**Exploratory analyses suggest less cognitive decline on nilvadipine treatment in very mild Alzheimer's disease subjects.**

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On behalf of the NILVAD Authorship Group

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**Abstract:**
**Background:** This study explores whether the baseline severity of Alzheimer's disease (AD) subjects in the NILVAD trial modified the effects of nilvadipine, a dihydropyridine calcium channel blocker shown to target multiple aspects of AD pathology in preclinical studies. **Methods**: Exploratory analyses were performed on the modified intention-to-treat (mITT) dataset (n = 497) to examine the response to nilvadipine in very mild, mild and moderate AD subjects. The main outcome measures included total scores and subscales of the Alzheimer's Disease Assessment Scale Cognitive 12 (ADAS-Cog 12) and the Clinical Dementia Rating Scale sum of boxes (CDR-sb). All statistical analyses were adjusted for the confounding effects of the apolipoprotein E (APOE) ε4 allele and education and effect modification by gender in order to examine the interactive effects of nilvadipine and AD severity over the 78-week study period. **Results**: Compared to their respective placebo controls, nilvadipine-treated very mild AD subjects showed lower decline whereas nilvadipine-treated moderate AD subjects showed greater decline on the ADAS-Cog 12 scores. The therapeutic effects of nilvadipine were observed for the memory trait (which included immediate word recall and delayed recall) in very mild AD subjects and the language trait (which included spoken language, word finding difficulties and language comprehension) in mild AD subjects. Moderate AD subjects on nilvadipine showed decline on several aspects of cognition. **Conclusion**: These exploratory analyses suggest that nilvadipine may have cognitive benefits for very early AD subjects. Future clinical trials of nilvadipine focused on such individuals are required to confirm these findings.

**Introduction:**

Alzheimer's disease (AD) is an untreatable neurodegenerative disease which currently affects nearly 5.3 million US citizens. By 2050, the prevalence of AD is expected to reach 13 million in the US alone and 100 million worldwide. The presence of amyloid plaques and neurofibrillary tangles in the brains are the key hallmarks of AD (1, 2), which are also accompanied by cerebrovascular disease and inflammation (3). Studies from the Alzheimer's Disease Neuroimaging Initiative (ADNI) suggest that the pathogenesis of AD could begin 30 years prior to the detection of clinical symptoms which is initially reflected as a decline in cerebrospinal fluid (CSF) amyloid-beta (Aβ)-42 and precedes brain amyloid accumulation. This is followed by an increase in CSF total-tau levels, then a rise of phosphorylated tau in CSF and finally the onset of detectable clinical symptoms of cognitive impairment (4). As such, clinically detectable stages of AD occur only after significant brain amyloid accumulation and correlate with tau abnormalities (4). Recent clinical trials have shown that moderate AD patients with established brain amyloid and tau pathologies are unresponsive to anti-amyloid therapeutic approaches, although some trials have shown potential benefits in mild and early stages AD patients (5-9). Thus, early and mild AD patients may be a more appropriate target population for anti-amyloid and anti-tau approaches.

Findings of the SYS-EUR trial of 2400 participants showed that intervention with nitrendipine a dihydropyridine (DHP) calcium channel blocker (CCB) similar in structure to nilvadipine) resulted in a reduction of AD incidence (10). Another small clinical trial of nilvadipine in mild cognitive impairment (MCI) patients showed reduced conversion to a diagnosis of AD in subjects treated with nilvadipine compared to those on amlodipine (11). Hence, not all DHP CCBs are equally beneficial for treating AD. In fact, *in vitro* screening of over 1000 DHPs using cell-based assays showed that inhibition of Aβ production is not a class effect of DHPs as only a minority of DHPs, such as nilvadipine and nitrendipine, demonstrated inhibitory action, while others had no effect, or even enhanced, Aβ production (12, 13). Preclinical studies in mouse models of AD have shown that nilvadipine improves cognitive function, lowers Aβ production, increases Aβ clearance across the blood-brain-barrier (BBB) and reduces tau hyper-phosphorylation and inflammation (12, 14-16). Interestingly, nilvadipine is a racemic compound, and examination of the individual isomers shows that while both (+)- and (-)-nilvadipine forms have beneficial effects on amyloid and tau pathologies (16), only the (+)-nilvadipine has the blood pressure lowering effects through antagonism of calcium channels. Therefore, we conclude that the preclinical anti-Alzheimer pathology effects of nilvadipine are not related to its CCB properties. In fact, we have shown that the mechanism of action of nilvadipine is as an inhibitor of spleen tyrosine kinase (Syk) which downregulates amyloid production, tau phosphorylation and inflammation (16). As such, nilvadipine may represent a novel multimodal disease-modifying therapy for AD. To evaluate this, a phase III multi-center, double-blinded, randomized, placebo-controlled clinical trial was conducted in Europe to test its efficacy in treating AD (the NILVAD trial).

In the NILVAD trial, mild and moderate AD subjects, when analyzed as a single population, did not benefit from nilvadipine treatment. However, pre-specified subgroup analyses indicated that, compared to placebo, nilvadipine-treated mild AD subjects (MMSE of 20 or above at baseline) showed cognitive benefits whereas moderate AD subjects showed worsening of cognition. These findings prompted further exploration of the effects of nilvadipine in AD patients at the earlier stages of the disease, when the brain amyloid burden and tau pathologies have not yet reached their maximum (4, 17-19). We propose that stratification of the study population by AD severity at baseline, would reveal cognitive benefits among mild and very mild AD subjects compared to moderate AD subjects. Thus, we conducted exploratory analyses to examine changes in the overall, and specific aspects of, cognition after nilvadipine intervention with an emphasis on understanding the treatment response in the mild AD population of the NILVAD trial. Despite major set-backs in the quest for AD treatments and with several pharmaceutical companies abandoning AD drug development, therapeutic efforts must continue to combat the unprecedented increases in the AD burden worldwide (20). As such, exploratory analyses of existing data are mandatory to help generate new hypotheses, inform researchers of reasons for unsuccessful outcomes and identify subpopulations which may have benefited from the intervention.

**Methods**

Study design and participants:

This 18-month phase III double-blind, placebo-controlled, randomized clinical trial was conducted in 9 countries across Europe (see elsewhere for additional details (21)) and funded by the European Commission under a Framework 7 Programme Health Theme collaborative project grant. The CONSORT flow-chart is

provided as Figure 1. The complete date range of the study from the recruitment to the completion of last patient is between 2013 to 2016. A separate Scientific Advisory Board, an independent Ethics Advisory Board and an independent Data Safety Monitoring Board were involved in the oversight of the trial. The study protocol and associated documents were approved by Independent Ethics Committees and Institutional Review Boards for all study sites.

Inclusion criteria for the study required that participants should be over the age of 50 and have a diagnosis of mild or moderate probable AD according to the established guidelines from the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association Inc. (NINCDS-ADRDA) and the Alzheimer's Association, and having a baseline Mini-Mental State Examination (MMSE) score of $\geq$12 and ≤ 27 (21). A total of 569 subjects were screened for eligibility and 511 were randomized into the trial with 253 assigned to the nilvadipine group (one dropped out due to blood pressure measurements being out of range) and 258 were assigned to placebo. Of these 510 subjects, 11 were lost to follow-up and 2 withdrew consent leaving 497 subjects in the modified intention-to-treat (mITT) dataset. Data from subjects in the mITT set were used for these additional exploratory analyses below. At baseline, each subject was randomly assigned to either 8mg of Nilvadipine or placebo, once a day and each study subject was required to take the capsule orally after breakfast for 78 weeks. The primary outcome measures were the 12-item Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-Cog 12) and the Clinical Dementia Rating scale sum of boxes (CDR-sb), and these tests were administered at four time-points (baseline, and weeks 13, 52 and 78). Apolipoprotein E (APOE) genotypes were available on a subset of subjects (n = 328).

