INTREPAD

A randomized trial of naproxen to slow progress of presymptomatic Alzheimer disease

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Abstract

Objective
To evaluate the safety and efficacy of low-dose naproxen for prevention of progression in presymptomatic Alzheimer disease (AD) among cognitively intact persons at risk.

Methods
Investigation of Naproxen Treatment Effects in Pre-symptomatic Alzheimer's Disease (INTREPAD), a 2-year double-masked pharmaco-prevention trial, enrolled 195 AD family history–positive elderly (mean age 63 years) participants screened carefully to exclude cognitive disorder (NCT-02702817). These were randomized 1:1 to naproxen sodium 220 mg twice daily or placebo. Multimodal imaging, neurosensory, cognitive, and (in ~50%) CSF biomarker evaluations were performed at baseline, 3, 12, and 24 months. A modified intent-to-treat analysis considered 160 participants who remained on-treatment through their first follow-up examination. The primary outcome was rate of change in a multimodal composite presymptomatic Alzheimer Progression Score (APS).

Results
Naproxen-treated individuals showed a clear excess of adverse events. Among treatment groups combined, the APS increased by 0.102 points/year (SE 0.014; $p < 10^{-12}$), but rate of change showed little difference by treatment assignment (0.019 points/year). The treatment-related rate ratio of 1.16 (95% confidence interval 0.64–1.96) suggested that naproxen does not reduce the rate of APS progression by more than 36%. Secondary analyses revealed no notable treatment effects on individual CSF, cognitive, or neurosensory biomarker indicators of progressive presymptomatic AD.

Conclusions
In cognitively intact individuals at risk, sustained treatment with naproxen sodium 220 mg twice daily increases frequency of adverse health effects but does not reduce apparent progression of presymptomatic AD.

Classification of evidence
This study provides Class I evidence that, for people who are cognitively intact, low-dose naproxen does not significantly reduce progression of a composite indicator of presymptomatic AD.

RELATED ARTICLES

Glossary

AD = Alzheimer disease; **AE** = adverse event; **APS** = Alzheimer Progression Score; **CI** = confidence interval; **INTREPAD** = Investigation of Naproxen Treatment Effects in Pre-symptomatic Alzheimer's Disease; **ITT** = intent-to-treat; **LP** = lumbar puncture; **MCI** = mild cognitive impairment; **mITT** = modified intent-to-treat; **MoCA** = Montreal Cognitive Assessment; **NSAID** = nonsteroidal anti-inflammatory drug; **p-tau** = phosphorylated tau; **PREVENT-AD** = Pre-symptomatic Evaluation of Novel or Experimental Treatments for Alzheimer's Disease; **RBANS** = Repeatable Battery for Assessment of Neuropsychological Status; **SAE** = serious adverse event; **t-tau** = total tau; **UPSIT** = University of Pennsylvania Smell Identification Test.

Alzheimer disease (AD) includes a decades-long period of presymptomatic biochemical, imaging, neurosensory, and subtle cognitive changes.[1,2] Because cognitive changes emerge gradually, AD prevention trials using cognitive endpoints typically follow thousands of individuals for several years. Improved efficiency may result from use of multimodal composite indicators of early AD pathogenesis as well as subtle cognitive decline. One such indicator, the Alzheimer Progression Score (APS),[3] assesses presymptomatic AD progression using Item Response Theory modeling. Predictive validity of the APS method has been demonstrated recently.[3]

Among potential preventive interventions in presymptomatic AD, nonsteroidal anti-inflammatory drugs (NSAIDs) have retained interest. Numerous observational studies have shown reduced incidence of AD in users of these drugs, at least in relatively young elderly.[4] However, NSAID prevention trials have failed to show benefit, and instead have caused harm to older individuals,[5] or those with imminent symptoms.[6,7] These findings suggest that NSAID prevention trials must screen (preferably younger-old) participants carefully for cognitive or other prodromal AD symptoms.[8] Such principles were applied when designing Investigation of Naproxen Treatment Effects in Pre-symptomatic Alzheimer's Disease (INTREPAD; NCT-02702817), a 2-year double-masked randomized trial of oral naproxen sodium 220 mg twice daily vs placebo for safety and efficacy against progression of AD-related change.

Methods

Primary research question

INTREPAD was designed to test the safety and efficacy of low-dose naproxen in reducing the rate of change of a composite indicator of presymptomatic AD. To that end, Class I evidence is provided here. An important additional objective of the work was to assess the practicality and utility of a more efficient approach to AD prevention trials using novel methods of recruitment, data collection, and analysis. A notable feature was the use of a composite primary efficacy outcome derived from a parallel observational program of cognitive, structural, and functional MRI, and neurosensory measures, along with CSF biomarkers.

