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ARTICLE

Safety, Tolerability, and Pharmacokinetics of the β-Site Amyloid Precursor Protein-Cleaving Enzyme 1 Inhibitor Verubecestat (MK-8931) in Healthy Elderly Male and Female Subjects

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β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) is required for the production of β-amyloid peptides, which are implicated in the etiology of Alzheimer's disease. The safety and pharmacokinetics of the BACE1 inhibitor verubecestat have previously been studied in young adults aged 19–45 years. In this randomized, placebo-controlled, phase I study (protocol MK-8931-006), we investigated the safety, tolerability, and pharmacokinetics of a single dose (100 mg) or multiple doses (30, 80, and 120 mg) once daily for 28 days of verubecestat in healthy elderly subjects. Safety end points were assessed at baseline and during the duration of the study period and indicated that verubecestat was generally well tolerated. Verubecestat pharmacokinetics were similar between healthy elderly male and female subjects and similar to those reported in healthy young males in previous studies. These data supported subsequent studies to assess the potential efficacy of verubecestat in subjects with Alzheimer's disease.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Pharmacological inhibition of β-amyloid synthesis may be of therapeutic benefit in patients with Alzheimer's disease. Preclinical studies have demonstrated that the oral β-site amyloid precursor protein-cleaving enzyme 1 inhibitor verubecestat is effective at lowering β-amyloid; however, safety and pharmacokinetic data are only available from healthy young adult subjects and elderly subjects with Alzheimer's disease.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Are the safety (including high-exposure safety), tolerability, and pharmacokinetic characteristics of verubecestat in elderly subjects (the target population for Alzheimer's disease therapies) compatible with its continued development?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Verubecestat was generally well tolerated in elderly subjects following single doses or multiple once-daily doses for up to 28 days. Pharmacokinetics were generally similar to those reported in young adult subjects.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ These data were critical in the design of subsequent phase III studies of verubecestat in elderly subjects with Alzheimer's disease and helped establish the upper margins for exposure to verubecestat.

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the leading cause of dementia[1] and the fourth-leading cause of death among high sociodemographic index populations globally.[2] Available therapies provide only modest and transient improvement in cognitive function without altering disease progression. There is clear clinical need for pharmacological agents that slow, halt, or reverse AD progression.

AD is characterized and definitively diagnosed by specific histopathological features in the brain, including neurovascular and parenchymal amyloid deposits (plaques) composed primarily of β-amyloid (Aβ) peptides, intraneuronal neurofibrillary tangles composed of hyperphosphorylated microtubule-associated protein tau and neuroinflammation.[3] According to the amyloid hypothesis, Aβ peptides are intimately involved in the etiology of AD via their aggregation to form toxic complexes (oligomers, fibrils, and plaques), which contribute to neuronal death.[4–7] Pathogenic Aβ peptides are produced by proteolytic cleavage of amyloid precursor protein (APP).[8] Mutations in the *APP* gene in familial AD lead to increased Aβ production,[9] whereas mutations in the *APP* gene that

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reduce Aβ production lead to a reduced risk of AD.[10] As a result, β-site APP-cleaving enzyme 1 (BACE1; also known as β-secretase), one of the enzymes responsible for APP cleavage to form Aβ, has been identified as a promising target for AD via Aβ depletion.[8] BACE1 inhibition blocks the amyloidogenic pathway at the initiation point, potentially stopping all downstream processes, particularly the production of aggregation-prone Aβ peptides.[8] BACE1-knockout mice have complete elimination of Aβ peptides in the brain and display only a mild phenotype with reduced peripheral nerve myelination and mild functional deficits.[11–14] Therefore, BACE1 inhibition in patients with AD should reduce Aβ peptide production, with the potential to modify AD progression.[15]

Verubecestat (MK-8931) is a potent inhibitor of BACE1.[16] In rats and monkeys, verubecestat administration resulted in a marked dose-dependent reduction of Aβ peptides in plasma and cerebrospinal fluid (CSF) and the cortex.[17] Similarly, in healthy subjects and subjects with AD, single doses (healthy subjects: 2.5–550 mg) and multiple doses (healthy subjects: 10–250 mg for 14 days; subjects with AD: 12–60 mg for 7 days) of orally administered verubecestat were generally well tolerated and reduced CSF levels of the APP metabolites Aβ40, Aβ42, and sAPPβ.[17] Initial clinical studies of verubecestat were limited to healthy young adults aged 19–45 years.[17] A subsequent phase III placebo-controlled clinical study (EPOCH: NCT01739348) investigating 12 mg and 40 mg verubecestat once daily for 78 weeks in subjects with mild to moderate AD found that CSF Aβ40, Aβ42, and sAPPβ were reduced in comparison with placebo; however, clinical progression of AD (based on cognition and functional assessments) was not slowed by these doses of verubecestat.[18] Verubecestat is cleared primarily by cytochrome P450 3A4 metabolism and its pharmacokinetics (PK) have been previously reported for healthy nonelderly adults,[17] including those of Japanese descent,[19] and adults with mild to moderate AD.[17] Because AD primarily affects the elderly[1] and because physiological changes associated with aging may lead to altered drug PK,[20] this study aimed to characterize the safety, tolerability, and PK of verubecestat in healthy elderly subjects and explore the impact of sex on verubecestat PK. Data obtained from this study helped guide the design of subsequent verubecestat studies in subjects with AD.

METHODS

Study design and subjects

This was a randomized, third-party–blind, placebo-controlled, sequential-panel, single-dose (panel A) and multiple-dose (panels A–E) study of verubecestat in healthy elderly subjects (protocol MK-8931-006 [P07829]). The study was conducted across the following two US sites: Arizona (site 001) and New Jersey (site 002). The study duration, including prestudy and poststudy evaluations, was ~12 months. Healthy adult men and women (of non–child-bearing potential) aged 65–85 years with body mass index 18–35 kg/m^2 at screening were eligible for enrollment. Other inclusion criteria included the absence of any clinically significant disease that would interfere with study evaluations and normal-range measurements of vital signs and sex-specific 12-lead electrocardiogram (ECG) conduction intervals (corrected QT interval: males ≤ 430 milliseconds, females ≤ 450 milliseconds; PR interval: ≤ 200 milliseconds). In response to a serious adverse event (SAE) that occurred in panel A (toxic encephalopathy in a 71-year-old female subject requiring hospitalization for 3 days), subjects in panels B–E were additionally required to have Mini Mental State Exam scores ≥ 28 for study inclusion.[21]

Exclusion criteria included any clinically significant disease that would interfere with study evaluations; any surgical or medical condition that may affect the absorption, distribution, metabolism, or excretion of any drug; or an estimated creatinine clearance of ≤ 60 mL/minute based on the Cockcroft–Gault equation. Further details of inclusion/exclusion criteria are given in the **Supplementary Material**.

