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A Randomized Exploratory Trial of an Alpha-7 Nicotinic Receptor Agonist (TC-5619) for Cognitive Enhancement in Schizophrenia

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This exploratory trial was conducted to test the effects of an alpha7 nicotinic receptor partial agonist, TC-5619, on cognitive dysfunction and negative symptoms in subjects with schizophrenia. In the United States and India, 185 outpatients (18–60 years; male 69%; 46% tobacco users) with schizophrenia treated with quetiapine or risperidone monotherapy were randomized to 12 weeks of placebo ($n=91$) or TC-5619 ($n=94$; orally once daily 1 mg day 1 to week 4, 5 mg week 4 to 8, and 25 mg week 8 to 12). The primary efficacy outcome measure was the Groton Maze Learning Task (GMLT; executive function) of the CogState Schizophrenia Battery (CSB). Secondary outcome measures included: CSB composite score; Scale for Assessment of Negative Symptoms (SANS); Clinical Global Impression-Global Improvement (CGI-I); CGI-severity (CGI-S); and Subject Global Impression-Cognition. GMLT statistically favored TC-5619 ($P=0.036$) in this exploratory trial. SANS also statistically favored TC-5619 ($P=0.030$). No other secondary outcome measure demonstrated a drug effect in the total population; there was a statistically significant drug effect on working memory in tobacco users. The results were typically stronger in favor of TC-5619 in tobacco users and occasionally better in the United States than in India. TC-5619 was generally well tolerated with no clinically noteworthy safety findings. These results support the potential benefits of TC-5619 and alpha7 nicotinic receptor partial agonists for cognitive dysfunction and negative symptoms in schizophrenia.
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INTRODUCTION

Schizophrenia is a heterogeneous mental disorder associated with different pathological features, including positive and negative symptoms (DSM IV-TR; American Psychiatric Association, 2000)) and cognitive dysfunction (Reichenberg and Harvey, 2007). The negative and cognitive symptoms may precede onset of positive symptoms by many years and persist despite the remission of psychosis (Addington and Addington, 1993; Fleischhacker, 2000; O'Carroll, 2000; Ojeda, 2000; Reichenberg *et al*, 2010; Tandon *et al*, 2009). Positive symptoms are highly responsive to antipsychotic medications in the majority of patients, but negative symptoms and cognitive dysfunction are much less effectively treated (Erhart *et al*, 2006; Gold, 2004; Marder and Fenton, 2004; Miyamoto *et al*, 2005; Stahl and Buckley, 2007) and hence may prevent many patients from functioning productively and independently (Gold, 2004; Stahl *et al*, 2007).

In contrast to psychotic symptoms in which the D2 receptor is a proven target, the pharmacology of cognitive and negative symptoms is not well defined. To this end, the National Institute of Mental Health sponsored the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative to develop a consensus battery of neuropsychological tests that could measure therapeutic effects of agents for cognitive dysfunction in schizophrenia (CDS; Gold, 2004; Kern *et al*, 2008; Nuechterlein *et al*, 2004; Nuechterlein *et al*, 2008). In the wake of the MATRICS initiative, molecular targets for the treatment of CDS have been proposed (Gray and Roth, 2007; Marder *et al*, 2004). Among the prioritized targets was the alpha7 neuronal nicotinic receptor (NNR; Freedman *et al*, 2008; Kucinski *et al*, 2011; Olincy and Stevens, 2007). Initially, preclinical studies indicated that antagonists of this receptor could induce sensory gating deficits in rodents that were similar to those observed in schizophrenia

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These data were previously presented at the International Congress of Schizophrenia Research (Colorado Springs, CO; April 2011); at the NCDEU Annual Meeting (Boca Raton, FL; June 2011); and at the American College of Neuropsychopharmacology Annual Meeting (ACNP; Hawaii; December 2011).
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(Luntz-Leybman *et al*, 1992). Shortly thereafter, genetic linkage was found for this deficit and the alpha7 NNR gene (Freedman *et al*, 1997). Post-mortem studies of individuals with schizophrenia further supported these findings by showing reduced expression of this receptor in the hippocampus (Freedman *et al*, 1995) and thalamic reticular nucleus (Court *et al*, 1999). Several alpha7 NNR agonists have shown promising results in preliminary trials by demonstrating efficacy against surrogate electrophysiological markers (EnVivo_Pharmaceuticals, 2009; Olincy *et al*, 2006) and cognitive and negative symptoms of schizophrenia (Freedman *et al*, 2008).

