The Alzheimer's Disease THErapy with NEuroaid II (ATHENE) Study: A Double-Blind Randomized Delayed-Start Trial to Assess the Safety and Efficacy of MLC901 (NeuroAiD™II) as an add-on Treatment to Cholinesterase Inhibitors or Memantine in Patients With Mild to Moderate Alzheimer's Disease

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Research

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Abstract

Background

Preclinical and clinical studies indicate a role for MLC901 (NeuroAiD^{TM}II) in Alzheimer's Disease (AD). We investigated its safety and efficacy as add-on therapy to standard treatment and evaluated a disease modifying effect in mild to moderate AD.

Methods

Mild-moderate probable AD patients by NINCDS-ADRDA criteria, stable on acetylcholinesterase inhibitors or memantine (n=125) were randomized to receive MLC901 (early starters) or placebo (delayed starters) for 6 months, followed by a further 6 months during which all patients received MLC901, in a delayed-start design. The primary outcome measure was serious adverse events at 6 months, secondary outcomes included the Alzheimer's Disease Assessment Scale - Cognitive subscale (ADAS-Cog) and other cognitive assessment scales.

Results

There was no significant difference in the risk of serious adverse events between early and delayed starters at month (M) 6 (22.6% vs. 27.0%, risk difference = -4.4%, 90% CI -16.9 to 8.3%). Furthermore, there was no significant difference in the risk of adverse events, including the occurrence of stroke or vascular events, between early and delayed starters throughout the 12-month study period. The early-starters differed significantly on ADAS-Cog from the delayed-starters at M9 (mean difference -3.36, 95% CI -5.64 to -1.09) and M12 (mean difference -2.35, 95% CI -5.45 to 0.74). Other cognitive assessment scales showed trends in favor of MLC901.

Conclusions

MLC901 is a safe adjunct to standard treatment for mild-moderate AD. There is no indication that the risk of any adverse events, including vascular, is increased with MLC901 in the study population. The cognitive outcomes provide support for a disease-modifying effect of MLC901 which requires confirmation in further studies.

Clinical trial registration: ClinicalTrials.gov, NCT03038035.
https://clinicaltrials.gov/ct2/show/NCT03038035

Background

Alzheimer's disease (AD) and other dementias cause a heavy economic and public healthcare burden worldwide [1-2]. Current approved treatment for AD comprises of acetylcholinesterase inhibitors (AChEIs) and the N-methyl-D-aspartate receptor antagonist memantine [3-4]. Higher doses are often required to achieve a stable effect leading to tolerability and compliance issues. Furthermore, these treatments are

symptomatic and do not alter the clinical course or appear to affect disease progression. Combining symptomatic treatments with disease-modifying therapies may be the optimal strategy for AD [5-8]. It is hypothesized that targeting either or both of the pathological hallmarks of AD, intracellular hyper-phosphorylated tau in neurofibrillary tangles (NFT) and extracellular b-amyloid plaques, may delay or halt progression of disease. While remarkable efforts have been made, no disease-modifying treatments for AD have been approved. Hence there is a need for further clinical trials with innovative and novel treatments that meet these needs.

MLC601 (NeuroAiD^{TM}) contains 9 herbal and 5 non-herbal components having neuroprotective properties in cellular and animal models of brain injury [9]. The second-generation simplified formulation, MLC901 (NeuroAiD^{TM}II) contains only the extracts from the 9 herbal components. Both MLC901 and MLC601 have similar pharmacological properties in pre-clinical models of brain injury, inducing neurogenesis and neuroproliferation in rodents and human stem cell cultures, promoting cell proliferation, neurite outgrowth and helping in the development of dense axonal and dendritic networks [9-12]. MLC601 modulated amyloid precursor protein (APP) processing towards a non-amyloidogenic pathway in human neuroblastoma cell cultures [13]. In addition, some of components of MLC601/901, specifically, *Radix Salviae miltiorrhizae*, *Radix Paeoniae rubra*, *Carthamus tinctorius*, *Radix Angelicae sinensis*, *Radix Astragali*, *Rhizoma chuanxiong* have been shown to ameliorate critical toxic events within the amyloid cascade [13]. Furthermore, MLC901 reduced tau phosphorylation at epitopes associated with NFT formation in stably transfected SH-SY5Y cells harboring the P301S mutation [14]. MLC901 showed positive effects on cognitive tasks in mice by promoting hippocampal neurogenesis, neuronal proliferation, differentiation and survival of young neurons contributing to its pro-cognitive effects [15]. Moreover, MLC901 was shown to activate ATP-dependent potassium channels (K_{ATP}) and to modulate neuro-inflammation [16 - 17]. Clinically, MLC601 as monotherapy showed better tolerability and comparable efficacy to AChEIs in patients with mild to moderate AD, vascular dementia and mild cognitive impairment [18 – 22]. Moreover, a recently published study suggested that MLC901 may be beneficial in vascular cognitive impairment no dementia (VCIND) patients with existing impairment in cognitive function [23].

The primary aim of this study was to investigate the safety of MLC901 as an add-on therapy in mild to moderate AD patients on stable standard symptomatic treatment and the secondary aim was to evaluate the efficacy of MLC901 as measured by ADAS-Cog and other cognitive assessment scales: Alzheimer's Disease Cooperative Study - Clinical Global Impression of Change (ADCS-CGIC), Alzheimer's Disease Cooperative Study - Activities of Daily Living (ADCS-ADL), Neuropsychiatric Inventory (NPI), and Mini Mental State Examination (MMSE) at M3, M6, M9 and M12.

Methods

Study Design

A delayed-start study design may be used to evaluate a treatment that slows the progression of disease by modifying the underlying pathology, rather than by attenuating symptoms. The delayed-start design consists of a double-blind, placebo-controlled phase, followed by a period when all patients on placebo (delayed start) as well as those on active treatment (early start) initially, are on active treatment for the rest of the study whilst blinded to the initial treatment allocation.

