Translational Development Strategies for TAK-063, a Phosphodiesterase 10A Inhibitor

Thomas A. Macek, PharmD, PhD, 1 Kazunori Suzuki, PhD, 2 Karen Asin, PhD, 1 and Haruhide Kimura, PhD 2

1 Takeda Development Center Americas, Inc., Deerfield, IL, USA
2 Takeda Pharmaceutical Company Limited, Fujisawa, Japan

*Corresponding author: 26-1, Muraoka-Higashi 2-chome, Fujisawa, Kanagawa, 251 - 8555.
Tel: +81-466-32-1859; Fax: +81-466-29-4423; Email address: haruhide.kimura@takeda.com

Significance Statement: The TAK-063 phase 1 program included a comprehensive translational development strategy with the main objective of determining whether the antipsychotic-like pharmacodynamic effects seen in nonclinical models would translate to human subjects. This strategy effectively guided clinical development, and findings in clinical studies were generally consistent with nonclinical findings. The approach to the TAK-063 development program provides a framework for the evaluation of new therapeutic approaches, even when relevant nonclinical models are not available.
Abstract

**Background:** TAK-063 is an inhibitor of phosphodiesterase 10A (PDE10A), an enzyme highly expressed in medium spiny neurons of the striatum. PDE10A hydrolyzes both cyclic adenosine monophosphate and cyclic guanosine monophosphate and modulates dopamine signaling downstream of receptor activation in both direct and indirect pathways of the striatum. TAK-063 exhibited antipsychotic-like effects in animal models; however, the translatability of these models to the clinical manifestations of schizophrenia and the meaningfulness for new targets such as PDE10A has not been established.

**Methods:** The TAK-063 phase 1 program included a comprehensive translational development strategy with the main objective of determining whether the antipsychotic-like pharmacodynamic effects seen in nonclinical models would translate to human subjects. To evaluate this objective, we conducted a single-rising dose study (84 healthy subjects), a positron emission tomography (PET) study (12 healthy subjects), a functional magnetic resonance imaging blood oxygen level-dependent (BOLD) study (27 healthy subjects), and a multiple-rising dose study that included people with schizophrenia (30 healthy Japanese subjects and 47 subjects with stable schizophrenia). In addition, assessments of cognition and electroencephalography (27 healthy subjects and 47 subjects with stable schizophrenia) were included.

**Results:** PDE10A engagement by TAK-063 was verified with a novel PET radiotracer for use in primates and humans. TAK-063 showed favorable pharmacokinetic and safety profiles in humans, and TAK-063 reduced ketamine-induced changes in electroencephalography and BOLD signaling in animal models and healthy human subjects. In addition, analogous effects on cognition were observed in animal models and human subjects.

**Conclusions:** Overall, the phase 1 results showed some consistent evidence of antipsychotic activity. This translational strategy may be valuable for future development of novel therapeutic approaches, even when relevant nonclinical models are not available.
INTRODUCTION

Antipsychotic agents can address the positive symptoms of schizophrenia via partial agonism or full antagonism of dopamine receptor-2 (D2), but have little effect on negative and cognitive symptoms (Geyer, 2008; Citrome, 2014). In addition, side effects such as weight gain, extrapyramidal syndromes, hyperprolactinemia, and sedation can limit the tolerability of these agents (Ginovart and Kapur, 2012). Therefore, more effective treatments with improved safety profiles are needed.

Phosphodiesterase 10A (PDE10A) inhibition has been under investigation recently as an alternative therapeutic approach for schizophrenia. PDE10A hydrolyzes both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Fujishige et al., 1999) and is primarily expressed in medium spiny neurons (MSNs) of the striatum (Seeger et al., 2003). In autoradiography studies using mouse, rat, monkey, or human brain sections, radiolabeled T-773, a PDE10A specific tracer, selectively accumulated in the striatum; however, this selective accumulation was not observed in brain sections of PDE10A-KO mice. These results indicate that PDE10A is also primarily expressed in MSNs of the human striatum (Harada et al., 2015b; Takano et al., 2015). Striatal outputs originating in MSNs are mainly divided into 2 pathways: the D2 receptor−expressing indirect pathway and the dopamine receptor 1 (D1)−expressing direct pathway (Suzuki et al., 2015). PDE10A is expressed in both pathways, and PDE10A inhibition alters the levels of second messengers induced by activation of D1 and D2 receptors. By acting downstream of dopamine receptors in specific neuronal populations, PDE10A inhibition has the potential to provide a viable treatment option without some of the shortcomings experienced with traditional dopamine receptor−focused approaches.

TAK-063 is a potent and selective inhibitor of PDE10A (Suzuki et al, 2015; Harada et al, 2015a) that increases striatal cAMP and cGMP levels, which in turn increase phosphorylation of key downstream substrates (Suzuki et al., 2015). TAK-063 demonstrated antipsychotic-like
effects in methamphetamine- or MK-801–induced hyperactivity and prepulse inhibition in rodents (Suzuki et al., 2016), improved function in multiple cognitive domains—including recognition memory, attention, impulsivity, working memory, and executive function—in rodent paradigms (Shiraishi et al., 2016), and was shown to displace the binding of the PDE10A positron emission tomography (PET) radioligands [³H]T-773 and [¹¹C]T-773 in rodents and nonhuman primates, respectively (Harada et al., 2015a; Takano et al., 2016a).

The development of PDE10A inhibitors faces several translational challenges. Compared with nonclinical models for dopamine receptor–targeting agents, nonclinical models for PDE10A inhibition and their relevance to clinical manifestations of schizophrenia have not been extensively characterized. Furthermore, assessing neurocognition in both animals and humans is particularly challenging with regard to construct validity and selection of measures (Green and Braff, 2001). To address these challenges, a comprehensive translational development strategy was utilized to examine if the findings in nonclinical studies of TAK-063 would extend to healthy human subjects and patients with schizophrenia.