All subjects were evaluated for a 28-day screening period to ensure that they complied with the inclusion/exclusion criteria. This study was conducted in accordance with Good Clinical Practice standards and applicable country and/or local statutes and regulations regarding ethical committee review, informed consent, and protection of human subjects participating in biomedical research; the protocol was approved by Celerion Institutional Review Board, Lincoln, NE.

Study objectives

The primary objective of this study was to evaluate the safety and tolerability of rising multiple oral doses of verubecestat in healthy elderly subjects. Primary safety end points included adverse events (AEs), physical examinations, vital signs (heart rate and blood pressure), 12-lead ECGs, clinical laboratory tests (hematology, serum chemistry, and urinalysis), Columbia Suicide Severity Rating Scale, and Mental Status Examination (MSE) obtained at prespecified time points.

Secondary study objectives were to obtain preliminary plasma PK data of verubecestat following single dose and multiple-dose administration. Other secondary objectives were to compare the single-dose PK parameters of verubecestat between males and females and between healthy elderly subjects and healthy young subjects (historical controls).

Randomization and drug administration

Five panels, each comprising eight males and eight females, were included in the study. Within each panel, subjects were randomized 3:1 to verubecestat or placebo according to a computer-generated allocation schedule. The study design is presented in **Figure 1**. Subjects in panel A were randomized to receive a single oral dose of 100-mg verubecestat or placebo on day 1 followed by a ≥21-day washout period to enable an interim PK analysis of clinical samples. Subjects were confined to the clinical research unit (CRU) until completion of the study-related procedures on day 3 and subsequently received multiple doses of 80-mg verubecestat or placebo once daily for 28 days. During the multiple-dose treatment phase, the subjects were confined to the CRU for the first 10 days of treatment and were subsequently treated for 17 days on

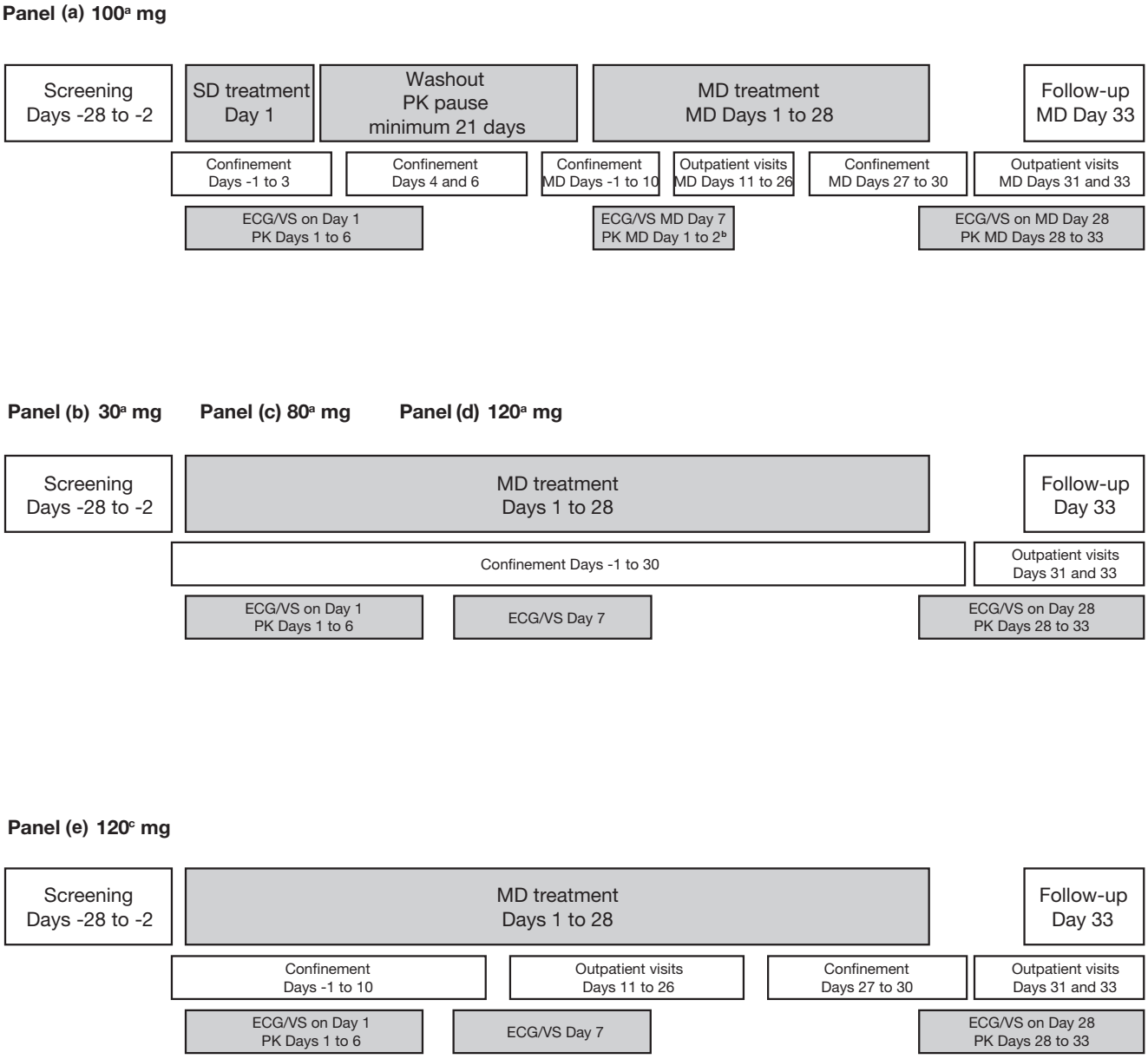

Figure 1 Study design. ECG, electrocardiogram; MD, multiple dose; PK, pharmacokinetics; SD, single dose; VS, vital signs. [a]Dose level may be reduced based on review of all available safety and PK data. [b]Only to be collected if dose for multiple-dose administration is changed relative to single-dose administration on day 1. [c]Dose level will not be greater than any dose found to be safe and well tolerated in previous panels.

