The Efficacy and Safety of a Selective Estrogen Receptor Beta agonist (LY500307) for Negative Symptoms and Cognitive Impairment Associated with Schizophrenia

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1.   **Background and Rationale**

Schizophrenia is a devastating mental illness affecting approximately one percent of the world's population (1). It is ranked by the World Health Organization as one of the 10 leading causes of disability worldwide (2). The unmet medical need associated with this illness is significant. Unemployment, lack of independent living and relative social isolation are experienced by the vast majority of schizophrenic patients. Negative symptoms (e.g., constricted emotional expression and social behavior) and cognitive impairment (e.g., working and verbal memory) are core clinical features of schizophrenia and significantly contribute to the functional disability associated with this illness (3). Both of these symptom domains are relatively unresponsive to approved treatments for schizophrenia, the first and second generation antipsychotic drugs, which has prompted a substantial focus on identifying new targets and developing novel therapeutic agents for these symptom complexes (4). A treatment that improves negative symptoms and/or cognitive impairment would have a major impact on the clinical landscape of schizophrenia by increasing the likelihood that patients with this illness would obtain higher levels of functioning and achieve an overall enhanced quality of life.

Estrogen's CNS Effects

Estrogen has well documented CNS effects that include neuroprotection, plasticity and improved cognitive performance (5, 6). The cognitive effects include improvement in working and verbal memory, (5) which is affected in schizophrenia (7-9). In fMRI studies, estrogen plasma levels have been correlated with cortical activation patterns produced by cognitive tasks in healthy females (10). Several neuroimaging studies have shown that treatment with estradiol in healthy women resulted in greater prefrontal activation during a working memory task compared to placebo (11-13). Similarly, neuroimaging studies in schizophrenic populations have demonstrated heightened task-related cortical activation associated with estrogen levels. For example, during a spatial rotation task, a positive correlation was found between cortical activation and estrogen levels in schizophrenic women (14).

The above clinical trial results of estrogen and its effects on brain systems described support the potential therapeutic role of estrogen for negative and cognitive symptoms associated with schizophrenia. However, estrogen has had limited therapeutic application for male and premenopausal female patients with schizophrenia because of safety concerns including uterine cancer, heart disease, and feminization in men.

Estrogen's Therapeutic Effects in Schizophrenia

Several lines of investigation have supported the potential therapeutic effects of estrogen for the negative and cognitive symptoms of schizophrenia. Women with schizophrenia have a later age of onset and less severe course of illness with fewer negative symptoms compared to males, which has led to the hypothesis that estrogen may have a neuroprotective effect for schizophrenia (15). Symptoms of schizophrenia are exacerbated during menopause and periods of the menstrual cycle when estrogen is low suggesting estrogen may have an "antipsychotic" effect (16). Additionally, low levels of estrogen have also been associated with more severe negative symptoms and cognitive deficits, including working and verbal memory impairments, in females with schizophrenia (16, 17). In a recent meta-analysis (18), estrogen therapy was shown to be effective in female patients with schizophrenia for both overall and negative symptoms (four randomized clinical trials, N=214 patients). In a separate analysis of trials assessing the efficacy of estradiol (three randomized clinical trials, N=170 female patients), even larger

effect sizes were found for overall and negative symptoms (18). In addition, clinical trials have shown that estradiol, as compared to placebo, improves cognition in females with schizophrenia (19, 20).

Rationale for Estrogen Receptor Beta as a Therapeutic Target for Schizophrenia

The effects of estrogen are mediated through two estrogen receptors – ER-alpha (ERα) and ER-beta (ERβ) which was discovered in 1996 (21-23). ERα is the primary receptor for estrogen's effects in reproductive systems. Unlike ERα, ERβ is essentially absent from adult pituitary and endometrium so agonists for this receptor subtype would not be associated with risks for chemical castration, feminization effects in men and uterine cancer (24). There is substantially greater ERβ than ERα receptor densities in the cerebral cortex and hippocampus (25), two brain regions implicated in the cognitive impairment and negative symptoms of schizophrenia (26). The hybridization signal for ERα mRNA in cerebral cortex and hippocampus is very weak while ERβ mRNA signal is robust in those regions (25). ERβ has been shown to mediate the cognitive-enhancing effects of estradiol on hippocampal-dependent spatial working memory tasks and, similarly, selective ERβ agonists improved spatial working memory and promoted hippocampal synaptic plasticity in wild-type but not ERβ null mutant mice (27). Cognitive deficits have been demonstrated in ERβ knock out studies and cognitive enhancement in preclinical studies of selective ERβ agonists (22, 28). ERβ mRNA is related to oxytocin and arginine vasopressin mRNAs in mice bred for extremes in social behavior (29) suggesting a possible role for ERβ in mediating negative symptoms. Selective ERβ agonists increase cortical GABA synthesis and increase removal of synaptic glutamate by astrocytes (21) which are plausible mechanisms to explain ERβ mediated therapeutic effects on cognitive and negative symptoms in schizophrenia. Indeed, decreased cortical GABA-ergic tone and altered cortical glutamatergic neurotransmission are among the most prominent pathophysiological hypotheses to account for the negative and cognitive symptoms of this illness (30-33). Because of ERβ's unique CNS properties, it has been proposed that ligands for this site be developed as potential therapeutics for CNS disorders including schizophrenia (23, 24).

LY500307, a selective ERβ agonist, as a potential therapeutic for schizophrenia

LY500307: Eli Lilly and Company (Lilly) has established research programs to synthesize and develop selective agonists for ERβ. Its lead compound is LY500307 which is a selective ERβ agonist with an excellent preclinical and Phase 1 profile (see below). Lilly's initial indication was for benign prostatic hypertrophy (BPH). ERβ is the predominate estrogen receptor subtype in prostate (34). In a BPH mouse model, orally administered LY500307 significantly reduced prostate weight without affecting circulating testosterone levels and it did not affect prostate weight in ERβ knock out animals. A large, multi-site Phase 2 study (N=441) of LY500307 failed to achieve its primary endpoint of improvement in the International Prostate Symptom Score. It was, however, safe and well tolerated with a paucity of adverse events and no clinical signs of ERα engagement such as suppression of testosterone levels.

2.   Preliminary Studies

LY500307 Preclinical Development

All of the LY500307 data presented in the application was obtained from Lilly data files (investigator brochures, study protocols, study summaries). LY500307 is an orally active, small molecule that was synthesized by Lilly to maximize binding to the ERβ receptor and minimize binding to the estrogen ERα receptor. LY500307 is highly potent and selective for ERβ. In competitive binding assays, its affinity for ERα is 2.04 nM, while its affinity for ERβ is 0.16 nM, representing an approximately 12-fold selectivity towards ERβ. LY500307 has negligible off target (non-estrogen receptor) binding.

Nonclinical **pharmacokinetic** studies in multiple species indicated that peak plasma concentrations (Cmax) of parent were observed from 0.5 to 8 hours after oral dosing. Following Cmax, plasma concentrations declined with elimination half-life values ranging between 3.3 and 7.8 hours for parent and between 5.9 and 21.4 hours for metabolites after oral administration. LY500307 was rapidly metabolized and the resulting metabolites were eliminated at a slower rate compared to the parent compound. LY500307 is highly bound (97.2% to 99.5%) to plasma proteins from mice, rats, monkeys and humans. The degree of plasma protein binding was independent of concentration over the ranges tested for all species. It is highly lipophilic and readily enters the CNS compartment with quantifiable levels of C-14-LY500307 in cerebrum, cerebellum, medulla and olfactory bulb. Direct glucoronidation and/or sulfation were the primary metabolic routes identified in mice, rats and monkeys.

The results of nonclinical **safety, pharmacology and toxicology** studies demonstrated an acceptable safety profile. Non-clinical toxicology studies support 24-week exposures in humans. No CNS, respiratory, or cardiovascular risks were identified at doses as high as 30 mg/kg in rats and mice and 1500 mg/kg in monkeys.   LY500307 has weak effects at hERG (IC-50 >1mM). In rat and monkey 1-, 6-, and 9-month toxicity studies, the NOEL-defining toxicity was consistent with the pharmacology of an estrogen agonist and is considered to be non-life-threatening, able to be monitored, and generally reversible. The NOEL was determined to be 1.0 mg/kg/day in the male rat and 1.5 mg/kg/day in the male monkey following 9 months of daily dosing.

Phase 1 Studies

In a **single dose escalating study (SDES) (BPAC),** healthy men (N=30, mean age: 44.3 years, age range: 23-60 years) were assigned to placebo and one of the following oral LY500307 dosing arms: 0.5, 5, 25, 125, 250, and 500 mg. In a **multiple dose escalating study (MDES) (BPAD),** healthy men (N=34, mean age: 49.5 years, age range: 21-62 years) were randomized to a 2-week treatment course of placebo and the following oral LY500307 dosing arms: 0.5, 5, 25, and 100 mg/day.

The **pharmacokinetic** concentration-time profiles of LY500307 post -single and -multiple oral doses indicates fast absorption, based on C-max within one hour following a single dose or multiple doses of LY500307 in most subjects, followed by a biexponential decline with a well-defined terminal elimination phase (Figure 1). The terminal t1/2 of LY500307 is approximately 15 hours. At steady state, the Cmax and daily exposure (area under the concentration versus time curve over the dosing interval) increased approximately 50% compared with those from a single dose or the first dose.

Figure 1. Pharmacokinetic concentration-time profiles of LY500307 post -single and -multiple oral doses
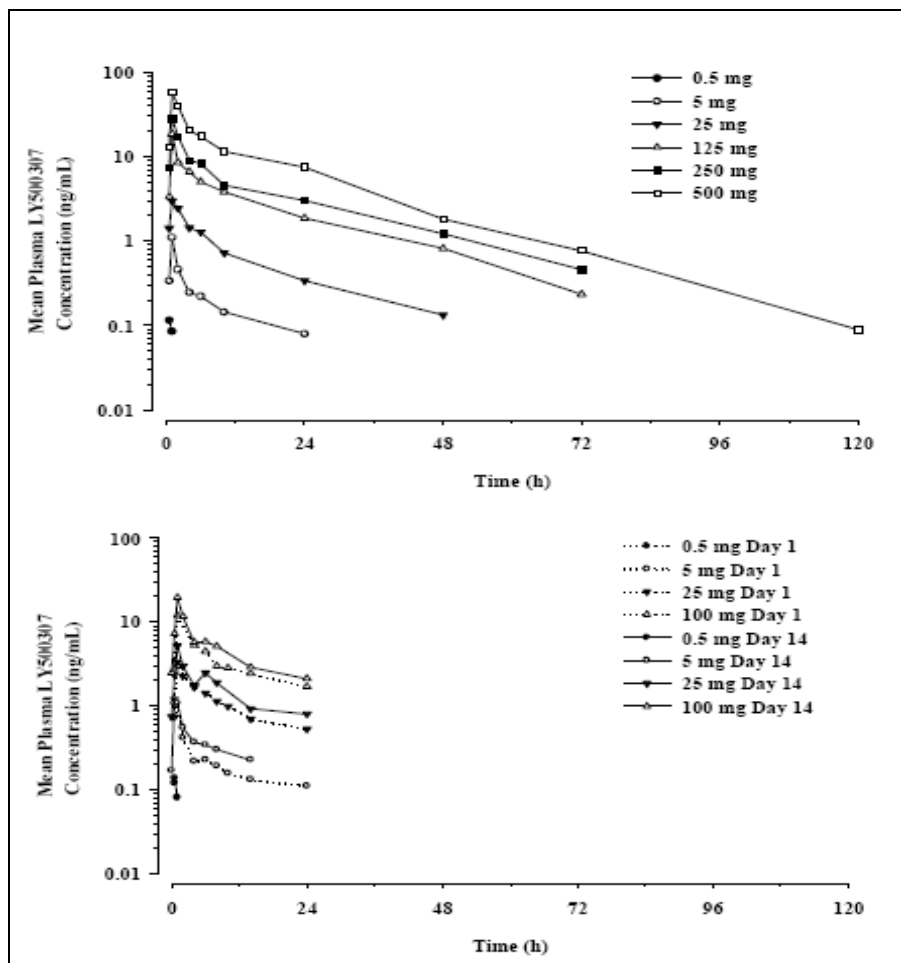
Safety in Phase 1 was assessed in the SDES (BPAC) and MDES (BPAD) described above as well as a single dose (25 mg) 14-C-LY500307 distribution study (N=6 healthy male subjects) (BPAF). Overall, LY500307 was well tolerated and associated with a paucity of adverse events (AEs). There were no serious AEs reported and no clinically significant safety concerns encountered in any of the three studies. All AEs reported in these studies were mild to moderate in severity and all were self-limiting without discontinuation of LY500307 dosing. Table 1 summarizes the AEs considered possibly/probably related to LY500307 from these studies. In addition, LY500307 produced no statistically significant changes in plasma cortisol, estradiol, thyroid-stimulating hormone (TSH) or prolactin. No significant effects were noted on vital signs, body weight or ECG recordings.

