Supplementary Materials

Preclinical to Human Translational Pharmacology of the Novel M1 Positive Allosteric Modulator MK-7622

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2. CORE PROTOCOL

2.1 OBJECTIVES AND HYPOTHESES

2.1.1 Primary

1. **Objective:** To evaluate the safety and tolerability of MK-7622 after administration of single rising oral doses to healthy young adult male subjects (Part I).

   Hypothesis: Single oral doses of MK-7622 are sufficiently safe and well tolerated, based on an assessment of clinical and laboratory evaluations and adverse experiences, in healthy young male subjects to permit continued clinical evaluation.

2. **Objective:** To obtain preliminary plasma pharmacokinetic data (e.g. AUC_{0-24}, C_{max}, t_{max}, $t_{1/2}$) of MK-7622 in the fasted state (Part I).

3. **Objective**: To evaluate the central nervous system pharmacodynamic effects of single doses of MK-7622 as evaluated by EEG (Part II).

   Hypothesis: At 2, 4 or 8 hours post dose, at least one generally well tolerated dose of MK-7622 produces an increase on sigma frequency, compared to placebo, as evaluated by qEEG in the eyes closed position (A mean increase of sigma at frontal and central electro-leads on the order of 20% is expected).

2.1.2 Secondary

1. **Objective:** To assess the effect of a standard high fat meal on the plasma pharmacokinetic parameters of MK-7622 (Part I).

   Estimation: The effects of a standard high-fat meal on the plasma pharmacokinetics (e.g. AUC and Cmax) of MK-7622 will be estimated and compared to the effect following a single oral dose of MK-7622 without food.

2. **Objective:** To compare the effects on resting maximal systolic and diastolic blood pressure and heart rate after administration of single dose of MK-7622 to placebo in healthy young male subjects (Part I).

   Estimation: The change in resting maximum MA5 systolic blood pressure and heart rate (i.e. maximum change from predose baseline in moving average of heart rate over 5hrs post dose) following single oral doses of MK-7622 will be estimated.

2.1.3 Exploratory

1. **Objective:** To obtain preliminary data on the urinary excretion of MK-7622 following single-dose administration (Part I).

2. **Objective:** To obtain preliminary plasma pharmacokinetic data on the N-oxide metabolite of MK-7622 following single-dose administration (Part I).

3. **Objective:** To explore the effects of single doses of MK-7622 on salivation flow as assessed by the amount (weight) of saliva change (Part I).

4. **Objective:** To explore the central nervous system pharmacodynamic effects of single doses of MK-7622 as evaluated by EEG on other frequency bands (e.g. delta, theta, alpha, beta, gamma).

   Estimation: The effects of single doses of MK-7622 on the power spectral density of waking EEG in the eyes closed condition will be estimated compared to placebo in the lower frequency bands (e.g. alpha, beta, delta, and theta) and higher frequency band (e.g. Gamma).

2.2 SUBJECT/PATIENT INCLUSION CRITERIA

a. Subject is a male 18 to 45 years of age at the prestudy (screening) visit.

b. The subject has a Body Mass Index (BMI) ≤30 kg/m^2 at the prestudy (screening) visit. BMI is calculated by taking the subject's weight in kg and dividing by the subject's height in meters, squared.

c. Subject is judged to be in good health based on medical history, physical examination, vital sign measurements, and laboratory safety tests (see Appendix 6.2) performed at the prestudy (screening) visit and/or prior to administration of the initial dose of study drug.

d. Subject has no clinically significant abnormality on electrocardiogram (ECG) performed at the prestudy (screening) visit and/or prior to administration of the initial dose of study drug.

e. Subject has been a nonsmoker and/or has not used nicotine or nicotine-containing products for at least approximately 6 months; subjects who have discontinued smoking or the use of nicotine/nicotine containing products for at least approximately 3 months may be enrolled in the study at the discretion of the investigator.

f. Subject understands the study procedures and agrees to participate in the study by giving written informed consent.

g. Subject is willing to comply with the study restrictions (see Section 3.2 for a complete summary of study restrictions).

2.3 SUBJECT/PATIENT EXCLUSION CRITERIA

a. Subject is under the age of legal consent.

b. Subject is mentally or legally incapacitated, has significant emotional problems at the time of prestudy (screening) visit or expected during the conduct of the study or has a history of a clinically significant psychiatric disorder over the last 10 years. Subjects

who have had situational depression may be enrolled in the study at the discretion of the investigator.

c. Subject has a history of any illness that, in the opinion of the study investigator, might confound the results of the study or poses an additional risk to the subject by their participation in the study.

d. Subject has an estimated creatinine clearance of ≤80 mL/min based on the Cockcroft-Gault equation; the Cockcroft-Gault equation is (for females multiply result by 0.85):

$$Cl_{Cr} = \frac{(140\text{-age[yr]})(\text{body wt [kg]})}{(72)(\text{serum creat [mg/dL]})}$$

When creatinine is measured in micromole/litre, use the following formula:

$$Cl_{Cr} = \frac{(140\text{-age[yr]})(\text{body wt[kg]})}{(72)(\text{serum creatinine [micromol/}\mathbf{L}\text{] x } 0.0113)}$$

An actual creatinine clearance, as determined by a 24-hour urine collection, may be used in place of, or in conjunction with, the Cockcroft-Gault equation; subjects who have an actual or estimated creatinine clearance up to 10% below of 80 mL/min may be enrolled in the study at the discretion of the investigator.

e. Subject has a history of stroke, chronic seizures, or major neurological disorder.

f. Subject has a history of clinically significant endocrine, gastrointestinal, cardiovascular, hematological, hepatic, immunological, renal, respiratory, or genitourinary abnormalities or diseases. Subjects with a history of uncomplicated kidney stones or childhood asthma may be enrolled in the study at the discretion of the investigator.

g. Subject has a history of neoplastic disease

h. Subject is unable to refrain from or anticipates the use of any medication, including prescription and non-prescription drugs or herbal remedies (such as St. John's Wort [hypericum perforatum]) beginning approximately 2 weeks (or 5 half-lives) prior to administration of the initial dose of study drug, throughout the study (including washout intervals between treatment periods), until the poststudy visit. There may be certain medications that are permitted.

i. Subject consumes excessive amounts of alcohol, defined as greater than 3 glasses of alcoholic beverages (1 glass is approximately equivalent to: beer [284 mL/10 ounces], wine [125 mL/4 ounces], or distilled spirits [25 mL/1 ounce]) per day. Subjects that consume 4 glasses of alcoholic beverages per day may be enrolled at the discretion of the investigator.

j. Subject consumes excessive amounts, defined as greater than 6 servings (1 serving is approximately equivalent to 120 mg of caffeine) of coffee, tea, cola, or other caffeinated beverages per day.

k. Subject has had major surgery, donated or lost 1 unit of blood (approximately 500 mL) or participated in another investigational study within 4 weeks prior to the prestudy (screening). The 4 week window will be derived from the date of the last study procedure (i.e. poststudy, AE follow-up, etc.) in the previous study to the prestudy/screening visit of the current study.

l. Subject has a history of significant multiple and/or severe allergies (including latex allergy), or has had an anaphylactic reaction or significant intolerability to prescription or non-prescription drugs or food.

m. Subject is currently a regular user (including "recreational use") of any illicit drugs or has a history of drug (including alcohol) abuse within approximately 12 months.

n. There is any concern by the investigator regarding the safe participation of the subject in the study or for any other reason, the investigator considers the subject inappropriate for participation in the study.

o. Subject has prominent artifact (s) on the screening EEG (e.g. excessive muscle activity), judged by the investigator as likely to interfere with EEG analysis.

p. Subject has a history of symptomatic orthostatic hypotension (including syncope, vasovagal reactions, frequent lightheadedness or frequent dizziness when standing).

q. Subject has a history of significant intraoral soft tissue disease (e.g. significant gingival inflammation, oral abrasions, trauma) (Part I).

2.4 STUDY DESIGN AND DURATION

2.4.1 Summary of Study Design

Part I is double-blind, randomized, placebo-controlled, single-ascending dose study to assess the safety, tolerability pharmacokinetics and pharmacodynamics of MK-7622 in healthy male subjects. Up to 24 subjects will be randomized to one of 3 panels (A-C). Panels A and B will be administered single-ascending oral doses of MK-7622 or placebo in alternating panels. There will be a food effect assessment in one of these panels. Panel C is single-ascending dosing in 8 subjects and its conduct is contingent upon the safety and pharmacokinetics (where available) of Panels A and B. Each panel will consist of 8 subjects who will receive MK-7622 or placebo in up to 5 treatment periods. Subjects in all panels will be assessed for the effects of MK-7622 on changes in power spectra densities in all frequency bands via qEEG in Period 5. In each period, 6 subjects will be randomized to receive MK-7622 and 2 subjects will receive placebo. A different pair of subjects will receive placebo in each period. The same subjects will receive MK-7622 or placebo in the periods where food effect is assessed. Part II is a double-blind,

randomized, placebo-controlled, crossover study to further assess the effects of MK-7622 on changes in power spectra densities via qEEG. Up to (**28**) subjects (Panel D) will be randomized to MK-7622 or placebo in a balanced crossover design. The total duration for the study is ~ 12 weeks (Part I) and 10 weeks (Part II).

