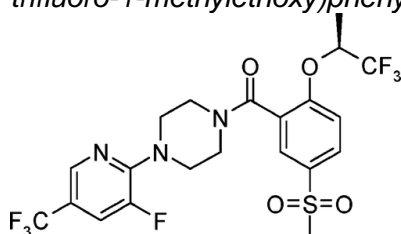


BITOPERTIN / RG1678 / RO4917838
F. Hoffmann–La Roche

Bitopertin is a glycine transporter type 1 (GlyT1) inhibitor that increases levels of the neurotransmitter glycine by inhibiting its reuptake from the synaptic cleft. Glycine acts as an obligatory co-agonist along with glutamate at NMDA receptors. Dysfunction of NMDA receptors may play a key role in the pathogenesis of schizophrenia and modulation of glutamatergic signalling via increased concentrations of glycine in the synaptic cleft may help potentiate NMDA receptor function and improve the symptoms of schizophrenia.

[4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone



Pharmacokinetic Properties

Chemical optimization in the class of benzoylpiperazines has led to the discovery of RG1678, a highly potent and selective GlyT1 inhibitor exhibiting excellent pharmacokinetic and in vivo efficacy profiles in nonhuman species.

parameter	species	
	rat (n = 2)	cynomolgus monkey (n = 2)
iv dose (mg/kg)	2 iv formulations: NMP/ hydroxypropyl γ -cyclodextrin	0.5 iv formulations: 30% captisol
CL ((mL/min)/kg) Total plasma clearance	4.32 (\pm 25%)	3.62 (\pm 9%)
V_{dss} (L/kg) Volume of distribution	3.58 (\pm 14%)	1.98 (\pm 8%)
po dose (mg/kg) po formulation: Tween-80, methylparaben, propylparaben, and hydroxyethylcellulose	3	2.8
C_{max} (ng/mL)	721 (\pm 13%)	517 (\pm 38%)
$AUC_{0-\infty}$ (ng·h/mL) Area under the curve extrapolated to infinity	8670 (\pm 16%)	7230 (\pm 2%)
$T_{1/2}$ (h) terminal	5.8 (\pm 25%)	6.4 (\pm 8%)
T_{max} (h)	1	5.5 (\pm 64%)
Oral bioavailability F (%)	78 (\pm 20%)	56 (\pm 3%)
brain/plasma	0.7	
PPB (% unbound) Plasma protein binding	3	3

*Pinard E, Alanine A, Alberati D, Bender M, Borroni E, Bourdeaux P, Brom V, Burner S, Fischer H, Hainzl D, Halm R, Hauser N, Jolidon S, Lengyel J, Marty HP, Meyer T, Moreau JL, Mory R, Narquizian R, Nettekoven M, Norcross RD, Puellmann B, Schmid P, Schmitt S, Stalder H, Wermuth R, Wettstein JG, Zimmerli D. Selective GlyT1 inhibitors: discovery of [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a promising novel medicine to treat schizophrenia. *J Med Chem.* 2010 24;53(12):4603-14. doi: 10.1021/jm100210p. PubMed PMID: 20491477.*

Distribution of GlyT1 in human brain (autoradiography)

The regional distribution of a novel PET tracer for the glycine transporter type 1 (GlyT1) in humans, RO5013853 binding sites was investigated at higher resolution by autoradiography using tritiated RO5013853. High binding density was observed in the pons, the superior and inferior colliculi, and various thalamic nuclei (data not shown). In the cerebellum, a higher density of [³H]RO5013853 binding sites was detected in the granular layer and a lower density in the molecular layer and the white matter. Moderate binding site density was seen in the caudate nucleus, the dentate gyrus, the putamen, and the CA 1–3 regions of the hippocampus. Low binding was observed in all cortical regions investigated. Co-incubation of the radioligand with the bitopertin (RG1678), completely abolished the binding, confirming the specificity of RO5013853 for GlyT1 (data not shown).

Following 12 days of once daily dosing with 175 mg bitopertin tracer binding is almost completely blocked (GlyT1 occupancy by bitopertin: 92% in the thalamus; unpublished data).

