

RESEARCH ARTICLE

Safety and tolerability of GRF6019 in mild-to-moderate Alzheimer's disease dementia

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Abstract

Introduction: This phase 2 trial evaluated the safety, tolerability, and feasibility of repeated infusions of the plasma fraction GRF6019 in mild-to-moderate Alzheimer's disease.

Methods: In this randomized, double-blind, dose-comparison trial, 47 patients were randomized 1:1 to receive daily infusions of 100 mL (n = 24) or 250 mL (n = 23) of GRF6019 for 5 consecutive days over two dosing periods separated by a treatment-free interval of 3 months.

Results: The mean (standard deviation [SD]) age of the enrolled patients was 74.3 (6.9), and 62% were women. Most adverse events (55%) were mild, with no clinically significant differences in safety or tolerability between the two dose levels. The mean (SD) baseline Mini-Mental State Examination score was 20.6 (3.7) in the 100 mL group and 19.6 (3.7) in the 250 mL group; at 24 weeks, the within-patient mean change from baseline was -1.0 points (95% confidence interval [CI], -3.1 to 1.1) in the 100 mL group and +1.5 points (95% CI, -0.4 to 3.3) in the 250 mL group. The within-patient mean change from baseline on the Alzheimer's Disease Assessment Scale-Cognitive subscale was -0.4 points (95% CI, -2.9 to 2.2) in the 100 mL group, while in the 250 mL group it was -0.9 points (95% CI, -3.0 to 1.2). The within-patient mean change from baseline on the Alzheimer's Disease Cooperative Study-Activities of Daily Living was -0.7 points in the 100 mL group (95% CI, -4.3 to 3.0) and -1.3 points (95% CI, -3.4 to 0.7) in the 250 mL

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group. The mean change from baseline on the Category Fluency Test, Clinical Dementia Rating Scale–Sum of Boxes, Alzheimer's Disease Cooperative Study–Clinical Global Impression of Change, and Neuropsychiatric Inventory Questionnaire was similar for both treatment groups and did not show any worsening.

Discussion: GRF6019 was safe and well tolerated, and patients experienced no cognitive decline and minimal functional decline. These results support further development of GRF6019.

KEYWORDS

aging, Alzheimer's disease, dementia, plasma, randomized clinical trial

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia, and no treatment or intervention has been shown to stop or slow disease progression.¹ The principal risk factor for late-onset AD is age,² with incidence increasing from 3.4 cases per 1000 person-years among people aged 65 to 74 to 36 per 1000 person-years in those 85 and older.³ Although the mechanisms are not well understood, the brain becomes more susceptible to developing AD with age. Therefore, addressing the aging process through therapeutics is expected to be a viable approach to delaying the onset and/or progression of AD.

Mice experience age-related brain changes similar to those that occur in aging human brains, as well as some of the pathological hallmarks of human AD.^{4–7} Experiments using heterochronic parabiosis or administration of whole plasma have demonstrated that plasma from young mice can partially reverse age-related changes in the brains of old mice.^{8–10} The Plasma for Alzheimer's Symptom Amelioration (PLASMA) study tested the feasibility of administering whole plasma (fresh frozen plasma [FFP]) from young donors in individuals with AD.¹¹ While patients who received FFP had improvements in some functional endpoints, the use of FFP is associated with risks of pathogen transmission and infusion reactions.^{12,13} Preclinical studies of plasma fractions (PFs) have shown that they are not only comparable to FFP with regard to the beneficial effects on brain aging in mice but have superior and longer-lasting effects on neurogenesis (manuscript under review). GRF6019 is a PF manufactured from pooled plasma from healthy donors. It is depleted of immunoglobulins and coagulation factors and has a lower risk of infusion reactions than FFP. PFs have a negligible risk of blood-borne pathogen transmission and do not require cross-matching or refrigeration like FFP.