Table 1. Summary of Adverse Events Possibly/Probably Related to LY500307 Treatment

Study ID	Adverse Event	Placebo	LY500307 Dose (mg)						
			0.5	5	25	100	125	250	500
I1A-MC-BPAC	Headache								1
	Insomnia	1							
I1A-MC-BPAD	Abdominal Pain		1	1					
	Frequent BM					1			
	Fatigue				1				
	Anorexia		2						
	Polydipsia		1						
	Muscle spasms				1				
	Headache	4	1		4	2			
	Lethargy		1						
	Psychomotor Hyperactivity		1						
	Somnolence		1						
	Insomnia		2						
	Erectile Dysfunction		1						
I1A-MC-BPAF	Abdominal Discomfort				1				
	Diarrhea				1				
I1A-JE-BPAG	None Reported								

Abbreviation: ID = identification; BM = bowel movement.
Data Source: Clinical Study Report for I1A-MC-BPAC; Clinical Study Report for I1A-MC-BPAD; Clinical Study Report for I1A-MC-BPAF; bpag_v1_tfls_01dec201

ERβ Selectivity and Effects on Testosterone: Non-selective estrogenic ligands (e.g., estrogen, estradiol, selective estrogen receptor modulators, SERMs) suppress the hypothalamus-pituitary-gonadal axis, resulting in decreases in testosterone and other reproductive hormone plasma levels (35, 36). The ERα, but not ERβ, receptor mediates the suppressing effects of these hormones (35, 36). Because testosterone suppression is mediated by ERα and it is associated with less intra-subject variability than other reproductive hormones (e.g., luteinizing hormone), plasma total testosterone levels have been used as a biomarker of central ERβ selectivity in clinical trials. Preclinical studies demonstrated dose-dependent effects of LY500307 on plasma total testosterone concentrations with engagement of ERα at the higher doses tested. In the SDES, plasma total testosterone levels demonstrated significant dose-dependent decreases at doses at 25 mg and above (i.e., 125 mg, 250 mg and 500 mg). Effects at 25 mg were small (12% change from baseline). Testosterone suppression was reversible in all cases. In the 14-day MDES, testosterone suppression did not occur at any dose including the highest dose tested which was 100 mg/day. The mean change from baseline in plasma total testosterone levels (nmol/dL) between the 100 mg/day assigned group (N=6) and placebo assigned group (N=8) across all time points ranged from a difference of -2.50 (p=0.205) to 0.99 (p=0.621). In the Phase 2 BPH study (see below), there were no effects observed on total testosterone or other reproductive hormones at any dose including 25 mg/day which was the highest dose tested. These data suggest that 25 mg is an ERβ selective dose. The MDES provide support for ERβ selectivity at doses up to 100 mg/day.

Drug-drug interaction: Based on data from definitive in vitro studies that assess cytochrome P450 (CYP) - mediated drug interactions and plasma concentrations of LY500307 from Phase 1 and Phase 2 trials, the risk for LY500307 to inhibit or induce major CYP isoform is low. No mechanism-based inactivation of any of the CYPs tested by LY500307 was found. These data suggest that LY500307 co-administration with most second generation antipsychotic medications and mood stabilizers (commonly metabolized by CYP3A4,5,6, CYP2C9,CYP1A, CYP2C19, or CYP2D6) is unlikely to lead to CYP-mediated DDI.

Phase 2 Study of BPH

LY500307 was tested as a potential treatment for BPH in an outpatient, multisite 24-week, randomized, placebo-controlled Phase 2 dose finding study in men 45 years and older (median ages for the treatment groups ranged from 63.55 to 67.47 years) with lower urinary tract symptoms and enlarged prostates secondary to BPH. Four hundred and forty one male subjects were enrolled and randomized to placebo or one of the following LY500307 treatment arms: 1, 3, 10, or 25 mg doses of LY500307. LY500307 failed to achieve its primary endpoint of improvement in the International Prostate Symptom Score. It was, however, well tolerated with a paucity of adverse events. The incidence rates of serious adverse events (SAEs), total AEs, and early terminations due to AEs were low and comparable between placebo and all LY500307 treatment arms. There was no evidence of effects on plasma total testosterone in any dosage arm. Total testosterone data are shown for all dosage arms at baseline (week 0), week 12 and week 24 in Table 2. Two consecutive post randomization plasma total testosterone values below 300 ng/dL, which is the lower limit of the normal range, was an *a priori* determined criterion for treatment discontinuation. Few subjects met this criterion and there was no relationship to study dose (placebo N=5, 1 mg N=3, 3 mg N=0, 10 mg N=2, and 25 mg N=4). There were no alterations in lipid levels or routine blood counts and no changes in weight and vital signs in subjects randomized to any LY500307 treatment dose. In addition, LY500307 had no effects on reported sexual function. The PK profile of LY500307 in BPH patients was similar to the PK profile in healthy men in the Phase 1 trials.

Table 2. Total testosterone (ng/dL) change from baseline in the Phase 2 BPH study (BPAE)

| | | | | | | | | | vs. Placebo | | |
Visit	Treatment	n	Mean	SD	Median	Min	Max	LSMean	LSMean Change	90% CI	p-value
Week 0	Placebo	83	517.1	211.4	495.0	184.0	1500.0	518.2			
	1 mg	82	508.2	170.5	489.5	233.0	1230.0	509.3	-8.9	(-61.8, 44.0)	.781
	3 mg	78	520.6	189.0	491.5	137.0	1026.0	519.0	0.7	(-52.9, 54.4)	.982
	10 mg	79	557.8	270.8	531.0	156.0	2534.0	559.0	40.8	(-12.5, 94.1)	.208
	25 mg	82	510.6	171.0	485.5	192.0	994.0	512.2	-6.0	(-58.9, 46.8)	.851
Week 12	Placebo	62	14.4	117.9	-1.0	-190.0	310.0	8.7			
	1 mg	62	-7.2	126.1	20.5	-513.0	206.0	-10.7	-19.5	(-54.61, 15.70)	.362
	3 mg	62	19.4	161.2	0.5	-313.0	836.0	19.1	10.4	(-24.82, 45.52)	.628
	10 mg	65	-1.3	99.4	-3.0	-394.0	174.0	6.8	-2.0	(-36.73, 32.82)	.926
	25 mg	68	34.5	134.5	32.5	-332.0	526.0	32.3	23.6	(-10.73, 57.93)	.258
Week 24	Placebo	57	-14.6	122.7	-27.0	-352.0	295.0	-15.4			
	1 mg	54	-12.5	166.4	7.3	-835.0	343.0	-19.5	-4.1	(-41.01, 32.72)	.853
	3 mg	57	-1.3	129.6	-3.0	-230.0	410.0	-0.7	14.7	(-21.73, 51.05)	.507
	10 mg	59	-24.9	112.2	-13.0	-296.0	282.0	-15.4	-0.0	(-36.11, 36.06)	>.999
	25 mg	63	24.6	136.7	4.0	-204.7	404.5	23.3	38.7	(3.22, 74.20)	.073

While preclinical models provided compelling support for LY500307's efficacy for BPH, it is unclear why the Phase 2 study failed to meet its primary efficacy endpoint. It was speculated that the study's highest dose of 25 mg/per day may have been too low and/or the advanced age of the BPH subjects may have contributed to the failure to demonstrate efficacy for BPH. It is well known that as men age, the hypothalamus-pituitary-gonadal sensitivity to estrogen suppression decreases (37).

Phase 1b/2a clinical trial in schizophrenia (blinded data)

The Phase 1b/2a clinical trial in schizophrenia is ongoing. Thirty of the thirty subjects have completed the trial. Investigators are blind to study drug assignment; however there have been no serious adverse events, no evidence of total testosterone suppression (as defined by a 50% reduction since the Visit 2 value) (see Table 3), no evidence of QTcB prolongations (see Table 4), and no adverse events (AEs) considered

"probably related" to study drug. There have been eight AEs determined to be "possibly related" to study drug as indicated in Table 5 below.

Table 3. Total Testosterone Levels (ng/dL) and Percent Changes in Testosterone from Baseline

Subject Number	Age	V1 (Screening)	V2 (Baseline)	V3 (2 weeks)		V4 (4 weeks)		V6 (8 weeks)	
		Value (ng/dL)	Value (ng/dL)	Value (ng/dL)	% Change	Value (ng/dL)	% Change	Value (ng/dL)	% Change
B4001	25	659	450	524	(+)16.4	764	(+)69.8	497	(+)10.4
B4002	22	449	569	516	(-)9.3	581	(+)2.1	606	(+)6.5
B4004	47	545	530	465	(-)12.3	505	(-)4.7	587	(+)10.8
B4006	51	912	736	770	(+)4.6	828	(+)12.5	616	(-)16.3
B4007	36	472	377	513	(+)36.1	436	(+)15.6	426	(+)13.0
B4009	35	512	423	506	(+)19.6	370	(-)12.5	481	(+)13.7
B4010	23	516	585	572	(-)2.2	553	(-)5.5	516	(-)11.8
B4012	24	780	796	900	(+)13.1	844	(+)6.0	882	(+)10.8
B4015	19	323	334	342[a]	(+)2.4	329	(-)1.5	436	(+)30.5
B4016	50	379	412	416	(+)1.0	398	(-)3.4	479	(+)16.3
B4017	53	449	435	457	(+)5.1	416	(-)4.4	421	(-)3.2
B4019	53	416	563	Not Assessed[b]	N/A	487	(-)13.5	536	(-)4.8
B4021	27	707	675	825	(+)22.2	756	(+)12.0	749	(+)11.0
B4022	26	570	575	682	(+)18.6	704	(+)22.4	660	(+)14.8
B4023	36	543	344	561	(+)63.1	396	(+)15.1	356	(+)3.5
B4024	30	486	530	643	(+)21.3	410	(-)22.6	606	(+)14.3
B4025	23	398	497	429	(-)13.7	643	(+)29.4	545	(+)9.7
B4026	55	753	855	779	(-)8.9	546	(-)36.1	546	(-)36.1
B4027	21	438	492	477	(-)3.0	446	(-)9.3	414	(-)15.9
B4028	28	430	175[h]	394	(+)125.1	243[g]	(+)38.9	357	(+)104.0
B4030	40	419	444	525	(+)18.2	514	(+)15.8	517	(+)16.4
B4031	33	348	400	496	(+)24.0	401	(+)0.3	492	(+)23.0
B4032	47	398	349	350	(+)0.3	382	(+)9.5	414	(+)18.6
B4033	37	351[c]	242[h]	300[d]	(+)24.0	317[f]	(+)31.0	326	(+)34.7
B4034	32	326	311	383	(+)23.2	312	(+)0.3	345	(+)10.9
B4035	25	312	397	522	(+)31.5	541	(+)36.3	495	(+)24.7
B4036	36	385	340	471	(+)38.5	362	(+)6.5	322	(-)5.3
B4039	19	628	710	671	(-)5.5	667	(-)6.1	654	(-)7.9
B4041	21	503	439	570	(+)29.8	559	(+)27.3	535	(+)21.9
B4042	30	337[e]	357	335	(-)6.2	432	(+)21.0	393	(+)10.1

a=Visit 3 occurred on 10.18.13 and sample was drawn at 09:10AM. Result was reported on 10.21.13 with a flagged value of 247 ng/dL. Subject returned to clinic on 10.22.13 and testosterone was redrawn at 07:37AM. Result was reported on 10.23.13 with a value of 342 ng/dL, within normal limits. Subject did not meet discontinuation criteria as retest value was above 300 ng/dL.

b=Evaluation of testosterone on 11.13.13 was cancelled by the lab due to lab accident. Testosterone sample was drawn and assessed within normal range with a value of 487 ng/dL at subsequent visit on 11.25.13. Therefore V3 testosterone was not assessed.

c=Visit 1 occurred on 02.10.14 and sample was drawn at 09:30 AM. Result was reported on 02.12.14 with a value of 302 ng/dL. Subject returned to clinic on 02.13.14 and testosterone was redrawn at 07:55 AM. Result was reported on 02.14.14 with a value of 351 ng/dL, within normal limits.

d=Visit 3 occurred on 03.13.14 and sample was drawn at 08:14 AM. Result was reported on 03.14.14 with a flagged value of 240 ng/dL. Subject returned to clinic on 03.19.14 and testosterone was redrawn at 07:40 AM. Result was reported on 03.20.14 with a value of 300 ng/dL, within normal limits. Subject did not meet discontinuation criteria as retest value was above 300 ng/dL.

e=Visit 1 occurred on 03.17.14 and sample was drawn at 09:29 AM. Result was reported on 03.18.14 with a value of 299 ng/dL. Subject returned to clinic on 03.19.14 and testosterone was redrawn at 08:38 AM. Result was reported on 03.20.14 with a value of 337 ng/dL, within normal limits. Subject did not meet discontinuation criteria as retest value was above 300 ng/dL.

f=Visit 4 occurred on 03.27.14 and sample was drawn at 07:42 AM. Result was reported on 03.28.14 with a value of 285 ng/dL. Subject returned to clinic on 04.03.14 and testosterone was redrawn at 07:27 AM. Result was reported on 04.04.14 with a value of 317 ng/dL, within normal limits. Subject did not meet discontinuation criteria as retest value was above 300 ng/dL.

g=Visit 4 occurred on 04.03.14 and sample was drawn at 07:13 AM. Result was reported on 04.04.14 with a value of 259 ng/dL. Subject returned to clinic on 04.09.14 and testosterone was redrawn at 08:26 AM. Result was reported on 04.10.14 with a value of 243 ng/dL.

h=Study inclusion is based on Visit 1 values because the result of Visit 2 values are received after study medication initiation/randomization.