Wong DF, Ostrowitzki S, Zhou Y, Raymont V, Hofmann C, Borroni E, Kumar A, Parkar N, Brašić JR, Hilton J, Dannals RF, Martin-Facklam M. Characterization of [(11)C]RO5013853, a novel PET tracer for the glycine transporter type 1 (GlyT1) in humans. Neuroimage. 2011 Dec 1. [Epub ahead of print] PubMed PMID: 22155032.

To investigate the relationship between the plasma concentration of bitopertin (RG1678) and brain GlyT1 occupancy, a PET study was done in 18 healthy male volunteers received up to 175 mg bitopertin once daily, for 10–12 days. Three PET scans, preceded by a single intravenous infusion of ~30 mCi [(11)C]RO5013853, were performed: at baseline, on the last day of bitopertin treatment, and 2 days after drug discontinuation. At baseline, regional volume of distribution (V(T)) values were highest in the pons, thalamus, and cerebellum (1.7–2.7 ml/cm³) and lowest in cortical areas (~0.8 ml/cm³). V(T) values were reduced to a homogeneous level following administration of 175 mg bitopertin.

Bitopertin steady-state plasma concentrations (mean of pre- and post-scan concentrations collected between 5 and 9 h post dose) increased in a dose-proportional manner. Following administration of 5, 15, 30, 60, or 175 mg bitopertin once daily for 10–12 days, plasma concentrations were 66.7±5.2, 146±26.8, 438±47.0, 561±135, and 1628±298 ng/ml, respectively (n = 3). Two days after drug discontinuation, concentrations decreased to approximately half that of the respective concentrations reported on the last day of treatment.

Martin-Facklam M, Pizzagalli F, Zhou Y, Ostrowitzki S, Raymont V, Brašić JR, Parkar N, Umbricht D, Dannals RF, Goldwater R, Wong DF. Glycine transporter type 1 occupancy by bitopertin: a positron emission tomography study in healthy volunteers. Neuropsychopharmacology. 2013 Feb;38(3):504–12. doi: 10.1038/npp.2012.212. Epub 2012 Nov 7. PubMed PMID: 23132267; PubMed Central PMCID: PMC3547202.

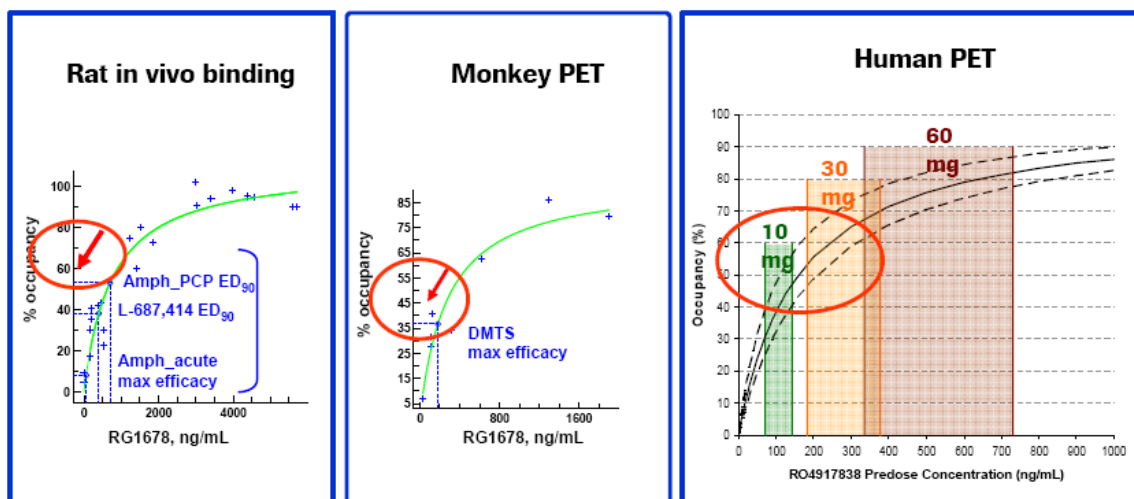
Selectivity profile

RG1678 was inactive (binding inhibition <45% at 10 μM) against 86 targets, including but not limited to glycine, glutamate, dopamine, GABA, serotonin, adrenaline, noradrenaline, muscarinic, adenosine, opioid, histamine, tachykinin, purinergic receptors, sodium, potassium, calcium, and chloride ion channels. In 21 functional assays, RG1678 did not inhibit enzymes such as monoamine oxidase, catechol-O-methyltransferase, acetylcholinesterase, phosphodiesterases (1–5), ATPase, adenylyl and guanylyl cyclases, and protein kinase C, and had no effect on the uptake or release of dopamine, serotonin (5-HT), or norepinephrine transporters.

