Efficacy and safety of adjunctive bitopertin versus placebo in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics: results from three phase 3, randomised, double-blind, parallel-group, placebo-controlled, multicentre studies in the SearchLyte clinical trial programme

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Summary

Background Many patients with schizophrenia require high doses of medication for their ongoing psychotic symptoms. Glutamate theories and findings from studies showing efficacy of sarcosine, an endogenous, non-selective glycine-reuptake inhibitor mediated by GlyT1, offer an alternative approach. We undertook the SearchLyte trial programme to examine the efficacy of bitopertin, a selective GlyT1-mediated glycine-reuptake inhibitor, as an adjunctive treatment to ongoing antipsychotic treatment.

Methods SearchLyte consisted of three phase 3, randomised, double-blind, parallel-group, placebo-controlled, multicentre studies done in outpatient clinics in Asia, Europe, and North and South America (TwiLyte done at 109 sites, NightLyte at 84, and MoonLyte at 87). Participants were male and female outpatients, aged at least 18 years, meeting DSM-IV criteria for schizophrenia with suboptimally controlled positive symptoms despite treatment with antipsychotics. Inclusion criteria included a Positive and Negative Syndrome Scale (PANSS) total score of at least 70 and antipsychotic treatment stability for the past 12 weeks before randomisation. Key exclusion criteria included meeting criteria for symptomatic remission or previous treatment with a GlyT1 inhibitor or any other investigational drug. After a screening or 4-week prospective stabilisation period, we randomly assigned participants (1:1:1) to a 12-week, double-blind treatment of either placebo or one of two fixed doses of oral, once-daily bitopertin (10 or 20 mg in TwiLyte and NightLyte; 5 or 10 mg in MoonLyte) added to their current antipsychotic medicine. After completion of 12 weeks' treatment, the study design allowed for additional double-blind treatment for 40 weeks to assess maintenance of the effect, followed by a randomised 4-week washout period to assess withdrawal effects. Subsequently, all patients were offered the opportunity to receive bitopertin treatment in a 3-year follow-up. The primary efficacy endpoint was the mean change from baseline in the PANSS Positive Symptom Factor Score (PSFS) at week 12, analysed in the modified intention-to-treat population. The trials were registered at ClinicalTrials.gov (numbers NCT01235520 [TwiLyte], NCT01235585 [MoonLyte], and NCT01235559 [NightLyte]).

Findings Between Nov 19, 2010, and Dec 12, 2014, we randomly assigned 1794 patients to treatment, of whom 1772 were treated and analysed. MoonLyte was discontinued in September, 2014, on the basis of results from futility analyses. Across studies and treatment arms, most patients completed 12 weeks of treatment (505 in TwiLyte, 517 in NightLyte, and 506 in MoonLyte). Only one study, NightLyte, met the primary endpoint where the PANSS PSFS significantly differed from placebo at week 12, and only in the 10-mg arm: mean difference in score –1·37, 95% CI –2·27 to –0·47; p=0·0028. Improvements from baseline for the bitopertin 20-mg arm in Nightlyte were not significant compared with placebo: –3·77, 95% CI –4·40 to –3·14; p=0·3142. Results from the other two studies also did not differ from placebo (TwiLyte 0·58, 95% CI –0·34 to 1·50, p=0·22 for 10 mg and 0·43, –0·49 to 1·36, p=0·36 for 20 mg; MoonLyte 0·06, 95% CI –0·79 to 0·92, p=0·88 for 5 mg and 0·44, –0·41 to 1·28, p=0·31 for 10 mg). Placebo responses varied across studies and might have contributed to the differences in efficacy between studies. Four deaths occurred during the 12-week treatment period, three in NightLyte (upper gastrointestinal haemorrhage, alcohol poisoning and related head injury, and a completed suicide) and one in MoonLyte (myocardial infarction in a patient with pre-existing risk factors). Only the death by suicide was deemed related to the study drug. The incidence of serious adverse events was low across treatment groups in all three studies; psychiatric disorders were the most frequently reported serious adverse events and the most frequent cause of adverse events leading to discontinuation.

Interpretation Only one of six active treatment arms across the three studies offered an advantage of adjunctive bitopertin over placebo for the treatment of suboptimally controlled symptoms of schizophrenia. The small

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improvement associated with bitopertin together with the varying placebo response suggests that adjunctive bitopertin treatment might offer only modest benefit to suboptimal responders to antipsychotics, if any.

Introduction

The course and outcome of schizophrenia is hetero-geneous.[1] Thus, although a subgroup of patients have a stable course of disease and show symptomatic improvement, a substantial proportion do not have such a favourable outcome and will display continuous psychotic symptoms. Current antipsychotics are effective for only about 50% of patients,[2,3] and approximately a third of patients are thought to be resistant to antipsychotic medication.[4] However, the classical dichotomy of effective versus resistant, or responders versus non-responders, is often based on arbitrary dichotomous cutoffs in clinical trials, which do not reflect the real-world heterogeneity of schizophrenia, and provide only an incomplete description of the variation in response.[5] In reality, a large proportion of patients show only a moderate response to treatment[6] and continue to present with some psychotic symptoms. These suboptimally controlled psychotic symptoms have been associated with a higher risk of relapse and admission to hospital[7] and a significant reduction in quality of life and functioning.[8,9] The treatment of these symptoms is challenging, and many of these patients are subject to polypharmacy or to high doses of medication despite no evidence of superior efficacy with these strategies.[10,11] An alternative approach to treating these symptoms is to target a different putative mechanism, such as the glutamatergic neurotransmission.

Evidence suggests that deficient N-methyl-D-aspartate (NMDA) glutamate receptor signalling is implicated in the pathophysiology of positive and negative symptoms of schizophrenia.[12–14] Therefore, increasing NMDA receptor function via pharmacological manipulation could provide an alternative therapeutic strategy for the treatment of psychotic symptoms that have not responded to traditional antidopaminergic mechanisms. The activation of the NMDA receptor requires simultaneous binding of glutamate and glycine.[15] Since glycine acts as an obligatory co-agonist at the NMDA receptor, increased transmission through this receptor can be obtained, either directly via a glycine agonist or indirectly through an increase in synaptic glycine concentrations. The increase in glycine can be achieved by inhibition of the glycine transporters, which regulate glycine concentrations by mediating its reuptake from the synaptic cleft. Findings from adjunctive studies[16–19] with glycine and D-serine, co-agonists at the NMDA

Research in context

Evidence before this study

Approximately 15–39% of patients with schizophrenia have suboptimally controlled psychotic symptoms that have a significant negative effect on their quality of life and functioning. We searched PubMed for English-language clinical studies of augmentation of antipsychotic treatment in schizophrenia published up to Dec 31, 2009. The search terms were "augmentation", "adjunctive antipsychotic", "partial", "suboptimal response", "NMDA", "glycine", and "glutamate". We found published reports of previous studies with adjunctive glycine and D-serine, co-agonists at the NMDA receptor glycine site, and sarcosine, a glycine-reuptake inhibitor that gave promising results in various symptom domains in patients with both acute schizophrenia exacerbation and stable schizophrenia. We repeated the literature search in May, 2016, for the update on adjunctive studies with glycine-reuptake inhibitors. Efficacy of adjunctive sarcosine in treatment of schizophrenia was shown, although not unequivocally and not in all schizophrenia domains. Some methodological limitations, mostly small sample sizes and too short durations for some studies, preclude the final assessment of this treatment strategy.

Added value of this study

We undertook the SearchLyte clinical trial programme, the first and largest of its kind, to examine the use of an adjunctive treatment with glycine-reuptake inhibitors.