Table 4. ECG QTcB's Values and Value Changes from
Baseline

Subject Number	V1 (Baseline)	V4		V6	
	Value (ms)	Value (ms)	Value Change Since Baseline	Value (ms)	Value Change Since Baseline
B4001	401	396	(-)5	390	(-)11
B4002	416	390	(-)26	391	(-)25
B4004	395	404	(+)9	408	(+)13
B4006	397	403	(+)6	380	(-)17
B4007	440	444	(+)4	405	(-)35
B4009	397	394	(-)3	417	(+)20
B4010	397	389	(-)8	401	(+)4
B4012	389	393	(+)4	419	(+)30
B4015	379	378	(-)1	420	(+)41
B4016	447	438	(-)9	456[a]	(+)9
B4017	417	419	(+)2	412	(-)5
B4019	402	398	(-)4	414	(+)12
B4021	408	408	0	402	(-)6
B4022	405	414	(+)9	408	(+)3
B4023	433	421	(-)12	429	(-)4
B4024	407	412	(+)5	404	(-)3
B4025	406	403	(-)3	402	(-)4
B4026	396	403	(+)7	396	0
B4027	402	390	(-)12	404	(+)2
B4028	390	386	(-)4	383	(-)7
B4030	399	410	(+)11	405	(+)6
B4031	430	409	(-)21	405	(-)25
B4032	409	404	(-)5	404	(-)5
B4033	440	418	(-)22	412	(-)28
B4034	406	410	(+)4	413	(+)7
B4035	397	402	(+)5	405	(+)8
B4036	420	438	(+)18	405	(-)15
B4039	408	402	(-)6	416	(+)8
B4041	370	397	(+)27	378	(+)8
B4042	449	408	(-)41	412	(-)37

ECG QTcB's Values and Value Chages from Baseline in Schizophrenia Patients Studied in Estrogen Receptor Beta Pro

a= Average of 3 QTcB's was 447ms

Table 5. Phase 1b/2a Clinical Trial Adverse Events "Possibly Related" to Study Drug (blinded data)

System	Specific AE	Start Date	Stop Date	Mild	Moderate	Severe
Neuropsychiatric	Somnolence	31-Mar-2014	a		X[d]	
	Head "tightness"	25-Dec-2013	a	X		
	Insomnia	27-Feb-2014	a	X		
Dermatologic	Rash, nonpruritic	06-Jan-2014	16-Jan-2014	X		
Endocrine	Unilateral breast tenderness [b,c]	04-Oct-2013	31-Oct-2013	X		
Gastrointestinal	Increased appetite	28-Feb-2014	a	X		
Genitourinary	Urinary frequency	02-Mar-2014	a		X	
	Delayed ejaculation	03-Sep-2013	14-Sep-2013	X		

a. Ongoing at the end of the end of the trial

b. This event occurred in the absence of decreases in total testosterone and at a time when a new antipsychotic medication was initiated

c. Prolactin values are as follows: 22-Aug-2013 = 25.7 ng/mL, on 20-Sept-2013 = 49.5 ng/mL, and on 17-Oct-2013 = 14.6 ng/mL.

d. Initially assessed as "mild" in severity on 28-Feb-2014, but increased to "moderate" in severity on 31-Mar-2014

3.   Specific Aims

Several lines of investigation have demonstrated that estrogen is an effective treatment for the negative and cognitive symptoms of schizophrenia. However, estrogen treatment itself is prohibitive for male and pre-menopausal female patients because of safety concerns that include uterine cancer, heart disease and feminization effects in men. The effects of estrogen are mediated through two estrogen receptors – ER-alpha (ERα) and ER-beta (ERβ) (24). Data suggest that selective estrogen receptor β (ERβ) agonists may be an effective treatment for the cognitive impairment and negative symptoms of schizophrenia without the major safety risks of estrogen therapy. The **primary objectives** of this application are to select the LY500307 dose that provides the best opportunity to assess full cortical target engagement and to assess efficacy of this dose for negative and/or cognitive symptoms in patients with schizophrenia. The aims are to determine if LY500307 is safe and well tolerated in this population and whether it elicits a sufficient efficacy signal to be advanced for further testing in schizophrenia. A single seamless, two-staged, Phase 1b/2a adaptive design will be used. The purpose of Stage 1 is to evaluate two LY500307 doses (25 mg/day and 75 mg/day) to determine which dose should be advanced to Stage 2 for continued dose assessment, cortical target engagement, and efficacy assessments.

Primary Aim 1: To determine if LY500307 demonstrates cortical target engagement as assessed by fMRI/N-back in frontal-parietal regions. Secondary measures of target engagement are fMRI episodic memory, Pseudo-Continuous Arterial Spin Labeling, Mismatch Negativity/evoked response potentials, Auditory Steady State Response, Auditory P300 and Quantitative EEG (QEEG).

Primary Aim 2: To determine if LY500307 is superior to placebo for one or more of the primary efficacy endpoints: negative symptoms (Negative Symptom Assessment Scale – 16-item total score), working memory (the composite score for the Letter Number Sequencing and Spatial Span tests) and verbal memory (Hopkins Verbal Learning Test).

Primary Aim 3: To determine if LY500307 reduces total testosterone (TT) plasma concentrations, which is indicative of loss of selectivity for ERβ and engagement of ERα, using the following criteria: Decrease in TT plasma concentrations of 50% from baseline in 50% of subjects per arm treated for two consecutive post-randomization values with LY500307 in Stage 1 and Stage 2 of the trial.

Primary Aim 4: To assess the safety of LY500307 by determining if there are SAEs, AEs "probably related to study drug," QTc prolongation, TT suppression (50% reduction from baseline) and to evaluate for other safety signals.

4. Study Design/Investigational Plan

A two-stage Phase 1b/Phase 2a adaptive ("drop the inferior dose") experimental design is ongoing that combines three studies (clinical dose optimization, cortical target engagement confirmation and efficacy and safety assessment) into a single clinical trial.

Stage 1 was conducted in year 1 and Stage 2 will be conducted in years 2 and 3. The goal of Stage1 was to identify and advance the highest dose that did not demonstrate a safety signal and had target selectivity as determined by lack of TT suppression. If these criteria were fulfilled at both doses, the larger of the two (75 mg/day dose) would be advanced to Stage 2. Furthermore, if there was no suggestion of ERα receptor activation (i.e., no pattern of TT decreases or feminization AEs) at either dose (25 mg/day and 75 mg/day), a third arm of 150 mg/day would be added to Stage 2 for evaluation. This scenario then results in the following three arms in Stage 2: placebo, 75 mg/day and 150 mg/day. The goals of stage 2 are to further assess LY500307 doses for safety and target selectivity, confirm cortical target engagement and assess efficacy.

In evaluating Stage 1 blinded safety and target selectivity (i.e., TT effects) data, the pattern of results (Tables 3, 4, and 5 above) suggest that both the 25 mg/day and 75 mg/day doses are safe and free of ERα receptor effects and would result in advancing the 75 mg/day dose to Stage 2. It should be noted that inspection of the blinded TT data from 30 completed subjects reveals that the TT suppression Go/No Go criterion, which requires five of 10 subjects per arm to demonstrate at least a 50% decrease in TT from baseline for "No Go," has been fulfilled as there has been no evidence of TT suppression.

The trial will be run "seamlessly" which means that the transition from Stage 1 to Stage 2 will be conducted without significant interruption and without breaking the study blind. The advantages of the two-stage seamless adaptive design are the efficient use of subjects, increased statistical power for final analysis and more rapid completion of the trial by minimizing the time between the end of Stage 1 and commencement of Stage 2. All data from the Stage 1 will be combined with Stage 2 data for the final analyses after the completion of Stage 2. In Stage 1, there are 10 subjects per arm (a total of 30 subjects).

Stage 2-Three Arm Dosing (Placebo, 75 mg/day, and 150 mg/day): During Stage 2 an additional 70 subjects will be randomized in a 1:1:1.5 ratio, with 20 assigned to placebo, 20 assigned to 75 mg/day and 30 assigned to 150 mg/day. Thus, a total of 100 subjects (30 subjects in Stage 1 plus 70 subjects in Stage 2) are required for this study. The final target engagement, efficacy, and safety analyses will include data from all 100 subjects and a dose-concentration-response analytic strategy that is highly sensitive to signal detection will be used.

Rationale for Stage 1 Doses

The selection of LY500307 25 mg/day dose for assessment in Stage 1 was based on the extensive preclinical, Phase 1 and Phase 2 BPH trial data sets. This dose was safe, well tolerated and selective for ERβ receptors without engagement of ERα receptors as demonstrated by the lack of effects on total testosterone levels (Table 3). The 75 mg/day dose was selected based on the Phase 1a multiple dose escalating study (MDES) that demonstrated 100 mg/day was safe, well tolerated and failed to affect

plasma testosterone levels. Safety including potential changes in testosterone levels was carefully monitored throughout the trial. If testosterone levels dropped below the normal lower limit of 300 ng/dL, a repeat value was assessed. If the retest value was also below 300 ng/dL, the subject met discontinuation criteria.

Rationale for Stage 2 Doses

The rationale for selecting 150 mg/day as the higher dose option is based on data from Lilly's Phase 1 14-day multi-dose escalating study (MDES) of LY500307 in healthy male volunteers. This study revealed that no dose, including the highest dose tested at 100 mg/day, suppressed testosterone or was associated with concerning adverse events. Lilly's Phase 1single dosing studies (BPAC and BPAF) reveal no AEs deemed to be "possibly/probably related" to LY500307 at 125 mg/day or 250 mg/day and only one AE (headache) at 500 mg/day (see Table 1 above). In addition to enhancing target engagement in the current Phase 1b/2a study, the 150 mg/day dose will allow for robust dose-concentration-response analyses of biomarker and efficacy endpoints across all arms tested (i.e., placebo, 25 mg/day, 75 mg/day, and 150 mg/day) (see Section 16 below).

Addressing Safety in Stage 2

Escalating doses of selective ERβ agonists have the potential for losing ERβ selectivity and commence the engagement of ERα receptors which may produce "estrogen-like" effects including adverse events associated with estrogen therapy. One such event is potential thromboembolic events (e.g., deep vein thrombosis, stroke, cardiovascular events). However, the overall rate of thromboembolic events associated with estrogen therapy is relatively low and risk factors for these events include past history of thromboembolic events, advanced age, greater duration of exposure and higher dose estrogen preparations (35, 38-41). We anticipate the risk to be somewhat reduced based on our study sample; the study sample is predominately young-to-middle aged (Stage 1 mean age of 33.9 years old) whereas advanced age is a risk factor for thromboembolic events during estrogen therapy.

A comprehensive safety plan is included in protocol (see Section 9 below) to ensure the safety of subjects participating in Stage 2 and that the risk for "estrogen-like" adverse events including thromboembolic events is mitigated. It has been demonstrated in Lilly's Single Dose Escalating Study (BPAC) that LY500307 related TT suppression occurs relatively rapidly. Therefore, potential cases of TT suppression in this study will be likely detected at week 1 (Visit 2.5), the first post-randomization TT assessment point. Importantly, this relatively short exposure interval and close monitoring of ERα engagement greatly reduces the possibility of experiencing adverse events, particularly an estrogen-like induced thromboembolic event. Moreover, related to the risk that higher dose estrogen preparations have greater thrombogenic potential than lower doses, this risk factor is addressed in the current protocol by purposely dosing LY500307 to avoid ERα receptor binding and thus negating the effects of full estrogen-like receptor activation that occurs with high dose estrogen preparations. In addition, ERα engagement is closely monitored with serial TT sampling. TT and safety will be assessed at screening, baseline, and weeks 1, 2, 4 and 8 during the treatment phase of the study.

Rationale for Primary Endpoints

Primary Aim 1: fMRI/N-back was selected as the primary biomarker of cortical target engagement because it has been shown to be sensitive to estrogen (11) and numerous studies have consistently demonstrated that this paradigm demonstrates abnormal frontal and parietal activation in schizophrenia.(42-45)

Primary Aim 2: The Negative Symptom Assessment Scale – 16-item (NSA-16) is a commonly used, validated instrument for assessing negative symptoms in schizophrenia clinical trials (46). The three cognitive tests were chosen because 1) the literature indicates they are sensitive to estrogen (5), 2) they reflect core cognitive impairment in schizophrenia (47) and 3) they are contained in the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB) which has been validated for schizophrenia treatment trials and endorsed by NIMH and FDA for studies of cognitive impairment associated with this illness (48). The Hopkins Verbal Learning Test has 6 alternative forms to mitigate practice effects and the Letter Number Sequencing and Spatial Span tests have minimal practice effects after the first follow up testing session.

Primary Aim 3: Both estrogen therapy and ERα agonists, but not selective ERβ agonists, robustly suppress plasma testosterone levels (35, 36). Thus, decreases in plasma testosterone concentrations from baseline levels in the LY500307 arms would reflect ERα engagement. Although schizophrenia and antipsychotic drugs are not directly associated with altered testosterone levels (49), antipsychotics may increase plasma prolactin levels which could secondarily reduce plasma testosterone levels (50). In addition, other factors such as smoking status, age and obesity may influence testosterone concentrations (51). Thus each subject will be used as his own control in assessing LY500307 potential effects on this hormone to address the effects of potential confounds.

NIMH Research Domain Criteria (RDoC) Initiative: The NIMH RDoC initiative, which is a component of the NIMH strategic plan, encourages the assessment of dimensions of observable behaviors and brain functions that may cut across traditional diagnostic categories (see NIMH/RDoC web page). RDoC principles have informed multiple aspects of the study design. While the DSM diagnosis of schizophrenia will be used for entry into the protocol, this heterogeneous disorder will also be characterized by dimensional measures of symptoms, cognitive processes and neurobiological function. This will enable the evaluation in secondary analyses whether these dimensional measures predict treatment response. Second, working memory and episodic memory outcomes will be evaluated across units of analysis, including behavioral, fMRI and ERPs. Three working memory paradigms recommended by the RDoC Working Memory Workshop were selected: N-back (which is coupled with fMRI), Letter Number Sequencing and Spatial Span. The ERP paradigms, P300 and MMN, both exemplify the construct of "Change Detection Tasks." Thus, the findings from this study will likely assist in the validation of the sensitivity of several key RDoC constructs to estrogen modulation across multiple levels of analysis.

Clinical Research Site

The **IU Psychotic Disorders Program (IUPDP),** which is directed by the PI (Breier) and part of the IU Department of Psychiatry, is located in Indianapolis, Indiana. IUPDP research personnel will manage the day-to-day activities of conducting the trial, including subject recruitment, consenting and screening subjects, conducting study visits, performing assessments, and study medication management. The IUPDP has three research psychiatrists, a fully dedicated study coordinator, two fully dedicated clinical research specialists, two fully dedicated subject recruiters, three fully dedicated research technicians, a data manager and three raters (1 PhD, 2 Masters level) who have been trained and have extensive experience in conducting the assessments in this application including the NSA-16 (46) and the cognitive tests used as primary outcome measures.

