Schizophrenia is a severe mental disorder characterized by cognitive deficits, and positive and negative symptoms. The development of effective pharmacological compounds for the treatment of schizophrenia has proven challenging and costly, with many compounds failing during clinical trials. Many failures occur due to disease heterogeneity and lack of predictive preclinical models and biomarkers that readily translate to humans during early characterization of novel antipsychotic compounds. Traditional early-phase trials consist of single- or multiple-dose designs aimed at determining the safety and tolerability of an investigational compound in healthy volunteers. However, by incorporating a translational approach, employing methodologies derived from preclinical strategies such as EEG measures and imaging, into the traditional Phase I program, critical information regarding a compound’s dose–response effects on pharmacodynamic biomarkers can be acquired. Furthermore, combined with the use of patients with stable schizophrenia in early-phase clinical trials, significant ‘de-risking’ and more confident ‘go/no-go’ decisions are possible.

Keywords: clinical trials early phase electroencephalography functional magnetic resonance imaging polysomnography PET schizophrenia translational

Schizophrenia is a severely debilitating psychiatric disorder consisting of positive and negative symptoms, cognitive impairment and social dysfunction, impacting approximately 1% of the general population. Currently marketed antipsychotics are effective primarily against positive symptoms (e.g., hallucinations), while the second-generation antipsychotics (SGAs) exhibit modest clinical benefit on negative symptoms (e.g., anhedonia) and even weaker effects on cognitive deficits [1,2]. Therefore, in addition to novel treatments that improve the overall symptomatology in schizophrenia, whether as add-on or monotherapy, there is currently a particular focus on drug development programs to identify novel compounds to treat negative symptoms and cognitive impairment. While numerous potential drug candidates representing a myriad of novel mechanisms have advanced from the preclinical stages, many have failed in early (Phase I) or later phase (Phase II or Phase III) trials. These failures were often due to toxicity, poor pharmacokinetics (PK) or lack of efficacy on primary clinical end points (e.g., change in clinical ratings scale score, in placebo-controlled trials). Despite a rich development pipeline, the majority of compounds currently in Phase III trials for the treatment of psychotic symptoms in schizophrenia, with a few exceptions, retain mechanisms influencing dopamine-2 (D_2)-receptor signaling, similar to currently marketed drugs [3]. The failures of novel antipsychotics beyond SGA therapies for schizophrenia illustrate the limitations of our traditional preclinical models in translating the effect of these new chemical entities from preclinical studies to patients with schizophrenia. Pharmacodynamic (PD) end points extending beyond traditional animal behavioral end points are needed, especially if these measures would translate into meaningful signals in early-phase healthy volunteer and patient trials. These improved models should deconstruct schizophrenia into endophenotypes or other robust clusters of observable phenomena, which are predictive of targeted (negative symptoms, cognition) antipsychotic efficacy. Proper selection and utilization of translational PD tools during early-phase clinical testing may therefore increase successful drug development of novel antipsychotic and/or more targeted therapeutics. These tools or biomarkers, when incorporated into Phase I trials, may provide translatable information including evidence of drug target engagement, signals suggestive of efficacy, improved detection of CNS toxicity and more robust PK–PD models [4–6]. There is also the real possibility that potentially efficacious drugs have failed clinical trials due to reductions in the signal detection of many larger scale Phase III trials [7,8]. The causes and solutions
for failed later phase trials, although relevant to improving a compound’s successful development, are beyond the scope of this article.

**Translational toolkit informing decision-making in early-phase CNS trials**

Traditional Phase I studies typically involve single (SAD) and multiple-ascending dose (MAD) trials in healthy volunteer subjects in order to establish safety, characterize PK and to identify the maximally tolerated dose (MTD). The probability of success for novel treatments of psychiatric disorders leaving Phase I trials is markedly lower than in other therapeutic areas, with only 8% of all compounds transitioning into Phase II trials [9]. To avoid costly failures associated with larger Phase II/III trials, many industry CNS drug development programs have increasingly incorporated translational medicine models, adaptive dose and PD modeling strategies into Phase I trials to gain detailed mechanistic and functional data to inform go/no-go decisions on a compound’s success while mitigating risk associated with developing the compound [10,11]. The challenges presented by drug development for schizophrenia have resulted in the search for novel PD methodologies that might readily translate from preclinical models of schizophrenia to patients, and provide informative data predictive of clinical efficacy [10]. In addition, the large heterogeneity of the illness at the molecular, genetic and clinical phenotype level [12] further support the use of biomarkers, adaptive design and stratification strategies to enhance signal and/or enrich populations to enhance response to new therapies. Therefore, early-phase development now tests the therapeutic potential of a novel therapy, leading to smaller, better designed proof-of-concept studies. The ‘Learn and Confirm’ is moved earlier into drug development, thereby accelerating the critical path to drug approval.

Utilizing novel study methodologies (e.g., adaptive design) with translational mechanistic or functional PD biomarkers during Phase I testing can provide valuable information in the characterization of antipsychotic compounds [13]. For example, while many Phase I studies use healthy volunteers to establish dose-limiting tolerability, the inclusion of patients with the target illness into the Phase I program may aid in dose-finding studies, as data have shown that patients with schizophrenia exhibit a higher tolerance of D₂ antagonists, D₂/D₃ partial agonists, antimuscarinic and α-blocking therapies compared with healthy volunteers, thus resulting in a significant shift in the dose–response curve [14,15]. Additional pharmacological mechanisms where tolerability differences between patients and healthy volunteers are apparent include ampakine modulators and H₃ antagonists [16]. Adding experimental and putative biomarker assessments based on PD considerations and targeted therapeutic focus (e.g., negative symptoms, cognitive symptoms) in patients with schizophrenia, as well as obtaining a clear indicator for CNS penetration and effect, are important strategies to increase the success of Phase II programs [17]. The potential to ‘de-risk’ a clinical development program frequently more than offsets the added complexity and cost required to conduct patient biomarker studies.

A translational tool kit informing the effect of a compound on various mechanistic and functional end points that may predict efficacious dose ranges and tolerability going in advance of a Phase II POC trial includes biochemical and ‘omic’, biomarkers, imaging (functional MRI (fMRI) or PET receptor occupancy studies, EEG, event-related potentials (ERPs) and cognitive assessments [13,18]). Many PD-based methodologies employed as part of the Phase I program are typically derived from preclinical behavioral animal models in vitro and animal behavioral studies. These measures and assessments can provide critical information related to the three pillars of compound survival (e.g., determination of drug exposure, target occupancy and functional modulation), potentially improving the success of these antipsychotic agents in later phase trials [17]. Typically, the use of multiple PD methodologies considered relevant to the therapeutic effects of the investigational compound are recommended, along with traditional PK assessments, providing corroborative confirmation of target engagement and modulation of relevant mechanisms of action. An additional challenge in the application of a toolkit approach is to determine the level of evidence deemed sufficient to move forward, or to kill the development plan. In general, early development trials relying on a single toolkit biomarker would not be sufficient to end a development program, with the exception being failure to demonstrate CNS penetration by PET occupancy or CSF measurements.