The programme consisted of three phase 3, multicentre, randomised, double-blind, parallel-group, placebo-controlled 12-week studies that investigated the efficacy and safety of bitopertin, a selective glycine-reuptake inhibitor, in patients with schizophrenia with suboptimally controlled symptoms who were being treated with antipsychotics. The results from only one of these studies showed a significant difference between bitopertin (10 mg per day) and placebo for both the primary and key secondary endpoints. Bitopertin was safe and well tolerated.

Implications of all the available evidence

After the SearchLyte programme, new guidelines were based on the programme design proposed by Roche at a meeting with the European Medicines Agency in 2011, when they endorsed the indication and the design and then updated their guidelines in 2012, which we were a part of as reviewers. In our studies, we noted that the addition of bitopertin to current antipsychotics offered no clinical advantage over placebo for the treatment of suboptimally controlled psychotic symptoms. Nevertheless, the encouraging evidence from studies with sarcosine and the positive outcome in one of the bitopertin groups in one of our studies warrant an independent, well designed study to assess the therapeutic benefits of augmentation strategies with glycine-reuptake inhibitors in the treatment of schizophrenia.

receptor glycine site, and sarcosine, an endogenous GlyT1-mediated glycine-reuptake inhibitor, were effective in various schizophrenia domains, supporting this approach. Bitopertin, a selective glycine-reuptake inhibitor, has shown antipsychotic-like activity in modulating both glutamatergic and dopaminergic neurotransmission in animal models[20] of schizophrenia. In animals and human individuals, a linear, dose-dependent increase in glycine concentrations occurred in cerebrospinal fluid after bitopertin administration.[21] Target engagement was confirmed in imaging studies in rats and baboons when binding of the selective glycine-reuptake inhibitor radioligand [3H]RO5013853 was blocked in animals pretreated with bitopertin in a dose-dependent fashion in the brain areas with highest GlyT1 expression.[22] Similarly, PET studies of the same radioligand in healthy human individuals investigated the relationship between the plasma concentration of bitopertin and brain GlyT1 occupancy to support dose selection—bitopertin plasma concentration was a reliable predictor of occupancy because the concentration–occupancy relationship was superimposable at steady state and 2 days after drug discontinuation.[23] Although findings from a recent phase 2 trial[24] showed the efficacy of bitopertin added to antipsychotics in patients with predominant negative symptoms, positive symptoms also improved (with bitopertin 10 mg and 30 mg) in a subgroup of patients with a median score equal to or greater than 18 on Positive and Negative Syndrome Scale Positive Symptom Factor Score (PANSS PSFS) at baseline.[24] Subsequently, it was expected that bitopertin would be efficacious in treatment of suboptimally controlled positive symptoms of schizophrenia when added to antipsychotics. Up to now, no similar trials of this size have been done that applied comprehensive measures to assess the clinical efficacy and safety of an adjunctive glycine-reuptake inhibitor in patients with stable schizophrenia with ongoing symptoms.

This Article reports the results of the first 12 weeks of the double-blind treatment of three studies (the SearchLyte trial programme) designed to assess the efficacy and safety of adjunctive treatment with bitopertin (5, 10, and 20 mg) in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics.

Methods

Study design and participants

SearchLyte consisted of three phase 3, randomised, double-blind, parallel-group, placebo-controlled, multi-centre studies (TwiLyte, MoonLyte, and NightLyte), done in outpatient clinics in Asia, Europe, and North and South America. NightLyte was done in 84 centres in Japan, the USA, China, Bulgaria, Czech Republic, Italy, and Russia; MoonLyte in 87 centres in the USA, Canada, Brazil, Poland, Spain, Chile, Lithuania, Slovakia, Taiwan, Turkey, Germany, Latvia, and the Netherlands; and in 109 centres in the USA, Russia, India, Argentina, Romania, South Korea,

Hungary, France, Mexico, Colombia, Sweden, the UK, and Australia. Before study initiation, the protocols were approved by local independent ethics committees or institutional review boards.

All participants were male and female outpatients aged at least 18 years meeting DSM-IV criteria for schizophrenia per structured clinical interview with suboptimally controlled positive symptoms despite treatment with antipsychotic agents (table 1).[25] Key inclusion criteria were: a Positive and Negative Syndrome Scale (PANSS) total score of at least 70; a score of 4 or more (moderate) on at least two of any of the following PANSS items: delusions, hallucinatory behaviour, suspiciousness, and unusual thought content; at least moderately ill as defined by the Clinical Global Impression–Severity (CGI-S) scale of positive symptoms with a score of at least 4; clinical stability for 16 weeks (4 months) before randomisation; and antipsychotic treatment stability for 12 weeks before

	Key inclusion criteria	Key exclusion criteria
Neuropsychiatric status		
PANSS	A score of ≥4 on at least two of: P1 (Delusions) P3 (Hallucinatory behaviour) P6 (Suspiciousness) G9 (Unusual thought content) A total PANSS score of ≥70	Met criteria for remission defined as: PANSS scores of ≤3 (mild or less) on all of the following items:[25] P1 (Delusions) P2 (Conceptual disorganisation) P3 (Hallucinatory behaviour) G5 (Mannerisms or posturing) G9 (Unusual thought content) N1 (Blunted affect) N4 (Social withdrawal) N6 (Lack of spontaneity)
CGI-S	A positive symptom score of ≥4 (at least moderately ill)	..
Treatment-resistant schizophrenia	..	Has treatment-resistant schizophrenia as judged by the treating physician or has failed two trials, meeting all of the following criteria: 6 weeks' duration of each trial Two different classes of antipsychotics Antipsychotic doses were ≥600 mg/day chlorpromazine or ≥6 mg/day risperidone equivalents No clinically significant response and absence of good social and occupational functioning in past 5 years
Retrospective antipsychotic treatment stability	12 weeks before randomisation	..
Medication		
Antipsychotics	A maximum of two antipsychotics where the sum of primary and secondary antipsychotics was ≤6 mg of risperidone equivalents or 600 mg of chlorpromazine equivalents, respectively	Current clozapine treatment Treatment with clozapine in the 6 months before randomisation
Medical status		
Haemoglobin concentration (g/L)	..	<120 g/L

PANSS=Positive and Negative Syndrome Scale. CGI-S=Clinical Global Impression–Severity scale.