**Exploratory analyses:**

The exploratory analyses of the NILVAD trial are restricted to the co-primary outcome measures of ADAS-Cog 12 and CDR-sb. For these studies, the mild AD group was further stratified by the baseline mini-mental status examination (MMSE) scores from 20 and above to examine treatment differences by a single point increase in MMSE scores as previously shown (7). Subsequently, the study population was divided into three subgroups to separate the moderate AD population (baseline MMSE scores of ≤ 19), mild AD (baseline MMSE scores between 20-24) and very mild AD (baseline MMSE scores ≥ 25) into distinct AD severity groups. The grouping of moderate, mild and very mild AD was based on the classification system previously utilized by Huntley and colleagues (22) and Folstein (23). These analyses also explored the potential impact of nilvadipine treatment on cognitive sub-scales of the ADAS-Cog 12 and CDR-sb tests. The ADAS-Cog 12 sub-scales are: immediate word recall, delayed recall, naming, following commands, constructional praxis, ideational praxis, orientation, word recognition, remembering test directions and instructions, spoken language, comprehension and word finding difficulty in spontaneous speech. The sub-scales of CDR-sb are: memory, orientation, judgment and problem solving, community affairs, home and hobbies and personal care. In addition, ADAS-Cog 12 sub-scales were further grouped into specific traits for memory, language and praxis based on the topography of tissue loss in AD depending on the stage of the disease as previously suggested by Verma and colleagues (24). Using this strategy sub-scales related to each trait were grouped together to generate a composite variable for each trait (Figure 2A).

**Statistical analyses:**

For these analyses, general demographic characteristics across AD subgroups by treatment within the modified intent to treat (mITT) dataset were compared using either ANOVA or the Chi-square test, as applicable. For these exploratory analyses, mixed linear model (MLM) regression was used to examine the main effects and the interactions between treatment, time (time points of study visits at 13 weeks, 52 weeks and 78 weeks) and the baseline MMSE scores, to stratify subjects by AD severity. As we were interested in the independent contributions of AD severity and treatment effect over time on the cognitive outcomes, these analyses were also adjusted to account for the effect modification by gender and confounding by APOE (coded as those with the presence of ε4, without ε4 and those with no genotype information since not all mITT subjects had APOE genotypes available) and confounding effects of age at which subject left education (referred to as "education" hereon). In addition, this model included interactions between time and APOE; treatment and APOE; time, treatment and APOE; as well as time and gender; treatment and gender; and time, treatment and gender; to account for the treatment effect modification observed in pre-specified analyses. Interactive terms were also included for education and time and for treatment and education to account for

education imbalance across AD severity subgroups. All of these variables were considered fixed factors. Subjects and country were treated as random factors. The autoregressive covariance structure was used in these MLM analyses. The outcome variables included change in the total ADAS-Cog 12 scores and change in composite scores from the ADAS-Cog 12 for different cognitive traits. Principal Component Analysis (PCA) was used to minimize multicollinearity and achieve dimension reduction for data from sub-scales from the ADAS-Cog 12 and CDR-sb for all visits. This method was used as an unsupervised procedure for achieving data reduction and for identifying treatment responses in subgroups of subjects based on their baseline severity. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test for sphericity was used to ensure adequacy for PCA analysis (KMO value of > 0.6 and Bartlett p value < 0.05). Variables with eigenvalues of ≥1 were retained and PCA components (Factors) were extracted using varimax with Kaiser normalization for rotation to simplify and clarify the data structure. Individual ADAS-Cog 12 and CDR-sb sub-scale having a correlation of > 0.4 within each PCA factor were then grouped according to their association with specific factor identified by PCA and then labeled as factors 1 through 4 (Figure 2B). These composite variables were used as the outcome measures for further analysis by MLM as described above. *Post-hoc* stratification were performed if the interaction terms for treatment, time and baseline AD severity showed a $p < 0.05$. Given the confounding by APOE genotypes or effect modification by gender, their influences were also examined by further stratifying the subjects by APOE genotype and baseline AD severity, and by gender and baseline AD severity. All analyses were conducted using SPSS version 24 (IBM, NY).

**Results**:

*General characteristics of the study population stratified by AD severity:*

Demographic characteristics of the AD subgroups stratified by treatment are presented in Table 1. There were no differences across AD subgroups for age, weight and for the time elapsed since the diagnosis of AD. Similarly, there were no differences in ethnicity and the presence of the APOE ε4 allele ($p > 0.05$). However, there were significant differences in education across AD subgroups by treatment. There were also significant differences in gender with females being overrepresented in the nilvadipine treated moderate AD group compared to all other groups. Mild AD subjects having MMSE ≥ 20 were further stratified using a 1-point incremental increase in the MMSE cut-off (Figure 3). This showed that very mild AD subjects with MMSE ≥ 25 benefited the most after nilvadipine treatment when compared to all other MMSE subgroups, as indicated by the reduced change from baseline on the ADAS-Cog 12. We therefore stratified the study population as described above to allow us to focus on very mild AD subjects along with mild and moderate AD subjects (MMSE = 20-24 and MMSE ≤19, respectively). Since our pre-specified analysis showed treatment effect modification by ε4 and gender on ADAS-Cog 12 change from baseline and because our exploratory analyses showed imbalances in education across AD subgroups by treatment, all subsequent analyses are adjusted for these confounders/effect modifiers.

*Changes in ADAS-Cog due to treatment are modified by baseline severity of AD:*

Regression analysis showed a significant interaction between nilvadipine treatment, baseline AD severity and time ($F = 2.56$, $p = 0.02$) on the change in ADAS-Cog 12 after adjusting for confounding factors described above. *Post-hoc* stratifications of the mean changes on ADAS-Cog 12 showed less decline after nilvadipine treatment among very mild AD subjects and a greater decline on ADAS-Cog 12 among nilvadipine treated individuals with moderate AD (Figure 4). There were no differences on the total ADAS-Cog 12 scores between nilvadipine and placebo treated individuals from the mild AD group. There were no differences on the CDR-sb total score with respect to the disease severity and treatment (data not shown). Since our previous pre-specified subgroup analyses showed an influence of the APOE ε4 allele and gender on the treatment outcome, supplementary figure 1 shows further stratification of changes in ADAS-Cog 12 in very mild, mild and moderate AD subjects by APOE ε4 status and gender.

*Responses to nilvadipine on memory and language traits of ADAS-Cog 12 depend on the initial severity of AD***.**

Regression analyses of the ADAS-Cog 12 sub-scales grouped as memory, language and praxis traits showed that the memory trait was significantly affected by the interaction between treatment, time and AD severity at baseline ($F = 2.18$, $p = 0.04$) after adjusting for the confounders/effect modifiers as above. *Post-hoc* stratifications show that only very mild AD subjects treated with nilvadipine had less decline on the memory trait compared to placebo treated very mild AD subjects (Figure 5A). There were no differences for the

memory trait between nilvadipine and placebo treated mild AD subjects, while a larger decline on the memory trait was noted for the moderate AD subjects treated with nilvadipine compared to placebo. Once adjusted, there was also a significant interaction between treatment, time and baseline AD severity on the language trait ($F = 2.1$, $p = 0.05$), with *post-hoc* stratifications showing less decline in the language trait for the nilvadipine treated mild AD group only, Figure 5B. There was no effect of AD severity, time and treatment on the praxis trait ($p > 0.05$).