TC-5619 (a benzofuran-substituted pyridylmethylquinuclidine) is a highly selective alpha7 NNR partial agonist with a Ki at the alpha7 NNR of 1 nM. It is 1000–10 000 times less potent at other NNRs and at other receptor subtypes (eg, 5HT3, opioid).

Two phase 1 studies of TC-5619 in healthy male volunteers showed that single doses of TC-5619 were well tolerated up to 406 mg (limited by orthostatic hypotension) and that multiple doses of 204 mg are well tolerated (Targacept, data on file). The half-life (20 h) and time to C_{max} (approximately 2 h) were independent of dose. Finally, the Cognitive Drug Research cognitive test battery revealed a statistically significant improvement in power of attention ($P<0.05$) in subjects taking 6.8 mg TC-5619 (Targacept Study TC-5619-CLP-002 (data on file)). Based on these clinical data as well as data from a preclinical model suggesting cognitive benefits at a human dose of approximately 3 mg (Hauser *et al*, 2009) (Targacept, data on file), a dose range of 1–25 mg was determined to be safe and conservatively chosen to encompass the anticipated efficacious dose range.

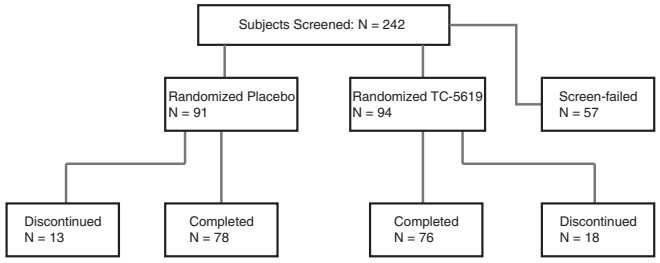

Figure 1 Subject disposition.

Tablee 1 Patient Features at Enrollment

	Placebo: *n* (%)	TC-5619: *n* (%)
Randomized subjects	91 (49%)	94 (51%)
Age	36.3 years	36.3 years
Male	63 (69%)	65 (69%)
Gender		
Female	28 (31%)	29 (31%)
Race		
Asian	60 (66%)	61 (65%)
African-American	25 (28%)	26 (28%)
Hispanic/Latino	2 (2%)	1 (1%)
Caucasian	4 (4%)	6 (6%)
Tobacco status		
User	41 (45%)	45 (48%)
Tobacco non-user	50 (55%)	49 (52%)
BMI	24.9	25.0
Completed HS or above	60 (66%)	62 (66%)

SUBJECTS AND METHODS

Subjects

The study was Institutional Review Board-reviewed, filed with the US Food and Drug Administration (FDA), and registered on www.clinicaltrials.gov (NCT01003379) before study initiation, and conducted according to the Guidelines of the Declaration of Helsinki (2008). It was conducted by the sponsor (Targacept) at 12 sites in India and 7 sites in the United States. Enrollment began in November 2009 and was completed in December 2010. A total of 185 subjects were enrolled and randomized (Figure 1; Table 1). Tobacco users were stratified approximately equally across the treatment arms; tobacco use was verified by urinary cotinine levels >500 ng/ml, and non-use by urinary cotinine levels <50 ng/ml. In all, 46% were tobacco users. In total, 154 subjects completed the study (Figure 1); the most frequent reason for discontinuation was withdrawal of consent (12 subjects). All subjects met DSM-IV criteria for schizophrenia, primarily paranoid type (92%). Subjects were required to be outpatients with: stable housing, availability of caregivers with personal contact at least four times weekly, and stable schizophrenia, defined as a lack of psychiatric hospitalization and no change in quetiapine or risperidone monotherapy dose for 2 months before screening and during the study. Quetiapine and risperidone were chosen for this preliminary adjunctive treatment study because they are prototypical atypical antipsychotics that are widely used in the clinic. Use of a wider range of underlying antipsychotic treatments will be adopted in future studies. Subjects could have: a score no >4 on the Positive and Negative Syndrome Scale (PANSS) items assessing delusions, hallucinations, unusual thought content, and conceptual disorganization; a score no >5 on the Calgary Depression Scale for Schizophrenia (CDSS); and no significant suicidality as assessed by the Columbia Suicide Severity Rating Scale (CSSRS). Subjects could have no other comorbid axis 1 or axis 2 DSM-IV disorder nor any unstable medical condition. All subjects signed a written informed consent.