an outpatient basis, returning to the CRU every morning for study-drug administration. On day 27, the subjects returned to the CRU for outpatient dosing in the morning. At the subjects' preference, they either remained in the CRU or left, returned in the afternoon, and remained confined until 48 hours post–last-dose. After discharge, the subjects returned to the CRU on an outpatient basis on days 31 and 33. The decision to progress to the multiple-dose treatment in panel A was based on acceptable safety, tolerability, and PK data following single-dose administration and historical data from healthy young subjects.[17]

Subjects in panels B–E received multiple oral doses of verubecestat (30, 80, 120, and 120 mg, respectively) or placebo once daily for 28 days. As a result of the protocol amendment described in the safety and tolerability assessments section, the subjects in panels B–D remained confined during the entire 28 days of dosing until 48 hours post–last-dose. During confinement, the subjects could leave the clinic during nonsleeping hours. After discharge, the subjects returned to the CRU on an outpatient basis on days 31 and 33. In panel E, the subjects were discharged from the CRU after completion of study-related procedures on day 7. The subjects were subsequently treated for 20 days on an outpatient basis, returning to the CRU every morning for study-drug administration. From day 27, dosing, confinement, and discharge procedures were as described

for subjects in the multiple-dose portion of panel A. During treatments in all five panels, the study drug was administered at approximately the same time each day with 240 mL of water. On serial PK-collection days, the subjects were required to fast ≥10 hours predose and 4 hours postdose. On all other dosing days, treatments were administered without regard to food. Verubecestat doses were administered orally in 100-mg and/or 10-mg capsules.

This was a third-party–blind (within-panel) study, meaning both subjects and study investigators were blinded to treatments by a randomization schedule administered by a third party. The third party was not involved with the study procedures, assessments, or data recording and was not permitted to reveal the randomized treatment-allocation code to anyone unless required to provide adequate medical care to the study subject.

Safety and tolerability assessments

The subjects were queried daily regarding the occurrence of AEs in a nonleading manner, and all reported AEs were documented. Safety monitoring took place throughout the study; assessments included hematology, blood chemistry and urine analysis, 12-lead ECGs, measurement of vital signs, physical examinations, Columbia Suicide Severity Rating Scale, and MSE. Prespecified events of clinical interest included an overdose of the study drug not associated with clinical symptoms or abnormal laboratory results, elevated aspartate aminotransferase or alanine aminotransferase (≥ 3 × upper limit of normal) associated with elevated bilirubin (≥ 2 × upper limit of normal) and alkaline phosphatase (< 2 × upper limit of normal), and clinically significant changes in behavior or mental status. All AEs reported by the subject or observed by the investigator were graded regarding maximum intensity, seriousness, action taken, and relationship to treatment. In response to the previously mentioned SAE that occurred in panel A, the study protocol was amended such that, as well as the addition of requiring a baseline Mini Mental State Exam score of ≥28 for inclusion in the study, there was a repeated evaluation of MSE for subjects in panels B–E, with subjects from panels B–D remaining confined to the CRU for the entire dosing period until 48 hours post–last-dose. The decision to proceed to the next panel of treatment was based on acceptable safety, tolerability, and PK data from the preceding panel and historical data from healthy young subjects.[17]

PK assessments

Blood samples for the determination of verubecestat plasma concentrations were collected from each subject in panel A predose and at selected timepoints over 120 hours following administration of a single 100 mg dose of verubecestat on day 1. For multiple-dose administrations in panels A–E (**Figure 1**), blood samples were obtained predose and at selected time points over 24 hours postdose on day 1; predose on days 3, 5, 7, and 14; and predose and at selected timepoints over 120 hours postdose on day 28.

Verubecestat plasma PK parameters were calculated by noncompartmental analyses using Phoenix WinNonlin (Certara USA Inc., Princeton, NJ, USA) software (version 6.3). Maximum concentration (C_{max}) and time to reach C_{max} were generated by Phoenix WinNonlin from the observed concentration–time data. Area under the concentration–time curve values (AUCs) were calculated using the linear-trapezoidal method for ascending concentrations and the log-trapezoidal method for descending concentrations. At least three consecutive timepoints in the terminal phase (excluding C_{max}) were used for determination of the apparent terminal elimination rate constant (λz) to enable apparent terminal half-life ($t_{1/2}$) to be meaningfully calculated ($t_{1/2} = \ln 2/\lambda z$). For multiple-dose data, the dosing interval (τ) and the day 28/day 1 AUC from time zero to 24 hours ($AUC_{0-24\ h}$) accumulation ratio (R) were used for effective $t_{1/2}$ determination using the following formula: effective $t_{1/2} = -\tau * \ln(2)/\ln(1 - (1/R))$, where it was assumed that steady state was attained by day 28.

Bioanalytical methods

Plasma samples were assayed for verubecestat by Merck (Oss, The Netherlands) using validated high-performance liquid chromatography tandem mass spectrometry. The lower limit of quantitation was 1.00 ng/mL (2.44 nM). The analytical range of quantitation was 1.00–1000.00 ng/mL (2.44–2442.48 nM).

Statistical analyses

For the assessment of safety and tolerability, the data from all subjects who received ≥ 1 dose of verubecestat or placebo were analyzed (all-subjects-as-treated population).

All AEs were tabulated. Descriptive statistics were provided for change from baseline in ECGs, vital signs, and laboratory end points. The per-protocol population included all subjects who complied with the protocol sufficiently to ensure data were likely to exhibit the effects of treatment. This population was used for analysis of PK results. Sex and age effects on verubecestat PK parameters were addressed using the 100 mg single-dose plasma PK of verubecestat in elderly male and female subjects obtained in panel A and those in young male subjects obtained from historical data.[17] Individual values of AUC from time zero to infinity ($AUC_{0-\infty}$) and C_{max} were natural-log–transformed and evaluated separately with a one-way analysis of variance model with population (elderly male, elderly female, and young male) as a fixed effect. Poolability of the PK data across the two elderly groups was assessed using the following criteria: (i) the 90% confidence interval (CI) of the geometric mean ratio (GMR) between the two groups constructed from the model was contained within the prespecified bounds of (0.50, 2.00), and (ii) the *P* value of the comparison between the two groups was > 0.05. If both criteria were satisfied, the PK data from the two groups would be pooled.