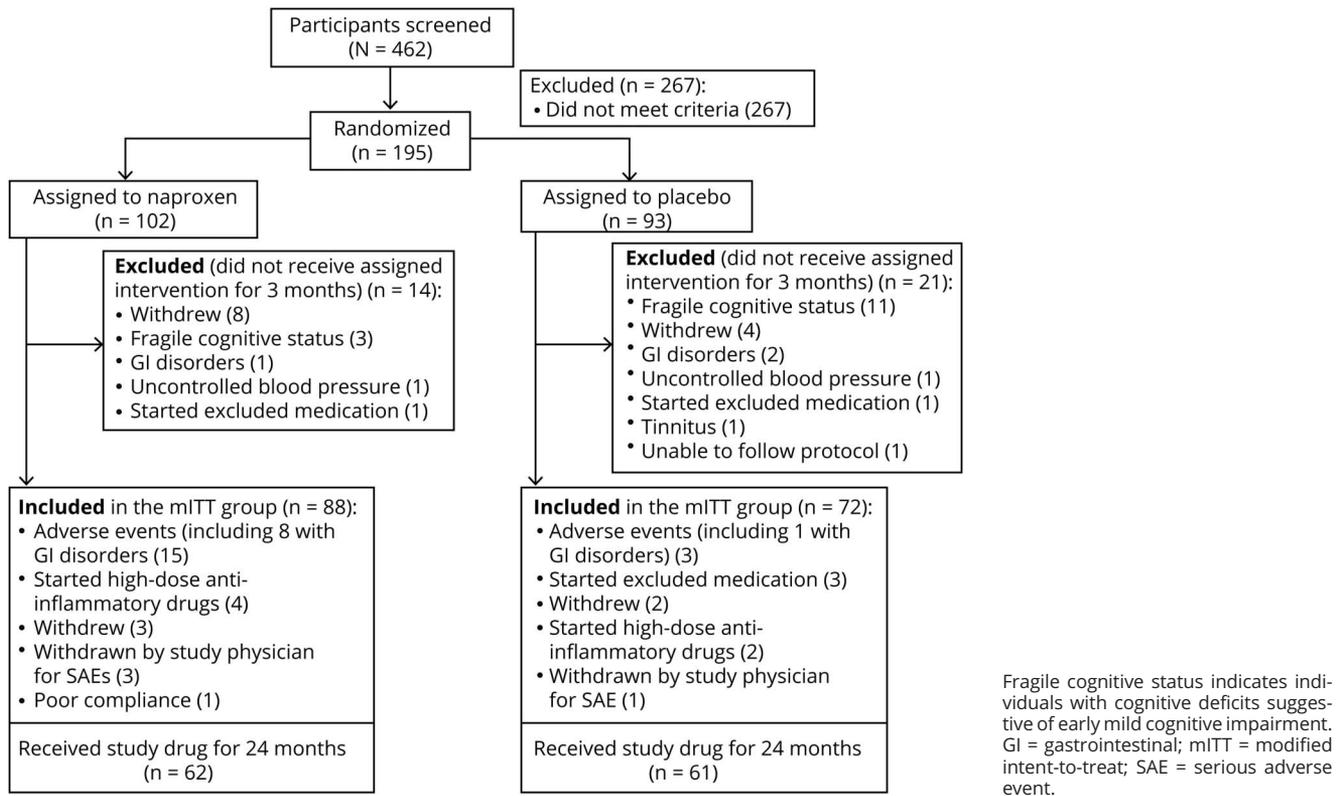### Standard protocol approvals, registrations, and patient consents

INTREPAD (NCT-02702817) was approved by the institutional review board of McGill University. Recruitment occurred between November 2011 and March 2015. Data gathering ended March 28, 2017. All participants provided written informed consent for each trial procedure. Data were collected at the Douglas Mental Health University Institute, an affiliate of McGill University (Montréal). All research procedures complied with the ethical principles of the Declaration of Helsinki. A Data Monitoring Committee reviewed all safety and efficacy data prepared by a contract (unmasked) statistician on October 20, 2016, and upon completion (June 26, 2017).

Overview of participants and trial regimen

We recruited 462 healthy older individuals with a parental or multiple-sibling history of AD for participation in Pre-symptomatic Evaluation of Novel or Experimental Treatments for Alzheimer's Disease (PREVENT-AD), an observational cohort study of healthy persons aged 55+ without evidence of cognitive deficit.[9] Among these, 195 eligible volunteers were randomized in INTREPAD to receive either low-dose naproxen sodium or placebo (figure 1). The primary efficacy analysis considered a modified intent-to-treat (mITT) group of 160 persons who remained on assigned treatments until their first follow-up evaluation 90 days after baseline. In all, 166 (85%) completed their participation per protocol (154 mITT and 12 others in the intent-to-treat (ITT) group with 2 years of follow-up and biomarker assessment). Participants who completed the trial on study treatments numbered 124.

Initial integrity of participants' cognitive abilities was evaluated by telephone interview, followed by in-person assessment using the Montreal Cognitive Assessment (MoCA) and the Clinical Dementia Rating scale.[10,11] In an early protocol change, we further verified intact cognitive status after baseline testing using the trial's principal cognitive assessment measure, the Repeatable Battery for Assessment of Neuropsychological Status (RBANS).[12] As a consequence, 14 enrollees (3 originally assigned to naproxen and 11 to placebo) were considered unsuitable for further participation because of notable cognitive deficits that had escaped detection at baseline, suggesting early mild cognitive impairment (MCI). Final

Figure 1 Consort flow

Fragile cognitive status indicates individuals with cognitive deficits suggestive of early mild cognitive impairment. GI = gastrointestinal; mITT = modified intent-to-treat; SAE = serious adverse event.

determination of cognitive eligibility relied for some on full neuropsychological evaluation.

Eligibility criteria

Other eligibility criteria included (1) at least 1 parent or 2 siblings with AD, (2) age ≥ 60 years, or ≥55 years if within 15 years of youngest-affected relative's onset, (3) health and social stability sufficient to enable participation for 5 years of longitudinal study, and (4) no contraindications to sustained treatment with naproxen sodium. Family history was ascertained from an expert's diagnosis of AD or, when necessary, via a brief, structured questionnaire (e-Methods; doi.org/10.5061/dryad.r58d342). Exclusion criteria included regular use (more than 4 doses per week) of corticosteroids, NSAIDs, other anti-inflammatory/immunosuppressant agents, or aspirin. A complete list of inclusion/exclusion criteria is available in the e-Methods.

Specification of primary efficacy outcome and initial power analysis

The original INTREPAD analysis plan called for a primary efficacy determination based on a composite of data that was not then specified. This composite was under development in data from the parallel (nontrial) parent PREVENT-AD Cohort, with near-identical characteristics.[9] Initial power analyses considered a subset of data from the public use ADNI database. We simulated an attempt to demonstrate a true 25% reduction in the slope of the CSF concentration of total tau

(t-tau) over 1 year, specifying 85% statistical power. This analysis suggested that the needed power could be provided by a sample of 228 aged cognitively normal participants assigned 1:1 to active drug vs placebo. We assumed that greater power would be provided by 2 (rather than 1) years of observation, and thus specified a target enrollment of 200 with an assumption that ∼80% would remain in an mITT primary analysis pool of persons who remained on study treatments through their first follow-up examination (but note below that results belied these assumptions).