An illustrative early clinical development plan for a new therapy for the Cognitive Impairment Associated with Schizophrenia is portrayed in Figure 1A. In the MAD study, healthy volunteers are traditionally used to establish MTD.
Figure 1. Illustrative ‘accelerated’ development ‘go/no-go’ plan of an add-on treatment CIAIS program incorporating various pharmacodynamic methodologies. (A) Illustrative example incorporating multiple PD methods into a nested SAD and MAD Phase I study program. In the SAD study, PD studies can be included as part of the SAD dosing cohorts, or separate PD-focused cohorts (1c) can be conducted at 2–3 dose levels once safety and tolerability data has been obtained from the MTD cohorts (1a). In addition, a comprehensive PD battery (1d) can be included as part of the SAD. As with the SAD, PD methods can be included as part of the MAD cohort panels (2a) where dosing may extend beyond the window for MTD determination to include additional PD measurements. Similarly, a PD battery (2b) can be included as a separate portion of the MAD to include multiple measurements. (B) Illustrative example of combining multiple PD methods into a PD ‘batter’. In this example, a novel investigational compound is being evaluated using a ketamine-reversal, crossover model where the test compound is administered prior to ketamine followed by fMRI, qEEG and cognitive/behavioral assessments scheduled depending upon PK parameters obtained from the SAD cohorts. Subjects are then assessed 7 days post-dose and then undergo repeat challenges.

Behav.: Behavior; Btry.: Btry.; D.: Dose; FE: Food effect; fMRI: Functional MRI; HNV: Healthy normal volunteer; I/E: I/E criteria; MAD: Multiple ascending dose; MTD: Multiple tolerated dose; PD: Pharmacodynamics; PK: Pharmcokinetics; qEEG: Quantitative EEG; SAD: Single ascending dose.
However, using an adaptive design approach, where dose level escalation is flexibly defined (delimited by toxicology limits), and the switch from healthy volunteers to patients is clearly defined in the protocol, result in a more rapid and less costly exploration of the drug in Phase I.

In addition to the traditional SAD cohorts, multiple PD methodologies selected from preclinical data and human disease models are incorporated across both the SAD and MAD studies. The role of translatable CNS ‘tool kit’ strategies that can be applied to a Phase I program (Figure 1A) or as part of a PD-based study to inform antipsychotic drug development is described below and illustrated in Figure 1B. The advantages and disadvantages of the various translational PD methodologies utilized in Phase I programs are listed in Table 1.

## CNS imaging (fMRI & PET)

fMRI may offer another tool to understand how newly developed compounds alter neural circuitry and functional connectivity in the CNS. This neural activity is indirectly measured by regional changes in blood-oxygen level dependent signal (BOLD), the most commonly used fMRI technique [19]. Neural activity in response to specific, reproducible and well-characterized stimuli can serve as a fingerprint of specific function for disease state or drug action [19]. Unlike other imaging modalities (e.g., PET), fMRI allows for a more global measure of target engagement since it does not rely on radioligands developed for specific predetermined receptor sites or enzymes [20]. Preclinical fMRI data may also reveal neural processing (both conscious or subconscious) that is more sensitive than behavioral assays, since it does not rely on motor-driven behavioral outcomes [19]. Translating this circuit-based approach into early phase trials may also reveal connectivity patterns that are indicative of off-target side effects such as sedation (intralaminar nuclei of thalamus and cortical regions) or nausea and emesis (nucleus tractus solitarius of brainstem) [19].

Some issues with fMRI have been uncertain reproducibility and test–retest reliability consistency, but academic and industry research collaborations have proposed a framework for fMRI in drug development, including standardized procedures for image acquisition and stimulus presentation, protocol optimization and best practices [21,22]. In the case of schizophrenia drug development, the Cognitive Neuroscience Test Reliability And Clinical applications for Schizophrenia (CNTRACS) Consortium have focused on optimizing psychometric data to measure cognitive effects of treatment in

<table>
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<tr>
<th>Methodology</th>
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<tr>
<td>Plasma</td>
<td>Mechanistic</td>
<td>Simultaneous measurement of PD biomarkers and PK</td>
<td>Invasive Volume limits, especially when combining exploratory PD measures and PK</td>
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<tr>
<td>CSF</td>
<td>Mechanistic</td>
<td>Provides surrogate of CNS penetration Simultaneous measurement of PD biomarkers and PK Aid in modeling between CSF and plasma compartments</td>
<td>Invasive Potential risk of AEs carrying over into AE assessments in MTD determination</td>
</tr>
<tr>
<td>EEG/ERP</td>
<td>Functional</td>
<td>Low cost; easily available Data can be used to provide PD and safety information</td>
<td>Lack of standardization in data analysis Low spatial resolution</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional</td>
<td>Noninvasive proof of concept Provide translatable circuitry data related drug action</td>
<td>Expensive technology Not high throughput</td>
</tr>
<tr>
<td>PET</td>
<td>Mechanistic</td>
<td>Provides data regarding CNS penetration and target engagement Dose-finding studies</td>
<td>Availability of radioligand tracer Half-life of radiotracer (e.g., 11C or 18F) may pose operational issues Expensive</td>
</tr>
<tr>
<td>Cognitive/behavioral measurements</td>
<td>Functional</td>
<td>Paper and computerized based ratings scales allow customizable batteries to be utilized based upon signal from preclinical data Aid in modeling cognitive/behavioral effects based upon PK</td>
<td>Single dose effects may not be detectable Results from lengthy cognitive batteries may difficult to interpret due to subject fatigue Practice and diurnal effects can be observed</td>
</tr>
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AE: Adverse event; CSF: Cerebral spinal fluid; ERP: Evoked related potential; fMRI: Functional MRI; MTD: Maximally tolerated dose.

schizophrenia [23]. The four key metrics (and task names) are related to goal maintenance and working memory (dot probe expectancy), relational and item encoding/retrieval (relational and item-specific encoding and retrieval), visual integration (jittered orientation visual integration) and gain of control (contrast–contract effect) [23].

The application of pharmacologic fMRI methods in antipsychotic drug development programs has been used in early-phase studies in healthy volunteers and in schizophrenic patients. These studies have utilized fMRI to examine drug-induced changes in resting-state BOLD signal for purposes of dose finding and proof of penetration, while others have examined drug-induced effects on BOLD signal during cognitive challenges to characterize networks involved in mediating a procognitive effect [24,25]. In addition, a number of resting state and task-induced paradigms exist to characterize the fMRI BOLD or arterial spin labeling changes to antipsychotic exposure. For example, Abbott et al. demonstrated that antipsychotics diminished neural activation in motor networks and default mode networks in patients with schizophrenia [26]. Using arterial spin labeling, a process by which magnetically labeled water passes through the cerebral vasculature and can be mapped during an MRI scan, Handley et al. demonstrated single-dose effect differences between first- and second-generation antipsychotics in healthy volunteers [27]. These different MRI paradigms can elucidate dose–response relationships and contribute to the PD modeling during Phase I studies.