Determination of sample size and power of study

For power calculations, the between-subject standard deviation (SD) of verubecestat $AUC_{0-\infty}$ on the log scale was estimated to be 0.147 (ln-μM·hour) based on data from a previous verubecestat study pooled across 80–300 mg doses. It was assumed that the variability for ln-$AUC_{0-\infty}$ in healthy elderly males and females was similar to that in healthy young males after single dosing.

With six elderly female and six elderly male subjects and a type I error α of 0.05, there was 99% probability that the

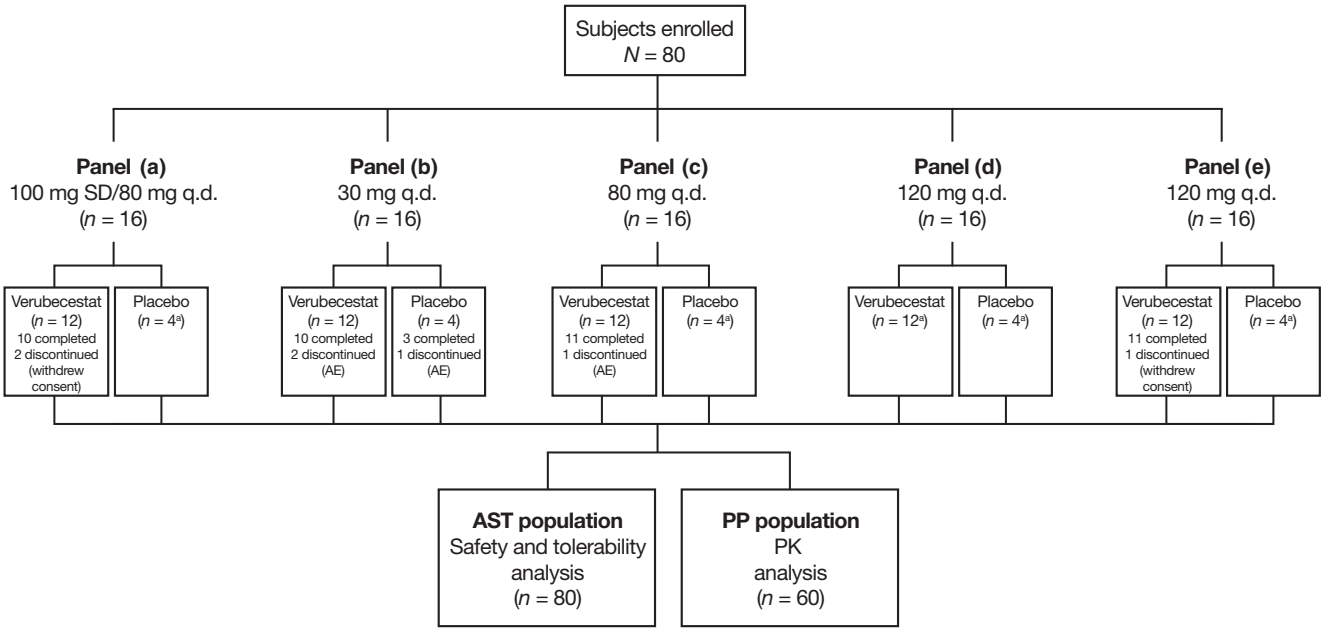

Figure 2 Study flow chart. AE, adverse event; AST, all subjects as treated; PP, per protocol; PK, pharmacokinetics; q.d., once daily; SD, single dose. [a]All subjects in this panel completed the study.

90% CI for the true GMR of $AUC_{0-\infty}$ (elderly female/elderly male) would be contained in 0.50–2.00 given that the true GMR is 1.00. The true $AUC_{0-\infty}$ GMR could be as low as 0.63 or as high as 1.59 and still have 80% probability that the 90% CI would be contained in 0.50–2.00. Meanwhile, with 12 elderly and six young male subjects, the true $AUC_{0-\infty}$ GMR could range from 0.61–1.65 and still have 80% probability that the 90% CI would be contained in 0.50–2.00.

RESULTS

Subjects

A total of 80 healthy elderly subjects (76 subjects at site 001; 4 subjects at site 002) were enrolled into the study in five sequential panels (**Figure 2**); subject characteristics are shown in **Table 1**. Of those enrolled, 73 subjects completed the study per protocol, 4 were discontinued due to AEs, and 3 withdrew consent (**Figure 2**). Each panel consisted of 16 subjects (8 males, 8 females), of whom 12 (6 males, 6 females) received oral verubecestat, and 4 (2 males, 2 females) received placebo. Mean age (years ± SD) for subjects who received verubecestat ranged from 69.1 ± 4.5 to 71.2 ± 4.0; for the 20 subjects who received placebo, the mean age was 69.7 ± 4.6.

Safety

During the single-dosing period of panel A, doses of 100 mg verubecestat were generally well tolerated in healthy elderly male and female subjects. Five subjects reported treatment-emergent AEs (TEAEs) following single-dose administration of verubecestat and one subject following single-dose administration of placebo. Among these, three subjects reported AEs considered verubecestat-related by the investigator (**Table 2**).

Multiple (30–120 mg) doses of verubecestat were also generally well tolerated. A total of 52 subjects (86.7%) reported TEAEs following multiple-dose administration of verubecestat and 16 subjects (80.0%) following multiple-dose administration of placebo. These included 50 subjects who reported AEs considered drug-related by the investigator (6 in panel A 80 mg verubecestat, 8 in panel B 30 mg verubecestat, 8 in panel C 80 mg verubecestat, 17 in panels D and E 120 mg verubecestat, and 11 following placebo (**Table 2**). A summary of all TEAEs based on system organ class is listed in **Table S1**.