2.4.2 Treatment Plan

In all panels, subjects will be admitted to the clinical research unit (CRU) from up to 24 hours prior to dosing of each treatment period, at the discretion of the investigator, and will remain in the unit until the completion of the 24 hour postdose procedures. Subjects will be asked to return to the CRU on all other study visits for procedures listed in the study flowchart.

Part I

In Panels A and B, subjects will receive single ascending oral doses of MK-7622 or placebo across 5 treatment periods in an alternating design. Panel A subjects will receive 1 mg, 5 mg, 20 mg and 80 mg of MK-7622 or placebo. Panel B subjects will receive 2 mg, 10 mg and 40 mg of MK-7622 or placebo. All doses will be administered in the fasted state with the exception of Panel B, Period 4, who will receive their dose following a standard high-fat breakfast. Two subjects in each panel will receive placebo instead of MK-7622 for each dose level according to a randomized allocation schedule (See Table 2-2). In Periods 2 (fasted) and 4 (fed) of Panel B, the same 6 subjects will receive a single oral dose of 10 mg MK-7622 and the same 2 subjects will receive placebo. In Period 5 of all panels, a safe and well tolerated dose will be administered and will not exceed the maximum dose given in any of the previous periods. Subjects within a panel will have a minimum of a 7 day washout between dosing periods. There will be a minimum of 72 hours between dose escalations for each alternating period of Panels A and B.

Table 2-2

Sample Allocation Schedule (Part I)

#of [a] Subjects	Period 1	Period 2	Period 3	Period 4	Period 5
Panel A (N)					
2	1 mg	5 mg	20 mg	Pbo	Pbo(qEEG)
2	1 mg	5 mg	Pbo	80 mg	≤80mg (qEEG)
2	Pbo	5 mg	20 mg	80 mg	≤80mg (qEEG
2	1 mg	Pbo	20 mg	80 mg	≤80mg (qEEG
Panel B (N)					
2	2 mg	Pbo	40 mg	Pbo(fed)	≤80mg (qEEG)
2	Pbo	10 mg	40 mg	10 mg(fed)	≤80mg (qEEG)
2	2 mg	10 mg	40 mg	10 mg(fed)	≤80mg (qEEG)
2	2 mg	10 mg	Pbo	10 mg(fed)	Pbo (qEEG)
Panel C (N)					
2	80 mg	Pbo	240 mg	300 mg	≤300mg(qEEG)
2	Pbo	160 mg	240 mg	300 mg	≤300mg(qEEG)
2	80 mg	160 mg	240 mg	Pbo	Pbo(qEEG)
2	80 mg	160 mg	Pbo	300 mg	≤300mg(qEEG)

[a] In each period, 6 subjects will be randomized to receive MK-7622 and 2 subjects to receive placebo. A different pair of subjects will receive placebo in each period.

[b] The assigned treatment for the 2 periods (fasted and fed) will be the same such that the same subjects will receive active drug or placebo in both periods. The (fasted /fed) periods may be switched to Panel A or C, if needed

Panel C is single-ascending dose in 8 subjects. Subjects will be sequentially administered 80 mg, 160 mg, 240 mg and 300 mg of MK-7622 or placebo across 5 treatment periods. Six (6) different subjects will receive MK-7622 and 2 different subjects will receive placebo for each dosing period. There will be a minimum of a 7 day washout between each dosing period.

Part II

Part II is a double-blind, randomized, placebo-controlled, crossover study to further assess the effects of MK-7622 on changes in power spectra densities via qEEG. Up to **(28)** subjects (Panel D) will be randomized to MK-7622 or placebo in a balanced crossover design (Table 2-3) in up to 4 treatment periods. Doses for Part II are defined in Table 2-3 and will not exceed the maximum safe and well-tolerated dose administered in Part I. There will be a minimum **9** day washout between dosing periods for all subjects.

Table 2-3

Sample Allocation schedule (Part II)

Subjects	Period 1	Period 2	Period 3	Period 4
Panel D				
n=7	**Low dose (10 mg)**	**Placebo**	**Intermediate dose (40 mg)**	**High dose (70 mg)**
n=7	**Intermediate dose (40 mg)**	**Low dose (10 mg)**	**High dose (70 mg)**	**Placebo**
n=7	**High dose (70 mg)**	**Intermediate dose (40 mg)**	**Placebo**	**Low dose (10 mg)**
n=7	**Placebo**	**High dose (70 mg)**	**Low dose (10 mg)**	**Intermediate dose (40 mg)**

In both parts, EEG will be recorded continuously from predose up to 12 hours postdose and recorded under controlled conditions at select time points.

2.5 LIST OF EFFICACY/PHARMACOKINETIC/IMMUNOGENICITY, ETC., MEASUREMENTS

Plasma and urine will be analyzed for MK-7622 concentration (and metabolites). For specific time points refer to the study flowchart. At anytime, not necessarily based on the preliminary analysis of the pharmacokinetic profile, the blood and the panel and period for urine sampling schedule may be adjusted.

In periods 1-4 of Part I, MK-7622 will be assessed for its effect on salivary flow by weight measurement in grams per minute.

In Period 5 of Part I and all of Part II, the pharmacological effect of MK-7622 within the central nervous system will be measured by awake EEG at multiple dose levels.

2.6 LIST OF SAFETY MEASUREMENTS

Physical examination, neurological examination, laboratory safety tests, vital signs and ECGs will be performed at various times throughout the study. MK-7622 induced dose-dependent increase on blood pressure (BP) and heart rate in monkeys. Ambulatory blood pressure will be frequently monitored via automatic BP measurement. Heart rate will be monitored via telemetry. Subjective assessment of nausea via use of a visual analog scale will be performed. Subjects will be assessed for adverse events during the study. Any safety procedures may be done at unscheduled time points, if deemed necessary by the investigator or sponsor.

2.7 STATISTICAL ANALYSIS PLAN SUMMARY

2.7.1 Statistical Methods

Part I

Safety:

Summary statistics and plots will be generated for the change from baseline values, as deemed clinically appropriate. Depending on the safety parameter, the difference from baseline will either be computed on the original scale (raw change from baseline) or on the log scale and back-transformed for reporting (percent change from baseline). Summary statistics for the raw laboratory safety tests, ECGs, and/or vital signs may also be computed, as deemed clinically appropriate. For VAS (Visual Analog Scale), the summary statistics of mean and standard error (SE) will be computed by dose and by time point. In addition, the summary statistics of mean and SE will be computed for changes from baseline.

Pharmacokinetics:

Pharmacokinetic parameter $AUC_{0-\infty}$ and C_{max} values will be log transformed and analyzed based on a linear mixed effects model containing a fixed effect for treatment and a random effect for subject.

To assess the effect of food on MK-7622, 90% confidence intervals will be constructed for the geometric mean ratios (fed/fasted) of MK-7622 $AUC_{0-\infty}$, and C_{max} at the dose level administered in the fed and fasted state from the same model as listed above.

Summary statistics will be reported for MK-7622 $AUC_{0-\infty}$, C_{max}, T_{max}, and $t_{1/2,}$ by treatment. A 95% confidence interval will be constructed for the geometric means of MK-7622 $AUC_{0-\infty}$, C_{max} at each dose level in the fed and fasted states. Descriptive Summary statistics will be provided for urinary excretion of intact MK-7622. In addition, median values will also be reported for T_{max} and the harmonic mean and jackknifed standard deviation of the harmonic mean will be reported for apparent half-life.

Dose Proportionality

An exploratory analysis will be conducted to preliminarily assess dose proportionality of MK-7622 $AUC_{0-\infty}$. The potential effects of panel will be first explored using a mixed effect model with ln(dose), panel, and ln(dose) by panel interaction as fixed effects and subject within panel as a random effect. If the ln(dose) by panel interaction is found to be statistically significant at the significance level of 0.05 level, slope will estimated separately for each panel. If the interaction term is not statistically significant but the main panel effect is, then an overall slope will be estimated across both panels based on a reduced model without the interaction term. In both cases, a plot containing observed PK data vs. dose and an estimated regression line on the raw scale will be provided

separately for each panel, together with a 95% Schéffe confidence band for the regression line. If the panel effect and the interaction term are both statistically insignificant, then a reduced model with subject as a random effect and ln(dose) as a covariate will be used to estimate the slope parameter associated with ln(dose). A plot of the observed PK data versus dose will be provided along with an overall estimated regression line on the raw scale and a 95% Schéffe confidence band. The assessment of the dose proportionality of MK-7622 C_{max} will be carried out in the same manner as that for MK-7622 $AUC_{0-\infty}$.

Preliminary data on the urinary excretion of MK-7622 following single-dose administration will be explored.

Preliminary plasma pharmacokinetic data on the N-oxide metabolite of MK-7622 following single-dose administration will be explored.

The effect of single doses of MK-7622 on salivation flow as assessed by the weight of the saliva specimen collected during a 1 minute period will be explored.