Our translational phase 1 program was designed with the following objectives: confirming PDE10A engagement by TAK-063, establishing the pharmacokinetic profile of TAK-063, and evaluating the pharmacodynamic effects of TAK-063 that were observed in nonclinical studies. To guide the decision to proceed to a phase 2 trial, Go/No Go criteria were prospectively established for each phase 1 study (Table 1). The intention of this strategy was to demonstrate that the effects of TAK-063 observed in our phase 1 program were consistent with the data from our nonclinical studies. The strategy also required that we demonstrate sufficient evidence for antipsychotic activity in our target population (patients with schizophrenia) as a requirement for proceeding with the clinical development of TAK-063. Our prospective criteria were met, and TAK-063 proceeded to a phase 2 trial.

MATERIALS AND METHODS
Nonclinical studies were mainly conducted at the Neuroscience Drug Discovery Unit of Takeda Pharmaceutical Company Limited and at the Karolinska Institute and King’s College London. Clinical studies were conducted at multiple sites in the United States and Sweden, and PET studies were conducted at Karolinska Institute (Stockholm, Sweden). Clinical studies were designed to be relatively similar to nonclinical counterparts.

**Positron-emission tomography studies**

\([^{11}\text{C}]\text{T-773, a novel PET radiotracer, was synthesized and developed for use in animals and humans from a series of candidate compounds (Harada et al., 2015a; Harada et al., 2015b; Stepanov et al., 2015; Takano et al., 2016a; Takano et al., 2016b). For PDE10A occupancy studies, displacement of non-radiolabeled T-773 and \([^{11}\text{C}]\text{T-773 were used as measurements to estimate PDE10A occupancy by TAK-063 (Takeda Pharmaceutical Company Limited; Fujisawa, Japan) (Kunitomo et al., 2014) in rodents (n=2 for 0.3 mg/kg dose; n=3 for other doses) and nonhuman primates (n=1 for each dose) (Harada et al., 2015a; Harada et al., 2015b; Takano et al., 2016a), respectively. Binding kinetics of \([^{11}\text{C}]\text{T-773 were analyzed in nonhuman primates in pretreatment and displacement studies (n=1 for each dose) (Takano et al., 2015). In a PDE10A occupancy study using rodents, TAK-063 (0, 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg) was orally administered to rats and 0.02 mg/kg of T-773 was administered by bolus intravenous injection via the lateral tail vein 90 minutes after TAK-063 administration. The rats were euthanized by cardiac perfusion with heparinized saline 30 minutes after T-773 injection, and the whole brains were isolated. The striatum and cerebellum were dissected from the brains. The concentration of T-773 was measured by mass spectrometry in each homogenate. Specific binding of T-773 (\(B_{SP}\)) to PDE10A in the rat striatum was represented as the difference between the T-773 concentration in striatum and that in cerebellum as a reference region because no specific binding of radiolabeled T-773 in the cerebellum was observed in the autoradiography studies using brain slices from multiple species including humans (Harada et al., 2015b; Takano et al., 2016a; Takano et al., 2016b).**
2015). PDE10A occupancy was calculated using the following equation: Occupancy (%) = 
\((B_{\text{SP,base}} - B_{\text{SP,drug}})/B_{\text{SP,base}} \times 100\), where \(B_{\text{SP,base}}\) and \(B_{\text{SP,drug}}\) are the concentrations at baseline (vehicle-treatment) and at drug-treatment, respectively. In a PDE10A occupancy study using nonhuman primates, 123-minute dynamic PET scans were performed with a High Resolution Research Tomograph (Siemens, Munich, Germany) after injection of \([^{11}\text{C}]T-773\) (141–166 MBq; specific radioactivity: >151 GBq/mmol; injected mass: less than 0.5 mg) under sevoflurane anesthesia. Two brain PET measurements were performed for each monkey. The first scan was made under baseline conditions and the next scan after intravenous administration. The doses of TAK-063 were 0.2, 0.8, and 1.6 mg/kg. TAK-063 was administered 35 minutes before the radioligand injection. The duration of the administration was 30 minutes using a syringe pump at a speed of 2 mL/kg/h. The administration of TAK-063 was finished 5 minutes before \([^{11}\text{C}]T-773\) injection. Arterial blood samples were taken continuously with an automated blood sampling system during the initial 3 minutes and manually after 3 minutes. Metabolite analysis of the radioligand was performed to evaluate the fraction of the parent compound. After reconstruction of the PET images, time activity curves were generated for various brain regions including the caudate, putamen, and cerebellum delineated on the MRI/PET co-registered images. PET data were analyzed using a 2-tissue compartment model with metabolite-corrected arterial plasma input function. Total distribution volume (VT) was calculated for each brain region. The specific binding part (VS) of the VT was calculated as the difference between the VTs of the target regions and cerebellum. PDE10A occupancy was calculated as the percentage change from baseline of the VS. PDE10A occupancy and dose were fitted to a hyperbolic function for determination of Kd (Takano A, et al., 2016a).

PET imaging was also conducted in 12 healthy volunteers to evaluate \([^{11}\text{C}]T-773\) kinetics in the brain and test-retest reproducibility of \([^{11}\text{C}]T-773\) (ClinicalTrials.gov ID: NCT02370602). Subsequently, PDE10A occupancy by TAK-063 was measured by TAK-063
displacement of \([^{11}C]T-773\) by 75-minute dynamic PET scan (n=3 for 30 mg and 100 mg doses; n=2 for 3 mg, 10 mg, and 1000 mg doses) (Takano et al., 2016b).