SAEs were reported by three subjects. One subject in panel A presented to the emergency room with toxic encephalopathy 3 days after withdrawing consent from the study. Symptoms included severe agitation and confusion that required hospitalization; the subject had a negative drug screen at screening and no history of alcohol abuse or Wernicke–Korsakoff syndrome, and a medical assessment, including computed tomography and magnetic resonance imaging of the brain, did not reveal any cause for the symptoms. Symptoms returned to baseline after 3 days, and the subject was subsequently discharged. The subject had received a single 100 mg dose of verubecestat and 80 mg verubecestat once daily for 13 days prior to withdrawing consent, and the subsequent SAE was considered to be study drug–related. In response to this SAE, the inclusion criteria for subjects in panels B–E were revised as described in the Methods section.

On day 6, one subject in panel B (30 mg verubecestat) fell in the shower after a brief loss of consciousness, leading to a head injury and hospitalization. This SAE of moderate syncope was considered study drug–related, and the subject was discontinued from the study. Another subject in panel B discontinued from the study as a result of an SAE of moderate cholecystitis on day 10 that required hospitalization, which was considered unrelated to the study drug.

Table 1 Subject characteristics

Subject characteristics	Panel A 100 mg SD/80 mg q.d. *n* (%)	Panel B 30 mg q.d. *n* (%)	Panel C 80 mg q.d. *n* (%)	Panel D 120 mg q.d. *n* (%)	Panel E 120 mg q.d. *n* (%)	Placebo q.d. *n* (%)
Subjects in population	12	12	12	12	12	20
Sex, *n* (%)						
Male	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	10 (50.0)
Female	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	10 (50.0)
Age, y						
Mean	69.1	69.7	70.2	69.4	71.2	69.7
Standard deviation	4.5	4.3	3.1	3.9	4.0	4.6
Median	68.0	69.5	69.5	68.0	71.0	69.5
Range	65–82	65–76	66–78	65–78	66–81	64–78
Weight (kg), all subjects						
Mean	75.4	76.8	81.7	79.0	80.0	75.8
Standard deviation	9.8	14.7	10.5	8.0	13.0	9.6
Median	72.7	73.1	81.8	79.0	83.3	75.9
Range	61.0–96.9	55.9–100.0	58.6–97.3	67.1–92.4	53.1–95.3	54.0–91.0
Weight (kg), males						
Mean	81.2	87.5	83.3	83.0	85.5	79.5
Standard deviation	9.9	11.3	9.5	8.3	7.3	6.9
Median	79.2	91.4	79.5	85.5	85.9	80.5
Range	70.9–96.9	69.4–100.0	72.0–97.3	71.0–92.4	71.9–93.4	68.5–87.2
Weight (kg), females						
Mean	69.5	66.2	80.1	75.1	74.6	72.2
Standard deviation	5.0	8.8	11.1	5.4	15.0	10.5
Median	72.1	65.1	82.4	78.3	74.0	72.8
Range	61.0–74.4	55.9–81.7	58.6–91.6	67.1–79.7	53.1–95.3	54.0–91.0
Race, *n* (%)						
American Indian or Alaskan Native	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
Black or African American	0 (0.0)	1 (8.3)	0 (0.0)	1 (8.3)	0 (0.0)	2 (10.0)
Multiracial	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
White	11 (91.7)	11 (91.7)	12 (100.0)	11 (91.7)	12 (100.0)	17 (85.0)
Ethnicity, *n* (%)						
Hispanic or Latino	3 (25.0)	1 (8.3)	3 (25.0)	0 (0.0)	0 (0.0)	4 (20.0)
Not Hispanic or Latino	9 (75.0)	11 (91.7)	9 (75.0)	12 (100.0)	12 (100.0)	16 (80.0)

Placebo is pooled over panels.
q.d., once daily; SD, single dose.

Four subjects discontinued from the study as a result of AEs. In addition to the two subjects described previously, one subject (80 mg verubecestat for 27 days) was discontinued because of a mild macular rash considered drug-related, and one subject (placebo) was discontinued from the study because of mild increased blood pressure considered unrelated to study drug.

There were two AEs of generalized erythematous macular rash with generalized pruritus that were assessed as study drug–related. In addition to the case of macular rash, one subject enrolled in panel E developed a diffuse moderate maculopapular rash and generalized pruritus after receiving 120 mg verubecestat for 28 days. The rash was resolving at the time of study completion. Additionally, four focal rashes were reported as verubecestat-related. In all instances, there were no systemic findings, including the absence of respiratory symptoms or the involvement of the oropharynx.

No events of clinical interest or deaths were reported during the study, and there was no evidence of suicidal ideation or behavior as assessed by the Columbia Suicide Severity Rating Scale. There were no consistent treatment-related changes in laboratory values, vital signs, physical examinations, or ECG safety parameters, and no dose-related changes in laboratory values, vital signs, or ECGs. There were no clinically significant or consistent findings related to the MSE in panels B–E, which was performed to assess changes in mental status during treatment in panels B–E.

PK

Following a single 100 mg dose of verubecestat, the geometric mean (GM) values for $AUC_{0-\infty}$ and C_{max} were 12.38 μM·hour and 495.2 nM, respectively, in the pooled elderly subjects (**Table 3**). Comparisons based on sex are shown in **Figure 3a**

Table 2 Subjects with drug-related TEAEs following single-dose and multiple-dose administrations of verubecestat or placebo