Experimental Drug and Matching Placebo

The experimental drug was a blend of TC-5619 and excipients in gelatin capsules. Placebo was manufactured to match the experimental drug TC-5619 in identical-appearing capsules in size, color, and shape.

Experimental Drug Protocol and Assessments

At screening, all subjects were trained to use the CogState Schizophrenia Battery (CSB). They also had assessments using the PANSS, Scale For Assessment of Negative Symptom (SANS), Subject Global Impression-Cognition

(SGI-Cog; a subject-rated seven-point Likert scale assessing three items: memory and learning, attention and concentration, and speed of thinking), and Clinical Global Impression-Severity (CGI-S) scales. Physical examination, vital signs, serum chemistry and hematology, urinalysis, urine drug screen for illicit drugs, and ECG were performed. Assessments were also made using the Abnormal Involuntary Movements Scale (AIMS), the CSSRS, and the CDSS.

On day 1, all subjects meeting eligibility criteria including absence of illicit drugs were randomized in a double-blind manner (subjects, caregivers, staff, sponsor were blinded) using a 1 : 1 allocation to receive either TC-5619 or placebo orally once daily, in addition to quetiapine or risperidone monotherapy. The random allocation method was based on a blinded sequence method applied by an independent statistician employed by an outside vendor. Approximately equal numbers of tobacco users and non-users were stratified and randomized to each cohort. Blood samples were taken before and approximately 3 h after study drug was administered in the clinic, and these were processed for PK analyses of TC-5619 and for measuring blood levels of quetiapine and risperidone. ECG and vital signs measurements were obtained just before each PK blood sample.

Subjects in the TC-5619 cohort were to take 1 mg TC-5619 qd from day 1 to week 4; 5 mg qd from week 4 to 8; and 25 mg qd from week 8 to 12. Subjects were instructed to take their daily dose of study drug each morning, at least 90 min before tobacco use (if a user) and at least 30 min before taking quetiapine or risperidone. Subjects returned for clinic visits at weeks 1, 4, 8, and 12 for safety and efficacy assessments. Subject compliance for TC-5619 was monitored by pill counts at each visit. Compliance with quetiapine, risperidone, and other concomitant medications was monitored by subject self-report. Urinary drug screens were used to monitor illicit drug use, and urinary cotinine levels discriminated tobacco use from non-use. There was a final clinic visit at week 14 for follow-up. Disposition of subjects is shown in Figure 1.

Plasma Levels of TC-5619, Quetiapine and Risperidone

Serial and sparse blood sampling from a subset of subjects ($n = 62$) was performed pre-dose and post-dose on day 1 and weeks 4, 8, and 12 to estimate TC-5619 PK parameters and to document plasma levels of the concomitant antipsychotic medication with and without TC-5619 co-administration. Sample analyses were performed using LC-MS/MS methods at Tandem Laboratories. All plasma samples were analyzed for TC-5619 levels (Salt Lake City, UT). All pre-dose samples were analyzed for either quetiapine or risperidone and 9-hydroxy-risperidone (active metabolite) levels (Trenton, NJ).

PK parameters were estimated for TC-5619, including C_{max}, T_{max}, and AUC to last collected sample (8 h post-dose) and the dosing interval (extrapolated to 24 h post-dose). Plasma levels of the antipsychotic medication in the pre-dose samples were examined by cohort.

Statistical Analyses

The prospectively defined primary efficacy outcome measure was the change from day 1 to weeks 4, 8, and 12 in the Groton Maze Learning Task (GMLT) of the CSB, as filed to FDA and to clinicaltrials.gov before study initiation. The CSB rather than other cognitive test batteries was used because of its shorter time for completion (20 min), its culturally neutral computerized tasks (important for India), and its assessment of the seven cognitive domains judged important to CDS by the MATRICS initiative. The GMLT was chosen because it measures executive function, the highest domain of cognitive function, and because CogState analyses of other alpha7 NNR agonists in previous studies showed that the GMLT was sensitive to effects produced by this pharmacological class.

