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Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial

Sandeep T Patil[1,13], Lu Zhang[1], Ferenc Martenyi[2], Stephen L Lowe[3], Kimberley A Jackson[4], Boris V Andreev[5], Alla S Avedisova[6], Leonid M Bardenstein[7], Issak Y Gurovich[8], Margarita A Morozova[9], Sergey N Mosolov[8], Nikolai G Neznanov[10], Alexander M Reznik[11], Anatoly B Smulevich[9], Vladimir A Tochilov[12], Bryan G Johnson[1], James A Monn[1] & Darryle D Schoepp[1,13]

Schizophrenia is a chronic, complex and heterogeneous mental disorder, with pathological features of disrupted neuronal excitability and plasticity within limbic structures of the brain. These pathological features manifest behaviorally as positive symptoms (including hallucinations, delusions and thought disorder), negative symptoms (such as social withdrawal, apathy and emotional blunting) and other psychopathological symptoms (such as psychomotor retardation, lack of insight, poor attention and impulse control)[1]. Altered glutamate neurotransmission has for decades been linked to schizophrenia, but all commonly prescribed antipsychotics act on dopamine receptors[2]. LY404039 is a selective agonist for metabotropic glutamate 2/3 (mGlu2/3) receptors[3] and has shown antipsychotic potential in animal studies. With data from rodents, we provide new evidence that mGlu2/3 receptor agonists work by a distinct mechanism different from that of olanzapine. To clinically test this mechanism, an oral prodrug of LY404039 (LY2140023) was evaluated in schizophrenic patients with olanzapine as an active control in a randomized, three-armed, double-blind, placebo-controlled study. Treatment with LY2140023, like treatment with olanzapine, was safe and well-tolerated; treated patients showed statistically significant improvements in both positive and negative symptoms of schizophrenia compared to placebo ($P < 0.001$ at week 4). Notably, patients treated with LY2140023 did not differ from placebo-treated patients with respect to prolactin elevation, extrapyramidal symptoms or weight gain. These data suggest that mGlu2/3 receptor agonists have antipsychotic properties and may provide a new alternative for the treatment of schizophrenia.

The amino acid analog LY404039 is a highly selective agonist for human and rat mGlu2/3 receptors (**Supplementary Table 1** online). LY404039 has no appreciable affinity for other glutamate receptor subtypes and transporters, or for nonglutamate receptors such as dopamine or serotonin that mediate the actions of atypical drugs such as olanzapine[3]. However, in humans, oral absorption of LY404039 was found to be low. LY2140023, a methionine amide of LY404039, was later discovered to be an effective oral prodrug in man (**Supplementary Fig. 1** online). Once absorbed, LY2140023 is efficiently hydrolyzed to produce the active mGlu2/3 receptor agonist LY404039 (**Supplementary Fig. 1**). This resulted in an estimated bioavailability of LY404039 49%, according to urine data. Unlike LY404039, LY2140023 has no appreciable affinity for rat or human mGlu2 or mGlu3 receptors (**Supplementary Table 1**) or for other central nervous system receptors.

To further explore the mechanism of action of LY2140023 in rodent models, we compared the active compound LY404039 to olanzapine in the mouse phencyclidine (PCP) model (**Fig. 1**). We examined the drugs' ability to block PCP-induced hyperlocomotion in both wild-type mice and mice lacking mGlu2/3 receptors. PCP treatment increased motor ambulations in both wild-type and mGlu2/3 knockout mice. LY404039 inhibited these PCP-induced effects, but this inhibition was lost in the mGlu2/3 knockouts. In contrast, olanzapine was equally effective in both wild-type and knockout animals. This shows that LY404039 exerts its effects through the activation of mGlu2/3 receptors, as opposed to the serotonergic and dopaminergic pathways used by olanzapine.

In the clinical proof-of-concept study, a total of 196 patients with considerable psychopathology of schizophrenia were enrolled and randomly assigned to receive LY2140023, placebo or olanzapine in a 3:2:1 ratio. All patients were hospitalized, gradually taken off any