*Principal component analysis of ADAS-Cog 12 sub-scales and CDR-sb show benefits of nilvadipine on memory and language in subjects with very mild and mild AD.*

The PCA analyses meeting the KMO-Bartlett's test criteria of sampling adequacy and data sphericity identified 4 factors. Supplementary table 1 shows correlations between these factors and different ADAS-Cog 12 and CDR-sb subscales. Among the 4 factors identified by PCA, there was a trend for an interaction between time, treatment and AD severity for factor 2 ($F = 1.98$, $p = 0.07$) loaded with ADAS-Cog 12 sub-scales related to spoken language ability, comprehension, and word finding difficulty in spontaneous speech. *Post-hoc* stratifications of the mean changes showed that factor 2 differed by treatment only in the mild AD group, see Figure 6. There was also an interaction between treatment, time and baseline AD severity for factor 3 ($F = 3.72$, $p = 0.001$) loaded with ADAS-Cog 12 sub-scales for the immediate and delayed word recall tasks. *Post-hoc* stratification showed that factor 3 differed by treatment only in the very mild AD group (See Figure 6). There were no significant differences between the groups for factor 1 which was loaded with all the sub-scales of CDR-sb along with several ADAS-Cog 12 sub-scales, or by factor 4, which was loaded with ADAS-Cog 12 sub-scales for orientation, word recognition task and remembering the test instructions.

**Discussion:**

Many clinical trials in combined populations of mild and moderate AD patients have failed to show benefits, possibly because different stages of AD reflect changes in the extent of the underlying amyloid and tau pathologies. Frequently the same drugs that failed in combined mild and moderate populations have shown suggestive benefits in early stages of the disease (5-8, 25) when the extent of amyloid and tau pathologies is considerably lower than for subjects in the moderate disease stage (4, 17, 18). Although analysis of the overall population showed no benefit of nilvadipine, our exploratory studies show that very mild AD subjects experienced less cognitive decline on the total ADAS-Cog 12 in the nilvadipine treated group compared to placebo. However, moderate AD subjects treated with nilvadipine showed a greater decline compared to their controls. An examination of different cognitive traits from the ADAS-Cog 12 sub-scales showed nilvadipine to have effects on the memory trait in the very mild AD group and the language domain in the mild AD group. However, moderate AD patients showed worsening of the memory trait after nilvadipine intervention. These findings suggest a differential impact of nilvadipine depending on the stage of the disease at which treatment is initiated.

The examination of changes over time in the total ADAS-Cog 12 scores by different stages of AD suggested that, after 78 weeks of treatment, very mild AD patients treated with nilvadipine scored nearly 4 points fewer than placebo controls. There were no differences between the placebo and nilvadipine treated subjects in the mild AD group and the moderate AD subjects treated with nilvadipine declined by 4 points compared to placebo. Placebo groups for very mild and mild AD declined at a similar rate whereas the moderate AD placebo group declined at nearly twice the rate during this period. These findings are largely consistent with those previously reported for placebo groups in other clinical trials as well as the fact that a lower decline is generally noted in mild AD versus moderate AD subjects (26, 27). In any of the AD severity categories, there were no correlations between blood pressure changes and total ADAS-Cog 12 changes (unpublished data). This is consistent with our preclinical studies showing that anti-Aβ effects of nilvadipine were not related to its blood pressure lowering ability (16). We did not observe an effect of nilvadipine treatment on the changes in CDR-sb. Since the CDR-sb is a global impression scale designed for staging of dementia rather than quantifying cognitive change over time. It is therefore possible that CDR-sb lacks the desired sensitivity to detect subtle cognitive changes due to high test-retest variability for detecting cognitive differences (28, 29). As such, given the observed benefits of nilvadipine were in very mild AD patients for memory, it is possible that CDR-sb was unable to reliably detect group differences.

As AD subpopulations in different stages of the disease demonstrate deficits in different aspects of cognition, ADAS-Cog 12 sub-scales were analyzed separately to estimate the effects of nilvadipine on

memory, language and praxis. These traits can be mapped to the underling brain tissue loss in AD in different stages of the disease. For instance, neuronal loss in early AD starts within the medial temporal lobe (MTL), which is primarily involved with memory functions. With the advancement of AD, further degeneration occurs within the parietal, frontal, and occipital lobes, which involve language processing and praxis (24, 30). Our exploratory analyses of cognitive traits suggested that benefits of nilvadipine were restricted to the memory trait in very mild AD subjects. In the mild AD cases, who are relatively more advanced compared to very mild cases, we observed a reduced decline on the language trait in nilvadipine treated subjects. There were no effects of nilvadipine on praxis for any of the AD subpopulations. In the moderate AD group, there was no specific domain accounting for the overall decrement in ADAS-Cog 12 with nilvadipine treatment, but rather there were trends for contributions from all domains. Over the 18 months, placebo-treated very mild AD subjects showed significant decline in memory. This is to be expected since very mild AD subjects initially have the most functional memory, which is rapidly lost in the early stages of the disease. The language trait remained largely preserved in very mild AD treated with placebo but continued to decline further in mild and moderate AD placebo groups. The praxis trait further declined in moderate AD on placebo (nearly a 3 point decline) with minimal decline (about 1 point) in both very mild and mild AD on placebo. This is again to be expected as loss of praxis occurs later with AD progression. Hence, these studies provide a framework for cognitive and functional decline on ADAS-cog 12 sub-scales in placebo treated AD patients over 18-months which is currently unavailable and require further investigation to understand the disease stage specific changes. Collectively, these data suggest that examination of appropriate cognitive domains relevant to the stage of AD might improve our ability to evaluate treatment effects in AD clinical trials.

Principal component analyses were performed using sub-scales from both the ADAS-Cog 12 and CDR-sb as an unsupervised dimension reduction approach to determine which specific cognitive domains from these tests segregate together and how nilvadipine treatment might impact these subdomains. The factor 1 loading primarily contained all of the CDR-sb sub-scales and also the orientation sub-scale from ADAS-Cog 12 and remained unaffected by nilvadipine treatment. However, factor 2 loading contained language and word finding abilities-related sub-scales from the ADAS-Cog 12 and, as with the trait analysis, benefit of nilvadipine was limited to the mild AD group only. Factor 3 was loaded with immediate and delayed recall showing less decline after treatment in the very mild AD group. The factor 4 loading contained orientation, word recognition and remembering test instructions which did not differ between the nilvadipine and placebo groups in any of the AD severity subpopulations. Collectively, these results suggest that memory was preserved in very mild AD and language was preserved in mild AD subjects after nilvadipine treatment.

We also stratified the study population by AD severity at baseline and by APOE ε4 carrier status, which suggested that non-carrier, very mild AD cases had lower decline on nilvadipine but the AD patients in the moderate stage showed cognitive decline on nilvadipine treatment. There were no changes among ε4 carriers at any of the disease stages. In the subpopulation that was genotyped for APOE, ε4 carriers were (unusually) as cognitively intact as non-carriers at baseline. Despite this, ε4 carriers did not benefit from nilvadipine treatment regardless of the initial disease stage, which is consistent with the literature. Similarly, stratification by gender showed that both males and females in very mild AD stages had less cognitive decline on nilvadipine compared to placebo treatment while females in the moderate AD stage showed a greater cognitive decline on nilvadipine treatment. With respect to gender, females at baseline had a 2 point higher ADAS-Cog 12 score and were over-represented in the moderate AD nilvadipine group compared to their male counterparts. Thus, in this data set gender may be a surrogate for disease severity since females experience a greater cognitive decline than males and some studies show poor response to certain therapies in AD females compared to AD males (31).