When the primary efficacy outcome (APS)[3] was fully specified, we performed a similar simulation in the parallel, nontrial PREVENT-AD Cohort. This simulation now relied on the Cohort's observed slope, random intercept variance, and error variance using a longitudinal random effects model. It suggested that 160 participants would afford 85% power to detect only a 50% difference in slope between 2 study arms, or 68% power to detect a 40% difference. Because the PREVENT-AD data did not include CSF biomarker measures, however, we expected that the trial's CSF assay results would provide a substantial increase in power, as had been the case in the observational BIOCARD study[13] (see Discussion).

Randomization, masking, and provision of study drug

Using randomization.com, participants were randomized 1:1 in 34 permuted blocks of 6 to identical-appearing tablets of

naproxen sodium 220 mg or placebo, both generously donated by Pharmascience (Montreal, Canada), for administration twice daily with meals. The Douglas Hospital pharmacy stored and prepared study drug in sealed dosage packets for each participant visit. Participants, the principal investigator, study staff, and all clinicians responsible for assessments or marker measures remained masked to treatment assignment until safety and efficacy analyses were complete. A protocol for interim unmasking was available but not needed. Only an external statistician (Daniel Morissette) had advance access to treatment assignment.

Assessment methods

Figure 2 shows the timeline for data gathering. Complete evaluations were performed at baseline and after 3, 12, and 24 months of treatment.

Safety

Follow-up interviews (on-site or by telephone) for adverse events (AEs) were administered ad hoc or, at a minimum, every 3 months using structured medical history and review-of-system questionnaires. On-site safety evaluation included routine laboratory studies, ECG, and a brief physical and neurologic examination. Potentially important incidental MRI and other newly discovered health risks were referred for expert review. Research nurses rated AEs using the Cancer Therapy Evaluation Program Common Terminology Criteria, version 3.0. AEs were graded as mild, moderate, or severe after physician review, and each AE was also assigned a Preferred Term and a System Organ Class using the Medical Dictionary for Regulatory Activities classification system, version 19.1. Serious AEs (SAEs) that were life-threatening or required hospitalization were reported in real time to the McGill Research Ethics Board. Relationship of AEs and SAEs to treatment (assuming assignment to naproxen) was assessed by a study physician. Elective surgeries were not considered SAEs.

Compliance

Participants were asked to bring unused supplies of drug to trial visits. Research nurses evaluated compliance at each visit, inquiring when indicated into reasons for missed doses.

Cognitive and neurosensory performance

At baseline, 3, 12, and 24 months, neuropsychological performance was measured using the RBANS, which evaluates 5 cognitive domains (immediate memory, delayed memory, attention, language, and visuospatial abilities) and a total summary score. The RBANS is available in 4 equivalent versions (for longitudinal assessment) in both French and English. Version A was administered at baseline, and alternate forms were used in random order at follow-up visits. We developed correction factors to improve version equivalence and scored results without correction for age (often used clinically; see e-Methods, doi.org/10.5061/dryad.r58d342).

At each visit's fMRI session, participants were also given alternate versions of an episodic memory task (encoding and retrieval of objects).[14] They were asked to identify whether items had been observed during the encoding period or were new at the retrieval session (details in e-Methods, doi.org/10.5061/dryad.r58d342). A correct response required a "hit" or correct rejection. Odor identification, a neurosensory faculty, was also tested using the 40-item "scratch and sniff" University of Pennsylvania Smell Identification Test (UPSIT).[15,16] The latter, comprising 40 items administered in 4 randomly ordered booklets, was available in both French and English.

Neuroimaging markers

Brain structural and functional MRI were performed at baseline and 3-, 12-, and 24-month visits on a Siemens TIM Trio 3T MRI system (Siemens Medical Solutions, Erlangen, Germany) using the Siemens standard 12-channel head coil (figure 2 and e-Methods, doi.org/10.5061/dryad.r58d342).

Figure 2 Trial timeline
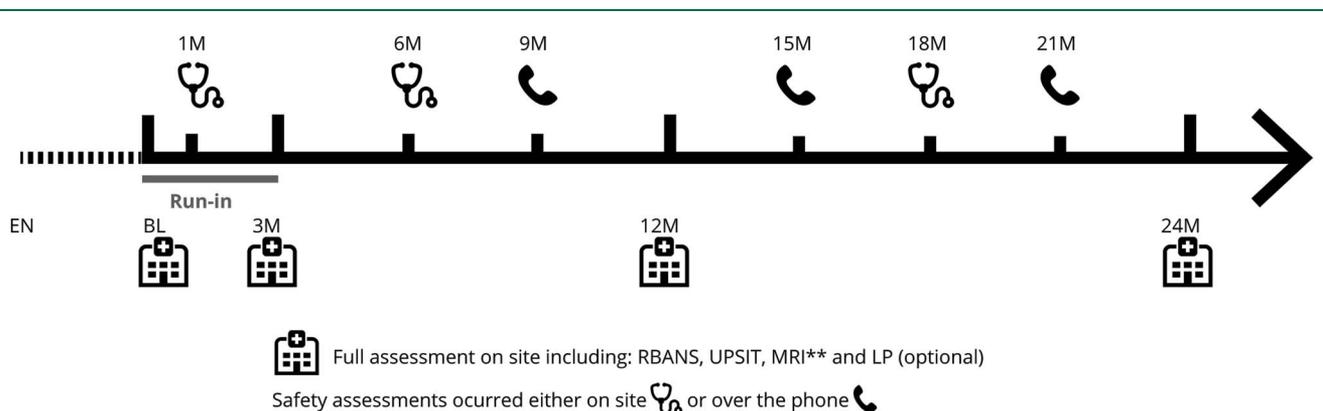
Full assessment (on site): baseline (BL), 3 months, 12 months, 24 months. Safety follow-up (on site): 1 month, 6 months, 18 months. Safety follow-up (telephone): 9 months, 15 months, 21 months. **T1-weighted (EN, BL, 3 months, 12 months, 24 months), fluid-attenuated inversion recovery (EN, 24 months), diffusion-weighted imaging (EN, 24 months), arterial spin labeling (BL, 3 months, 12 months, 24 months), resting-state fMRI (BL, 3 months, 12 months, 24 months), gradient echo quantitative T2* task (BL, 3 months, 12 months, 24 months), task fMRI (BL, 3 months, 12 months, 24 months). EN = enrollment; LP = lumbar puncture; RBANS = repeatable battery for assessment of neuropsychological status; UPSIT = University of Pennsylvania Smell Identification Test.