Hence, the Alzheimer's disease THErapy with NEuroaid (ATHENE) study randomized patients to be (a) early starters: MLC901 from 0-12 months, or (b) delayed starters: placebo from 0-6 months, and MLC901 from 6-12 months, resulting in two treatment periods: a double-blind placebo-controlled phase from 0-6 months, followed by an early vs delayed-start phase during which all patients received MLC901 from 6-12 months. During the entire study period of 12 months, study personnel and patients were blinded to each patient's allocation as an early or delayed starter. The trial protocol is registered in ClinicalTrials.gov (NCT03038035) and has been published [24]. Briefly:

Primary Objective:

1. To test the hypothesis that the proportion of patients experiencing serious adverse events (SAEs) within the first 6 months after randomization among patients on standard treatment, will be no larger in those who receive MLC901 than in patients receiving placebo.

Secondary Objectives:

2. To test the following hypotheses: that (a) add-on MLC901 will show no increase in occurrence of any adverse event (AE) or discontinuation of treatment during 6 months of usage in patients with AD on standard treatment, (b) add-on MLC901 will be superior to standard treatments alone in cognitive change from baseline to M6 as measured by Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-Cog) and other cognitive assessments, (c) add-on MLC901 will show long-term safety, with no increase in occurrence of SAEs and AEs, during 1 year of usage in patients with AD on standard treatment, and (d) early starters will show less disease progression on cognitive assessments compared with delayed starters.

Data from the first 6-month placebo-controlled phase of the study was used to test hypotheses 1, 2a and 2b while data from the 12-months study period was used to test hypotheses 2c and 2d.

The ADAS-Cog is often used in clinical trials because it can measure incremental improvements or declines in cognitive function. The ADAS-Cog is a reliable, valid and responsive measure used in clinical trials across the Asian region and a 3-point ADAS-Cog change was shown to be an appropriate Minimal Clinically Relevant Change (MCRC) in patients with AD [25 - 27].

Study Participants

Patient eligibility was based on the following criteria. Inclusion criteria were male or female patients, aged ≥50 years old, diagnosed with probable AD according to the National Institute of Neurological and

Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) criteria, Mini–Mental State Examination (MMSE) score of 8 to 26, receiving AChEI or memantine or both during the prior 4 months and on a recommended stable dose defined in the study protocol for the prior 2 months [24]. Exclusion criteria were intake of any investigational product within 60 days or 5 half-lives prior to study entry, whichever was longer, and presence of any serious medical or psychiatric condition which might jeopardize the patient by participation in the study or may hamper ability to perform and complete study procedures.

Study Treatment and Blinding

MLC901 was provided in capsule form containing 400 mg of dry extracts from nine herbs (*Radix Astragali*, *Radix Salviae miltiorrhizae*, *Radix Paeoniae rubra*, *Rhizoma chuanxiong*, *Radix Angelicae sinensis*, *Carthamus tinctorius*, *Prunus persica*, *Radix polygalae*, and *Rhizoma Acori tatarinowii*). Matching placebo capsules contained dextrin, turmeric, carmine, and caramel and had the same appearance as the active treatment. Both MLC901 and placebo were provided by Moleac Pte Ltd.

The detailed protocol was approved by the independent institutional review board (IRB), namely NHG (National Healthcare Group) Domain Specific Review Board (DSRB), Singapore, and the study was performed according to the Good Clinical Practice guidelines and the Declaration of Helsinki. After obtaining informed consent, patients were randomly assigned to either MLC901 (400 mg capsules) 2 capsules 3 times a day or matching placebo for 6 months in a double-blind manner.

All patients who completed the first 6 months of the study were offered the opportunity to continue treatment (MLC901 400 mg capsules, 2 capsules 3 times a day) for 6 additional months (with placebo patients switching to MLC901). Standard treatment for AD were continued according to the treating physician's judgement. Investigators and patients/caregivers remained blinded to initial treatment allocation, i.e., early or delayed starters.

Assessments and Outcomes

Patients were assessed at baseline, M1 (±7 days), M3 (±14 days), M6 (±14 days) during the first 6 months (placebo-controlled phase), and M7 (±7 days), M9 (± 14 days), and M12 (±14 days) during the next 6 months (early vs delayed-start phase). M1 and M7 assessments were performed by telephone calls, while other visits were in-person.

At baseline, data were collected on demography, medical history, and concomitant medications. Occurrence of any AE/SAE, dose of standard treatment for AD (AChEI, memantine) and compliance to study treatment were ascertained at all timepoints. Vital signs and physical examination were performed at baseline, M3, M6, M9 and M12. Safety laboratory investigations and ECGs were performed at M6 and M12. For the efficacy endpoints, ADAS-Cog, Alzheimer's Disease Cooperative Study - Clinician's Global Impression of Change (ADCS-CGIC), Alzheimer's Disease Cooperative Study - Activities of Daily Living

Scale (ADCS-ADL), Neuropsychiatric Inventory (NPI), and MMSE were performed at baseline, M3, M6, M9 and M12.

Statistics

Sample Size Calculation

Based on data from earlier trials, it was assumed that the proportion of patients stable on standard AD treatment experiencing SAEs was 5%. To achieve a power of 80% and a 5% type I error, 118 subjects (59 per group) were required to conclude non-inferiority of MLC901 against placebo in the proportion of subjects experiencing SAEs at a non-inferiority margin of 10%. The sample size also provided >80% power to detect a difference of 3 points (28 versus 25) on the ADAS-Cog between early and delayed starters using a standard deviation of 6 at a 5% level of significance. Hence it was planned to recruit 150 subjects to allow for a 20% dropout rate.

Statistical Analyses

The safety analyses were performed on the “as-treated” population which consisted of all patients with documented intake of at least one dose of MLC901 or placebo. The primary endpoint was the proportion of patients experiencing SAEs within the first 6 months of treatment (double-blind placebo-controlled phase). We calculated the differences in proportions of patients who experienced an outcome between early and delayed starters with their 90% confidence intervals (CI) using the method of Miettinen and Nurminen [28, 29] and compared them to a pre-specified non-inferiority margin of 10%. We assessed secondary safety endpoints by comparing SAEs and AEs in groups of comparable treatment duration: (a) delayed starters (6-12 months) vs. delayed starters (0-6 months), (b) early starters (6-12 months) vs. delayed starters (0-6 months), (c) early starters (0-12 months) vs. delayed starters (0-12 months), where time in the brackets indicates the time period of data for comparisons.