With failures of most AD trials in satisfying the efficacy outcomes in mixed AD populations, exploratory analyses of existing data are required to understand this apparent lack of efficacy and to identify sub-populations which may have been affected by putative interventions. The NILVAD trial was designed for the analysis of a mixed mild and moderate AD population and therefore further stratification of the study population into very mild, mild and moderate AD was unplanned and therefore exploratory. As such, these subgroup analyses were likely underpowered, particularly when considering the confounding effects of gender and APOE. Nevertheless, analyses adjusted for these factors continue to suggest an interaction between baseline AD severity and response to nilvadipine on the total ADAS-Cog 12 scores. Together, these findings suggest a differential response to nilvadipine treatment in AD based on the underlying severity of the disease. This finding is also consistent with the results of several other experimental AD treatments whereby only very early

stage AD subjects demonstrated benefit, such as Solanezumab (5, 8, 25), aducanumab (6) and LipiDiDiet trials (7). Consequently, the Alzheimer's therapeutic field is increasingly targeting the early stages of AD (32). The possible benefit in the very mild AD group identified by these exploratory analyses warrants further studies of nilvadipine treatment in prodromal/very early stage AD patients.

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**Conflict of Interest declaration**
Dr. Mullan is the Chief Executive Officer of Archer Pharmaceuticals, Dr. Crawford is the Chief Operating Officer of Archer Pharmaceuticals. Both Drs. Mullan and Crawford have commercial interests in nilvadipine.
Drs. Mullan, Crawford, Lawlor, Abdullah and Kennelly are listed as inventors on a pending patent.

## Figure legends:

**Figure 1: CONSORT flow-chart:**
Flow-chart of the main study from which data on exploratory analysis were obtained.

**Figure 2: Grouping of ADAS-cog and CDR-sb sub-scales.** (2A) Sub-scales of the ADAS-cog 12 test were grouped based on traits for memory, language and praxis to account for the topography of tissue loss in AD depending on the stage of disease. (2B**)** Sub-scales of the ADAS-cog 12 and the CDR-sb were analyzed by PCA which resulted in grouping of sub-scales into four factors that explained most of the variance into the dataset. A composite variable was then generated which included sub-scales identified in each factor by PCA and were named factors 1 through 4. Note, factor 1 also contains orientation sub-scale from the ADAS-Cog in addition to the ones from CDR-sb.

**Figure 3: Further stratification of the mild AD by increasing the increment of MMSE scores by 1 starting from ≥ 20 up to ≥ 25**. Mean ± SE (for MMSE ≥ 20 n = 154 for nilvadipine, n = 157 for placebo; ≥ 21 n = 125 for nilvadipine and 136 for placebo; ≥ 22 n = 103 for nilvadipine and n = 117 for placebo; ≥ 23 n = 70 for nilvadipine and n = 99 for placebo; ≥ 24 n = 57 for nilvadipine and n = 68 for placebo; ≥ 25 n = 36 for nilvadipine and n = 44 for placebo). Mean change from baseline for the total ADAS-Cog 12 scores show lowest decline among nilvadipine treated subjects compared to placebo-treated subjects with MMSE score of ≥ 25.

**Figure 4: Nilvadipine-treated very mild AD subjects show lower cognitive decline compared to controls on the ADAS-Cog 12 test**. Mean ± SE (n = 82 for moderate AD (MMSE ≤ 19) on nilvadipine, n = 94 for moderate AD on placebo, n = 118 for mild AD (MMSE 20-24) on nilvadipine, n = 113 for mild AD on placebo, n = 36 for very mild AD (MMSE ≥ 35) on nilvadipine and n = 44 for very

mild AD on placebo) for the change in ADAS-Cog 12 scores. There was a significant effect for the interaction between treatment, time and baseline AD severity as assessed by MMSE scores after correcting for the confounding effects of APOE, gender and education, $p < 0.05$. (4A) Stratifications show very mild AD subjects treated with nilvadipine were lower by 3.5 points on the ADAS-Cog 12 compared to placebo. (4B) Mild AD subjects treated with nilvadipine were similar to their placebo controls. (4C). However, moderate AD subjects on nilvadipine were higher by about 3.7 points on the ADAS-Cog at 78 weeks compared to those on placebo.

**Figure 5: Very mild AD subjects show lower decline on memory trait whereas mild AD subjects show lower decline on language after nilvadipine treatment compared to placebo.** Mean ± SE (n = 82 for moderate AD on nilvadipine, n = 94 for moderate AD on placebo, n = 118 for mild AD on nilvadipine, n = 113 for mild AD on placebo, n = 36 for very mild AD on nilvadipine, n = 44 for very mild AD on placebo) for the change in memory, language and praxis traits of grouped ADAS-cog 12 sub-scales. (5A) There was a significant effect for the interaction between treatment, time and baseline AD severity on the memory trait, $p < 0.05$. Stratifications show that very mild AD subjects on nilvadipine had a lower decline (about 2 points) on the memory trait compared to their controls. (5B) There was also a significant interaction between treatment, time and baseline AD severity for the language trait, $p < 0.05$. Stratifications show that mild AD subjects on nilvadipine had a lower decline on the language trait compared to the mild AD subjects on placebo. (5C) There was no effect seen for the praxis trait, $p > 0.05$.

**Figure 6: Nilvadipine-treated mild and very mild AD groups show less decline for PCA Factors 2 and 3 respectively.** Mean ± SE (n = 82 for moderate AD on nilvadipine, n = 94 for moderate AD on placebo, n = 118 for mild AD on nilvadipine, n = 113 for mild AD on placebo, n = 36 for very mild AD on nilvadipine, n = 44 for very mild AD on placebo) for the change in Factors 1, 2, 3, and 4. (6A) There were no differences between any of the groups for Factor 1. (6B) A marginally significant interaction between time, treatment and AD severity was observed for Factor 2, $p = 0.07$ and subsequent stratifications show that only mild AD subjects on nilvadipine had less decline compared to their placebo controls. (6C) There was a significant interaction between time, treatment and AD severity for Factor 3, $p < 0.05$. Once stratified lower decline was limited with the very mild AD group only. (4D) There were no significant differences seen between groups for Factor 4.

**Supplemental figure 1: Changes in total ADAS-Cog 12 stratified by baseline AD severity and APOE ε4 carrier status.** Mean ± SE (n = 32 for moderate AD on nilvadipine, n = 33 for moderate AD on placebo, n = 47 for mild AD on nilvadipine, n = 48 for mild AD on placebo, n = 15 for very mild AD on nilvadipine, n = 19 for very mild AD on placebo). Moderate non-carrier AD subjects treated with nilvadipine showed decline over the 78-week period whereas mild or moderate ε4 carrier AD subjects treated with nilvadipine were similar to their placebo controls.

**Supplemental figure 2: Changes in total ADAS-Cog 12 stratified by baseline AD severity and gender.** Mean ± SE (n = 82 for moderate AD on nilvadipine (71% female), n = 94 for moderate AD on placebo (60% female), n = 118 for mild AD on nilvadipine (65% females), n = 113 for mild AD on placebo (61% females), n = 36 for very mild AD on nilvadipine (47% females), n = 44 for very mild AD on placebo (48% females). Moderate females appear to decline more after nilvadipine treatment compare to placebo. Both very mild female and male AD subjects appear to do better on nilvadipine compared to their respective placebo treated controls.