Table 1: Key inclusion and exclusion criteria for the TwiLyte, MoonLyte, and NightLyte studies
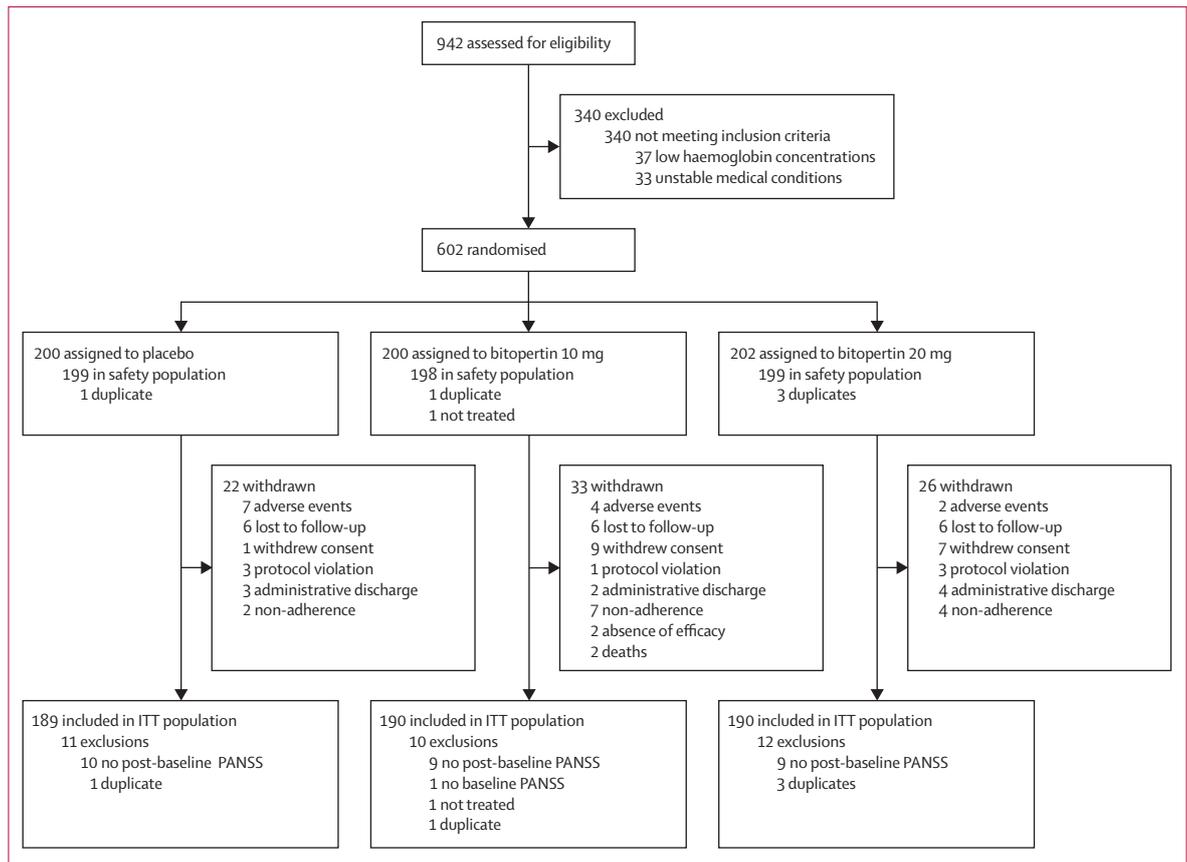
Figure 1: Trial profile (NightLyte)
ITT=intention-to-treat. Note that for the numbers given for exclusions from the ITT population, some participants had more than one reason.

randomisation. Clinical stability was defined by no signs of exacerbation of schizophrenia (no admissions to hospital) or incarceration in prison, and no evidence of an increased level of psychiatric care (including cognitive behaviour rehabilitation or individual psychotherapy) in the 16 weeks before randomisation, and no change in antipsychotic dosing supported by documentation for the 12 weeks before randomisation. Adjunctive anticholinergics, anxiolytics, β blockers, and sodium valproate (from mood stabilisers) were allowed provided that the current dose was unchanged for the 12 weeks before randomisation. Key exclusion criteria were: meeting criteria for symptomatic remission;[25] previous treatment with a GlyT1 inhibitor or any other investigational drug; electroconvulsive therapy; concurrent or past treatment with clozapine (within 6 months before randomisation); maximum antipsychotic dose more than 6 mg/day of risperidone equivalents or more than 600 mg/day of chlorpromazine equivalents; and a blood haemoglobin concentration of less than 120 g/L or a history of haemoglobinopathy (table 1; full inclusion and exclusion criteria and antipsychotic conversion table guidance are in the appendix). Participants were mostly patients who were being seen at the outpatient clinics (and invited to

participate); some were recruited via advertisement. Participants gave written informed consent.

Randomisation and masking
Each study comprised three treatment arms. Placebo and a bitopertin dose of 10 mg, previously shown to be efficacious in negative symptoms of schizophrenia in per-protocol populations,[24] were common for all three studies. Improvement of negative symptoms with a higher dose in a phase 2 study[24] and model-based simulation of the relationship between the dose and efficacy suggested a maximal effect around 20 mg. This supported the choice of a bitopertin 20-mg dose for two of the studies (TwiLyte and NightLyte). Similarly, based on the efficacy exposure relationship, bitopertin 5 mg was tested in the MoonLyte study as the minimal effective dose. Therefore, after a screening or 4-week prospective stabilisation period, we randomly assigned patients (1:1:1) to a 12-week, double-blind treatment of either placebo or one of the two fixed doses of oral, once-daily bitopertin (bitopertin 10 or 20 mg in TwiLyte and NightLyte; bitopertin 5 or 10 mg in MoonLyte) added to their current antipsychotic medicine.

Bitopertin and placebo tablets were matched in shape, taste, and colour to ensure that the investigators, patients,
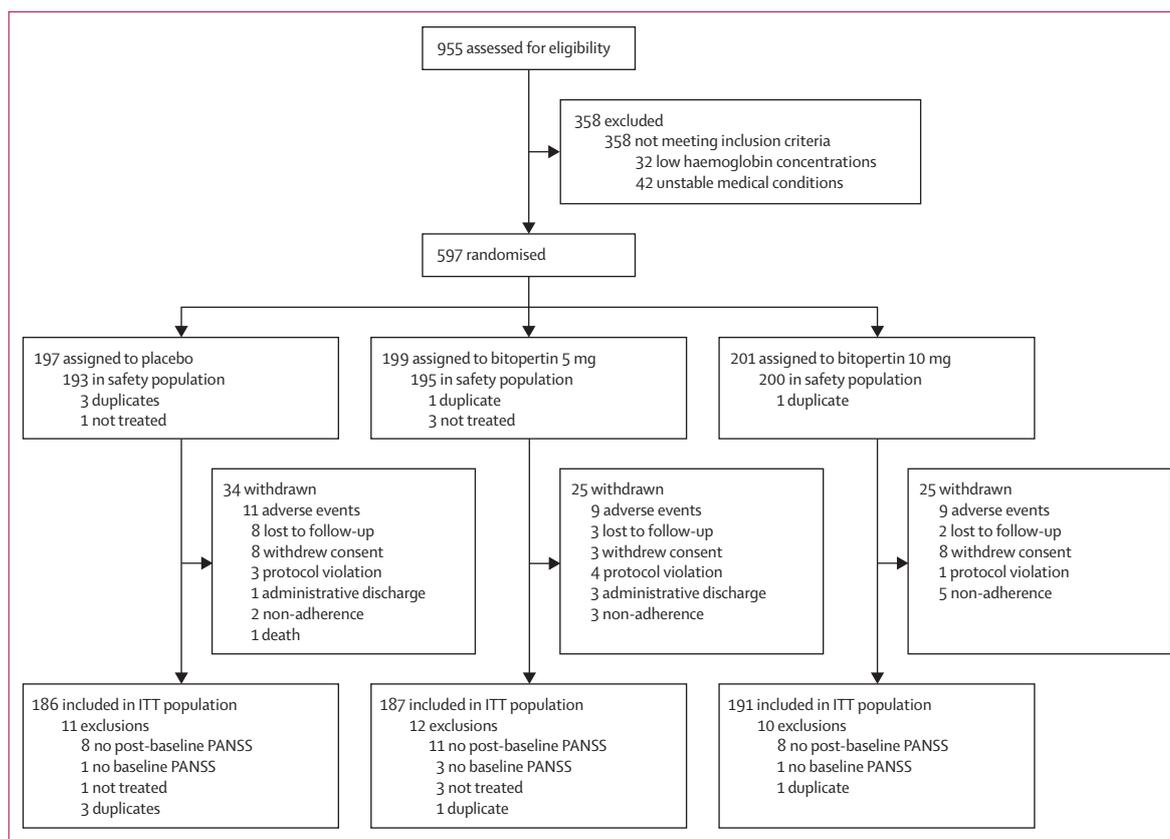
Figure 2: Trial profile (MoonLyte)
ITT=intention-to-treat. Note that for the numbers given for exclusions from the ITT population, some participants had more than one reason.

and Roche remained masked to the study treatments. We did the randomisation centrally through the use of a voice or web interactive system. Treatment was allocated by stratified block randomisation with the following three factors: (1) geographical region (North America, eastern Europe, western Europe, China [NightLyte only], Japan [NightLyte only], and others); (2) type of background antipsychotic medication at randomisation (typical or atypical); and (3) age (≤65 years or >65 years). The password-protected and encrypted electronic master randomisation list was kept by a clinical supply department in a secure system. The study centres, Roche monitors, project statisticians, or other Roche team members or designees had no access to the code. Adherence was assessed based on the quantity of the study drug dispensed and the quantity returned at each visit.