The efficacy endpoints (ADAS-Cog, ADCS-CGIC, ADCS-ADL, NPI, MMSE) were analyzed based on the intention-to-treat (ITT) population. Missing data were imputed using the last observation carried forward (LOCF) method. We compared the mean difference (MD) of change from baseline in ADAS-Cog and the other cognitive tests between early and delayed starters at the specified time points using a two-sample t-test with a two-sided significance level of 5%. The 11-item ADAS-Cog was categorized into cognitive domains of memory, praxis and language [25]. Additionally, a per-protocol (PP) analysis that included patients having ≥70% treatment compliance and without major protocol deviations was performed, as well as sensitivity analyses using the ITT population without LOCF and after adjustment for potential confounders. As AD is a progressive neurodegenerative condition, the ADCS-CGIC between early and delayed starters was compared at different time points using the Mann-Whitney U test and the proportions of patients with improvement/no change versus deterioration by the Chi-squared test.

We used SAS® (version 9.4) in performing all analyses.

Trial Oversight

An independent Data Safety Monitoring Board (DSMB) assessed the blinded safety data and monitored the progress of the study at completion of 12-month follow-ups of 30, 60 and 90 patients. On each occasion the DSMB advised the study team to continue the study as planned. Towards the end of the study, based upon safety data available, the IRB expressed concerns related to high rates of stroke and vascular events (VE) in the study, and as a measure of precaution, decided that the study be discontinued and that remaining patients be taken off study treatment. Therefore, in compliance with this decision, the 14 study patients still participating to the trial at that date, all in the 6-12 month delayed-start phase, were discontinued from study treatment and from further study follow-up (except for reporting of any stroke or VE experienced up to 12 months following start of study medication). However, at the end of the study and after full analysis of the complete dataset, it was concluded that the risk of stroke and VE was not higher in MLC901 treated patients as shown below.

Results

From December 2016 to November 2018, 136 patients with AD were screened and 125 were found eligible and randomized (MLC901 n=62, placebo n=63). The study design diagram and flow of patients in the study are presented in Figure 1.

Baseline Characteristics

The mean age of the overall study population was 78.6 ± 6.7 years with 87 (69.6%) women. Ninety (72%) had hypertension, 87 (69.6%) hyperlipidemia, 45 (36%) diabetes mellitus, and 23 (18.4%) a history of previous stroke. Standard AD treatments were: Donepezil 101 (80.8%), Rivastigmine 18 (14.4%), Galantamine none (0%) and Memantine 9 (7.2%). There was higher educational attainment in the delayed starter group (p=0.003) (Table 1). More patients in early starter group had vascular risk factors at baseline. (Table 1). The standard treatment for AD at baseline and throughout the study were similar between groups.

Safety

The number of study capsules consumed and number of dosing days were similar between early and delayed starters. Treatment adherence rate was 76% in both groups.

The numbers of events and patients experiencing SAEs and AEs by study phase are summarized in Table 2 and by system organ classification in Supplement Table A. In the placebo-controlled phase (0-6 months) of the study, 14 (22.6%) patients on MLC901 experienced 22 SAEs while 17 (27%) on placebo experienced 19 SAEs with a risk difference (RD) of -4.4% and a 90% confidence interval (CI) of -16.9 to 8.3%. The upper limit of the CI range is less than the pre-specified non-inferiority margin of 10% (Figure 2). None of the SAEs were considered related to study treatment. Five deaths were reported in the placebo-controlled phase, 2 were on MLC901 (both from pneumonia) and 3 on placebo (from pneumonia, lung cancer, and ruptured aortic dissection). Similarly, in the placebo-controlled phase, 38 (61.3%) patients on MLC901 experienced 79 AEs compared to 38 (60.3%) patients on placebo who experienced

67 AEs. One (1.6%) patient on MLC901 and none on placebo had AE that led to discontinuation of study medication (RD 1.6%; 95% CI -4.3 to 8.6%).

Among early starters, the proportions of patients experiencing SAE and any AE reduced in the delayed-start phase (6-12 months) compared to the placebo-controlled phase (0-6 months). Figure 2 shows comparable risks of SAE, any AE, and major VE for within-group and between-group comparisons. Two other deaths occurred in the delayed-start phase of the study (due to myocardial infarction and pneumonia). Overall, the 6 AEs in 5 (8.1%) patients considered possibly related to MLC901 were all gastrointestinal (i.e., nausea and giddiness, vomiting, acute gastroenteritis, diarrhea, constipation), non-serious, and resolved spontaneously or after interruption of the study drug.

There were no significant changes from baseline or between-group differences in vital signs, ECG, hematology and biochemistry laboratory parameters over the whole duration of the study (data not shown).

An analysis was performed to address the concern of the IRB regarding major VE in the study. In this analysis, major VE was defined as any stroke, myocardial infarction, aortic aneurysm, hypertensive urgency and/or death due to vascular cause. Overall, 7 patients in the study experienced a major VE (5 ischemic strokes, 1 myocardial infarction, 1 ruptured thoracic aortic aneurysm). As the total duration of exposure to MLC901 (82.0 person-years) was much longer than placebo (28.6 person-years), we compared the incidence rate of major VE between MLC901 and placebo. The calculated incidence rate of major VE was 6.1 (95% CI 2.3, 13.3) per 100 person-years of exposure to MLC901 compared to 7.0 (95% CI 1.4, 22.3) per 100 person-years for placebo. For stroke alone, the incidence rates were 4.9 (95% CI: 1.6, 11.6) and 3.5 (95% CI: 0.3, 16.2) per 100 person-years for MLC901 and placebo, respectively. All 7 patients had combinations of two or more vascular risk factors, including hypertension, hyperlipidemia, diabetes mellitus type 2, chronic kidney disease stage 3, sick sinus syndrome and atrial fibrillation. Two patients with atrial fibrillation were not anticoagulated due to clinical contraindications. Evidence of VE evaluated by CT/MRI brain and CT angiogram showed vascular occlusion or multiple acute strokes in patients who experienced ischemic strokes. Blinded assessment performed for each event showed underlying causes considered plausible reasons for the occurrence of stroke or VE. No increase in the risk of major VE in early starters compared to delayed starters was found at any phase of the study (Table 2 and Figure 2): the early starters had a total of 4 major VE, 2 in the first 6 months and 2 in the second 6 months whilst the delayed starters had 3 major VE, 2 in the first 6 months whilst on placebo and 1 in the second 6 months.