**Electroencephalogram studies**

Extensively validated techniques such as electroencephalogram (EEG) were utilized to assess the effects of TAK-063 on neurological deficits associated with schizophrenia (Light and Swerdlow, 2015). EEG studies were performed in awake rats (n=4 for each dose; a recording electrode and a reference electrode were positioned on the prefrontal cortex and the cerebellum, respectively) and monkeys (n=6 for each dose; EEG montage was obtained using frontal and occipital cortex electrodes) (Tomimatsu et al., 2016). After 5 minutes of baseline recording, rats were treated with vehicle or TAK-063 (0.03–3 mg/kg orally) and monkeys were administered vehicle or TAK-063 (0.2 or 0.8 mg/kg/h intravenously for 30 minutes) after 5 minutes of baseline recording and a 15-minute non-treatment phase. Rats received 10 mg/kg of ketamine subcutaneously 90 minutes after TAK-063 administration, and EEG signals in rats were filtered at 0.1–1 kHz, amplified, and digitized at 500 Hz. Monkeys received 1 mg/kg ketamine intramuscularly 10 minutes after TAK-063 administration, and EEG signals in monkeys were filtered (0.5–100 Hz), amplified, and digitized online. Fast Fourier transformations were performed, and the total power in gamma (30–80 Hz) frequency was calculated for both rats and monkeys.

EEG studies were also conducted in subjects with stable schizophrenia (SSS) who were on stable antipsychotic monotherapy and received 3, 10, 30, or 100 mg TAK-063 during a 7-day multiple-rising dose study (Macek et al., 2016a); according to Goldsmith P et al (Goldsmith P et al, 2017), SSS can be defined as an individual on stable antipsychotic monotherapy for ≥1 month before screening, who had a Clinical Global Impression of Severity (CGI-S) score ≤4, and a total Positive and Negative Symptom Scale (PANSS) score ≤70 at screening and check-in (day −1) (Macek et al., 2016a, Goldsmith et al. 2017). Additionally, EEG was assessed in
healthy subjects who received 3, 10, and 30 mg TAK-063 in an incomplete crossover, ketamine-challenge, functional magnetic resonance imaging (fMRI) study (Yurgelun-Todd et al., 2019, unpublished observations; ClinicalTrials.gov ID: NCT01892189), and EEG recordings were made after the imaging battery was complete, which was approximately 5 hours after administration of study medication (n=14 for 3 mg and 10 mg doses; n=15 for 30 mg dose). In the multiple-rising dose study, 10-minute samples of 19-channel EEG recordings were taken while subjects were either (i) seated with eyes closed (resting condition) or (ii) presented with high-frequency auditory stimuli at 40 Hz in blocks (40-Hz condition) (n=9 for placebo; n=7 for 100 mg dose). Digital filtering techniques were used to remove artefacts, and data were subsequently transformed using the Fast Fourier method to obtain the following bands: delta (0–3.5 Hz), theta (4–7.5 Hz), alpha (8–12 Hz), beta (13–25 Hz), global gamma (30–50 Hz), and high gamma (40–50 Hz). Spectral power in each frequency band was quantified in topographic displays as well as in 5 extracted regions: frontal, central, parietal, occipital, and temporal, each as median of a cluster of electrodes per subject and per time point. The timing and extent of gamma band amplitude increases due to auditory stimulation were also examined during 30-, 40-, and 50-Hz high-frequency stimulation EEG recordings.

**Pharmacological magnetic resonance imaging (phMRI) and fMRI blood oxygen level-dependent (BOLD) studies**

PhMRI studies in Sprague-Dawley rats were conducted at King’s College London (Tomimatsu et al., 2016). The ketamine-challenge fMRI study was a 3-period incomplete crossover study that enrolled 27 healthy volunteers. In this clinical study, subjects were randomized to receive single oral administrations of placebo and 2 of 3 doses of TAK-063 (3, 10, or 30 mg) with an approximately 1-week washout period between administrations. Subanesthetic ketamine doses were administered intravenously approximately 4 hours after the administration of TAK-063 to achieve a plasma concentration of 75 ng/mL (Macek et al., 2016b).
Changes in BOLD signals pre-ketamine and post-ketamine were compared with placebo. EEG recordings were made after the imaging battery was complete, approximately 5 hours after administration of study medication, and were generally similar to the EEG methods used in the multiple-rising dose study. fMRI BOLD signal was assessed during resting state and a working memory activation task (the expectancy AX Continuous Performance Test paradigm for 15 minutes) (Yurgelun-Todd et al., 2019). BOLD echo planar images were obtained during a 20-minute resting-state sequence using a 3T Siemens Verio scanner (TR=2 s, TE=28 ms, 40 slices, 3 mm slice thickness). fMRI images were analyzed using SPM8 and Matlab. Percentage signal change for resting state data was calculated between pre-ketamine and post-ketamine infusion based on a priori regions of interest using SPM’s Anatomy Toolbox.

**Single-rising dose/multiple-rising dose pharmacokinetics, safety, and tolerability**

The single-rising dose study was a double-blind, placebo-controlled study in 84 healthy Japanese subjects (HJS) and non-Japanese subjects who received 3- (n=11), 10- (n=11), 30- (n=11), 100- (n=11), 300- (n=11), or 1000-mg (n=11) TAK-063 or placebo (n=18; 3 patients per cohort) (Tsai et al., 2016). The multiple-rising dose study was a double-blind, placebo-controlled study in 77 subjects (n=30 HJS and n=47 SSS) who were administered placebo (n=15) or 3- (n=8), 10- (n=8), or 20-mg (n=8) TAK-063 (HJS) or 3- (n=7), 10- (n=8), 20- (n=7), 30- (n=8), or 100-mg (n=8) TAK-063 (SSS) (Goldsmith et al., 2017; ClinicalTrials.gov ID: NCT01879722). The dose levels selected for investigation in the multiple-rising dose study were based on the safety findings from the single-rising dose study. In both studies, validated liquid chromatography-tandem mass spectrometry was used to determine TAK-063 concentrations (Tsai et al., 2016; Goldsmith et al., 2017). Safety parameters including adverse events (AEs) and clinical laboratory tests were examined at specific intervals during the studies (Tsai et al., 2016; Goldsmith et al., 2017).

**Cognition studies**
Cognitive studies were conducted in Institute of Cancer Research mice, Long-Evans rats, and hooded Lister rats. The performance of experimentally naïve rats, phencyclidine (PCP)-treated rats and mice, and MK-801–treated rats was evaluated in tasks assessing recognition memory, spatial working memory, attention and impulsivity, and executive function (Shiraishi et al., 2016).

