Replication Trial To Confirm Reversal of Cognitive Decline with Bryostatin for Advanced Alzheimer's Patients in the Absence of Memantine

Richard Thompson, PhD[1]; Lee Jen Wei, PhD[2]; Alan J. Tuchman, MD[3]; and Daniel L. Alkon, MD[3]
[1]Johns Hopkins University, Baltimore, MD, USA, [2]Harvard University, Boston, MA, USA, [3]Neurotrope, Inc., New York, NY, USA.

ABSTRACT

Background: Bryostatin, pre-clinically shown to induce synaptogenesis and prevent neuronal death, is being evaluated in a double-blind, placebo-controlled confirmatory trial as a treatment for advanced Alzheimer's disease patients with a dose regimen of 20 µg of bryostatin compared to placebo. 108 subjects were randomized in a 1:1 treatment allocation. Subjects on Namenda (memantine) were excluded. Namenda was previously shown to block sustained cognitive improvement observed in a recently completed Phase II trial. (cf. Farlow et al., 2019 [6]).

Methods: In the present confirmatory trial, each patient received two initial doses of study drug, bryostatin 20 µg or placebo, administered by infusions 1 week apart, followed by doses every 2 weeks; a total of 7 doses over 12 weeks. The primary endpoint is Severe Impairment Battery (SIB) score at 13 weeks versus placebo.

Analysis: In comparing 20 µg bryostatin with placebo, the difference in SIB change from baseline, a positive result would require at least a 4.0 point difference in SIB scores with 83% confidence interval. The initial primary efficacy analysis will use ANCOVA tests with appropriate imputation methods to correct for patient drop-outs. Additional efficacy analyses will be done at weeks 5, 9 and 15. As a secondary analysis, patients in the Mini Mental State Exam, version 2 (MMSE-2) 4-9 and 10-15 stratification groups will be analyzed by group following the same methods used for the primary analysis.

Hypothesis: With repeated measures of SIB over time, the 20 µg bryostatin group should show early benefit starting at week 5, with continued improvement, as previously demonstrated, sustained for the entire study follow-up. Positive results of these new analyses would be consistent with previous analyses that indicated persistence of bryostatin's efficacy, even 30 days after all dosing was completed. Bryostatin, in the absence of memantine, should show efficacy in treating the cognitive decline in advanced Alzheimer's patients.

CONTACT

Daniel L. Alkon, MD.
Neurotrope
Email: dalkon@neurotrope.com

INTRODUCTION

In a number of pre-clinical studies, activators of PKC epsilon, such as the marine macrocyclic lactone, bryostatin, have been shown to increase synaptic numbers via synaptic growth factors such as BDNF, NGF, and IGF.[1,2] These PKC epsilon activators have also shown anti-apoptotic, anti-amyloid and anti-hyperphosphorylated tau, and cognitive enhancement efficacies. Specific enzymatic pathways in pre-clinical studies were demonstrated to mediate such effects.

Electron and confocal microscopy demonstrated bryostatin-enhanced PKCε activity as expressed by the generation of new synaptic connections (synaptogenesis) among hippocampal neuronal networks of cultured rats. In the confocal studies, increased red (pre-synaptic synaptophysin), green (post-synaptic PSD-95) and yellow (Merged = synapse formation) indicated loci of synapse formation. In addition to reducing Aβ and hyperphosphorylated tau, bryostatin induces synaptogenesis, enhances cognition, and prevents neuronal death.

A pharmacokinetic study with Alzheimer's patients demonstrated a peak activation of PKC within one hour of infusion onset, closely associated with a measured rise to peak of bryostatin blood levels.[3,5] Furthermore, compassionate use trials showed marked improvements in AD patients with advanced disease.[5]

METHODS AND MATERIALS

In a previous trial, [6] we conducted a double-blind, randomized, phase 2 trial, in which bryostatin was administered by intravenous infusion to patients with advanced Alzheimer's disease for 12 weeks. Adults aged 55-85 with cognitive deficits present for at least 2 years, MMSE-2 of 4-15, inclusive, were considered eligible for this trial. Patients were randomized equally into the 20 µg bryostatin, 40 µg bryostatin or placebo treatment arms. 264 patients were screened at 27 clinical sites in the United States. Of these, a total of 147 were randomized and 141 were treated with at least one dose of bryostatin. 135 subjects were analyzed as the Full Analysis Set (FAS) based on the modified intention to treat (mITT) principle. 113 of those randomized had 13 week outcome data, and were analyzed at the Completer Analysis Set (CAS). In the current confirmatory trial, there are two treatment arms: 20 µg bryostatin and placebo, randomized 1:1, stratified as in the first trial by disease severity of MMSE-2 4-9 or 10-15, with a final total number of 95 completed patients. Patient participation has recently been completed in the current trial and data is now being finalized for analysis.

In both trials, the primary safety outcome was treatment emergent adverse events (TEAE). The primary efficacy endpoint was the change in Severe Impairment Battery (SIB) scores at 13 weeks from baseline. Secondary SIB assessments at 5, 9, and 15-weeks were also assessed. In the current trial the treatment effect will also be evaluated separately for the two stratification cohorts as secondary efficacy analyses.