Preclinical characterization of PFs has established a well-defined timecourse of beneficial effects in old mice.¹⁰ Within hours of receiving a single dose (150 μ L) of PF, an increase in neuronal activity was observed in multiple brain regions. After repeated dosing over 5 to 7 consecutive days, old mice had a reduction in neuroinflammation, increased synaptic density, improved spatial learning in the Barnes and Y mazes, and increased neurogenesis in the dentate gyrus.¹⁰ Based on these robust effects, we designed a trial to test the safety, tolerabil-

ity, and feasibility of repeated infusions of GRF6019 in individuals with mild-to-moderate AD.

2 | METHODS

2.1 | Trial design

ALK6019-201 was a 24-week, Phase 2, randomized, double-blind, dose-comparison trial of GRF6019 in patients with probable AD at nine research sites in the United States between March 2018 and May 2019. There were two dosing periods (Weeks 1 and 13); during each period, patients received one daily intravenous infusion of 100 or 250 mL GRF6019 (administered over ~2 hours for both doses) for 5 consecutive days and were required to remain in inpatient units for continuous safety monitoring. Because this was primarily a safety, tolerability, and feasibility trial designed to obtain safety and comparative dose data, there was no placebo control arm. The trial was conducted in accordance with the principles of Good Clinical Practice, under IND 17594, and was approved by institutional review boards at the participating sites. Written informed consent was provided by patients and/or their legally authorized representatives, and patients received a stipend for study visits and optional procedures if completed.

2.2 | Participants

Eligibility for enrollment required a diagnosis of probable AD according to National Institute on Aging–Alzheimer's Association (NIA-AA) criteria, age 60 to 90 years, and a Mini-Mental State Examination (MMSE) score of 12 to 24. All patients underwent medical and neurologic evaluations at screening and had a dedicated trial partner (eg, family member) who had frequent contact with the patient and provided support to ensure compliance with study requirements. Medical history, age, sex, race, ethnicity, height/weight, marital status, family size, and socioeconomic information were collected. The total study duration for each patient was ~7 months, including a 30-day screening period.

2.3 | Randomization and blinding

Using a web-based, centralized interactive response system, patients were randomly assigned in a 1:1 ratio to receive infusions of 100 or 250 mL of GRF6019 according to a computer-generated schedule. Permuted block randomization was used with a mixed block size of two and four with stratification by sex. The randomization codes were generated by a statistician who was not an employee of the sponsor and who had no involvement in the study other than generation and maintenance of the randomization codes. Study personnel (except for the unblinded infusion nurse, study pharmacists, and staff responsible for study drug accountability) remained blinded to the dose assignments until after database lock (Appendix A in supporting information). Blinding was maintained to ensure that reporting and assessment of adverse events and other safety signals were not biased based on a knowledge of the dose received, and to enable detection of potential differences between the two dose levels in terms of symptomatic and disease-slowng effects.

2.4 | Dose selection

GRF6019 dose levels were based on safety data collected from clinical experience in humans and scaling from efficacy in nonclinical studies. In mice, GRF6019 shows beneficial effects on age-related brain changes when a dose of 150 μ L is given, with less robust effects at lower doses. Because GRF6019 contains ~500 plasma proteins that are believed to exert most of their effects in the circulation, isometric scaling using the ratio of dose volume to total blood volume was considered the most appropriate scaling method and yielded a human equivalent dose of 413 mL. As there are potential risks of repeated infusions of GRF6019 in older patients (eg, volume overload), doses of 100 and 250 mL were chosen for this initial trial to assess safety and tolerability. A higher 500 mL dosing arm was planned; however, based on regulatory feedback, enrollment in this arm was stopped and these patients ($n = 5$) were withdrawn from the study before completing the first dosing period and not included in the final analysis. Higher doses and various dosing regimens will be evaluated in future trials.