[1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, USA. [2]Lilly Medical Center, Kolblgasse 8-10, A-1030 Vienna, Austria. [3]Lilly-National University of Singapore Centre for Clinical Pharmacology, Level 6 Clinical Research Centre (MD11), 10 Medical Drive, 117597, Singapore. [4]Lilly-Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK. [5]St. Petersburg State University, St. Petersburg and Gatchinskiy Regional Psychiatric Hospital, Nikolskoe 188357, Russia. [6]Serbsky State Scientific Center of Social and Forensic Psychiatry, 23, Kropotkinskiy per. Moscow 123367, Russia. [7]Moscow State University of Medicine and Dentistry based at Moscow Psychiatry Hospital no. 15, Moscvorechie ul. 7, Moscow 115522, Russia. [8]Moscow Research Institute of Psychiatry, Poteshnaya ul. 3, Moscow 107076, Russia. [9]State Institution "Mental Health Scientific Research Center of the Russian Academy of Medical Sciences", Kashyrskoye sh. 34, Moscow 115522, Russia. [10]Bekhterev Research Psychoneurological Institute, Bekhtereva ul. 3, St. Petersburg 192019, Russia. [11]Moscow Regional Psychiatry Hospital #5, Abramtsevskoye sh. 1a, Khotkovo, Moscow region 142601, Russia. [12]Mechnikov State Medical Academy, Naberezhnaya reki Mojki, 126, St. Petersburg 190121, Russia. [13]Present addresses: Takeda Global Research and Development Center, Inc., One Takeda Parkway, Deerfield, Illinois 60015, USA (S.T.P.); Merck and Company, Inc., 351 Sumneytown Pike, UG/4CDS013, North Wales, Pennsylvania 19454, USA (D.D.S.). Correspondence should be addressed to F.M. (Martenyi_Ferenc@lilly.com) or J.A.M. (Monn_James_A@lilly.com).

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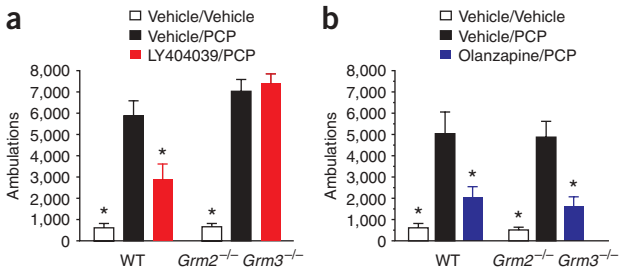

Figure 1 Blockade of phencyclidine-induced hyperlocomotion by LY404039, but not by olanzapine, is dependent on expression of Grm2 and Grm3 in mice. (**a**,**b**) LY404039 (10 mg per kg body weight subcutaneously) or saline vehicle (**a**) or olanzapine (1 mg per kg body weight subcutaneously.) or saline vehicle (**b**) were administered 30 min before phencyclidine (7.5 mg per kg body weight intraperitoneally) or saline vehicle in either wild-type mice or mice lacking expression of mGlu2/3 receptors, and motor activity (ambulations) was measured for 1 h. Data presented are mean + s.e.m. for each experiment ($n = 7$–8 animals per group). *$P < 0.05$ compared to the Vehicle/PCP control group.

pretrial antipsychotic medications, and treated in a double-blind manner for 4 weeks. Overall, the treatment groups did not differ with respect to patient characteristics and baseline severity of illness (**Supplementary Table 2** online). The mean (s.d.) baseline Positive and Negative Symptom Scale (PANSS) total score was 94.8 (11.4) and the mean (s.d.) baseline PANSS negative score was 25.9 (4.0), reflecting relatively severe overall psychopathology.

In all, 118 patients completed 4 weeks of the planned study treatment (**Supplementary Fig. 2** online). A significantly higher number of patients ($P < 0.001$) discontinued treatment from the placebo group (58.7%) than from the LY2140023 (33.7%) and olanzapine (20.6%) groups (**Supplementary Table 2**). A considerable number of the patients who discontinued from the placebo group (26 out of 37) did so because of lack of efficacy. Overall, rates of discontinuation resulting from adverse events were not significantly different across the three treatment groups ($P = 0.66$). There was no statistically significant difference among the three treatment groups regarding the use of benzodiazepine or anticholinergic medications.

Treatment with 40 mg LY2140023 twice daily or 15 mg olanzapine once daily for 4 weeks resulted in statistically significant improvements in PANSS total scores as well as in other outcome measures such as Clinical Global Impression–Severity (CGI-S) scores and PANSS positive and negative subscores compared to placebo (**Table 1**). The LY2140023 group and the olanzapine group showed rapid onset of efficacy, with statistically significant effects manifesting at week 1 ($P < 0.05$, **Fig. 2a–d**). Significant improvements in all efficacy measures were sustained with $P < 0.001$ at subsequent weeks after week 1 through the end of the study (**Fig. 2a–d**). Additional *post hoc* efficacy analysis is available for review in **Supplementary Table 3** online. A patient showing a 25% or more decrease in PANSS total score was defined as a responder. After 4 weeks of treatment, both the LY2140023 group (32.0%, $P < 0.001$) and the olanzapine group (41.2%, $P < 0.001$) showed significantly greater response rates than the placebo group (3.2%).