Procedures

The assigned study treatment, added to current antipsychotic, was taken orally, once daily.

At baseline and week 12, we measured the PANSS PSFS, the PANSS total score, PANSS subscores and factor scores, the CGI-S and Clinical Global Impression scales of improvement (CGI-I) of positive and overall symptoms, and the change in the Personal and Social Performance (PSP) scale total score. In an exploratory biomarker discovery programme, we analysed serum samples from patients who gave consent in the phase 2 study[24] for a few proteins that were then assessed for potential correlation with treatment response to bitopertin. Complement factor H-related protein 1 (CFHR1) was identified as a candidate predictive biomarker, because patients from the phase 2 study with high concentrations of CFHR1 seemed to have a greater magnitude of response to bitopertin. Given that the expected proportion of patients who carried this marker was 55% to 70%, we also assessed PANSS PSFS in the subpopulation of patients with a high CFHR1 serum concentration at baseline. Additionally, we assessed clinical response at 12 weeks using four definitions based on reports in the scientific literature:[26] (1) an improvement of 20% or more from baseline in PANSS PSFS, (2) improvements rated much or very much on the CGI-I overall, (3) improvements rated much or very much on the CGI-I positive symptoms, and (4) an improvement of 20% or more from baseline in PANSS PSFS and improvements rated much or very much on the CGI-I positive symptoms.

All efficacy and safety assessments were obtained by the site raters at baseline and at each visit every 4 weeks.
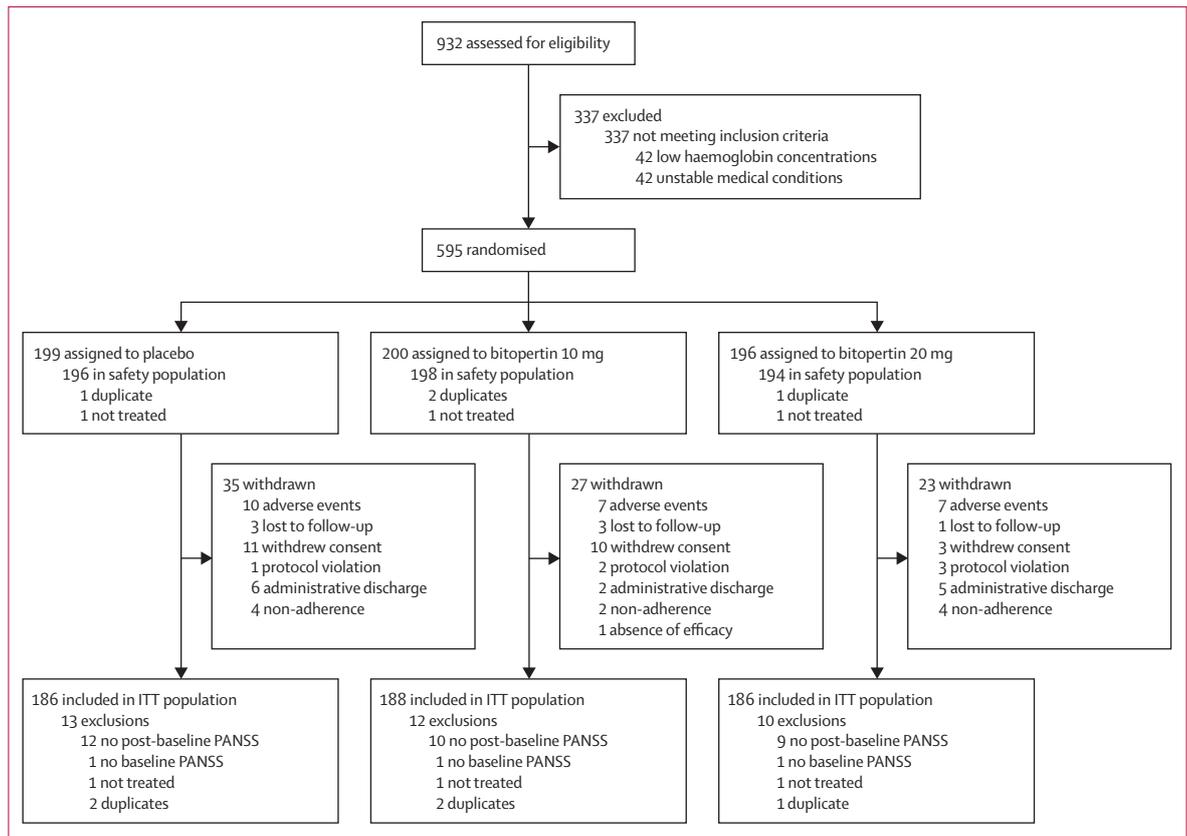
Figure 3: Trial profile (TwiLyte)

ITT=intention-to-treat. Note that for the numbers given for exclusions from the ITT population, some participants had more than one reason.

Comprehensive rater training consisting of online assessment modules, interview skills and scoring assessments, and an in-study assessment programme comprising Video Enhancement of Rater Interviewing for Independent Evaluation of Data (VERIFIED) to identify discrepant scoring and unusual scoring patterns. Site data monitoring was used to ensure quality of efficacy assessments, reduce variability, and ensure consistency across the sites. Inter-rater reliability was done for the PANSS scale and two PANSS subscales created based on Marder factor analysis, namely PANSS Marder Negative Factor and PANSS Marder Positive Factor calculating κ statistics for active raters. Overall kappa was excellent (for 195 active NightLyte raters, the overall κ was 0·891; for 208 active MoonLyte raters, the overall κ was 0·897; and for 220 active TwiLyte raters, the overall κ was 0·883; rater surveillance methodology details are in the appendix).

After completion of 12 weeks' treatment (pivotal part), the study design allowed for additional double-blind treatment for 40 weeks to assess maintenance of the effect, followed by a randomised 4-week washout period to assess possible withdrawal effects. Subsequently, all patients were offered the opportunity to receive bitopertin treatment in a 3-year, long-term extension follow-up (appendix).

Outcomes

The primary efficacy endpoint was the mean change from baseline in the PANSS PSFS at week 12. Secondary endpoints assessed at week 12 included the mean change from baseline in PANSS total score, PANSS subscores and factor scores, the change in CGI-S and CGI-I of positive and overall symptoms, and the change in PSP scale total score. The mean change from baseline in the PANSS PSFS in the subpopulation of patients with a high CFHR1 serum concentration was added as a key secondary measure, along with clinical response.