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Figure 1

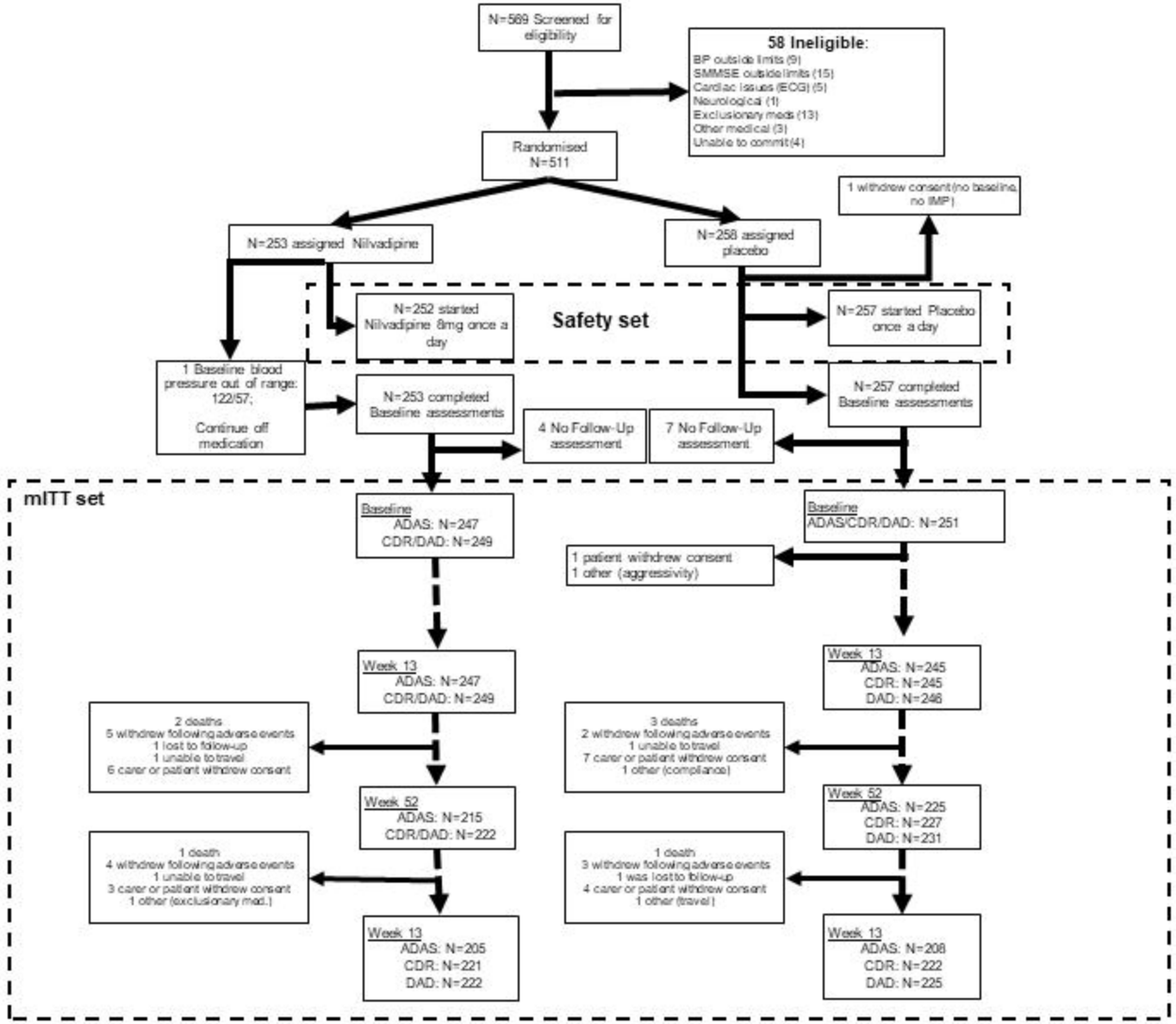

N=569 Screened for eligibility
58 Ineligible:
BP outside limits (9)
SMMSE outside limits (15)
Cardiac issues (ECG) (5)
Neurological (1)
Exclusionary meds (13)
Other medical (3)
Unable to commit (4)
Randomised N=511
1 withdrew consent (no baseline, no IMP)
N=253 assigned Nilvadipine
N=258 assigned placebo
N=252 started Nilvadipine 8mg once a day
Safety set
N=257 started Placebo once a day
1 Baseline blood pressure out of range: 122/57;
Continue all medication
N=253 completed Baseline assessments
N=257 completed Baseline assessments
4 No Follow-Up assessment
7 No Follow-Up assessment
mITT set
Baseline
ADAS: N=247
CDR/DAD: N=249
Baseline
ADAS/CDR/DAD: N=251
1 patient withdrew consent
1 other (aggressivity)
Week 13
ADAS: N=247
CDR/DAD: N=249
Week 13
ADAS: N=245
CDR: N=245
DAD: N=246
2 deaths
5 withdrew following adverse events
1 lost to follow-up
1 unable to travel
6 carer or patient withdrew consent
3 deaths
2 withdrew following adverse events
1 unable to travel
7 carer or patient withdrew consent
1 other (compliance)
Week 52
ADAS: N=215
CDR/DAD: N=222
Week 52
ADAS: N=225
CDR: N=227
DAD: N=231
1 death
4 withdrew following adverse events
1 unable to travel
3 carer or patient withdrew consent
1 other (exclusionary med.)
1 death
3 withdrew following adverse events
1 was lost to follow-up
4 carer or patient withdrew consent
1 other (travel)
Week 13
ADAS: N=205
CDR: N=221
DAD: N=222
Week 13
ADAS: N=208
CDR: N=222
DAD: N=225

