay unsuitable for mouse PK studies, since the volume is approximately 38% of the total blood volume in a 25 g mouse. Limiting the total blood volume to 200 μl makes the snapshot PK method amendable to both mouse and rat studies. Although the implementation of CARRS relied heavily on automation of the analytical process, using ultra-fast LC/MS/MS techniques [14,28], the implementation of snapshot PK at GNF addressed additional automation in compound and sample logistics, as well as data analysis. Tighter integrations with the rest of the drug discovery components could potentially lead to continuous improvement in the future.

Interestingly, the low and high threshold dose-normalized AUC values in snapshot PK studies of 2 and 10 (min μg/ml)/(mg/kg) [27] were close to those determined by simulation and empirical evaluation in the rapid rat screen methodology (CARRS) [15], though the snapshot PK threshold values were published before the CARRS values. Unit conversion of the CARRS threshold values

500 and 2000 h ng/ml at a dose of 10 mg/kg gave the dose-normalized AUC values of 3 and 12 (min μg/ml)/(mg/kg), demonstrating that oral exposure screening data are interpreted consistently across the laboratories. There are also similarities in the distribution of compounds into low, moderate and high oral exposure categories. In snapshot PK studies, the distribution of 1305 compounds in low, moderate and high oral exposure categories was 45, 28 and 27%, respectively. Similarly, the CARRS assay of near 5300 compounds resulted in low, moderate and high exposure of 50, 25 and 25% of all compounds tested [15]. The agreements are remarkable given that the screened compounds originated from two distinct chemical libraries at two separate companies. It suggests that modern pharmaceutical chemical libraries have commonalities in the distribution of physical–chemical properties of the compounds.

We evaluated the reliability of the snapshot PK study design by comparing the oral exposure category in snapshot PK with that obtained in full PK studies. Of 177 compounds studied in full PK studies, 2/3 was placed in the correct oral exposure category while nearly 1/3 deviated by one category. Only 2% of compounds deviated by two categories. Similarly, of 100 compounds tested in full PK studies, the CARRS assay predicted the compounds in the right exposure category in 100% of cases if low exposure in CARRS, 76% of cases if moderate in CARRS or 89% of cases if high in CARRS, respectively. These results suggest that both the snapshot PK study and CARRS predict the correct exposure category in a large majority of cases. Importantly, the snapshot PK studies filtered out 86% of all compounds, thus only allowing 14% of the compounds to enroll in full PK studies. In an analysis of CARRS assay data, investigators reported discarding 50% of 5298 compounds with an $AUC_{0–6\ hours}$ value below the lower cutoff value [15]. Thus, both the snapshot PK and CARRS assays were highly effective in prioritizing discovery compounds for further evaluations.

The snapshot PK study design is currently used by scientists at GNF in all stages of discovery, from scaffold selection to lead optimization and candidate selection. In the early phase of the medicinal chemistry, discovery process chemists are using snapshot PK data to prioritize among several chemical scaffolds. Their goal is to focus the efforts on a few promising chemical prototypes. Rapid rodent PK studies may be combined with *in vitro* ADME assays to increase the efficiency of compound section at this stage in drug discovery. Preliminary data in our laboratory (not shown) suggest that PAMPA and microsomal stability may be used to predict poor snapshot PK. It should be noted that since these two *in vitro* assays only model passive diffusion and hepatic metabolism by membrane-bound enzymes, their utility may not be extended to predicting good PK. As discovery programs progress into lead optimization, structurally similar compounds are submitted for snapshot PK studies to determine if minor modifications to molecules result in improved PK properties. Serial submissions of different compounds from specific scaffolds into the snapshot PK model enable scientists to establish structure–activity relationship (SAR) around the structural modifications. Interesting compounds identified in snapshot PK studies are followed up with full PK studies in 14% of all cases. The effective triage of compounds in snapshot PK studies allows scientist to spend minimal effort on poor compounds while focus their attention on compounds with favorable PK properties.

Conclusions

The snapshot PK approach reviewed here has similarities with the CARRS assay, which both have advantages over several existing *in vivo* PK screening strategies. Compared to full PK studies, these rapid PK screens offer significant savings in the in-life portion by standardizing dosing and study protocols, eliminating study arms, reducing animal usage and limiting the number of sampling points. The snapshot PK approach is predominantly used in mice (90%) with a minority (10%) in rats, while CARRS is exclusively applicable to rats. Unlike cassette dosing, snapshot PK and CARRS studies use a single compound dosing strategy which avoids potential drug–drug interactions. The snapshot PK screen is conducive to automated compound submission and sample preparation, unattended LC/MS/MS method development, regression analysis and data reporting. Completely automated sample processing and analyses ensure that snapshot PK studies can be done in a high-throughput fashion. Application of the snapshot PK screen to more than a thousand discovery compounds at GNF proved this method to be an efficient *in vivo* PK tool to triage discovery compounds.

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