In addition, translational protocols such as ketamine-induced changes in neural networks in rodents have been extrapolated to healthy volunteers. For example, Driesch et al. determined that ketamine infusion produced an increase in global-based connectivity and was associated with schizophrenia-like symptoms as assessed by the Positive and Negative Symptoms Scale (PANSS) [28]. In addition, reversal of ketamine-induced changes in frontal and thalamic BOLD fMRI signals have been performed in healthy volunteers using drugs such as lamotrigine and risperidone, compounds shown in preclinical rodent models to attenuate the increased BOLD signal associated with glutamate antagonism [29].

Wise and Preston have attempted to characterize the value of fMRI in CNS drug development as part of early-phase clinical program decision-making, and how to use these new datasets to improve go/no-go decision-making [24]. They contend that programs employing fMRI methodology in Phase I could possibly save US$10 million per project when comparing the average risk-adjusted cost of CNS development programs with and without it [24]. Thus, fMRI, when appropriately applied in early-phase trials, could theoretically improve drug development programs to treat schizophrenia by providing key information related to dose determination, adverse events profiling and PD signals potentially predicting efficacy.

Unlike MRI, PET imaging has been utilized as both a mechanistic and functional biomarker in antipsychotic drug development, allowing translation from preclinical models into humans [30]. PET imaging has a number of advantages in Phase I development programs that include the use of biologically ubiquitous elements (e.g., $^{11}\text{C}$), which exhibit a wide range of half-lives, allowing the ability to label almost any novel compound or protein target with specificity and to conduct repeat scans in subjects [31]. During Phase I drug development programs, most PET studies evaluating antipsychotic therapies have been used to determine the $EC_{50}$ values, the plasma concentration associated with 50% receptor occupancy in the $E_{max}$ model, in an effort to aid in dose selection and dosing interval prior to going into Phase II studies [32]. While studies have shown that healthy normal volunteers differ from patients with schizophrenia with respect to determination of the MTD, many PET studies have been safely conducted in healthy volunteers [33,34]. In addition, establishment of $EC_{50}$ values of novel antipsychotics using healthy volunteers has been reported to be similar to those of patients with schizophrenia, despite differences in underlying disease state and exposure to prior antipsychotic therapy, indicating that healthy subjects could be used in lieu of patients with schizophrenia during exploratory PET studies [35–37].

To define optimal effective dosing among various antipsychotics, PET imaging has been utilized to determine the degree of $D_{2}$-receptor blockade threshold between clinical efficacy and extrapyramidal symptoms [30]. For example, Nyberg et al. utilized $[^{11}\text{C}]$-raclopride to determine the minimal effective dose of risperidone in patients with schizophrenia [38]. Risperidone 6 mg/day was found to produce 79–85% $D_{2}$-receptor occupancy, while 3 mg/day resulted in 53–78% $D_{2}$-receptor occupancy [38]. Higher $D_{2}$-receptor occupancy (>80%) was associated with the highest incidence of extrapyramidal symptoms [38]. Similar approaches were...
used for olanzapine, loxapine, ziprasidone and others \cite{39-41}.

In addition to the role of PET imaging as a functional biomarker to establish target engagement and dose ranging, PET imaging has been applied as a functional assay to characterize metabolic patterns of drug response using $[^{18}\text{F}]$-deoxyglucose-PET \cite{42}. Studies of antipsychotics using $[^{18}\text{F}]$-deoxyglucose-PET have not only identified regional differences in cerebral metabolic patterns among the different antipsychotics, but have also found correlations in differences in cerebral metabolism and improvement in positive, negative and cognitive symptoms or adverse effects \cite{43-46}.

Imaging modalities such as fMRI and PET represent minimally invasive, readily translatable approaches to characterizing mechanistic and functional PD correlations between preclinical models and humans. In coupling these imaging techniques with traditional PK measurements, novel antipsychotic compounds can be evaluated on key PD parameters such as dose selection, dose interval, CNS penetration and target engagement. In addition, fMRI and PET imaging permit characterization of a novel compound’s interaction with complex neuronal circuitry that may predict clinical response or adverse event profiles in later-phase studies.

Cognitive & behavioral testing

Neurocognitive impairments in a number of functional domains are core features associated with schizophrenia and have been attributed to dysregulation in neurotransmitters such as acetylcholine, dopamine and glutamate \cite{47}. These include deficits in memory, attention, processing speed, working memory, executive function and social functioning \cite{48,49}. The severity of cognitive impairment in schizophrenia has been associated with poor treatment outcomes and risk of relapse, while imaging studies have demonstrated functional and structural abnormalities associated with impairment in specific domains of schizophrenia \cite{50-52}. Recognizing the clinical and societal importance of these cognitive impairments in schizophrenia, key opinion leaders from academia, industry and governments developed guidelines for the design of clinical trials and recommended domain-specific cognitive assessment batteries (e.g., MATRICS Consensus Cognitive Battery) for evaluation of novel therapeutics on cognitive function \cite{53-55}.

In addition, significant efforts have been made to optimize preclinical assays in rodent and primates so that they may readily translate into clinical assessments. Preclinical models, such as the 5-choice serial reaction time task for attention and executive function, demonstrate high sensitivity and translatability from rodents to humans \cite{56}.

Early clinical trials with SGAs suggested beneficial effects in cognition; however, results from NIH-sponsored CATIE demonstrated modest improvements in cognitive outcomes with SGAs, but no statistically significant differences between treatments when compared with first-generation antipsychotics (FGAs) \cite{1,57}. These findings have led many antipsychotic drug development programs to explore detection of changes in domain-specific cognitive function during early phase studies.

While cognitive batteries such as the MATRICS Consensus Cognitive Battery are considered the gold standard for US FDA registration trials for antipsychotics (e.g., Phase III trials), the employment of easier-to-administer cognitive assessments that map to MATRICS domains and behavioral ratings scales during early-phase studies can provide useful information related to cognitive processes and safety outcomes (Table 2). In first-in-human studies, utilization of simple cognitive or behavioral tests can be used as safety assessments informing potential adverse effects on cognition or mood, particularly for compounds exhibiting CNS penetration. These may include simple tasks of motor function (reaction time task), attention (digit-symbol substitution), working memory (n-back) or executive function (Stroop task). These tasks can serve to identify clinically meaningful CNS effects that may limit subsequent development. Second, while Phase I studies are not traditionally powered to demonstrate a statistically significant effect of treatment versus placebo, these assessments can provide metrics that will inform statistical/sample size decisions regarding cognitive changes in subsequent Phase II POC trials.

In addition, for cognitive domains demonstrating a treatment trend, examination of PK/PD relationships can be performed to characterize dose–exposure effects informing later-phase dose selections for outcome measures.

Selection of a particular cognitive battery during Phase I studies should be tailored to the pharmacologic mechanism and targeted neurological circuits underlying the behavioral effects identified from preclinical studies. Consideration of the type and extent of changes in specific cognitive domains from single- and multiple-dose studies in preclinical models can aid in determining translatable cognitive assessments in...
similar human trials. For example, single-dose improvements in a number of specific cognitive domains have been reported in both healthy volunteers and in patients receiving procognitive drugs [58–60]. Another important consideration is the availability of normative data from the specific cognitive battery whereby data from individual treatment effects can be compared [61]. Data obtained from PK or exploratory PD studies can be used to support dose-finding studies predictive of efficacy and possibly useful dose range when transitioning from healthy normal volunteers to patients with schizophrenia. For example, data from the CATIE trial showed that antipsychotic doses predictive of >80% D2-receptor occupancy based upon plasma concentrations using population PK modeling increased the risk of cognitive impairment and predicted performance on vigilance tasks [62]. Although beyond the scope of this article, the assessment of cognition or function in patients receiving novel therapies is probably best assessed under a stimulating environment encompassing cognitive loading.