Secondary outcomes included the CSB composite score, and scores for SANS, CGI-S, Clinical Global Impression-Global Improvement (CGI-I), and SGI-Cog. Additional analyses included individual items of the CSB.

Efficacy analysis was based on a mixed model repeated-measures methodology. The model considered change from baseline to weeks 4, 8, and 12 as the response variable and included fixed effects for treatment, visit and treatment by visit interaction as well as baseline score and a random effect for center. Values reported here use a 2-tailed test with an alpha of 0.05 in order to determine statistical significance. As this was an exploratory study, within-company success criteria for drug development discussions were prospectively defined as P-values < 0.10 (one-tailed) as specified in the FDA-submitted protocol. Outcomes were not corrected for multiplicity in this exploratory study. For a number of CogState tasks (detection, identification, 1-card learning, and 1-back), data integrity criteria were specified *a priori* to identify responses that reflect poor subject cooperation. The analysis excluded subjects who failed to meet these criteria.

Sample size calculations showed that 60 subjects should complete week 12 of the study to have a 90% power of detecting a signal using a one-tailed test with an alpha of 0.10. As this study examined the efficacy of TC-5619 in both a tobacco user and a non-user cohort, 120 subjects were needed (60 per cohort) in order to be able to detect a signal in either of these cohorts.

RESULTS

Efficacy

Primary efficacy outcome measure. A blinded review by CogState during the study's treatment phase indicated a potentially greater-than-expected variance in the GMLT data, and analysis after database lock confirmed the existence of a positive skew to the GMLT data in the intent to treat (ITT) population (sample skewness $g_1 = 1.03$ in all patients at baseline). Consequently, a log (10) transformation of the GMLT data was performed ($g_1 = -0.02$). The treatment effect was statistically significant in favor of TC-5619 ($P = 0.036$, Week 4; Table 2), and the magnitude of the effect did not diminish following 12 weeks of randomized treatment (Cohen's d for week 12 compared with day 1 = 0.40). The treatment effect in US patients (mean difference = 0.082, 0.040, and 0.080 at 4, 8, and 12 weeks) was greater than the effect in Indian patients (mean difference = 0.020, 0.009, and 0.011 at 4, 8, and 12 weeks); this effect was significant at weeks 4 and 12 for US patients ($P = 0.0072$, $P = 0.0044$, respectively) but not for the Indian

Table 2 Results of Primary, Secondary, and CogState Analyses, Total Population (Tobacco Users and Non-Users)

Task	Week 4[a]	Week 8[a]	Week 12[a]
GMLT log (10) transformed	0.046 (0.022); $P=$ **0.036**	0.023 (0.021); $P=0.262$	0.037 (0.021); $P=0.081$
SANS	0.78 (1.18); $P=0.510$	1.71 (1.44); $P=0.236$	3.70 (1.68); $P=$ **0.030**
CGI-I	0.15 (0.09); $P=0.098$	0.08 (0.12); $P=0.506$	0.15 (0.14); $P=0.300$
SGI-Cog	0.05 (0.28); $P=0.856$	0.01 (0.36); $P=0.982$	0.66 (0.39); $P=0.092$
CGI-S	−0.02 (0.06); $P=0.652$[b]	−0.01 (0.06); $P=0.912$[b]	0.01 (0.09); $P=0.900$
CogState composite score[c]	−0.066 (0.066); $P=0.324$[b]	−0.062 (.068); $P=0.358$[b]	0.030 (0.067); $P=0.652$
CogState detection[c]	0.007 (0.015); $P=0.614$[b]	0.006 (0.014); $P=0.700$[b]	0.002 (0.015); $P=0.866$[b]
CogState identification[c]	−0.005 (0.011); $P=0.622$	−0.014 (0.012); $P=0.222$	−0.017 (0.011); $P=0.126$
CogState 1-card learning[c]	−0.008 (0.019); $P=0.680$[b]	−0.024 (0.019); $P=0.202$[b]	−0.019 (0.020); $P=0.318$[b]
CogState 1-back[c]	0.007 (0.014); $P=0.618$[b]	0.005 (0.015); $P=0.706$[b]	−0.024 (0.014); $P=0.084$
CogState ISLT[c]	−1.121 (0.597); $P=0.060$[b]	−0.536 (0.605); $P=0.374$[b]	0.556 (0.602); $P=0.354$
CogState social-emotional cognition[c]	0.013 (0.020); $P=0.518$	0.015 (0.020); $P=0.434$	−0.002 (0.020); $P=0.908$[b]
Trail-making test A (TMT A) Cognition processing	0.82 (2.55); $P=0.750$[b]	5.22 (2.58); $P=0.046$[b]	4.25 (2.56); $P=0.104$[b]
Trail-making test B (TMT B) Visual searching and processing	3.35 (5.39); $P=0.540$[b]	2.31 (5.46); $P=0.676$[b]	1.22 (5.40); $P=0.824$[b]
Digit-symbol substitution test (DSST) Speed of processing	−0.71 (1.21); $P=0.562$[b]	−0.76 (1.22); $P=0.536$[b]	−1.91 (1.22); $P=0.122$[b]

