2. LFAN Synopsis

Clinical Study Report Synopsis: Study H6L-MC-LFAN

Title of Study: Effect of γ-Secretase Inhibition on the Progression of Alzheimer's Disease: LY450139 (Semagacestat) versus Placebo

Number of Investigators: This multicenter study included 178 principal investigators.

Study Centers: This study was conducted at 178 study centers in 19 countries.

Publications Based on the Study: Eric Siemers, David Henley, Karen Sundell, Gopalan Sethuraman, Robert Dean, Kristin Wrobleski, Richard Mohs. 2011. Evaluating semagacestat, a gamma secretase inhibitor, in a Phase III trial. PL-03-02 Alzheimer's & Dementia: The Journal of the Alzheimer's Association Vol. 7, Issue 4, Supplement, Pages S484-S485

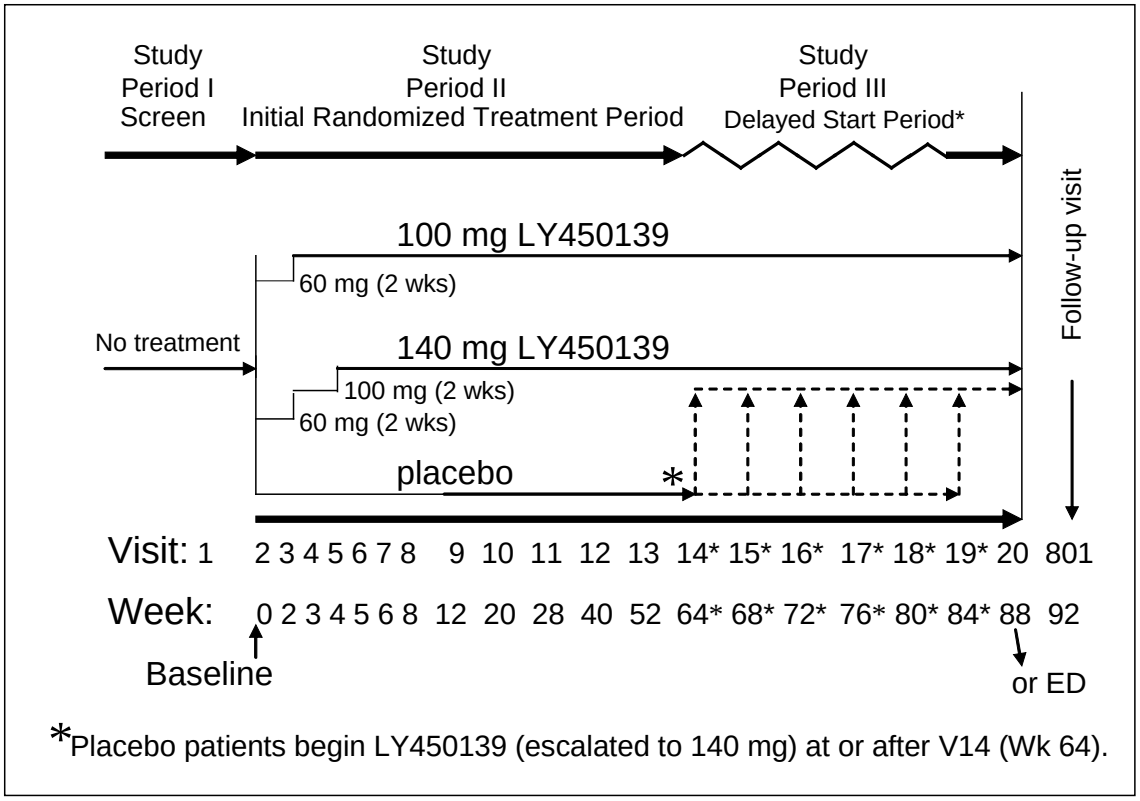
Length of Study:	**Phase of Development:** 3
First patient enrolled (assigned to therapy): 08 April 2008 Date of early study drug dosing cessation: 17 August 2010 Last patient completed: 08 April 2011	