Sample Size Determination

Thirty subjects will be randomly assigned to three arms (1:1:1) in Stage 1. In Stage 2, 70 additional subjects will be randomized to placebo, the selected LY500307 arm from Stage 1, or the 150 mg/day arm. Thus a total of 100 subjects will be randomized for the study. In a study of chronic schizophrenia,

Shekhar et al (52) reported significant effects of the mixed M1/M4 agonist xanomeline (N=20) on verbal and working memory (p<0.05) and trend effects of negative symptoms (p=0.08) with effect sizes up to 0.9 (52). Based on the estimates from a cross-over study of a nicotinic agonist in patients with schizophrenia (53), it is reasonable to expect an improvement in the working memory score with a mean of 4.5 and a standard deviation of 7.3 using data from the less effective of two doses in that study. Thus for this study, we assume a treatment effect size of 0.61 between the placebo group and the LY500307 treated group. The power estimate based on the variance of the change is conservative for the proposed analyses that actually use baseline outcome values as covariates. We will test the treatment effects on the three primary efficacy measures (negative symptoms, working memory and verbal episodic memory) at the end of Stage 2 with one-tailed tests at an overall alpha=0.1 (see *Stage 2 Efficacy Analysis* in Section 16.0). Adjusting for multiple comparisons, the test for each of the three individual efficacy outcomes will be performed at alpha=0.0333. The fMRI data will be tested at alpha=0.05. With 30 subjects each in the placebo, 75mg and 150 mg groups across the entire study, 10 subjects assigned to 25 mg/day from Stage 1, and the employment of the dose-concentration-response analytic strategy, we will have >80% power to detect a beneficial treatment effect on each outcome for any effect size $\geq$0.61 (measured in SD of the change).

5.   Study Population (Inclusion/Exclusion Criteria)

Medically stable male subjects with a confirmed diagnosis of schizophrenia as determined by the Structured Clinical Interview for DSM-IV-TR (SCID) (54) interview will be enrolled in the trial. The rationale for including only males is based on the lack of data on females in the Lilly LY500307 Phase 1 and 2 data sets. If the results of the current proposal are positive, Phase 1 studies in females with schizophrenia could be rapidly conducted and females could then be potentially included in future Phase 2 and 3 trials.

Clinical trial guidelines established by the NIMH sponsored MATRICS initiative (55) was used for the proposed trial. As recommended by MATRICS, subjects will meet DSM IV-TR diagnostic criteria for schizophrenia and will be clinically stable prior to enrollment. Enrolling stable subjects will help to isolate changes in cognitive and negative symptoms apart from changes in other symptoms and clinical features. Extrapyramidal signs may confound interpretation of therapeutic effects on cognition and negative symptoms so only subjects without significant EPS will be enrolled. Treatments with anticholinergic properties will be monitored throughout the trial using the Anticholinergic Cognitive Burden Scale (ABS) (56). Guidelines developed for assessing the efficacy of agents for cognitive impairment associated with schizophrenia do not recommend excluding patients based on their cholinergic medication scores. The 1:1:1.5 randomization used in this study will mitigate the preferential effects of medication properties on the experimental arm. Analyses are based on change from baseline cognitive testing which will mitigate preferential effects of medication properties on the experimental arm. Previously reported studies demonstrating positive effects of agents on cognition in schizophrenia did not screen out patients based on ABS scores. Therefore, the ABS will be used to monitor psychotropic medications based on their anticholinergic effects and not used to determine inclusion/exclusion. MATRICS recommends adding the investigational compound to a stable regimen of antipsychotic drug therapy to further minimize the likelihood that changes in psychosis and other clinical features of schizophrenia during the study will compromise the interpretation of primary drug effects on cognition. The MATRICS guidelines do not recommend requiring minimum cut off scores on cognitive tests as inclusion criteria. However, a minimum level of negative symptoms will be required for inclusion in the current study.

Inclusion Criteria:

1. 18 to 65 years of age at study entry
2. Male
3. DSM IV-TR diagnosis of schizophrenia as confirmed by Structured Clinical Interview for DSM-IV-TR (SCID)
4. Outpatient or inpatient status
5. Mild to moderate overall disease severity as defined by a CGI-S score of less than or equal to 4 (moderately ill) at randomization
6. Moderate levels of negative symptoms as defined by a PANSS negative symptom sub-score $\geq$ 11.
7. Clinical stability as defined by:
    a. No exacerbation of illness leading to an intensification of treatment in the opinion of the investigator within four weeks prior to randomization

Exclusion Criteria:
1. Subjects with current acute, serious, or unstable medical conditions, including, but not limited to: inadequately controlled diabetes, asthma, COPD, severe hypertriglyceridemia, recent cerebrovascular accidents, acute systemic infection or immunologic disease, unstable cardiovascular disorders, malnutrition, or hepatic, renal gastroenterologic, respiratory, endocrinologic, neurologic, hematologic, or infectious diseases
2. Known or suspected history of prostate cancer, breast cancer, or other clinically significant neoplastic disease (other than squamous cell or basal cell carcinoma of skin)
3. Known or suspected history of thromboembolic events including deep venous thrombosis, stroke, venous thromboembolism, pulmonary embolism, paresis or paralysis that may be thrombogenic in origin.
4. Subjects currently receiving testosterone replacement therapy or drugs that influence the hypothalamus-pituitary-gonadal axis.
5. Subjects who have clinically significant extrapyramidal signs (EPS) as defined by a score of >20 on the Simpson-Angus Scale (SAS) (57)
6. Clinically significant electrocardiogram (ECG) abnormality, including, but not limited to, a corrected QT interval (Bazett's; QTcB) >450 msec. Repeat ECGs may be conducted at the discretion of the principal investigator.
7. Subjects with known medical history of Human Immunodeficiency Virus positive (HIV+) status
8. Subjects with an active seizure disorder
9. Subjects with implanted pacemaker, medication pump, vagal stimulator, deep brain stimulator, TENS unit, ventriculoperitoneal shunt, or other contraindication to undergoing an MRI scan
10. Known IQ less than 70 based on medical history
11. Current DSM IV-TR diagnosis of substance dependence (excluding caffeine and nicotine)
12. Subjects who test positive for (1) Hepatitis C virus antibody or (2) Hepatitis B surface antigen (HBsAg) with or without positive Hepatitis B core total antibody
13. Subjects with moderate to severe renal impairment as defined by creatinine clearance (CrCl) < 60 ml/min (measured by the Cockcroft-Gault equation) at screening. Repeat evaluation may be conducted at the discretion of the Principal Investigator.
14. Subjects with hepatic impairment as defined by liver transaminases or total bilirubin > 3 × upper limit of normal (ULN). Repeat evaluation may be conducted at the discretion of the Principal Investigator.
15. Subjects considered a high risk for suicidal acts – active suicidal ideation as determined by clinical interview OR any suicide attempt in 30 days prior to screening
16. Subjects who have participated in a clinical trial with any pharmacological treatment intervention for which they received study-related medication in the four weeks prior to randomization OR subjects currently receiving treatment (within 1 dosing interval plus four weeks) with an investigational depot formulation of an antipsychotic medication
17. Subjects who demonstrate overtly aggressive behavior or who are deemed to pose a substantial risk of danger in the Investigator's opinion

6.   Subject Recruitment

Subjects will be recruited through referring community mental health centers, treatment providers, invited to participate if they are included in our registry, and self-referrals through advertisement and word-of-mouth.

7.   Study Procedures

The following assessments and procedures will be administered according to the study procedures table (Attachment A). All assessments will be completed by study personnel trained to administer the instruments and will be based on interviews with the subject or questionnaires completed by the subject.

8.   Clinical Assessments and Procedures

Primary Efficacy Outcome Measures:

Negative Symptom Assessment Scale – 16-item (NSA-16), a primary outcome measure, is used to help clinicians rate behaviors (not psychopathology) commonly associated with negative symptoms of schizophrenia. The scale rates subjects on 16 "anchors," is a semi-structured, clinical interview, and each item is rated from 1 to 6. The total score is the sum of the 16 specific items and ranges from 16 to 96; a higher score indicates greater severity of illness. In addition, there is a global rating which represents the overall assessment of a subject's negative symptoms. The rating should not be an average of any particular behavior, but a gestalt of everything observed in the interview (58).

Hopkins Verbal Learning Test-Revised, Wechsler Memory Scale-Third Ed, Spatial Span, and Letter-Number Sequencing are cognitive tests for verbal memory (Hopkins) and working memory (Spatial Span and Letter-Number Sequencing combined as a single composite score). These tests are part of the MCCB (48) which has proven reliability for clinical trials of cognitive impairment associated with schizophrenia. For each cognitive test, a multi-item score will be derived based on the raw item values according to the MCCB scoring manual. Each of the individual item raw scores is standardized to age- and gender-corrected t-scores (mean=50, standard deviation=10). Each working memory test will be converted in to a single domain score.

Secondary Outcome Measures:

MATRICS Consensus Cognitive Battery (MCCB) will be administered in its entirety at baseline, Visit 4, and the end of study. It is a reliable cognitive battery (test-retest reliability ~0.7 for most tests, (48). Small but statistically significant practice effects have been noted with speed of processing and problem-solving subtests (1/5th of standard deviation). To overcome these practice effects, alternate forms of MCCB will be implemented in this study. The total average administration time of MCCB is approximately 75 minutes. MCCB is comprised of seven cognitive domains and 10 related tests (Trail Making Test: Part A; Brief Assessment in Cognition in Schizophrenia: Symbol Coding; Hopkins Verbal Learning Test-Revised; Wechsler Memory Scale-Third Ed: Spatial Span; Letter-Number Sequencing; Neuropsychological Assessment Battery: Mazes; Brief Visuospatial Memory Test-Revised; Category Fluency: Animal Naming; Mayer-Salovey-Caruso Emotional Intelligence Test: Managing Emotions; and Continuous Performance Test-Identical Pairs). For each cognitive test, a multi-item score will be derived based on the raw item values according to the MCCB scoring manual. Each of the individual item raw scores is standardized to age- and gender-corrected t-scores (mean=50, standard deviation=10). Each test

will be converted in to a domain score, with Working Memory and Speed of Processing domains consisting of 2 and 3 tests, respectively.

Note: At day 14 and 42 only the Hopkins Verbal Learning Test-Revised, Wechsler Memory Scale-Third Ed: Spatial Span, and Letter-Number Sequencing will be administered. At day 0, 28, and 56, the entire MCCB will be administered and the 3 tests that constitute primary outcome measures will be administered prior to the administration of the remainder of the MCCB so test administration order is maintained throughout the trial for the cognitive primary outcome measures.

Clinical Global Impression Severity Scale (CGI-S) (59) will be used for repeated evaluations of global psychopathology. The CGI-S scale is widely used in schizophrenia research and is a single 7-point Likert scale rating severity of psychopathology on a scale of 1 (normal, not ill) to 7 (very severely ill).

Clinical Global Impression Change Scale (CGI-I) (59) is used to assess the clinical change as compared to symptoms at baseline using a 7-point Likert scale, ranging from very much improved (1) to very much worse (7).

The Positive and Negative Syndrome Scale (PANSS) (60) is an assessment instrument for general psychopathology and positive symptoms. The PANSS contains 30 items that assess symptoms of psychotic disorders including positive, negative and general psychopathology. The PANSS was chosen because of its widespread use in clinical studies of psychosis, and its demonstrated reliability in assessing psychopathology across diverse patient populations.

Personal and Social Performance Scale (PSP) (61) is a 100-point, single item, clinician rated scale to assess 4 domains of functioning, including personal and social relationships, socially useful activities, self-care and disturbing and aggressive behaviors.

Quality of Life Scale (QLS) (62) assesses multiple dimensions of quality of life. It was developed specifically for schizophrenia populations and is commonly employed in clinical trials.

Barnes Akathisia Scale (BARS) (63) is used to rate drug-induced akathisia. Symptoms are rated on a 4-point scale, with 0 being no akathisia and 3 being severe akathisia. A global clinical assessment of akathisia is then scored on a 6-point scale, with 0 being no evidence of akathisia and 5 being severe akathisia.

Simpson Angus Scale (SAS) (57) is a 10-item scale used to evaluate the presence and severity of drug induced Parkinsonism. Each item is rated from 0 to 4.

Calgary Depression Scale for Schizophrenia (CDSS) (64) is a nine-itemed structured interview specifically developed for assessment of depression in patients with schizophrenia. Items are scored from 0 (absent) to 3 (severe).

Columbia Suicide Severity Rating Scale (C-SSRS) (65) captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period. The definition of behavioral suicidal events used in this scale is based on those used in the Columbia Suicide History Form (66). During the baseline assessment, questions are in relation to lifetime experiences and all subsequent questioning is in relation to the last assessment.

Anticholinergic Cognitive Burden Scale (ABS) (56) was developed by an interdisciplinary group of pharmacy and medical professionals using a systematic review identifying drugs with anticholinergic activity established in the literature. Drugs are categorized based on their clinically relevant cognitive

effects. Summed scores reflect the total clinically relevant anticholinergic effects on cognition of all medications in a subject's drug regimen, with higher scores denoting higher burden.

MRI Methods

MRIs will be performed at the IU Center for Neuroimaging using an integrated 90-minute exam including structural and functional MRI (fMRI). All scans will be completed on a newly installed (June 2012) research-dedicated Siemens MAGNETOM Skyra 3T 70cm bore whole body scanner using a 32-channel head coil. Scans will include structural series for registration with functional tasks, to examine white matter integrity, and to rule out incidental findings, and fMRI and MR perfusion measures. FMRI tasks will focus on episodic and working memory.

Figure 2. N-Back Task

N-Back Task

0 back *(target=C)*
C Q Z G C R D C G S J F C H P T

1 back
B X X A G G L F G N N T C P P H

2 back
K S B S Q M M G M R C J C F Q F

Positioning and motion: Subjects are instructed to remain still during scanning and deformable foam cushioning is used to stabilize the head. Real time image reconstruction and processing are used for quality assurance at the time of scanning. If there is any problem the scan is repeated. For fMRI, minor subvoxel-level translation or rotation is adjusted during post-processing. Noise: Noise-attenuating headphones and ear stopples provide excellent noise reduction and permit adequate auditory perception. **Longitudinal repositioning**: We will employ Siemens AutoAlign scanner software to help ensure maximum reproducibility. AutoAlign, which uses a 3D atlas for automated slice positioning, appears robust and to yield repeatable and accurate repositioning (67). **Structural Imaging Sequences** include: (1) Sagittal survey 3-plane localizer (2) Sagittal T1-weighted MP-RAGE 3D anatomical volume (3) 3D fluid attenuated inversion recovery (FLAIR) and (4) Diffusion tensor imaging (DTI). **Functional MRI Sequences** include: Echo-planar functional MRI and GRE Field map.

fMRI activation tasks: fMRI activation tasks will include Visual-Verbal 2-Back, Scene Encoding and Recognition, and Resting Connectivity paradigms, shown by our group and others to demonstrate reliable activation for the cognitive functions and brain regions of interest (68-78). We have successfully implemented all tasks in multiple studies of patients with psychosis and other clinical populations, as well as in pharmacological imaging studies, and have detected task-related activation in expected brain regions.