[a]Values in each cell are: (mean difference TC-5619 *vs* placebo (SEM); two-tailed *P*-value). Values in bold indicate statistical significance (two-tailed alpha, $P<0.05$), favoring TC-5619.

[b]Result favored placebo, typically not significant.

[c]Depicts results in data set meeting predefined data integrity criteria.

Table 3 Results of Primary, Secondary, and CogState Analyses[a] in Tobacco Users

	Week 4[a]	Week 8[a]	Week 12[a]
GMLT log (10) transformed	0.059 (0.026); $P=$ **0.028**	0.038 (0.027); $P=0.172$	0.074 (0.024); $P=$ **0.004**
SANS	2.74 (2.10); $P=0.196$	4.00 (2.45); $P=0.108$	4.86 (2.58); $P=0.066$
CGI-I	0.25 (0.14); $P=0.094$	0.24 (0.16); $P=0.150$	0.18 (0.20); $P=0.378$
SGI-Cog	−0.13 (0.47); $P=0.786$[b]	0.24 (0.49); $P=0.626$	0.65 (0.52); $P=0.218$
CGI-S	0.02 (0.09); $P=0.868$	0.08 (0.10); $P=0.448$	0.07 (0.14); $P=0.614$
CogState composite score[c]	0.007 (0.099); $P=0.942$	−0.029 (0.101); $P=0.772$[b]	0.129 (0.100); $P=0.196$
CogState detection[c]	−0.014 (0.021); $P=0.508$	−0.000 (0.021); $P=0.982$	−0. 013 (0.021); $P=0.530$
CogState identification[c]	−0.021 (0.016); $P=0.194$	−0.011 (0.017); $P=0.522$	−0.026 (0.016); $P=0.114$
CogState 1-card learning[c]	−0.008 (0.027); $P=0.770$[b]	−0.021 (0.028); $P=0.444$[b]	0.012 (0.028); $P=0.676$
CogState 1-back[c]	0.005 (0.021); $P=0.790$[b]	−0.009 (0.021); $P=0.658$	−0.042 (0.020); $P=$ **0.040**
CogState ISLT[c]	0.13 (0.89); $P=0.888$	0.33 (0.90); $P=0.716$	1.71 (0.89); $P=0.054$
CogState social-emotional Cognition[c]	−0.010 (0.028); $P=0.736$[b]	−0.009 (0.029); $P=0.750$[b]	−0.019 (0.027); $P=0.496$[b]
TMT A cognition processing	−0.05 (3.78); $P=0.990$	5.43 (3.81); $P=0.160$[b]	5.02 (3.77); $P=0.190$[b]
TMT B visual searching and processing	11.73 (8.02); $P=0.150$[b]	1.62 (8.15); $P=0.844$[b]	6.86 (7.95); $P=0.396$[b]
DSST speed of processing	−0.03 (1.78); $P=0.986$[b]	−0.71 (1.80); $P=0.696$[b]	−0.97 (1.78); $P=0.592$[b]

[a]Values in each cell are: (mean difference TC-5619 *vs* placebo (SEM); two-tailed *P*-value). Values in bold indicate statistical significance (two-tailed alpha, $P<0.05$), favoring TC-5619.

[c]Depicts results in data set meeting predefined data integrity criteria.