Objectives: Semagacestat has a novel mechanism of action as a functional inhibitor of γ-secretase with the ability to inhibit the synthesis of amyloid-β (Aβ) potentially slowing the underlying rate of disease progression. The primary objective of this study was to test the hypothesis that semagacestat given orally would slow the decline of AD as compared with placebo. The primary objective was assessed using a mixed-model repeated measures (MMRM) analysis of 2 coprimary outcomes, the Alzheimer's Disease Assessment Scale—Cognitive subscore (ADAS-Cog_{11}) and the Alzheimer's Disease Cooperative Study—Activities of Daily Living Inventory (ADCS-ADL), in which the specific hypothesis was that the change at the end of the initial treatment phase for semagacestat would be significantly less than that for placebo. This was evaluated at Week 76 of the 88-week trial, but investigators and patients were blinded to the timing of the primary endpoint. The secondary objectives of the study were: 1) to test the hypothesis that semagacestat is a disease-modifying medication independent of acute symptomatic effects using a "delayed-start" study design in which placebo patients began receiving double-blind semagacestat after 76 weeks in the study. The timing of the delayed start was blinded to investigators and patients; they were informed only that "sometime after 64 weeks," all patients would receive active semagacestat treatment; 2) to provide supporting evidence that semagacestat is a disease-modifying compound by assessment of multiple biomarkers; 3) to compare the safety of semagacestat and placebo; 4) to characterize population pharmacokinetics (PK) of semagacestat, explore potential factors that may have influenced variability of PK, and explore the association of PK variables with efficacy, biomarkers, and safety parameters; 5) to test the hypothesis that semagacestat would slow the rate of decline of AD using extended versions of the ADAS-Cog_{11} and the Mini-Mental State Examination (MMSE) compared with placebo; and, 6) to assess global clinical benefit of treatment with semagacestat.

Patients may have chosen to participate in the assessment of exploratory hypotheses relating to various biomarkers of disease progression.

Study Design: Study H6L-MC-LFAN (LFAN) was a multicenter, randomized, double-blind, placebo-controlled, Phase 3 study comparing semagacestat 140 mg, semagacestat 100 mg, and placebo in approximately 1500 patients with mild to moderate AD. Patients who met entry criteria were randomized in a 1:1:1 ratio (500 per treatment arm) to 1 of the 3 treatment groups: semagacestat 100 mg once daily, semagacestat 140 mg once daily, or placebo once daily. Patients were randomized by site and by mild or moderate Mini-Mental State Examination (MMSE) scores (for the purposes of this study, moderate was defined as scores including 16 through 19, and mild as including 20 through 26). The primary hypothesis being tested was that semagacestat would slow the rate of cognitive and functional decline in AD as compared with placebo. In addition, sometime after 64 weeks of treatment, patients receiving placebo began receiving semagacestat (escalated to 140 mg/day) for the remainder of the study. This "delayed-start" design feature evaluated whether patients originally treated with placebo improved during this

period such that their cognitive scores subsequently approximate those of the patients initially treated with semagacestat. The absence of such an improvement—in other words, maintaining a statistical difference between patients initially assigned to semagacestat and placebo following the delayed start—would have supported the conclusion that semagacestat slowed disease progression and did not affect simply symptoms of disease. Patients and investigators were blinded to the timing of the delayed start of active treatment for the placebo-treated patients. The study design is shown in the figure below.



Abbreviations: ED = early discontinuation; wk = week

Number of Patients:

Planned: 1500; Actual: 1537

Randomized: 501 semagacestat 140 mg, 507 semagacestat 100 mg, 529 placebo

Completed: 189 semagacestat 140 mg, 153 semagacestat 100 mg, 121 placebo

Diagnosis and Main Criteria for Inclusion: Patients must have met National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria for probable AD, had a Modified Hachinski Ischemia Scale score of ≤4, had an MMSE score of 16 through 26, had a Geriatric Depression Scale (GDS) score of ≤6 (on the staff-administered short form), had a magnetic resonance imaging (MRI) or computerized tomography (CT) scan within the past 2 years on file with the investigator with no findings inconsistent with a diagnosis of AD, and been at least 55 years old. Patients must have had adequate literacy (in the investigator's opinion) to complete the protocol-specified questionnaires. If female, must have been post-menopausal, as evidenced by a lack of menstruation for at least 12 consecutive months or by having had a bilateral oophorectomy. Concurrent treatment with cholinesterase inhibitors or memantine was permitted if doses remained stable throughout the study. The full list of inclusion/exclusion criteria may be found in the Site Identification Questionnaire.

Study Drug, Dose, and Mode of Administration:
Semagacestat, taken orally, starting at 60 mg once-daily for 2 weeks and then escalating to 100 mg once daily; patients randomized to 140 mg once daily were escalated again after taking 100 mg once daily for 2 weeks. At any point after Week 4 (Visit 5) of the study (when patients randomized to semagacestat are taking 140 mg once daily or 100 mg once daily), patients were allowed to dose-reduce (from 140 mg/day to 100 mg/day or from 100 mg/day to 60 mg/day) in the case of AEs that appeared to be drug-related and that, in the opinion of the site PI, would not have allowed continued participation in the study. Each patient received 3 tablets per day.