AE	Panel A 100 mg SD/80 mg q.d. *n* (%)	Panel B 30 mg q.d. *n* (%)	Panel C 80 mg q.d. *n* (%)	Panel D 120 mg q.d. *n* (%)	Panel E 120 mg q.d. *n* (%)	Placebo q.d. *n* (%)
Subjects with drug-related TEAEs following single-dose administration of verubecestat[a]						
Subjects in population	12	–	–	–	–	4
With one or more AEs	3 (25.0)	–	–	–	–	0 (0.0)
With no AEs	9 (75.0)	–	–	–	–	4 (100.0)
Musculoskeletal and connective tissue disorders	2 (16.7)	–	–	–	–	0 (0.0)
Muscle spasms	1 (8.3)	–	–	–	–	0 (0.0)
Myalgia	1 (8.3)	–	–	–	–	0 (0.0)
Nervous system disorders	2 (16.7)	–	–	–	–	0 (0.0)
Somnolence	2 (16.7)	–	–	–	–	0 (0.0)
Subjects with drug-related TEAEs following multiple-dose administrations of verubecestat						
Subjects in population	12	12	12	12	12	20
With one or AEs	6 (50.0)	8 (66.7)	8 (66.7)	6 (50.0)	11 (91.7)	11 (55.0)
With no AEs	6 (50.0)	4 (33.3)	4 (33.3)	6 (50.0)	1 (8.3)	9 (45.0)
Cardiac disorders	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Eye disorders	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)
Gastrointestinal disorders	1 (8.3)	4 (33.3)	5 (41.7)	4 (33.3)	3 (25.0)	7 (35.0)
General disorders and administration site conditions	1 (8.3)	2 (16.7)	2 (16.7)	3 (25.0)	9 (75.0)	2 (10.0)
Hepatobiliary disorders	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
Investigations	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
Metabolism and nutrition disorders	0 (0.0)	0 (0.0)	1 (8.3)	1 (8.3)	0 (0.0)	0 (0.0)
Musculoskeletal and connective tissue disorders	3 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (25.0)	0 (0.0)
Nervous system disorders	5 (41.7)	3 (25.0)	3 (25.0)	5 (41.7)	5 (41.7)	5 (25.0)
Psychiatric disorders	1 (8.3)	3 (25.0)	4 (33.3)	4 (33.3)	4 (33.3)	0 (0.0)
Renal and urinary disorders	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
Respiratory, thoracic, and mediastinal disorders	0 (0.0)	0 (0.0)	1 (8.3)	1 (8.3)	1 (8.3)	1 (5.0)
Skin and subcutaneous tissue disorders	1 (8.3)	3 (25.0)	2 (16.7)	2 (16.7)	5 (41.7)	1 (5.0)
Vascular disorders	0 (0.0)	1 (8.3)	2 (16.7)	1 (8.3)	0 (0.0)	0 (0.0)

AE, adverse event; q.d., once daily; SD, single dose; TEAE, treatment-emergent adverse event.
[a]Every subject is counted a single time for each applicable row and column. A system organ class or specific AE appears in this table only if its incidence in one or more of the columns was > 0% in one or more treatment groups, after rounding. For panel A, the single dose was 100 mg q.d.

and **Table 3**. The elderly female/elderly male GMR for $AUC_{0-\infty}$ was 1.31 (90% CI 1.03–1.67) and for C_{max} was 1.30 (90% CI 0.97–1.73), whereas the median time to reach C_{max} and apparent terminal $t_{1/2}$ were comparable between sexes (**Table 3**).

Exposure in healthy elderly (pooled male and female) subjects was compared with historical data from healthy young male subjects, and the arithmetic mean verubecestat plasma concentration–time profiles following single 100 mg administration are presented in **Figure 3b**. The elderly/young male GMR for $AUC_{0-\infty}$ was 1.31 (90% CI 1.07–1.61) and for C_{max} was 0.95 (90% CI 0.74–1.22).

Following multiple dosing of verubecestat, the GM accumulation ratio day 28/day 1 $AUC_{0-24\,h}$ ranged from 1.72–2.20 (**Table 4**), in agreement with expectations based on the apparent terminal $t_{1/2}$. The GM day 28 $AUC_{0-24\,h}$ was 3.85, 10.1, and 15.5 μM·hour following 30 mg, 80 mg, and 120 mg once-daily doses of verubecestat, respectively, in healthy elderly subjects. The plasma concentration–time profile and the arithmetic mean concentration at 24 hours postdose vs. time profile following multiple-dose administration of verubecestat are presented in **Figure 3c,d**, respectively. Accumulation in trough concentrations was evident during the first week of once-daily dosing; there was no consistent trend of substantive further increases in concentrations during the second and third weeks of dosing. The GM effective $t_{1/2}$ ranged from 19–27 hours, supportive of once-daily dosing and consistent with a time to steady state of ~ 1 week. The GM apparent terminal $t_{1/2}$ ranged from 23–26 hours (**Table 4**).

Table 3 Summary statistics for plasma pharmacokinetics following single oral dose of 100 mg verubecestat

Population	$AUC_{0-\infty}$, μM·hour[a]	C_{max}, nM[a]	T_{max}, hour[b]	Apparent terminal $t_{1/2}$, hour[c]
Young male[d] (n = 6)	9.43 (7.69–11.50)	521.2 (407–668)	1.5 (1.0, 4.0)	20.3 (6.7)
Elderly male (n = 6)	10.80 (8.82–13.20)	434.9 (340–557)	3.5 (1.0–8.0)	22.0 (11.7)
Elderly female (n = 6)	14.18 (11.60–17.40)	563.8 (440–722)	3.0 (2.0–8.0)	22.2 (5.4)
Pooled elderly (n = 12)	12.38 (10.70–14.30)	495.2 (416–590)	—	—

Comparison	$AUC_{0-\infty}$[e]	C_{max}[e]
Sex: elderly female vs. elderly male	1.31 (1.03–1.67)	1.30 (0.97–1.73)
Age: pooled elderly vs. young male	1.31 (1.07–1.61)	0.95 (0.74–1.22)

%CV, percentage coefficient of variation; $AUC_{0-\infty}$, area under the concentration–time curve from time zero to infinity; CI, confidence interval; C_{max}, maximum concentration; GMR, geometric least-squares mean ratio between populations; $t_{1/2}$, half-life; T_{max}, time to C_{max}.
[a]Geometric mean back-transformed from log scale (95% CI). [b]Median (minimum, maximum). [c]Geometric mean (%CV). [d]Historical data from verubecestat 100-mg dose. [e]GMR (90% CI).

DISCUSSION

The amyloid hypothesis implicates Aβ peptides in the etiology of AD, and it is hypothesized that Aβ blockade may confer beneficial effects in AD.[4–7,15] Thus, preventing generation of Aβ peptides by inhibition of BACE1 (the first step in the processing of APP to Aβ) is a logical target to test the amyloid hypothesis, and the BACE1 inhibitor verubecestat is in clinical development for AD.[16] The safety and PK of verubecestat were previously established in healthy young adults.[17] Furthermore, reductions in Aβ in CSF by verubecestat have been demonstrated in healthy adults and subjects with AD and found to be consistent with those in animal models.[16,17] As AD is predominantly a disease of the elderly,[1] the current study was designed to investigate the safety, tolerability, and PK of verubecestat in elderly subjects relative to young male subjects and assess the impact of sex on verubecestat PK.