Prolactin levels steadily declined from baseline to endpoint in all three groups (**Fig. 2e**). Mild to moderate changes in body weight were observed in the LY2140023 and olanzapine groups; there was no significant change in the placebo group. After 4 weeks of treatment, a mean 0.51-kg weight reduction from baseline was observed in the LY2140023 group (**Fig. 2f**). A moderate but statistically significant weight gain was observed in the olanzapine group (0.74 kg, $P = 0.017$, **Fig. 2f**) relative to the placebo group.

Overall, 40 mg LY2140023 given twice daily was safe and well tolerated in this study, with most treatment-emergent adverse events being mild to moderate in severity and not treatment-limiting (as assessed by physician's clinical report). There was no statistically significant difference in the percentage of patients discontinuing because of adverse events in any of the treatment groups (**Supplementary Table 2**). The most common treatment-emergent adverse events in the LY2140023 group were insomnia, affect lability ($P = 0.038$), nausea, headache, somnolence and blood creatine phosphokinase increase. In the olanzapine group, treatment-emergent adverse effects included elevation in blood triglyceride levels ($P = 0.005$), insomnia, weight gain ($P = 0.034$), somnolence, akathisia, agitation and periodontitis ($P = 0.03$, **Table 2**).

No significant treatment-emergent dyskinesia, akathisia or parkinsonism was identified by categorical analysis of commonly used extrapyramidal symptom–scaled scores in any of the treatment groups. Simpson-Angus Scale, Barnes Akathisia Scale and Abnormal Involuntary Movement Scale (items 1–7) scores showed no significant difference between the three treatment groups (**Supplementary Table 4** online). There were no clinically significant alterations in vital signs, laboratory analytes or electrocardiograms associated with LY2140023 treatment during the dosing period.

Our data provides strong new evidence for the role of glutamate modulation in treating psychosis, and specifically for mGlu2/3 receptor activation as a viable therapeutic approach to treat schizophrenia.

Table 1 Change from baseline in severity-of-illness scores after 4 weeks of treatment

Measure	LY2140023 ($n = 97$)	Placebo ($n = 62$)	Olanzapine ($n = 34$)	LY2140023 vs. placebo	Olanzapine vs. placebo
PANSS total	−13.17 (<0.001)	7.63 (0.034)	−19.12 (<0.001)	−20.8 (<0.001)	−26.7 (<0.001)
	[−18.9, −7.4]	[0.6, 14.7]	[−26.8, −11.5]	[−26.8, −14.8]	[−34.2, −19.3]
PANSS positive	−4.62 (<0.001)	1.73 (0.16)	−6.91 (<0.001)	−6.35 (<0.001)	−8.64 (<0.001)
	[−6.6, −2.7]	[−0.7, 4.1]	[−9.4, −4.4]	[−8.3, −4.4]	[−11.1, −6.2]
PANSS negative	−3.33 (<0.001)	1.36 (0.15)	−3.87 (<0.001)	−4.68 (<0.001)	−5.23 (<0.001)
	[−4.8, −1.8]	[−0.5, 3.2]	[−5.8, −1.9]	[−6.2, −3.2]	[−7.1, −3.4]
CGI-S	−0.62 (<0.001)	0.35 (0.11)	−0.89 (<0.001)	−0.97 (<0.001)	−1.24 (<0.001)
	[−1.0, −0.3]	[−0.1, 0.8]	[−1.3, −0.4]	[−1.3, −0.6]	[−1.7, −0.8]

Values are least-squares means (*P*-value) [confidence interval] calculated using MMRM analysis. *n*, number of patients with baseline and at least one postbaseline measure. Two-sided *P*-values were calculated for change-from-baseline scores with 95% confidence interval; one-sided *P*-values were calculated for comparisons with placebo with 90% confidence interval.

