Significant Cognitive Improvement with Bryostatin for Advanced Alzheimer's Patients in the Absence of Memantine

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ABSTRACT

Background: Bryostatin, pre-clinically shown to induce synaptogenesis and prevent neuronal death, was evaluated via a double-blind, placebo-controlled trial with two doses (20 µg and 40 µg) of bryostatin compared to placebo. 147 subjects were randomized in a 1:1:1 treatment allocation. Each patient received initial doses 1 week apart, followed by every 2 weeks, for 11 weeks. The endpoints were severe impairment battery (SIB) scores at 0, 5, 9, 13, and 15 weeks (30 days after the last dose).

Methods: Because PKC epsilon, the target of bryostatin, controls NMDA function that is blocked by memantine, a pre-specified exploratory analysis of the treatment effect of bryostatin for patients stratified by memantine usage, as a baseline therapy, was of particular interest. The initial primary efficacy analysis used a mixed model (MMRM) to handle missing data. However, for the post-hoc analyses, since there were few missing observations for patients not taking memantine, the complex MMRM modeling for analysis was not needed. Moreover, to avoid large potential intra-patient SIB variation over time, for the post-hoc analyses, we considered the change in average score collected during week 13-15 from baseline as the endpoint. The treatment effect estimate was based on the simple, transparent two-sample t-statistic for assessing the between-group-difference.

Results: In comparing 20 µg bryostatin with placebo for patients not taking memantine, the difference in SIB change from baseline was 6.1 points with 95% confidence interval of (1.5,10.7) and p = 0.012, suggesting 20 µg bryostatin was highly significantly better than placebo. On the other hand, there was no treatment effect for memantine-dosed patients. Furthermore, with repeated measures of SIB over time for patients not taking memantine, the 20 µg bryostatin group showed early benefit starting at week 5 and the positive trend was sustained for the entire study follow-up. The results of these new analyses are consistent with, although clearer than, previous analyses that indicated persistence of bryostatin's reversal of cognitive decline even 30 days after all dosing was completed.

Conclusion: Bryostatin, without memantine, shows potential as a modifier of Alzheimer's disease – to be confirmed by additional studies.

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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 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.

Figures 1 and 2 Confocal microscopy demonstrates bryostatin-enhanced PKCε activity as expressed by the generation of new synaptic connections (synaptogenesis) among hippocampal neuronal networks of cultured rats. In these figures, increased red (pre-synaptic synaptophysin), green (post-synaptic PSD-95) and yellow (Merged = synapse formation) indicate 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

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.

A total of 264 patients were screened at 27 clinical sites in the United States. Of these, a total of 147 were randomized and treated with at least one dose of bryostatin. A total of 141 subjects were analyzed as the Full Analysis Set (FAS) based on the modified intention to treat (mITT) principle. A total of 113 (76·9% of those randomized) had 13 week outcome data, and were analyzed at the Completer Analysis Set (CAS).

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 considered.

The primary endpoint of the change in SIB at 13 weeks from baseline was analyzed using the Mixed Model for Repeated Measures (MMRM). The results were considered statistically significant at a one-sided alpha level of 0·10. In contrast, the treatment effect estimate was based on the two-sample t-statistic for group difference in the post-hoc analyses that considered patients with and without memantine – as specified in an exploratory analysis of the Statistical Analysis Plan. However, since there were few missing observations for patients without memantine, the complex MMRM modeling for analysis was not needed. Moreover, to avoid large potential intra-patient SIB variation over time, for the present analysis, we considered the change in average score collected during week 13-15 from baseline as the endpoint. More conservatively, the post-hoc analyses assessed statistical significance with two-sided p-values ≤ 0·05.

RESULTS

Safety: Overall, patients in the 20 µg treatment arm 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, 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.

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. 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).

Cultured Rat Hippocampal Neuronal Networks

Red = Presynaptic staining (Synaptophysin) **Green** = Postsynaptic staining (PSD-95)
Yellow (Yellow) = Merged, Synaptic Formation

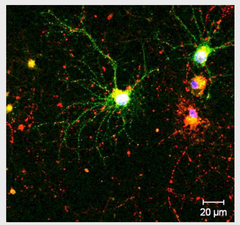

Figure 1. Treated with Aβ Oligomers.

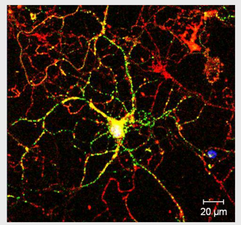

Figure 2. Treated with Bryostatin & Aβ Oligomers.

RESULTS

Post-hoc Analyses: Memantine patients not receiving concurrent memantine (without memantine) in the 20 µg 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; see **Figure 3**). This significant difference persisted after controlling for baseline SIB and baseline MMSE-2 strata in an ANCOVA model.

A 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. 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.

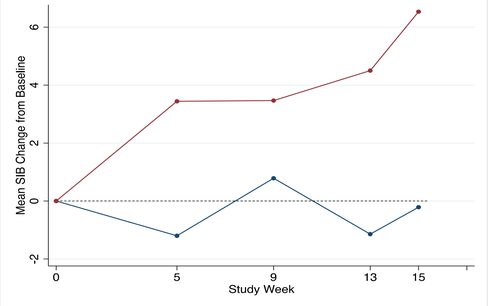

Figure 3. Mean SIB Changes (Unadjusted) from Baseline for the FAS. Without Memantine Patients for the Placebo arm (Blue), and the 20 µg treatment arm (Red).

DISCUSSION

Based on the results of this Phase 2 clinical trial, bryostatin in the 20 µg arm reversed SIB decline as compared to the placebo arm, suggesting a potential utility of this drug to improve cognitive function, and not only 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.

Post-hoc analyses of SIB over time in the patients without memantine demonstrated an even greater improvement effect as compared to the original data sample. 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. In contrast, evidence presented here suggests that **bryostatin safely produces sustained *reversal* of cognitive decline** 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 pronounced in the absence of exposure to memantine. A confirmatory study is currently underway to further establish the efficacy of bryostatin, in the absence of memantine, to treat and/or reverse disease progression of advanced AD patients.

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