Emraclidine, a novel positive allosteric modulator of cholinergic M4 receptors, for the treatment of schizophrenia: a two-part, randomised, double-blind, placebo-controlled, phase 1b trial

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Summary

Background Emraclidine is a novel, brain-penetrant, highly selective M4 receptor positive allosteric modulator in development for the treatment of schizophrenia. We aimed to evaluate the safety and tolerability of multiple ascending doses of emraclidine in patients with schizophrenia.

Methods We conducted a two-part, randomised, phase 1b trial in the USA. Eligible participants were aged 18–50 years (part A) or 18–55 years (part B) with a primary diagnosis of schizophrenia per the Diagnostic and Statistical Manual of Mental Disorders 5th edition, as confirmed by the Mini International Neuropsychiatric Interview, and extrapyramidal symptom assessments indicating normal to mild symptoms at screening. Part A evaluated the safety and tolerability of emraclidine in five cohorts of participants with stable schizophrenia who received ascending oral doses of emraclidine 5–40 mg (40 mg was administered as 20 mg twice daily) or placebo at a single US site. Part B was a double-blind, randomised, placebo-controlled study that enrolled adults with acute schizophrenia across five US sites; participants were randomly assigned (1:1:1) to receive emraclidine 30 mg once daily, emraclidine 20 mg twice daily, or placebo for 6 weeks (doses established in part A). The primary endpoint was safety and tolerability, assessed in the safety population (participants who received at least one dose of emraclidine or placebo). This trial is now complete and is registered with ClinicalTrials.gov, NCT04136873.

Findings Between Sept 23, 2019, and Sept 17, 2020, 118 patients were assessed for eligibility and 49 were randomly assigned across five cohorts in part A. 44 participants completed the study, with 36 participants receiving emraclidine and eight receiving placebo. The two highest doses tested were selected for part B. Between Oct 12, 2020, and May 7, 2021, 148 patients were assessed for eligibility and 81 were randomly assigned to emraclidine 30 mg once daily (n=27), emraclidine 20 mg twice daily (n=27), or placebo (n=27) in part B. Incidence of adverse events (14 [52%] of 27 participants in the emraclidine 30 mg once daily group, 15 [56%] of 27 in the emraclidine 20 mg twice daily group, and 14 [52%] of 27 in the placebo group), clinical assessments, and weight changes were similar across groups. The most common adverse event was headache (15 [28%] of 54 participants in the emraclidine groups, seven [26%] of 27 in the placebo group). Modest, transient increases in blood pressure and heart rate in emraclidine groups observed at treatment initiation diminished over time and were not considered clinically meaningful by week 6.

Interpretation These data support further investigation of emraclidine as a once-daily treatment for schizophrenia without need for titration and with a potentially favourable side-effect profile.

Funding Cerevel Therapeutics.

Introduction

Many of the currently available antipsychotics used in the treatment of schizophrenia directly interact with dopamine receptors to reduce dopamine signalling as, historically, the symptoms of schizophrenia were thought to be driven by dopaminergic hyperactivity in the mesolimbic pathway.[1,2] While currently available drugs are effective in managing psychotic episodes in most patients, they have a modest effect on negative and cognitive symptoms of schizophrenia, both of which are commonly present throughout the disease course and substantially impact functioning.[3] Antipsychotics are also associated with a range of side-effects, including extrapyramidal symptoms, prolactin elevation (and its related adverse effects), metabolic abnormalities, and weight gain, which can impair functioning, quality of life, and treatment adherence.[4–8] For example, although antipsychotic use is associated with decreased all-cause and cardiovascular mortality in schizophrenia, evidence suggests that antipsychotics might also contribute to obesity, metabolic disorders, and heart disease.[9–11] Medication non-adherence is a consistent predictor of

Research in context

Evidence before this study

Schizophrenia is a chronic, distressing mental illness that is among the most disabling and economically catastrophic medical disorders. Despite the availability of numerous medications, approximately one-third of patients with schizophrenia do not respond to current treatments. Effective management of positive, negative, and cognitive impairment symptoms with a reduced side-effect profile represents an unmet clinical need. Preclinical studies have suggested that M4 muscarinic receptor agonism is a possible treatment strategy for symptoms of schizophrenia. We searched PubMed on Nov 24, 2021, for clinical trials, with no language or date restrictions, using the terms "schizophrenia" and "muscarinic," and found a total of 38 clinical trials conducted and published to date, of which only two investigated muscarinic agonists for the treatment of schizophrenia. The first study was a pilot study of the M1/M4 receptor agonist xanomeline, which found improvements in symptom severity but an association with increased incidence of gastrointestinal adverse events compared with placebo. More recently, a double-blind, phase 2 trial in patients with acute schizophrenia investigated a combination of xanomeline and the peripheral anticholinergic trospium (to circumvent potential peripheral cholinergic adverse effects associated with xanomeline monotherapy), titrated over 1 week.

Added value of this study

This two-part, phase 1b study was designed to assess the safety and tolerability of emraclidine, a novel, highly selective M4 muscarinic receptor positive allosteric modulator in patients with schizophrenia. The first part of the study (part A) was a multiple ascending-dose design to establish safety, tolerability, and appropriate dosing frequency based on pharmacokinetics. The second part of the study (part B) assessed the safety and tolerability of emraclidine 30 mg once daily and 20 mg twice daily compared with placebo. Both doses showed generally favourable safety profiles, with a similar incidence of adverse events and no clinically meaningful differences compared with placebo in general clinical safety measures, extrapyramidal symptoms, or weight changes after 6 weeks of treatment. In both parts of the study, emraclidine administration was associated with small, transient increases in blood pressure and heart rate that diminished over time and were not considered clinically meaningful versus placebo after 6 weeks of treatment.