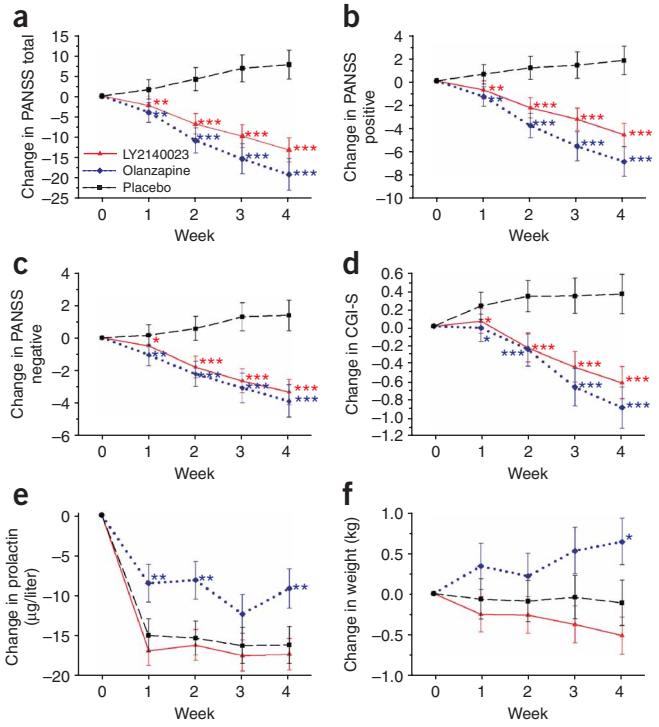

Figure 2 Weekly change in efficacy measures from baseline determined using MMRM analysis. (**a**) PANSS total score. (**b**) PANSS positive score. (**c**) PANSS negative score. (**d**) CGI-S. (**e**) Prolactin level. (**f**) Weight. Data show least-squares means and s.e.m. at each week. Statistical significance is calculated versus placebo (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$).

Table 2 Treatment-emergent adverse events

Event	LY2140023 ($n = 98$)	Placebo ($n = 63$)	Olanzapine ($n = 34$)	Overall *P*-value
Patients with ≥1 event	51.0	49.2	55.9	0.83
Insomnia	12.2	15.9	8.8	0.68
Affect lability	8.2	0.0	2.9	0.038
Nausea	6.1	3.2	0.0	0.35
Headache	5.1	1.6	0.0	0.32
Somnolence	4.1	0.0	5.9	0.16
Blood CPK increased	4.1	0.0	0.0	0.24
Anxiety	3.1	6.3	0.0	0.29
Abdominal pain	3.1	1.6	0.0	0.83
Vomiting	3.1	0.0	0.0	0.29
Akathisia	3.1	3.2	5.9	0.77
Myocardial ischemia	3.1	3.2	0.0	0.71
Weight decreased	3.1	1.6	0.0	0.83
Asthenia	3.1	0.0	0.0	0.29
Agitation	2.0	11.1	5.9	0.040
Blood triglycerides increased	0.0	0.0	8.8	0.005
Weight increased	0.0	1.6	5.9	0.034
Periodontitis	0.0	0.0	5.9	0.030

Values are percentages of events occurring at >3% in the LY2140023 treatment group, or at a statistically significant level. Two-sided *P*-values were calculated from Fisher's exact test.

LY2140023 is the first investigational agent that acts on metabotropic glutamate receptors to be studied in humans for schizophrenia treatment. This agent is thought to work in part by reducing the presynaptic release of glutamate at limbic synapses where these receptors are expressed[4,5]. However, other agents that directly or indirectly modulate glutamate neurotransmission have previously been explored in schizophrenia. The anticonvulsant drug lamotrigine does not act directly on glutamate receptors, but instead attenuates the release of glutamate in the brain. This agent has a different preclinical profile from LY404039 in that it does not block PCP-induced hyperlocomotion in rats, but has other pharmacological effects (such as reversal of prepulse inhibition of startle) suggestive of antipsychotic activity in humans[6]. A recent summary of results in human trials of lamotrigine[7] suggests that it might be used as an adjunctive therapy for some patients, particularly to treat positive symptoms, but additional well-powered and controlled trials are necessary to substantiate these results. Glycine site agonists or partial agonists for the *N*-methyl-D-aspartate receptor (D-serine, glycine, D-cycloserine) have also been studied in schizophrenia as adjunctive therapy[8,9]. Of these, D-serine is more potent than glycine in activating *N*-methyl-D-aspartate receptors and has demonstrable efficacy as an add-on pharmacotherapy in treatment-resistant schizophrenic patients[10]. Our results indicate that the mGlu2/3 receptor agonist prodrug LY2140023 given at 80 mg per day is effective, well-tolerated and safe as a potential monotherapy for positive, negative and other symptoms in patients with schizophrenia. In contrast to the aforementioned agents, LY2140023 produced a statistically significant improvement as compared to the placebo control in all efficacy readouts measured at endpoint (PANSS total, PANSS positive, PANSS negative and CGI-S scores).