2.5 | Outcome measures

The primary endpoints were frequency of treatment-emergent adverse events (AEs) and feasibility/tolerability of each dose level (as measured by the number of patients completing 5 and 10 infusions). Secondary safety assessments included changes from baseline in clinical laboratory tests, vital signs, electrocardiogram (ECG) readings, and Columbia Suicide Severity Rating Scale (C-SSRS),¹⁴ and changes on safety magnetic resonance imaging (MRI) scans to assess potential amyloid-related imaging abnormalities. Secondary efficacy endpoints included the MMSE,¹⁵ Alzheimer's Disease Assessment Scale-Cognitive Subscale 11-item version (ADAS-Cog11),^{16,17} Grooved Pegboard Test,¹⁸ Category Fluency Test,¹⁹ Clinical Demen-

RESEARCH IN CONTEXT

- 1. Systematic review:** Aging is the principal risk factor for late-onset Alzheimer's disease (AD), and modulating biological mechanisms underlying aging through administration of young plasma proteins may slow disease progression. In a previous pilot trial, whole plasma administration was associated with improvement in functional endpoints in patients with AD. The plasma fraction GRF6019 shows superior effects in animal models compared to whole plasma and was hypothesized to be beneficial in AD.
- 2. Interpretation:** This phase 2 trial in 47 patients with mild-to-moderate AD showed that infusions of GRF6019 were safe and well tolerated, and that infusions were feasible. Patients experienced no cognitive decline and minimal functional decline over 24 weeks.
- 3. Future directions:** These results support further trials of longer duration, wider dose range, and with a placebo control arm to determine whether GRF6019 or similar plasma fractions confer cognitive and/or functional benefits in patients with AD.

tia Rating Scale Sum of Boxes (CDR-SB),^{20,21} Alzheimer's Disease Cooperative Study-Activities of Daily Living scale, 23-item version (ADCS-ADL₂₃),²² ADCS-Clinical Global Impression of Change (ADCS-CGIC),^{23,24} and Neuropsychiatric Inventory Questionnaire (NPI-Q)²⁵ (see Table 1 for assessment timepoints). Secondary feasibility endpoints included patient compliance with the study visit schedule and procedures, patient retention, and the success of blinding. An additional secondary efficacy endpoint, a tablet-based cognitive evaluation, the Savonix Neurocognitive Assessments and Digit Span test, was performed throughout the study (Appendix B in supporting information).

Exploratory endpoints included volumetric MRI (vMRI), resting-state functional MRI (rsfMRI), and pseudo-continuous or pulsed arterial spin labeling (ASL) MRI, which were conducted in conjunction with safety MRI and performed at baseline, after the first dosing period (Day 6), and at the end of the study (24 weeks; Appendices C, D, and E in supporting information). For key analyses, the vMRI acquired after the first dosing period was not used, as it was too early to detect differences. Plasma proteomics from the evaluable population, and cerebrospinal fluid biomarkers in patients who consented to lumbar punctures, were evaluated and will be detailed in a forthcoming publication.

2.6 | Statistical analysis

All safety analyses were performed on the Safety Set (all patients who received at least one partial or whole infusion of GRF6019).

TABLE 1 Timepoints for cognitive and functional assessments

Week	-4 to -1	1-5	6	4	8	13	85-89	16	20	24	
Day	Baseline	(Dosing Period 1)		28	56	85	(Dosing Period 2)	90	112	140	168
MMSE	X		X					X			X
ADAS-Cog11	X		X	X		X ^a		X	X		X
CDR-SB	X			X					X		X
ADCS-ADL ₂₃	X			X	X				X	X	X
ADCS-CGIC	X			X					X		X
NPI-Q	X			X	X				X	X	X
Category Fluency Test	X			X					X		X
Grooved Pegboard	X			X					X		X
Savonix Full Battery	X		X	X	X	X		X	X	X	X
Savonix Brief Battery		X ^a					X ^a				

Abbreviations: ADAS-Cog11, Alzheimer's Disease Assessment Scale-Cognitive Subscale 11-item version; ADCS-ADL₂₃, Alzheimer's Disease Cooperative Study-Activities of Daily Living scale, 23-item version; ADCS-CGIC, Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; CDR-SB, Clinical Dementia Rating Scale Sum of Boxes; MMSE, Mini-Mental State Exam; NPI-Q, Neuropsychiatric Inventory Questionnaire.