Alberati D, Moreau JL, Lengyel J, Hauser N, Mory R, Borroni E, Pinard E, Knoflach F, Schlotterbeck G, Hainzl D, Wettstein JG. Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. Neuropharmacology. 2012 Feb;62(2):1152–61. doi: 10.1016/j.neuropharm.2011.11.008. Epub 2011 Nov 27. PubMed PMID: 22138164.

Level of receptor occupancy determines efficacy

~50% sufficient for full efficacy in all animal models



Optimal predicted human dose: 10–30 mg range

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Pharmacologic Profile

In vitro, RG1678 noncompetitively inhibited [³H]glycine uptake in cells stably expressing hGlyT1b and mGlyT1b, with IC₅₀ values of 25 ± 2 nM and 22 ± 5 nM, respectively (*n* = 6) and competitively displaced [³H]ORG24598 binding with a K_i of 8.1 nM at human hGlyT1b in membranes from Chinese hamster ovary cells. RG1678 had no effect on hGlyT2-mediated [³H]glycine uptake up to 30 μM concentration. There is no significant species difference in the pharmacology for RG1678 based on the ability of the compound to displace [³H]ORG24598.

In hippocampal CA1 pyramidal cells, RG1678 enhanced NMDA-dependent long-term potentiation (LTP) at 30 nM (213 ± 18%; *n*=7), 100 nM (269 ± 44%, *n*=13) but not at 300 nM (152 ± 14%; *n* = 9).

In vivo, RG1678 dose-dependently increased striatal levels of glycine measured by microdialysis in rats. Administration of RG1678 (1–30 mg/kg p.o.) produced a long-lasting (>3h) dose-dependent increase in extracellular glycine levels (2.5-fold over basal levels at 30 mg/kg; 80–100 min after drug administration). The lack of effect on the striatal extracellular levels of D/L-serine (data not shown) indicated that RG1678 did not affect other neutral amino acid transporters and provided *in vivo* evidence of its selectivity for the GlyT1 transporter.

A similar dose-dependent increase in glycine concentration was observed in the CSF of rats treated with RG1678 (1–10 mg/kg p.o.), 3 h after drug administration. Interestingly, the level of CSF glycine increase 3h after RG1678 dosing was very similar to the increase in the microdialysis experiment at the same time point.

In mice, RG1678 (0.3–3 mg/kg oral) dose-dependently and significantly attenuated hyperlocomotion induced by the psychostimulant D-amphetamine (2 mg/kg).

RG1678 (1–10 mg/kg, oral) dose-dependently and significantly decreased the NMDA receptor glycine site antagonist, L-687,414 (50 mg/kg s.c.)-induced hyperlocomotion in mice, with comparable potency at 0.5, 2.5, and 4.5 h after administration, but was no longer significantly active 24 h post-dose

RG1678 (3 and 10 mg/kg) also prevented the hyper-response to D-amphetamine challenge in rats treated chronically with phencyclidine, an NMDA receptor open-channel blocker.

In the latter experiment, a decrease in *ex vivo* striatal [³H]raclopride binding was observed suggesting an increase in synaptic dopamine.

Together, the data show that RG1678 is a potent, selective, noncompetitive and reversible inhibitor of GlyT1. The physiologic consequence of this inhibition is increased extracellular and CSF glycine, leading to enhanced NMDA receptor function and consequent normalization of perturbed dopaminergic and glutamatergic transmission. Thus, the results from these studies lead to the suggestion that inhibitors of GlyT1 such as RG1678, which is currently under investigation in multiple clinical trials, may eventually provide benefit to patients with schizophrenia.