Olanzapine at a dose of 15 mg daily served as a positive control for both the experimental conditions and the study design, as well as for established treatment-responsiveness in the trial population. Because this was a proof-of-concept study meant to test the efficacy of LY21400023 in the treatment of schizophrenia, no prospective analysis to compare its treatment response to that of olanzapine was planned. However, exploratory *post hoc* analysis (**Supplementary Table 3**) does not show a statistically significant difference between the primary efficacy measures of the two active treatment groups. Though the results from this trial show that LY2140023 can be beneficial in the treatment of schizophrenia, its optimal therapeutic dose has not yet been determined. The adverse event profile of LY2140023 did not include prolactin increase or worsening of extrapyramidal symptoms (EPS). A mild reduction in body weight and body mass index was noted in patients treated with LY2140023 (**Fig. 2f** and **Supplementary Fig. 3**). Although affect lability seems to represent the most important potential adverse event, it should be noted that this outcome was observed primarily at one clinical site. According to the investigator at the site, some patients with severe affective flattening and withdrawal not only exhibited an increase in emotional response, but also showed spontaneous emotional fluctuations (such as memories of past emotionally important events). In general, the patients seemed to be more emotional than before. In future trials we will attempt to closely follow and increase our understanding of this potentially interesting observation.

Results from studies in animals lacking mGlu2/3 receptors suggest that the antipsychotic effect of LY2140023 may be mechanistically distinct from that of olanzapine. Agonists of mGlu2/3 receptors, such as LY354740, LY379268 or MSG0028, can block the expression of PCP-induced behaviors in animals[11]. The ability of mGlu2/3 receptor agonists to reverse PCP-induced behaviors in either normal or monoamine-depleted rats provides further evidence of a distinct mechanism[11,12].

Notably, LY404039 and other mGlu2/3 agonists do not directly interact with dopamine or serotonin (5-HT2A) receptors. However, 'functional' 5-HT2A receptor antagonism in the prefrontal cortex may

represent a common mechanism shared by clinically effective atypical antipsychotics and mGlu2/3 receptor agonists, and may contribute to the antipsychotic actions of LY2140023[11–13]. Atypical antipsychotics such as clozapine and olanzapine block 5-HT2A receptors (in addition to the D2 dopamine receptors), and this action has been linked to improvements in the control of negative and cognitive symptoms[14]. The prefrontal cortex appears to be important in mediating this action of atypical antipsychotic drugs[13]. In the prefrontal cortex of rats, mGlu2/3 receptors show an overlapping cellular distribution with 5-HT2A receptors[15]. Activation of 5-HT2A receptors evokes the asynchronous presynaptic release of glutamate from thalamocortical glutamate afferents[16,17]. In the prefrontal cortex, mGlu2/3 receptor agonists (like atypical antipsychotics) block 5-HT2A receptor–mediated release of glutamate[15]. *In vivo* administration of mGlu2/3 receptor agonists such as LY354740 or LY379268 blocks the *in vivo* behavioral and neurochemical effects of psychotogens that act through 5-HT2A receptor activation (for example, the hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane, or DOI)[18,19]. Like these other mGlu2/3 compounds, LY404039 has also been shown to suppress 5-HT2A receptor–mediated increases in glutamate neurotransmission[3].

Blockade of limbic dopamine neurotransmission has been linked to the actions of all antipsychotic drugs. Preclinical evidence suggests that selective blockade of mesolimbic dopamine neurotransmission may be a mechanistically distinct but common feature of both atypical antipsychotics and mGlu2/3 receptor agonists. Amphetamine induces an increase in locomotor activity in rodents as a result of increased dopaminergic activity in mesolimbic pathways (nucleus accumbens). In rodents, all known clinically effective antipsychotics reverse locomotor activation induced by amphetamine, and this has been linked to the antagonism of D2 dopamine receptors in mesolimbic dopamine pathways[20]. Glutamate neurons are also important regulators of dopaminergic tone in the nucleus accumbens[21]. Agonists of mGlu2/3 receptors reduce glutamate release in the nucleus accumbens[22] and indirectly modulate (reduce) dopamine transmission as well[23]. *In vivo* activation of mGlu2/3 receptors selectively attenuates locomotor behaviors (ambulations and rearing) induced by amphetamine. As in the PCP model, these effects of mGlu2/3 receptor agonists are also selectively reversed by an mGlu2/3 receptor antagonist[24].

Progressive neurodegeneration has been hypothesized to be a feature of schizophrenia pathophysiology. In this regard, mGlu2/3 receptor agonists are neuroprotective in several preclinical models of apoptotic and necrotic neuronal degeneration[25]. The consequence of these findings in schizophrenic patients is unknown and may be an important feature to explore in the future.