In the multiple-rising dose study, cognitive function was examined in HJS receiving 3-, 10-, or 20-mg TAK-063 and in SSS receiving 3-, 10-, 20-, 30-, or 100-mg TAK-063. Cognitive function and postural sway were assessed using the Cognitive Drug Research test battery on days 1, 2, 4, 6, and 7 predose and 2 and 6 hours postdose, and statistical analyses were conducted (Macek et al., 2016c).

The study protocols were approved by the institutional review boards at all participating institutions, and study procedures were conducted in accordance with the principles outlined in the Declaration of Helsinki. All patients provided written informed consent before any study procedures were performed.

RESULTS

Single- and multiple-rising dose pharmacokinetics

The objectives of the single- and multiple-dose pharmacokinetic studies were to determine the pharmacokinetics of single and multiple doses of TAK-063 in healthy subjects and subjects with schizophrenia, and to confirm that the half-life was suitable for once- or twice-daily dosing. Because food may affect the rate of absorption and pharmacokinetics of drugs administered orally, we evaluated the food effects on pharmacokinetic parameters of 100 mg TAK-063 in the single-rising dose study. TAK-063 exposures were increased slightly under fed vs non-fed conditions in HJS, and exposure increased in a dose-dependent manner (Tsai et al., 2016). As in the nonclinical studies, TAK-063 exhibited slower absorption and increased oral
bioavailability in the fed state. In humans, the mean elimination half-life of TAK-063 under fasting conditions ranged from 15–25 hours across dose groups. In the multiple-rising dose study, TAK-063 exposure (maximum serum concentration [C\text{max}] and area under the curve [AUC]₀–₂⁴) increased dose proportionally up to 30 mg in SSS and up to 20 mg in HJS on day 1 (Goldsmith et al., 2017). On day 7, C\text{max} (~100 ng/mL) and AUC₀–₂⁴ (~1000 ng*h/mL) in the 20-mg TAK-063 group were comparable to the pharmacologically active exposures in nonclinical studies (Goldsmith et al., 2017; Tohyama et al., 2018). Taken together, the dose-exposure relation from the single- and multiple-rising dose studies supported once- or twice-daily dosing and achieved target plasma concentrations of TAK-063, leading to the decision of continued clinical development (Table 1).

Single- and multiple-rising dose safety and tolerability

It has been reported that, in pharmacokinetic studies of antipsychotics, there may be differences in the tolerability of these agents in healthy volunteers vs patients with schizophrenia (Cutler NR, 2001). In the single- and multiple-rising dose studies, TAK-063 was generally safe and well tolerated in the single- and multiple-rising dose clinical studies. No serious or dose-limiting AEs, and no clinically significant changes in hematology, clinical laboratory tests, or electrocardiograms were reported in either HJS or SSS (Tsai et al., 2016; Goldsmith et al., 2017) – similar to nonclinical studies, there were no increases in prolactin or glucose (Tsai et al., 2016, Goldsmith et al., 2017, Suzuki et al., 2015); the most frequent AE in both studies was somnolence. In the multiple-rising dose study, most TAK-063–related AEs in SSS occurred at doses of 30 mg or higher, and most extrapyramidal syndrome (EPS) events in SSS were of mild or moderate severity. In HJS, most TAK-063–related AEs were mild or moderate in severity, and 1 subject in the 20-mg TAK-063 group experienced EPS (Goldsmith et al., 2017). Although the durations of the single- and multiple-rising dose studies were relatively brief, TAK-063 did
not produce signs of adverse metabolic effects in HJS or SSS (Tsai et al., 2016; Goldsmith et al., 2017).

As reported in these studies, the safety and tolerability data supported once-daily dosing, and the incidence of EPS was acceptable at TAK-063 doses of 30 mg or less (Tsai et al., 2016; Goldsmith et al., 2017), leading to the decision to continue clinical development (Table 1).

**Positron-emission tomography studies**

In mice, [³H]T-773 accumulated in brain regions with high PDE10A expression (Harada, et al., 2015b), while Pde10a-knockout mice showed no [³H]T-773 or [¹¹C]T-773 binding in the brain (Harada, et al., 2015b; Toth et al., 2016) (Supplementary Fig. S1a). Similarly, [¹¹C]T-773 accumulated in the striatum of monkeys (Harada et al., 2015b) (Supplementary Fig. S1b). In agreement with these nonclinical studies, [¹¹C]T-773 accumulated to high levels in the striatum of healthy subjects (Takano et al., 2015; Takano et al., 2016b) (Supplementary Fig. S1c) and showed distribution patterns similar to those observed in animal models. Similar to mice and monkeys, displacement of [¹¹C]T-773 by TAK-063 was demonstrated in healthy subjects (Takano et al., 2016a; Takano et al., 2016b; Toth et al., 2016).

In nonclinical studies, potential antipsychotic and pro-cognitive effects were demonstrated at a PDE10A occupancy of approximately 26% in animal models (Suzuki et al., 2015; Harada et al., 2015a; Suzuki et al., 2016; Shiraishi et al., 2016). The objective of the clinical PET study was to determine whether a level of occupancy consistent with nonclinical studies could be achieved. Using concentration:occupancy modeling, similar occupancies were predicted to be achieved at steady state at doses of 10 mg TAK-063 (Macek et al., 2016b). Upon confirmation of PDE10A engagement and achievement of approximately 30% PDE10A...
occupancy in human subjects, the decision to proceed to clinical development was consistent with the Go/No Go criteria developed for the compound (Table 1).