2-Back Visual-Verbal working memory task: Verbal working memory has been widely studied in terms of cognitive components and models (79-83), neural circuitry of the frontal lobes and basal ganglia (84), and its disruption in brain disorders such as schizophrenia (45, 85), MS (78, 86), and MTBI (70, 71), as well as in relation to estrogen status (11, 12, 87, 88). Subjects view a string of consonant letters (except L, W, and Y). The working memory load is varied parametrically via three task conditions: 0-back, 1-back, and 2-back (Figure 2). The 0-back control condition has a minimal working memory load; subjects are asked to decide if the current letter matches a single target letter that is specified before the epoch begins. In the 1-back condition, they are asked to decide if the current letter matches the previous one. During the 2-back condition, the task is to decide whether the letter currently presented matches the letter that has been presented two back in the sequence. In our previous studies, this task has been highly robust in producing bilateral frontal and parietal activation in healthy young and elderly controls and sensitive to differences in patient groups (70, 71, 77, 78). Task length 6:09.
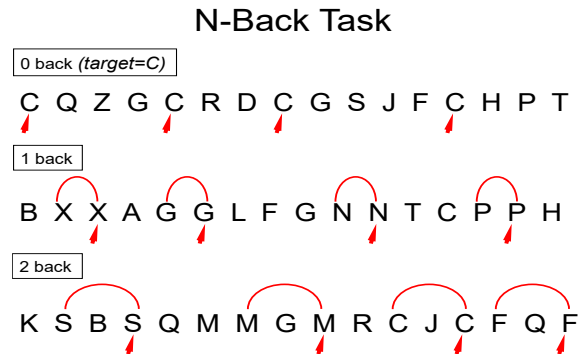
Scene Encoding Task

Figure 3. Scene Encoding

Scene Encoding and Recognition tasks: These tasks utilize stimuli and a task design modified from that used by Detre et al. in episodic memory studies in temporal lobe epilepsy (68, 69, 74). In their published studies and Drs. Saykin and McDonalds' initial work, these tasks elicit reliable bilateral MTL activation in healthy children, adolescents, and adults, with a slight right hemisphere predominance. During the blocked-design encoding trial subjects attempt to encode complex scenes for later recognition (Figure 3).

The control condition consists of repetition of a single degraded version of one of the encoding stimuli. During the event-related recognition trial the subject views target scenes intermixed with foils, and is asked to decide which were presented during the encoding trial. Task length for each trial is 5:15.

Pseudo-Continuous Arterial Spin Labeling (pCASL) perfusion images will also be acquired to quantify any drug related changes in cerebral blood flow. Subjects are asked to lie still and keep their eyes closed. Task length 5:14.

Functional Imaging Stimulation and Physiological Monitoring Procedures: All stimuli are presented through an MR-safe and compatible headphone and projector system (Psychology Software Tools, Inc.). Experimental tasks are presented by PC interface and are programmed using the Presentation software package. Responses are recorded electronically through an MRI compatible fiber optic button response system (Current Designs, Inc.). This custom device is also connected to the sync pulse output of the scanner's gradient amplifier and sends a TTL signal to the PC marking the time each volume is acquired for synchronization between scans, event-related fMRI stimuli, and button responses. A comprehensive physiological monitoring system will be used with synchronized digital recording including pulse oximeter, respiratory belt, HR and BP measures, which will be available for analysis in relation to BOLD fMRI and PASL time series.

Event Related Potentials (ERP) Methods

Electroencephalography (EEG) will be recorded from a 32 channel, silver/silver-chloride scalp electrode montage using Neuroscan Inc. SYNAMPS-2 bioamplifier with a bandpass of .1 to 200 Hz (24 dB/octave roll off). Electrode impedance will be maintained below 5 kOhms. EEG will be recorded continuously during the stimulus paradigms. Three minute periods of resting EEG will be recorded for eyes open and eyes closed prior to the ERP paradigms. All three ERP measures are highly sensitive to schizophrenia, with an effect sizes (d) > 0.7 (7, 89). **Auditory Steady State Response (ASSR)** which synchronizes to the frequency and phase of periodic auditory stimuli, will be used to test auditory pathway integrity and possible ERβ modulation of glutamate and GABA transmission (90, 91). ASSR power in the gamma range (40 to 59 Hz) is usually reduced in schizophrenia (d > .8; 10). Gamma frequency entrainment will be obtained to a series of 100 click trains (40 Hz; 1000 ms duration; 500 ms ISI; 90 dB). After digital filtering (.1 to 55 Hz), ocularcorrection and artifact rejection, averaging (100 ms baseline and 1000 ms post-stimulus epoch) and baseline correction, we will measure event-related spectral perturbation (ERSP; mean power relative to baseline) at Fz to 40 click train stimulation (92), and predict larger ERSP values in treated patients. Phase locking factor (inter-trial coherence) will also be measured, since this measure of synchrony is often affected in schizophrenia as well (7). In addition, we will obtain **QEEG** recordings during the same ERP/MMN testing sessions. This measure is one of the most commonly used indices to confirm CNS penetrance and cortical activity in early stage CNS drug development. Increased average alpha power (8 to 13 Hz) across the set of electrode sites determined by QEEG in LY500307 treated subjects compared to placebo treated subjects will be the measure of activity utilized in this study. **Mismatch Negativity** (**MMN**) is an ERP component elicited by a deviant tone interpolated in a series of standard tones which indexes pre-attentive sensory memory, and is generated by the auditory and frontal cortices (89). MMN amplitude is reduced in schizophrenia and has been correlated with level of psychosocial function in schizophrenia and degree of grey matter loss in frontal cortex and Heschl's gyrus

(93). MMN will be recorded to infrequent 1.2 kHz tones (0.05 probability, 50 ms duration) amid frequent 1.0 kHz tones, with a 300 ms ISI and total of 1,600 tone presentations. After digital filtering (.01 to 24 Hz), ocular correction and artifact rejection, averaging by condition (100 ms baseline and 300 ms post-stimulus epoch) and baseline correction, the standard ERP will be subtracted from the deviant ERP. MMN will be measured as the most negative sample in the difference waveform between 100 and 300 ms period at Fz, and it is predicted that MMN will be increased in treated subjects. **Auditory P300 component** indexes working memory during stimulus discrimination and is decreased in schizophrenia with an effect size of about 0.9 (89). ERPs will be elicited by infrequent target tone-pips of 40-ms duration presented pseudo-randomly, interspersed among frequent low-pitched tones (1000 Hz, 90 dB) with 1 s ISIs and 600 trials. Subjects will respond to infrequent target tones using a button press. Target ERP trials will be processed off line, using digital filtering with a low pass of 24 Hz, ocular correction, and artifact rejection for trials with data points exceeding $\pm$ 100 microvolts prior to averaging trials within each condition.   After averaging using the epoch -100 to 600 ms after stimulus onset, P300 latency will be measured at the largest positive voltage sample between 280 and 600 ms at the Pz electrode site of the target ERP relative to baseline. Peak amplitude will be measured as the mean voltage between + 20 ms about the peak latency at the Pz electrode site. It is predicted that P300 peak voltage will be greater, and P300 latency shorter in treated subjects. Total ERP testing time: about one hour.

9.   Safety Assessments and Procedures

Vital Signs: Vital signs will be assessed at study visits per Study Procedures Table (Attachment A). Blood pressure, heart rate, and oxygen saturation will be taken in a seated position or supine position after a rest period of five minutes.

Medical History: The subject's lifetime medical history will be taken during the screening period. Medical history includes previous and current diseases.

Physical Examination: A physical examination including a neurological examination, an assessment for breast tenderness and gynecomastia, thrombosis (e.g., calf tenderness, shortness of breath), and sexual dysfunction.

Electrocardiograph (ECG): A supine, 12 lead ECG will be performed according to the Study Procedures Table (Attachment A). Potentially clinically significant ECG abnormalities will be interpreted by a local cardiologist at the discretion of the Principal Investigator

10.   Criteria for Repeat Assessments, Rescreening, and Discontinuation

Repeat Assessments

Screening Window
-Screening assessments can be repeated within the screening window under the same screening number with the exception of eligibility criteria related rating scales/questionnaires. Subject diagnosis confirmation will not be repeated.

-Morning (6am-12pm) Total Testosterone (TT) Plasma Levels: As noted, TT will be assessed during screening.

Post Randomization
>   -Morning (6am-12pm) Total Testosterone (TT) Plasma Levels: See Section 12, *Stage 2 Dosing*
>   *Downward Titration*

Rescreening
Subjects who screen fail may be rescreened one time, under a new screening number. If a subject is rescreened, all screening assessments (with the exception of the diagnosis confirmation) must be repeated and the stability criteria timelines must be met.

Discontinuation
Subjects will be discontinued under the following circumstances:
- ECG: Any subject with an absolute QTc of 450 msec or greater or a change from baseline of 60 msec or greater during the active treatment phases
- Total Testosterone Suppression: Subjects with a 50% reduction from baseline post dosing downward titration (See Section 12) will have values reassessed within one week of receipt. If the retest value is also a 50% reduction from baseline, the subject will be discontinued.
- Study Medication Non-Compliance: Significant noncompliance is defined as missing > 7 consecutive days of study medication or > 20 cumulative doses during the entire study.

If a subject discontinues from the study, discontinuation assessments will be at the discretion of the principal investigator.

A subject may withdraw from the study medication at any time at his own request, or may be withdrawn at any time at the discretion of the principal investigator for safety, behavioral, or administrative reasons.

11.   Laboratory Assessments

Laboratory assessments (blood and urine) will be collected at time points specified in Study Procedures Table (Attachment A) and analyzed by a local laboratory with the exception of the urine dipstick assessments which will be collected and analyzed onsite.

A total of 26 mL of blood will be collected at Visit 1, 14 mL at Visit 2, 10 mL of blood at Visits 2.5 and 3, 17 mL at Visit 4 and 23 mL of blood at Visit 6.

Laboratory assessments to be completed:

complete blood count with differential (CBC w/diff)
comprehensive metabolic panel (CMP)
hepatitis panel
hemoglobin A1c (HgbA1c)
lipid panel
testosterone plasma levels (Free and Total)
thyroid stimulating hormone level (TSH)
urine toxicology screen
Luteinizing Hormone
Prolactin

Pharmacokinetics (PK): Pharmacokinetic (PK) blood samples to determine LY500307 plasma concentration will be obtained according to the Attachment A. The exact time of the blood draws and dosing will be recorded.

12. Study Medication

Study medication will be taken by mouth once daily. During Stage 1 doses will consist of 25 mg, 75 mg, and placebo. During Stage 2, placebo, LY500307 75 mg/day, and LY500307 150 mg/day will be administered as six blinded study drug pills per day. LY500307 and matched placebo will be supplied by Lilly for the study. Placebo capsules will look like LY5000307, with matching shape, taste, and color. All study medication supplies will be labeled, stored, reconciled, and destroyed according to applicable regulatory requirements. A Certificate of Analysis, the expiry date, and a statement of GMP compliance will be supplied by Lilly.

Stage 2 Dosing Downward Titration

If total testosterone suppression is confirmed during the study (a 50% reduction from baseline post randomization), subjects will be blindly switched to the next lowest dosing arm for the remainder of the trial. Thus, a subject randomized to 150 mg/day will be titrated down to 75 mg/day, and those randomized to 75 mg/day will be titrated down to placebo, and those randomized to placebo will continue on placebo.

Stage 2 Continued Dose Assessment

The first 10 subjects randomized to 150 mg/day will be reviewed by the Data Safety Monitoring Board (Section 15 below) to determine the safety of further randomizations at this dose. If five or more of 10 subjects randomized to 150 mg/day meet the TT suppression criteria as described in Section 12 above, that dose will be dropped from Stage 2 and all future newly randomized subjects will be randomized to placebo and 75 mg/day in a 1:1 fashion. (Note: ERα receptor engagement by LY500307 insures full engagement of ERβ receptors. Thus, if 150 mg/day proves to be a TT suppressing dose, these data provide support that the 75 mg/day dose is fully activating ERβ receptors and therefore increases confidence that the primary aim of this study [i.e., assessing the ERβ receptor as a target for innovative therapeutics for schizophrenia] will be achieved).

Treatment Compliance

Compliance with medications will be assessed at each study visit by direct questioning and study medication count of unused study medication and packaging to be returned at each visit. Study medication dispensing records will be obtained.

13. Concomitant Medication

See Attachment B.

14. Adverse Events

Adverse events (AEs), especially those for which the relationship to study medication is not "unrelated," will be followed up until they have returned to baseline status or stabilized at the discretion of the

principal investigator. If after the follow-up period, return to baseline or stabilization cannot be established an explanation will be recorded in the source documentation.

15. Steering Committee and Data Safety Monitoring Board

The **Steering Committee** will serve as the operational governing board for the project. The Steering Committee will monitor and review reports from the Data Safety Monitoring Board (described below) and any serious AEs. It will oversee the conduct of the trial and progress towards meeting study milestones. It will meet following an award notice and quarterly during the conduct of the trial. The Steering Committee will be chaired by the study PI and will include the CTSI Director, senior statistician, senior pharmacologist, Lilly representative *ex officio,* NIH Project Scientist, NIH Program Officer, and two external scientists with expertise in early stage clinical trials and statistics. The **Steering Committee** will be charged with the review of all relevant data at the end of Stage 1 with the purpose of determining if Go/No go criteria has been met. The relevant data include adverse events considered "probably" and "possibly" related to study drug or study procedure, QTcB values, and TT levels.