Efficacy

ADAS-Cog

Among early starters, ITT analysis showed that the mean ADAS-Cog score gradually improved over time and peaked at M9 (Figure 3A). By contrast, delayed starters showed progressive decline in mean ADAS-

Cog scores, with a more rapid decline observed at M9 and M12. A divergence in mean change in scores started at M3 which became significant at M9 (MD -3.36, 95% CI -5.64, -1.09; p = 0.01).

PP analysis showed similar results with differences between early and delayed starters at different time points reaching significance at M9 (p = 0.01) and at M12 (p = 0.03) (Figure 3B). Further sensitivity analyses using the ITT population without LOCF as well as after adjusting for potential confounding factors, showed similar patterns of the differences over time between early and delayed starters (Supplement Table B).

ADCS-CGIC

At 3 months, 11.7% of patients improved on ADCS-CGIC, 63.3% had no change, and 25% deteriorated in early starters compared to 5%, 55% and 40%, respectively, in delayed starters (p= 0.044) (Figure 4). These percentages were 8.5%, 59.3% and 32.2%, respectively, in early starters compared to 10.0%, 41.7% and 48.4% in delayed starters(p=0.121) at 6 months. The between-group difference in the proportion of patients who improved or had no change was 15.0% (95% CI -1.7, 30.6) at M3 and 16.1% (95% CI -1.4, 32.4) at M6 in favor of MLC901, however, the difference was lost by M9 and M12.

ADCS-ADL, NPI, MMSE

While the mean differences in change from baseline ADCS-ADL, NPI and MMSE scores did not reach statistical significance at a level of 0.05, they were generally in favor of MLC901 (Supplement Table C). In particular, the trajectory patterns of mean change from baseline on MMSE paralleled that observed on ADAS-Cog.

Discussion

In this study, MLC901 was shown to be safe as an add-on treatment to standard therapy in patients with mild to moderate AD. While ATHENE is the first trial to combine MLC901 with standard treatment in AD, previous randomized controlled trials of MLC901 and its precursor, MLC601, in stroke and traumatic brain injury demonstrated a similar safety profile [27 - 32]. Of note, a 4-year safety study with MLC601 confirmed these results in AD [19]. When used as monotherapy in patients with AD, MLC601 was shown to be associated with lower AEs than AChEIs [18 – 20]. Nevertheless, it is important to assess the safety and potential interaction of MLC901 taken concomitantly with standard AD treatments, which themselves may have significant side effects. Moreover, a delayed-start study design allowed more patient-years of exposure data to the study drug [36]. The results of ATHENE, thus, contributes further to the long-term safety data of MLC901, particularly in AD patients who were stable on their regular doses of AChEI or memantine, showing that MLC901 is safe and well tolerated up to at least 12 months of intake with very few AEs leading to discontinuation of treatment. As in other studies, the most common AEs considered possibly related to MLC901 were gastrointestinal, none of which were considered serious.

After reviewing all VEs reported as SAEs, the IRB made the decision to terminate the study due to concern regarding the rate of stroke and major VE that occurred in the study. Subsequently, a formal analysis was performed which showed that the observed rate of stroke and other VEs in ATHENE was no more than expected in an AD population. Additionally, no significant difference in the risk of VE between MLC901 and placebo was observed despite an imbalance of vascular risk factors at baseline. Many of the study patients have vascular risk factors for stroke and other VE at baseline which is in line with studies showing that patients with AD have more vascular risk factors than non-demented controls [37, 38]. Furthermore, patients with AD dementia have higher rates of stroke compared to non-AD dementia patients even after adjusting for risk factors [39]. The incidence of ischemic stroke in AD cases and non-AD controls has been shown to be 37.8 and 23.2 per 1,000 person-years, respectively [39, 40]. By comparison, the ATHENE study had about 40 ischemic strokes for every 1000 person-years. Hence, there was no evidence that MLC901 increased the risk of stroke and VE in this study population.

The ATHENE study allowed assessment of the potential benefit of adding MLC901 as long-term therapy in patients already on standard treatment for AD. The placebo-controlled phase in the first 6 months of the study showed a gradual divergence of mean ADAS-Cog scores between early and delayed starters at M3 and M6 after initiation of treatment in favor of MLC901. Although the differences did not reach statistical significance, they may suggest a delayed treatment effect. It is noteworthy that patients entered the study only after being on standard AD treatment for at least 4 months and on a stable dose in the last 2 months. They may be at or near the peak of their response to standard AD treatment by the time of inclusion [41 - 43], leaving less room to exhibit an additive effect. This is supported by the observation that the mean ADAS-Cog score in the placebo-treated patients started to decline from M3 onwards. Additionally, more patients in the MLC901-treated group were deemed stable or improved (based on ADCS-CGIC) at M3 and M6.

In addition to allowing more person-years of safety observation of MLC901, the study had a randomized delayed-start design [44 - 46] which allowed for the opportunity to observe possible disease modifying effects. From M6, the MLC901 “early starters” continued to improve on mean ADAS-Cog score at M9 before declining at M12. The “delayed starters”, on the other hand, exhibited an earlier and more rapid decline at months 9 and 12. The difference between treatment arms did not show a clear trajectory of approaching each other, suggesting either a prolonged symptomatic effect of MLC901 or slowing of disease progression by early treatment with MLC901. The effect was more apparent among patients who were compliant to the study medication and study completers as shown in the PP analysis.

These clinical findings are relevant and consistent with the reported preclinical pharmacological properties and clinical studies of MLC901 and MLC601 in AD. Using a model of human neuroblastoma cell line SH-SY5Y, MLC601 was shown to significantly increase the level of soluble APPα secreted into the media and decrease the level of full-length APP, implying a modulatory effect on APP processing towards a non-amyloidogenic pathway [13]. In experiments on stably transfected cell culture model expressing tau harboring the P301S mutation, MLC901 reduced tau phosphorylation at epitopes recognized by the AT8, AT270 and PHF-13 antibodies, induced serine9 phosphorylation of glycogen synthase kinase 3β, and

decreased the activation of cyclin-dependent kinase 5 [14]. Apart from β-amyloid and neurofibrillary tangles, the roles of K_{ATP} channels and neuroinflammation in the pathogenetic processes have been elucidated [47 - 50]. In preclinical studies, MLC901 was demonstrated to activate K_{ATP} channels, modulate inflammation processes and protect against glutamate-induced cell death in *in vitro* and *in vivo* studies [16, 17, 51].