^aPerformed prior to the start of each infusion.

Efficacy analyses were conducted using the Evaluable Set, which included all patients who completed at least five infusions and Visit 8. The sample size was based on the statistical approximation described by Hanley (aka the "Rule of Threes"); the upper bound of the 95% confidence interval (CI) for the rate of an unreported AE is at most 7.5% (3/number of patients receiving active drug [\sim 40 patients]).²⁶ The study was not powered to detect statistically significant changes from baseline or differences in cognitive and functional endpoints between the dose levels; however, using available data from analysis of the secondary endpoints, a descriptive summarization and appropriate 95% confidence limits were developed. For each efficacy endpoint, a paired *t*-test was conducted to evaluate within-patient changes from baseline. Between-group differences were assessed by analysis of covariance, including baseline and sex as the covariates.

All analysis dataset preparations and statistical analyses were performed using SAS version 9.4 or higher. No imputation for missing data was performed, and no sensitivity analyses were conducted to compare patients with complete assessments to those with incomplete assessments.

3 | RESULTS

3.1 | Disposition and baseline characteristics

A total of 89 patients were screened; of these, 47 were randomized and received 100 or 250 mL of GRF6019 (Figure 1). Attrition was low, with 40 patients (85%) completing all 10 infusions and 43 patients (92%) completing at least five infusions. There was no meaningful difference in discontinuation reasons between the two doses. Patients' demo-

graphics and other baseline characteristics were well balanced across the two dose groups (Table 2).

3.2 | Safety

In the safety population ($n = 47$), 38 patients experienced at least one treatment-emergent AE: 18 (75%) in the 100 mL group and 20 (87%) in the 250 mL group (Table 3). Two patients (8%) in the 100 mL group and one patient (4%) in the 250 mL group withdrew due to AEs. Common AEs (occurring in $\geq 5\%$ of patients in either group) were headaches, diarrhea, falls, arthralgia, transient changes in blood pressure (BP), transient laboratory abnormalities, and infusion-site extravasation or bruising. Most AEs (55%) were mild in intensity. Eleven patients experienced AEs that were moderate in intensity (Table F.1 in supporting information). The number of AEs of moderate intensity that were considered related or possibly related to GRF6019 by either the investigator or the sponsor was similar in the two dose groups.

There were no deaths. Two patients experienced serious AEs (SAEs). One patient had a hypersensitivity infusion reaction with elevated BP after starting the first infusion (Day 1). The patient's systolic BP increased from 140 to 228 mm Hg within 10 minutes; this patient had a history of difficult-to-treat hypertension that was being managed with four anti-hypertensive medications. The patient had been randomized to receive 250 mL but had only received 5 to 10 mL of the first infusion when the event started. This SAE was therefore assessed as related to GRF6019 but not to dose as it would have occurred regardless of the dose. The other patient who experienced an SAE was randomized to the 250 mL arm and had a recent history of deep vein thrombosis (DVT) and pulmonary embolism (PE) that was not disclosed at screening. Ten weeks after receiving the last infusion of dosing period two, the patient