Electroencephalogram studies

Findings from clinical, pharmacological, and genetic studies suggest that N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis has been proposed to help understand the etiology and pathophysiology of schizophrenia (Snyder et al., 2013). In fact, NMDA receptor antagonists such as ketamine, phencyclidine, and MK-801 transiently induce schizophrenia-like symptoms in rats and monkeys, and sub-anesthetic doses of ketamine are an accepted means of modeling schizophrenia symptoms in healthy human subjects (Abi-Saab et al., 1998; Lahti et al., 2001; Frohlich and Van Horn, 2014). TAK-063 has shown antipsychotic-like and pro-cognitive effects in NMDA antagonist-induced rodent models of schizophrenia (Suzuki et al., 2015; Shiraishi et al., 2016); we therefore used ketamine to assess the effect of TAK-063 in these studies. Oral administration of TAK-063 was shown to reduce ketamine-induced increases in resting gamma power in rat nonclinical models. Similarly, ketamine-induced increases in gamma power were reduced by intravenous administration of 0.2 and 0.8 mg/kg TAK-063 in awake monkeys (Tomimatsu et al., 2016).

As in rats and monkeys, ketamine-induced increases in gamma power in the human ketamine challenge study were significantly attenuated in healthy human subjects who received a single dose of 30-mg TAK-063 during the eyes-closed task, which approximates the steady state exposures of 20 mg (Macek et al., 2016b) (Supplementary Fig. S2).

Deficits in gamma power have been reported in people with schizophrenia and are thought to be reflective of impairments in cognition (Minzenberg et al., 2010). To explore these effects on EEG activity in patients with schizophrenia, the effects of TAK-063 on EEG in SSS were assessed as an exploratory endpoint during the phase 1 multiple-rising dose study. TAK-
063 increased gamma power in several brain regions of SSS after 40-Hz stimulation on day 1 (Fig. 1) but not on day 7. These generally consistent results in ketamine studies across healthy human subjects, rodents, and nonhuman primates suggest that TAK-063 attenuated changes in EEG are associated with schizophrenia and met the “Go” criteria established for the compound.

**phMRI and fMRI studies**

Ketamine is reported to induce aberrant cortical activation that is similar to that associated with schizophrenia (Hunt and Kasicki, 2013; Driesen et al., 2013). PhMRI and fMRI signals are changed by ketamine treatment (Deakin et al., 2008; Chin et al., 2011). In rats, subcutaneous administration of ketamine (10 mg/kg) increased the BOLD signal across many brain regions, and TAK-063 reversed the ketamine-induced BOLD signal changes in the cortex, brainstem, and cerebellum (Tomimatsu et al., 2016).

To extend the phMRI findings in rodents, TAK-063 was investigated in ketamine-treated healthy human subjects (Yurgelun-Todd et al., 2016). Compared with placebo, TAK-063 reduced ketamine-induced increases in BOLD signal across all brain regions analyzed during the working memory task (Fig. 2, Supplementary Fig. S3) in a dose-dependent manner (Yurgelun-Todd et al., 2016). The most consistent reversal of ketamine-induced effects in fMRI was observed in the 30-mg TAK-063 dose group, which approximates the steady state exposure of 20 mg (Macek et al., 2016b). These outcomes indicate that TAK-063 exhibited antipsychotic-like effects in nonclinical models of psychosis and in healthy human subjects experiencing schizophrenia-like symptoms, consistent with the “Go” criteria established for the compound (Table 1).

**Cognition studies**

As described above, NMDA receptor antagonists, such as PCP and MK-801, have been known to cause schizophrenia-like symptoms including cognitive deficits in clinical and
preclinical studies. We attempted to evaluate cognitive functions using animal models based on
the NMDA receptor hypofunction hypothesis; however, accurate assessment of cognitive
performance in some tests was affected by NMDA receptor antagonist–induced behavioral
change such as increased locomotor activity. Thus, in such cases we used naïve animals. We
focused on evaluating the effects of TAK-063 on multiple cognitive domains associated with
schizophrenia such as recognition memory, spatial working memory, attention and impulsivity,
and executive function using naïve rats, PCP-treated rats and mice, and MK-801–treated rats
(Shiraishi et al., 2016).

TAK-063 increased recognition memory during the novel object recognition task and
increased accuracy rates while decreasing impulsive responses during a 5-choice serial
reaction time task in naïve rats. Of note, increase in dopamine transmission is typically
associated with increased impulsivity. Importantly, dopamine or dopamine receptor agonist is
thought to affect various neural circuits in the brain; however, PDE10A inhibition by TAK-063
increases striatal cyclic nucleotide levels in both indirect and direct pathways of striatum, which
would potentially produce the effect of D2 receptor antagonism along with D1 receptor agonism.
Thus, the pharmacological effect of PDE10A inhibition is different from that of dopamine or
dopamine receptor agonist. In PCP-treated mice and MK-801–treated rats, TAK-063 reduced
spatial working memory deficits as assessed by maze tests. An attentional set-shifting task
using sub-chronic PCP-treated rats was used to assess executive function, and during this task
TAK-063 reversed cognitive deficits in extradimensional shifts (Shiraishi et al., 2016). These
pro-cognitive effects were observed at 0.3 mg/kg TAK-063, a dose that achieves PDE10A
occupancy and exposures that are consistent with preclinical antipsychotic-like efficacy.

Consistent with the findings in rats, improvements in cognition were observed in subjects
with schizophrenia in the phase 1 multiple-rising dose study (Fig. 3) (Macek et al., 2016c).
Improvements over placebo were observed in the 3-mg TAK-063 group in measures of
attention-reaction time variability and in the tapping task in the 3- and 30-mg groups. Improvements in the quality of working memory were observed in the 3- and 10-mg groups, and improvements in executive function were observed in the 3-mg TAK-063 group. These findings were consistent with the “Go” criteria for the compound.