STRENGTHS AND LIMITATIONS

The main strength of this study is the randomized, double-blind, placebo-controlled trial design followed by delayed-start phase. The target recruitment was achieved and the vast majority (95%) of the subjects in the double-blind phase of the study opted to participate in the delayed-start extension phase. Attrition due to withdrawal of consent and to follow-up was minimal.

However, we acknowledge some potential limitations in this study. The primary analysis on safety was achieved as planned, and efficacy results were supported by consistency in sensitivity analyses. Each phase of the study was planned to last only 6 months primarily to investigate safety. However, AD, being a chronic progressive disease, may require study follow ups of more than a year to sufficiently assess the full effects of disease modifying treatments. The diagnosis of AD as study entry requirements was not based on neuroimaging or cerebrospinal fluid biomarkers of amyloid burden, hence not all study subjects may have AD pathology. Future clinical trials of MLC901 in AD are warranted and these studies should utilize AD biomarkers, larger sample sizes, longer follow-up and sensitive assessments to detect disease modification such as the CDR (Clinical Dementia Rating) - sum of boxes.

Conclusions

The ATHENE study showed MLC901 to be a safe adjunct to standard treatment with AChEI or memantine. The results are encouraging and together with the body of evidence available from the preclinical studies suggests a potential disease-modifying effect of MLC901 by slowing disease progression. A larger and longer study using biomarkers for AD is needed for confirmation.

Abbreviations

AD: Alzheimer’s Disease; AChEIs: Acetylcholinesterase Inhibitors; ADAS-Cog: Alzheimer's Disease Assessment Scale - Cognitive subscale; ADCS-ADL: Alzheimer’s Disease Cooperative Study - Activities of Daily Living Scale; ADCS-CGIC: Alzheimer’s Disease Cooperative Study - Clinician's Global Impression of Change; ADRDA: Alzheimer’s Disease and Related Disorders Association; APP: Amyloid Precursor Protein; ATHENE: Alzheimer’s disease THErapy with NEuroaid; CDR: Clinical Dementia Rating; CI: Confidence Interval; DSMB: Data Safety Monitoring Board; DSRB: Domain Specific Review Board; IRB: Institutional Review Board; ITT: Intention-To-Treat; LOCF: Last Observation Carried Forward; MD: Mean Difference; MMSE: Mini-Mental State Examination; NFT: Neurofibrillary tangles; NHG: National Healthcare Group; NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke and

the Alzheimer's Disease and Related Disorders Association; NPI: Neuropsychiatric Inventory; PP: Per-Protocol; RD: Risk Difference; SAE: Serious Adverse Events; VE: Vascular Events

Declarations

Ethics approval and consent to participate

The study was conducted according to the principles of the International Conference on Harmonization - Good Clinical Practice. Before study initiation, Institutional Review Board (IRB) approval was obtained from the NHG (National Healthcare Group) Domain Specific Review Board (DSRB), Singapore. All subjects or their legally authorized representatives provided written informed consent for participation in the study. The ATHENE study was registered at ClinicalTrials.gov (NCT03038035).

Consent for publication

Not applicable.

Availability of data and materials

Individual de-identified participant data used in the analysis are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' details and contributions

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Tables

Table 1. Baseline characteristics of randomized patients. Values are in mean (SD) or n (%).

Variable	Early starters (N=62)	Delayed starters (N=63)
Age, years	78.9 (6.8)	78.3 (6.6)
Women	44 (71.0)	43 (68.3)
Ethnicity		
Chinese	58 (93.5%)	53 (84.1%)
Malaysian	3 (4.8%)	3 (4.8%)
Indian	1 (1.6%)	6 (9.5%)
Mixed	0	1 (1.6%)
Education level		
Primary	48 (77.4%)	37 (58.7%)
Secondary	12 (19.4%)	11 (17.5%)
Tertiary	2 (3.2%)	15 (23.8%)
Living situation		
Lives alone	3 (4.8%)	5 (7.9%)
Lives with partner/spouse	25 (40.3%)	25 (39.7%)
Lives with children/relative/friend	26 (41.9%)	29 (46.0%)
Misc. (lives with group/maid/others)	8 (5.6%)	4 (6.3%)
Medical history		
Cerebrovascular event	14 (22.6%)	9 (14.3%)
Ischemic or TIA	14 (22.6%)	7 (11.1%)
Hemorrhagic	0	2 (3.2%)
Hypertension	47 (75.8%)	43 (68.3%)
Hyperlipidemia	47 (75.8%)	40 (63.5%)
Ischemic heart disease	13 (21.0%)	14 (22.2%)
Diabetes Mellitus	26 (41.9%)	19 (30.2%)
ADAS-Cog	31.1 (11.9)	29.3 (9.5)
ADCS-ADL23	44.8 (15.8)	46.1 (15.2)
NPI	11.1 (13.9)	11.0 (11.7)
MMSE	14.9 (4.2)	15.9 (3.9)

Variable	Early starters (N=62)	Delayed starters (N=63)
Standard treatment for Alzheimer's disease		
Standard AD Treatment		
Donepezil alone	48 (77.4%)	50 (79.4%)
Rivastigmine alone	10 (16.1%)	8 (12.7%)
Memantine alone	3 (4.8%)	3 (4.8%)
Donepezil + Memantine	1 (1.6%)	2 (3.2%)

Abbreviations: AD: Alzheimer's Disease; ADAS-Cog: Alzheimer's Disease Assessment Scale - Cognitive subscale; ADCS-ADL23: Alzheimer's Disease Cooperative Study – Activities of Daily Living Inventory; MMSE: Mini-Mental State Examination; NPI: Neuropsychiatric Inventory; TIA: Transient ischemic attack,

Table 2. Summary of serious adverse events and all adverse events by study phase.