Alberati D, Moreau JL, Lengyel J, Hauser N, Mory R, Borroni E, Pinard E, Knoflach F, Schlotterbeck G, Hainzl D, Wettstein JG. Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. Neuropharmacology. 2012 Feb;62(2):1152-61. doi: 10.1016/j.neuropharm.2011.11.008. Epub 2011 Nov 27. PubMed PMID: 22138164.

In a subsequent proof-of-mechanism study in healthy volunteers, once-daily oral doses of 3–60 mg bitopertin over 10 days resulted in a dose-dependent increase in glycine levels in CSF of a similar magnitude, as previously seen in rats treated with RG1678 (1–10 mg/kg p.o.).

Hofmann C, Alberati D, Banken L, Boetsch C, Ereshefsky L, Jhee S et al(2011). Glycine transporter type 1 (GlyT1) inhibitor RG1678: proof of mechanism of action in healthy volunteers. Schizophr Bull 37(Suppl 1): 306.

Adverse Effect

Bitopertin doses of 5–175 mg administered for 10–12 days in 18 healthy male volunteers were well tolerated with no serious adverse effects. There were several re-occurring adverse effects at 175 mg (headache, dizziness, fatigue, constipation, and insomnia). The most frequently observed effects, considered as possibly or probably associated to bitopertin, were dizziness (one of three and two of three subjects after 60 and 175 mg, respectively) and fatigue (two of three and one of three subjects after 60 and 175 mg, respectively). After 60 mg dizziness occurred as a unique event, whereas after 175 mg dizziness was reported as a recurrent event in both subjects. Dizziness occurred at the beginning of treatment (day 1–day 4) and ceased under treatment, indicating tolerance.

Martin-Facklam M, Pizzagalli F, Zhou Y, Ostrowitzki S, Raymont V, Brašić JR, Parkar N, Umbricht D, Dannals RF, Goldwater R, Wong DF. Glycine transporter type 1 occupancy by bitopertin: a positron emission tomography study in healthy volunteers. Neuropsychopharmacology. 2013 Feb;38(3):504-12. doi: 10.1038/npp.2012.212. Epub 2012 Nov 7. PubMed PMID: 23132267; PubMed Central PMCID: PMC3547202.

A multiple-dose, randomized, double-blind, double-dummy, placebo-controlled, parallel-group study using bitopertin 30 mg (n = 56) or bitopertin 175 mg (n = 56) once daily for 10 days was conducted for thorough QT studies to assess the effects of bitopertin on cardiac repolarization and proarrhythmic potential. Multiple dosing with bitopertin 30 mg or 175 mg

did not affect the corrected QT interval (QTcF) in these healthy predominantly white male volunteers (mean age, 31.8 years).

Peak bitopertin plasma concentrations were achieved ~4 hours after dosing. The terminal elimination t(1/2) was ~53 hours. No safety or tolerability concerns were noted with bitopertin at either dose. Dizziness, nausea, and blurred vision were more common in the bitopertin 175-mg group compared with the bitopertin 30-mg or placebo groups.

Hofmann C, Banken L, Hahn M, Swearingen D, Nagel S, Martin-Facklam M. Evaluation of the effects of bitopertin (RG1678) on cardiac repolarization: a thorough corrected QT study in healthy male volunteers. *Clin Ther.* 2012 Oct;34(10):2061-71. doi: 10.1016/j.clinthera.2012.08.010. Epub 2012 Sep 12. PubMed PMID: 22980315.



GlyT-1: placebo-like safety profile

Adverse events by body system during treatment

	Placebo	RG1678		
		10 mg/day	30 mg/day	60 mg/day
% Patients with ≥ one AE	40	41	41	46
Nervous system	11	12	14	31
Infectious	10	13	5	10
Psychiatric	11	6	12	9
General disorder	6	2	6	5
Musculo-skeletal-connective	1	2	4	5