In the present trial, the primary endpoint of the change in SIB at 13 weeks from baseline will be analyzed with ANCOVA and appropriate imputation methods. The results will be considered statistically significant at a two-sided alpha level of 0·05 Conservatively, the post-hoc analyses will assess statistical significance with two-sided p-values ≤ 0·05. Based on the results of the previous Phase II trial, it is anticipated that the patients in the present confirmatory trial who received the 20 µg Bryostatin treatment will show a significant sustained improvement in the SIB scores when compared to patients in the Placebo cohort who received only a vehicle infusion.

RESULTS of INITIAL PHASE 2 TRIAL

Safety: Overall, patients in the 20 µg treatment arm previously demonstrated minimal differences in their safety profiles from patients taking placebo. Both groups had similar numbers of TEAEs (28 events in the placebo group versus 30 events in the 20 µg group). In contrast, patients in the 40 µg treatment arm had significantly greater TEAEs (24 patients, with 57 events-51·1% of total) than patients in either of the other treatment arms.

Efficacy: Based on the MMRM model results of the previous trial, patients who received the 20 µg dose, but not patients receiving the 40 µg dose, showed a sustained improvement throughout the trial duration in mean change SIB from baseline as compared to the placebos.

In the previous trial, among the FAS patients, treatment difference between the placebo arm and the 20 µg treatment arm was significant at week 5 at the alpha 0.1 cut point (difference [80% CI] = 2·96 [0·58, 5·34], p=0·056). For this FAS patient sub-set, there were no statistically significant differences between the 20 µg bryostatin treatment arm and the placebo group at either week 9 or week 13. By week 13, those in the 20 µg showed an increase in mean (SD) SIB of 1·16 (1·15) from baseline, while the placebo mean (SD) SIB decreased by -0·79 (1·33) points from baseline during this same time period (SIB difference [80% CI] = 1·94 [-0·31, 4·19] points, p=0·134).

A greater treatment effect on the SIB was seen in the CAS patients among those in 20 µg bryostatin arm (Fig. 2). At week 13, the mean (SD) SIB increased by 1·51 (1·12) points from baseline in the 20 µg arm, while placebos showed a decrease in their mean (SD) SIB scores from baseline of -1·12 (1·39), a difference that was statistical significance at the alpha 0·10 cut-point (SIB difference [80% CI] = 2·63 [0·35, 4·91] points, p=0·070). At week 5, there was a statistically significant change in mean SIB scores from baseline among 20 µg bryostatin patients versus the placebos (SIB difference [80% CI] = 4·00 [1·63, 6·38] points, p=0·016). In Fig. 2, control groups with and without memantine were combined.

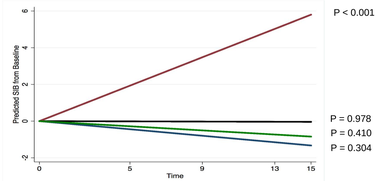
Secondary Analyses: The Statistical Analysis Plan (SAP) contained a secondary analysis to analyze efficacy of bryostatin in patients not receiving concurrent memantine. The 20µg bryostatin arm showed a consistent and sustained benefit of SIB improvement from baseline over the entire trial. In contrast, the placebo patients without memantine showed further decline in cognitive function over time. Among the patients without memantine, the mean SIB change at the average of week 13 and week 15 time points from baseline was statistically greater in the 20 µg bryostatin arm as compared to the placebo arm based on the two sample t-test (SIB difference [95% CI] = 6·1 [1·5, 10·7] points; p = 0·012). This significant difference persisted after controlling for baseline SIB and baseline MMSE-2 strata in an ANCOVA model. Unlike memantine, standard of care Aricept baseline therapy did not affect the 20 µg bryostatin benefit of SIB improvement.

Another secondary analysis used the non-parametric method of Wei and Lachin[4] to simultaneously test for treatment group differences in SIB from baseline at the multiple time points of week 5, 9, and 13. The method of Wei and Lachin gave results that were consistent with those described above (p=0.039 based on the Wilcoxon statistic with equal weighting of outcomes).

A tertiary analysis considered trends of SIB outcomes over time (Fig. 1). MMRM was used in the trend analysis to provide consistency with the analysis of the whole patient sample. No increase in SIB measures over time for the placebo patients was found. In contrast, SIB slopes for the 20 µg bryostatin patients without memantine were highly significant in this analysis, giving a slope (95% CI) = 0·36 (0·08, 0·64) SIB points per week in the random intercept model, and a slope (95% CI) = 0·41 (0·21, 0·61) points per week in the random slope model. The model interaction terms, which indicate a difference in treatment effect by arm, were highly significant in both mixed models considered (p < 0.001).

Sustained positive results from the Wei and Lachin method and the trend analysis support the conclusion of the primary post-hoc efficacy analysis of a superior treatment effect for bryostatin at the 20 µg dose versus placebo.