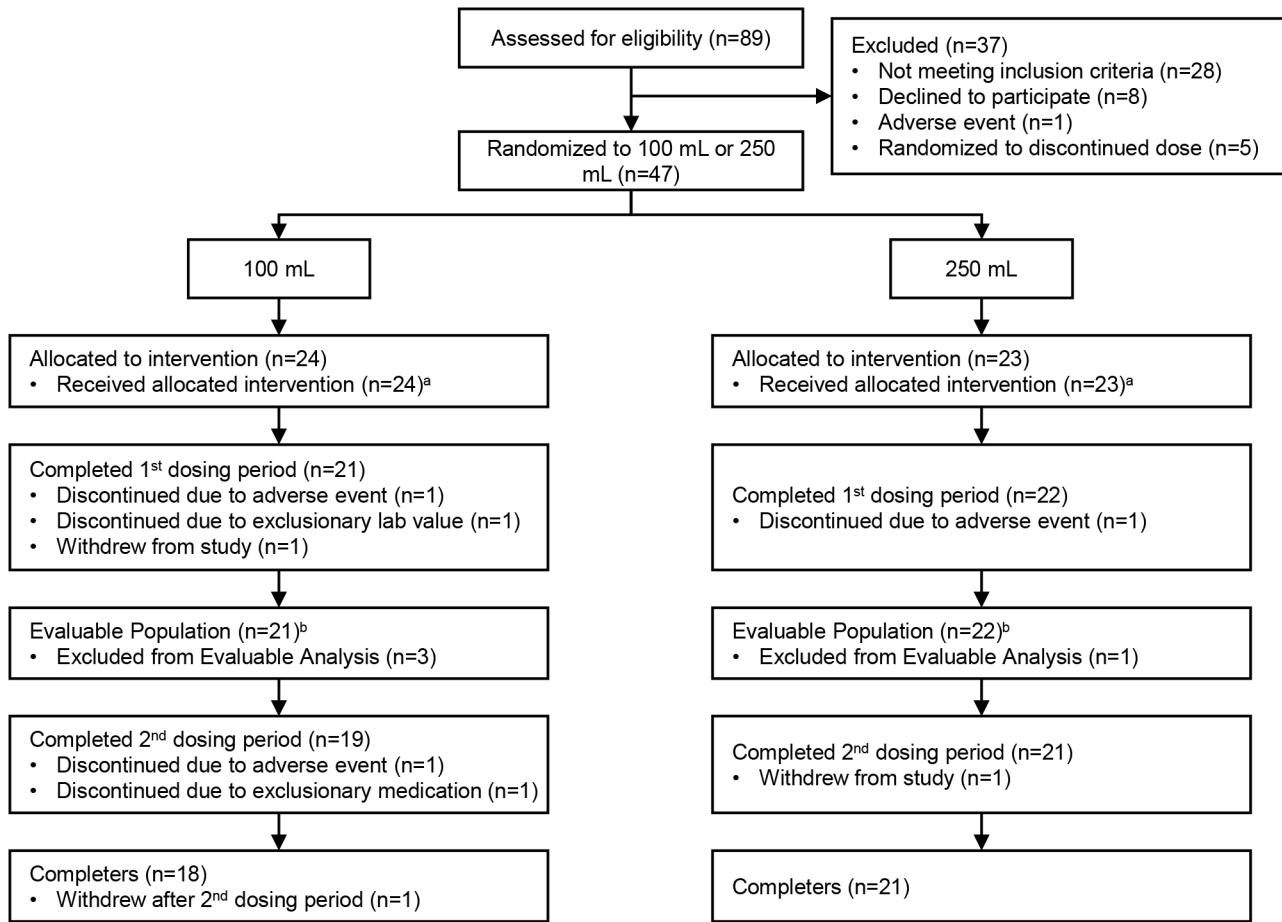


FIGURE 1 Study flow diagram. **a**, Safety analyses were performed on the Safety Set, which included all patients who received at least one partial or whole infusion of GRF6019 (100 mL $n = 24$; 250 mL $n = 23$). **b**, Efficacy analyses were conducted using the Evaluable Set, which included all patients who completed at least five infusions and all Visit 8 assessments (100 mL $n = 21$; 250 mL $n = 22$)

had a DVT with PE. Given the prior history and temporal separation (69 days) between the last infusion of GRF6019 and the DVT/PE, this SAE was assessed as unrelated to GRF6019.