Figure 2A

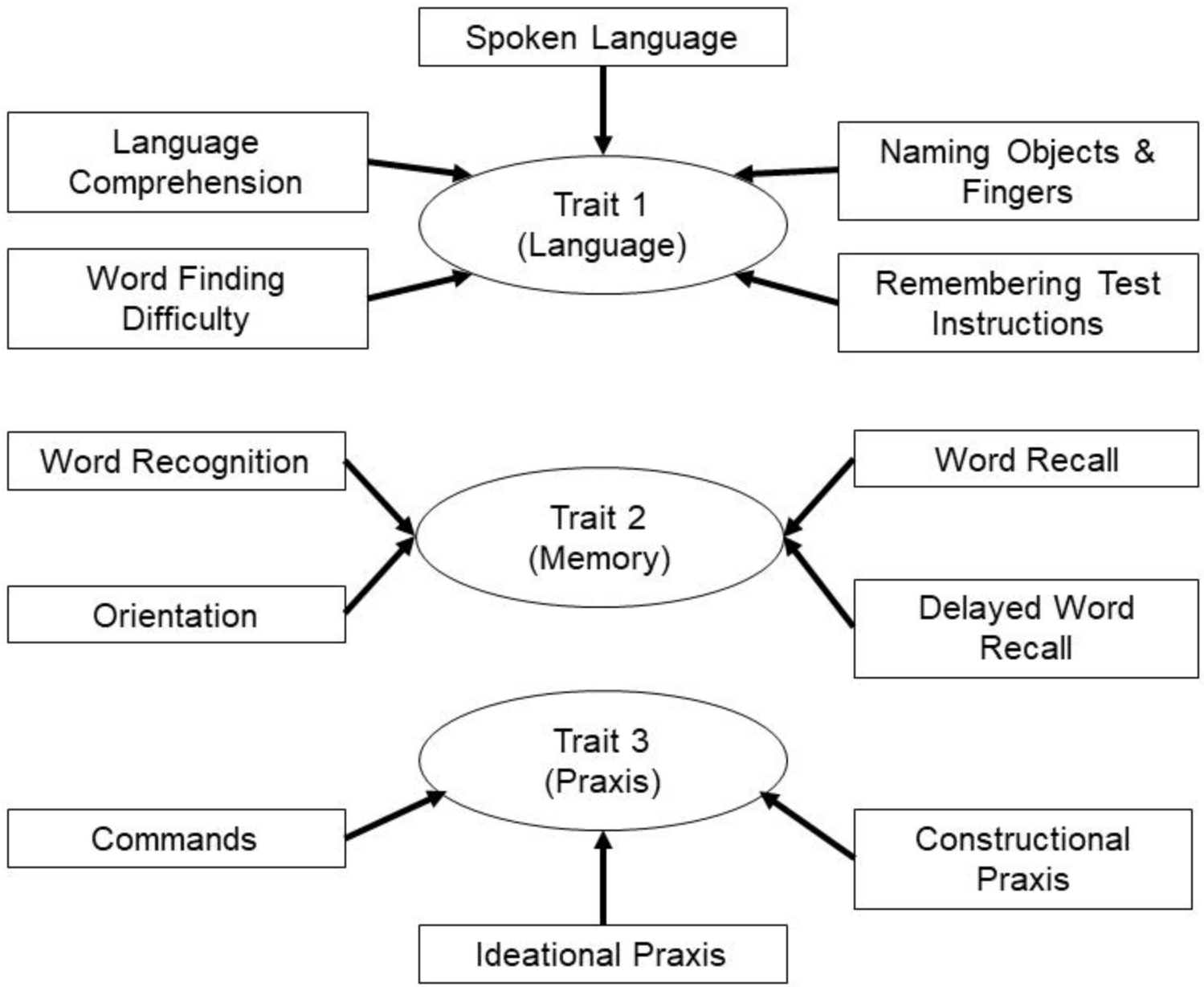

Spoken Language
Language Comprehension
Naming Objects & Fingers
Trait 1 (Language)
Word Finding Difficulty
Remembering Test Instructions
Word Recognition
Word Recall
Trait 2 (Memory)
Orientation
Delayed Word Recall
Trait 3 (Praxis)
Commands
Constructional Praxis
Ideational Praxis

Figure 2B

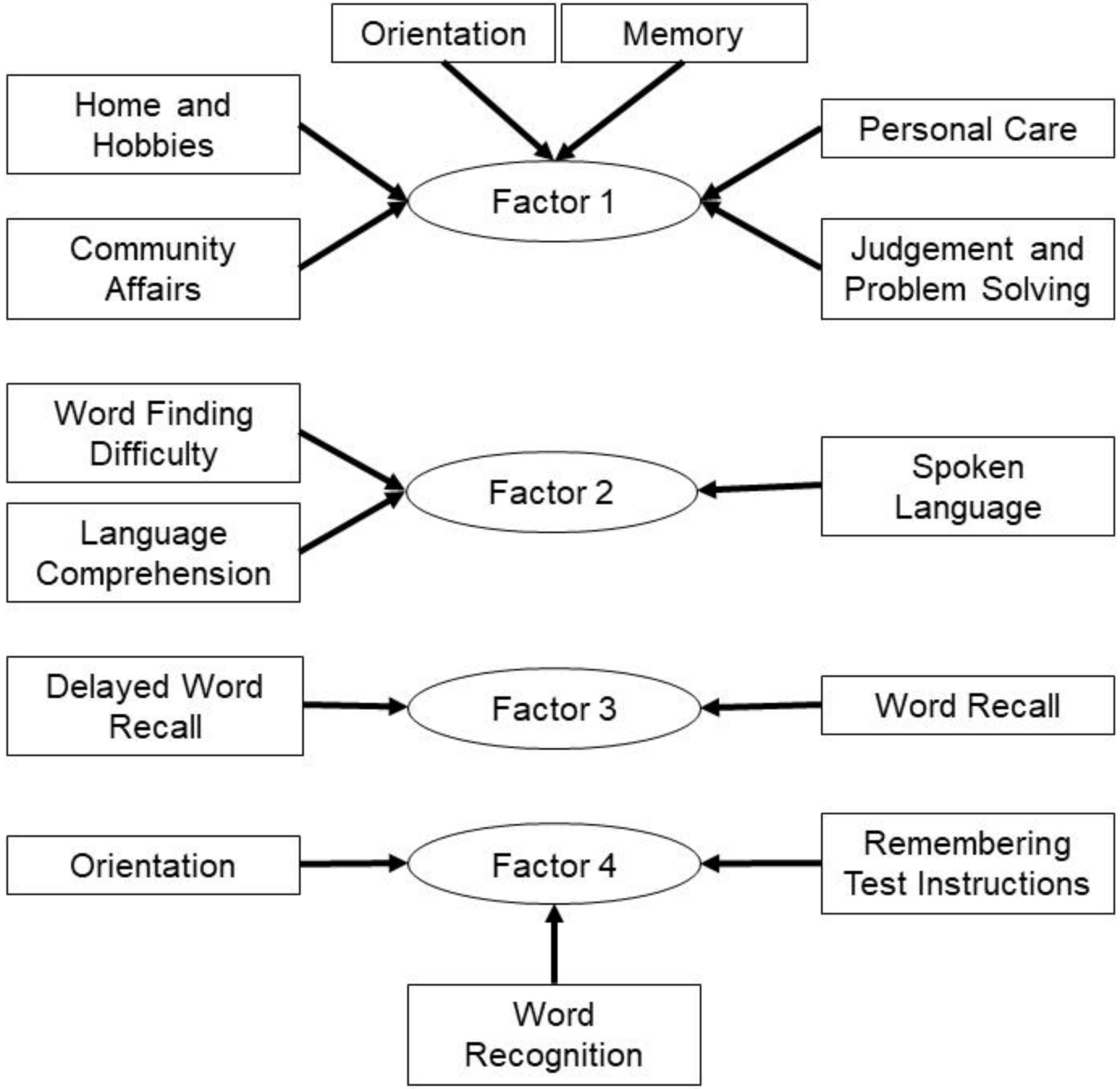

Orientation
Memory
Home and Hobbies
Community Affairs
Factor 1
Personal Care
Judgement and Problem Solving
Word Finding Difficulty
Language Comprehension
Factor 2
Spoken Language
Delayed Word Recall
Factor 3
Word Recall
Orientation
Factor 4
Remembering Test Instructions
Word Recognition