Using conventional pipelines, averages of gray matter density were calculated for 78 regions based on T1-weighted images using the SPM12 toolbox to define probabilistic gray matter maps. Cortical thickness was estimated from T1-weighted images using version 1.12 of the CIVET pipeline.[17] Brain volumes were computed from the same images using a volumetric pipeline.[18] Regional cerebral blood flow was evaluated using quantitative pipelines from single-echo pseudo-continuous arterial spin labeling acquisitions.[19]

CSF biomarkers

A subset of 93 participants in the mITT pool volunteered for up to 4 serial lumbar punctures (LPs) over the 2-year trial interval following their clinical and imaging evaluations. After an overnight fast, LPs were performed by P.R.-N. using an introducer and the Sprotte 24-gauge atraumatic needle. Samples of 20–30 mL were withdrawn by syringe and aliquoted (500 μL) into propylene cryotubes for storage at −80°C. We followed procedures specified by the BIOMARK-APD consortium of the EU Joint Program in Neurodegenerative Disease to measure CSF concentrations of the AD biomarkers $A\beta_{1-42}$, t-tau, and phosphorylated tau (p-tau) with the Innotest ELISA assay kit (Fujirebio, Ghent, Belgium).

APOE genotype

APOE genotype was determined using RT-PCR amplified DNA and the PyroMark Q96 pyrosequencer (Qiagen, Toronto, Canada), as described previously.[16]

Primary efficacy outcome: The composite APS

The primary efficacy outcome was annual rate of change in the APS using marker weights estimated beforehand in the nontrial PREVENT-AD Cohort. The APS is a composite that incorporates multimodal imaging, neurosensory, cognitive, and CSF markers, based on an assumption that change in each of these arises from a single underlying latent process (AD pathogenesis). Its scores are scaled as a standard normal distribution, with higher scores denoting increasing severity. Constituent measures are summarized in table 1. At each measurement, a uniform scheme of weightings for individual markers yielded a composite summary score. All available data were used to estimate individual scores, and missing data were accommodated in a process that essentially averaged over missing values. To verify robustness to missing data, Gross et al.[20] had used iterative leave-one-out analyses in a similar outcome measure comprising 6 markers. Leoutsakos et al.[3] also examined effects of missing CSF data on the APS, noting similarly satisfactory findings. The APS approach had been validated using data from the BIOCARD study,[13] before being incorporated into INTREPAD efficacy analyses. In BIO-CARD, the APS approach showed substantial abilities to predict subsequent conversion to MCI or AD dementia.[3] Analyses there also showed temporal measurement invariance. Before applying the PREVENT-AD cohort-derived marker weights to the analysis of INTREPAD, we demonstrated that they provided excellent performance in the trial sample.[3] However, the trial data (table 1) included 2 variables

Table 1 Variables included in the Alzheimer Progression Score

Measures	Variables
Cognitive measures	RBANS attention index score
	RBANS immediate memory index score
	Item recognition task
Neurosensory measure	UPSIT total score
Gray matter density measures	Bilateral entorhinal cortex
	Bilateral lingual cortex
	Bilateral putamen
Gray matter cortical thickness measures	Right superior parietal gyrus
	Right superior dorsal frontal gyrus
Cerebral blood flow measures	Right rostral anterior cingulate cortex
Brain volume variables	Bilateral hippocampus volumes
	Lateral ventricular volume
CSF measures	Total tau concentrations (pg/mL)
	β-amyloid$_{1-42}$ concentrations (pg/mL)

Abbreviations: RBANS = Repeatable Battery for Assessment of Neuropsychological Status; UPSIT = University of Pennsylvania Smell Identification Test.

(CSF t-tau and $A\beta_{1-42}$ levels) that were not available from nontrial participants. We incorporated these measures only after verifying that doing so did not materially alter the weights for the remaining variables. Full specification of variables and APS parameters preceded unmasking of treatment assignment.

Statistical analysis

We followed a statistical analysis plan finalized on June 8, 2017, by J.L. and the PREVENT-AD Research Group. To assure consideration to potentially important AEs occurring during the first 3 months of the trial, safety analyses were based on the full intention-to-treat population (n = 195). These analyses were based on summary listings of AEs, using a χ^2 test for pairwise comparisons. The baseline characteristics of the treatment groups were compared pro forma using Fisher exact (for sex), χ^2 (for number of *APOE* ε4 alleles), and 2-sample t tests (for age, education, parental age at AD onset, MoCA score, and APS).

The primary efficacy analysis was based on the mITT sample of participants who had remained on treatment through at least one follow-up (3-month) assessment (n = 160). Secondary outcomes were rate of change in cognition (RBANS total score), olfaction (UPSIT score), and CSF biomarkers of AD ($A\beta_{1-42}$, t-tau, p-tau, t-tau/$A\beta_{1-42}$, and p-tau/$A\beta_{1-42}$ ratio)

extending over the 24 months of treatment. For both primary and secondary efficacy analyses, we used longitudinal linear random effects models (random intercepts) to assess between-group differences in rates of change. We performed an additional post hoc analysis that included baseline APS score as a covariate. We also constructed additional exploratory models to test treatment effects on APS rate of change after first assigning participants into tertiles based on their baseline CSF $A\beta_{1-42}$.