Pharmacodynamics:

Maximum MA_5: Placebo data will be pooled across panels for the analysis. Estimated means and 95% confidence intervals for maximum moving average change from baseline will be obtained for each treatment based on a linear mixed effects model containing fixed effects for treatment, panel, and a random effect for subject within panel. Point estimates and 90% CIs for the difference between each dose of MK-7622 and placebo will be provided.

qEEG: Summary statistics will be provided for the effect of single doses of MK-7622 on central nervous system pharmacodynamic effects as evaluated by EEG. Placebo data will be pooled across panels. Individual values for power spectra in the eyes closed position at low and high frequency bands will be log transformed and evaluated separately with a linear mixed effects model containing fixed effects for treatment, time, and treatment by time interaction and a random effect for subject within treatment.

Part II

Primary Hypothesis (qEEG on Sigma Band)

A linear mixed-effect model appropriate for a four period, randomized, balanced crossover study will be used to evaluate the **sigma** frequency band power data. Prior to statistical analysis, individual values of **sigma** frequency band power at 2, 4, and 8 hour time points will be **averaged on all frontal and central leads, then** natural log-transformed and evaluated with a model that includes fixed factors for period, treatment, eyes (open or closed), time, treatment by time interaction, and treatment by time by eyes interaction, and a random factor for subject. A test for first order carryover will be provided.

A step-down approach will be used to address the primary hypotheses that there is an **increase** for at least one dose of MK-7622 in **sigma** frequency band power during awake EEG in the eyes closed condition, as measured by spectral analysis (placebo versus MK-7622). Started from the highest dose, the mean treatment difference (Highest dose of MK-7622 - placebo) and 90% confidence interval for the treatment difference at 2 hours will be computed using the mean square error from the above mixed model and referencing a t distribution. This difference and confidence interval will be back-transformed to obtain the estimated percent change in **sigma** frequency band power. If the confidence interval lies completely **above zero**, then the procedure will be repeated for each descending dose until a dose does not meet the criteria outlined.

Sigma power data at the 4 and 8 hours time points will be analyzed in the same way as for the 2-hour time point. The hypothesis will be supported if the confidence interval lies **above zero** for at least one dose at one time point.

Exploratory

A similar model will be used to estimate the effects of single doses of MK-7622 on the power spectral density of waking EEG in the eyes closed condition for the alpha, beta, delta, gamma, theta, and other time points of the **sigma** frequency bands power. **Separate models will be used for different electrode leads.** Point estimates and 90% CIs for the difference between each dose of MK-7622 and placebo at each time point and band will be provided.

Power:

Part I

Safety: Power calculations for comparison of adverse experience rates are too imprecise to be clinically meaningful because the actual rates are not even approximately known. However, if an adverse experience occurs at a rate of 1% or 10% for a given MK-7622 dose, then the chance of observing such an adverse event among 6 recipients will be 6% or 47%, respectively. At the end of the study, for a particular adverse event, if none are observed in any of the 6 recipients for a given dose, then the true incidence is 24% with 80% confidence (32% with 90% confidence).

Estimation of Maximum MA_5

The precision of the estimates of MK-7622 maximum MA_5 of SBP, DBP, and heart rate obtained from this study can be assessed by calculating the half-width of the 90% confidence intervals expected for the given sample size and assumed variability.

The calculations below are based on a between-subject standard deviation of SBP, DBP, and heart rate of 5.12, 4.45, and 4.35 obtained from MK-0634 study PN008 and a correlation of 0.5 for the within-subject standard deviation.

Assuming a between-subject standard deviation of 5.12, 4.45, and 4.35 for SBP, DBP, and heart rate, respectively, and a correlation of 0.5 for the within-subject standard deviation, then with 6 subjects on active drug and 14 subjects on placebo, the half-width of the 90% confidence interval of maximum MA_5 SBP, DBP, and heart rate will be 3.53, 3.07, and 3.00, respectively. The lower and upper 90% confidence limits for the true mean difference of SBP, DBP, and heart rate will be given by OBS - 3.53 and OBS + 3.53, OBS - 3.07 and OBS + 3.07, OBS- 3.00 and OBS + 3.00, respectively, where OBS is the observed mean. Thus, for example, if the observed mean difference (active - placebo) in SBP was 0, the 90% confidence interval would be -3.53 to 3.53.

Part II

Primary Hypothesis (qEEG on **Sigma** band)

Assuming **28** subjects completing the crossover study, and a one-sided test at alpha=0.05, there will be **92%** probability to detect the difference between MK-7622 and placebo in **sigma** frequency band power during awake EEG, given a true **20**% **increase** of MK-7622 versus placebo. These calculations are based on an assumption of a within-subject log-scale standard deviation for MK-7622 EEG power with eyes closed at **sigma** frequency **averaged on all frontal and central leads** of 0**.2184,** as obtained from MK-7**622PN001 Part I.**

Methods S2. Protocol for Clinical Pharmacology Study 002

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2. CORE PROTOCOL

2.1 OBJECTIVES AND HYPOTHESES

2.1.1 Primary

Objective

(1) To evaluate the safety and tolerability of multiple daily oral doses of MK-7622 in healthy young males.

Hypothesis

(1) Multiple oral doses of MK-7622 are sufficiently safe and well tolerated, based on an assessment of clinical and laboratory adverse experiences (AEs), to permit continued clinical investigation.

2.1.2 Secondary

Objective

(1) To obtain plasma pharmacokinetic data (e.g., $AUC_{(0-24)}$, C_{max}, T_{max}, and $t_{½}$) and to estimate the accumulation ratio (Day 10 AUC_{0-24hr} / Day 1 AUC_{0-24hr}) of MK-7622 and N-oxide metabolite following 10 days of multiple daily doses of MK-7622 in healthy male subjects.

(2) To obtain CSF concentrations data for MK-7622 and N-oxide metabolite following approximately 10 days of multiple daily doses of MK-7622 in healthy male subjects.

(3) To assess the effects of single and multiple dose MK-7622 on blood pressure and heart rate in healthy adult male subjects.

Hypothesis

(1) The highest MK-7622 dose levels achieve a post dose CSF concentration of at least 2 nM

Estimation

(1) The change from predose baseline in resting maximum MA5 systolic blood pressure and hear rate (i.e. maximum change from baseline in moving average of heart rate over 5 hours post dose) following once daily oral dosing of MK-7622 for 10 days will be estimated.

2.1.3 Exploratory

Objective

(1) To assess the effects of multiple dose MK-7622 on saliva production in healthy male subjects.

2.2 SUBJECT/PATIENT INCLUSION CRITERIA

a. Subject is a male 18 to 45 years of age at the prestudy (screening) visit.

b. The subject has a Body Mass Index (BMI) ≤30 kg/m^2 at the prestudy (screening) visit. BMI is calculated by taking the subject's weight in kg and dividing by the subject's height in meters, squared.

c. Subject is judged to be in good health based on medical history, physical examination, vital sign measurements, and laboratory safety tests (see Appendix 6.2) performed at the prestudy (screening) visit and/or prior to administration of the initial dose of study drug.

d. Subject has no clinically significant abnormality on electrocardiogram (ECG) performed at the prestudy (screening) visit and/or prior to administration of the initial dose of study drug.

e. Subject has been a nonsmoker and/or has not used nicotine or nicotine-containing products for at least approximately 6 months; subjects who have discontinued smoking or the use of nicotine/nicotine containing products for at least approximately 3 months may be enrolled in the study at the discretion of the investigator.

f. Subject understands the study procedures and agrees to participate in the study by giving written informed consent.

g. Subject is willing to comply with the study restrictions (see Section 3.2 for a complete summary of study restrictions).

h. Subject has a normal fundoscopic exam (Panels C and D).

2.3 SUBJECT/PATIENT EXCLUSION CRITERIA

a. Subject is under the age of legal consent.

b. Subject is mentally or legally incapacitated, has significant emotional problems at the time of prestudy (screening) visit or expected during the conduct of the study or has a history of a clinically significant psychiatric disorder over the last 10 years. Subjects who have had situational depression may be enrolled in the study at the discretion of the investigator.

c. Subject has a history of any illness that, in the opinion of the study investigator, might confound the results of the study or poses an additional risk to the subject by their participation in the study.

d. Subject has an estimated creatinine clearance of ≤80 mL/min based on the Cockcroft-Gault equation; the Cockcroft-Gault equation is:

$$Cl_{Cr} = \frac{(140\text{-age[yr]})(\text{body wt [kg]})}{(72)(\text{serum creat [mg/dL]})}$$

When creatinine is measured in micromole/litre, use the following formula:

$$Cl_{Cr} = \frac{(140\text{-age[yr]})(\text{body wt[kg]})}{(72)(\text{serum creatinine [micromol/}\mathbf{L}\text{]} \times 0.0113)}$$

An actual creatinine clearance, as determined by a 24-hour urine collection, may be used in place of, or in conjunction with, the Cockcroft-Gault equation;

e. Subject has a history of stroke, chronic seizures, or major neurological disorder.

f. Subject has a history of clinically significant endocrine, gastrointestinal, cardiovascular, hematological, hepatic, immunological, renal, respiratory, or genitourinary abnormalities or diseases. Subjects with a history of uncomplicated kidney stones or childhood asthma may be enrolled in the study at the discretion of the investigator.