Administration of single (100 mg) and multiple (30–120 mg) oral doses of verubecestat was generally well tolerated in healthy elderly male and female subjects. Drug-related AEs were reported by 3 of 12 subjects following single-dose administration and by 39 of 60 subjects following multiple-dose administration of verubecestat. Incidences of macular rash and delirium were considered AEs of concern and included as events of clinical interest in subsequent studies. No consistent effects on vital signs or ECG parameters were observed at any dose, and there were no consistent trends or dose-related changes in laboratory evaluations. Overall, there was no evidence of dose-dependent TEAEs that would limit further development of verubecestat as a therapeutic agent for the treatment of AD in elderly subjects. Verubecestat $AUC_{0-24\ h}$ in the 80 and 120 mg panels was 2.0- and 3.1-fold increased, respectively, relative to the 40-mg group in EPOCH (5.01 μM·hour), the highest verubecestat dose evaluated in phase III,[18] thus establishing a margin of safety to clinically relevant doses. The safety and tolerability profile at those doses in this study compared with EPOCH suggests a lack of strong dose or exposure dependency in the safety profile of verubecestat and that factors that elevate verubecestat exposures on the order of twofold to threefold are unlikely to substantially alter tolerability.

Verubecestat GM $AUC_{0-\infty}$ and C_{max} in elderly female subjects following a single 100-mg dose were ~ 31% and 30% higher, respectively, than in elderly male subjects, whereas the median time to reach C_{max} and GM apparent terminal $t_{1/2}$ were similar. Verubecestat GM C_{max} following single-dose administration in elderly subjects was similar to that previously observed in healthy young males, whereas $AUC_{0-\infty}$ was ~ 31% increased.[17] The differences in apparent drug clearance and apparent volume of distribution in elderly compared with nonelderly populations[20] and in females compared with males[22] may contribute to the minor differences in PK parameters observed herein; however, further investigations are needed to determine whether the observed PK differences are direct effects of age and sex or indirect effects of other factors such as differences in body weight.

The overall PK profile of verubecestat is supportive of once-daily dosing. Specifically, following multiple-dose, once-daily administration of verubecestat to healthy elderly subjects, the effective $t_{1/2}$ ranged from 19–27 hours; the GM apparent terminal $t_{1/2}$ ranged from 23–26 hours. Verubecestat exposure increased with dose in an approximately dose-proportional manner from 30–120 mg. Accumulation in trough concentrations was evident during the first week of once-daily dosing with no consistent trend of changes thereafter, which, together with the observed $t_{1/2}$, is consistent with steady state being achieved by day 7. The GM accumulation ratio day 28/day 1 $AUC_{0-24\ h}$ following 30–120 mg verubecestat once-daily dosing ranged from 1.72–2.20, in agreement with expectations from the GM apparent terminal $t_{1/2}$.

In conclusion, single and multiple oral doses of verubecestat were generally well tolerated in healthy elderly male and female subjects, as indicated by assessment of clinical and laboratory evaluations and AEs, and therefore continued clinical evaluation in this age group is warranted. These data supported the initiation of further studies to robustly test the mechanism by which verubecestat exerts its effects. Verubecestat was subsequently shown to reduce CSF levels of Aβ and sAPPβ following single dose and multiple-dose administration in subjects with AD.[17] Together, these findings provided input into the protocol design and dosing regimen