Version 9.4 of the SAS (SAS Institute, Cary, NC) statistical package and R (Core Team 2014) were used for analyses except for post hoc exploratory analyses, which were performed using MATLAB. APS scores were calculated using STAT, R, and MPLUS.[21] All statistical tests were 2-sided. A p value ≤0.05 was considered to indicate statistical significance.

Data sharing

All de-identified data and related documentation from this trial are available upon request to qualified researchers without limit of time, subject a standard data sharing agreement. The PREVENT-AD program is currently developing a less restrictive approach to data sharing through the Canadian Open Neuroscience Platform.

Results

Enrollment and study completion data

The naproxen- and placebo-assigned groups included 102 and 93 participants. The mITT analysis groups included 88 and 72 of these. The main reasons for exclusion of the remaining 35 were apparent ineligibility (discovered typically following review of baseline cognitive testing; n = 14), early appearance of intolerable adverse effects (n = 6), or voluntary withdrawal (many reasons given; n = 12). Compliance-to-completion rates (24 months on study drug) were 62% for participants

assigned to naproxen and 66% for those given placebo ($p = 0.579$; figure 1). After the 3-month run-in, the most common cause for drug discontinuation was occurrence of new AEs (12 naproxen-assigned and 3 placebo-assigned individuals; figure 1).

Baseline characteristics

There were no important differences between naproxen and placebo-treated groups with respect to age, sex, or education. All participants were Caucasian, and there were no substantial imbalances across groups in *APOE* ε4 status, parental age at AD onset, or MoCA score. As noted below, however, we observed an unexpected difference by treatment assignment in baseline APS values in the mITT pool (table 2).

Concomitant medications

Of the 102 naproxen-assigned persons in the ITT pool, 85 (83%) initiated use of concomitant medicines, regularly or for short intervals, over the interval of the trial. The comparable figure for those assigned to placebo was 66/93 (71%; $p = 0.04$; table e-3, doi.org/10.5061/dryad.r58d342). The apparent imbalance was attributable principally to initiation of lipid-lowering drugs during the trial but, notably, a similar imbalance was not evident in the mITT analysis pool. Baseline or anytime use of such drugs was also similar across the treatment arms in the mITT group and, accordingly, post hoc statistical adjustment for their use brought no appreciable change in the primary outcome results.

Safety outcomes

Table 3 shows the frequency of AEs occurring in ≥10% of either treatment arm. Gastrointestinal AEs prompted study drug discontinuation in 9 naproxen-assigned and 3 placebo-assigned participants. Constipation, dyspnea, hypertension, and petechiae were also substantially more common in persons receiving naproxen. However, as expected, this group reported pain less frequently. Overall, reports of any AE were

Table 2 Baseline characteristics of Investigation of Naproxen Treatment Effects in Pre-symptomatic Alzheimer's Disease (INTREPAD) participants

Characteristics	Total	Naproxen	Placebo	*p* For difference
No. of participants	195	102	93	NS
Age, y, mean ± SD	63.3 ± 5.6	64.0 ± 5.9	62.6 ± 5.0	NS
Male sex, n (%)	51 (26)	25 (25)	26 (28)	NS
Education, y, mean ± SD	15.2 ± 3.4	15.0 ± 3.2	15.5 ± 3.6	NS
Parental age at AD onset, y, mean ± SD	73.3 ± 7.7	72.8 ± 7.6	73.8 ± 7.8	NS
MoCA score (out of 30), mean ± SD	28.0 ± 1.6	28.0 ± 1.5	28.0 ± 1.6	NS
***APOE* ε4 carriers, n (%)**	73 (37)	37 (36)	36 (39)	NS
Baseline data (mITT)	n = 160	n = 88	n = 72	
APS	−0.09 ± 0.86	0.021 ± 0.8	−0.214 ± 0.9	0.06

Abbreviations: AD = Alzheimer disease; APS = Alzheimer Progression Score; mITT = modified intent-to-treat; MoCA = Montreal Cognitive Assessment.
No observable difference between the 2 groups, except for APS at baseline.

Table 3 Adverse events (AEs)

	Naproxen (n = 102)	Placebo (n = 93)	p Value
AEs			
Constipation	19 (19)	6 (6)	0.011
Dyspnea	23 (23)	10 (11)	0.028
Heartburn	31 (30)	17 (18)	0.05
Peripheral edema	25 (25)	14 (15)	0.1
Hypertension	19 (19)	8 (9)	0.04
Petechiae	12 (12)	2 (2)	0.009
Pain	20 (20)	28 (30)	0.09
Serious AEs	8 (8)	2 (2)	0.07
Vascular/cardiac events	4[a]	1	
Cancers	1	1	
Musculoskeletal/ injuries	3	—	

AE table represents n (%) of participants. If an individual had the same adverse experiences multiple times during the trial, he or she was counted only once.

[a] Two SAEs in this category were judged to have a clear or possible relationship with study drug. For detailed description of SAEs, see e-Results (doi.org/10.5061/dryad.r58d342).

more common in naproxen-assigned persons (98% vs 89%; $p = 0.015$). The principal contributors to this excess were vascular disorders, which were more common in the naproxen-assigned group ($p = 0.023$).

Of 10 participants reporting SAEs over 2 years (table e-4, doi.org/10.5061/dryad.r58d342), 8 had been assigned to naproxen. Three SAEs required study drug discontinuation, while 2 prompted treatment interruption. A further 3 participants were unable or unwilling (some upon physician's advice) to continue participation. No suspected unexpected serious adverse reaction occurred during the trial.

Among blood safety measures, both hemoglobin and hematocrit decreased in persons assigned to naproxen, but not in placebo-treated individuals. Between-group comparison of these measures showed strong differences at all time points except at 24 months (figures e-1 and e-2, doi.org/10.5061/dryad.r58d342). No differences were found in the 2 treatment groups with respect to other safety measurements (weight, pulse rate, systolic and diastolic blood pressure).