Implications of all the available evidence

These data support further development of emraclidine as a potential treatment for schizophrenia with once-daily dosing without need for titration, and suggest that it has a favourable side-effect profile for patients.

relapse and has been shown to increase total annual mean health-care costs by US$20787 among patients with uncontrolled symptoms of schizophrenia when compared with controls without schizophrenia.[12,13] Additionally, an estimated one-third of patients with schizophrenia do not respond to current available treatments.[14] Novel therapeutic approaches are needed to balance the efficacy of treatment against side-effect profiles that might contribute to long-term comorbidities, low rates of long-term treatment adherence, cycles of relapse, and health-care costs.[15]

The original dopamine hypothesis of schizophrenia suggests that presynaptic striatal dopaminergic dysregulation (ie, at the stage of dopamine synthesis, storage, or release) might serve as an opportunity for novel therapeutic intervention.[16] M4 muscarinic acetylcholine receptor (mAChR) activation has emerged as a novel potential treatment mechanism that avoids dopamine D2 receptor blockade.[1,17] Presynaptic mAChRs can act as autoreceptors on striatal cholinergic interneurons to inhibit acetylcholine release and modulate local dopamine release.[18] Stimulating M4 mAChRs on D1 receptor-expressing striatal spiny neurons also inhibits striatal dopamine release by evoking release of the endocannabinoid 2-arachinonoylglycerol from these neurons, resulting in inhibition of dopamine release by stimulating cannabinoid receptor type 2 receptors on dopamine terminals.[19] Reduced striatal dopamine release via mAChR-targeting compounds accompanies antipsychotic-like activity in preclinical models, including amphetamine-induced hyperlocomotion and prepulse inhibition of startle assays.[19–21] M4 receptor activation might also have antipsychotic and procognitive effects by reducing cortical glutamatergic hyperactivity which can be a feature of schizophrenia, particularly early in its course.[22,23] In humans, the M1/M4 agonist xanomeline has shown efficacy for symptoms of psychosis in patients with Alzheimer's disease and Positive and Negative Syndrome Scale (PANSS) scores in patients with schizophrenia; however, xanomeline development was stalled due to gastrointestinal and cardiovascular side-effects that are likely to be related to peripheral M1 or non-selective cholinergic agonism.[24,25] The positive results in patients with acutely exacerbated schizophrenia receiving a combination of xanomeline plus the peripheral anticholinergic trospium (which reduced xanomeline's mostly M1 agonism-related, peripheral side-effects) further substantiated the efficacy of M4 agonist activity for the treatment of schizophrenia.[26] An alternative approach to reducing peripheral side-effects associated with M1/M4 agonism with xanomeline could be specific activation of M4 receptors; however, previous development of M4 selective agonists has been halted due to differential potency between preclinical models and human M4 receptors, and solubility issues.[27]

Emraclidine is a novel, brain-penetrant, highly selective M4 muscarinic receptor positive allosteric modulator that

has shown a beneficial early clinical profile and is being developed for the treatment of schizophrenia.[28] We aimed to evaluate the safety and tolerability of multiple ascending doses of emraclidine in patients with schizophrenia.

Methods

Study design

We conducted a two-part, randomised, phase 1b trial in the USA. Part A of the trial was a multiple ascending-dose study in patients with stable schizophrenia to evaluate the safety and tolerability of five doses of emraclidine. Part B was a randomised, double-blind, placebo-controlled study in patients with acutely exacerbated schizophrenia that focused on safety, tolerability, pharmacokinetics, and pharmacodynamics of the two doses identified in part A that were considered safe and generally well tolerated, emraclidine 30 mg once daily and 20 mg twice daily.

The trial was approved by the Aspire Institutional Review Board (Santee, CA, USA) and was conducted in accordance with the Council for International Organizations of Medical Sciences International Ethical Guidelines and the principles of Good Clinical Practice. Both parts of the trial were inpatient studies conducted at US private research centres (one site in part A, five sites in part B; appendix p 4). Participants were recruited via site-specific databases, referrals from outside physicians, and local advertising contracted by individual sites. All study participants or their legally authorised representatives provided written informed consent. The protocol is available in the appendix (pp 14–132).

Participants

Detailed inclusion and exclusion criteria, including medical history and concurrent disease information, are provided in the appendix (pp 67–73). In both parts, participant eligibility criteria included a primary diagnosis of schizophrenia per the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5), as confirmed by the Mini International Neuropsychiatric Interview, and extrapyramidal symptom assessments indicating normal to mild symptoms at screening. Additional eligibility criteria for part A included age 18–50 years, Clinical Global Impression of Severity (CGI-S) score of 4 or lower (normal to moderately ill), and PANSS total score of 80 or lower. Additional eligibility criteria for part B included age 18–55 years, CGI-S score of 4 or greater (moderately to severely ill), PANSS total score of 80 or greater, a score of 4 or greater for at least two positive subscale items (delusions, conceptual disorganisation, hallucinatory behaviour, or suspiciousness of persecution), history of relapse or exacerbation of symptoms when not receiving antipsychotic treatment, and current acute exacerbation or relapse of symptoms with onset within the previous 2 months.

Exclusion criteria for both parts included a current DSM-5 diagnosis other than schizophrenia; history of resistance to antipsychotic treatment, history of no response to clozapine, or history of response to clozapine treatment only; extrapyramidal symptoms being treated with a medication requiring dose modification or new treatment within the 6 months before enrolment; serious risk of suicide, or medical history, concurrent disease, or clinical assessment that would increase risk of adverse events or preclude the evaluation of drug safety or study results; abnormal results on a 12-lead electrocardiogram (ECG); abnormal blood pressure or current treatment with antihypertensive medications; and history of moderate to severe substance or alcohol-use disorder as per DSM-5 criteria within the 12 months before enrolment. Additional exclusion criteria for part A included hospitalisation due to psychosis within the past 6 months, and change in antipsychotic medication due to a psychotic event within the past 3 months. Additional exclusion criteria for part B included hospitalisation for more than 14 days for the current episode of schizophrenia (excluding hospitalisations for psychosocial reasons), and a first episode of schizophrenia.

Randomisation and masking

Randomisation for both parts of the study was performed using a third-party, computer-generated randomisation code (IQVIA; Durham, NC, USA). In part A, participants in each cohort were randomly assigned (4:1) to receive emraclidine (at one of five dose levels) or matching placebo. In part B, participants were randomly assigned (1:1:1) to receive emraclidine 30 mg once daily, emraclidine 20 mg twice daily, or matching placebo with an identical number of tablets. Treatment was masked in both parts such that participants, raters for clinician-administered scales, investigators, and other site personnel were unaware of treatment assignments (appendix p 2). The treatment assignment for part A was based on provided randomisation codes and was performed by an independent, unmasked pharmacist who had no involvement in other study procedures. The treatment assignment for part B was performed by a validated Interactive Response Technology system. Sponsor representatives were unmasked to treatment allocations in part A for the purposes of dose escalation and safety monitoring. The sponsor was masked to treatment allocation in part B.

Procedures

Participants in both parts A and B were screened over a 3-week period and were admitted to the clinic before day –1 if washout of prohibited medications (including antipsychotic agents, antidepressants, mood stabilisers, and varenicline) was needed (appendix p 77). In part A, participants received oral placebo or oral emraclidine at a dose of 5 mg once daily for 14 days; 10 mg once daily for 14 days; 20 mg once daily for 14 days; 30 mg once daily for 14 days; or 5 mg twice daily for 3 days, 10 mg twice daily for 4 days, and 20 mg twice daily for 21 days, for a total of 28 days of inpatient treatment. In part B,

participants received oral emraclidine 30 mg once daily, emraclidine 20 mg twice daily, or placebo for 42 days of inpatient treatment without dose titration; participants assigned to receive emraclidine 30 mg once daily received a morning dose of emraclidine and an evening dose of placebo.