Figure 3

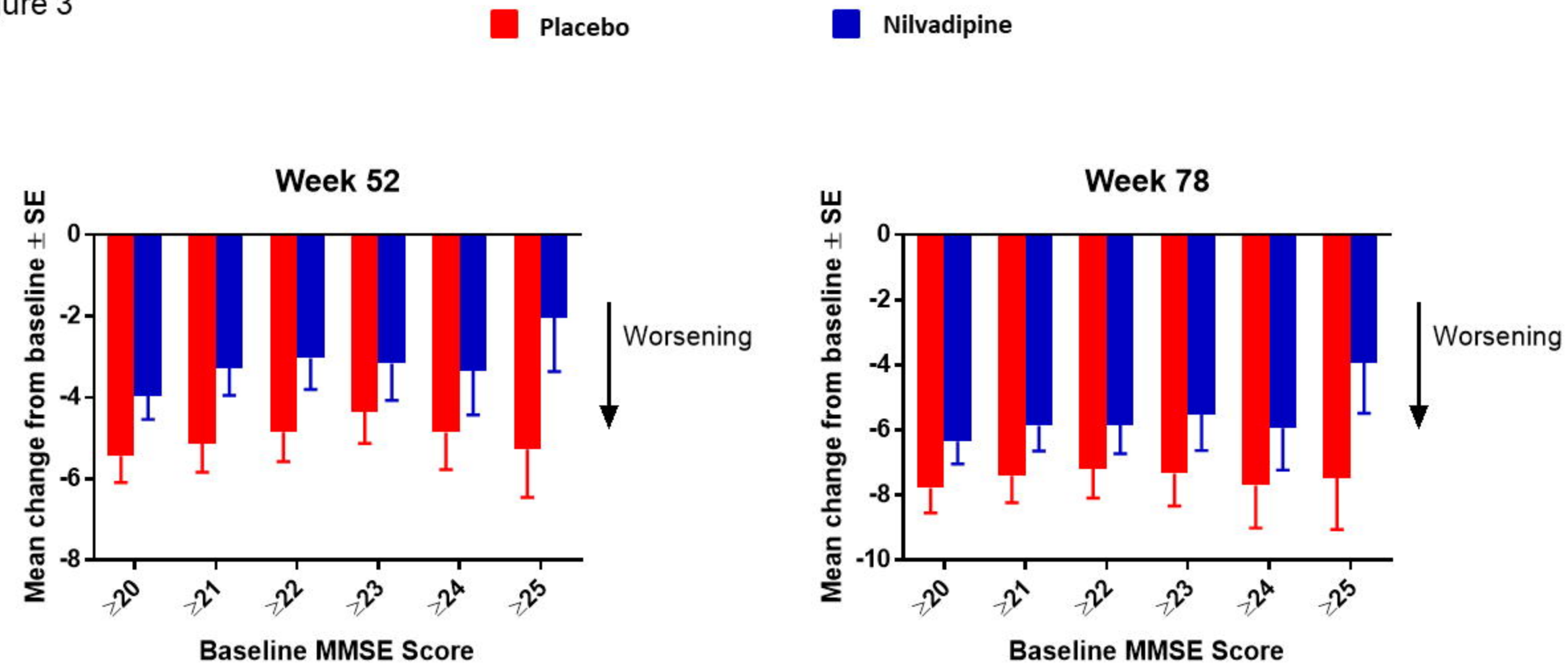
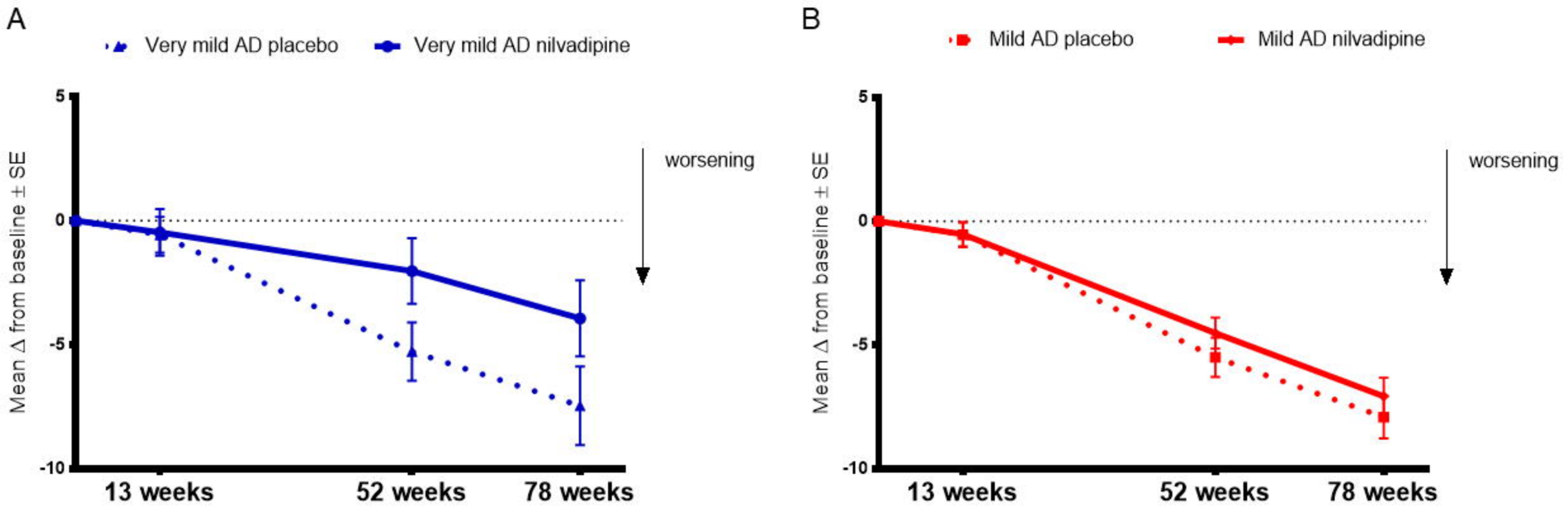
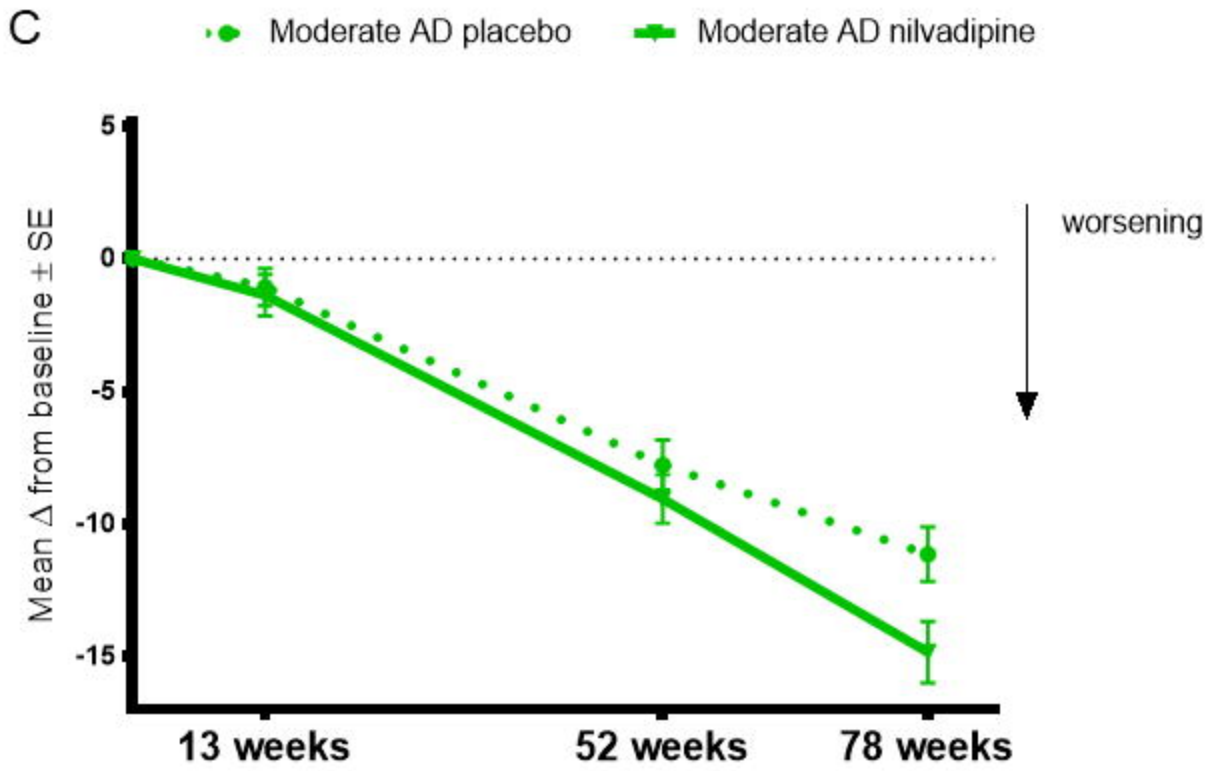