[b]Result favored placebo, typically not significantly.

patients ($P>0.4$ for all visits). The test for country (United States or India) by treatment interaction was not statistically significant ($P=0.1577$).

This effect of TC-5619 was also typically greater in tobacco users (Table 3).

Secondary efficacy outcome measures. Statistically significant results favoring TC-5619 in the ITT population were found in the SANS ($P=0.030$, week 12; Cohen's d for week 12 compared with day 1 = 0.26; Table 2). In the SGI-Cog, although the total score was not statistically

significant, the attention/concentration item favored TC-5619 ($P = 0.042$). There was no drug effect identified in the ITT population in the other secondary outcome measures. The effect of TC-5619 was typically stronger in tobacco users (Table 3). The result in the SANS was driven by the avolition/apathy ($P = 0.110$), anhedonia ($P = 0.062$), and affective flattening ($P = 0.036$) items.

CogState analyses, Trail-Making Test (TMT) A and B, Digit-Symbol Substitution Test (DSST). No measures in the CogState test battery, nor in the TMT or DSST, were statistically significant at the 0.05 level in the ITT population (Table 2).

A subgroup analysis of these data by tobacco use (Table 3) showed statistical significance in favor of TC-5619 in tobacco users on the 1-back test (ONB, a test of working memory: $P = 0.040$, week 12; Table 3).

Safety and Tolerability

Adverse events (AEs). In all, 26% of subjects in the placebo cohort and 32% of subjects in the TC-5619 cohort reported AEs. All AEs were mild or moderate in severity. AEs that were present in at least three subjects in either cohort were: constipation (2% placebo *vs* 4% TC-5619); nausea (0% placebo *vs* 5% TC-5619); body temperature increased (2% placebo *vs* 3% TC-5619); decreased appetite (5% placebo *vs* 4% TC-5619); somnolence (2% placebo *vs* 3% TC-5619); (worsening of) schizophrenia (2% placebo *vs* 3% TC-5619); headache (2% placebo *vs* 3% TC-5619); and insomnia (0% placebo *vs* 3% TC-5619). Nausea did not lead to discontinuation, and it was mild ($n = 4$) or moderate ($n = 1$) in severity.

Serious adverse events (SAEs). Two SAEs were reported in the study, one in the placebo group (gastritis) and one in the TC-5619 group (acute exacerbation of schizophrenia). Both were considered not drug related by the investigator. The subject with gastritis became dehydrated and was hospitalized to receive intravenous fluids. The subject with acute exacerbation of schizophrenia stopped taking quetiapine and a week later was found wandering in the streets and acting bizarrely. He was hospitalized and treated.

AEs leading to discontinuation. Seven AEs led to discontinuation, three in the placebo group and four in the TC-5619 group. All were considered not drug related by the investigator. In the placebo group, the events were: gastritis (moderate), dermatitis (mild), and worsening schizophrenia (moderate). In the TC-5619 group, the events were: gastroenteritis (moderate), exacerbation of schizophrenia (two, moderate), and exacerbation of psychotic symptoms (moderate).

Results of the CSSRS and CDSS. The CSSRS detected very low levels of suicidality during the trial between any visits, and all changes were in the placebo cohort. The CDSS showed no meaningful change either within or between cohorts in the very low level of depression during the study.

Physical examination, AIMS, vital signs, laboratory analytes, and ECG. There were no clinically meaningful changes between cohorts in physical examination, vital signs, orthostatic blood pressure changes, or urine or serum laboratory measurements. The AIMS revealed no difference between cohorts in the very low mean severity of involuntary movements (AIMS score 0.1 in both cohorts on day 1 and at week 12).

There was no clinically meaningful difference in any ECG parameters or interpretations between cohorts. Mean QTcF values changed by a small and similar way in the cohorts between the beginning (placebo: 394.4 ms pre-dose day 1; TC-5619: 392.9 ms pre-dose day 1) and end of the study (placebo: +1.6 ms post-dose week 12; TC-5619: +0.8 ms post-dose week 12). No QTcF values were > 480 ms in either cohort.