**DISCUSSION**

The translational strategy for TAK-063 was designed to guide the development of the compound, despite the lack of validated nonclinical models for evaluating PDE10A inhibition in schizophrenia. The main objective was to demonstrate that the effects of TAK-063 were consistent in nonclinical and clinical studies, and the decision to move into phase 2 development was contingent on consistency between phase 1 results and critical preclinical data. The findings with TAK-063 generally were consistent, with analogous pharmacodynamic effects in nonclinical models and human subjects across studies of EEG, fMRI BOLD, and cognition. TAK-063 also exhibited pharmacokinetics in humans that were supportive of our targeted daily dosing profile. Based on the concordance between nonclinical and clinical studies, achievement of pharmacologically active exposures, and favorable safety and tolerability profiles in the single- and multiple-rising dose studies, a 20-mg TAK-063 dose was selected for a phase 2 study. Some impairments in cognition were noted in both healthy subjects and subjects with schizophrenia (Tsai et al. 2016; Macek et al., 2016c), which may have been due to the somnolent effects of TAK-063; these effects appeared to decrease over time (Macek et al., 2016c). Interestingly, correlation between improvements in cognition and EEG measures were observed in patients with schizophrenia who received TAK-063 (Macek et al., 2017a). TAK-063 increased gamma power in the EEG (in frontal, central, and parietal regions), and improved cognitive domains such as reaction time variability, quality of working memory, and tapping tasks in SSS (data not shown).
There remains a need for rigorous translational development strategies to evaluate pharmacodynamic effects observed in humans relative to those observed in nonclinical models in the development of antipsychotics. Many animal models used to evaluate antipsychotics were not prospectively developed to recapitulate psychosis observed in humans. Commonly used models, such as PCP- or methamphetamine-induced inhibition of hyperlocomotion by dopamine antagonists, are considered valid because dopamine antagonists used clinically to treat schizophrenia exert pharmacological effects in these models.

The relevance of nonclinical models of cognition to the specific cognitive impairments observed in schizophrenia is poorly defined, and using these models to characterize cognitive effects of drugs represents a challenge. Because of the limitations in models for developing antipsychotic and other central nervous system–related therapies, it is imperative to conduct human studies to demonstrate consistency between these data and those generated in nonclinical experiments.

The comprehensive translational strategy used for TAK-063 effectively guided clinical development, and findings in clinical studies were generally consistent with nonclinical findings. Therefore, TAK-063 was advanced into a phase 2, proof-of-concept study of 20-mg TAK-063 vs placebo in patients with an acute exacerbation of psychotic symptoms. The 20-mg dose was chosen based on the signals detected in nonclinical studies and phase 1 data, and was considered to be the highest dose that was well tolerated and that had demonstrated pharmacodynamic effects consistent with potential antipsychotic effects (Macek et al., 2017b).

In conclusion, our approach to the TAK-063 development program provides a framework for the evaluation of new therapeutic approaches, even when relevant nonclinical models are not available.

**Funding**
This work was supported by Takeda Development Center Americas, Inc.

Acknowledgments

Medical writing assistance was provided by Stephanie Agbu, PhD, and Jake Edelstein, PhD, of inVentiv Medical Communications, LLC, a Syneos Health™ group company, and supported by Takeda Development Center Americas, Inc.

Statement of Interests

Kazunori Suzuki and Haruhide Kimura are employees of Takeda Pharmaceutical Company Limited, Fujisawa, Japan. Thomas A. Macek and Karen Asin were employees of Takeda Development Center Americas, Inc., Deerfield, IL, at the time of this study.
References

Abi-Saab WM, D'Souza DC, Moghaddam B, Krystal JH (1998) The NMDA antagonist model for schizophrenia: promise and pitfalls. Pharmacopsychiatry 31 Suppl 2:104-109. doi:10.1055/s-2007-979354.

Chin CL, Upadhyay J, Marek GJ, Baker SJ, Zhang M, Mezler M, et al (2011) Awake rat pharmacological magnetic resonance imaging as a translational pharmacodynamic biomarker: metabotropic glutamate 2/3 agonist modulation of ketamine-induced blood oxygenation level dependence signals. J Pharmacol Exp Ther 336(3):709-715. doi:10.1124/jpet.110.173880.

Citrome L (2014) Unmet needs in the treatment of schizophrenia: new targets to help different symptom domains. J Clin Psychiatry 75 Suppl 1:21-26. doi:10.4088/JCP.13049su1c.04.

Cutler NR (2001) Pharmacokinetic studies of antipsychotics in healthy volunteers versus patients. J Clin Psychiatry 62 Suppl 5:10-13; discussion 23-24.

Deakin JF, Lees J, McKie S, Hallak JE, Williams SR, Dursun SM (2008) Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. Arch Gen Psychiatry 65(2):154-164. doi:10.1001/archgenpsychiatry.2007.37.

Driesen NR, McCarthy G, Bhagwagar Z, Bloch M, Calhoun V, D'Souza DC, et al (2013) Relationship of resting brain hyperconnectivity and schizophrenia-like symptoms produced by the NMDA receptor antagonist ketamine in humans. Mol Psychiatry 18(11):1199-1204. doi:10.1038/mp.2012.194.

Frohlich J, Van Horn JD (2014) Reviewing the ketamine model for schizophrenia. J Psychopharmacol 8(4):287-302. doi:10.1177/0269881113512909.

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, et al (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J Biol Chem 274(26):18438-18445.
Geyer MA (2008) Developing translational animal models for symptoms of schizophrenia or bipolar mania. Neurotox Res 14(1):71-8. doi:10.1007/BF03033576.

Ginovart N, Kapur S (2012) Role of dopamine D(2) receptors for antipsychotic activity. Handb Exp Pharmacol (212):27-52. doi:10.1007/978-3-642-25761-2_2.

Goldsmith P, Affinito J, McCue M, Tsai M, Roepcke S, Xie J, et al (2017) A randomized multiple dose pharmacokinetic study of a novel PDE10A inhibitor TAK-063 in subjects with stable schizophrenia and Japanese subjects and modeling of exposure relationships to adverse events. Drugs R D 17(4):631-643. doi:10.1007/s40268-017-0214-8.

Green MF, Braff DL (2001) Translating the basic and clinical cognitive neuroscience of schizophrenia to drug development and clinical trials of antipsychotic medications. Biol Psychiatry 49(4):374-384.

Harada A, Suzuki K, Kamiguchi N, Miyamoto M, Tohyama K, Nakashima K, et al (2015a) Characterization of binding and inhibitory properties of TAK-063, a novel phosphodiesterase 10A inhibitor. PLoS One 10(3):e0122197. doi:10.1371/journal.pone.0122197.