Reference Therapy, Dose, and Mode of Administration: Because the semagacestat dose strengths were a different size and color, a "triple-dummy" design was used, in which patients received placebo tablets with semagacestat tablets to match the strength(s) that was/were not being dosed. Each patient received 3 tablets per day.

Duration of Treatment:
Semagacestat Frequency: 88 weeks
Placebo Frequency: 64 weeks – followed by escalation to semagacestat 140 mg once-daily for 24 weeks

Variables:
Safety: Adverse events were collected at every visit, regardless of relationship to study drug. These events were captured as actual terms and coded to Medical Dictionary for Regulatory Activities (MedDRA) terms. Routine physical, skin and neurological examinations, including fundoscopy, were performed. Vital signs (including temperature) were taken at all visits, with blood pressure and pulse measured supine and standing at designated visits and sitting only at all other visits. Twelve-lead ECGs were obtained in triplicate. Laboratory evaluations, including chemistry, hematology, special hematology, special drug monitoring (for patients taking certain concomitant medications), and urinalysis panels, were collected at regular intervals.
Efficacy: The Alzheimer's Disease Assessment Scale—Cognitive subscale (ADAS- Cog_{11}) and Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL) cognitive testing instruments were used as primary measures in this study. Secondary clinical outcome measures included: The Clinical Dementia Rating Scale (CDR); Neuropsychiatric Inventory (NPI); Resource Utilization in Dementia—Lite questionnaire (RUD-Lite); the EuroQoL-5D (EQ-5D) Proxy; and, Mini-Mental State Examination (MMSE). Plasma samples for assessment of Aβ were collected from all patients. Patients may have chosen to participate in the assessment of additional biomarkers, including FDG-PET, vMRI, amyloid imaging and CSF via study addenda. Information about these assessments is provided in the addenda.
Bioanalytical: Blood samples were collected to measure the concentration of donepezil, study drug, and study drug metabolites (as appropriate).
Pharmacokinetic: All plasma semagacestat concentration-time data was pooled and evaluated by a population PK approach. A covariate screen of patient-specific factors was included in the analyses based on those factors investigated in previous and ongoing PK analyses and those appropriate for the target population. An exploratory analysis to investigate a relationship between plasma semagacestat and the proximal secondary endpoint (plasma Aβ) at Weeks 0, 12, and 52 was also conducted. Other analyses of efficacy and safety outcome measures may also have been assessed as scientifically appropriate and warranted by available data.

Evaluation Methods:
Statistical: The primary objective of this study was to test the hypothesis that semagacestat given orally would slow the decline of AD as compared with placebo. The primary objective was assessed using a mixed-model repeated measures (MMRM) analysis of 2 coprimary outcomes, the Alzheimer's Disease Assessment Scale—Cognitive subscore ($ADAS\text{-}Cog_{11}$) and the Alzheimer's Disease Cooperative Study—Activities of Daily Living Inventory (ADCS-ADL), in which the specific hypothesis is that the change at the end of the initial treatment phase for LY450139 will be significantly less than that for placebo. Change from baseline difference in the MMSE scores was also analyzed using a repeated measures mixed model analysis.
Safety: Safety was assessed by summarizing and analyzing TEAEs, laboratory analytes, vital signs, and ECGs. Unblinded data on safety-related endpoints and SAEs were periodically reviewed by the Data Monitoring Committee (DMC). In addition, for safety reasons, the DMC evaluated ADAS-Cog results after approximately 50% of the patients had at least 12 months of treatment. A blinded Clinical Endpoint Committee (CEC) reviewed and adjudicated specific safety endpoints.

Summary and Conclusions:
Semagacestat is a γ-secretase inhibitor that was being studied in two pivotal Phase 3 clinical trials (LFAN and LFBC) to determine effectiveness in slowing the progression of AD by decreasing the production of suspected neurotoxic species of amyloid beta peptide. During a protocol pre-specified interim analysis for cognitive safety in this study, the external DMC discovered statistically significant dose-dependent cognitive and functional worsening in semagacestat-treated patients compared with placebo-treated patients. Dosing with semagacestat was halted in both Phase 3 trials and the open label extension study and patients were given the option to continue in the respective amended studies to continue to have their cognition, function and traditional safety measures assessed for a period of 7 months after dosing cessation. In LFAN, the cognitive and functional decline of patients initially treated with semagacestat was not different than patients initially treated with placebo in the 7-month Safety Follow-Up Period, suggesting that semagacestat-treated patients did not continue to worsen beyond what was expected for mild to moderate AD patients after dose cessation; however, cognition and function did not return to placebo levels and therefore were not "reversed" during the Safety Follow-Up Period.