Generally, the rate of EPS reported in patients treated with atypical antipsychotics is significantly lower than that in patients treated with typical antipsychotics, but the risk of EPS is not entirely eliminated. EPS can contribute to noncompliance and thereby can reduce the effectiveness of antipsychotic treatments[26]. Encouragingly, the LY2140023-treated group showed no additional worsening of EPS, but instead showed a marginal decrease in Barnes akathisia scores ($P = 0.099$) when compared to the placebo group. Akathisia is a highly uncomfortable manifestation of EPS, and is vaguely defined as a syndrome of severe inner restlessness accompanied by an inability to avoid purposeless movements. It is most commonly a disturbing acute adverse event, but can be a persistent phenomenon in some patients who have undergone chronic antipsychotic therapy.

Hyperprolactinemia is frequently noted in patients with schizophrenia treated with typical antipsychotics, and mild but transient hyperprolactinemia has been reported even with the prototypical atypical antipsychotic agent clozapine. Similarly, in a well-controlled dose-response study in which potential changes in prolactin concentrations were examined over time, a mild but transient dose-related elevation in prolactin was noted in the olanzapine group[27] at week 2. Although the clinical implications of elevated prolactin are unknown, patients with chronically elevated prolactin have reported endocrinal disturbances (amenorrhea, galactorrhea, infertility, osteoporosis or sexual dysfunction). In some cases, persistent, modest elevations in prolactin have been noted with chronic olanzapine treatment. In our study, patients treated with LY2140023 and olanzapine experienced a mean reduction in serum prolactin. Preclinical literature shows that LY379268 blocks stress or α-methyl-*para*-tyrosine–induced prolactin secretion in rats[28]. Because this was an acute, 4-week study, the full clinical importance of changes in prolactin levels warrant further investigation.

In summary, the 'glutamate hypothesis of schizophrenia' suggests that drugs that correct or modulate disrupted glutamate pathways may be useful to treat negative and positive symptoms of the disorder. Here, we demonstrate that an agent that modulates glutamate neurotransmission through selective agonism of mGlu2/3 receptors has antipsychotic activity in patients with schizophrenia. Limitations of this study include a relatively short treatment period and limited comparisons with other established antipsychotics. Additional larger and longer-term studies are warranted to further support and optimally characterize the benefits and the potential risks of this approach to treat schizophrenia.

METHODS

Nonclinical studies. All animal experiments were approved by the Eli Lilly and Company Institutional Animal Care and Use Committee. The generation of separate mGlu2 and mGlu3 receptor knockout mice by homologous recombination and the measurement of behavioral parameters of PCP-induced hyperlocomotion in mice are explained in the **Supplementary Methods** online.

Clinical proof-of-concept study. The phase 2, double-blind, parallel, placebo-controlled, fixed-dose study was conducted at ten investigative sites in Russia with enrollment from 15 August 2005 to 17 June 2006. Informed consent was obtained from all patients participating in the study. This study was approved by the centralized Ethical Review Board (ERB) in the Russian Federation per the local regulations. The documents submitted for the ERB approval included the Informed Consent Document, Case Report Forms, study protocol and Clinical Investigator Brochure translated into the Russian language as necessary. Throughout the trial the ERB was regularly updated on any newly emerging safety findings. We chose Russia for this project based on our previous experience with a well-qualified and experienced principle investigator (PI) and supportive staff, and the availability of a large, homogeneous patient population. Relatively few sites (ten) were required for an inpatient trial of this size, and because this was not a multinational study, only one language was required for the rating scales, thus reducing possible external sources of heterogeneity and variation. All of the participating PIs and the supporting study staff received in-depth International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use–Good Clinical Practice (ICH-GCP) training from individuals specialized in GCP education before the start of the study. In addition, key documents, such as those for ICH-GCP training, were translated into Russian. The guidelines and requirements for the informed consent were emphasized before the study began and were monitored throughout the study. The investigators or qualified persons from the study team provided information to patients about the trial, and all patients had ample opportunity to discuss all aspects of their participation either with the PI or a co-investigator or with an independent person (such as a family member or their referring psychiatrist or physician). None of the patients participating in this trial were under guardianship.

The primary objective of study was to determine whether LY2140023 administered for 28 d was superior to placebo in the treatment of patients with schizophrenia as measured by the PANSS[29] total score. In this study, olanzapine (15 mg/d) served as an active control. The secondary outcome measures included PANSS subscores and CGI-S[30]. The tertiary outcomes measured to assess potential adverse events included such readouts as movement disorders and prolactin level changes. Patients were men and nonchildbearing women 18–65 years of age who met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) criteria for schizophrenia as confirmed by the modified Structured Clinical Interview for DSM–IV (SCID). All had symptoms that were poorly controlled without medication (option 1) or with medication (option 2), and were willing to remain inpatients in the clinical unit for the duration of the study (8–12 weeks). Nonchildbearing women were defined as those who had ligated fallopian tubes or no uterus, or who were devoid of any ovarian function as a result of natural causes or surgery. A woman was presumed to be infertile due to ovarian failure if she had been amenorrheic for 12 months or longer and had a follicle-stimulating hormone (FSH) value of >30 IU/l. Additional information on inclusion and exclusion criteria is available (**Supplementary Methods**).