Harada A, Suzuki K, Miura S, Hasui T, Kamiguchi N, Ishii T, et al (2015b) Characterization of the binding properties of T-773 as a PET radioligand for phosphodiesterase 10A. Nucl Med Biol 42(2):146-154. doi:10.1016/j.nucmedbio.2014.09.005.

Hunt MJ, Kasicki S (2013) A systematic review of the effects of NMDA receptor antagonists on oscillatory activity recorded in vivo. J Psychopharmacol 27(11):972-986. doi:10.1177/0269881113495117.

Kunitomo J, Yoshikawa M, Fushimi M, Kawada A, Quinn JF, Oki H, et al (2014) Discovery of 1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one (TAK-063), a highly potent, selective, and orally active phosphodiesterase 10A (PDE10A) inhibitor. J Med Chem 57(22):9627-9643. doi:10.1021/jm5013648.
Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA (2001) Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 25(4):455-467. doi:10.1016/S0893-133X(01)00243-3.

Light GA, Swerdlow NR (2015) Future clinical uses of neurophysiological biomarkers to predict and monitor treatment response for schizophrenia. Ann N Y Acad Sci 1344:105-119. doi:10.1111/nyas.12730.

Macek TA, McCue M, Johnstone J, Boeijinga P (2016a) TAK-063 increases gamma synchrony in subjects with schizophrenia. 5th Schizophrenia International Research Society Conference. Abstract T155. Florence, Italy; April 2-6.

Macek TA, Goldsmith P, Tsai M, McCue M, Affinito J, Suzuki K, et al (2016b) M56 drug development strategies for schizophrenia using a novel PDE10A inhibitor: TAK-063. NPJ Schizophr 2(16008):20.

Macek TA, McCue M, Xie J, Wesnes K (2016c) Abstract T154: The effects of TAK-063 on cognition in a multiple dose, phase 1 study in healthy Japanese volunteers and subjects with schizophrenia are consistent with its somnolent effects. NPJ Schizophr 2(16007):57.

Macek T, McCue M, Ogrinc F, Hanson E, Goldsmith P, Affinito J, et al (2017a) M20. A phase 2, randomized, double-blind, placebo-controlled, parallel-group, 6-week study to evaluate the efficacy and safety of TAK-063 in subjects with an acute exacerbation of schizophrenia. Schizophrenia Bull 43(suppl_1):S218-S.

Macek TA, Suzuki K, Asin K, Kimura H, eds (2017b) Translational development strategies utilized in the development of an inhibitor of PDE10A (TAK-063). Neuropsychopharmacology: Nature Publishing Group, London, England.
Minzenberg MJ, Firl AJ, Yoon JH, Gomes GC, Reinking C, Carter CS (2010) Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. Neuropsychopharmacology 35(13):2590-2599. doi:10.1038/npp.2010.150.

Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, et al (2003) Immunohistochemical localization of PDE10A in the rat brain. Brain Res 985(2):113-126.

Shiraishi E, Suzuki K, Harada A, Suzuki N, Kimura H (2016) The phosphodiesterase 10A selective inhibitor TAK-063 improves cognitive functions associated with schizophrenia in rodent models. J Pharmacol Exp Ther 356(3):587-595. doi:10.1124/jpet.115.230482.

Stepanov V, Miura S, Takano A, Amini N, Nakao R, Hasui T, et al (2015) Development of a series of novel carbon-11 labeled PDE10A inhibitors. J Labelled Comp Radiopharm 58(5):202-208. doi:10.1002/jlcr.3284.

Suzuki K, Harada A, Shiraishi E, Kimura H (2015) In vivo pharmacological characterization of TAK-063, a potent and selective phosphodiesterase 10A inhibitor with antipsychotic-like activity in rodents. J Pharmacol Exp Ther 352(3):471-479. doi:10.1124/jpet.114.218552.

Suzuki K, Harada A, Suzuki H, Miyamoto M, Kimura H (2016) TAK-063, a PDE10A inhibitor with balanced activation of direct and indirect pathways, provides potent antipsychotic-like effects in multiple paradigms. Neuropsychopharmacology 41(9):2252-2262. doi:10.1038/npp.2016.20.

Snyder MA, Gao WJ. (2013) NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. Front Cell Neurosci 7:31. doi: 10.3389/fncel.2013.00031.

Takano A, Stepanov V, Gulyas B, Nakao R, Amini N, Miura S, et al (2015) Evaluation of a novel PDE10A PET radioligand, [(11) C]T-773, in nonhuman primates: brain and whole body PET and brain autoradiography. Synapse 69(7):345-355. doi:10.1002/syn.21821.
Takano A, Stenkrona P, Stepanov V, Amini N, Martinsson S, Tsai M, et al (2016b) A human [(11)C]T-773 PET study of PDE10A binding after oral administration of TAK-063, a PDE10A inhibitor. Neuroimage 141:10-17. doi:10.1016/j.neuroimage.06.047.

Takano A, Stepanov V, Nakao R, Amini N, Gulyas B, Kimura H, et al (2016a) Brain PET measurement of PDE10A occupancy by TAK-063, a new PDE10A inhibitor, using [11 C]T-773 in nonhuman primates. Synapse 70(6):253-263. doi:10.1002/syn.21896.

Tohyama K, Sudo M, Morohashi A, Kato S, Takahashi J, Tagawa Y (2018) Pre-clinical characterization of absorption, distribution, metabolism and excretion properties of TAK-063. Basic Clin Pharmacol Toxicol doi:10.1111/bcpt.12964.

Tomimatsu Y, Cash D, Suzuki M, Suzuki K, Bernanos M, Simmons C, et al (2016) TAK-063, a phosphodiesterase 10A inhibitor, modulates neuronal activity in various brain regions in phMRI and EEG studies with and without ketamine challenge. Neuroscience 339:180-190. doi:10.1016/j.neuroscience.2016.10.006

Toth M, Haggkvist J, Stepanov V, Takano A, Nakao R, Amini N, et al (2015) Molecular imaging of PDE10A knockout mice with a novel PET radiotracer: [(11)C]T-773. Mol Imaging Biol 17(4):445-449. doi:10.1007/s11307-015-0822-z.