Outcomes

The primary endpoint was safety and tolerability. Safety and tolerability assessments in both parts A and B included physical and neurological examinations, standard 12-lead ECG, vital sign measurements, clinical laboratory tests, the Columbia-Suicide Severity Rating Scale, assessments of extrapyramidal symptoms (Simpson-Angus Scale, Abnormal Involuntary Movement Scale, and Barnes Akathisia Rating Scale), and adverse events. Adverse events of special interest were defined in the protocol as: events that resulted in the discontinuation of treatment with study drug; events that satisfied the criteria for suspected Hy's Law (alanine aminotransferase or aspartate aminotransferase >3 times the upper limit of normal [ULN], serum bilirubin ≥2×the ULN, and alkaline phosphatase <2×the ULN); events of heart rate of greater than 120 beats per min, confirmed by ECG; events of confirmed corrected QT interval using Fridericia's formula of greater than 500 milliseconds or an increase of greater than 75 milliseconds from baseline; and events of mean triplicate blood pressure reading of greater than 160 mm Hg systolic or 100 mm Hg diastolic, with confirmation. In part B, blood pressure and heart rate were measured daily 2 h after the morning dose and 2 h after the evening dose during the treatment period.

The secondary endpoint was pharmacokinetics of emraclidine in participants in part B following administration of emraclidine 20 mg twice daily or 30 mg once daily. Blood samples were collected at specified timepoints (appendix pp 30–55) and plasma emraclidine concentrations were determined by a validated liquid chromatography with tandem mass spectrometry method (lower limit of quantification of 0·100 ng/mL).

Part B included predefined exploratory pharmacodynamic endpoints, including the PANSS, CGI-S, and the Brief Assessment of Cognition in Schizophrenia (BACS) symbol coding test. The PANSS consisted of three subscales (positive symptoms, negative symptoms, and general psychopathology) containing 30 symptom constructs, with each rated on a 7-point scale where 1 indicates the absence of symptoms and 7 indicates extremely severe symptoms;[29] PANSS total scores range from 30 to 210. The Marder negative symptom subscore, with scores ranging from 7 to 49, was also assessed.[30] The CGI-S was administered by an investigator responding to the question "Considering your total clinical experience with this particular population, how ill is the subject at this time?", with responses ranging from 1 (normal, not ill at all) to 7 (among the most extremely ill subjects). The BACS consisted of participants matching the

numerals 1–9 to symbols on an electronic platform over a 90-s period, and the number of correct numerals over time was scored on a range from 0 to 110.[31]

Statistical analysis

Part A was composed of five cohorts, with planned enrolment of ten participants with stable schizophrenia (eight receiving emraclidine and two receiving placebo) per cohort, based on the adequacy of a previous single-dose trial designed to characterise the single-dose pharmacokinetics and safety and tolerability of emraclidine at each dose level. In part B, planned enrolment included 75 participants with schizophrenia with an acute exacerbation or relapse of psychotic symptoms (appendix p 2).

All participants who received at least one dose of emraclidine or placebo were included in the safety population for the primary analysis. Adverse events were coded according to the Medical Dictionary for Regulatory Activities and summarised using descriptive statistics. In part B, the weekly means of the daily measurements after day 1 were used as the basis of analysis to reduce the variability of daily data for heart rate and blood pressure assessments. A linear mixed model for repeated measures was used to compare each active treatment with placebo; treatment, study week, and the interaction between treatment and study week were included as fixed effects, baseline as a covariate, and participant within study day as a repeated measure with an unstructured covariance matrix. The least-square means of difference between each dose and placebo were derived from the model. Standard pharmacokinetic parameters were estimated using non-compartmental methods and summarised using descriptive statistics.

In part B, participants in the safety population with at least one pharmacodynamic assessment after baseline were included in the pharmacodynamic analysis population. The change from baseline in PANSS total score, PANSS subscales (including a post-hoc Marder negative factor analysis), and CGI-S were analysed using a mixed model for repeated measures, with both treatment-group visit and treatment-by-visit interaction as fixed effects, and participant as a random effect, with baseline score as a covariate with an unstructured covariance matrix. The mixed model for repeated measures approach was prespecified in the statistical analysis plan before database lock. A Cohen d value was also derived as the ratio of the estimated difference to the population SD at each visit, estimated from the model. For responder analysis, the percentage of participants who had changes of 20% or greater, 30% or greater, and 50% or greater in PANSS total score and the percentage of participants who had improvements of 1 or more, 2 or more, and 3 or more points on CGI-S score from baseline were assessed. A χ^2 test was used to compare the proportion of participants who had a response in each active treatment group versus the placebo group; if expected counts were

less than 5, then a Fisher's exact test was used. As the pharmacodynamic assessments were exploratory, no formal sample size determination or hypothesis testing procedure with multiplicity control was planned. The p values derived from the mixed model for repeated measures or the χ^2 test were intended to provide a gauge of the strength of signal versus noise and were thus labelled nominal. The study was not statistically powered for the exploratory endpoints. Statistical analyses were done with SAS (version 9.4).

This trial is registered with ClinicalTrials.gov, NCT04136873.

Role of the funding source
The funder of the study was involved in study design, data collection, data analysis, data interpretation, writing of the report, and the decision to submit for publication.

Results
In part A, between Sept 23, 2019, and Sept 17, 2020, 118 patients were assessed for eligibility and 49 were enrolled and randomly assigned across five cohorts. Five participants withdrew consent before study completion and 44 completed the study; 36 received emraclidine and eight received placebo (appendix p 11; protocol deviations in part A are described in the appendix [p 3]). The majority of participants were male (≥70% in all treatment groups) and Black (≥57% in all treatment groups), with a mean age of 38 years (SD 8·1)

in all emraclidine groups and 35 years (6·4) in all placebo groups; baseline characteristics are shown in the appendix (p 5). There were no serious adverse events or deaths and no clinically relevant findings in clinical laboratory assessments, physical examinations, or ECGs. Across all emraclidine groups, 16 (41%) of 39 participants had adverse events; the most frequently reported events were headache (four [10%]), dizziness (two [5%]), insomnia (two [5%]), and increased weight (two [5%]; appendix p 6). In both cases of increased weight (both in the emraclidine 10 mg once daily group), the observed weight gain mostly occurred before treatment initiation and was not considered related to treatment by the investigator. Additionally, there was no indication of treatment effects on extrapyramidal symptoms or suicidal ideation or behaviour. Administration of emraclidine was associated with small, transient increases in both systolic and diastolic blood pressure across the 5 mg, 10 mg, 20 mg, and 30 mg once daily doses and the 20 mg twice daily dose; none of the increases were considered clinically significant or associated with adverse events. The two highest doses tested (emraclidine 30 mg once daily and 20 mg twice daily) were further investigated in part B.