In addition to the cognitive assessments mentioned above, several behavioral ratings scales can be utilized during Phase I trials to assess a CNS compound’s effect on behavioral domains such as sedation/drowsiness, sleep quality, mood states and extrapyramidal symptom liability in healthy volunteers and patients with schizophrenia (Table 2). For example, many patients with schizophrenia are often dual diagnosed with polysubstance abuse, which can dramatically alter response to treatment and worsen outcome [63]. Thus, during a Phase I trial of a novel procognitive compound that may exhibit stimulating properties as demonstrated during preclinical studies, the incorporation of ratings such as the Addiction Research Center Inventory and Profile of Mood States that bracket Cmax based on PK data obtained during the SAD study may provide important data so that dose-dependent effects on cognition and mood can be dissociated prior to Phase II.

As an illustration, the development of selective dopamine-1 (D1)-receptor agonists for cognitive impairment in schizophrenia has gained interest among many in industry. Selective D1 agonists have been shown to improve cognition and prefrontal perfusion in rodent models and in schizophrenia [64–66]. In addition to modulating working memory, preclinical studies suggest that D1-receptor activation can modulate drug craving of cocaine-seeking behavior and suppress cocaine self-administration [67]. Rodent studies with the novel D1 agonist, ABT-431, demonstrated suppression of motivation to seek cocaine and masked the reinforcing effects of cocaine self-administration [68]. Similarly, in a small trial of regular cocaine users, ABT-431

| Table 2. Neurocognitive and behavioral ratings scales utilized in early phase studies to evaluate novel antipsychotic compounds. |
|-----------------|---------------------|
| **Cognitive/behavioral scale** | **Domain** |
| MMSE | Mental status |
| DMS | Working memory |
| WMS-R digit span | Working memory |
| Spatial span | Working memory (visuospatial) |
| Paired associates | Working memory |
| WCST | Executive function |
| CPT-IP | Executive function (attention) |
| CVLT/RVLT | Episodic memory (verbal) |
| WMS-R | Episodic memory (visual) |
| WMS-R logical memory | Episodic memory |
| Trails making A/B | Processing speed |
| WAIS-R digit symbol | Processing speed |
| **Sedation/sleepiness** | |
| ESS | Sedation |
| KSS | Sedation |
| PSQI | Sleep quality |
| LSEQ | Sleep quality |
| **Mood/behavior** | |
| POMS | Mood states |
| ACR-I-49 | Subjective drug effects |
| CADSS | Dissociative symptoms |
| PSI | Psychotomimetic effects |
| **Motor/extrapyramidal** | |
| AIMS | Dyskinesias |
| ESRS (or ESRS-A) | Extrapyramidal symptoms |
| SAS | Parkinsonian symptoms |
| BARS | Akathisia symptoms |
| **Symptomatology** | |
| PANSS | Psychopathology ratings scale |
| BPRS | Psychopathology ratings scale |

ACR-I-49: Addiction Research Center Inventory; AIMS: Abnormal Involuntary Movement Scale; BARS: Barnes Akathisia Rating Scale; BPRS: Brief Psychiatric Ratings Scale; CADSS: Clinician Administered Dissociative Symptom scale; CPT-IP: Continuous performance test-identical pairs subtest; CVLT: California Verbal Learning test; DMS: Delayed match to sample test; ESRS: Extrapyramidal Symptom Rating Scale; ESS: Epsworth sleepiness scale; KSS: Karolinska sleepiness scale; LSEQ: Leeds Sleep Quality Evaluation Questionnaire; MMSE: Mini-mental state exam; PANSS: Positive and Negative Symptom Scale; POMS: Profile of Mood States; PSI: Psychotomimetic states inventory; PSQI: Pittsburgh sleep quality index; RVLT: Rey’s Verbal Learning Test; SAS: Simpson–Angus Scale; WAIS-R: Wechsler Adult Intelligence test-Revised; WCST: Wisconsin Card Sort Test; WMS-R: Wechsler Memory Scale-Revised. Data taken from [87].
demonstrated a trend to decrease cocaine craving, but not the frequency of use [69,70]. These results demonstrate the myriad of pharmacologic effects that D₁ agonists may produce in patients that are translatable from preclinical models to humans. Therefore, as part of a Phase I study using patients with schizophrenia, the inclusion of ratings scales that can assess single or multiple dose effects on mood or abuse potential such as the POMS or ARCI-49 respectively, could be used to characterize the effect of a novel D₁ compound on mood and drug effect.

In addition to the use of various ratings scales assessments to characterize behavior or cognition during the Phase I study, several PD methods can be incorporated into one larger PD-focused battery as part of either the SAD or MAD, as demonstrated in Figure 1A. These assessments can be performed in a placebo-controlled fashion with or without using a pharmacologic challenge strategy. One potentially translatable PD battery consists of a pharmacologic-reversal model, similar to those utilized in preclinical rodent models to screen characterized compounds for many neuropsychiatric disorders [71,72]. Pharmacologic reversal models using drugs such as ketamine, scopolamine and amphetamine have been used safely in healthy volunteers to simulate behavioral or cognitive impairments associated with schizophrenia [73–76]. For example, the leading hypotheses behind schizophrenia include a hyperdopaminergic state within the mesolimbic pathway and hypogluta matergic deficits impacting thalamocortical neurotransmission [77]. The N-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, has been shown to transiently induce range of psychomimetic and cognitive effects that are similar to symptoms associated with schizophrenia [74]. Unlike amphetamine, subanesthetic doses of ketamine have been shown to produce positive and negative symptoms, including cognitive deficits in domains such as working memory, attention and executive function by disruption of glutamate signaling [18,74].

As illustrated in Figure 1B, a PD battery can be designed as a double-blind (investigational drug vs placebo), randomized crossover study separated by a 7-day washout. Ketamine is administered as a bolus followed by a continuous infusion to create a stable altered state allowing a number of PD measurements to be conducted. Ratings scales such as the Positive and Negative Symptom Scale, the Clinician Administered Dissociative Symptom Scale and the Brief Psychiatric Ratings Scale have all demonstrated induction of psychotomimetic symptoms in response to ketamine [74,78]. In addition, plasma samples measuring ketamine and the investigational compound are obtained for comparisons of PK parameters with behavioral and cognitive effects. Several medications from different pharmacology have been evaluated safely in healthy volunteers using variations of this paradigm [75,78,79]. While this model represents an easily translatable method similar to that conducted in preclinical models of schizophrenia, several compounds with proven antipsychotic efficacy (e.g., haloperidol) have failed to reverse ketamine-induced changes in PD outcome measures [29,80].