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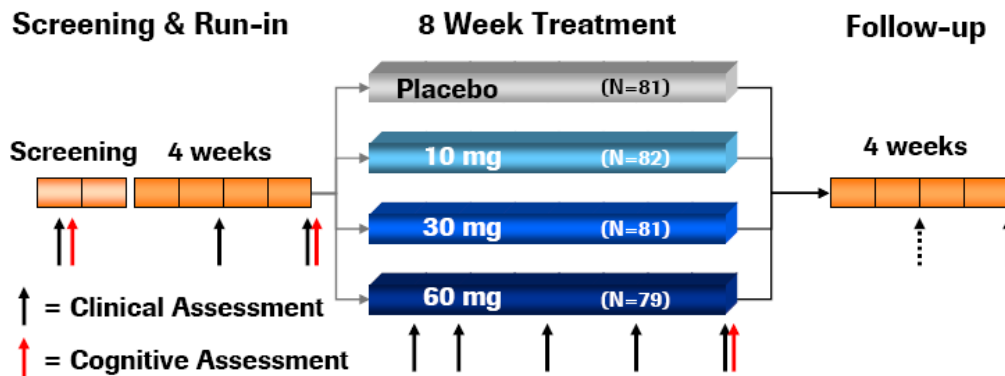
Clinical Trials

Phase II

Roche reported in January 2010 results from a phase II trial with RG1678. In a 323 schizophrenic patient phase II proof of concept trial, RG1678 demonstrated robust, consistent and clinically meaningful improvement in negative symptoms (personal and social performance) from baseline within 8 weeks (from -4.86 in the placebo group to -6.65 in the treatment group) with trend improvement and functioning and without significant motor side effects, a symptom cluster that the standard D₂ antagonist therapies do not impact. In addition, 83% of patients on RG1678 described an improvement of negative symptoms on the CGI-I1 vs 66% on placebo. A trend was also seen in the subgroup of patients with moderately high positive symptoms which was supportive of potential in positive symptoms control. As opposed to earlier studies, the clinical dose of RG-1678 was selected based upon a PET study of glycine-site occupancy, with dose chosen to prevent activation-related NMDAR desensitization. The study demonstrated, first, that inhibition of GlyT1-mediated transport does indeed lead to increased CNS glycine levels, and second, that resultant allosteric NMDAR via the glycine modulatory site may be therapeutically beneficial.

GlyT-1 in negative symptoms of schizophrenia

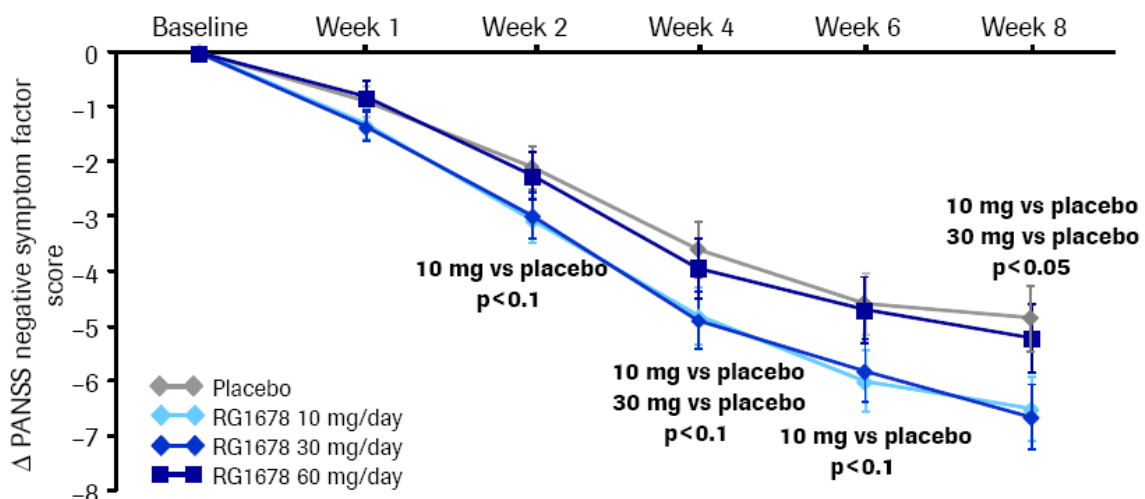
Phase 2 Proof-of-Concept study design



<p>Population</p> <ul style="list-style-type: none"> Patients with predominant negative symptoms, stabilised on antipsychotic medication 	<p>Primary endpoint</p> <ul style="list-style-type: none"> -PANSS Negative Symptom Factor Score Negative symptoms <p>Secondary endpoints</p> <ul style="list-style-type: none"> -CGI-S/I for Negative Symptoms Clinical assessment -PSP Function -CNSVitalSigns Cognition <p style="text-align: right;">75</p>
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GlyT-1 in negative symptoms of schizophrenia