Primary efficacy outcome

Analyses of the primary outcome are summarized in figures 3 and 4. Among the combined treatment groups, the APS showed a clear increase over the 2-year trial period ($\beta = 0.101$ standard units/year; SE = 0.014; 95% confidence interval [CI] 0.074–0.130; $p < 0.001$). However, this change did not differ meaningfully between naproxen- and placebo-assigned participants after 3, 12, or 24 months of treatment. A longitudinal linear random effects model for APS showed a slight increase, if anything, in rate of change among naproxen-assigned persons, but this was well within chance expectation (time-by-treatment interaction $\beta = +0.019$ APS units/year for naproxen vs placebo; SE = 0.03; 95% CI –0.037 to +0.074; $p = 0.51$). The APS ratio for rate of change comparing naproxen- vs placebo-assigned mITT participants was 1.16 (bootstrapped 95% CI 0.64–1.96).

Secondary efficacy analyses

We used similar linear random effects models in the mITT sample to evaluate treatment-related differences in rate of change of RBANS total index score, UPSIT score, and CSF AD biomarkers ($A\beta_{1-42}$, t-tau, p-tau, t-tau/$A\beta_{1-42}$, and p-tau/$A\beta_{1-42}$). None of these comparisons suggested any benefit of

**Figure 3** Treatment effects on Alzheimer Progression Score (APS)
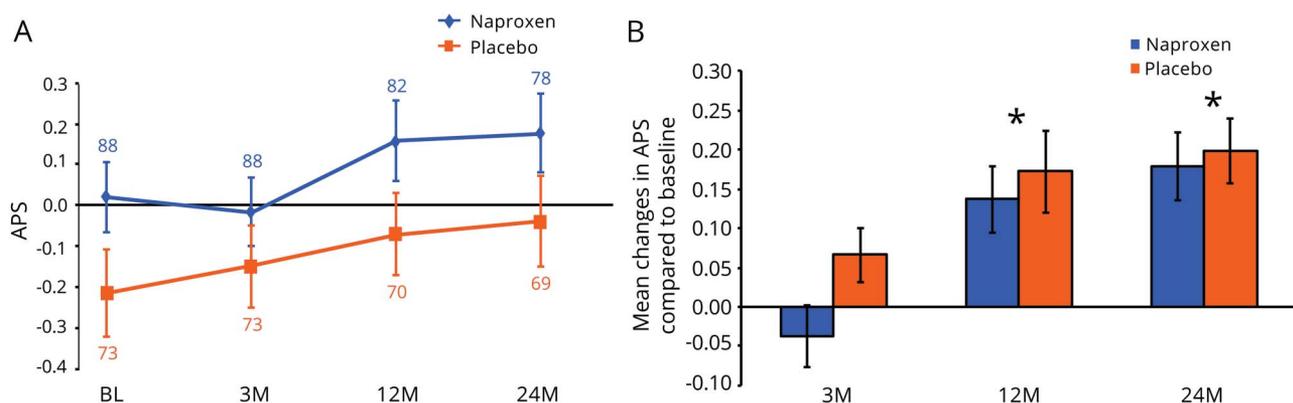
Results for primary and exploratory secondary outcomes are represented. (A) There was no meaningful difference in APS rate of change between treatment groups. (B) Mean change from baseline (± standard error of the mean) in APS did not differ between the 2 treatment groups at any time during the treatment interval. However, APS for both groups increased after 12 and 24 months (*$p < 0.05$). Data are represented as point estimates (group means) with error bars (standard error of the mean). BL = Baseline; M = months.