In addition to measuring changes in the reversal of various cognitive or behavioral ratings, other PD methods such as qEEG and fMRI can be paired to provide a comprehensive battery capable of exploring multiple drug points of a novel antipsychotic during the ketamine-reversal paradigm. As discussed below, EEG and fMRI methods have been used to characterize the cognitive and behavioral effects of ketamine and to evaluate the effects of novel antipsychotics [28,81]. In addition, an important new frontier of drug development is to combine cognitive remediation or cognitive–behavioral therapies with cognitive-enhancing drugs, or with CSF or plasma biomarkers [82]. For example, plasma-based biomarker (such as brain-derived neurotrophic factor) changes as a result of cognitive remediation programs for patients with schizophrenia suggest that the PD and biomarker approaches discussed in this article are also applicable to nondrug interventions [83].

The use of cognitive ratings in drug development can be host to a number of methodologic problems that include appropriate selection of instrument and controls, timing and environmental surroundings during administration of assessments, practice effects, and the administration and interpretation of various tests by unqualified individuals [84]. Because of the procedurally intensive nature of Phase I studies, it is recommended that use of brief cognitive tests (~15–20 min) are selected, frequently using computerized tests to aid in the facilitation of administration. These assessments (whether administered live or by computer) need to be administered in a quiet space, free from distractions, with specific attention to meals, use of sedating or other cognitive impairing drugs (i.e., benzotropine), and cigarette breaks. Practice effects related to a subject’s repeated performance of a cognitive assessment and diurnal variation related to the timing of administration of the tests have been reported.
and may differ significantly from healthy volunteers to patients [59, 85, 86]. Therefore, consistency in the timing of cognitive test administration can help diminish the variability observed in cognitive performance. The inclusion of repeated trials of testing during baseline has been recommended to reduce practice effects [84, 87]. Additional strategies to enhance signal detection include the selection of patients phenotyped (or genotyped) for a specific deficit relevant to drug effect, and/or the inclusion of a healthy control group to serve as a calibration of the magnitude of change of a cognitive measure can improve the interpretability of small sample size Phase I PD readouts.

The use of cognitive and behavioral testing during a Phase I study provides critical PD information in the characterization of CNS compounds. These measures have historically served to characterize adverse cognitive or behavioral toxicities associated with novel CNS compounds and when compared with PK data, can provide key information related to dose-limiting response effects. In addition, cognitive and behavioral ratings, when appropriately selected based upon informed preclinical data and administered during Phase I studies, may provide relevant findings suggestive of an efficacy signal to inform dose selection. To be clear, these techniques rarely in and of themselves predict the best dose; rather, these studies can suggest a dose (or range of doses) likely to produce relevant efficacy in later Phase studies.

EEG & polysomnography methodology

The neuronal pathways responsible for generating the electroencephalography (EEG) signals are similar between species due to similarities in anatomical and functional organization of the CNS, allowing the use of EEG methodology as a readily translatable PD biomarker between preclinical models and humans [88, 89]. Numerous EEG studies have been conducted in schizophrenia, demonstrating deficits in EEG frequency band power (e.g., decreased δ and increased β band power), synchronous activity in ERPs (e.g., auditory evoked potentials; P50, P300 response), measures of sensory gating (e.g., PPI) have similarly been identified in preclinical models of schizophrenia as well as in patients [90–92]. Freedman and Adler used an auditory-paired click auditory-evoked potentials procedure to investigate the sensory gating hypotheses in schizophrenia [93, 94]. This research has been recently reviewed [95]. The general conclusion from numerous studies is that there is a consistent difference in the amplitude of the response to the first compared with the second click in closely timed paired auditory stimuli. The lack of relative lack of as decrement to the second tone in a pair has been interpreted as reflecting deficient sensory gating in schizophrenics. It is clear that technical factors vary significantly across studies making direct comparisons difficult, but this finding has been replicated and is generally accepted.

The effect of drugs on measures of cortical gating, attention and cognitive processing using EEG and EP/ERP methods is well documented. For example, Olincy et al. carried out a P50 auditory EP study involving administration of two doses of an α7 nicotinic (α7nAChR) agonist to a group of 12 nonsmoking schizophrenics [96]. Changes in the P50 component were measured as well as effects on neurocognitive testing performance. Of interest, the drug produced inhibition of the response to the second click in the paired stimuli, moving the response toward that seen with normals [96]. This study showed the applicability of the P50 paired stimuli decrement as a biomarker assessing change in the CNS associated with a specific compound. In addition, these investigators studied dose response of the α7 nicotine agonist. It was noted that the magnitude of changes in response could help predict the most effective dose [96]. However, P50 studies are technically difficult to conduct, since the signal is weak and influenced by the psychiatric mental status of the patient. Enrichment studies selecting patients with abnormal P50 are possible in small Phase I or POC studies, but are not feasible in larger studies due to the high screen fail rate in identifying patients who meet published criteria. Enrichment strategies of high versus low working memory or characterizing patients by their COMT genotype can result in a clearer signal readout, especially when considering the small sample size used in early development trials. These measures can be useful in larger clinical trials, as a post hoc stratification exploratory objective. Auditory evoked potentials have also been studied in pre-attentive processes, where the patient’s mental status is not likely to affect signal processing. Mismatch negativity (MMN) is an auditory event-related potential procedure using presentation of standard and deviant stimuli. Deviant stimuli, either differing in pitch or duration generate a potential when the stimulus violates the regularity of the recent auditory past, that is, echoic memory. A peak is usually
seen in the 100–240-ms range after a deviant stimulus. Shelley et al. first studied this ERP in schizophrenia and demonstrated a reduced MMN response [97]. A recent meta-analysis concludes that deficits in MMN generation are a robust feature in chronic schizophrenia and suggest maximal effects are due to deviance in stimulus duration rather than frequency characteristics [98].

The auditory P300 procedure is similar to the MMN procedure, except that individuals are instructed to attend to and identify deviant or rare tones in a series of standard tones. Auditory P300 involves active detection of target stimuli in a series of standard auditory stimuli. A recent meta-analysis of findings from studies using measures of P50 and P300 confirms the presence of ERP deficits in schizophrenia. These authors conclude that findings involving auditory ERPs are robust and comparable with regard to size of effects using neuroimaging and neuropsychological testing methods [99].

Another method used to study sensory processing in schizophrenia is the auditory steady-state paradigm (aSSR). With the aSSR procedure an individual is presented with trains of brief, repeated auditory stimuli presented at various rates while an EEG recording is done simultaneously. In a normal aSSR, an individual’s EEG synchronizes to the frequency of the auditory stimuli. In general, the aSSR amplitude increases monotonically over a 200 ms period beginning approximately 40 ms after the train onset and at train offset the aSSR stops within 50 ms [97,100].

Using the aSSR paradigm, it has been shown that individuals with schizophrenia have trouble supporting γ activity. In a study by Kwon et al., patients with schizophrenia and age-matched controls were presented with trains of clicks at various frequencies (20, 30 and 40 Hz) while their EEG were recorded [101]. The results showed that in patients with schizophrenia, EEG exhibited a decreased power at 40-Hz stimulation compared to controls. In addition, the EEGs of individuals with schizophrenia were slower than controls to show synchronization to the clicks and were slower to desynchronize after the clicks stopped [101]. More recently, Hamm et al. showed that schizophrenics displayed decreased synchronization to 80-Hz frequencies as well [102]. In addition, several studies have shown that γ frequency oscillations are modulated by a number of cognitive processes such as attention and working memory, reflecting an integration of neural circuitry [103,104].