	Placebo-controlled Phase (0-6 months)		Delayed-start Phase (6-12 months)		Both Phases (0-12 months)	
	Early starters N=62	Delayed starters N=63	Early starters N=59	Delayed starters N=60	Early starters N=62	Delayed starters N=63
Serious Adverse Events (SAE)						
Subjects with any SAE, n (%)	14 (22.6%)	17 (27.0%)	10 (16.9%)	14 (23.3%)	21 (33.9%)	25 (39.7%)
Total number of SAEs	22	19	14	18	36	37
Seriousness criteria of events						
Death	2	3	0	2	2	5
Inpatient/prolonged hospitalization	20	15	14	16	34	31
Important medical event	0	1	0	0	0	1
Causality to study product						
Not related	22	19	14	18	36	37
Action taken on medications, n (%)						
Study product withdrawn	1 (1.6%)	0	1 (1.7%)	1 (1.7%)	2 (3.2%)	1 (1.6%)
Change in standard treatment	1 (1.6%)	4 (6.3%)	0	0	1 (1.6%)	4 (6.3%)
All Adverse Events (AE)						
Subjects with any AE, n (%)	38 (61.3%)	38 (60.3%)	29 (49.2%)	28 (46.7%)	47 (75.8%)	47 (74.6%)
Total number of AEs	79	67	56	46	135	113
Causality to study product						
Possibly related	3	0	3	0	6	0
Not related	76	67	53	46	129	113

	Placebo-controlled Phase (0-6 months)		Delayed-start Phase (6-12 months)		Both Phases (0-12 months)	
	Early starters N=62	Delayed starters N=63	Early starters N=59	Delayed starters N=60	Early starters N=62	Delayed starters N=63
Action taken on medications, n (%)						
Study product withdrawn	1 (1.6%)	0	1 (1.7%)	1 (1.7%)	2 (3.2%)	1 (1.6%)
Change in standard treatment	1 (1.6%)	4 (6.3%)	2 (3.4%)	0	2 (3.2%)	4 (6.3%)
Major Vascular Events (VE)						
Subjects with any VE, n (%)	2 (3.2%)	2 (3.2%)	2 (3.4%)	1 (1.7%)	4 (6.5%)	3 (4.8%)
Total number of VEs	2	2	2	1	4	3

Abbreviations: AE: Adverse events; SAE: Serious adverse events; VE: Vascular events

Figures

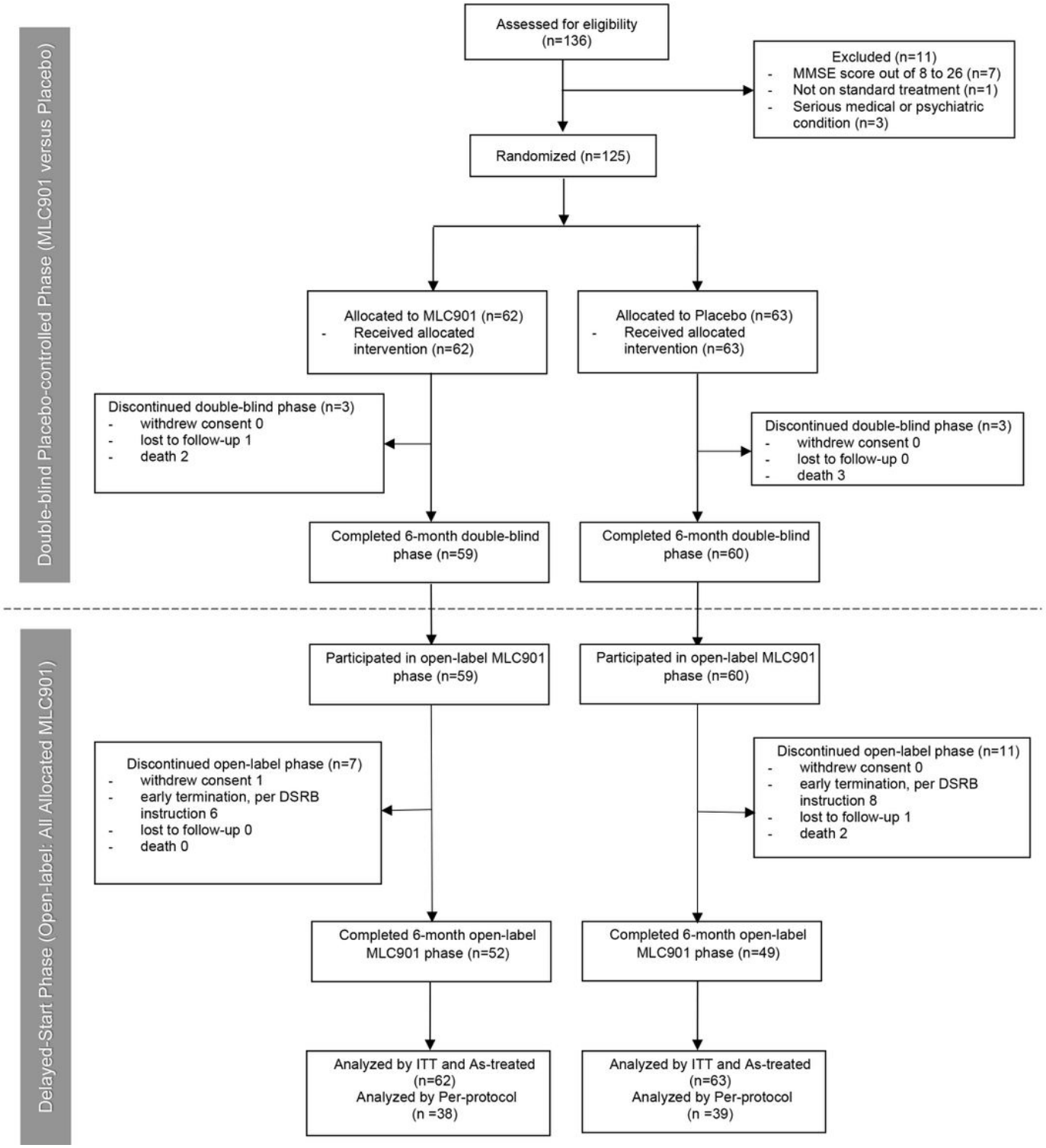

Figure 1

Study Design and Patient Flow (CONSORT Diagram)