Steering Committee, Eli Lilly scientists and NIH scientists and program officers determined that the Stage 1 sample size (10 subjects per arm) was too small to draw conclusions about cortical target engagement and efficacy and therefore these related endpoints will not be considered in the Go/No Go decision. The IU Adult Psychiatry **Data Safety Monitoring Board** (DSMB) will be responsible for data and safety monitoring. DSMB is responsible for reviewing study procedures, AEs, safety mailings (if applicable), enrollment, active subject progress, drop-out rates, and ongoing conduct of the research. The DSMB members can ask questions and make comments and/or recommendations to the investigators. The IRB is notified of significant findings by way of the DSMB meeting minutes at the time of continuing review. DSMB members consist of Department of Psychiatry faculty members who are not investigators on this trial. Data on the number of subjects enrolled and the number of AEs will be reviewed by the Board at least quarterly and more frequently if needed. The resulting report will be issued to the IU IRB at least at the time of continuing review or more frequently by request. Any unanticipated events will be immediately directed to the PI who will follow the IRB reporting procedures.

The DSMB will be responsible for reviewing all safety and testosterone data for the first 10 subjects treated with 150 mg/day in Stage 2 to determine if it is safe to further randomize subjects to this dose (as described in Section 12 above).

16. Statistical Methods

Data Management

Data management will be performed by the Indiana CTSI Design and Biostatistics Program under the direction of Dr. Lourens. The study database will be set up in OnCore clinical trial software system for data acquisition and management, including establishing a trial eCRF, procedures for randomization and biomarker acquisition and tracking. All procedures will conform to FDA GCP requirements. Data will be quality checked and entered into data analytic programs on a continual basis throughout the conduct of the study to be certain analyses of all relevant variables are available on the established timeline for the ADC to make the Go/No go decision at the end of Stage 1.

Stage 1 Dose Selection Analyses

Go/No go criteria: At the end of Stage 1, relevant data will be provided to the **Steering Committee** to make the Go/No go decision. Two LY500307 doses (25 mg/day or 75 mg/day) will be evaluated in Stage 1 to determine which dose will be advanced to Stage 2 for continued dose assessment, cortical target engagement and efficacy assessment analyses. Advancement to Stage 2 will be determined according to the following Go/No go criteria: 1) Lack of a safety signal as defined by SAEs, concerning AEs deemed to be probably related to study medication, absolute QTcB of 450 msec or greater or a change from baseline of 60 msec or greater during the active treatment phases, and 2) lack of TT suppression as determined by a decrease in total testosterone plasma concentrations from baseline levels of at least 50% in at least 5 of 10 subjects per LY500307 dosing arm for two of three consecutive post-randomization values. Testosterone suppression reflects ERα engagement and lack of ERβ selectivity.

Stage 2 Continued Dose Assessment, Cortical Target Engagement, and Efficacy Assessment Analyses

Data collected from both stages will be combined for a final analysis. Providing the 150 mg/day dose is not dropped from Stage 2 because of TT suppression, a dose-concentration-response analysis will be used as the primary analytic approach with a secondary approach being based on intent-to-treat principles. If the 150 mg/day dose is dropped from the Stage 2 of the trial, intent-to-treat will be the primary analytic approach used and dose-concentration-response will be the secondary approach. Both methods are described subsequently:

Dose-Concentration-Response Analyses: A simultaneous Pharmacokinetic (PK)/Pharmacodynamic (PD) modeling approach will be employed. Each subject's LY500307 blood concentration measurements will be utilized in conjunction with a population PK model that was previously developed for this compound internally at Lilly. This PK model, based on assumptions derived from thermodynamic laws, will be used to generate individual-specific exposures, specifically the area under the concentration time curve generated from empirical Bayes estimates for PK parameters for each subject conditional on their concentration measurement. The subject-specific exposure estimate from the model will then be compared to the observed responses at the 8 week time point to construct a dose-concentration-response model. The use of individualized concentration exposure values will increase the range of observations utilized in comparison to a strict dose-response analysis. The dose-response analysis is limited to four points (placebo (i.e., zero dose), 25 mg/day, 75 mg/day and 150 mg/day) to define the relationship with response.

It is anticipated that at any given dose there will be variability in the observed individual concentration exposures. The dose-concentration-response analysis will provide additional "points" that will be shared across subjects to assist in defining the nature of the concentration response relationship. This will be particularly important if the relationship has an emax or sigmoid emax shape with multiple parameters to be identified. The four concentration response relationship types will be explored; linear, exponential, emax and sigmoid emax, and the models will be implemented using a linear or nonlinear mixed effects modeling framework in NONMEM VII to facilitate sharing of data across subjects while preserving within-subject correlation.

There will be two tiers to the examination of whether there is a concentration effect related signal. The first "test" is based on the change in the objective function value when a concentration factor is included in the model as either a linear or exponential effect. That is, is there any detectable effect of incorporating the individualized concentration (we will use the area under the curve derived from the Empirical Bayes Estimates of the PK parameters for each subject). An alpha level for significance of 0.05 will be used. If there is a significant difference in the evaluation at test 1, the additional model structures will be

evaluated to determine what "shape" appears to best describe the relationship between concentration and response.

As stated above, four concentration response relationship types will be compared (linear, exponential, emax, sigmoid emax). These are standard PD relationships and, in particular, the emax and sigmoid emax models are based on assumptions that derive from receptor occupancy theory and thermodynamic laws similar to the PK model. These models will be constructed and tested utilizing a linear/nonlinear mixed effects modeling approach. Objective function value differences, information criteria (e.g. AIC) and simulation based model performance evaluation techniques such as visual predictive checks will be utilized to evaluate model performance and guide model selection for the concentration effect relationship. Once a model is selected, the responses for a particular dosage can be simulated, incorporating between subject variability as well as parameter uncertainty identified using the modeling approach described above. These simulations will generate a distribution of predicted responses for dosages of interest (including placebo as the zero dose condition). The simulated/predicted responses can be examined to evaluate whether or not there is overlap in the distributions by percentile and provide an answer as to whether or not a particular dosage administered to subjects results in a distinguishable difference in response from placebo. In addition, the model can be used to examine the effect of increasing the numbers of subjects to distinguish drug effect from placebo at any number of dosages under different trial conditions.

Intent-to–Treat Analyses: Point estimates and the corresponding confidence intervals for the primary study endpoints (biomarkers and efficacy measures) at week 8 will be obtained. Statistical tests will be performed based on one-sided test at the 10% level of significance with an adjustment for multiple comparisons for the 3 primary efficacy outcome measures with each primary measure tested at an alpha threshold of $p<0.0333$ as noted above. One-tailed tests are planned because the main interest of this study is to determine if LY500307 is superior to placebo and thus has produced a sufficient efficacy signal in one or more of the three primary efficacy outcome measures to warrant further assessment in subsequent larger, confirmatory trials. It is therefore a priority of this data analytic plan to avoid a "false negative" (i.e., falsely concluding LY500307 lacks efficacy) that may lead to prematurely terminating further development of the compound.

A "per protocol" analysis will restrict the analysis to subjects who receive minimal exposure to the intervention, have a minimal number of longitudinal evaluations, and/or are free of major protocol violations. A "completer" analysis will only include subjects who completed treatment without exiting or prematurely terminating from their assigned treatment. Because subjects may be removed differentially across the treatment arms in these analyses, both approaches are vulnerable to differential selection bias and will be interpreted cautiously.

Repeated Measures Analyses: Most of the study outcomes are observed repeatedly at well-defined time-points post-randomization, so the primary analyses are based on mixed models for repeated measurements (MMRM). This includes the mixed effects models for continuous outcomes (normally transformed if necessary) as implemented in PROC MIXED in SAS in which subjects are included as random effects. The fixed-effects predictors include the overall mean, the treatment effect, the time effect, the treatment × time interaction plus selected covariates. Initially, time will be treated as a categorical variable to avoid arbitrary assumptions on the nature of the trend over time. Hypotheses will be tested by deriving contrasts among the model parameters, including between-group differences at the end of study, and the effect size of treatment will be estimated accordingly. This primary analysis will be applied to each of the primary and secondary outcomes.

Covariates in Regression Models: The baseline value of the outcome, centered about its sample mean, will be included in each analysis. The baseline value tends to correlate with post-randomization

outcomes, and this maneuver reduces residual variance, thus increasing statistical power and the precision of the estimated treatment effect. Otherwise, only a small number of prognostic factors, e.g. age, found to be imbalanced at baseline, will also be entered into the model.

Missing Data: For this study, missing data falls in to one of two general categories: The first is data that is missing because of missed visits or early termination. The second is related to incomplete data. The former will not affect the results of the mixed-effects model, which is unbiased if missing-at-random assumptions are met; sensitivity analyses will also be performed as described below. The problem of incomplete data in composite measures will be addressed in the case of the cognitive measures by following MATRICS Neurocognition Committee guidelines for imputation (94). For other instruments, total and subscale scores will be imputed using the average of the available items as long as 80% or more of the individual items are available. A low dropout rate is anticipated because subjects will have met a priori clinical stability criteria before randomization and those agents will not be altered throughout the course of the active treatment phase. For drop-outs, sensitivity analyses will be performed using a variety of imputed values, including best-case and worst-case scenarios, as well as multiple imputations if drop-out is higher than expected.

Other Confounders Arising Post-Randomization: Analyses will be conducted to investigate: (a) adherence to the assigned study medication, (b) co-administration of psychotropic medications, and (c) discontinuation from the study intervention, and logistic regression analysis will be applied to investigate these factors. First, it will determine whether these effects occur differentially across the treatment arms suggesting that a differential selection bias may have influenced the results. Then, whether baseline characteristics are predictive of any behavior will be evaluated. This will include demographic characteristics, initial severity as measured by the baseline values of the outcome variables, and clinical characteristics such as diagnoses and medications taken at baseline. Finally, these confounding factors will be included as covariates in the primary analyses to investigate whether the conclusions on treatment effects are altered.

Pharmacokinetics (PK): These data will be used in post hoc analyses to potentially explain inter-subject variability and toxicity.

Brain Imaging Analyses

Quality control, preprocessing and statistical analyses for all scans (see "MRI Methods") will follow established protocols as in our previous publications (e.g., working memory (95-97), episodic memory (98), Resting DMN connectivity (99), PASL perfusion (100), DTI (101, 102), structural MRI (103-105)). Region of interest (ROI) predictions for LY500307 treated vs. placebo effects are as follows: working memory (increase, frontal & parietal), episodic verbal encoding/retrieval (increase, hippocampal & DLPFC) and resting state (increase DMN & hippocampal/DLPFC connectivity). PCASL perfusion may show parallel increases to fMRI (above). DTI white matter tractography and gray matter density/cortical thickness will be examined for possible drug effects such as improvement in GM and WM markers. Additional exploratory analyses will assess whether baseline imaging variables predict treatment response and relationships with cognition and ERP measures (below) will also be evaluated.

Event Related Potentials Analyses

The data analytic plan for the three ERP measures (P300, MMN, and ASSR ERSP) will follow the repeated measures approach outlined above for other secondary variables and described in our previous protocols (7, 8, 92). Each variable is listed with the predicted effect on the LY500307 treated compared to the placebo treated control group: P300 amplitude (increased), P300 latency (decreased), MMN amplitude (increased), ASSR ERSP power (increased). In exploratory analyses, it will be tested whether

baseline ERP values predict response on the primary and secondary clinical outcome measures in the treated groups.

17.   Privacy and Confidentiality Issues

Confidentiality will be protected by ensuring all research staff have been properly trained in confidentiality and human subject research procedures, coding all subject information when possible, and by securing subject files in a locked filing cabinet or on secured databases with access available only to the principal investigator and research staff. Furthermore, data entered into a computer database will only use subject codes on secured computers that will be password protected with access available only to the principal investigator and research staff. Any screening information obtained from potential research subjects who subsequently do not participate in the research study will be destroyed.

18.   Record Retention

Paper copies of medical records and source documentation will be kept for seven years after the study is closed with the IRB. One year after study closure, the documents will be shipped to the Indiana University Department of Psychiatry long-term storage facility until destruction.

ATTACHMENT A

Study Procedures Table

Visit	1	2	2.5	3	4	5	6 /ET
Day		0	7	14	28	42	56
Visit Window (days)	0-30	5-9	5-9	11-17	11-17	11-17	
Informed Consent	X						
SCID & Demographics	X						
Med/Psych/Medication History	X						
Physical Exam	X	X[g]	X[g]	X[g]	X[g]	X[g]	X
Vitals	X	X	X	X	X	X	X
ECG	X				X		X
BARS & SAS	X	X			X		X
NSA-16 & CDSS		X		X	X	X	X
CGI-I				X	X	X	X
CSSRS[f]	X	X	X	X	X	X	X
CGI-S	X	X		X	X	X	X
PANSS, PSP, EQ-5D, & Quality of Life		X			X		X
Current Substance Use Habits[h]		X		X	X	X	X
Hopkins Verbal Learning Test-Revised, Wechsler Memory Scale-Third Ed: Spatial Span, and Letter-Number Sequencing[a]				X		X	
MCCB		X			X		X
MRI		X					X
ERP		X			X		X
Labs (CBC w/ diff, CMP, Luteinizing Hormone, Prolactin)[c,e]	X	X[b]			X		X
Testosterone (Total and Free)[c,e]	X	X	X	X	X		X
Urine Toxicology		X					X
Hepatitis Panel	X						
TSH, Lipid Panel, and HgbA1c[c,e]	X						X
PK Blood Draw[d,e]			X	X	X		X
Adverse Event (AE's)[i]		X	X	X	X	X	X
Concomitant Medication & ABS	X	X	X	X	X	X	X
Medication Dispensation		X		X	X	X	
Medication Accountability				X	X	X	X

[a]Hopkins Verbal Learning Test-Revised, Wechsler Memory Scale-Third Ed: Spatial Span, and Letter-Number Sequencing are included in the MCCB. At Visit 3 and 5 they will be administered separately from the full MCCB.

[b]If Visit 2 occurs within 14 days from Visit 1, the following labs will NOT be repeated at Visit 2: Luteinizing Hormone, CBC w/ diff, CMP, Prolactin

[c]Labs and baseline assessments to be obtained pre-dose

[d]PK samples will be collected from all randomized subjects. PK samples will be collected at Visits 2.5, 3, 4, and 6. The exact time of the blood draws and dosing will be recorded.