In part B, between Oct 12, 2020, and May 7, 2021, 148 patients were assessed for eligibility and 81 were enrolled and randomly assigned to emraclidine 30 mg once daily (n=27), emraclidine 20 mg twice daily (n=27), or placebo (n=27; figure 1). Two of these 81 participants
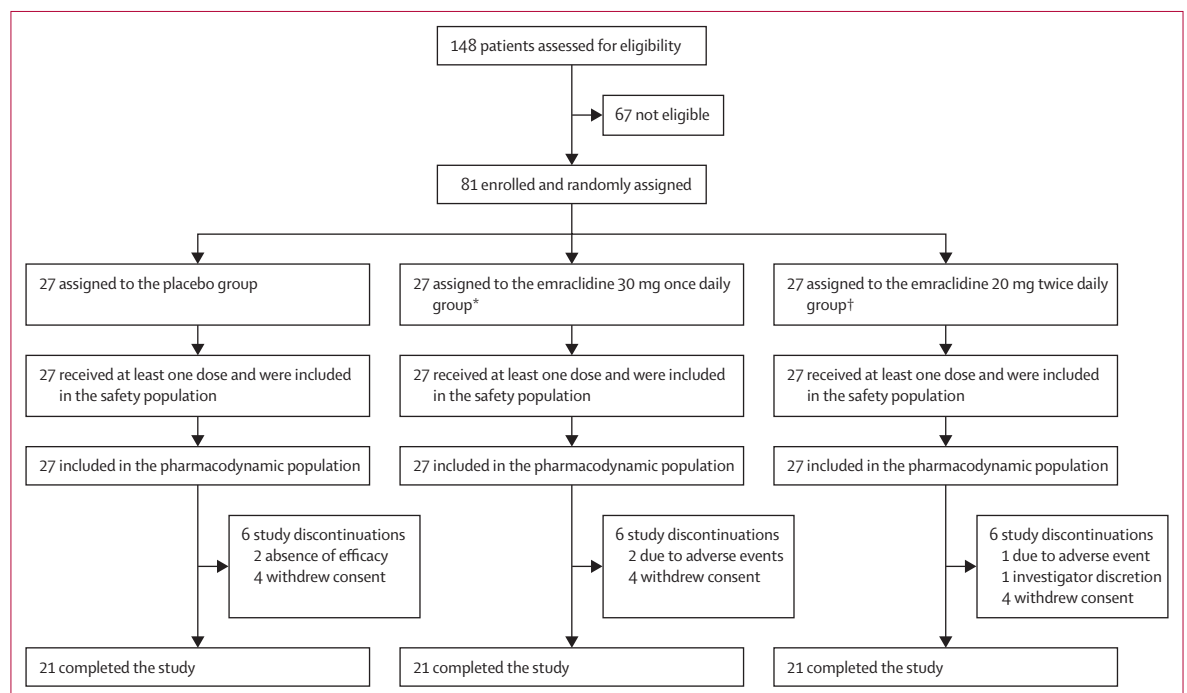
Figure 1: Trial profile for part B
*One participant was randomly assigned to receive emraclidine 30 mg once daily despite having an exclusionary screening heart rate of lower than 50 beats per min; this participant was included in the safety and pharmacodynamic analyses. †One participant was randomly assigned to receive emraclidine 20 mg twice daily despite having an exclusionary diastolic blood pressure of greater than 80 mm Hg at baseline; this participant was included in the safety and pharmacodynamic analyses.

were randomly assigned to treatment despite not meeting eligibility criteria (one due to screening heart rate <50 beats per min, one with diastolic blood pressure >80 mm Hg); both participants were included in the safety and pharmacodynamic analysis sets per protocol definitions (additional protocol deviations in part B are described in the appendix [p 3]). Altogether, 18 participants discontinued; four participants in each treatment group withdrew consent, two in the placebo group discontinued due to absence of efficacy, three who received emraclidine discontinued due to adverse events, and one in the emraclidine 20 mg twice daily group discontinued at the investigator's discretion due to unwillingness to comply with protocol procedures. Most participants were male (≥70% across all treatment groups) and Black (≥63% across all treatment groups), with a mean age of 40 years (SD 9; table 1). At baseline, the mean PANSS total score was 95 (range 80–114) and the mean CGI-S score was 5 (range 4–6), indicating moderate to severe illness. The mean time since disease onset was 19 years (SD 10) and most participants (69 [85%]) had been hospitalised at least once.

The overall incidence of adverse events and treatment-related adverse events was similar across the placebo and emraclidine treatment groups (14 [52%] of 27 participants in the emraclidine 30 mg once daily group, 15 [56%] of 27 in the emraclidine 20 mg twice daily group, and 14 [52%] of 27 in the placebo group had adverse events; table 2). Most adverse events were mild or moderate in severity and there were no deaths associated with adverse events. Serious adverse events were reported in three participants, including one case of COVID-19 (in the emraclidine 20 mg twice daily group), one accidental cocaine overdose (in the emraclidine 30 mg once daily group), and one exacerbation of schizophrenia (in the emraclidine 30 mg once daily group); none of these serious adverse events were considered by the investigator to be related to study treatment. The events of accidental cocaine overdose and exacerbation of schizophrenia resulted in study discontinuation; an additional participant in the emraclidine 20 mg twice daily group discontinued due to an adverse event of psychotic disorder (concurrent with the serious adverse event of COVID-19), which was not considered related to study treatment by the investigator (appendix p 3).