We assessed safety and tolerability through reporting of adverse events, measurement of clinical laboratory variables, electrocardiograms, vital signs, and physical and neurological examinations. We assessed the development or worsening of motor symptoms using the abbreviated Extrapyramidal Symptom Rating Scale-A (ESRS-A). We used the Columbia Suicide Severity Rating Scale (C-SSRS) to assess suicidal ideation and behaviour during the study. Treatment was to be withdrawn for patients with a confirmed haemoglobin concentration of less than 100 g/L or a confirmed decrease of 25% or more in haemoglobin concentrations from baseline at any time during the study. An external independent data monitoring

	TwiLyte			MoonLyte			NightLyte		
	Placebo (n=196)	Bitopertin 10 mg once a day (n=198)	Bitopertin 20 mg once a day (n=194)	Placebo (n=193)	Bitopertin 5 mg once a day (n=195)	Bitopertin 10 mg once a day (n=200)	Placebo (n=199)	Bitopertin 10 mg once a day (n=198)	Bitopertin 20 mg once a day (n=199)
Sex									
Male	123 (63%)	129 (65%)	121 (62%)	133 (69%)	124 (64%)	145 (73%)	134 (67%)	113 (57%)	114 (57%)
Race									
White	114 (58%)	114 (58%)	112 (58%)	128 (66%)	126 (65%)	139 (70%)	79 (40%)	77 (39%)	83 (42%)
Black	30 (15%)	29 (15%)	29 (15%)	42 (22%)	51 (26%)	39 (20%)	30 (15%)	33 (17%)	30 (15%)
Asian	40 (20%)	44 (22%)	36 (19%)	9 (5%)	6 (3%)	11 (6%)	86 (43%)	86 (43%)	85 (43%)
Other	12 (6%)	11 (6%)	17 (9%)	14 (7%)	12 (6%)	11 (6%)	4 (2%)	2 (1%)	1 (<1%)
Age (years)	39·8 (12·2)	40·8 (11·4)	41·4 (11·6)	41·9 (12·3)	43·4 (12·2)	40·7 (12·6)	39·7 (12·7)	40·2 (12·4)	39·1 (12·2)
PANSS Positive Symptom Factor Score	18·63 (3·93)	19·15 (3·61)	19·40 (4·10)	19·10 (3·99)	19·08 (4·13)	19·26 (3·58)	19·35 (3·98)	19·27 (4·23)	19·13 (4·15)
PANSS total	55·95 (10·23)	55·89 (9·85)	57·34 (10·49)	56·55 (10·56)	56·92 (11·12)	(n=199) 56·30 (11·32)	58·09 (11·39)	57·56 (11·53)	57·48 (11·43)
CGI-S positive score	4·41 (0·56)	4·41 (0·56)	4·49 (0·60)	4·49 (0·56)	4·51 (0·57)	4·53 (0·62)	4·60 (0·60)	4·49 (0·56)	4·58 (0·64)
CGI-S overall	4·32 (0·55)	4·32 (0·56)	4·40 (0·65)	4·44 (0·58)	4·47 (0·62)	4·47 (0·66)	4·55 (0·62)	4·45 (0·55)	4·57 (0·66)
Primary antipsychotics	(n=194)	(n=196)	(n=189)	(n=192)	..	..	(n=198)	(n=196)	(n=197)
Atypical	161 (83%)	159 (81%)	154 (81%)	159 (83%)	155 (79%)	159 (80%)	181 (91%)	171 (87%)	174 (88%)
Typical	33 (17%)	37 (19%)	35 (19%)	33 (17%)	40 (21%)	41 (21%)	17 (9%)	25 (13%)	23 (12%)

Data are n (%) or mean (SD). For all analyses of PANSS data, the scores were transformed into 0–6 points to express absence as 0 instead of 1–7. Positive Symptom Factor Score conversion: displayed baseline value 8 points (eight PANSS items); PANSS total score conversion: displayed baseline value 30 points (30 PANSS items). PANSS=Positive and Negative Syndrome Scale. CGI-S=Clinical Global Impression–Severity.

Table 2: Demographic and baseline disease characteristics of the TwiLyte, MoonLyte, and NightLyte studies (safety population)

committee reviewed the safety data throughout the programme duration.

Statistical analysis

We selected a sample size of 200 patients per treatment arm (a total of 600 per study) for the primary efficacy variable to achieve 80% power at the α level of 0·025 (two-sided) to maintain an overall type I error rate of 0·05 (two-sided), adjusting for multiple comparisons for each treatment group against the placebo group. The power calculation was based on simulations of the mixed-effects model repeated measures (MMRM) analysis planned for the primary efficacy variable and included three post-baseline visits, an assumed treatment difference (SD) of 2·1 (6·0) at 12 weeks with increasing magnitude of treatment difference over the 12-week period, and an overall dropout rate of 25%. We analysed each study separately.

All of the efficacy endpoints were analysed using the modified intention-to-treat (ITT) population, which included all randomly assigned patients who received at least one dose of bitopertin and had at least one post-baseline assessment of the primary efficacy variable. For the assessment of differences in the mean change from baseline in PANSS PSFS at week 12 between each group receiving bitopertin or placebo treatment, we used an MMRM analysis with an unstructured variance–covariance matrix. The model included independent variables of randomised treatment, assessment week relative to randomisation (time), and treatment by time interaction as factors, and the baseline PANSS PSFS as a covariate. Time was treated as a repeated variable within the patient. We also assessed efficacy in the CFHR1-high subgroup using the same model. For all PANSS data analysis, scores were transformed from a 1–7 point scale into a 0–6 point scale, with 0 expressing absence and 6 extreme. Additionally, we did a preplanned subgroup analysis of the primary efficacy endpoint in the ITT population according to race, region, sex, and primary antipsychotic.

We analysed prespecified dichotomous secondary endpoints using the Cochran-Mantel-Haenszel (CMH) test including the randomisation stratification factors as stratification variables for the analysis (geographical region of the study centres, type of primary antipsychotic background medication at randomisation, and age group). To control the type I error for multiple testing of the primary endpoint, we used a two-stage gatekeeping approach. Multiple testing adjustments were not applied to the secondary efficacy endpoints. Unadjusted p values are reported in this paper.

For the biomarker subgroup analysis, patients were retrospectively stratified to one of two subgroups according to their CFHR1 serum concentration at baseline: CFHR1—high (patients with CFHR1 serum concentration ≥11·07 µg/mL) and CFHR1-low (patients with CFHR1 serum concentration <11·07 µg/mL).
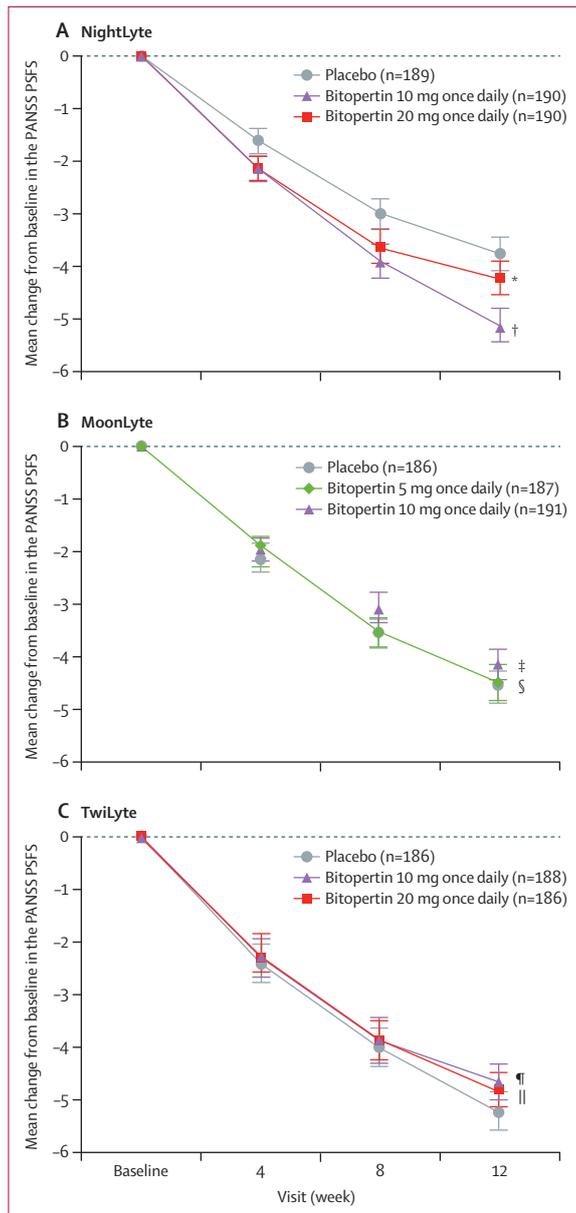
Figure 4: **Change in PANSS PSFS score from baseline to week 12 (ITT population)**
Data are adjusted mean (SE) by visit based on mixed-effects model repeated measure analysis. PANSS scores were transformed from a 1–7 point into a 0–6 point scale, with 0 expressing absence and 6 extreme. ITT=intention to treat. PANSS PSFS=Positive and Negative Syndrome Scale Positive Symptom Factor Score. *p=0·3142 bitopertin 20 mg once daily versus placebo. †p=0·0028 bitopertin 10 mg once daily versus placebo. ‡p=0·31 bitopertin 10 mg once daily versus placebo. §p=0·88 bitopertin 5 mg once daily versus placebo. ¶p=0·22 bitopertin 10 mg once daily versus placebo. ||p=0·36 bitopertin 20 mg once daily versus placebo.