All participating patients with schizophrenia were outpatients before their temporary hospitalization for worsening symptoms. They typically had had a chronic course of schizophrenia of more than 15 years, had ages consistent with the mean age (40.5 years) and had past history of disease exacerbations leading to temporary hospitalizations. The primary reason for limiting the trial to inpatients was to ensure patient safety, given the investigational status of the drug. An inpatient setting allowed for close observation of any clinical worsening and quick intervention with a rescue medication if needed. No therapeutically stable patients were included in the trial or weaned from effective treatment. Additional information on antipsychotic tapering and past medications is available (**Supplementary Methods**).

Statistical analysis. The study was planned to have at least 78 patients in the LY2140023 group and 52 patients in the placebo group, which would provide approximately 86% power to detect a treatment difference of 11 in the change-from-baseline PANSS total score at a one-sided, 5% significance level. The sample size was determined using two-sample *t*-tests with unadjusted significance levels, assuming a s.d. of 22.2.

All efficacy and safety analyses were performed on all randomized patients according to the treatment to which the patients were assigned (intent-to-treat basis). For change-from-baseline analyses, randomized patients with a baseline measurement and at least one measurement after baseline were included.

The primary analysis model for the primary efficacy measure PANSS-total score was a mixed-effects with repeated measures model (MMRM). The dependent variable was the change-from-baseline measurement taken after baseline. The model included baseline as a covariate, with site, patient option, treatment, visit and treatment-by-visit interaction as fixed effects, and used an unstructured covariance matrix. The primary contrast was that between LY2140023 and placebo at week 4. The same analysis model was applied to all secondary efficacy measures (PANSS positive score, PANSS negative score and CGI-S) and the EPS scores. For the responder rate analysis of PANSS total score, the Cochran-Mantel-Haenszel test, controlling for the site, was performed at each visit, and the last-observation-carried-forward test was used to impute any subsequent missing data.

The change-from-baseline values of body weight and prolactin level were analyzed using the MMRM. The model included baseline as a covariate, with patient option, treatment, visit and treatment-by-visit interaction as fixed effects, and used the compound symmetry covariance structure. The site effect was not considered in the model for these variables. The treatment-emergent adverse events were compared among treatment groups using Fisher's exact test.

The tests of efficacy between treatments were conducted at a one-sided α level of 0.05, as the study was powered at a one-sided, 5% significance level because of the proof-of-concept nature of the compound development stage. However, *post hoc* analysis based on a two-sided test for efficacy are provided in the supplementary material (**Supplementary Table 3**). The tests of changes from baseline and all safety tests (including EPS) were conducted at a 0.05 two-sided α level. All analyses were performed without adjustment for multiplicity.

Details of software, procedure and additional statistical methods are available (**Supplementary Methods**).