The most commonly reported adverse event in the emraclidine treatment groups was headache (15 [28%] of 54 participants in the emraclidine groups, seven [26%] of 27 in the placebo group). The incidence of gastrointestinal adverse events was low and similar across treatment groups (five [19%] of 27 participants in the emraclidine 30 mg once daily group, two [7%] of 27 in the emraclidine 20 mg twice daily group, and four [15%] of 27 in the placebo group). Overall, six participants had adverse events of special interest related to heart rate and blood pressure (three in the placebo group and three in the emraclidine 20 mg twice daily group). No participants

	Placebo group (n=27)	Emraclidine 30 mg once daily group (n=27)	Emraclidine 20 mg twice daily group (n=27)
Age, years			
Mean (SD)	41 (10)	41 (8)	38 (10)
Median (range)	41 (22–55)	41 (24–55)	37 (23–55)
Sex			
Male	19 (70%)	23 (85%)	21 (78%)
Female	8 (30%)	4 (15%)	6 (22%)
Race			
Asian	1 (4%)	0	0
Black or African American	17 (63%)	20 (74%)	19 (70%)
White	9 (33%)	7 (26%)	0
Multiple	0	0	1 (4%)
Hispanic or Latino	2 (7%)	2 (7%)	2 (7%)
Weight, kg*			
Mean (SD)	89·3 (16·1)	84·5 (13·4)	84·5 (14·6)
Median (range)	88·6 (59·1–130·6)	86·7 (62·1–109·9)	82·8 (62·5–120·0)
BMI, kg/m²*			
Mean (SD)	29·0 (4·8)	27·6 (4·5)	28·0 (5·0)
Median (range)	28·9 (21·0–37·7)	27·2 (19·9–36·2)	27·1 (19·0–41·4)
Time since initial onset, years	20 (11)	19 (9)	16 (11)
Previous hospitalisations			
0	3 (11%)	3 (11%)	6 (22%)
1–3	14 (52%)	16 (59%)	12 (44%)
≥4	10 (37%)	8 (30%)	9 (33%)
PANSS total score	93 (8·8)	93 (7·3)	97 (7·9)
Change in PANSS total score from screening to baseline	0 (6·7)	2 (5·0)	0 (7·5)
PANSS positive symptom subscale score	24 (2·7)	25 (3·0)	26 (2·6)
PANSS negative symptom subscale score	23 (3·3)	22 (3·7)	24 (3·8)
CGI-S score	5 (0·6)	5 (0·5)	5 (0·7)

Data are n (%) or mean (SD) unless otherwise stated. CGI-S=Clinical Global Impression of Severity. PANSS=Positive and Negative Syndrome Scale. *Before dosing on day –1.

Table 1: Baseline characteristics of participants in part B

who had adverse events of special interest related to heart rate and blood pressure were symptomatic and no additional adverse events associated with these events were reported. Additionally, no adverse events related to extrapyramidal symptoms were reported.

The least-square mean differences from baseline in supine systolic and diastolic blood pressure were modestly increased at various timepoints in both the emraclidine 30 mg once daily and 20 mg twice daily groups compared with the placebo group (figure 2A; appendix pp 7–8, 12). The largest increases in supine systolic and diastolic blood pressure were observed for the day 1 or week 1 average (using the 2 h after morning dose timepoint). The differences in supine blood pressure compared with the placebo group trended downward over the 6-week treatment period for both emraclidine dose groups. The difference compared with the placebo group in week 6 average (days 36–42) mean change from

baseline in systolic blood pressure was 1·2 mm Hg (SE 2·2) in the emraclidine 30 mg once daily group and

	Placebo group (n=27)	Emraclidine 30 mg once daily group (n=27)	Emraclidine 20 mg twice daily group (n=27)
Any adverse event	14 (52%)	14 (52%)	15 (56%)
Adverse events related to study drug	10 (37%)	7 (26%)	12 (44%)
Adverse events of special interest	3 (11%)	2 (7%)	4 (15%)
Serious adverse events	0	2 (7%)	1 (4%)
Adverse events leading to study discontinuation	0	2 (7%)	1 (4%)
Adverse events that occurred in at least 5% of participants receiving emraclidine where percent incidence was greater with emraclidine than with placebo			
Headache	7 (26%)	8 (30%)	7 (26%)
Nausea	1 (4%)	2 (7%)	2 (7%)
Back pain	1 (4%)	2 (7%)	1 (4%)
Blood creatinine phosphokinase increased	0	1 (4%)	2 (7%)
Dizziness	0	1 (4%)	2 (7%)
Dry mouth	0	3 (11%)	0
Somnolence	0	1 (4%)	2 (7%)

Data are n (%).

***Table 2:* Adverse events in part B**

Figure 2: **Changes in blood pressure and heart rate in participants in part B**
Change from baseline in least-square mean supine systolic blood pressure (A) and heart rate (B) at 2 h after the morning dose (left) and 2 h after the evening dose (right). Apart from for day 1, blood pressure least-square mean change from baseline is reported as a weekly average. Assessments at 2 h after the evening dose for the emraclidine 30 mg once daily group were taken after a placebo dose. Error bars are SE.

0·9 mm Hg (2·1) in the emraclidine 20 mg twice daily group, and was not considered clinically meaningful. The difference compared with the placebo group in week 6 average mean change from baseline in diastolic blood pressure was –0·1 mm Hg (SE 1·3) in the emraclidine 30 mg once daily group and 0·1 mm Hg (1·3) in the emraclidine 20 mg twice daily group, and was not considered clinically meaningful. The emraclidine 30 mg once daily and 20 mg twice daily treatment groups showed increases in least-square mean supine heart rate on day 1 compared with the placebo group (figure 2B); the differences in mean change from baseline compared with the placebo group decreased over the treatment period in both emraclidine dose groups (week 6 mean difference compared with placebo of 4·4 beats per min [SE 2·1] in the emraclidine 30 mg once daily group and 5·3 beats per min [2·1] in the emraclidine 20 mg twice daily group).

There were no meaningful changes in ECG intervals across treatment groups aside from the modest, asymptomatic heart rate elevations. Additionally, there were no meaningful changes in mean clinical chemistry, haematology, or urinalysis results from baseline to day 42 across all treatment groups. Weight change from baseline to day 42 was similar across all treatment groups, with a mean change of 1·4 kg (SD 4·3) in the emraclidine 30 mg once daily group, 1·7 kg (3·1) in the emraclidine 20 mg twice daily group, and 1·6 kg (4·0) in the placebo group. The percentage of participants whose bodyweight increased by 7% or more from baseline to day 42 was similar across treatment groups (three [15%] of 27 in the emraclidine 30 mg once daily group, three [14%] of 27 in the emraclidine 20 mg twice daily group, and four [19%] of 27 in the placebo group). There were no meaningful changes in the Columbia-Suicide Severity Rating Scale, Simpson-Angus Scale, Abnormal Involuntary Movement Scale, and Barnes Akathisia Rating Scale safety scores in any treatment group.