g. Subject has a history of neoplastic disease

h. Subject is unable to refrain from or anticipates the use of any medication, including prescription and non-prescription drugs or herbal remedies (such as St. John's Wort [hypericum perforatum]) beginning approximately 2 weeks (or 5 half-lives) prior to administration of the initial dose of study drug, throughout the study (including washout intervals between treatment periods), until the poststudy visit. There may be certain medications that are permitted.

i. Subject consumes excessive amounts of alcohol, defined as greater than 3 glasses of alcoholic beverages (1 glass is approximately equivalent to: beer [284 mL/10 ounces], wine [125 mL/4 ounces], or distilled spirits [25 mL/1 ounce]) per day. Subjects that consume 4 glasses of alcoholic beverages per day may be enrolled at the discretion of the investigator.

j. Subject consumes excessive amounts, defined as greater than 6 servings (1 serving is approximately equivalent to 120 mg of caffeine) of coffee, tea, cola, or other caffeinated beverages per day.

k. Subject has had major surgery, donated or lost 1 unit of blood (approximately 500 mL) or participated in another investigational study within 4 weeks prior to the prestudy (screening). The 4 week window will be derived from the date of the last study procedure (i.e. poststudy, AE follow-up, etc.) in the previous study to the prestudy/screening visit of the current study.

l. Subject has a history of significant multiple and/or severe allergies (including latex allergy), or has had an anaphylactic reaction or significant intolerability to prescription or non-prescription drugs or food.

m. Subject is currently a regular user (including "recreational use") of any illicit drugs or has a history of drug (including alcohol) abuse within approximately 12 months.

n. There is any concern by the investigator regarding the safe participation of the subject in the study or for any other reason; the investigator considers the subject inappropriate for participation in the study.

o. Subject has a history of symptomatic orthostatic hypotension (including syncope, vasovagal reactions, frequent lightheadedness or frequent dizziness when standing).

p. Subject has a history of significant intraoral soft tissue disease (e.g. significant gingival inflammation, oral abrasions, trauma).

q. Subject has a history of clinically significant deep venous thrombosis (DVT), thrombophlebitis, or coagulopathy (Panel C and D only).

r. Subject has a history or signs/symptoms of lumbar spine/disc disease including but not limited to scoliosis, herniation, or previous spinal surgery or any other contraindication to lumbar puncture (Panel C and D only).

s. Subject has a history of a recent infection (e.g. meningitis, encephalitis) (within past month), significant immunodeficiency and/or hospitalization (within past year) for any reason (Panel C and D only).

t. Subject has recent history (within the past year) of migraine headaches (Panel C and D only).

u. Subject has a family or personal history of hemophilia or any coagulopathy (Panel C and D only).

v. Subject has a hypersensitivity to lidocaine (Panel C and D only).

2.4 STUDY DESIGN AND DURATION

2.4.1 Summary of Study Design

This is a double-blind, randomized, placebo-controlled, sequential-panel rising multiple-dose study to assess the safety, tolerability, multiple-dose pharmacokinetics and pharmacodynamics of MK-7622 in young healthy male volunteers. Up to 32 subjects will be randomized to one of 4 panels (Panels A to D). All panels will consist of 8 subjects; 6 subjects will be randomized to receive active drug and 2 will be randomized to receive placebo. Plasma for MK-7622 and N-oxide metabolite will be obtained predose on Day 1, and at prespecified time points according to the study flowchart. Cerebral spinal fluid (CSF) will be collected for MK-7622 concentration and N-oxide metabolite levels per the study flow chart. Safety will be monitored throughout the study by repeated clinical and laboratory evaluations.

The total duration for this study is ~ 12-14 weeks.

2.4.2 Treatment Plan

In all panels, subjects will be admitted from up to 24 hours prior to dosing of each treatment panel, at the discretion of the investigator, and will remain in the unit until the completion of the 24 hour postdose procedures on Day 10. Subjects will be asked to return to the CRU for all other study procedures as outlined in the study flowchart.

Multiple oral doses of MK-7622 as a capsule or placebo will be administered in the morning after an 8 hour fast on Days 1, 5, 6, 9 and 10. All remaining treatment days, subjects will be given MK-7622 or placebo regardless of food. All doses will be administered with 240 mL of water as follows:

Panel A: 10 mg MK-7622 or placebo once daily for 10 days

Panel B: 20 mg MK-7622 or placebo once daily for 10 days

Panel C: 30 mg MK-7622 or placebo once daily for 10 days

Panel D: 40 mg MK-7622 or placebo once daily for 10 days

There will be a minimum 4 day break between panels. An evaluation of the pharmacokinetics of Panel A (10 mg dose) will occur prior to dosing of Panel C (30 mg dose) and a pause will occur between Panels C and D to permit analysis and review of MK-7622 pharmacokinetics from Panels A-C to ensure adherence to the eCTA exposure (AUC)cap for MK-7622 of 23 μM *hr.

Within each panel, 6 of 8 subjects will receive MK-7622 and 2 of 8 subjects will receive placebo. Allocation to active drug or placebo will be done in a blinded fashion according to a computer-generated allocation schedule similar to the sample schedule in Table 2-1.

Table 2-1

Sample Allocation Schedule

Panel	Sample Size (N)	Treatment [a]
A	6	10 mg
	2	Placebo
B	6	20 mg
	2	Placebo
C	6	30 mg
	2	Placebo
D	6	40 mg
	2	Placebo

[a] The suggested doses in any panel may be adjusted downwards based on the safety, tolerability and pharmacokinetic data from previous panels of the study.

2.5 LIST OF EFFICACY/PHARMACOKINETIC/IMMUNOGENICITY, ETC., MEASUREMENTS

Plasma and CSF will be analyzed for MK-7622 concentration (and metabolites). For specific time points refer to the study flowchart. At anytime, not necessarily based on the preliminary analysis of the pharmacokinetic profile, the blood or CSF sampling schedule may be adjusted. (e.g. preliminary PK results). Only 1 CSF sample will be obtained from each subject at the highest dose levels (Panels C and D) in the study.

In all panels, MK-7622 will be assessed for its effect on salivary flow by weight measurement in grams per minute.

2.6 LIST OF SAFETY MEASUREMENTS

Physical examination, neurological examination, laboratory safety tests, vital signs and ECGs will be performed at various times throughout the study. MK-7622 induced dose-dependent increase on blood pressure (BP) and heart rate in monkeys. Blood pressure and heart rate will be frequently monitored via automatic BP measurement device. Subjective assessment of nausea via use of a visual analog scale will be performed. Bond and Lader VAS will be administered according to the study flow chart to assess for potential CNS effects of MK-7622. Subjects will be assessed for adverse events during the study. Any safety procedures may be done at unscheduled time points, if deemed necessary by the investigator or sponsor.

2.7 STATISTICAL ANALYSIS PLAN SUMMARY

Methods:

Safety: Summary statistics and plots will be generated for the change from baseline values, as deemed clinically appropriate. Depending on the safety parameter, the difference from baseline will either be computed on the original scale (raw change from baseline) or on the log scale and back-transformed for reporting (percent change from baseline). Summary statistics for the raw laboratory safety tests, ECGs, and/or vital signs may also be computed, as deemed clinically appropriate.

Pharmacokinetics (Plasma): Pharmacokinetic parameters $AUC_{0\text{-}24hr}$ and C_{max} will be log transformed and analyzed based on a linear mixed effects model containing fixed effects for treatment, day (Day 1 and Day 10) and treatment by day interaction and a random effect for subject. Summary statistics will be reported for MK-7622 $AUC_{0\text{-}24hr}$, C_{max}, T_{max}, and $t_{½}$ by treatment and day. A 95% confidence interval (CI) will be constructed for the geometric means of MK-7622 $AUC_{0\text{-}24hr}$ and C_{max} at each dose level on Day 1 and Day 10. The accumulation of MK-7622 will be assessed through the construction of a 90% CI for the geometric mean ratio (Day 10 / Day 1) of $AUC_{0\text{-}24hr}$ and C_{max}. In addition, median values will also be reported for T_{max} and the harmonic mean and jackknifed standard deviation of the harmonic mean will be reported for apparent half-life.

Time to Steady State (Pharmacokinetics): Two methods will be used in the assessment of time to steady state. For the primary method, the effective rate of drug accumulation will be obtained for each subject from their accumulation ratio, and this value will be used to estimate the approach to steady state. Modeling of the trough plasma concentrations (C_τ) collected during multiple-dose administration will also be performed as a secondary approach.

Assessment of Dose Proportionality: The assessment of dose proportionality will be conducted using Day 10 data only in an exploratory manner. The primary assessment of dose proportionality for MK-7622 $AUC_{0\text{-}24hr}$ will be performed using the power law model with ln(AUC) as the dependent variable and ln(dose) as an explanatory variable. A point estimate of the slope associated with ln(dose) will be provided together with a 95% confidence interval from the power law model. A plot of the observed PK data versus dose will be provided along with an estimated regression line on the raw scale and a 95% Schéffe confidence band. The assessment of the dose proportionality of MK-7622 C_{max} will be carried out in the same manner as that used for MK-7622 AUC.