[e]All blood samples for clinical laboratories will be drawn in the morning after an 8-hour overnight fast

[f]Baseline version of CSSRS will be conducted at the screening visit/Visit 1. The "Since Last Visit" version will be used on subsequent visits.

[g]Brief physical examination will be performed at Visits 2-5 including a general examination assessing for health changes, an assessment for breast tenderness, gynecomastia, sexual dysfunction, and thrombogenic events

[h]Lifetime substance use will be captured at Visit 1.

[i]Pre-existing medical conditions will be tracked as AE's at the discretion of the principal investigator.

ATTACHMENT B
Concomitant Medication Table

Medication	Allowed	Notes
Amphetamines (e.g., methylphenidate, dextroamphetamine)	Yes-Conditional	Stable dose, no changes or additions for one week prior to screening and during the trial
Androgens	No	
Antiemetics with dopamine blocking properties (eg., metoclopramide, domperidone, others)	No	
Antiepileptic mood stabilizers	Yes	Stable dose, no changes or additions for one week prior to screening
Antihistamines, nonsedating (eg., loratidine, fexofenadine, cetirizine)	Yes	
Antihistamines, sedating (e.g. diphenhydramine, hydroxyzine, meclizine, benztropine)	Yes	Stable dose OR No use within 8 hours prior to cognitive assessments
Antipsychotic medications	Yes	
Barbiturates	No	
Benzodiazepines	Yes	Stable dose OR No use within 8 hours prior to cognitive assessments
Bupropion	Yes	Stable dose, no changes or additions for one week prior to screening
Corticosteroids (except topical, intranasal, or inhaled)	No	
Decongestants (eg., pseudophedrine)	Yes	Stable dose OR No use within 8 hours prior to cognitive assessments
Dicyclomine	No	
Estrogens	No	
Herbal medications or Over the Counter medications w/ primary CNS activity	No	
Ketamine	No	
Lithium	Yes	Stable dose, no changes or additions for one week prior to screening
MAOIs	Yes	Stable dose, no changes or additions for one week prior to screening, patient must maintain low tyramine diet
Methadone	No	
Mirtazepine	Yes	Stable dose, no changes or additions for one week prior to screening
Muscle Relaxants	Yes	Stable dose OR No use within 8 hours prior to cognitive assessments
Nicotine Replacement	Yes	
Opiates	No	
Progestins	No	
Benzodiazepine derivative sleeping medications (eg., zolpidem)	Yes	Stable dose OR No use within 8 hours prior to cognitive assessments
SNRIs	Yes	Stable dose, no changes or additions for one week prior to screening
SSRIs (note: Citalopram must be at dose of 40 mg or less)	Yes	Stable dose, no changes or additions for one week prior to screening
Tricyclic antidepressants	Yes	Stable dose, no changes or additions for one week prior to screening
Trazodone	Yes	Stable dose OR No use within 8 hours prior to cognitive assessments

ATTACHMENT C

References

1. Robins LN, Helzer JE, Weissman MM, Orvaschel H, Gruenberg E, Burke JD, Jr., et al. Lifetime prevalence of specific psychiatric disorders in three sites. Arch Gen Psychiatry. 1984;41(10):949-58. PubMed PMID: 6332590.
2. WHO. The World Health Report 2001: Mental Health: New Understanding, New Hope. World Health Organization Geneva; 2001.
3. Breier A, Schreiber JL, Dyer J, Pickar D. National Institute of Mental Health longitudinal study of chronic schizophrenia. Prognosis and predictors of outcome. Arch Gen Psychiatry. 1991;48(3):239-46. PubMed PMID: 1671741.
4. Harvey PD, Keefe RS. Studies of cognitive change in patients with schizophrenia following novel antipsychotic treatment. Am J Psychiatry. 2001;158(2):176-84. PubMed PMID: 11156796.
5. Pompili A, Arnone B, Gasbarri A. Estrogens and memory in physiological and neuropathological conditions. Psychoneuroendocrinology. 2012;37(9):1379-96. doi: 10.1016/j.psyneuen.2012.01.007. PubMed PMID: 22309827.
6. McEwen B. Estrogen actions throughout the brain. Recent Prog Horm Res. 2002;57:357-84. PubMed PMID: 12017552.
7. Brenner CA, Krishnan GP, Vohs JL, Ahn WY, Hetrick WP, Morzorati SL, et al. Steady state responses: electrophysiological assessment of sensory function in schizophrenia. Schizophr Bull. 2009;35(6):1065-77. doi: 10.1093/schbul/sbp091. PubMed PMID: 19726534; PubMed Central PMCID: PMC2762626.
8. Carroll CA, Boggs J, O'Donnell BF, Shekhar A, Hetrick WP. Temporal processing dysfunction in schizophrenia. Brain Cogn. 2008;67(2):150-61. doi: 10.1016/j.bandc.2007.12.005. PubMed PMID: 18262701; PubMed Central PMCID: PMC2512257.
9. O'Donnell BF. Cognitive impairment in schizophrenia: a life span perspective. Am J Alzheimers Dis Other Demen. 2007;22(5):398-405. doi: 10.1177/1533317507304745. PubMed PMID: 17959875.
10. Dietrich T, Krings T, Neulen J, Willmes K, Erberich S, Thron A, et al. Effects of blood estrogen level on cortical activation patterns during cognitive activation as measured by functional MRI. NeuroImage. 2001;13(3):425-32. doi: 10.1006/nimg.2001.0703. PubMed PMID: 11170808.
11. Dumas JA, Kutz AM, Naylor MR, Johnson JV, Newhouse PA. Increased memory load-related frontal activation after estradiol treatment in postmenopausal women. Hormones and behavior. 2010;58(5):929-35. doi: 10.1016/j.yhbeh.2010.09.003. PubMed PMID: 20849856; PubMed Central PMCID: PMC2982897.
12. Joffe H, Hall JE, Gruber S, Sarmiento IA, Cohen LS, Yurgelun-Todd D, et al. Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. Menopause. 2006;13(3):411-22. PubMed PMID: 16735938.
13. Smith YR, Love T, Persad CC, Tkaczyk A, Nichols TE, Zubieta JK. Impact of combined estradiol and norethindrone therapy on visuospatial working memory assessed by functional magnetic resonance imaging. J Clin Endocrinol Metab. 2006;91(11):4476-81. doi: 10.1210/jc.2006-0907. PubMed PMID: 16912129; PubMed Central PMCID: PMC1861832.
14. Mendrek A, Lakis N, Jimenez J. Associations of sex steroid hormones with cerebral activations during mental rotation in men and women with schizophrenia. Psychoneuroendocrinology. 2011;36(9):1422-6. doi: 10.1016/j.psyneuen.2011.03.016. PubMed PMID: 21514059.
15. Lewine R. Gender and schizophrenia. Handbook of schizophrenia. 1988;3:379-97.
16. Riecher-Rossler A, Hafner H, Stumbaum M, Maurer K, Schmidt R. Can estradiol modulate schizophrenic symptomatology? Schizophr Bull. 1994;20(1):203-14. PubMed PMID: 8197416.

17. Hoff AL, Kremen WS, Wieneke MH, Lauriello J, Blankfeld HM, Faustman WO, et al. Association of estrogen levels with neuropsychological performance in women with schizophrenia. Am J Psychiatry. 2001;158(7):1134-9. PubMed PMID: 11431236.
18. Begemann MJ, Dekker CF, van Lunenburg M, Sommer IE. Estrogen augmentation in schizophrenia: A quantitative review of current evidence. Schizophrenia research. 2012;141(2-3):179-84. doi: 10.1016/j.schres.2012.08.016. PubMed PMID: 22998932.
19. Ko YH, Joe SH, Cho W, Park JH, Lee JJ, Jung IK, et al. Estrogen, cognitive function and negative symptoms in female schizophrenia. Neuropsychobiology. 2006;53(4):169-75. doi: 10.1159/000093780. PubMed PMID: 16763376.
20. Bergemann N, Parzer P, Jaggy S, Auler B, Mundt C, Maier-Braunleder S. Estrogen and comprehension of metaphoric speech in women suffering from schizophrenia: results of a double-blind, placebo-controlled trial. Schizophr Bull. 2008;34(6):1172-81. doi: 10.1093/schbul/sbm138. PubMed PMID: 18156639; PubMed Central PMCID: PMC2632488.
21. Tan XJ, Dai YB, Wu WF, Kim HJ, Barros RP, Richardson TI, et al. Reduction of dendritic spines and elevation of GABAergic signaling in the brains of mice treated with an estrogen receptor beta ligand. Proc Natl Acad Sci U S A. 2012;109(5):1708-12. doi: 10.1073/pnas.1121162109. PubMed PMID: 22307635; PubMed Central PMCID: PMC3277134.
22. Bodo C, Rissman EF. New roles for estrogen receptor beta in behavior and neuroendocrinology. Front Neuroendocrinol. 2006;27(2):217-32. doi: 10.1016/j.yfrne.2006.02.004. PubMed PMID: 16603234.
23. Nilsson S, Koehler KF, Gustafsson JÅ. Development of subtype-selective oestrogen receptor-based therapeutics. Nature Reviews Drug Discovery. 2011;10(10):778-92.
24. Koehler KF, Helguero LA, Haldosen LA, Warner M, Gustafsson JA. Reflections on the discovery and significance of estrogen receptor beta. Endocr Rev. 2005;26(3):465-78. doi: 10.1210/er.2004-0027. PubMed PMID: 15857973.
25. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J Comp Neurol. 1997;388(4):507-25. PubMed PMID: 9388012.
26. Breier A, Buchanan RW, Elkashef A, Munson RC, Kirkpatrick B, Gellad F. Brain morphology and schizophrenia. A magnetic resonance imaging study of limbic, prefrontal cortex, and caudate structures. Arch Gen Psychiatry. 1992;49(12):921-6. PubMed PMID: 1449382.
27. Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, et al. Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression. J Biol Chem. 2002;277(27):24353-60. doi: 10.1074/jbc.M201829200. PubMed PMID: 11986316.
28. Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA. Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. Horm Behav. 2008;54(5):726-34. doi: 10.1016/j.yhbeh.2008.07.013. PubMed PMID: 18775724.
29. Murakami G, Hunter RG, Fontaine C, Ribeiro A, Pfaff D. Relationships among estrogen receptor, oxytocin and vasopressin gene expression and social interaction in male mice. Eur J Neurosci. 2011;34(3):469-77. doi: 10.1111/j.1460-9568.2011.07761.x. PubMed PMID: 21749489.
30. Wolkowitz OM, Breier A, Doran A, Kelsoe J, Lucas P, Paul SM, et al. Alprazolam augmentation of the antipsychotic effects of fluphenazine in schizophrenic patients. Preliminary results. Arch Gen Psychiatry. 1988;45(7):664-71. PubMed PMID: 3289524.
31. Breier A, Malhotra AK, Pinals DA, Weisenfeld NI, Pickar D. Association of ketamine-induced psychosis with focal activation of the prefrontal cortex in healthy volunteers. Am J Psychiatry. 1997;154(6):805-11. PubMed PMID: 9167508.
32. Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, et al. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology. 1997;17(3):141-50. doi: 10.1016/S0893-133X(97)00036-5. PubMed PMID: 9272481.

33.   Adler CM, Malhotra AK, Elman I, Goldberg T, Egan M, Pickar D, et al. Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. American Journal of Psychiatry. 1999;156(10):1646-9.

34.   McPherson SJ, Ellem SJ, Simpson ER, Patchev V, Fritzemeier KH, Risbridger GP. Essential role for estrogen receptor beta in stromal-epithelial regulation of prostatic hyperplasia. Endocrinology. 2007;148(2):566-74. doi: 10.1210/en.2006-0906. PubMed PMID: 17068134.

35.   McLeod DG. Hormonal therapy: historical perspective to future directions. Urology. 2003;61(2 Suppl 1):3-7. PubMed PMID: 12667881.

36.   Coss CC, Jones A, Parke DN, Narayanan R, Barrett CM, Kearbey JD, et al. Preclinical characterization of a novel diphenyl benzamide selective ERalpha agonist for hormone therapy in prostate cancer. Endocrinology. 2012;153(3):1070-81. doi: 10.1210/en.2011-1608. PubMed PMID: 22294742.

37.   Veldhuis JD, Iranmanesh A. Short-term aromatase-enzyme blockade unmasks impaired feedback adaptations in luteinizing hormone and testosterone secretion in older men. J Clin Endocrinol Metab. 2005;90(1):211-8. doi: 10.1210/jc.2004-0834. PubMed PMID: 15483079; PubMed Central PMCID: PMC1315304.

38.   Rosendaal FR, Van Hylckama Vlieg A, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. Journal of thrombosis and haemostasis : JTH. 2003;1(7):1371-80. PubMed PMID: 12871270.

39.   Magliano DJ, Rogers SL, Abramson MJ, Tonkin AM. Hormone therapy and cardiovascular disease: a systematic review and meta-analysis. BJOG : an international journal of obstetrics and gynaecology. 2006;113(1):5-14. doi: 10.1111/j.1471-0528.2005.00797.x. PubMed PMID: 16398764.

40.   Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA : the journal of the American Medical Association. 2007;297(13):1465-77. doi: 10.1001/jama.297.13.1465. PubMed PMID: 17405972.

41.   Eischer L, Eichinger S, Kyrle PA. The risk of recurrence in women with venous thromboembolism while using estrogens: a prospective cohort study. Journal of thrombosis and haemostasis : JTH. 2014. doi: 10.1111/jth.12528. PubMed PMID: 24548536.

42.   Jansma JM, Ramsey NF, van der Wee NJ, Kahn RS. Working memory capacity in schizophrenia: a parametric fMRI study. Schizophrenia research. 2004;68(2-3):159-71. doi: 10.1016/S0920-9964(03)00127-0. PubMed PMID: 15099600.

43.   Lowe GD, Sandercock PA, Rosendaal FR. Prevention of venous thromboembolism after major orthopaedic surgery: is fondaparinux an advance? Lancet. 2003;362(9383):504-5. doi: 10.1016/S0140-6736(03)14149-9. PubMed PMID: 12932379.