Emraclidine pharmacokinetic profiles were characterised by rapid absorption, with a median time to reach maximum concentration of 1 h for both treatments (appendix p 13). Steady-state emraclidine exposures (calculated as area under the concentration-time curve) were associated with high variability (coefficient of variation >80%) with greater between-participant variability observed in the 20 mg twice daily group than in the 30 mg once daily group. The mean emraclidine maximum plasma concentration and area under the concentration-time curve values were slightly lower in the 20 mg twice daily group than in the 30 mg once daily group. However, there was a high degree of overlap observed in individual exposure values across both dose groups.

Both the 30 mg once daily and 20 mg twice daily doses of emraclidine were associated with antipsychotic activity as assessed by the PANSS total score. By day 21, least-square mean reductions in the PANSS total score were 14·20 points (SE 2·55) in the emraclidine 30 mg once daily group, 9·22 (2·61) in the 20 mg twice daily group,

and 5·60 (2·49) in the placebo group (figure 3A); the least-square mean difference between the emraclidine 30 mg once daily group and the placebo group was significant at day 21 (difference versus placebo –8·61; nominal p=0·018; Cohen's *d* –0·68). By the end of trial treatment (day 42), the least-square mean reduction in the PANSS total score from baseline in the placebo group was 6·77 points (SE 3·82); treatment with emraclidine 30 mg once daily resulted in a least-square mean reduction from baseline of 19·5 points (3·91; difference versus placebo –12·7 points; nominal p=0·023; Cohen's

d –0·68). Emraclidine 20 mg twice daily resulted in a least-square mean reduction from baseline in PANSS total score of 17·9 points (SE 3·93) and a least-square mean reduction of 11·1 points compared with the placebo group (nominal p=0·047; Cohen's *d* –0·59) at day 42. The proportion of participants with a reduction of 30% or greater in total PANSS score from baseline was higher in both emraclidine treatment groups than in the placebo group. When compared with placebo, the percentage of participants with a reduction of 30% or greater in total PANSS score was significantly higher in the emraclidine
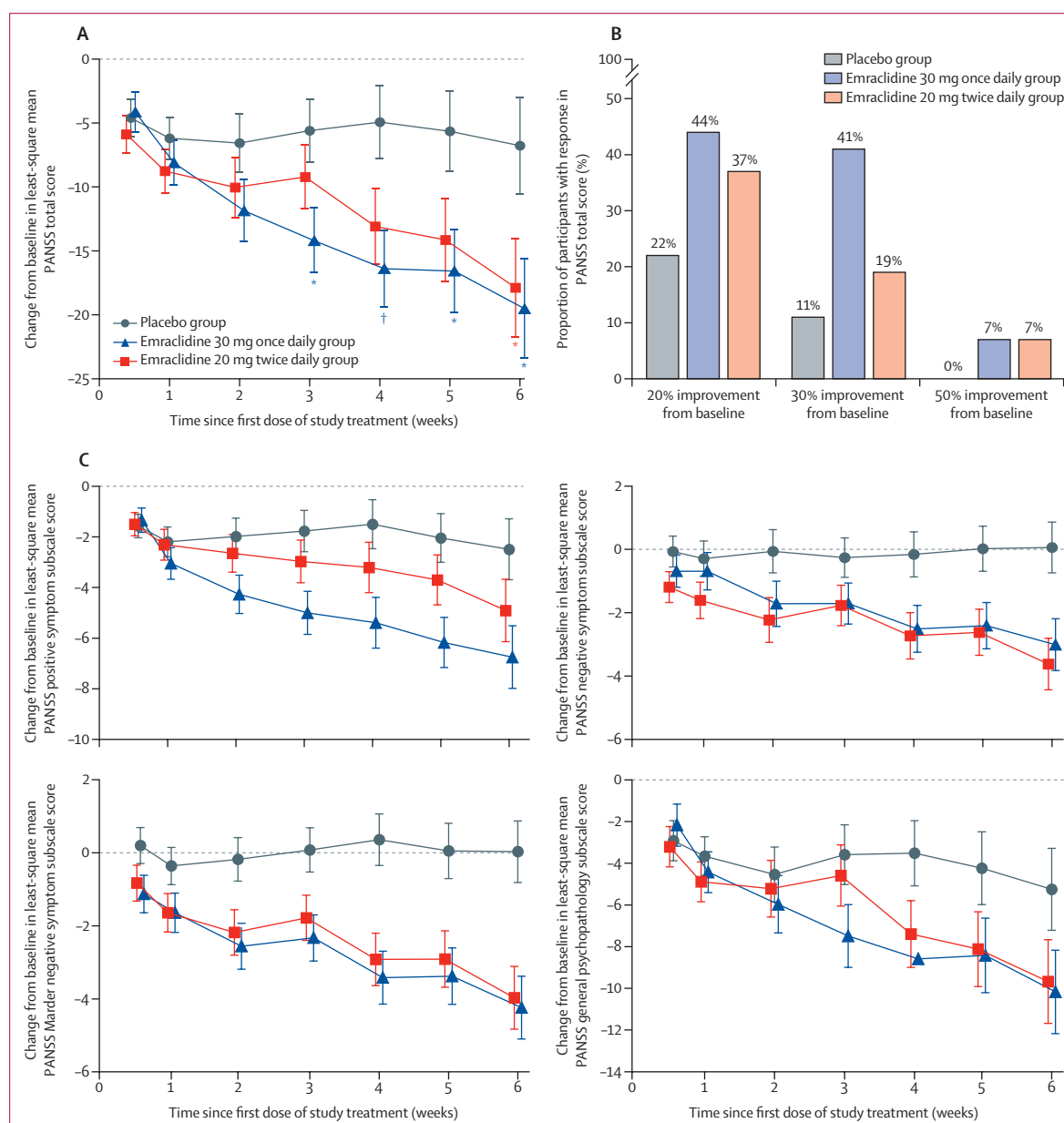
Figure 3: PANSS scores in participants in part B
(A) Change in mean PANSS total over time. (B) Proportion of participants with a response in PANSS score. (C) Change in PANSS subscale scores over time. Error bars show SE. PANSS=Positive and Negative Syndrome Scale. *Nominal p<0·05. †Nominal p<0·01.

A

B

Figure 4: **CGI-S scores in participants in part B**
(A) Change in mean CGI-S score over time. (B) Proportion of participants with a response in CGI-S score. Error bars show SE. CGI-S=Clinical Global Impression of Severity. *Nominal p<0·05. †Nominal p<0·01.