Pharmacokinetics (CSF): Individual CSF concentration values of MK-7622 and N-oxide metabolite will be log transformed, and the mean and 90% confidence interval calculated, based on a t-distribution. These values will be back-transformed to obtain a two-sided 90% confidence interval for the geometric mean concentration in CSF. The secondary hypothesis that the highest MK-7622 dose level achieves a post dose CSF concentration of at least 2 nM will be supported if the lower limit of this 90% confidence

interval (equivalent to the lower limit of a one-sided 95% confidence interval) is greater than 2 nM.

Pharmacodynamics

Maximum MA_5: Placebo data will be pooled across panels for the analysis. Estimated means and 95% confidence intervals of the Maximum MA_5 in resting systolic blood pressure and heart rate will be obtained for each treatment based on a linear mixed effects model containing fixed effects for treatment, day (Days 1, 5, and 10) and treatment by day interaction and random effect for subject.. Point estimates and 90% CIs for the difference between each dose of MK-7622 and placebo on each day will be provided.

Saliva: Placebo data will be pooled across panels for the analysis. Estimated means and 95% confidence intervals of saliva measurements will be obtained for each treatment based on a linear mixed effects model containing fixed effects for treatment, day (Days 1, 5, and 10), time, treatment by day, and treatment by day by time interaction and random effect for subject. Point estimates and 90% CIs for the difference between each dose of MK-7622 and placebo on each day at each time point will be provided.

Power:

Safety: Power calculations for comparison of adverse experience rates are too imprecise to be clinically meaningful because the actual rates are not even approximately known. However, if an adverse experience occurs at a rate of 1% or 10% then the chance of observing such an adverse event among 6 subjects receiving that dose will be 6% or 47%, respectively. If no AE of a given type is observed in any of the 6 subjects at a given dose, then with 80% (90%) confidence, the true incidence of the adverse experience at that dose is at most 24% (32%). **Pharmacokinetics (CSF):** Since no reliable estimates of CSF variability are available, no meaningful power statements can be made in the assessment of the first secondary hypothesis. **Pharmacodynamics (Maximum MA5):** The precision of the estimates of MK-7622 maximum MA5 of SBP and heart rate obtained from this study can be assessed by calculating the half-width of the 90% confidence intervals expected for the given sample size and assumed variability. The calculations below are based on a between-subject standard deviation of SBP and heart rate of 4.57 and 4.36 obtained from MK-7622 study PN001. Assuming a between-subject standard deviation of 4.57 and 4.36 for SBP and heart rate, respectively, then with 6 subjects on active drug and 8 subjects on placebo, the half-width of the 90% confidence interval of maximum MA5 SBP and heart rate will be 4.40 and 4.20, respectively. The lower and upper 90% confidence limits for the true mean difference of SBP and heart rate will be given by OBS – 4.40 and OBS + 4.40, OBS – 4.20 and OBS + 4.20, respectively, where OBS is the observed mean. Thus, for example, if the observed mean difference (active - placebo) in SBP was 0, the 90% confidence interval would be -4.40 to 4.40.

Methods S3. Protocol for Clinical Pharmacology Study 003

Merck & Co., Inc. policy on posting of redacted study protocols on journal websites is described in Guidelines for Publication of Clinical Trials in the Scientific Literature on the www.merck.com website.

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This report may include approved and non-approved uses, formulations, or treatment regimens. The results reported may not reflect the overall profile of a product. Before prescribing any product mentioned in this report, healthcare professionals should consult local prescribing information for the product approved in their country.

Hypotheses

1. The combination of donepezil and MK-7622 attenuates the scopolamine-induced impairment of psychomotor function and information processing to a greater extent than donepezil alone as measured by the reaction time in the Detection test. Specifically, a difference of 0.06 in $\log_{10}$ transformed reaction time between MK-7622 + donepezil and donepezil alone is targeted.

2. Single doses of MK-7622 in co-administration with donepezil 10 mg is sufficiently safe and well tolerated in healthy young male subjects, based on an assessment of clinical and laboratory adverse experiences (AEs), to permit continued clinical investigation.

2.1.2 Secondary

Part I

Objective

1. To determine whether donepezil 10 mg attenuates scopolamine-induced impairment in psychomotor function and information processing as measured by the Detection test of the CogState Early Phase Battery.

Hypothesis

1. Donepezil 10 mg attenuates scopolamine-induced impairment of psychomotor function and information processing as measured by the reaction time in the Detection test. Specifically, a difference of 0.06 in $\log_{10}$ transformed reaction time between donepezil + scopolamine and scopolamine alone is targeted.

Part II

Objective

1. To evaluate the effect of single-dose MK-7622 on the pharmacokinetics (e.g., $AUC_{0\text{-}24hr}$, C_{max} and T_{max}) of single-dose donepezil in healthy male subjects.

2. To determine the effects of MK-7622 + donepezil compared to donepezil alone on scopolamine-induced cognitive impairment as measured by other modules of the CogState Early Phase Battery.

Hypothesis

1. The $AUC_{0\text{-}24hr}$ of donepezil following a single 10 mg oral dose of donepezil is similar following co-administration with a single dose of MK-7622 to that observed when donepezil is given alone [i.e., the true $AUC_{0\text{-}24hr}$ GMR of donepezil with and without MK-7622 is contained within the interval (0.50, 2.00)].

Estimation

1. The effects MK-7622 + donepezil compared to donepezil alone on scopolamine-induced cognitive impairment as measured on the Identification (peak effect), CPAL (peak effect), and Groton Maze Learning Test (GMLT; time-weighted average of errors 1-4 hours) tasks will be estimated.

2.1.3 Exploratory Objectives

Part I and Part II

1. To compare cognitive assessments obtained using CogState Early Phase Battery and CANTAB.

2.2 SUBJECT/PATIENT INCLUSION CRITERIA

Demographics

a. Subject is a male 18 to 45 years of age at the prestudy (screening) visit. Male subjects must agree to use a medically acceptable method of contraception during the study and for 120 days after the last dose of study drug. If their partner is of non-childbearing potential, pregnant, or a same-sex partner, males must agree to use a condom and no additional method of contraception is required. If their partner is of child-bearing potential, males should use a condom with spermicide. Spermicides alone are not an acceptable method of contraception. Their partner must additionally be using one of the following methods: hormonal contraception, intra-uterine device, diaphragm with spermicide, cervical cap with spermicide or female condom with spermicide.

b. The subject has a Body Mass Index (BMI) $\leq$ 32 kg/m^2 at the prestudy (screening) visit. BMI is calculated by taking the subject's weight in kg and dividing by the subject's height in meters, squared.

Medical history, physical examinations, laboratory safety tests and ECG measurements

c. Subject is judged to be in good health based on medical history, physical examination, vital sign measurements, and laboratory safety tests (see Appendices 6.1 and 6.2) performed at the prestudy (screening) visit and/or prior to administration of the initial dose of study drug.

d. Subject has no clinically significant abnormality on electrocardiogram (ECG) performed at the prestudy (screening) visit and/or prior to administration of the initial dose of study drug.

e. Subject has normal or corrected to normal visual and auditory acuity.

Diet/Activity/Other

f. Subject has been a nonsmoker and/or has not used nicotine or nicotine-containing products for at least approximately 6 months; subjects who have discontinued smoking or the use of nicotine/nicotine containing products for at least approximately 3 months may be enrolled in the study at the discretion of the investigator.

g. Subject understands the study procedures and agrees to participate in the study by giving written informed consent.

h. Subject is willing to comply with the study restrictions (see Section 3.2 for a complete summary of study restrictions).

2.3 SUBJECT/PATIENT EXCLUSION CRITERIA

Medical history, physical examinations, laboratory safety tests and ECG measurements

a. Subject is under the age of legal consent.

b. Subject is mentally or legally incapacitated, has significant emotional problems at the time of prestudy (screening) visit or expected during the conduct of the study or has a history of a clinically significant psychiatric disorder over the last 10 years. Subjects who have had situational depression may be enrolled in the study at the discretion of the investigator.

c. Subject has a current diagnosis of or history of bipolar illness, schizophrenia or Attention Deficit Hyperactivity Disorder (ADHD) as verified by the subject during assessment of psychiatric history.

d. Subject works a night shift and is not able to avoid night shift work within 3 days before each treatment visit (or before the study start and during the study).

e. Subject has a history of any illness that, in the opinion of the study investigator, might confound the results of the study or poses an additional risk to the subject by their participation in the study.

f. Subject has an estimated creatinine clearance of ≤80 mL/min based on the Cockcroft-Gault equation; the Cockcroft-Gault equation is (for females multiply result by 0.85):

$$Cl_{Cr} = \frac{(140\text{-age[yr]})(\text{body wt [kg]})}{(72)(\text{serum creat [mg/dL]})}$$

When creatinine is measured in micromole/litre, use the following formula:

$$Cl_{Cr} = \frac{(140\text{-age[yr]})(\text{body wt[kg]})}{(72)(\text{serum creatinine [micromol/L]} \times 0.0113)}$$

An actual creatinine clearance, as determined by a 24-hour urine collection, may be used in place of, or in conjunction with, the Cockcroft-Gault equation; subjects who have an actual or estimated creatinine clearance up to 10% below 80 mL/min may be enrolled in the study at the discretion of the investigator.

g. Subject has a history of stroke, chronic seizures, major neurological disorder or any history of head trauma with loss of consciousness greater than 30 minutes during the previous 10 years prior to the prestudy (screening) visit.

h. Subject has a history of clinically significant endocrine, gastrointestinal, cardiovascular, hematological, hepatic, immunological, renal, respiratory, or genitourinary abnormalities or diseases. Subjects with a history of uncomplicated kidney stones or childhood asthma may be enrolled in the study at the discretion of the investigator.

i. Subject has a history of neoplastic disease.

j. Subject has a current diagnosis of or a prior history of glaucoma or has a first degree relative diagnosed with glaucoma (glaucoma is contraindicated for the use of scopolamine).

k. Subject is color blind.

l. Subject has known hypersensitivity to scopolamine or donepezil.