CFHR1 was measured using the Roche Diagnostic Elecsys CFHR1 assay. We identified the cutoff by measuring the baseline concentration of CFHR1 in 216 patients enrolled into the phase 2 study who consented to the collection and use of serum samples for the exploratory biomarker analysis, and for whom an untouched aliquot of the serum sample from the baseline visit was available. The natural CFHR1 cutoff was identified as 11·07 µg/mL, which separated patients into two groups: those without a deletion of CFHR1 and patients with a heterozygous deletion of CFHR1.

We analysed demographic characteristics, baseline disease characteristics, and safety in the safety population, which included all patients who received at least one dose of bitopertin. Safety variables, including exposure to study drug, adverse events, laboratory tests, and vital signs, were summarised by treatment group using descriptive statistics. The studies were registered on ClinicalTrials. gov (numbers NCT01235520 [TwiLyte], NCT01235585 [MoonLyte], and NCT01235559 [NightLyte]).

Role of the funding source
The funder of the study had a role in study design, study execution, data collection, data analysis, data interpretation, and preparation, writing, review, and approval of the report, and the decision to submit for publication. The corresponding author had full access to all the data in the study.

Results
Between Nov 19, 2010, and Dec 12, 2014, we randomly assigned 1794 patients to treatment, of whom 1772 were treated and analysed (figures 1–3). MoonLyte was discontinued in September, 2014, based on results from futility analyses (because there were no immediate safety concerns, the sites were allowed to terminate over a 4–6 week period depending on the timing of the last visit before the decision to stop was communicated). Across studies and treatment arms, most patients completed 12 weeks of treatment (>80% of patients in each group; 505 in TwiLyte, 506 in MoonLyte, and 517 in NightLyte; figure 1).

There were slightly more female patients than male patients in the bitopertin arms in NightLyte compared with the other two studies (table 2). Because NightLyte was done in seven countries with many sites in China and Japan, a higher percentage (about 40%) of patients were Asian compared with the other two studies.

For the primary outcome of the mean change from baseline in PANSS PSFS at week 12, a significant difference from placebo was noted only in the bitopertin 10-mg arm in NightLyte (mean difference in score –1·37, 95% CI –2·27 to –0·47, p=0·0028; figure 4; appendix).This study showed the least improvement in adjusted means in the placebo arm (–3·77, 95% CI –4·40 to –3·17) compared with the response in the placebo arms in MoonLyte (–4·59, –5·20 to –3·98) and TwiLyte (–5·25, –5·90 to –4·59; appendix). Improvements from baseline in PANSS PSFS for the bitopertin 20-mg arm in NightLyte, were of smaller magnitude (–4·22, 95% CI –4·85 to –3·60) compared with bitopertin 10 mg (–5·14, –5·78 to –4·50) and were not significant compared with placebo: –3·77,–4·40 to –3·14; p=0·3142 (appendix). PANSS PSFS scores at week 12

from the other two studies also did not differ from placebo (TwiLyte 0·58, 95% CI –0·34 to 1·50, p=0·22 for 10 mg and 0·43, –0·49 to 1·36, p=0·36 for 20 mg; MoonLyte 0·06, 95% CI –0·79 to 0·92, p=0·88 for 5 mg and 0·44, –0·41 to 1·28, p=0·31 for 10 mg; figure 4).

Consistent with the results in the PANSS PSFS, the PANSS total score was only significantly different from placebo at 12 weeks in the bitopertin 10-mg arm in NightLyte (–2·79, 95% CI –5·17 to –0·41, p=0·0217; figure 5, appendix). Significant treatment effects in PANSS PSFS in the CFHR1-high subgroup occurred only in the bitopertin 10-mg group in NightLyte (–1·58, 95% CI –2·72 to –0·44; p=0·0069 compared with placebo) and not in the 20-mg group (–0·06, –1·16 to 1·03, p=0·9099). This effect was not significant after adjustment for multiple testing (appendix).

Improvements in PANSS total scores in the other treatment arms across all three studies were not significant (appendix). Prespecified subgroup analyses of the primary endpoint in the ITT population by sex, primary antipsychotic type, race, or geographical region did not reveal any consistent differences between either doses of bitopertin compared with placebo (appendix).

Improvements in PSP total scores in all treatment arms across all three studies were noted (mean difference from baseline in PSP total score in the bitopertin 10-mg arm in the NightLyte study 1·80, 95% CI 0·00 to 3·60, p=0·0505) but were not significantly different from placebo (appendix). A significantly greater percentage of patients treated with bitopertin 10 mg in the NightLyte study had a clinical response (≥20% improvement from baseline in PANSS PSFS) compared with placebo (53% vs 45·0%, p=0·0387; appendix). By contrast, rates of clinical response in the other treatment arms across all three studies were noted but were not significant compared with placebo (MoonLyte 51·3% vs 53·8%, p=0·6230; TwiLyte 51·1% vs 52·2%, p=0·7585) for the 10-mg dose versus placebo, respectively (appendix). There was no significant difference in clinical response rates between the bitopertin treatment arms and placebo in all three studies as assessed by the other definitions of clinical response (appendix).

Overall, bitopertin 5, 10, and 20 mg once daily over the 12-week treatment period were generally well tolerated when added to atypical and typical antipsychotics (table 3). Four deaths occurred during the 12-week treatment period, three cases in NightLyte (all receiving bitopertin 10 mg: due to upper gastrointestinal haemorrhage caused by a duodenal ulcer, alcohol poisoning and related head injury, and a completed suicide, the death by suicide being the only one deemed related to the study drug by the principal investigator) and one in MoonLyte (a myocardial infarction in a patient with pre-existing risk factors receiving placebo).

The incidence of serious adverse events was low across treatment groups in all three studies and higher in the placebo arms of TwiLyte and MoonLyte, and similar
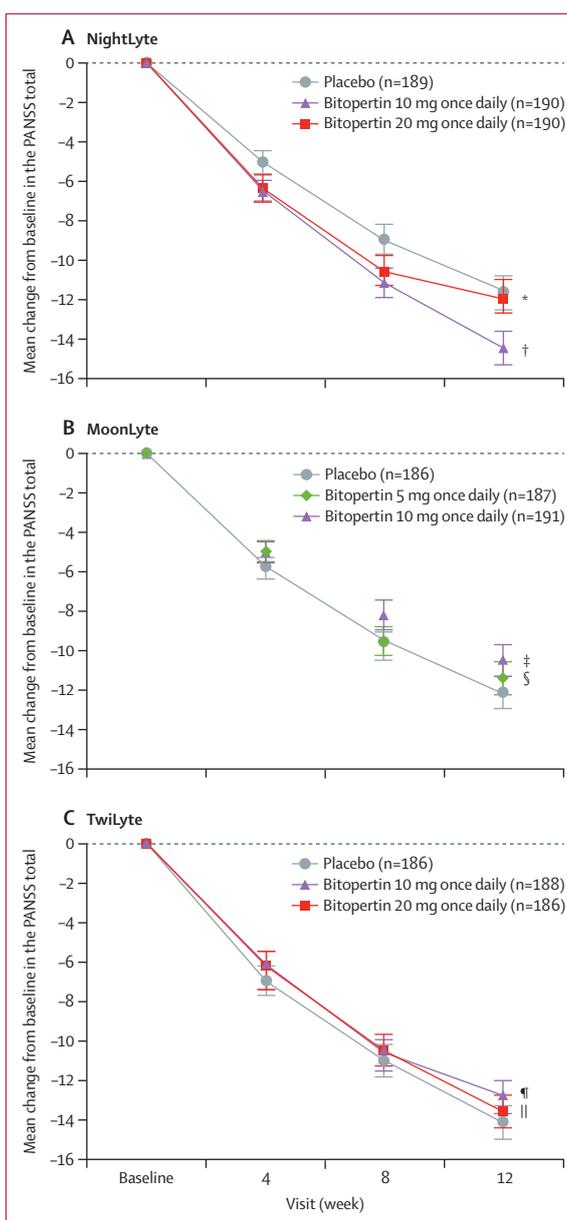