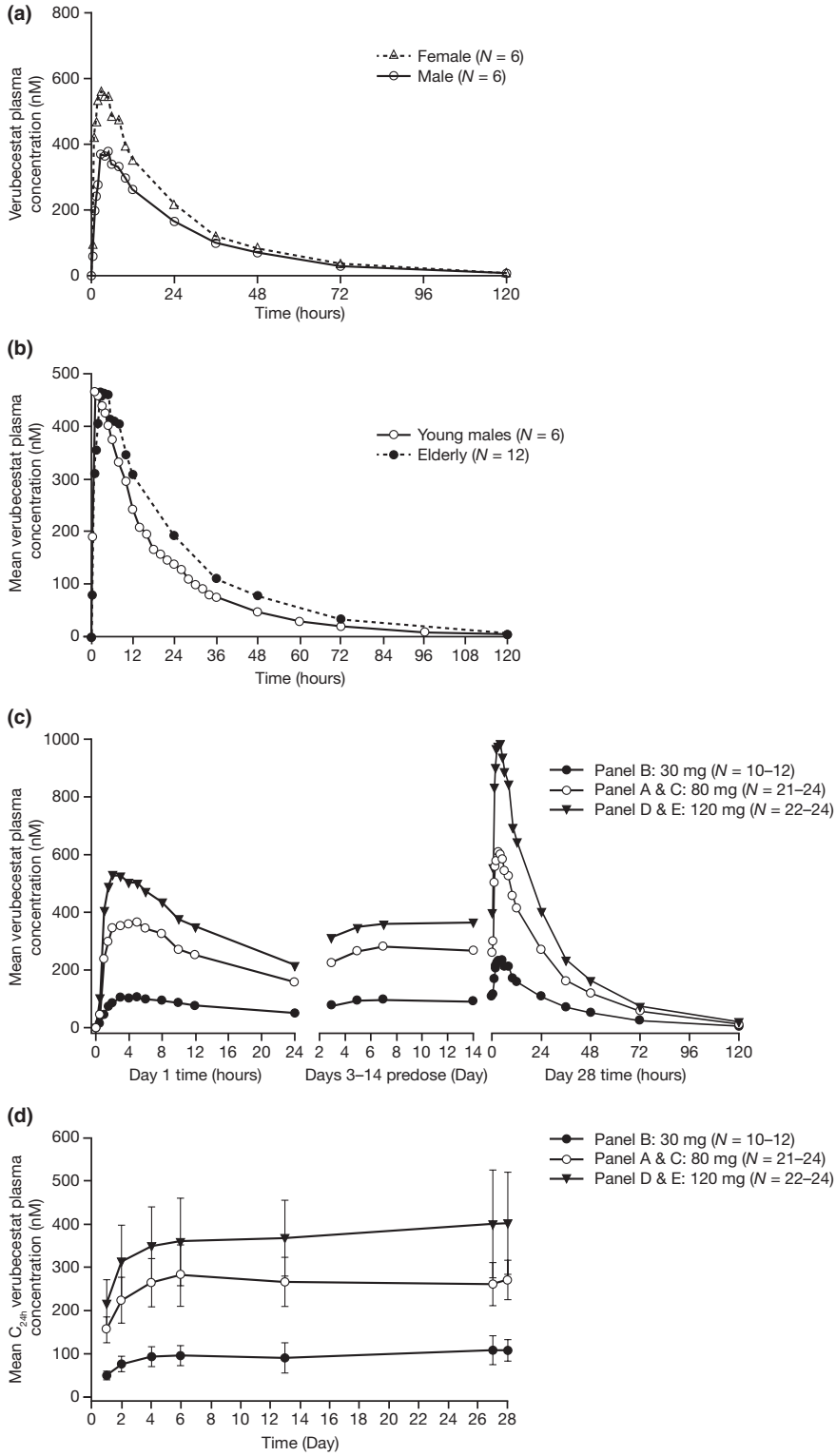

Figure 3 Arithmetic mean verubecestat plasma concentration–time profiles after (**a**) single 100-mg administration in elderly female and male subjects; (**b**) single 100-mg administration in young males and pooled elderly subjects; (**c**) multiple once-daily doses of 30 mg, 80 mg, and 120 mg for 28 days in elderly female and male subjects; and (**d**) multiple-dose, once-daily 30-mg, 80-mg, and 120-mg administration in healthy elderly subjects. Error bars in **d** show ± standard deviation. $C_{24\,h}$, concentration at 24 hours postdose.

Table 4 Summary statistics for plasma pharmacokinetics following multiple-dose administration of verubecestat

		$AUC_{0-24\,h}$, μM·hour		C_{max}, nM		T_{max}, hour		Apparent terminal $t_{1/2}$, hour		Effective $t_{1/2}$, hour	
Verubecestat dose	***N***	**GM**	**95% CI[a]**	**GM**	**95% CI[a]**	**Median**	**Min, max**	**GM**	**%GCV**	**GM**	**%GCV**
First day (day 1)											
30 mg (panel B)	12	1.75	1.55–1.97	113	98.2–130	4.50	3.00, 10.00	—	—	—	—
80 mg (panel A)	12	5.90	5.11–6.81	397	344–459	3.49	1.50, 5.00	—	—	—	—
80 mg (panel C	12	5.89	5.36–6.46	388	353, 426	5.01	1.05, 8.00	—	—	—	—
80 mg (pooled)	24	5.89	5.45–6.37	392	363–425	4.49	1.05, 8.00	—	—	—	—
120 mg (panel D)	12	8.52	7.39–9.82	549	461–654	3.50	1.00, 8.00	—	—	—	—
120 mg (panel E)	12	7.97	6.87–9.26	577	482–692	2.00	1.00, 3.00	—	—	—	—
120 mg (pooled)	24	8.24	7.49–9.07	563	501–633	2.00	1.00, 8.00	—	—	—	—
Last day (day 28)											
30 mg (panel B)	10	3.85	3.24–4.57	237	194–289	3.99	1.50, 5.00	26.36	11.00	27.37	19.03
80 mg (panel A)	10	9.96	8.71–11.4	642	544–758	3.54	1.00, 8.00	23.54	9.04	19.18	30.84
80 mg (panel C)	11	10.2	9.20–11.2	645	588–707	3.02	1.50, 8.00	23.47	9.42	19.68	10.25
80 mg (pooled)	21	10.1	9.35–10.9	643	592–699	3.02	1.00, 8.00	23.51	9.01	19.44	21.76
120 mg (panel D)	12	17.5	14.7–20.9	1110	902–1360	3.00	1.50, 5.03	24.10	12.26	24.82	21.08
120 mg (panel E)	11	13.6	11.6–15.8	932	754–1150	2.00	1.00, 5.02	22.51	9.39	18.84	24.50
120 mg (pooled)	23	15.5	13.7–17.5	1020	886–1170	3.00	1.00, 5.03	23.32	11.28	21.75	26.53
Accumulation (day 28/day 1)											
30 mg (panel B)	10	2.20	2.04–2.39	2.10	1.89–2.33	—	—	—	—	—	—
80 mg (panel A)	10	1.74	1.55–1.97	1.67	1.49–1.88	—	—	—	—	—	—
80 mg (panel C)	11	1.76	1.69–1.82	1.70	1.63–1.78	—	—	—	—	—	—
80 mg (pooled)	21	1.75	1.66–1.85	1.69	1.60–1.78	—	—	—	—	—	—
120 mg (panel D)	12	2.06	1.91–2.22	2.02	1.80–2.25	—	—	—	—	—	—
120 mg (panel E)	11	1.72	1.59–1.87	1.64	1.45–1.85	—	—	—	—	—	—
120 mg (pooled)	23	1.89	1.78–2.01	1.83	1.68–1.99	—	—	—	—	—	—

%GCV, percentage geometric coefficient of variation; $AUC_{0-24\,h}$, area under the concentration–time curve from time zero to 24 hours; CI, confidence interval; C_{max}, maximum concentration; GM, geometric mean; max, maximum; min, minimum; $t_{1/2}$, half-life; T_{max}, time to C_{max}.
[a]90% CI for accumulation ratios.

of the EPOCH study[18] and a phase III study in subjects with prodromal AD (APECS: NCT01953601) and provided insight into higher exposure safety margins for verubecestat.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Supplementary Material.

Table S1. Drug-related TEAEs following multiple-dose administration of verubecestat or placebo based on system organ class. Every subject is counted a single time for each applicable row and column. A system organ class or specific adverse event appears in this table only if its incidence in one or more of the columns was > 0% in one or more treatment groups, after rounding. For panel A, the multiple dose was 80 mg. Placebo is pooled over panels. q.d., once daily; SD, single dose; TEAE, treatment-emergent adverse event.

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Conflict of Interest. J.P., J.A.S., B.W., and M.F.D. are current employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, and may own stock and/or stock options in Merck & Co., Inc., Kenilworth, NJ. M.F., J.T., and M.D.T. were employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, at the time the study was conducted and may own stock and/or stock options in Merck & Co., Inc., Kenilworth, NJ. D.S. and B.W. declared no competing interests for this work.

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Data Accessibility Statement. The data sharing policy of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, including restrictions, is available at http://engagezone.msd.com/

ds_documentation.php. Requests for access to the clinical study data can be submitted through the EngageZone site or via email to dataaccess@merck.com.

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