Pharmacokinetics

TC-5619 exposure levels and pharmacokinetic parameters. TC-5619 exposure, measured by steady-state concentrations (pre-dose (1.5–41.9 ng/ml) and at T_{max} (15.3–142 ng/ml)) and $AUC_{24\,h}$ (129–1504 ng × hr/ml, estimated by extrapolation from 8-h collection time point), increased in a dose-dependent manner over the dose range. T_{max} (1.0–1.8 ng/ml) remained independent of dose. An analysis of the PK parameters of tobacco users and non-users was similar and could not account for differences in the effect of TC-5619 observed in the two sub-populations in the study.

Antipsychotic exposure levels. Plasma levels of the concomitant antipsychotic were monitored in some subjects ($n = 62$) to ensure that a significant pharmacokinetic drug interaction could not contribute to observed efficacy. Levels of quetiapine and the 'active moiety' of risperidone (risperidone + 9-hydroxy-risperidone levels; (Risperdal prescribing information)) are presented (Table 4). Subjects were allowed to take any approved dose of antipsychotic, which contributed to the variability in levels. In addition, several subjects in each antipsychotic subgroup did not have quantifiable antipsychotic levels, indicating either low doses or noncompliance with antipsychotic therapy.

Subjects receiving TC-5619 appeared to have lower (range of mean values: 208 to 284 nM) quetiapine levels than subjects receiving placebo (range of mean values: 512–743 nM). The number of subjects in the analyzed quetiapine subgroup is small and differences in quetiapine doses or compliance across the two subgroups may have contributed to the observed differences in quetiapine levels between the cohorts. Levels of active moiety of risperidone were similar among subjects receiving placebo and TC-5619. These findings indicate that a drug interaction did not contribute to the effects observed in this study, because a lower quetiapine level in the TC-5619 group would be anticipated to produce smaller rather than greater beneficial effects in this cohort.

DISCUSSION

There has been great interest and much effort to develop novel medications for the treatment of the cognitive and

Table 4 Mean (SD) Plasma Levels of the Co-Administered Antipsychotic Following Oral Doses of Placebo or TC-5619

	Visit			
	Day 1	**Week 4**	**Week 8**	**Week 12**
	Placebo + quetiapine			
Quetiapine	$n=7$	$n=6$	$n=6$	$n=6$
C_{trough} (nM)	630 (1144)	512 (633)	587 (598)	743 (634)
No. subjects BLQ[a]	6	6	5	6
	TC-5619 + quetiapine			
Quetiapine	$n=8$	$n=8$	$n=8$	$n=7$
C_{trough} (nM)	284 (313)	208 (139)	254 (170)	243 (229)
No. subjects BLQ[a]	6	5	5	4
Risperidone	Placebo + risperidone			
Active moiety[b]	$n=7$	$n=6$	$n=5$	$n=8$
C_{trough} (nM)	79.3 (39.3)	60.2 (47.7)	87.0 (66.2)	58.3 (42.0)
No. subjects BLQ[a]	8	9	9	6
Risperidone	TC-5619 + risperidone			
Active moiety[b]	$n=13$	$n=12$	$n=10$	$n=11$
C_{trough} (nM)	66.6 (63.5)	68.1 (64.5)	99.2 (62.0)	83.5 (69.3)
No. subjects BLQ[a]	5	5	5	5

[a]Number of additional subjects for whom pre-dose samples were obtained but on analysis appeared to have no antipsychotic level (levels were below the limit of quantitation (BLQ)).

[b]Active moiety represents the levels of risperidone + 9-hydroxy-risperidone.

negative symptoms for which antipsychotic drugs are ineffective (Gray *et al*, 2007; Miyamoto *et al*, 2005). In this context, nicotinic cholinergic receptors have emerged as priority targets and particularly the alpha7 and alpha4 beta2 NNRs (Freedman *et al*, 2008; Kucinski *et al*, 2011; Olincy *et al*, 2007; Ripoll *et al*, 2004). Although studies of the latter have yielded mixed results (Buchanan *et al*, 2008), studies of the former, using DMX-B and EVP6124, have shown therapeutic potential in subjects with schizophrenia (EnVivo_Pharmaceuticals, 2009, 2011; Freedman *et al*, 2008; Olincy *et al*, 2006). The results of this exploratory trial with TC-5619 are generally consistent with those of prior studies of alpha7 NNR partial agonists, and indicated statistically significant effects on the primary efficacy outcome (GMLT, executive function) and on some secondary efficacy outcomes including SANS (negative symptoms). The results on the CSB task assessing working memory were statistically significant in favor of TC-5619 in tobacco users.