Figure 5: Change in PANSS total score from baseline to week 12 (ITT population)
Data are adjusted mean (SE) by visit based on mixed-effects model repeated measure analysis. PANSS scores were transformed from a 1–7 point into a 0–6 point scale, with 0 expressing absence and 6 extreme. ITT=intention to treat. PANSS=Positive and Negative Syndrome Scale. *p=0·90 bitopertin 20 mg once daily versus placebo.†p=0·02 bitopertin 10 mg once daily versus placebo. ‡p=0·16 bitopertin 10 mg once daily versus placebo. §p=0·55 bitopertin 5 mg once daily versus placebo. ¶p=0·28 bitopertin 10 mg once daily versus placebo. ||p=0·65 bitopertin 20 mg once daily versus placebo.

among the three arms of NightLyte (appendix). Psychiatric disorders were the most frequently reported serious adverse event and the most frequent cause of adverse events leading to discontinuation. There were also no differences in the incidence of extrapyramidal symptoms between bitopertin and placebo (less than 2% across all

	TwiLyte			MoonLyte			NightLyte		
	Placebo (n=196)	Bitopertin 10 mg once a day (n=198)	Bitopertin 20 mg once a day (n=194)	Placebo (n=193)	Bitopertin 5 mg once a day (n=195)	Bitopertin 10 mg once a day (n=200)	Placebo (n=199)	Bitopertin 10 mg once a day (n=198)	Bitopertin 20 mg once a day (n=199)
Number of adverse events	133	114	114	145	152	146	104	117	124
Number of patients with at least one adverse event	68 (35%)	60 (30%)	69 (36%)	86 (45%)	83 (43%)	75 (38%)	59 (29·6%)	70 (35%)	68 (34%)
Number of serious adverse events	7	3	1	7	4	6	1	2	2
Number of patients with at least one serious adverse event	7 (4%)	3 (2%)	1 (<1%)	7 (4%)	4 (2%)	6 (3%)	1 (0·5%)	2 (1%)	2 (1%)
Number of patients with at least one adverse event leading to withdrawal	11 (6%)	10 (5%)	7 (4%)	11 (6%)	9 (5%)	10 (5%)	7 (3·5%)	5 (3%)	5 (3%)
Psychiatric disorder adverse events leading to withdrawal	5 (3%)	5 (3%)	2 (1%)	7 (4%)	5 (3%)	5 (3%)	5 (2·5%)	1 (<1%)	4 (2%)

Data are n (%) and include adverse events with onset or worsening from the day of first dose of study drug up to week 12, or the last dose day plus 1 day for patients who withdrew before week 12. The investigator text for adverse events was encoded using MedDRA version 16.1.

Table 3: Overall adverse event profile up to week 12 in the TwiLyte , MoonLyte, and NightLyte studies (safety population)

three studies). No adverse effects were noted regarding vital signs, metabolic syndrome, or weight.

Consistent with bitopertin's mode of action, and as expected based on results from previous studies,[24,27–30] a dose-dependent gradual reduction from baseline in mean haemoglobin concentrations occurred in the bitopertin groups through week 16, with the greatest decreases in the bitopertin 20-mg group. After week 16 (ie, beyond 120 days of the erythrocyte lifecycle), haemoglobin concentrations gradually recovered. The proportions of patients with decreases in haemoglobin of more than 10 g/L and more than 20 g/L were greater in the bitopertin groups than in the placebo group at visits up to week 52. In NightLyte, a confirmed haemoglobin concentration of less than 100 g/L or a confirmed decrease of 25% or more from baseline was noted in eight patients (4%) in the bitopertin 20-mg group during the period from baseline to week 52. Of these eight patients, four were withdrawn from study treatment and reported as adverse events according to the protocol. In MoonLyte between baseline and week 52 for this safety variable, one alert (0·5%) occurred for one patient in the placebo group, four alerts (2·2%) for two patients in the bitopertin 5-mg group, and four alerts (2·1%) for one patient in the bitopertin 10-mg group. Three patients were then withdrawn from the study, while the fourth stayed at the discretion of the investigator. In TwiLyte this safety signal was noted in six patients, two (1%) in the placebo group, one (<1%) in the bitopertin 10-mg group, and three (2%) in the bitopertin 20-mg group. All six patients were withdrawn from the study. There were no associated clinical symptoms reported in any of the withdrawn subjects across all studies.

Discussion

Despite encouraging evidence from previous studies,[24] only one out of six active treatment arms of bitopertin across three large, multicentre, randomised, double-blind, placebo-controlled trials offered significant improvement over placebo in suboptimally controlled psychotic symptoms of schizophrenia. On the basis of these data, to conclude whether bitopertin is efficacious in schizophrenia patients with suboptimally controlled symptoms is impossible. In an attempt to uncover the reasons for the apparent absence of efficacy of bitopertin, all data were reviewed and analysed.

Bitopertin is a glycine reuptake inhibitor proposed to mediate its effects by enhancing the NMDA receptor hypofunction that is thought to underlie the pathophysiology of schizophrenia. The mixed results from our study throw into question the assumption derived from preclinical studies[20,23,31] and an earlier adjunctive phase 2 study,[24] which suggested that the increased amounts of glycine mediated by bitopertin might enhance NMDA transmission and provide an additional pathway to treat patients with schizophrenia. Nevertheless, findings from previous small-scale studies showed significant improvements in positive symptoms when D-serine and D-alanine were added to antipsychotics.[15,18,19] Furthermore, the largest and most consistent improvement occurred with the non-selective GlyT1 inhibitor sarcosine. The adjunctive 2-g/day sarcosine treatment led to improvement of overall symptoms and functioning that was superior to both the placebo and adjunctive D-serine in 60 patients with chronic schizophrenia treated for 6 weeks.[32] A similar trend in the reduction of positive symptoms, although limited by a small number of patients, occurred in a study by Amiaz and colleagues.[33] Strzelecki and colleagues in another study[34] noted an improvement in PANSS total over 6 months of –13·7 points compared with placebo –1·8 (p=0·00487) when antipsychotic treatment was augmented with 2 g/day of sarcosine. The sample size was restricted to 50 patients. By contrast, in a different study, the improvement in overall clinical symptoms with adjunctive sarcosine was similar to placebo, although this study enrolled a chronic, older, less treatment-responsive population.[35] The only evidence of promising treatments based on glycine-reuptake inhibitors in schizophrenia in

our programme came from the NightLyte study, in which bitopertin 10 mg significantly reduced the PANSS PSFS and PANSS total score compared with placebo. However, this result was not supported by results in the TwiLyte and MoonLyte studies.