A. Cognitive and sensory measures
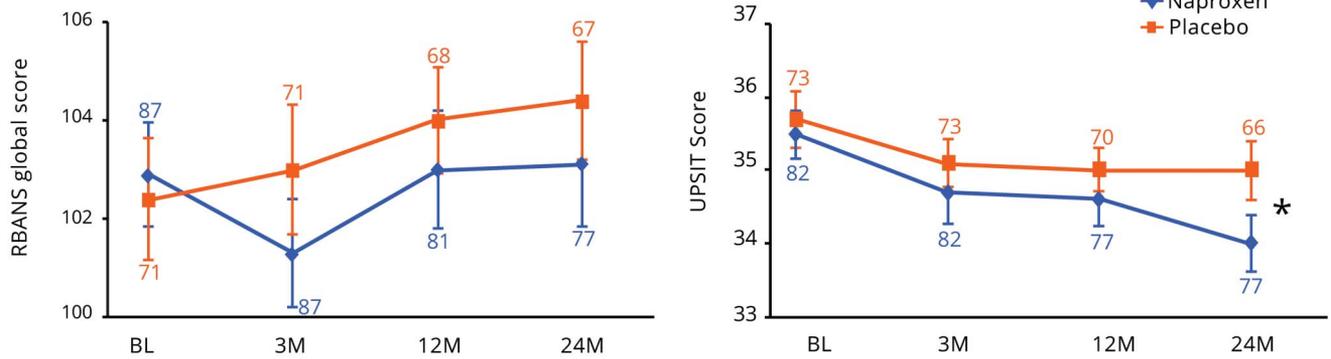
B. Fluid biomarker measures
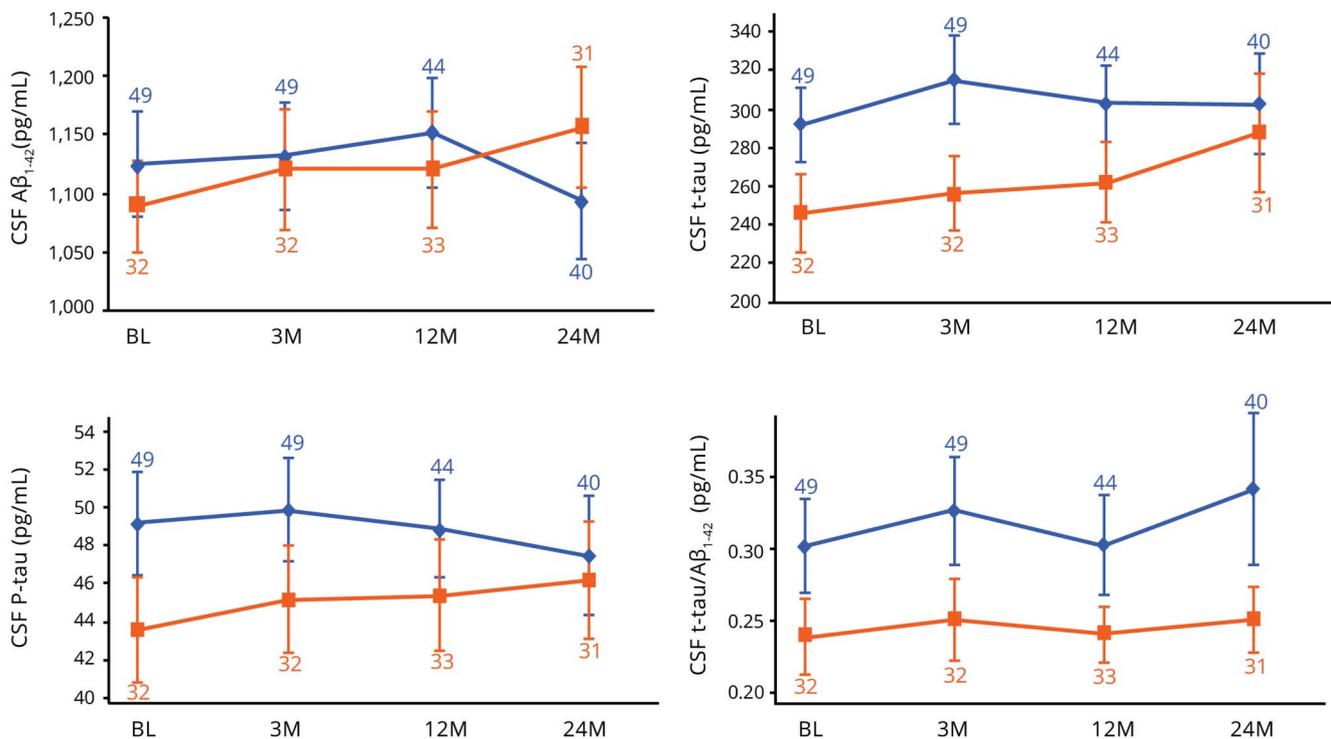
Linear mixed effect models did not indicate any difference between naproxen- and placebo-assigned groups in rate of change of (A) cognitive or neurosensory or (B) biological markers of Alzheimer disease. University of Pennsylvania Smell Identification Test (UPSIT) scores decreased over the 2-year trial period for the whole group (*$p < 0.05$). Data are represented as point estimates (group means) with error bars (standard error of the mean). BL = baseline; p-tau = phosphorylated tau; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; t-tau = total tau.

naproxen treatment. Indeed, UPSIT results suggested a trend toward harm ($\beta = -0.320$, SE = 0.196; 95% CI -0.704 to $+0.064$; $p = 0.102$; figure 2B). Over the treatment interval, 4 INTREPAD enrollees (3 naproxen-assigned and 1 placebo-assigned) developed MCI[22] or other suggestive cognitive deficiency sufficient to prompt discontinuation of study drug.

Exploratory efficacy analyses

Addition of covariates for *APOE* ε4 carrier status, age at baseline, years of education, and sex, as well as their interaction with time, did not materially affect the above findings.

As noted above, we observed that the naproxen-assigned group had higher baseline APS scores than placebo-assigned participants. This finding prompted us to carry out a post hoc addition of a term for baseline APS in the model used for the primary endpoint analysis. Addition of this term brought no consequential change from the primary endpoint results. We also analyzed whether higher baseline APS was associated with an increased rate of change in the outcome, regardless of treatment assignment. No such association was found. After partitioning participants into tertiles of $A\beta_{1-42}$ concentration and testing for a triple interaction with time and treatment

assignment, we also observed no effect of naproxen treatment on rate of change in APS among the different CSF Aβ$_{1-42}$ tertiles.

Discussion

To assess the potential of the common NSAID naproxen sodium for prevention of AD, we tested this agent in INTREPAD, a 2-year randomized placebo-controlled trial among cognitively healthy persons at increased risk of dementia. We observed a clear increase over time in the trial's primary efficacy outcome (the APS composite representing several imaging metrics, a neurosensory modality, 3 cognitive measures, and, when available, CSF biomarkers). Assignment to naproxen produced an unambiguous increase in adverse events but no meaningful alteration in the rate of change for this primary efficacy outcome. Overall, our results suggest 2 points for discussion: (1) evaluation of a design intended to increase efficiency of AD prevention trials and (2) the trial's safety and efficacy results, and their ethical implications.

We employed several design features intended to improve efficiency for INTREPAD and, by implication, other AD prevention trials. First, we attempted to enrich participants' proportion of persons vulnerable to AD by requiring that enrollees have an affected parent or multiple siblings.[23] Enrichment by requirement of a first-degree relative with AD is not novel, but the INTREPAD family history criteria were stronger. Especially in today's environment of greater longevity and increased awareness of AD-affected status (thus, ready identification), this more restrictive method is increasingly practical. It would seem easier to implement than a requirement of amyloid pathology demonstrable by PET[24] or CSF analysis, or even homozygosity at the APOE ε4 risk allele.[25] How this enhanced family history method of enrichment compares with the latter, more costly or invasive methods is unknown. However, we have recently shown that proximity to an index relative's age at symptom onset is related to increased AD biomarker load.[26] This same metric was associated in a separate cohort with brain changes predictive of time to symptom onset.[27] Given the method's reduced costs and subject burden, a cost/benefit comparison with other methods probably deserves consideration.