From a safety perspective, most of the safety findings in the LFAN study were predicted by earlier toxicology and pre-clinical studies and with Phase 1 healthy subject pharmacology studies and Phase 2 clinical studies in patients with AD. These studies suggested the possibility for QTc prolongation, induction of donepezil metabolism and other drugs metabolized by CYP3A4/5, gastrointestinal effects, rash, hair and skin depigmentation, renal tubular effects, and hepatic enzyme elevations. However, in large part due to the brevity of the earlier clinical studies, AEs that might require longer exposure to manifest were not identified. For example, rates of infection and neoplasms were not elevated with semagacestat treatment in Phase 1 or 2 studies. Also weight loss was not identified as a risk though, again, exposures were not of sufficient length to see such changes.

A detailed description of the potential mechanisms of the safety findings with semagacestat is beyond the scope of this document but several key points are of note. First, there are multiple substrates for gamma secretase and inhibition of cleavage of (and therefore decreased signaling of) one or more of these may account for these findings. At the time semagacestat was discovered, only the transmembrane protein Notch was known to be an alternate substrate for gamma secretase (in addition to APP). There are substantial data now on the effects of Notch signaling and the inhibition thereof. In fact, the literature suggests that inhibiting Notch signaling may lead to altered hematopoiesis (altering numbers of immune cells such as B- and T-lymphocytes and increasing rates of infection) as well as affecting cell differentiation including of melanocyte stem cells into melanocytes and melanoblasts (leading to lack of melanin in hair and skin). Notch signaling is also likely to affect differentiation of GI epithelial cells (leading to goblet cell hyperplasia and diarrhea), increase keratinocyte proliferation (which, when combined with environmentally induced mutations, allows these mutations to accumulate and increases the rate of squamous cell and basal cell carcinoma of the skin), and decrease differentiation of monocytes into dendritic cells (known as Langerhan cells in skin).

While some of the safety findings with semagacestat may be explained by inhibiting Notch cleavage, the mechanisms underlying other safety findings remain unclear. For example, the mechanism for the effect on renal tubular reabsorption that could explain decreases in phosphorus, uric acid, potassium and calcium and glycosuria remains unknown. Also, the mechanism behind the rashes seen with semagacestat is also unclear. Finally, the mechanism behind the increased hepatic enzymes such as ALT, AST and GGT could be an adaptive response of the liver to semagacestat but the etiology of the decreases in bilirubin also remains unknown.

Finally, the mechanism underlying the cognitive and functional worsening seen with semagacestat remains unknown. Several possibilities exist including effects on levels of fragments normally produced by cleavage of APP by gamma secretase such as amyloid precursor protein intracellular domain (AICD) (decreased with γ-secretase inhibition) or the c-terminal 99 amino acid fragment of APP (c99) (increased with γ-secretase inhibition). AICD levels may have involvement in gene transcription, apoptosis, development and cytoskeletal dynamics. Other possible explanations for the cognitive and functional worsening seen with semagacestat treatment include the apparent increase in Aβ1-42/1-40 ratio, inhibition of Notch signaling, and/or inhibition of another substrate of γ-secretase. One other known substrate with data to suggest a role in cognition is EphA4. Gamma secretase is responsible for cleavage of EphA4 to produce intracellular domain (EICD) which is responsible for neuronal synaptic plasticity when environmental stimulus is applied. Inoue and colleagues demonstrated that a γ-secretase inhibitor was associated with decreased EICD and decreased dendritic spine density. Additionally, Bittner and colleagues demonstrated that semagacestat decreased dendritic spine density in wild-type mice but not in APP-deficient mice suggesting that the decrease in dendritic spine density was mediated by APP.

In conclusion, semagacestat was being studied as a potential disease modifying therapy for AD and had a robust biomarker signature leading into Phase 3 development. However, dosing was halted early in this Phase 3 study due to greater rates of cognitive and functional decline in the semagacestat-treated patients compared to placebo-treated patients. Patients were followed to assess reversibility of the worsened cognition and function and of changes in traditional safety measures. While the rates of cognitive and functional decline in semagacestat-treated patients were not different than placebo-treated patients in the 7-month Safety Follow-Up Period, the cognitive and functional scores did not return to placebo levels and therefore were not “reversed.” However, changes in all traditional safety findings were reversed with the exception of a few laboratory changes. Importantly, the rate of skin cancer with previous semagacestat exposure was the same as placebo in the follow-up period suggesting that patients exposed to semagacestat did not have permanently increased risk for skin cancer.