Patients with schizophrenia have been shown to exhibit deficits in steady state and event-related γ oscillations reflecting deficits in attention and memory [105]. Furthermore, some antipsychotics that are thought to enhance cognition have shown clear γ frequency shifts in power as exposure is increased [103,106].

Another method that may be useful in understanding neuronal mechanisms underlying schizophrenia is EEG coherence. EEG coherence is computed between signals from different scalp locations and offers a measure of similarity of activity in the two regions. This measure has been used as a measure of brain connectivity and may be particularly useful in evaluating synchrony of γ activity between or among regions.

During Phase I studies, EEG/ERP paradigms can be applied to either single or multiple dose studies with healthy volunteers or in patients as electrophysiologic measures predictive of clinical effect [88,107]. EEG measurements, especially when combined with PK sampling, allow the characterization of PK/PD relationships of centrally acting drugs and can aid in discerning treatment-emergent adverse effects or proconvulsive effects of antipsychotic compounds [108,109]. For example, γ frequency changes associated with nonselective NMDA antagonism or specific NR2A antagonists have been identified using qEEG in preclinical rodent models of schizophrenia and have been shown to correlate with cognitive dysfunction in schizophrenia [110–112].

Several studies have applied EEG/ERP methodology as part of single-dose studies of antipsychotics in healthy normal volunteers [113–115]. As an example, single-dose administration of olanzapine to healthy controls produced increases in θ-band activity associated with treatment-emergent somnolence, although no changes in P300 component were seen compared with placebo [114]. Similarly, risperidone in conjunction with a novel 5-HT3 antagonist administered to healthy controls resulted in an increased EEG α and β frequency [116]. Similar to methods involving reversal of scopolamine or ketamine-induced deficits in cognition utilized in preclinical studies for screening antipsychotic compounds, clinical pharmacology trials utilizing these compounds have been shown to induce similar deficits in EEG band activity and ERP, and may be reversed by traditional and novel mechanism antipsychotic compounds [81,117–120].

Current theories of schizophrenia emphasize that core aspects of the pathophysiology are due to deficits in the coordination of distributed processes that involve various cortical regions. γ
Use of translational PD biomarkers in early-phase clinical studies for schizophrenia

**Synchrony abnormalities seen in schizophrenia** offer a potential objective measure of this coordination. In addition, if it can be demonstrated that medication can normalize aSSR and/or MMN responses, then these indices may also be useful in ascertaining optimal dose.

In addition to EEG, polysomnography (PSG) studies have documented a number of sleep abnormalities in patients with schizophrenia that have been associated with cognitive impairment and diminished quality of life [121]. In particular, the most common sleep abnormality, a decrease in slow-wave sleep, has been correlated to deficits in consolidation of procedural and declarative learning [121]. Many currently available antipsychotics improve sleep abnormalities in schizophrenia via 5-HT2A blockade [122]. For example, the SGA olanzapine, a potent 5-HT2A antagonist, has been reported to increase slow-wave sleep and was positively correlated with an increase in verbal memory consolidation [123]. Thus, the use of PSG in Phase I studies may serve as a useful biomarker for novel treatments for schizophrenia capable of inducing changes in slow-wave sleep reflective of positive changes in cognition [124]. Conversely, drugs that are procognitive and increase arousal could readily delay sleep onset and reduce sleep efficiency. Given the close inter-relationship between normal physiologic sleep and cognitive function, characterization of a drug’s effects on PSG can play a role in the go/no-go decision process [125].

In addition to PSG studies, a quantitative physiologic characterization of the sedative effects of CNS active compounds can be performed using methods such as the multiple sleep latency test, a technique measuring the speed at which a person falls asleep during the day (and is observed repeatedly over time, since subjects are not permitted to remain asleep). When coupled with various psychometric tests assessing attention, concentration and psychomotor processing, a robust model for sleepiness and sedative effects on cognitive function, typically as a function of concentration time course over the dosing interval, can be built. Sedation is one of the primary adverse events associated with many CNS active compounds, and early characterization of the severity of these effects is an important tolerability assessment in early phase studies [124].

These methods can be employed during Phase I studies to examine electrophysiologic changes characteristic of procognitive or CNS sedative/sleep disruptive effects that may worsen cognition or primary symptomatology. Electrophysiologic techniques offer noninvasive strategies to directly also measure temporal PK and PD relationships between drug exposure and its effect on CNS physiology.

### CSF & plasma biomarkers

A central tenant to the success of any CNS drug discovery program is the demonstration of achievable drug concentrations at a target site of action within the brain, which is believed to be therapeutic. Challenges for any novel CNS compound in reaching the brain include physiochemical properties, PKs and ability to penetrate the blood–brain barrier. While some drug development programs may utilize PET imaging methods to determine CNS penetration and occupancy at the intended site of action,
the use of this methodology requires availability of site-specific radioligands. Other methods, such as CSF sampling, can be incorporated into Phase I SAD or MAD studies as either a single or continuous sampling method to evaluate dose-dependent effects on PD biomarkers (Figure 2). While CSF sampling can provide indirect evidence of a compound’s direct target engagement, this method most commonly provides information regarding the compound’s CNS penetration, action on specific PD biomarkers related to the compound’s pharmacology, modeling of PK/PD relationships with drug concentration from plasma, and has been performed successfully with limited adverse events in healthy volunteers and in patients [126–129]. In addition, scaling and refinement of CSF/plasma models from rodents have evolved permitting extrapolation of preclinical rodent data to humans [126,130].

CSF sampling via single or continuous indwelling lumbar catheterization (dynambridging) has been utilized to characterize PK/PD relationships of a CNS compound and the impact on biomarkers of interest in a number of neuropsychiatric disorders including Alzheimer’s disease (AD) [128,131], schizophrenia [132–133], major depression [134,135] and post-traumatic stress disorder [136,137]. CSF dynambridging studies may also have increasing importance in schizophrenia drug development as novel biomarkers emerge related to specific phenotypic domains associated with the illness that may respond to drug effect (e.g., cognitive impairment, suicidality) [138–140].

One CSF biomarker, amyloid-β (Aβ) has been commonly used as a mechanistic biomarker in the evaluation of disease-modifying compounds in AD. Some investigators have found similar reductions in CSF Aβ levels in samples from older patients with schizophrenia [141,142]. Novel muscarinic receptor agonists and allosteric modulators selective at the muscarinic-1 (M₁) and muscarinic-4 (M₄) receptor subtypes have been shown to lower Aβ levels, improve cognition and have subsequently been investigated as pro-cognitive treatments for schizophrenia and AD [143–147]. In a 4-week clinical study of the selective M₁ agonist talsaclidine in patients with Alzheimer’s disease, CSF Aβ42 levels were reduced by an average of 16% compared with placebo, with no change in Aβ40 levels [148]. While the utility of CSF Aβ levels as a biomarker in schizophrenia is limited, translation of the preclinical effects of M₁ selective compounds on Aβ have been shown in in vitro and in CSF studies, therefore potentially representing a surrogate biomarker of a compound’s pharmacologic effect centrally [149].