Diet/Activity/Other

m. Subject is unable to refrain from or anticipates the use of any medication, including prescription and non-prescription drugs or herbal remedies (such as St. John's Wort [hypericum perforatum]) beginning approximately 2 weeks (or 5 half-lives) prior to administration of the initial dose of study drug, throughout the study (including washout intervals between treatment periods), until the poststudy visit. There may be certain medications that are permitted.

n. Subject consumes excessive amounts of alcohol, defined as greater than 3 glasses of alcoholic beverages (1 glass is approximately equivalent to: beer [284 mL/10 ounces], wine [125 mL/4 ounces], or distilled spirits [25 mL/1 ounce]) per day. Subjects that consume 4 glasses of alcoholic beverages per day may be enrolled at the discretion of the investigator.

o. Subject consumes excessive amounts, defined as greater than 6 servings (1 serving is approximately equivalent to 120 mg of caffeine) of coffee, tea, cola, or other caffeinated beverages per day.

p. Subject has had major surgery, donated or lost 1 unit of blood (approximately 500 mL) or participated in another investigational study within 3 months prior to the

prestudy (screening). The 3 month window will be derived from the date of the last dose in the previous study to the prestudy/screening visit of the current study.

q. Subject has a history of significant multiple and/or severe allergies (including latex allergy), or has had an anaphylactic reaction or significant intolerability to prescription or non-prescription drugs or food. This includes a documented or subject-verified allergy to scopolamine, donepezil or MK-7622 [see the product circulars for scopolamine (Hyoscine Hydrobromide) and donepezil (Aricept®) included as Attachments].

r. Subject is currently a regular user (including "recreational use") of any illicit drugs or has a history of drug (including alcohol) abuse within approximately 12 months.

s. There is any concern by the investigator regarding the safe participation of the subject in the study or for any other reason, the investigator considers the subject inappropriate for participation in the study.

2.4 STUDY DESIGN AND DURATION

2.4.1 Summary of Study Design

This is a randomized, double-blind, double-dummy, placebo-controlled, 2-part crossover study in healthy male subjects to evaluate the effect of donepezil and MK-7622 in attenuating the cognitive impairment associated with scopolamine administration.

Subjects in Part I will not participate in Part II of the study. In Part I, subjects will be randomized to Treatments A-E in a 5-period complete crossover design. In Part II, 20 of 24 subjects will be randomized to receive Treatments F and G in a 2-period complete crossover. The remaining 4 subjects will be randomized to receive Treatment H in both periods of Part II. This will ensure the investigator will remain blinded.

At the Screening Visit, subjects will complete a full medical evaluation (physical exam, ECG, vital signs and laboratory assessments), an alcohol breath test, neurological exam, a Bond and Lader VAS and psychiatric history within 28 days of the first treatment visit. At Visit 2, the Familiarization Visit, subjects will undergo 2 practice sessions of 2 computerized cognitive test batteries, the CogState Early Phase Battery and the CANTAB battery (therefore, 4 total practice sessions), to minimize potential practice effects at subsequent treatment visits. The Part I Treatment Visits (V3-7) and the Part II Treatment Visits (V3-4) will occur at least every 14 days. Subjects will be admitted to the unit the morning before dosing (approximately -29 hours with respect to scopolamine administration) and will have the same series of laboratory and safety assessments as at the screening visit with the exception that there will be no neurological exam or serology testing. Subjects will also undergo a Bond and Lader VAS, urine drug screen and alcohol breath test. Subjects will be given a standard lunch, dinner and snack and will then be required to fast from midnight until breakfast is served on the following day.

A standard light breakfast will be served at -5 hr following administration of the baseline cognitive batteries. Administration of MK-7622 or placebo and donepezil or placebo will be at -3.5 hours and will be administered with 250 mL of water. Administration of scopolamine will begin at 0 hours. A standard light lunch will be served following scopolamine administration. All meals and snacks administered in the Research Unit will be standard for each subject. The computerized cognitive testing will occur several times throughout the day. CogState module assessments will occur at -5, 1, 2, 3, 4 and 6 hours and each testing session will take approximately 19 minutes to complete. Following at least a 5 minute rest, CANTAB module assessments will occur at -5, 2, 4 and 6 hours and each testing session will take approximately 12 minutes to complete (except predose [-5hr] and 4 and 6 hr postdose, which will take approximately 19 minutes to complete). Subjects will stay overnight in the CRU on the evening of dosing Day 1. On Day 2, following a discharge assessment, a taxi may be available to take subjects home. A follow-up phone call will be made to each subject 48 hours following dosing to ensure the patient is feeling well.

Safety will be monitored throughout the study by clinical and laboratory evaluations.

2.4.2 Treatment Plan

Subjects who meet the study requirements will be assigned to treatments according to a computer-generated random allocation schedule as presented in Table 2-1 for Part I and Table 2-2 for Part II..

Table 2-1

Sample Allocation Schedule (Part I)

Sequence	N	Period 1	Period 2	Period 3	Period 4	Period 5
1	3	A	B	D	E	C
2	3	B	C	E	A	D
3	3	C	D	A	B	E
4	3	D	E	B	C	A
5	3	E	A	C	D	B
6	3	A	C	B	E	D
7	3	B	D	C	A	E
8	3	C	E	D	B	A
9	3	D	A	E	C	B
10	3	E	B	A	D	C

Treatment A: scopolamine 0.5 mg + donepezil placebo + MK-7622 placebo
Treatment B: scopolamine 0.5 mg + donepezil placebo + MK-7622 1 mg
Treatment C: scopolamine 0.5 mg + donepezil placebo + MK-7622 10 mg
Treatment D: scopolamine 0.5 mg + donepezil placebo + MK-7622 70 mg
Treatment E: scopolamine 0.5 mg + donepezil 10 mg + MK-7622 placebo

Table 2-2

Sample Allocation Schedule (Part II)

Sequence	N	Period 1	Period 2
1	10	F	G
2	10	G	F
3	4	H	H
Treatment F: scopolamine 0.5 mg + donepezil 10 mg + MK-7622 Placebo Treatment G: scopolamine 0.5 mg + donepezil 10 mg + MK-7622 dose to be determined (1, 10 or 70 mg) Treatment H: scopolamine 0.5 mg + donepezil Placebo + MK-7622 Placebo			

2.4.3 Protocol Modifications Allowed During the Conduct of the Study

Because this is a Phase I assessment of MK-7622 in humans and the pharmacokinetic, pharmacodynamic and safety profiles of the compound are still being elucidated, this protocol is written with some flexibility to accommodate the inherent dynamic nature of Phase I clinical studies.

Please refer to Section 3.2.3.6 (Protocol Details) for examples of modifications permitted within the protocol parameters.

2.5 LIST OF EFFICACY/PHARMACOKINETIC/IMMUNOGENICITY, ETC., MEASUREMENTS

2.5.1 Pharmacokinetic Measurements

Blood will be collected for analyses of scopolamine, donepezil and MK-7622. See study flow chart for timing of pharmacokinetic samples.

2.5.2 Efficacy Measurements

The efficacy assessments for this study include CogState modules and CANTAB modules (exploratory). The CogState modules will be administered to all subjects at the intervals indicated in the study flow chart. Together, the CogState modules take approximately 19 minutes to complete, and are comprised of tasks that cover the following cognitive domains: executive function, psychomotor function/information processing, visual attention and visuospatial associative learning ability. For each cognitive task a single primary outcome measure has been identified to minimize experimental error rates (see Table 2-3).

Table 2-3

CogState Modules

Task Name	Task Code	Time to complete (mins)	Unit of Measurement	Description	Cognitive Domain
Groton Maze Learning Task (GMLT)	GMLT	10	Total number of errors	Total number of errors made in completing the 5 learning trials	Executive Function: problem solving and reasoning
Detection	DET	2	Log_{10} milliseconds	Speed of performance; mean of the Log_{10} transformed reaction times for correct responses	Psychomotor function/Information processing
Identification	IDN	2	Log_{10} milliseconds	Speed of performance; mean of the Log_{10} transformed reaction times for correct responses	Visual attention
Continuous Paired Associate Learning	CPAL	5	Total number of errors	Inaccuracy of performance; total number of errors when completing the test	Visuospatial associative learning ability

As an exploratory endpoint, CANTAB modules will also be administered to all subjects at the intervals indicated in the study flow chart. Together, the CANTAB modules take approximately 12-19 minutes to complete and are comprised of tasks that cover the following cognitive domains: psychomotor function/information processing, visual attention, spatial information and working memory (predose [-5hr], 4 and 6 hr postdose only) and visuospatial associative learning ability. For each cognitive task a single primary outcome measure has been identified to minimize experimental error rates (see Table 2-4).