44.   Schlosser R, Gesierich T, Kaufmann B, Vucurevic G, Hunsche S, Gawehn J, et al. Altered effective connectivity during working memory performance in schizophrenia: a study with fMRI and structural equation modeling. NeuroImage. 2003;19(3):751-63. PubMed PMID: 12880804.

45.   Weinberger DR, Mattay V, Callicott J, Kotrla K, Santha A, van Gelderen P, et al. fMRI applications in schizophrenia research. NeuroImage. 1996;4(3 Pt 3):S118-26. doi: 10.1006/nimg.1996.0062. PubMed PMID: 9345536.

46.   Axelrod BN, Goldman RS, Alphs LD. Validation of the 16-item Negative Symptom Assessment. J Psychiatr Res. 1993;27(3):253-8. PubMed PMID: 7905033.

47.   Heinrichs RW, Zakzanis KK. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology. 1998;12(3):426-45. PubMed PMID: 9673998.

48.   Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD, et al. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. Am J Psychiatry. 2008;165(2):203-13. doi: 10.1176/appi.ajp.2007.07010042. PubMed PMID: 18172019.

49.   Ceskova E, Prikryl R, Kasparek T. Testosterone in first-episode schizophrenia. Neuro Endocrinol Lett. 2007;28(6):811-4. PubMed PMID: 18063925.

50. Konarzewska B, Wolczynski S, Szulc A, Galinska B, Poplawska R, Waszkiewicz N. Effect of risperidone and olanzapine on reproductive hormones, psychopathology and sexual functioning in male patients with schizophrenia. Psychoneuroendocrinology. 2009;34(1):129-39. doi: 10.1016/j.psyneuen.2008.08.015. PubMed PMID: 18838228.

51. Miner MM, Khera M, Bhattacharya RK, Blick G, Kushner H. Baseline data from the TRiUS registry: symptoms and comorbidities of testosterone deficiency. Postgrad Med. 2011;123(3):17-27. doi: 10.3810/pgm.2011.05.2280. PubMed PMID: 21566412.

52. Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dube S, Mallinckrodt C, et al. Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. Am J Psychiatry. 2008;165(8):1033-9. doi: 10.1176/appi.ajp.2008.06091591. PubMed PMID: 18593778.

53. Freedman R, Olincy A, Buchanan RW, Harris JG, Gold JM, Johnson L, et al. Initial phase 2 trial of a nicotinic agonist in schizophrenia. Am J Psychiatry. 2008;165(8):1040-7. doi: 10.1176/appi.ajp.2008.07071135. PubMed PMID: 18381905.

54. First MB, Spitzer RL, Gibbon M, Williams JBW. SCID-I/P2007.

55. Buchanan RW, Davis M, Goff D, Green MF, Keefe RS, Leon AC, et al. A summary of the FDA-NIMH-MATRICS workshop on clinical trial design for neurocognitive drugs for schizophrenia. Schizophr Bull. 2005;31(1):5-19. doi: 10.1093/schbul/sbi020. PubMed PMID: 15888422.

56. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: a review and practical application. Aging Health. 2008;4(3):311-20.

57. Simpson GM, Angus JW. A rating scale for extrapyramidal side effects. Acta Psychiatr Scand Suppl. 1970;212:11-9. PubMed PMID: 4917967.

58. Alphs LD, Summerfelt A, Lann H, Muller RJ. The negative symptom assessment: a new instrument to assess negative symptoms of schizophrenia. Psychopharmacol Bull. 1989;25(2):159-63. PubMed PMID: 2602512.

59. Guy W. ECDEU assessment manual for psychopharmacology, revised 1976 Rockville, MD: National Institute of Mental Health. Psychopharmacology Research Branch.217-331.

60. Kay SR, Flszbein A, Opfer LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia bulletin. 1987;13(2):261.

61. Morosini P, Magliano L, Brambilla L, Ugolini S, Pioli R. Development, reliability and acceptability of a new version of the DSM-IV Social and Occupational Functioning Assessment Scale (SOFAS) to assess routine social funtioning. Acta Psychiatrica Scandinavica. 2000;101(4):323-9.

62. Heinrichs DW, Hanlon TE, Carpenter WT, Jr. The Quality of Life Scale: an instrument for rating the schizophrenic deficit syndrome. Schizophr Bull. 1984;10(3):388-98. PubMed PMID: 6474101.

63. Barnes TR. A rating scale for drug-induced akathisia. Br J Psychiatry. 1989;154:672-6. PubMed PMID: 2574607.

64. Addington D, Addington J, Maticka-Tyndale E. Assessing depression in schizophrenia: the Calgary Depression Scale. Br J Psychiatry Suppl. 1993(22):39-44. PubMed PMID: 8110442.

65. Posner K, Brent D, Lucas C, Gould M, Stanley B, Brown G, et al. Columbia-Suicide Severity Rating Scale (C-SSRS)2008.

66. Oquendo MA, Halberstam B, Mann JJ. Risk factors for suicidal behavior. Rev Psychiatry. 2003;22:103-29.

67. Benner T, Wisco J, Kouwe A, Fischl B, Sorensen A. Accuracy and repeatability of automatic slice positioning compared with manual slice positioning. ISMRM2004. p. 2243.

68. Detre JA, Maccotta L, King D, Alsop DC, Glosser G, D'Esposito M, et al. Functional MRI lateralization of memory in temporal lobe epilepsy. Neurology. 1998;50(4):926-32.

69. Killgore WD, Glosser G, Casasanto DJ, French JA, Alsop DC, Detre JA. Functional MRI and the Wada test provide complementary information for predicting post-operative seizure control. Seizure. 1999;8(8):450-5.

70.   McAllister TW, Saykin AJ, Flashman LA, Sparling MB, Johnson SC, Guerin SJ, et al. Brain activation during working memory 1 month after mild traumatic brain injury: a functional MRI study. Neurology. 1999;53(6):1300-8. PubMed PMID: 10522888.

71.   McAllister TW, Sparling MB, Flashman LA, Guerin SJ, Mamourian AC, Saykin AJ. Differential working memory load effects after mild traumatic brain injury. Neuroimage. 2001;14(5):1004-12. PubMed PMID: 11697932.

72.   McDonald BC, Saykin AJ, Williams JM, Assaf BA. fMRI Wada test: prospects for presurgical mapping of language and memory. In: Faro SH, Mohamed FB, editors. Functional MRI: Basic Principles and Clinical Applications. New York: Springer; 2006. p. 278-314.

73.   McDonald BC, Saykin AJ. Functional magnetic resonance imaging in neurosurgical planning for temporal lobe epilepsy: language, memory, and seizure outcome. In: Hillary FG, DeLuca J, editors. Functional Neuroimaging in Clinical Populations. New York: Guilford Press; 2007. p. 185-218.

74.   Rabin ML, Narayan VM, Kimberg DY, Casasanto DJ, Glosser G, Tracy JI, et al. Functional MRI predicts post-surgical memory following temporal lobectomy. Brain. 2004;127(Pt 10):2286-98. Epub 2004/08/27. doi: 10.1093/brain/awh281awh281 [pii]. PubMed PMID: 15329352.

75.   Saykin AJ, Flashman LA, Frutiger S, Johnson SC, Mamourian A, Moritz C, et al. Semantic processing and memory in Alzheimer's disease: fMRI activation patterns. J Int Neuropsychol Soc. 1998;4:49.

76.   Saykin AJ, Flashman LA, McAllister TW, Johnson SC, Guerin SJ, Brown CJ, et al. Semantic, phonological and episodic memory processing in schizophrenia: Functional MRI activation patterns indicate a need for new models of dysfunction. Schizophr Res. 1999;36:233.

77.   Saykin AJ, Wishart HA, Rabin LA, Flashman LA, McHugh TL, Mamourian AC, et al. Cholinergic enhancement of frontal lobe activity in mild cognitive impairment. Brain. 2004;127(Pt 7):1574-83. Epub 2004/05/14. doi: 10.1093/brain/awh177awh177 [pii]. PubMed PMID: 15140813.

78.   Wishart HA, Saykin AJ, McDonald BC, Mamourian AC, Flashman LA, Schuschu KR, et al. Brain activation patterns associated with working memory in relapsing-remitting MS. Neurology. 2004;62(2):234-8. PubMed PMID: 14745059.

79.   Baddeley AD. Working Memory. Oxford: Clarendon Press; 1986.

80.   Baddeley A. Working memory. Science. 1992;255(5044):556-9.

81.   Baddeley AD, Hitch GJ. Developments in the concept of working memory. Neuropsychology. 1994;8:485-93.

82.   Baddeley AD. Working Memory. In: Gazzaniga MS, editor. The Cognitive Neurosciences. Cambridge, MA: MIT Press; 1995.

83.   Baddeley AD. Recent developments in working memory. Current Opinion in Neurobiology. 1998;8(2):234-8.

84.   Goldman-Rakic PS. Regional and cellular fractionation of working memory. Proc Natl Acad Sci U S A. 1996;93(24):13473-80. PubMed Central PMCID: PMC9703.

85.   Goldman-Rakic PS, Selemon LD. Functional and anatomical aspects of prefrontal pathology in schizophrenia. Schizophrenia Bulletin. 1997;23(3):437-58.

86.   Rao SM. Neuropsychology of multiple sclerosis: a critical review. Journal of Clinical & Experimental Neuropsychology. 1986;8(5):503-42.

87.   Dumas JA, Saykin AJ, McDonald BC, McAllister TW, Hynes ML, Newhouse PA. Nicotinic versus muscarinic blockade alters verbal working memory-related brain activity in older women. Am J Geriatr Psychiatry. 2008;16(4):272-82. PubMed PMID: 18378552; PubMed Central PMCID: PMCNIHMS128558PMC3114443.

88.   Shaywitz SE, Shaywitz BA, Pugh KR, Fulbright RK, Skudlarski P, Mencl WE, et al. Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. Jama. 1999;281(13):1197-202. PubMed PMID: 10199429.

89.   Luck SJ, Kappenman ES. The Oxford Handbook of Event-Related Potential Components: Oxford University Press, USA; 2011.

90.   Vohs JL, Chambers RA, Krishnan GP, O'Donnell BF, Berg S, Morzorati SL. GABAergic modulation of the 40 Hz auditory steady-state response in a rat model of schizophrenia. Int J Neuropsychopharmacol. 2010;13(4):487-97. doi: 10.1017/S1461145709990307. PubMed PMID: 19627651; PubMed Central PMCID: PMC2882653.

91.   Vohs JL, Chambers RA, O'Donnell BF, Krishnan GP, Morzorati SL. Auditory steady state responses in a schizophrenia rat model probed by excitatory/inhibitory receptor manipulation. Int J Psychophysiol. 2012;86(2):136-42. doi: 10.1016/j.ijpsycho.2012.04.002. PubMed PMID: 22504207; PubMed Central PMCID: PMC3443327.

92.   Rass O, Forsyth JK, Krishnan GP, Hetrick WP, Klaunig MJ, Breier A, et al. Auditory steady state response in the schizophrenia, first-degree relatives, and schizotypal personality disorder. Schizophrenia research. 2012;136(1-3):143-9. doi: 10.1016/j.schres.2012.01.003. PubMed PMID: 22285558; PubMed Central PMCID: PMC3298621.

93.   Rasser P, Schall U, Todd J, Michie P, Ward P, Johnston P, et al. Gray matter deficits, mismatch negativity, and outcomes in schizophrenia. Schizophrenia bulletin. 2011;37(1):131-40.

94.   Nuechterlein K, Green M. MCCB: Matrics Consensus Cognitive Battery. Los Angeles: MATRICS Assessment. 2006.

95.   Dumas JA, Kutz AM, McDonald BC, Naylor MR, Pfaff AC, Saykin AJ, et al. Increased working memory-related brain activity in middle-aged women with cognitive complaints. Neurobiology of Aging. 2012.

96.   McAllister TW, McDonald BC, Flashman LA, Ferrell RB, Tosteson TD, Yanofsky NN, et al. Alpha-2 adrenergic challenge with guanfacine one month after mild traumatic brain injury: Altered working memory and BOLD response. International Journal of Psychophysiology. 2011;82(1):107-14.

97.   McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Alterations in Brain Activation During Working Memory Processing Associated With Breast Cancer and Treatment: A Prospective Functional Magnetic Resonance Imaging Study. Journal of Clinical Oncology. 2012;30(20):2500-8.

98.   Dumas JA, McDonald BC, Saykin AJ, McAllister TW, Hynes ML, West JD, et al. Cholinergic modulation of hippocampal activity during episodic memory encoding in postmenopausal women: a pilot study. Menopause (New York, NY). 2010;17(4):852.

99.   Luo D WY, Huang H, Sheng J, Cheng H, Saykin AJ, Shen L. , editor. Detecting common connectivity modules from complex brain networks. OHBM'12: 18th Annual Meeting of the Organization for Human Brain Mapping; 2012; Beijing, China.

100.   Wang Y, Saykin AJ, Pfeuffer J, Lin C, Mosier KM, Shen L, et al. Regional reproducibility of pulsed arterial spin labeling perfusion imaging at 3T. NeuroImage. 2011;54(2):1188-95.

101.   Wang Y, West JD, Flashman LA, Wishart HA, Santulli RB, Rabin LA, et al. Selective changes in white matter integrity in MCI and older adults with cognitive complaints. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2012;1822(3):423-30.

102.   Cheng H, Wang Y, Sheng J, Sporns O, Kronenberger WG, Mathews VP, et al. Optimization of seed density in DTI tractography for structural networks. Journal of neuroscience methods. 2011.

103.   Flashman LA, McAllister TW, Andreasen NC, Saykin AJ. Smaller brain size associated with unawareness of illness in patients with schizophrenia. American Journal of Psychiatry. 2000;157(7):1167-9.

104.   McDonald BC, Conroy SK, Smith DJ, West JD, Saykin AJ. Frontal Gray Matter Reduction after Breast Cancer Chemotherapy and Association with Executive Symptoms: A Replication and Extension Study. Brain, Behavior, and Immunity. 2012.

105.   Risacher SL, Shen L, West JD, Kim S, McDonald BC, Beckett LA, et al. Longitudinal MRI atrophy biomarkers: relationship to conversion in the ADNI cohort. Neurobiology of aging. 2010;31(8):1401-18.