Other CSF biomarkers that have been investigated for both diagnostic and drug efficacy studies in schizophrenia include monoamine metabolites, various neuropeptides and the S100b protein. The S100b calcium-binding protein is involved in cell proliferation, differentiation and calcium homeostasis and has been proposed as a biomarker of astrocyte activation [154]. S100b protein is elevated in schizophrenia patients when compared with control groups, but its specificity remains uncertain [150]. In particular, S100b has been shown to be increased in acute stages of schizophrenia, particularly in patients with residual or predominantly negative symptoms [151]. Similarly, increased serum levels of S100b have been correlated with cognitive impairment in patients with chronic schizophrenia [152]. The S100b receptor, receptor for advanced glycation end products, has become a focus of drug development in schizophrenia. Using an inhibitor of receptor for advanced glycation end products, Steiner et al. increased recovery time in schizophrenia and subsequently normalized serum levels of S100b [153]. These results point to the potential of CSF or plasma levels of S100b as a drug-modifiable biomarker in schizophrenia.

Changes in neurotransmitter metabolic levels have also been sampled in CSF as a biomarker for validation of PD effects in response to pharmacologic challenge involving novel glutamatergic modulating compounds. In a study of rats administered a metabotropic glutamate-2 receptor (mGluR2) positive allosteric modulator (PAM), the histamine metabolite tele-methylhistamine was found in CSF, which mirrored a reduction of extracellular histamine in the medial prefrontal cortex measured by microdialysis [154]. In another study, novel glycine-transporter-1 inhibitors (GlyT1), which have been shown to exhibit antipsychotic and pro-cognitive effects in preclinical models of schizophrenia, were shown to increase CSF glycine levels [155,156]. These new compounds developed to modulate the glutamate system may provide more new targets for biomarker discovery in schizophrenia.

Others have measured CSF biomarkers such as monoamine and various neuropeptides along with plasma drug levels to describe the relationship between drug exposure, changes on measurable biomarkers related to its pharmacology and therapeutic effects with several antipsychotics. In a 4-week open-label trial in patients...
with schizophrenia treated with quetiapine 600 mg/day, CSF monoamine levels and plasma quetiapine levels were obtained at baseline and at the end of 4 weeks [157]. Significant inverse correlations were found between the change in PANSS negative subscale score and change in CSF 5-HIAA and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels. An increase in CSF homovanillic acid, 5-HIAA and MHPG levels were also significantly correlated with improvements in positive and negative symptoms that similarly correlated with quetiapine plasma levels [157]. Caution should be taken in interpreting the above results as while significant correlations were observed, there were relatively low correlation coefficients calculated and the sample size was small. Despite the limitations of this study, similar increases in CSF monoamine levels and neuropeptides such as neuropeptide Y and corticotropin-releasing hormone, and change in clinical effect have been reported with FGA [132,158], and with a few SGA and third-generation antipsychotics [159–161].

In addition to CSF sampling, the use of plasma sampling for PK and/or PD characterization of novel compounds is often a key secondary objective of any Phase I program. While there are no validated plasma biomarkers of schizophrenia, a number of candidate biomarkers have been identified to include inflammatory mediators, cytokines, neurotropic factors and acute-phase proteins [162]. Measurement of these biomarkers as it relates to a compound’s activity derived from preclinical data may yield useful information related to dose response and toxicity. Conversely, plasma PK concentrations of an investigational compound have been utilized to estimate D₂ receptor occupancy where PET imaging may be unavailable clinically or only performed in preclinical studies, although limitations between plasma drug concentration and D₂ occupancy do exist [163,164].

For novel antipsychotics exhibiting traditional D₂ antagonist profiles, the use of elevations in serum prolactin has been proposed as a proxy biomarker of antipsychotic response. In a small case report series, Agarwal et al. reported a significant negative correlation between serum prolactin and global clinical improvement as measured by the CGI-I scale in patients receiving mono- or combination antipsychotic therapy [165]. However, the dose-dependent effect of prolactin elevations and D₂ antagonism may not be correlated with clinical response, especially for second- and third-generation antipsychotics. For example, comparison of several dose-dependent effects of FGA and SGAs on prolactin levels and therapeutic dose found no significant relationships between the 5HT₂/D₂ ratio and the prolactin dose equivalence and therapeutic dose [166]. While hyperprolactinemia is an undesirable adverse effect of DA-modulating antipsychotics, the use of prolactin as a biomarker of antipsychotic efficacy or adverse effects may be limited to those with a D₂ modulating effect.

Plasma biomarkers related to glutamatergic dysfunction in schizophrenia have yielded mixed results. In a study of 108 Japanese patients with schizophrenia, plasma glutamatergic-amino acid levels failed to be correlated with measures of cognitive function [167]. However, others have shown that plasma levels of glycine or glycine-serine ratios predicted change in a negative symptoms subscore (ablation) after 6 weeks of clozapine treatment [168]. A recent meta-analysis of case-controlled studies showed that serum serine levels were significantly higher in schizophrenia versus controls, where CSF serine, glycine, alanine and aspartate levels did not differ [169].

Changes in other plasma biomarkers, such as brain-derived neurotropic factor, cytokines (e.g., interleukins) and monoamine metabolites, such as homovanillic acid, MHPG and their ratios, have been shown to be associated with response to antipsychotic therapy [170–172]. The usefulness of these biomarkers in Phase I trials involving novel antipsychotics remains unclear; however, selection of the appropriate biomarker based upon preclinical data stemming from the known mechanism of action, may aid in characterizing dose–response relationships and toxicities to inform later phase trials [13].

Schizophrenia likely represents a polygenic, non-Mendelian disorder exhibiting variable penetrance, thus making identification of genetic loci challenging [173]. Pharmacogenetic (PGx) and pharmacogenomic studies have mainly focused on the monoamine targets related to the pharmacology of conventional antipsychotics and have seldom been applied a priori during Phase I trials. Similarly, FDA guidance on clinical pharmacogenetics in early phase studies only provides recommendations for pharmacogenetics approaches as exploratory studies intended to establish genetic hypotheses that may be applied to later Phase III trial designs [174]. Pharmacogenetic data collected during Phase I trials can provide information related to differences in PK/PD phenotypes that can characterize drug-exposure effects and PK variability that can be included in subsequent
population PK/PD models as the compound advances through the development phases [175]. In addition, novel approaches such as convergent functional genomics, while primarily applied to the identification and prioritization of candidate genes for a specific disorder, combine data derived from gene expression studies, genome-wide association studies (GWAS) studies and preclinical studies performed in transgenic animals, and may be beneficial in establishing a genetic connectome that could be used to profile novel antipsychotics [173,176].