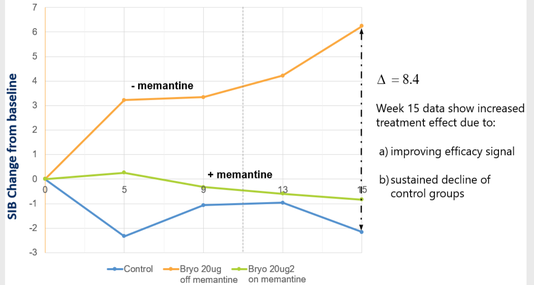
Group SIB Trends Over Time



Red = 20 µg bryostatin, in the absence of memantine
Black = placebo in the absence of memantine
Green = 20 µg bryostatin in the presence of memantine
Blue = placebo in the presence of memantine

MMRM was used in the trend analysis to provide consistency with the analysis of the whole patient sample. Based on the statistical trend analysis, only the 20 µg bryostatin in the absence of memantine group shows a significant positive SIB trend (e.g. SIB improving over time). The treatment-by-time interaction, indicating a difference in treatment effect by arm, was highly significant (p < 0.001). This significant positive trend indicates a treatment effect of bryostatin for this patient group only. These data further indicate that the patients in the memantine-free group improved throughout the 15 week protocol.

SIB BY VISIT: 30 DAYS POST DOSING IN COMPLETERS (CAS) ON AND OFF MEMANTINE



SIB Improvement signals are clear with repeated doses of Bryostatin in the absence of memantine. No such improvement was apparent with SOC memantine.

DISCUSSION

Based on the results of the previous Phase 2 clinical trial, bryostatin in the 20µg arm improved SIB performance as compared to the placebo arm, suggesting a potential utility of this drug to improve cognitive function, and to provide symptomatic relief and delay of cognitive decline of patients with moderately severe to severe AD. Null results of the effect of SIB at the 40 µg dose of bryostatin as compared to placebo suggests a lack of efficacy due to prolonged down regulation of PKC that typically follows higher and/or longer levels of PKC activation.

In addition, the safety profile of exposure to bryostatin was similar between those in 20 µg bryostatin arm and the placebo arm, while those in the 40 µg bryostatin arm presented with more TEAEs as compared to those in the other two treatment arms. Greater side-effects in the 40 µg bryostatin arm also led to more dropouts in this arm as compared to other study participants.

Secondary analyses of SIB over time in the patients without memantine demonstrated a greater improvement effect as compared to the FAS data. The principle targets of bryostatin, PKC isozymes, are known to regulate NMDA receptor functions, which are blocked by memantine. Therefore, it is not surprising that the blockade of the NMDA receptor could offset most if not all of the bryostatin treatment effect.

.

CONCLUSIONS

Previous trials with neurotransmitter agonists and/or antagonists have delayed and/or slowed the rate of cognitive decline in advanced AD patients. A recent A Beta antibody trial with prodromal and early AD patients have also suggested a reduction in the rate of decline, although this was not confirmed in a follow-up trial. In contrast, evidence would suggest that bryostatin safely produces sustained cognitive improvement at the 20 µg dose at least four weeks after the termination of the dosing protocol at week 11. This sustained SIB improvement was more evident in the absence of exposure to memantine. The confirmatory study currently underway will help further examine the efficacy of bryostatin in the absence of memantine to treat and/or reverse disease progression of advanced AD patients.

REFERENCES

1 Sun, MK, Hongpaisan, J, Alkon, DL: Post-ischemic PKC activation rescues retrograde and anterograde long-term memory. *Proc Nat. Acad. Sci. USA* 2009 106 (34): 14676–14680.
2 Sen A, Alkon DL, Nelson TJ Apolipoprotein E3 (ApoE3) but Not ApoE4 Protects against Synaptic Loss through Increased Expression of Protein Kinase Cε. *J Biol Chem* 2012, 287(19), 15947-15948.
3 Sun MK, Nelson TJ, Alkon DL. Towards universal therapeutics for memory disorders. *Trends Pharmacol Sci.* 2015, pii: S0165-6147(15)00076-0. doi: 10.1016/j.tips.2015.04.004.
4 Wei LJ, Lachin JM. Two Sample Asymptotically Distribution Free Tests for Incomplete Multivariate Observations. *J Am Stat Assoc*. 1984 79:653-661.
5 Nelson, TJ, Sun, MK, Lim, C, et al., Bryostatin Effects on Cognitive Function and PKCε in Alzheimer's Phase IIa and Expanded Access Trials, *J Alzheimers Dis*. 2017 58: 521-535.
6 Farlow, M., Thompson,R., Wei, L.J.,Tuchman,A., Genier,E.,Crockford, D.,Wilke,S.,Benison, J., and Alkon, D.L., A Randomized, Double-Blind,Placebo-Controlled, Phase II Study Assessing Safety, Tolerability, and Efficacy of Bryostatin in the Treatment of Moderately Severe to Severe Alzheimer's Disease,*J Alzheimers Dis*. 2019 67 (2): 555-570.