Table 2-4

CANTAB Modules

Task Name	CogState Correlate	Time to complete (mins)	Unit of Measurement	Description	Cognitive Domain
Motor Screening	N/A			Screen for visual, movement and comprehension difficulties ***to be administered at -5h (predose)r only***	N/A
Simple Reaction Time	Detection	5	Log_{10} milliseconds	Speed of performance; mean of the Log_{10} transformed reaction times for correct responses	Psychomotor function/Information processing
Choice Reaction Time	Identification		Log_{10} milliseconds	Speed of performance; mean of the Log_{10} transformed reaction times for correct responses	Visual attention
Spatial Working Memory	N/A	7	Total number of errors	Spatial information, working memory **** to be administered at -5h (predose), 4hr and 6 hr rpostdose only***	Working memory
Paired Associate Learning	Continued Paried Associate Learning	7	Total number of errors	Inaccuracy of performance; total number of errors when completing the test	Visuospatial associative learning ability

2.6 LIST OF SAFETY MEASUREMENTS

Physical examinations, neurological exams, Bond and Lader VAS vital signs (heart rate, blood pressure, respiratory rate, and temperature), 12-lead ECG, laboratory safety tests, and monitoring of clinical adverse experiences will be performed as outlined in the study flowchart. These procedures may also be performed at unscheduled time points if deemed clinically necessary by the primary investigator.

2.7 STATISTICAL ANALYSIS PLAN SUMMARY

Methods: Primary: Part I MK-7622 + Scopolamine vs. Scopolamine alone: To address the first primary hypothesis in Part I, natural log_{10} transformed peak effect for detection test will be examined in a linear mixed effects model appropriate for a five-period crossover with fixed factors for treatment and period, and a random factor for subject. A one-sided test procedure will be used to evaluate the primary hypothesis. Because no monotonic assumption can be made, Hochberg's step-up method will be applied to preserve the overall alpha level at 0.05. P-values for the difference

(Scopolamine + MK-7622) – Scopolamine will be calculated for each MK-7622 dose. The p-values obtained from each dose will be ranked over all of the doses in ascending order $P_{(3)} \leq P_{(2)} \leq P_{(1)}$. These p-values will be compared in up to 3 steps: If the maximum p-value $P_{(1)}$ is less than or equal to $\alpha = 0.05$, then stop and it will be concluded that the one-sided hypothesis has been met for all 3 doses. If the maximum p-value $P_{(1)}$ is greater than $\alpha = 0.05$, it will be concluded that the one-sided test for the dose associated with that p-value has not been met. The next largest p-value $P_{(2)}$ will then be compared with $\alpha/2 = 0.025$. If $P_{(2)}$ is less than or equal to 0.025, then stop and it will be concluded that the one-sided hypothesis has been met for the 2 remaining doses. If the next largest p-value $P_{(2)}$ is greater than $\alpha = 0.025$, it will be concluded that the one-sided test for the dose associated with that p-value has not been met. The last p-value $P_{(3)}$ will then be compared with $\alpha/3 = 0.017$. If $P_{(3)}$ is less than or equal to 0.017, then it will be concluded that the one-sided hypothesis has been met for the last dose. The first primary hypothesis that Scopolamine-induced impairment in psychomotor function and information processing is attenuated by one of three doses of MK-7622 (1, 10, 70 mg) as measured by the Detection task, will be supported for at least one dose that is deemed to have mean difference (MK-7622 +Scopolamine) – Scopolamine less than 0 based on the above procedures. ***Estimation of Identification, CPAL, and GLMT***: A similar model will be used for peak effect on the Identification and CPAL tasks, and time-weighted average of errors 1-4 hours of GMLT. The mean treatment differences (MK-7622 (1, 10, and 70 mg) + Scopolamine) – Scopolamine and (Donepezil + Scopolamine) – Scopolamine and their 90% percent confidence intervals for the true mean between-treatment differences will be reported. The GMLT $TWA_{1\text{-}4\ hrs}$ may be natural log transformed. If so, the natural log scale treatment differences for (MK-7622 (1, 10, and 70 mg) +Scopolamine) – Scopolamine and (Donepezil + Scopolamine) – Scopolamine will be exponentiated and reported as a mean % change along with 90% confidence intervals for the true mean % change. **Part II Donepezil + MK-7622 + Scopolamine vs. Donepezil + Scopolamine:** To address the first primary hypothesis in Part II, natural $\log_{10}$ transformed peak effect for detection test will be examined in a linear mixed effects model appropriate for a two-period crossover with fixed factors for sequence, treatment and period, and a random factor for subject within sequence. The mean treatment difference (Donepezil + MK-7622 + Scopolamine) – (Donepezil + Scopolamine) and 90% confidence interval for the treatment difference will be computed using the mean square error from the mixed model and referencing a t distribution. This difference and confidence interval will be back-transformed to obtain the estimated percent change. The first primary hypothesis, that the combination of donepezil and MK-7622 attenuates the scopolamine-induced impairment of psychomotor function and information processing to a greater extent than donepezil alone as measured by the reaction time in the Detection test, will be supported if the upper bound of the 90% confidence interval for mean difference (Donepezil + MK-7622 + Scopolamine) – (Donepezil + Scopolamine) is less than zero. **Safety (Part I and Part II):** Summary statistics and plots will be generated for the change from baseline values in the vital signs, ECG parameters, and selected laboratory safety parameters, as deemed clinically appropriate. Depending on the safety parameter, the difference from baseline will either be computed on the original scale (raw change from baseline) or on the log scale and back-transformed for reporting (percent change from baseline). Summary statistics for the raw laboratory safety tests, ECGs, and/or vital signs may also be computed, as

deemed clinically appropriate. Summary statistics will be provided for VAS scales. **Secondary: Part I Donepezil + Scopolamine vs. Scopolamine alone:** To address the secondary hypothesis in Part I, natural $\log_{10}$ transformed peak effect for detection test for scopolamine alone and the combination of scopolamine and donepezil will be examined in a linear mixed effects model with fixed factors for treatment and period, and a random factor for subject. The mean treatment difference (Donepezil + Scopolamine) – Scopolamine and 90% confidence interval for the treatment difference will be computed using the mean square error from the mixed model and referencing a t distribution. This difference and confidence interval will be back-transformed to obtain the estimated percent change. The secondary hypothesis, that donepezil 10 mg attenuates scopolamine-induced impairment of psychomotor function and information processing as measured by the reaction time in the Detection test, will be supported if the upper bound of the 90% confidence interval for mean difference (Donepezil + Scopolamine) – Scopolamine is less than zero. **Part II Effect of MK-7622 on Donepezil:** The secondary hypothesis in Part II will be addressed by comparing $AUC_{0\text{-}24hr}$ values from administration of donepezil alone to those obtained from coadministration of donepezil and MK-7622. Individual $AUC_{0\text{-}24hr}$ values will be natural log-transformed and evaluated with a mixed effects model with sequence, treatment and period as fixed effects and subject within sequence as random effect. A two-sided 90% confidence interval for the true mean difference ((Donepezil + MK-7622) - Donepezil alone) in ln-AUC will be calculated using the mean square error from the above mixed effects model and referencing a t-distribution. The confidence limit will then be exponentiated to obtain a confidence interval for the true geometric mean $AUC_{0\text{-}24hr}$ ratio (Donepezil + MK-7622/Donepezil alone). The hypothesis that there is no clinically important difference in donepezil $AUC_{0\text{-}24hr}$ with and without coadministration of MK-7622 will be supported if the 90% CI for the $AUC_{0\text{-}24hr}$ GMR (Donepezil + MK-7622/Donepezil alone) is contained within the interval [0.5, 2.00]. C_{max} may be analyzed in a similar fashion. Summary statistics may be provided for T_{max}. ***Estimation of Identification, CPAL, and GLMT:*** A similar model will be used for peak effect on the Identification and CPAL tasks, and time-weighted average of errors 1-4 hours of GMLT. The mean treatment differences (Donepezil + MK-7622 +Scopolamine) – (Donepezil + Scopolamine) and 90% percent confidence intervals for the true mean between-treatment differences will be reported. The GMLT $TWA_{1\text{-}4\ hrs}$ may be natural log transformed. If so, the natural log scale treatment differences for (Donepezil + MK-7622 +Scopolamine) – (Donepezil + Scopolamine) will be exponentiated and reported as a mean % change along with 90% confidence intervals for the true mean % change. **Exploratory (Part I and Part II)**: The cognitive assessments obtained using CANTAB will be analyzed in a similar model and in a similar fashion as that for the cognitive assessments obtained using CogState Early Phase Battery. Side by side plots showing the results from tests from the two batteries that measure the same effect maybe provided to compare the results. In addition, each of the corresponding cognitive assessments obtained using CANTAB and CogState Early Phase Battery at Hour -5 in the first treatment period will be analyzed in a model with fixed effects of test battery and random effect of subjects. Ninety-five percent confidence intervals for the between-battery differences will be provided.