Consistent with the results from the PANSS scores, CGI-S scores improved following treatment with emraclidine. By the end of trial treatment (day 42), CGI-S score least-square mean reductions from baseline were 1·25 points (SE 0·24) in the emraclidine 30 mg once daily group, 0·96 (0·24) in the emraclidine 20 mg twice daily group, and 0·36 (0·24) in the placebo group (figure 4A). The least-square mean differences compared with placebo for the emraclidine 30 mg once daily group were significant at both day 42 (−0·89 points, nominal p=0·010; Cohen's d −0·77) and day 21 (−0·63, nominal p=0·003; Cohen's d −0·86). The least-square mean difference compared with placebo for the emraclidine 20 mg twice daily group at day 21 was −0·35 (nominal p=0·10; Cohen's d −0·47) and at day 42 was −0·60 (nominal p=0·082; Cohen's d −0·52). The percentage of participants with 2 points or greater and 1 point or greater improvements was higher in both emraclidine treatment groups than in the placebo group (figure 4B). No changes in the BACS symbol coding test were observed between the placebo and treatment groups.

Discussion

This two-part study investigated the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple ascending doses of emraclidine, a muscarinic M4 receptor positive allosteric modulator, in patients with schizophrenia. Results from part A (a multiple ascending-dose study) showed that emraclidine had a favourable safety profile with both once-daily dosing (5–30 mg) and twice-daily dosing (20 mg titrated). On the basis of the results from part A, part B further investigated emraclidine doses of 30 mg once daily and 20 mg twice daily in patients with schizophrenia who had an acute exacerbation of psychotic symptoms. In both parts of the study, no clinically meaningful findings relative to placebo were observed in clinical laboratory assessments, physical examinations (including changes in weight), or ECGs; additionally, there was no indication of effect of treatment on extrapyramidal symptoms or suicidal ideation or behaviour. Transient, modest increases in heart rate and blood pressure associated with emraclidine admini-stration observed in both part A and part B were asymptomatic, decreased over time, and were not considered clinically meaningful versus placebo after 6 weeks of treatment in this trial. A study designed to characterise the effects of emraclidine treatment on blood pressure and heart rate is ongoing (NCT05245539). The overall incidence of adverse events in part B was similar across the placebo and emraclidine treatment groups, with most adverse events considered mild to moderate in severity. In part B, emraclidine plasma exposures were similar between the 30 mg once daily group and the 20 mg twice daily groups, and clinically meaningful improvements[32] in symptoms were consistently observed in PANSS and CGI-S exploratory pharmacodynamic endpoints compared with placebo.

30 mg once daily group (three [11%] of 27 participants vs 11 [41%] of 27; nominal p=0·028; figure 3B). Reductions in PANSS total scores were corroborated by reductions in the PANSS positive, negative, Marder negative, and general psychopathology subscale scores compared with placebo (figure 3C). Least-square mean differences from placebo on the PANSS subscales at day 42 were −4·27 (nominal p=0·016; Cohen's d −0·72) in the emraclidine 30 mg once daily group and −2·43 (nominal p=0·17; Cohen's d −0·41) in the 20 mg twice daily group for the PANSS positive symptoms subscale; −3·07 (nominal p=0·009; Cohen's d −0·80) and −3·68 (nominal p=0·002; Cohen's d −0·96) for the PANSS negative symptoms subscale; −4·27 (nominal p<0·001; Cohen's d −1·05) and −4·00 (nominal p=0·001; Cohen's d −0·98) for the PANSS Marder negative symptom subscale; and −4·92 (nominal p=0·085; Cohen's d −0·52) and −4·43 (nominal p=0·12; Cohen's d −0·47) for the PANSS general psychopathology subscale.

Emraclidine exposures at 30 mg once daily combined with pharmacodynamic outcomes suggest that once-daily dosing of emraclidine without titration has potential for management of acute psychotic and negative symptoms in patients with schizophrenia. Of note, most schizophrenia spectrum disorder guidelines recommend dose titration when prescribing antipsychotics, and administering the minimum necessary dose to patients with acute schizophrenia, to avoid unnecessary adverse effects.[33] In a retrospective chart review of 149 patients admitted to an inpatient psychiatric facility who received dose titration of antipsychotics (including risperidone, paliperidone, olanzapine, quetiapine, and aripiprazole), doses were titrated every 2 days on average, with the final dose reached in an average of 3 days.[34] Ultimately, dose titration rates of antipsychotics are based in clinical discretion and patient monitoring, and dose selection in the acute setting might be complicated by latency to therapeutic effect.[2]

In the current study of emraclidine, which is highly selective for M4 over M1 and the other muscarinic receptors, improvements in the PANSS total scores and subscales were observed, but adverse events were similar between placebo and emraclidine treatment groups, with no clinically meaningful difference in incidence of gastrointestinal adverse events, as has been seen in previous studies of muscarinic receptor agonists not highly selective for M4.[24–26] However, the small sample size of our study might limit power to detect differences in safety and tolerability, and most participants were Black and male (reflecting the patient population at the investigating site locations), which might affect generalisability of the study data. Additionally, although initial pharmacodynamic data with emraclidine suggest a clinically meaningful benefit, a formal hypothesis test with a primary endpoint was not prespecified for part B of the trial, and this study was not formally powered for assessment of antipsychotic activity. Moreover, part B was conducted over 6 weeks of treatment; long-term effects of emraclidine still need to be determined. Overall, additional data from larger randomised controlled trials and open-label extension studies with wider geographical representation are warranted.

These results support selective targeting of the M4 muscarinic receptor via positive allosteric modulation as a novel treatment approach with a favourable safety profile and minimal side-effects, with the potential for once-daily dosing without titration regimens. Further research is warranted to confirm the efficacy, safety, and tolerability of emraclidine in schizophrenia.

Contributors
JR, ML, PI, RS, and SD conceptualised the study. IC, JR, LF, ML, PP, RS, SD, SP, and SV designed the study. LF, SV, and DPW acquired study data. IC, LF, ML, PI, SD, SP, and SV analysed study data. All authors participated in data interpretation, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. Additionally, all authors accessed and verified the data and approved the manuscript for submission and final publication.

References
1   McCutcheon RA, Abi-Dargham A, Howes OD. Schizophrenia, dopamine and the striatum: from biology to symptoms. *Trends Neurosci* 2019; **42:** 205–20.
2   Keepers GA, Fochtmann LJ, Anzia JM, et al. The American Psychiatric Association practice guideline for the treatment of patients with schizophrenia. *Am J Psychiatry* 2020; **177:** 868–72.

3 Correll CU, Schooler NR. Negative symptoms in schizophrenia: a review and clinical guide for recognition, assessment, and treatment. *Neuropsychiatr Dis Treat* 2020; **16:** 519–34.

4 Huhn M, Nikolakopoulou A, Schneider-Thoma J, et al. Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: a systematic review and network meta-analysis. *Lancet* 2019; **394:** 939–51.