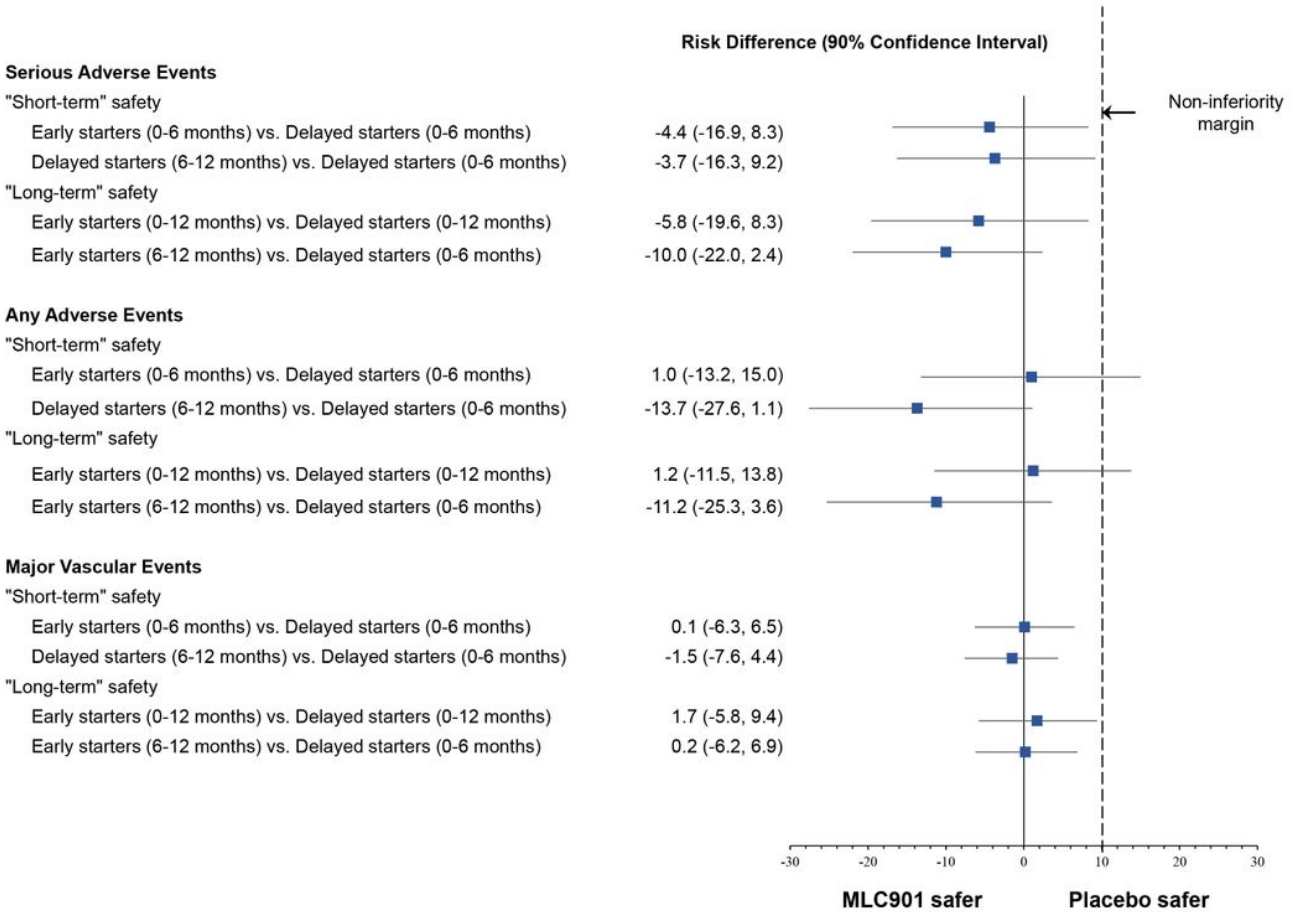

Figure 2

Risk differences and 90% confidence intervals of the proportions of serious adverse events, any adverse events, and major vascular events between early and delayed starters in the double-blind phase (0-6 months), delayed-start phase (6-12 months), and overall (0-12 months).