Although TC-5619 produced a statistically significant benefit in the ITT population on executive function as measured by the GMLT, it did not produce a statistically significant benefit in the ITT population on the other six cognitive domains as measured in the CSB. Interestingly, despite the absence of a statistically significant benefit of TC-5619 on the other six cognitive domains in the ITT population, there were statistically significant effects of TC-5619 on some of these other domains (eg, working memory, as measured by the identification task) in the tobacco user cohort. The reason(s) for stronger effects of TC-5619 on executive function than on other cognitive domains is (are) unknown.

TC-5619 was generally well tolerated. Except for nausea, there were similar numbers of AEs, SAEs, and AEs leading to discontinuation. There were no clinically meaningful differences between cohorts in suicidality, depression, physical examination, AIMS, vital signs, lab measurements of serum or urine analytes, or ECG.

No differences in the drug levels between tobacco users and non-users were observed to account for differences in efficacy between the two sub-populations. Antipsychotic levels across the TC-5619 and placebo groups were similar for risperidone, and we do not believe that the difference in quetiapine would account for the effects observed.

It is intriguing that the positive effects of TC-5619 were stronger in tobacco users. This finding may pertain to observations that tobacco use is considerably higher in patients with schizophrenia than in the general population (de Leon *et al*, 1995; Goff *et al*, 1992; Hughes *et al*, 1986), suggesting that these patients derive an NNR-mediated therapeutic benefit from nicotine.

It is possible that the relatively small number of subjects in the study population caused this finding in tobacco users to arise by chance. However, if this observation is confirmed by subsequent studies, at least three hypotheses may be invoked to explain this finding. First, alpha7 NNRs may be upregulated by tobacco use. In patients with schizophrenia who smoke, mRNA encoding the alpha7 NNR subtype was shown to be upregulated by 250% in comparison with patients who did not smoke, and there were a greater number of functional alpha7 NNRs in smokers (Mexal *et al*, 2010). This finding suggests that there could be more alpha7 receptors in tobacco-users on which TC-5619 could act. Second, nicotine increases blood–brain barrier permeability to small molecules in preclinical models (Hawkins *et al*, 2004; Manda *et al*, 2010), and hence a greater brain concentration of TC-5619 may be present in tobacco users. Third, nicotine can act at NNR subtypes other than alpha7 (eg, alpha4 beta2) to enhance the effect of TC-5619 (Targacept, data on file).

These observed differences between tobacco users and non-users further underscore the complex and incompletely understood pharmacodynamic mechanisms by which alpha7 NNR agonists work (Buchanan and Schwarcz, 2011). Recent studies have demonstrated how differences in dosing and schedule of administration, in addition to variation in the intrinsic activities of alpha7 NNR agonists, can influence cognitive and neurophysiological responses (Castner *et al*, 2011; Tregellas *et al*, 2011). If future studies confirm that TC-5619 or other alpha7 NNR agonists work primarily in tobacco-using patients, it will be important to determine the pharmacodynamic basis of this effect.

There is weak evidence suggesting that the positive effects of TC-5619 on CogState items might be stronger in the United States than India, although the difference was not statistically significant. The modest difference between the effects in the US subjects compared with Indian subjects

could arise from the greater tobacco use in the US subjects (60%) than in the Indian subjects (38%). If the apparent difference in the magnitude of effect between the US and Indian subjects is confirmed in future studies that equalize the percentage of tobacco users in the two geographic populations, it may instead reflect a cultural effect and stem from relatively less familiarity of Indian subjects to computer-based tests.

A number of caveats should be considered in the interpretation of this study. First, the number of tobacco users in the study was relatively small (46% of the enrolled population), so the findings need to be replicated in larger studies. Second, approximately two-thirds of enrolled subjects in this study were from sites in India, and therefore the findings need to be tested in other regions to assess translation into other populations, cultures, and ways of medical practice.

Nevertheless, these encouraging findings support the role of alpha7 NNR partial agonists like TC-5619 to treat the residual psychopathology of schizophrenia. An effective treatment for these symptoms could be of great value for patients, their families and caregivers, and for the society and economy that could benefit from enhanced productivity of these individuals.

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