Placebo
Nilvadipine
Week 52
Mean change from baseline ± SE
0
-2
-4
-6
-8
≥20
≥21
≥22
≥23
≥24
≥25
Baseline MMSE Score
Worsening
Week 78
Mean change from baseline ± SE
0
-2
-4
-6
-8
-10
≥20
≥21
≥22
≥23
≥24
≥25
Baseline MMSE Score
Worsening

Figure 4




ADAS-Cog 12

Figure 4




ADAS-Cog 12

Figure 5

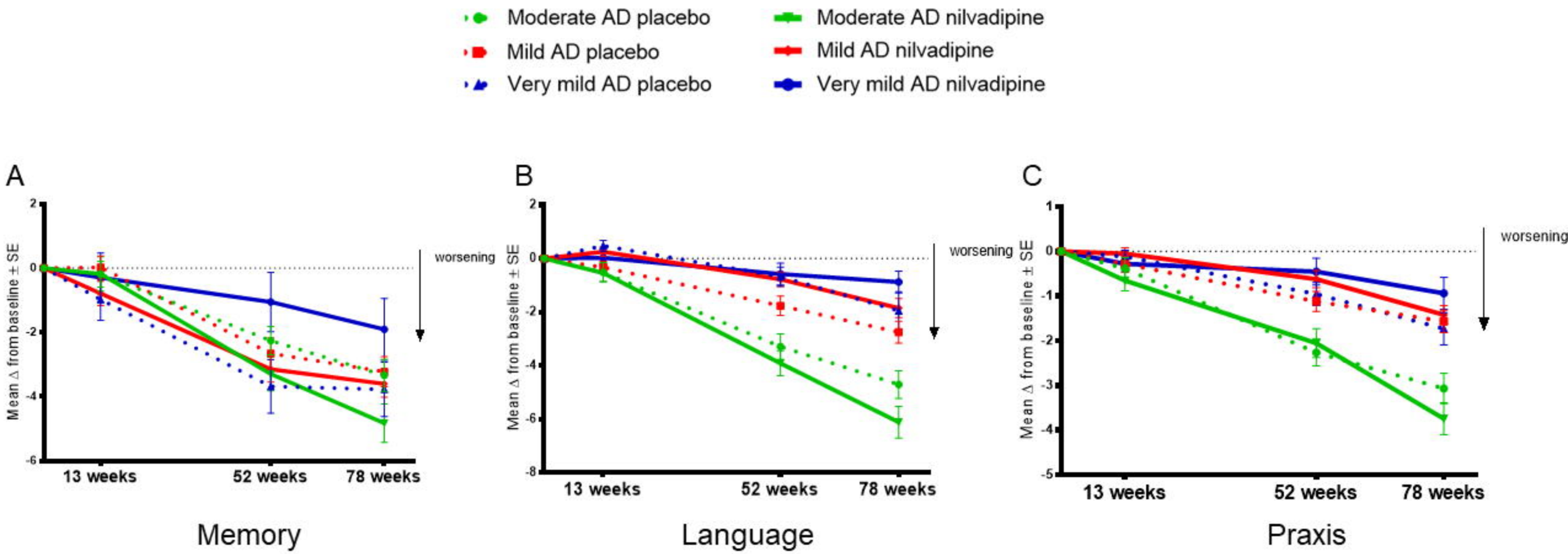
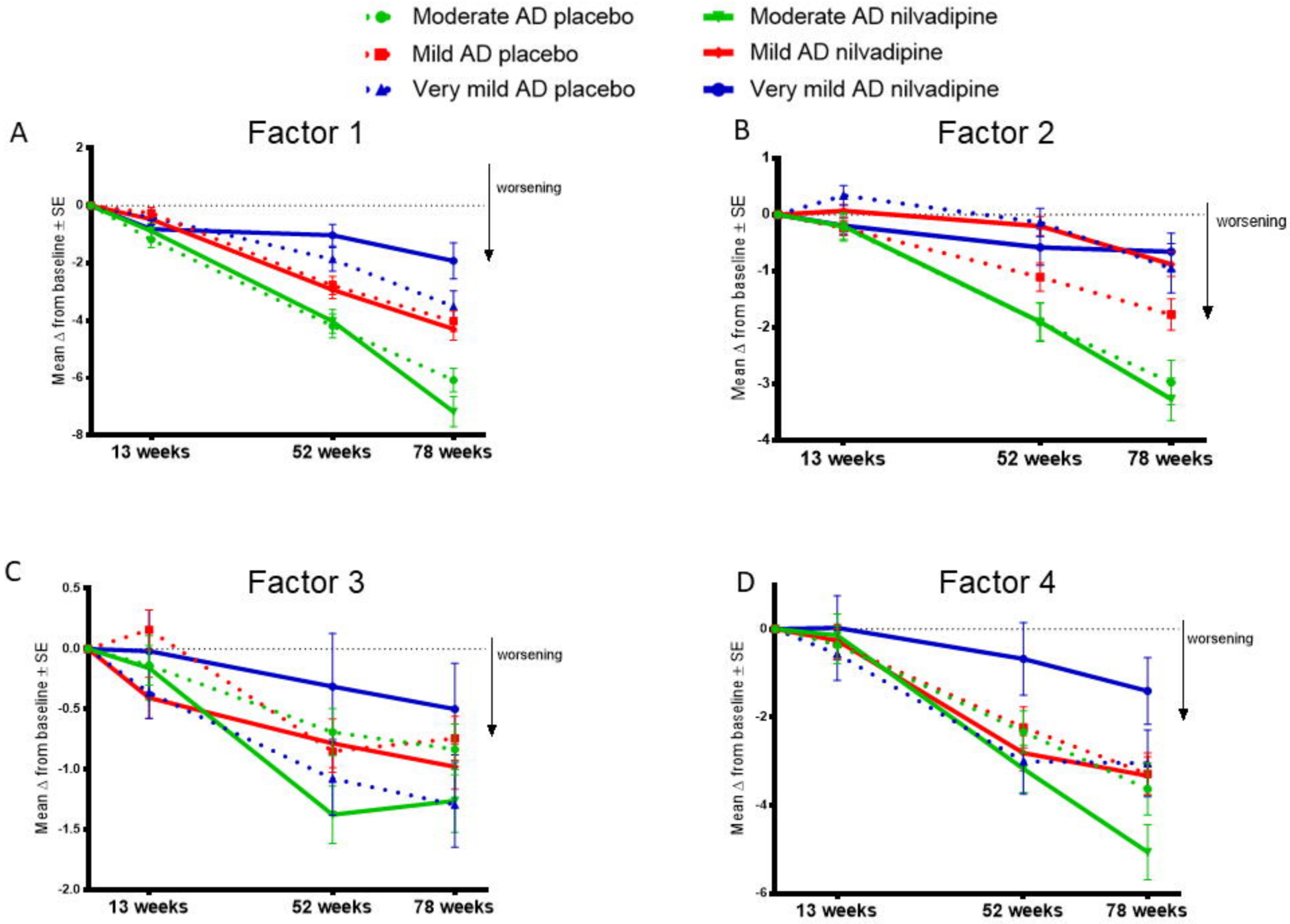

Moderate AD placebo
Mild AD placebo
Very mild AD placebo
Moderate AD nilvadipine
Mild AD nilvadipine
Very mild AD nilvadipine
A
B
C
Mean Δ from baseline ± SE
worsening
13 weeks
52 weeks
78 weeks
Memory
Language
Praxis

Figure 6


Moderate AD placebo
Mild AD placebo
Very mild AD placebo
Moderate AD nilvadipine
Mild AD nilvadipine
Very mild AD nilvadipine
A
Factor 1
B
Factor 2
C
Factor 3
D
Factor 4
Mean Δ from baseline ± SE
worsening
13 weeks
52 weeks
78 weeks