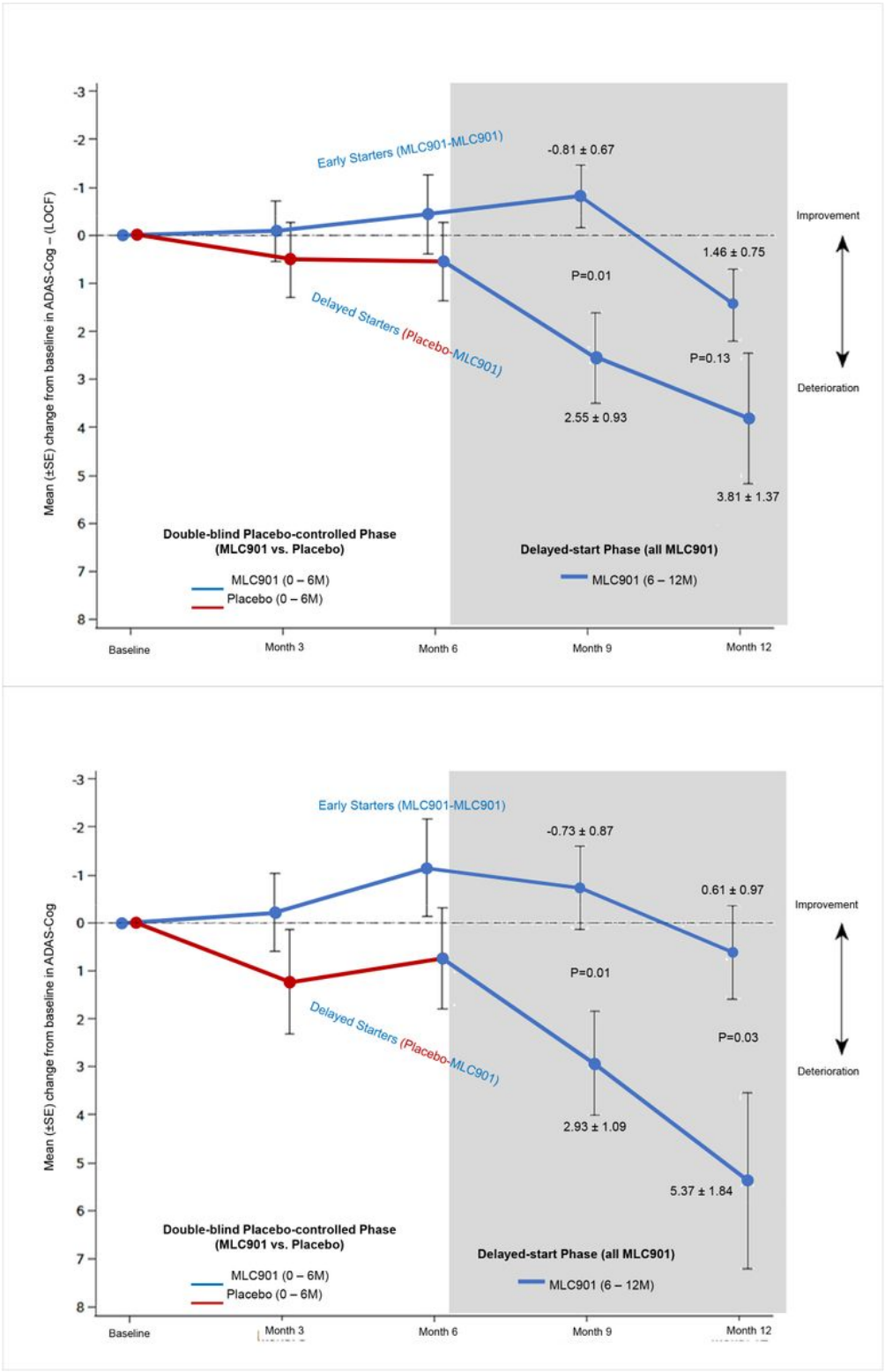

Figure 3

Comparison of mean change in Alzheimer's Disease Assessment Scale – Cognitive subscale (ADAS-Cog) score between early starters and delayed starters in the (A) intention-to-treat and (B) per-protocol populations.

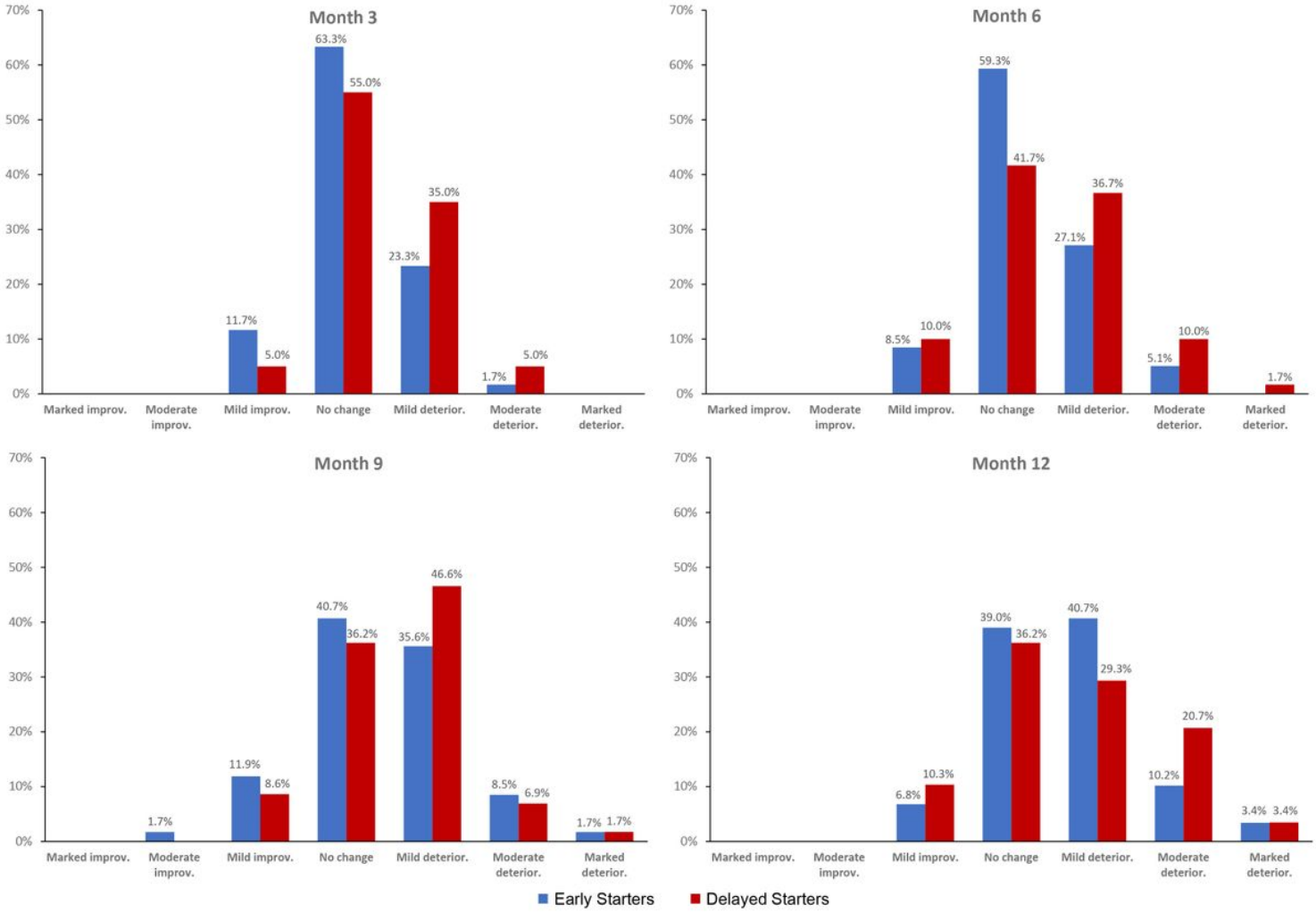

Figure 4

Comparison of Alzheimer's Disease Cooperative Study - Clinician's Global Impression of Change (ADCS-CGIC) scores between early and delayed starters at months 3, 6, 9 and 12.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ATHENEalzresCONSORT2010Checklist.pdf
- SupplementTables.docx