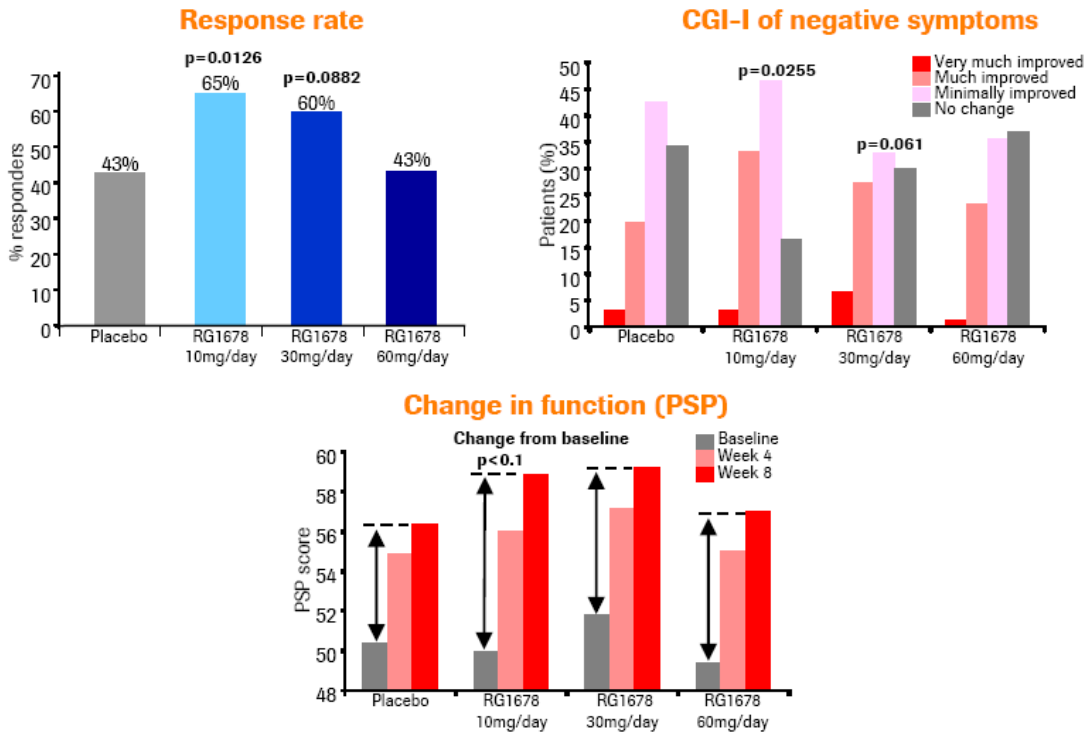
Significant reduction in negative symptom factor score*



Effect Size (Week 8): 10 mg = 0.37, 30 mg = 0.40

*PP population

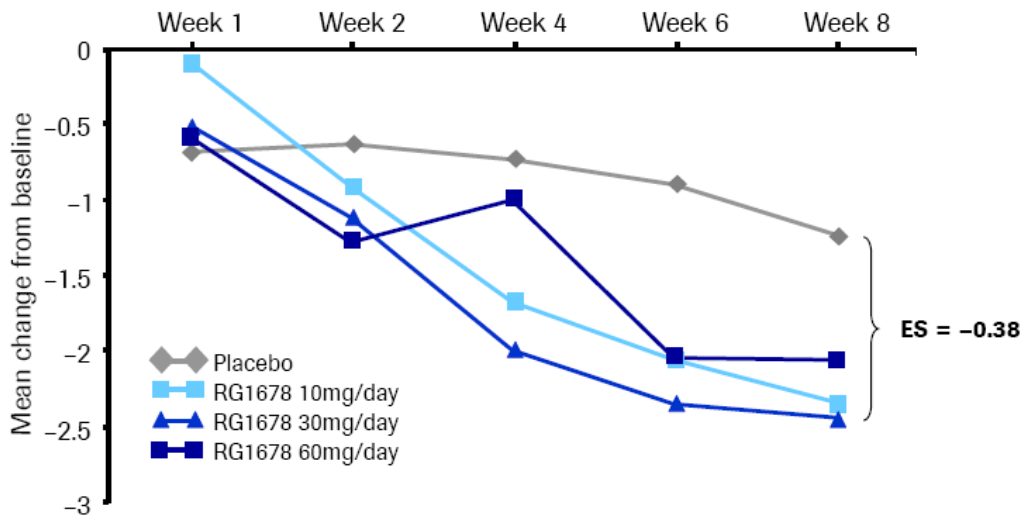
Consistent effects on all measured outcomes*