Power: Primary Part I MK-7622 + Scopolamine Vs. Scopolamine alone on Reaction Time: With 30 subjects complete for this design and a log scale within-subject standard deviation of 0.0624 observed from MK-7128 PN013, alpha=0.017 and a one-tailed test, there will be 93% power to detect a difference of 0.06 in log transformed reaction time between MK-7622 + scopolamine and scopolamine alone. ***Estimation of Identification, CPAL, and GMLT:*** The precision of the estimates of (MK-7622 + Scopolamine) – Scopolamine and (Donepezil + Scopolamine) - Scopolamine in the peak effect on the Identification test, CPAL, and time-weighted average of errors 1-4 hours of GMLT obtained from this study can be assessed by calculating the half-width of the 90% confidence intervals expected for the given sample size and assumed variability. With 30 subjects and a log scale within-subject standard deviation of 0.0555 for peak of identification obtained from MK-7128 PN013, the half width of the 90% confidence intervals for mean differences (MK-7622 + Scopolamine) – Scopolamine and (Donepezil + scopolamine) - Scopolamine in the peak effect on the Identification test is 0.024 . With 30 subjects and a within-subject standard deviation of 13.2170 for peak of CPAL obtained from MK-7128 PN013, the half width of the 90% confidence intervals for mean differences (MK-7622 + Scopolamine) – Scopolamine and (Donepezil + scopolamine) - Scopolamine in the peak effect on the CPAL tasks is 5.65. With 30 subjects and a log scale within-subject standard deviation of 0.1998 for time-weighted average of errors 1-4 hours of GMLT obtained from MK-5757 PN006, the half width of the 90% confidence intervals for mean differences (MK-7622 + Scopolamine) – Scopolamine and (Donepezil + scopolamine) - Scopolamine of time-weighted average of errors 1-4 hours of GMLT is 0.085 . **Part II Donepezil + MK-7622 + Scopolamine Vs. Donepezil + Scopolamine on Reaction Time** With 20 subjects in active treatments complete for this design and a log scale within-subject standard deviation of 0.0624 observed from MK-7128 PN013, alpha=0.05 and a one-tailed test, there will be 90% power to detect a difference of 0.06 in log transformed reaction time between (Donepezil + MK-7622 + scopolamine) and (Donepezil + scopolamine). **Safety (Part I and Part II)** Power calculations for comparison of adverse experience rates are too imprecise to be clinically meaningful because the actual rates are not even approximately known. However, if an adverse experience occurs at a rate of 1% or 10% then the chance of observing such an adverse event among 30 (Part I) and 20 (Part II) subjects receiving the treatment will be 26% (18%) and 96% (88%) respectively. At the end of study, for a particular adverse event, if none are observed in any of the 30 (20) subjects at a given dose, then with 80% or 90% confidence, the true incidence of the adverse experience is at most 65% (8%), 7% (11%) respectively. **Secondary Part I Donepezil + Scopolamine vs. Scopolamine alone** With 30 subjects complete for this design and a log scale within-subject standard deviation of 0.0624 observed from MK-7128 PN013, alpha=0.05 and a one-tailed test, there will be 98% power to detect a difference of 0.06 in log transformed reaction time between Donepezil + scopolamine and scopolamine alone. **Part II Effect of MK-7622 on Donepezil** With 20 subjects complete in this design and assumed true within-subject standard deviation for Donepezil ln-$AUC_{0\text{-}24hr}$ is similar to that for Donepezil ln-$AUC_{0\text{-}240hr}$ of 0.1005 pooled from E2020-A001-015 and E2020-A001-016 obtained in Aricept ODT FOI, alpha=0.05, then there is at least 0.99 probability that the 90% confidence interval for the geometric mean $AUC_{0\text{-}24hr}$ ratio (Donepezil + MK-7622/Donepezil alone) is contained within the interval [0.5, 2.00], given that the true geometric mean ratio is 1.0.

The true geometric mean ratio can be between 0.54 and 1.84 and still have 0.80 probability of supporting the hypothesis. ***Estimations of Identification, CPAL, and GMLT*:** The precision of the estimates of (MK-7622 + Donepezil + Scopolamine) – (Donepezil + Scopolamine) in the peak effect on the Identification test, CPAL, and time-weighted average of errors 1-4 hours of GMLT obtained from this study can be assessed by calculating the half-width of the 90% confidence intervals expected for the given sample size and assumed variability. With 20 subjects and a log scale within-subject standard deviation of 0.0555 for peak of identification obtained from MK-7128 PN013, the half width of the 90% confidence interval for the mean difference (MK-7622 + Donepezil + Scopolamine) – (Donepezil + Scopolamine) in the peak effect on the Identification test is 0.03. With 20 subjects and a within-subject standard deviation of 13.2170 for peak of CPAL obtained from MK-7128 PN013, the half width of the 90% confidence interval for the mean difference (MK-7622 + Donepezil + Scopolamine) – (Donepezil + Scopolamine) in the peak effect on the CPAL tasks is 7.25. With 20 subjects and a log scale within-subject standard deviation of 0.1998 for time-weighted average of errors 1-4 hours of GMLT obtained from MK-5757 PN006, the half width of the 90% confidence interval for the mean difference (MK-7622 + Donepezil + Scopolamine) – (Donepezil + Scopolamine) of time-weighted average of errors 1-4 hours of GMLT is 0.11.

Methods S4. M1 FLIPR Shift Assay Methods

M1 FLIPR Shift Assay Methods

Cell Culture

The human M1 receptor was stably expressed in CHO cells, grown at 37° C, 5% CO_2, 95% relative humidity in CHO basal medium (DMEM, 10% FBS, 25 mM HEPES, 100 units/ml Pen/Strep, 2 mM L-glutamine, 1 mM Na-pyruvate, 1x non-essential amino acids) with appropriate selection conditions (500 µg/ml Geneticin and 250 µg/ml Zeocin). The cells were expanded prior to freezing in multiple vials referred to as "Assay Ready Frozen" (ARF) cells, and stored in liquid nitrogen (LN_2).

FLIPR™ ACh Shift Assay

CHO-hM1 ARF cells were taken from LN_2 storage, thawed rapidly at 37° C, washed with CHO basal medium and dispensed in 384 well black, clear bottom plates at ~20,000 cells per well. After incubation overnight at 37° C, 5% CO2, 95% relative humidity, the medium was removed and replaced with 25 µl per well of Calcium 5 dye buffer (Calcium 5 reagent [Molecular Devices] in HBSS, 20 mM HEPES, 2.5 mM probenecid), followed by incubation at 37° C for 52 min., and subsequently 8 min. at room temperature. The plates were then loaded into the FLIPR™ instrument where 25 µl of 0.1% DMSO was added to the cells while detecting fluorescence (excitation 470-495 nm/emission 515-575 nm) for 5 min., followed by addition of 25 µl of test compound plus acetylcholine (ACh), and continued detection of fluorescence for 5 min. For further data analysis, a max-min value was determined for each well by subtracting the minimum fluorescence signal observed immediately prior to compound addition from the maximum fluorescence signal observed in the time following compound addition.

ACh was prepared by serial, 3.5 fold dilution of 10 mM DMSO stock solution into neat DMSO. The test compounds for the assay were prepared by serial, 3.16 fold dilution of 10 mM DMSO stock solutions into neat DMSO. Fifty nanoliters of the diluted ACh and 500 nl of diluted test compounds are then added to 75 µl assay buffer (HBSS, 20 mM HEPES, pH 7.4) in a matrix fashion such that each concentration of test compound was assayed with a complete dose range of ACh.

Data Analysis

Analysis of the FLIPR Shift experimental data involved import of the FLIPR max-min values from 2 or more independent experiments, and the ACh and test compound dosage information, into GraphPad Prism (version 7.02). The combined data for a given test compound plus ACh were then analyzed using global curve fitting to an operational model of receptor allosterism (Zhang and Kavana, 2015), using the following set of equations:

A=X

Eapp=kai*KA*KB+tauA*A*(KB+aa*bb*B)+tauB*B*KA

Kapp=KA*KB+A*KB+KA*B+aa*A*B

Y=100/(1+Kapp^n/Eapp^n)

Where A(=X) and B are, respectively, the ACh and test compound concentrations; kai is a measure of basal (constitutive) activity; aa and bb are, respectively, the modifier for affinity (alpha) and efficacy (beta); tauA and tauB are, respectively, ligand efficacy for A and B; KA and KB are, respectively, the dissociation constant for A and B. Y is the percent maximal response multiplied by 100.

In the analysis, certain parameters were constrained as follows: kai=0; bb=1; KA=42666. The 0% and 100% responses were set to Y=0 and Y=the average max, respectively. The average max was defined as the average of all max-min values for the top ACh concentration of every curve in the data set for that compound. The allosteric parameters for the test compounds (KB, alpha, and tauB), ± the standard error, as calculated by GraphPad Prism, are reported.

Reference

Zhang R, Kavana M. Quantitative Analysis of Receptor Allosterism and its Implication for Drug Discovery. Expert Opin Drug Discov. 2015 July; 10(7): 763-80.