Tsai M, Chrones L, Xie J, Gevorkyan H, Macek TA (2016) A phase 1 study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of TAK-063, a selective PDE10A inhibitor. Psychopharmacology (Berl) 233(21-22):3787-3795. doi:10.1007/s00213-016-4412-9.

Yurgelun-Todd D, Renshaw P, Goldsmith P, Xie J, Uz T, Macek TA (2016) Abstract T156: The PDE-10A inhibitor TAK-063 reverses ketamine-induced changes in fMRI BOLD signal. NPJ Schizophr 2(16007):58.
| Study                  | Objectives                                                                 | Go Criteriaa |
|-----------------------|-----------------------------------------------------------------------------|--------------|
| PET study             | Proof of principle: confirm target engagement in humans                    | Target occupancy >30% in PET study |
|                       | • Assess central exposure, biodistribution, and target engagement of TAK-063 |              |
|                       | • Assist in dose selection for phase 2                                      |              |
| fMRI BOLD study       | Proof of mechanism: confirm PDE10A inhibition in humans                     | Evidence of changes in fMRI in TAK-063 alone, baseline BOLD measurements, or during ketamine challenge |
|                       | • Assist in dose selection for multiple-rising dose study                   |              |
|                       | • Confirm findings from rats and monkeys demonstrating that TAK-063 ameliorates ketamine-induced fMRI BOLD effects |              |
| SRD PK study          | Characterize PK following single and multiple dosing of TAK-063            | Half-life amenable to BID or QD dosing |
|                       | Achieving clinical exposures comparable to minimally effective dose in animals (~1000 ng·h/mL) |              |
| SRD/MRD safety study | Establish safety in subjects with schizophrenia                            | No intractable dystonia or other extrapyramidal syndromes |
|                       | • Assess changes in exploratory endpoints with regard to symptoms of schizophrenia, EEG, and cognitive batteries | No unmanageable hematological effects such as severe leukopenia or agranulocytosis |
|                       |                                                                             | No significantly elevated prolactin, cholesterol, triglycerides, glucose, or weight gain |
| MRD study             | Evaluate tolerability and multiple-dose pharmacokinetics in healthy subjects and subjects with stable schizophrenia | No severe or serious adverse effects that limit patient adherence, contribute to significant withdrawals due to adverse events, or limit patient exposures to less than the minimum effective dose |
|                       | Evaluate effects on measures of cognition and neurophysiological measures in patients with schizophrenia | Demonstration of meaningful effects on EEG or other potential measures of pharmacodynamic effects (PPI) |

aAll “Go” criteria were met.

Abbreviations: BID, twice a day; BOLD, blood oxygen level-dependent; EEG, electroencephalogram; fMRI, functional magnetic resonance imaging; MRD, multiple-rising dose; PET, positron emission tomography; PK, pharmacokinetic; PPI, pre-pulse inhibition; QD, once a day; SRD, single-rising dose.
Figure legends

Fig. 1. Modulation of gamma power by TAK-063 in subjects with stable schizophrenia.

Abbreviations: NS, not significant; PBO, placebo.

Fig. 2. Attenuation of ketamine-induced changes in BOLD signal by TAK-063 in healthy human subjects.

Abbreviations: ASL, arterial spin labeling; BOLD, blood oxygen level-dependent; MPRage, 3-dimensional magnetization-prepared rapid gradient-echo; SPM, statistical parametric mapping.

Fig. 3. Effects of TAK-063 on cognition in subjects with stable schizophrenia.

Abbreviations: SSS, subjects with stable schizophrenia.
| Day | 30 Hz | 40 Hz | 50 Hz |
|-----|-------|-------|-------|
| Placebo | ![Image](https://example.com/placebo_30Hz.png) | ![Image](https://example.com/placebo_40Hz.png) | ![Image](https://example.com/placebo_50Hz.png) |
| P<0.05 vs predose (frontal, central, parietal, occipital ↓) | P<0.05 vs predose (frontal, parietal ↓) | P<0.05 vs predose (temporal, occipital ↓) |
| TAK-063 (100 mg) | ![Image](https://example.com/tak_30Hz.png) | ![Image](https://example.com/tak_40Hz.png) | ![Image](https://example.com/tak_50Hz.png) |
| NS vs PBO; P<0.05 vs predose (frontal ↑) | P<0.05 vs PBO (central, parietal, occipital ↑) | NS vs PBO; NS vs predose |
| 1.33 | 1.33 | 1.33 |
| 0.04 | 0.04 | 0.04 |
Figure 2

Within group SPM analysis for Working Memory Task after ketamine infusion. Activation on the left is with pre-treatment with placebo. Activation on the right is after pre-treatment with 10 mg TAK-063.
Figure 3

**Reaction Time Variability - SSS**

Cohen's d Effect Sizes for Differences Between Each Dose and Placebo at Each Time Point

Reaction Time Variability: a composite score created from the addition of variability scores in reaction times from 3 attention tests. Reflects the ability to intently focus attention with minimal levels of fluctuation.

**Quality of Working Memory - SSS**

Cohen's d Effect Sizes for Differences Between Each Dose and Placebo at Each Time Point

Quality of Working Memory: a composite score created by combining accuracy measures from 2 working memory tasks. Reflects the ability to hold articulatory and spatial information on-line, which is crucial for everyday tasks which involve executive function.

**Tapping Task - SSS**

Cohen's d Effect Sizes for Differences Between Each Dose and Placebo at Each Time Point

Tapping Task: The ability to press a response button as rapidly as possible for 1 min which reflects psychomotor control.

**Executive Function Task - SSS**

Cohen's d Effect Sizes for Differences Between Each Dose and Placebo at Each Time Point

Executive Function Task (EFT): a measure which reflects the ability to rapidly switch between different processing rules.