PP population; Response rate: • £0% improvement in NSFS; PSP=Personal and Social Performance; CGI-I=Clinical Global Impression-Improvement

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GlyT-1 in positive symptoms of schizophrenia Phase II: trend in reduction of positive symptoms



*PP population; patients with baseline positive symptom factor score • 18 (median split 30-35 patients/arm)

www.roche.com/irp101209.pdf

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For data on the Roche clinical trial with RG1678, see www.roche.com/irp101209.pdf or www.clinicaltrials.gov

Umbricht D, Yoo K, Youssef E, Dorflinger E, Martin-Facklam M, Bausch A, Arrowsmith R, Alberati D, Marder SR, Santarelli L. Glycine transporter type 1 (GLYT1) inhibitor RG1678: Positive results of the proof-of-concept study for the treatment of negative symptoms in schizophrenia. In ACNP, 49th Annual Meeting, Miami Beach, FL. Neuropsychopharmacology. 2010;35:S320–S321.

Umbricht D, Martin-Facklam M, Pizzagalli E, Youssef E, Yoo K, Doerflinger E, Bausch A, Arrowsmith R, Alberati D, Santarelli L. Glycine transporter type 1 (GLYT1) inhibition RG1678: Results of the proof-of-concept study for the treatment of negative symptoms in schizophrenia. Schiz. Bull. 2011;37 Suppl. 1:324.

PHASE III Trial

Six Phase III trials are planned to start by the end of 2010. NCT01192880 will randomise 630 patients from the US, China, Bulgaria, Czech Republic and Japan, with persistent, predominant negative symptoms of schizophrenia, on stable treatment with antipsychotics, to daily oral doses of RG1678 or matching placebo for 52 weeks, followed by an optional treatment extension for up to 3 years. NCT01192906 and NCT01192867 are similarly designed studies taking place in the US, which also aim to each enrol 630 patients.

Three other studies (NCT01235559, NCT01235520 and NCT01235585) will measure the effect of RG1678 on positive symptoms factor score assessed by Positive and Negative Syndrome Scale (PANSS).



GlyT-1 in phase III: exploring two indications *Negative symptoms and sub-optimally controlled patients*

	Negative symptoms of schizophrenia (3 trials)		Patients with sub-optimally controlled symptoms of schizophrenia (3 trials)	
	2x	1x	2x	1x
No. of patients	N=620 1:1:1 randomisation	N=620 1:1:1 randomisation	N=600 1:1:1 randomisation	N=600 1:1:1 randomisation
Primary endpoint	PANSS negative symptoms factor score at week 24		PANSS positive symptoms factor score at week 12	
Design	ARM A: 10 mg GlyT-1 ARM B: 20 mg GlyT-1 ARM C: placebo	ARM A: 5 mg GlyT-1 ARM B: 10 mg GlyT-1 ARM C: placebo	ARM A: 10 mg GlyT-1 ARM B: 20 mg GlyT-1 ARM C: placebo	ARM A: 5 mg GlyT-1 ARM B: 10 mg GlyT-1 ARM C: placebo
Status	FPI Q4 2010; Expect data 2013			

Two new indications, study designs and patient populations agreed with health authorities in US (SPA), Europe and Japan

GlyT-1 development: optimizing the data quality

Synergies in study design and patient recruitment



Studies for negative symptoms and partial responders developed in parallel at the same clinical sites:

- High unmet medical need in both indications
- Creates broad safety data base
- Synergy in recruitment: reduced risk of rater inflation/deflation