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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1. Andreasen, N.C. & Carpenter, W.T., Jr. Diagnosis and classification of schizophrenia. *Schizophr. Bull.* **19**, 199–214 (1993).
2. Tamminga, C.A. & Lahti, A.C. The new generation of antipsychotic drugs. *Int. Clin. Psychopharmacol.* **11**, 73–76 (1996).
3. Rorick-Kehn, L.M. *et al.* Pharmacological and pharmacokinetic properties of a structurally-novel, potent, selective mGlu2/3 receptor agonist: *in vitro* characterization of LY404039. *J. Pharmacol. Exp. Ther.* **321**, 308–317 (2007).
4. Moghaddam, B. & Adams, B.W. Reversal of phenylcyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* **281**, 1349–1352.
5. Schoepp, D.D. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther.* **299**, 12–20 (2001).
6. Large, C.H., Webster, E.L. & Goff, D.C. The potential role of lamotrigine in schizophrenia. *Psychopharmacology (Berl.)* **181**, 415–436 (2005).
7. Kremer, I. *et al.* Placebo-controlled trial of lamotrigine added to conventional and atypical antipsychotics in schizophrenia. *Biol. Psychiatry* **56**, 441–446 (2004).
8. Tuominen, H.J., Tiihonen, J. & Wahlbeck, K. Glutamatergic drugs for schizophrenia: a systematic review and meta-analysis. *Schizophr. Res.* **72**, 225–234 (2005).
9. Goff, D.C. *et al.* A six-month, placebo-controlled trial of D-cycloserine co-administered with conventional antipsychotics in schizophrenia patients. *Psychopharmacology (Berl.)* **179**, 144–150 (2005).
10. Heresco-Levy, U. *et al.* D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. *Biol. Psychiatry* **57**, 577–585 (2005).
11. Schoepp, D.D. & Marek, G.J. Preclinical pharmacology of mGlu2/3 receptor agonists: novel agents for schizophrenia? *Current Drug Targets. CNS & Neurological Disorders.* **1**, 215–225 (2002).
12. Swanson, C.J. & Schoepp, D.D. The group II metabotropic glutamate agonist LY379268 and clozapine reverse phencyclidine-induced behaviors in monoamine-depleted rats. *J. Pharmacol. Exp. Ther.* **303**, 919–927 (2002).
13. Aghajanian, G.K. & Marek, G.J. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Research. Brain Res. Brain Res. Rev.* **31**, 302–312 (2000).
14. Meltzer, H.Y. *Neuropsychopharmacology: The Fifth Generation of Progress* (eds. Davis, K.L., Charney, D., Coyle, J.T. & Nemeroff, C.) 819–831 (Lippincott Williams & Wilkins, 2002).
15. Marek, G.J., Wright, R.A., Schoepp, D.D., Monn, J.A. & Aghajanian, G.K. Physiological antagonism between 5-hydroxytryptamine(2A) and group II metabotropic glutamate receptors in prefrontal cortex. *J. Pharmacol. Exp. Ther.* **292**, 76–87 (2000).
16. Aghajanian, G.K. & Marek, G.J. Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* **36**, 589–599 (1997).
17. Marek, G.J., Wright, R.A., Gewirtz, J.C. & Schoepp, D.D. A major role for thalamocortical afferents in serotonergic hallucinogen receptor function in the rat neocortex. *Neuroscience* **105**, 379–392 (2001).
18. Gewirtz, J.C. & Marek, G.J. Behavioral evidence for interactions between a hallucinogenic drug and group II metabotropic glutamate receptors. *Neuropsychopharmacology* **23**, 569–576 (2000).
19. Zhai, Y. *et al.* Group II metabotropic glutamate receptor modulation of DOI-induced c-fos mRNA and excitatory responses in the cerebral cortex. *Neuropsychopharmacology* **28**, 45–52 (2003).
20. Ellenbroek, B.A. Treatment of schizophrenia: a clinical and preclinical evaluation of neuroleptic drugs. *Pharmacol. Ther.* **57**, 1–78 (1993).

21. Svensson, L., Zhang, J., Johannessen, K. & Engel, J.A. Effect of local infusion of glutamate analogues into the nucleus accumbens of rats: an electrochemical and behavioural study. *Brain Res.* **643**, 155–161 (1994).
22. Xi, Z.X. *et al.* Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. *J. Pharmacol. Exp. Ther.* **303**, 608–615 (2002).
23. Hu, G., Duffy, P., Swanson, C., Ghasemzadeh, M.B. & Kalivas, P.W. The regulation of dopamine transmission by metabotropic glutamate receptors. *J. Pharmacol. Exp. Ther.* **289**, 412–416 (1999).
24. Cartmell, J., Monn, J.A. & Schoepp, D.D. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus D-amphetamine motor behaviors in rats. *J. Pharmacol. Exp. Ther.* **291**, 161–170 (1999).
25. Flor, P.J., Battaglia, G., Nicoletti, F., Gasparini, F. & Bruno, V. Neuroprotective activity of metabotropic glutamate receptor ligands. *Adv. Exp. Med. Biol.* **513**, 197–223 (2002).
26. Fleischhacker, W.W., Meise, U., Gunther, V. & Kurz, M. Compliance with antipsychotic drug treatment: influence of side effects. *Acta Psychiatr. Scand. Suppl.* **382**, 11–15 (1994).
27. Crawford, A.M., Beasley, C.M., Jr. & Tollefson, G.D. The acute and long-term effect of olanzapine compared with placebo and haloperidol on serum prolactin concentrations. *Schizophr. Res.* **26**, 41–54 (1997).
28. Johnson, M.P. & Chamberlain, M. Modulation of stress-induced and stimulated hyperprolactinemia with the group II metabotropic glutamate receptor selective agonist, LY379268. *Neuropharmacology* **43**, 799–808 (2002).
29. Kay, S.R., Opler, L.A. & Fiszbein, A. Positive and negative syndrome scale (PANSS) manual. (Multi-Health Systems, North Tonawanda, New York, USA, 1986).
30. Guy, W. ECDEU assessment manual for psychopharmacology, revised version. (US Department of Health, Education and Welfare, Bethesda, Maryland, USA, 1976).