Second, we selected the composite APS as the trial's primary outcome. Relative to any single cognitive or biomarker indicator, composite outcomes of this sort should logically offer greater inference, especially in relatively early-stage presymptomatic AD. We chose the APS in preference to several similar composite indicators that had been less extensively validated.[28,29] While additional efforts to validate the APS are warranted, it has shown demonstrable utility for evaluation of presymptomatic AD progress. Specifically, in BIOCARD, a 1 standard unit increase in APS predicted a 5-fold greater hazard of diagnostic progression over time.[3] In the parallel PREVENT-AD cohort, we performed several simulations to compare the statistical power of the APS to that of its constituent markers. The APS provided more information than did any single endpoint, including all cognitive measures, and it also offered improved performance over a simple summing of z scores of its individual components (data not shown). We therefore suggest that the APS or similar multimodal composites show promise for prevention trials like INTREPAD.

Nonetheless, post hoc analyses suggested that our methods resulted in a trial with substantially less statistical power than had been originally projected. Our original power estimates were based in part on expectation that considerable information would be contributed by CSF biomarker data, available from more than half of the participants. That turned out not to be the case and, accordingly, the trial outcome's CI was sufficiently broad to suggest a notable possibility of type II error. Now having data on rate of change in the trial's outcomes, we can estimate that 2,250 person-years of observation (e.g., a sample of 1,125 followed over 2 years, or 563 followed for 4 years) would have been required for 80% power to detect a 30% reduction in the rate of APS change. Although much higher than originally estimated, this number still represents a considerable improvement over requirements of conventional prevention trial designs. For example, the ongoing A4 trial will follow ∼1,150 persons over ∼4.6 years (5,290 person-years) for its primary cognitive outcome.[24,30] Similarly, the TOMMORROW trial had originally estimated a requirement of 5,800 persons over ∼4 years (29,200 person-years).[31] By comparison, even ignoring costs of PET scans in the former or serial detailed psychometric assessments in the latter, and assuming costs proportional only to person-years of observation, the INTREPAD design may achieve cost savings of 57%–90% over traditional methods.

The INTREPAD safety results affirm prior data suggesting that, even in relatively low dosage among younger elderly persons, naproxen and other NSAIDs provoke harm in several health outcomes.[32–34] Our findings that these risks can be held within bounds by careful monitoring does not obviate the ethical concern that NSAID treatments are potentially harmful and should be given for AD prevention only if they produce substantial reduction of AD risk. The INTREPAD results provide no evidence for such a reduction. This result is especially salient, inasmuch as the trial sample was relatively young for this sort of work, and chosen for a favorable risk/benefit balance in relation to NSAID treatment. Specifically, participants were on average ∼10 years younger than their affected parent or first-affected sibling, and were meticulously screened for incipient cognitive disorder.[35] These attributes appear to weaken arguments that earlier NSAID trials failed to show benefit because their aging samples were too old or too near the cusp of symptom onset.[36–38]

The null efficacy outcomes of INTREPAD are reinforced by several observations. The CI around the trial's efficacy rate ratio suggests 95% certainty that the true treatment-related reduction of AD risk in this trial (or, presumably, in similar

samples) does not exceed 36%. This conclusion is buttressed by a consistent absence of apparent benefit on any of the trial's secondary or exploratory outcomes. These results recall observations in ADAPT[8] and prior NSAID treatment and prevention trials with null or negative results. While we cannot exclude possible benefits of NSAIDs in middle life, we can now suggest that such benefits would be nearly impossible to demonstrate in randomized prevention trials.

As regards our further objective of testing or demonstrating methods for improving efficiency in AD prevention trials, we offer several observations. Our enrichment strategy requiring parental or multiple-sibling family history of AD might have been improved by a further requirement specifying participants' age in relation to their index relatives' onsets. While evidently more informative than any single biomarker, or a composite of only a few such markers, our outcome could today probably be improved by incorporation of newer salient markers of preclinical disease. These might include threshold values for the traditional CSF biomarkers $A\beta_{1-42}$, t-tau, or p-tau, or possibly neurofilament light chain as a precondition to enrollment. Power estimation and sample size requirement for such work should be clearer now than when we began this work. In this last sense, INTREPAD may be viewed as providing an approximate benchmark for work with samples having similar baseline characteristics.

In all events, this work has left us with extreme pessimism regarding any possible role of NSAIDs in AD prevention. Instead, our results may suggest reconsideration of inflammatory diseases (or a proinflammatory diathesis) as a possible explanation for the reduced AD incidence among NSAID users in observational studies.[8]

Appendix 1 Authors

Name	Location	Role	Contribution
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Jeannie-Marie Leoutsakos, PhD	John Hopkins University, Baltimore, MD	Author	Development of primary outcome Alzheimer Progression Score, data analysis
Cécile Madjar, MSc	McGill Centre for Integrative Neuroscience, McGill University, Montreal, Canada	Author	Data management, conceptualization of database, support for data analysis
Marie-Élyse Lafaille-Magnan, PhD	McGill University, Montreal, Canada	Author	Major role in data acquisition (olfaction), study design (randomization)
Melissa Savard, MSc	McGill University, Montreal, Canada	Author	Data analysis
Pedro Rosa-Neto, MD, PhD	McGill University, Montreal, Canada	Author	Major role in data acquisition (lumbar punctures)
Judes Poirier, PhD	McGill University, Montreal, Canada	Author	Major role in data acquisition (laboratory methods), study design, revising the manuscript
Pierre Etienne, MD	McGill University, Montreal, Canada	Author	Major role in data acquisition (supervision of safety monitoring), data analysis and interpretation, study design, drafting the manuscript
John Breitner, MD, MPH	McGill University, Montreal, Canada	Author	Study conceptualization and design, interpretation of results, drafting and revising the manuscript, study funding

Appendix 2 Coinvestigators

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