We have considered a few possible reasons for the differences in the outcomes between the studies. Placebo responses varied across studies and might have contributed to the differences in efficacy between studies. The NightLyte study showed the least improvement in the placebo arm, compared with MoonLyte and TwiLyte, thus reducing the likelihood of separation of placebo from bitopertin in two of the three SearchLyte trials. A similar outcome was reported in three add-on studies[29,30] involving 1867 patients with schizophrenia, in which bitopertin showed no significant improvement on the negative symptoms of schizophrenia after 24 weeks when added to conventional antipsychotic medication. These results were also accompanied by a large placebo effect, as were findings from a previous bitopertin monotherapy study in acute exacerbation of schizophrenia,[36] where the active comparator (olanzapine) also failed to separate from placebo, limiting the interpretability of the study. This finding is also consistent with results from a phase 3 trial[37] with another glycine transporter inhibitor (Org 25935), where results with two doses of the agent did not differ from placebo. In the absence of an established active positive control in the negative symptom indication, to what extent the high placebo response might have contributed to the absence of significant findings with Org 25935 is unclear.[37] A strong placebo response is not uncommon in clinical trials in patients with schizophrenia. Depending on the definition of response (≥20%, ≥30%, or ≥50% improvement), it ranges from 0 to 41%, with an average response rate of 25%.[38] In comparison, response rates (≥20% improvement from baseline) in our three studies ranged from 45% (NightLyte) to 53·8% (MoonLyte). The sources of placebo responses are diverse and include patient beliefs and expectations regarding treatment, as well as positive associations with a clinical setting.[39] The expectation from the mechanism of action of bitopertin and increased clinical attention over the course of the trial might account for meaningful improvements in all treatment arms including placebo. Additionally, a large sample size with a large number of study sites and fewer academic sites have been recognised as factors contributing to the high placebo response in randomised, controlled, clinical trials of antipsychotics.[40,41] The three trials in this report were done across 280 sites around the world where the smallest number of sites (84) and countries (seven) was in NightLyte, the only study with a positive result. This large study population was possibly less sensitive to drug–placebo differences, as shown in a meta-analysis of multicentre antidepressant trials.[42]

Although differences in placebo response among the three studies might account for the absence of a significant difference from placebo in the primary endpoint in the TwiLyte and MoonLyte studies, other factors, such as baseline characteristics, sampling issues, and an attenuated drug response, should also be considered as reasons. With respect to demographic characteristics, the NightLyte study had about 10% more male participants in the placebo arm compared with the bitopertin arms. However, in findings from the scientific literature, the percentage of male participants assigned to receive placebo in antipsychotic trials was not a significant contributor to placebo response.[43] Regional differences also existed, with a higher proportion of females (43%) in bitopertin arms and fewer white patients (40%) in NightLyte. Differences in responses to antipsychotic treatment between sexes have been reported in the past.[43–45] The analysis of the differences in the efficacy of antipsychotics in the EUFEST study[46] revealed robust improvement among women in positive and overall pathology, particularly those receiving olanzapine. Likewise, results from the SOHO study[46] showed greater improvement with clozapine and first-generation antipsychotics among women.[47] Differences in exposure might account for the better response of women in these studies.

Although the variability between responses in patients from Asian ethnic groups compared with patients from white and African-American groups could explain the positive outcome in the NightLyte study, analysis of bitopertin exposure was consistent across the studies and identified no effects of race or body-mass index on the primary outcome (appendix). The mean age, ranging from 39 to 43 years, was similar among studies and similar to results from other schizophrenia trials. By contrast with many global trials characterised by a large placebo effect driven regionally (usually in the USA),[43] NightLyte had a similar placebo effect between regions.

In the prespecified subgroup analysis by race, region, sex, and primary antipsychotic, as well as the CFHR1-high group, results were consistent across subgroups within NightLyte. Furthermore, post-hoc analyses of all three studies showed no consistent differences in the outcome based on other baseline characteristics. Placebo response was variable across the sites and regions, and no consistent differences in results were apparent in patients based on the background antipsychotic.

Baseline symptom severity or type of antipsychotic used in SearchLyte were also in line with findings from other clinical trials, and the mean risperidone-equivalent dose (4·2 mg) was similar across the studies. This trial programme also explored the results of bitopertin when patients were stratified by serum concentrations of CFHR1. However, across the three studies there was no evidence of improved efficacy in the CFHR1-high subgroup. These results argue against the use of this predictor biomarker, at least in this patient population.

Sampling issues, such as the inclusion of patients with less pronounced positive symptoms who would still meet the inclusion criteria, should be considered for such trials. Nevertheless, close monitoring of patients and

profiles by regions in terms of PANSS subscore baseline severity revealed similar profiles among the three studies for positive and negative symptoms with pronounced positive symptoms as required. A non-significant difference was noted in NightLyte where excitement or hostility and anxiety or depression were slightly higher in the 10-mg group.

Finally, although bitopertin response in the main 10-mg arm was also more pronounced in the NightLyte study compared with MoonLyte and TwiLyte, the absolute improvement across the studies was similar and fairly modest, suggesting an attenuated drug effect as a possible contributing factor for the apparent absence of efficacy.

The heterogeneity of the schizophrenia syndrome is also a major obstacle to identifying an overall group effect, because some biological subtypes might be less responsive than others. To investigate whether any subgroups of patients differed in response, we did several post hoc analyses of the effect in patients with strong negative or strong disorganised symptoms in addition to positive symptoms. Subsequent analysis identified a subgroup of patients across all three studies with factors that showed greater treatment effect in the initial analyses, in the 10-mg arm. These patients were taking one antipsychotic and had a lower severity of symptoms on the PANSS disorganised thought/cognitive factor score (<12 in 0–6 scoring) and little PANSS fluctuation defined as <15% change in PANSS total score from screening to baseline in the pre-randomisation evaluation period (data not shown). Although significant effects on the primary endpoint occurred with 10 mg in NightLyte, those did not occur in the other two studies. Greater effects in this subgroup than in the overall ITT population also occurred with 20 mg but were not significant.

Similar to results from other studies where bitopertin was used,[24,29,30] the overall incidence of adverse events was very low. During the 12-week treatment period, no serious adverse event led to discontinuation of treatment. The number of serious adverse events was higher in the placebo groups than in any of the bitopertin groups across the three studies. The inhibition of GlyT1 might cause a reduction in the intake of glycine by erythrocyte precursors, with a consequent reduction in the synthesis of haemoglobin.[48] A reduction of haemoglobin concentration in the bitopertin group is therefore expected with the use of this drug. However, across studies the reduction was gradual, dose-related, and led to a single discontinuation of treatment during the 12-week period.

Finally, patients enrolled in the trial improved in a clinically meaningful way after 12 weeks of treatment on average in all treatment arms, including placebo. These clinically meaningful improvements highlight the importance of psychosocial interventions in the management of schizophrenia.

We noted several study limitations. The study design, although approved by all health authorities worldwide, did not include an active comparator. There are no approved drugs to treat suboptimally controlled symptoms, and augmentations are made either with other antipsychotics or mood stabilisers for which there is no strong evidence. Likewise, there are no marketed glycine-reuptake inhibitors that could have been used in a global programme. Additionally, study entry criteria allowed for a broad range of suboptimally controlled patients with different severity and chronicity of disease; novel mechanisms of action require better phenotypic distinction between responders and non-responders. Earlier-phase trial results would have been beneficial to understand if a more targeted population of patients with recent onset of illness, as suggested by reports from other studies[49,50] related to glutamatergic mechanisms, would have been more appropriate.

In conclusion, the addition of bitopertin to current antipsychotics over 12 weeks in patients with suboptimally controlled symptoms of schizophrenia was well tolerated but did not show a beneficial effect over placebo in treatment of psychotic symptoms. The presence of a significant result in one of the trials (bitopertin 10 mg) was of interest, but we were unable to identify a specific subset, trial design feature, or a biomarker that could either predict or explain why this result was only observed in one trial. On the basis of findings from these trials and other reported trials of glycine reuptake inhibitors, therapeutic prospects of this augmentation strategy should be further assessed.

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