Within the majority of PGx studies, polymorphisms at dopamine and serotonin receptors have been the most replicated associations with response phenotypes in schizophrenia thus far, but other possible gene targets have been recognized in GWAS [177]. Postsynaptic dopamine receptor subtype genes DRD2 and DRD3 have been associated with antipsychotic efficacy. The DRD2 functional polymorphism allele -141-C Del has been associated with lower expression DRD2 being associated with antipsychotic efficacy, while another SNP allele 311Cys showed less improvement [178,179]. A DRD3 9Gly variant is associated with better efficacy, reportedly related to its higher affinity for dopamine [180]. An enzyme responsible for the metabolic degradation of dopamine, catechol-O-methyltransferase 158Met allele has also been associated with improved cognitive function during antipsychotic therapy [181]. These pharmacogenetic association studies suggest that dopaminergic genes have been the most widely studied candidate genes since nearly all FDA-approved antipsychotics have D2 antagonism as the primary mechanism of action.

In addition to D2 antagonism, most atypical or second-generation antipsychotics also have 5-HT2B antagonism; therefore serotonin polymorphisms have also been investigated with respect to antipsychotic response. Several HTR2A SNPs had already been investigated for antidepressant response, including T102C (rs6313), -1438G/A, Thr25Asn, C516T and His452Tyr [182]. An extensive review of psychiatric HTR2A PGx studies highlighted associations between 102C, -1438G, 452Tyr SNPs and antipsychotic treatment resistance, but these findings have not been consistently replicated [183,184].

One of the largest GWAS to investigate antipsychotic response genotyped over 700 patients from the CATIE trial cohort [185]. The top statistical SNP result of this study was found in an intergenic region on chromosome 4p15 and mediated ziprasidone’s effect on positive symptoms. Several other genes appeared to mediate the effect of atypical antipsychotics (risperidone or olanzapine) on negative symptoms, including ANKSIB (tyrosine kinase signaling), CNTNAP5 (cell adhesion and communication) and TRPM1 (Ca2+ channels) [179].

Transcriptional profiling is another technique to identify biomarkers that may prove useful in early phase schizophrenia drug trials. For example, 14-3-3 proteins are involved in intracellular signaling, cell division, cell differentiation, apoptosis, ion channel functioning and neurotransmission [186]. In rat brains, 14-3-3 proteins associate and colocalize with GABA receptors, DISC1, CHRNA and TPH2 [187]. These proteins may influence neurotransmission by regulating exocytosis or phosphorylation of synaptic proteins, including NMDAR2 receptors [188]. 14-3-3 proteins gene expression studies have demonstrated that YWHA, -B, -E, -G, -H, -Q and -Z levels are decreased in the dorsolateral prefrontal cortex of schizophrenia patients [187]. In monkeys haloperidol increases YWHAB expression in the dorsolateral prefrontal cortex [189]. These proteins may influence neurotransmission by regulating exocytosis or phosphorylation of synaptic proteins, including NMDAR2 receptors [188]. Glutamate decarboxylase (GAD67) enzyme expression is increased by both clozapine and haloperidol in rat neocortex [190]. Another glutamatergic association found in rats is that haloperidol and olanzapine induce striatal upregulation of mGlu4 and mGlu5, respectively, while both antipsychotics also increase expression of dopamine reuptake transporter and neurotensin [191]. The sensitivity and specificity of these antipsychotic-induced gene expression patterns are largely undetermined, but some research groups have attempted to use gene expression fingerprinting algorithms to identify and predict opioid, antidepressant or antipsychotic efficacy profiles [192]. Through classification tree and random forest methods, Gunther et al. determined that four gene markers (pentaxin 3, integrin linked kinase, ectonucleoside triphosphate diphosphohydrolase 6 and GPCR CG50207) were sufficient to provide high resolution between these therapeutic classes [192]. These biomarkers could potentially reveal a novel compound’s downstream effects and thereby predict efficacy in early phase antipsychotic drug trials.

Biomarkers obtained from CSF and plasma during early phase studies can provide useful information related to an investigational
compound’s effect on mechanistic or functional end points related to its pharmacology. While specific biomarkers predictive of antipsychotic response have yet to be validated, strategies such as genomic or metabolomic profiling may represent translatable methods for use in early-phase studies. For example, in a small study of acutely psychotic patients with schizophrenia, a biomarker profile was identified and associated with prediction of relapse [193]. In addition, while the majority of patients’ demonstrated improvement in response to treatment, specific molecular profiles were identified that were associated with symptom severity and response [193]. When combined with other translational methodologies, such as qEEG or imaging during a Phase I study, these combined approaches can significantly aid in the translation of preclinical data and help characterize the dose–response PK/PD relationships prior to Phase II or III trials. Successful examples, such as the GlyT1 inhibitor RG1678 (bitopertin), incorporated the use of combined CSF sampling and PET imaging along with plasma PK data to predict clinically effective doses prior to the initiation of Phase II studies, and demonstrates the translatability of these approaches and utility in Phase I trials [13,33].

Conclusion & recommendations
While Phase I trials often focus on the safety and tolerability of an investigational agent, novel methodological strategies should be employed during this learn phase of drug development to improve PK/PD knowledge and support the three pillars of drug development that can inform key decisions related to a compound’s subsequent clinical development plan. Novel strategies, including the addition of imaging, cognitive testing and EEG/PSG in Phase I, can provide information regarding CNS penetration, extent of exposure and target engagement, and provide surrogate signals related to cognitive improvement that can inform subsequent dose selections in later Phase II studies. Although significant challenges exist in developing and translating these strategies from preclinical models of schizophrenia to humans, as well as from healthy volunteers to patients with schizophrenia, the wider use of these early-phase methodological approaches will aid in our identification, understanding and validation of PD biomarkers. It is believed that these approaches will increase the efficiency (cost and speed) of drug development by more accurately nominating candidates for global development that have a greater likelihood of successful progression to regulatory approval and to meaningfully improve the lives of patients with schizophrenia.

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Executive summary

Heading
- Novel imaging methods such as PET and fMRI permit characterization of pharmacodynamic target engagement and functional effects.

Heading
- Specialized cognitive and behavioral testing in Phase I studies of novel antipsychotics can provide useful data on effects on cognition and other safety measures such as tolerance and abuse liability.

Heading
- EEG studies, including polysomnography, serve as readily translatable methods that allow full characterization of CNS compounds with respect to CNS penetration and precognitive effects of novel compounds.

Heading
- CSF and plasma biomarkers while not fully characterized in schizophrenia may permit the characterization of pharmacodynamic effects of novel antipsychotics, especially when combined with traditional pharmacokinetic measures.

Heading
- Employment of novel pharmacodynamic biomarker strategies (e.g., EEG and fMRI) incorporated into a Phase I program, selected based upon data derived from preclinical studies, may aid in informing go/no-go decisions transitioning to Phase II.

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Use of translational PD biomarkers in early-phase clinical studies for schizophrenia

Review

Recent advances in the field of functional genomics have provided new insights into the molecular mechanisms underlying mental health disorders, including schizophrenia. The use of convergent functional genomics, which integrates data from multiple sources, has become a powerful approach for identifying potential targets for therapeutic intervention.

**Key Points**

- Identification of blood biomarkers for psychosis using convergent functional genomics.
- Use of convergent functional genomics to identify biomarkers associated with illness severity and treatment outcomes.
- Toward understanding generic risk for differential antipsychotic response in individuals with schizophrenia.
- Genome-wide pharmacogenomic analysis of response to treatment with antipsychotics.
- Identification of candidate genes for psychosis using convergent functional genomics.

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