5 Kane JM, Kishimoto T, Correll CU. Non-adherence to medication in patients with psychotic disorders: epidemiology, contributing factors and management strategies. *World Psychiatry* 2013; **12:** 216–26.

6 Pillinger T, McCutcheon RA, Vano L, et al. Comparative effects of 18 antipsychotics on metabolic function in patients with schizophrenia, predictors of metabolic dysregulation, and association with psychopathology: a systematic review and network meta-analysis. *Lancet Psychiatry* 2020; **7:** 64–77.

7 Solmi M, Murru A, Pacchiarotti I, et al. Safety, tolerability, and risks associated with first- and second-generation antipsychotics: a state-of-the-art clinical review. *Ther Clin Risk Manag* 2017; **13:** 757–77.

8 Tandon R, Lenderking WR, Weiss C, et al. The impact on functioning of second-generation antipsychotic medication side effects for patients with schizophrenia: a worldwide, cross-sectional, web-based survey. *Ann Gen Psychiatry* 2020; **19:** 42.

9 Correll CU, Detraux J, De Lepeleire J, De Hert M. Effects of antipsychotics, antidepressants and mood stabilizers on risk for physical diseases in people with schizophrenia, depression and bipolar disorder. *World Psychiatry* 2015; **14:** 119–36.

10 Taipale H, Tanskanen A, Mehtälä J, Vattulainen P, Correll CU, Tiihonen J. 20-year follow-up study of physical morbidity and mortality in relationship to antipsychotic treatment in a nationwide cohort of 62,250 patients with schizophrenia (FIN20). *World Psychiatry* 2020; **19:** 61–68.

11 Rojo LE, Gaspar PA, Silva H, et al. Metabolic syndrome and obesity among users of second generation antipsychotics: a global challenge for modern psychopharmacology. *Pharmacol Res* 2015; **101:** 74–85.

12 Pilon D, Patel C, Lafeuille MH, et al. Economic burden in Medicaid beneficiaries with recently relapsed schizophrenia or with uncontrolled symptoms of schizophrenia not adherent to antipsychotics. *J Manag Care Spec Pharm* 2021; **27:** 904–14.

13 Alvarez-Jimenez M, Priede A, Hetrick SE, et al. Risk factors for relapse following treatment for first episode psychosis: a systematic review and meta-analysis of longitudinal studies. *Schizophr Res* 2012; **139:** 116–28.

14 Correll CU, Brevig T, Brain C. Patient characteristics, burden and pharmacotherapy of treatment-resistant schizophrenia: results from a survey of 204 US psychiatrists. *BMC Psychiatry* 2019; **19:** 362.

15 Olivares JM, Sermon J, Hemels M, Schreiner A. Definitions and drivers of relapse in patients with schizophrenia: a systematic literature review. *Ann Gen Psychiatry* 2013; **12:** 32.

16 Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: version III—the final common pathway. *Schizophr Bull* 2009; **35:** 549–62.

17 Field JR, Walker AG, Conn PJ. Targeting glutamate synapses in schizophrenia. *Trends Mol Med* 2011; **17:** 689–98.

18 Threlfell S, Clements MA, Khodai T, et al. Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J Neurosci* 2010; **30:** 3398–408.

19 Foster DJ, Wilson JM, Remke DH, et al. Antipsychotic-like effects of M4 positive allosteric modulators are mediated by CB2 receptor-dependent inhibition of dopamine release. *Neuron* 2016; **91:** 1244–52.

20 Byun NE, Grannan M, Bubser M, et al. Antipsychotic drug-like effects of the selective M4 muscarinic acetylcholine receptor positive allosteric modulator VU0152100. *Neuropsychopharmacology* 2014; **39:** 1578–93.

21 Bubser M, Bridges TM, Dencker D, et al. Selective activation of M4 muscarinic acetylcholine receptors reverses MK-801-induced behavioral impairments and enhances associative learning in rodents. *ACS Chem Neurosci* 2014; **5:** 920–42.

22 Krystal JH, Anticevic A, Yang GJ, et al. Impaired tuning of neural ensembles and the pathophysiology of schizophrenia: a translational and computational neuroscience perspective. *Biol Psychiatry* 2017; **81:** 874–85.

23 Yang D, Günter R, Qi G, Radnikow G, Feldmeyer D. Muscarinic and nicotinic modulation of neocortical layer 6a synaptic microcircuits is cooperative and cell-specific. *Cereb Cortex* 2020; **30:** 3528–42.

24 Bodick NC, Offen WW, Levey AI, et al. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. *Arch Neurol* 1997; **54:** 465–73.

25 Shekhar A, Potter WZ, Lightfoot J, et al. Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am J Psychiatry* 2008; **165:** 1033–39.

26 Brannan SK, Sawchak S, Miller AC, Lieberman JA, Paul SM, Breier A. Muscarinic cholinergic receptor agonist and peripheral antagonist for schizophrenia. *N Engl J Med* 2021; **384:** 717–26.

27 Wood MR, Noetzel MJ, Melancon BJ, et al. Discovery of VU0467485/AZ13713945: an M4 PAM evaluated as a preclinical candidate for the treatment of schizophrenia. *ACS Med Chem Lett* 2016; **8:** 233–38.

28 Kane J, Keefe R, Walling D, et al. CVL-231: a novel positive allosteric modulator of the cholinergic M4 receptor for the treatment of schizophrenia. Schizophrenia International Research Society; April 17–21, 2021 (abstr F29).

29 Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 1987; **13:** 261–76.

30 Marder SR, Davis JM, Chouinard G. The effects of risperidone on the five dimensions of schizophrenia derived by factor analysis: combined results of the North American trials. *J Clin Psychiatry* 1997; **58:** 538–46.

31 Keefe RS, Goldberg TE, Harvey PD, Gold JM, Poe MP, Coughenour L. The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. *Schizophr Res* 2004; **68:** 283–97.

32 Hermes ED, Sokoloff D, Stroup TS, Rosenheck RA. Minimum clinically important difference in the Positive and Negative Syndrome Scale with data from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). *J Clin Psychiatry* 2012; **73:** 526–32.

33 Shimomura Y, Kikuchi Y, Suzuki T, Uchida H, Mimura M, Takeuchi H. Antipsychotic treatment strategies for acute phase and treatment resistance in schizophrenia: a systematic review of the guidelines and algorithms. *Schizophr Res* 2021; **236:** 142–55.

34 Vadiei N, Chien J, Enwereji J, Myslinski B, Guzman A. Start low, go fast? Antipsychotic titration patterns at an inpatient psychiatric hospital. *Ment Health Clin* 2020; **10:** 275–81.