There was no difference in the magnitude of mean BP changes across the dose levels; however, because transient increases and decreases in BP were commonly observed AEs, a comparison of BP changes above or below certain thresholds was performed by dose (Table F.2 in supporting information). Because there were few patients who had changes in BP that were considered clinically significant or that reached the thresholds outlined in Table F.2, it is not possible to assess whether BP increases are more likely to occur at the 250 mL dose versus the 100 mL dose.

Nine patients experienced AEs related to abnormal laboratory values (Table F.3 in supporting information). The most common laboratory AEs were increases in amylase and lipase ($n = 3$); other abnormalities occurred in single subjects. None of these laboratory AEs was accompanied by signs or symptoms, and all were transient and returned to baseline by the end of the study.

None of the patients had clinically meaningful changes in body temperature, heart rate, respiration, body weight, 12-lead ECG, C-SSRS scores, or safety MRI parameters.

3.3 | Efficacy

The two dose groups were similar in baseline cognitive and functional status (Table 4). In the evaluable population (both doses combined), there was no cognitive decline after 24 weeks, minimal functional decline, and no worsening on the NPI-Q (Figure 2). The Savonix Neurocognitive Battery showed no worsening in performance in tests of impulse control, focus, attention, processing speed, flexible thinking, or executive function (Appendix B in supporting information).

Although the study was not statistically powered to detect differences between the dose levels, some endpoints suggested that a difference between the 100 and 250 mL groups may exist. On the MMSE, the within-patient mean change from baseline at 24 weeks was -1.0 points (95% CI, -3.1 to 1.1) in the 100 mL group and $+1.5$ points (95% CI, -0.4 to 3.3) in the 250 mL group, with a least squares (LS) mean between-group difference of 2.5 (95% CI, -0.3 to 5.3). Consistent with this, the within-patient mean change from baseline on the ADAS-Cog11 was -0.4 points (95% CI, -2.9 to 2.2) in the 100 mL group, while in the 250 mL group it was -0.9 points (95% CI, -3.0 to 1.2 ; a reduction in ADAS-Cog11 score indicates improvement), with a between-group LS mean difference of -0.5 (95% CI, -3.7 to 2.7). During the study, the 250 mL

TABLE 2 Patient demographics and baseline characteristics^a

Patients (safety population)	100 mL (n = 24)	250 mL (n = 23)	Overall (n = 47)
Age, years, mean (SD)	75.9 (6.3)	72.7 (7.2)	74.3 (6.9)
Sex, no. (%)			
Women	15 (63)	14 (61)	29 (62)
Men	9 (38)	9 (39)	18 (38)
Race, ^b no. (%)			
Asian	0	1 (4)	1 (2)
Black or African American	1 (4)	3 (13)	4 (9)
White	23 (96)	19 (83)	42 (89)
Hispanic or Latino, no. (%)	8 (33)	7 (30)	15 (32)
Body mass index (kg/m ²), mean (SD)	25.7 (4.7)	26.5 (3.3)	26.1 (4.1)
Duration of AD, mean (SD)	4.2 (3.9)	4.5 (4.4)	4.4 (4.1)
MMSE total score, mean (SD)	20.6 (3.7)	19.6 (3.7)	20.1 (3.7)
≤20 (moderate AD), no. (%)	8 (33)	11 (48)	19 (40)
>20 (mild AD), no. (%)	16 (67)	12 (52)	28 (60)
Cholinesterase inhibitor, no. (%)	14 (58)	11 (48)	25 (53)
Memantine, no. (%)	9 (38)	6 (26)	15 (32)

Abbreviations: AD, Alzheimer's disease; MMSE, Mini-Mental State Exam; SD, standard deviation.

^aSmoking status, marital status, family size, longest held career, and annual household income were also collected (data on file) and were similar across the two dose groups.

^bRace and ethnicity data were gathered to characterize the patients and were self-reported by the patient and/or their trial partner via interview with the investigator/study coordinator.

TABLE 3 Treatment-emergent adverse events

Treatment-emergent adverse events ^a	No. (%)	
	100 mL (n = 24)	250 mL (n = 23)
Patients with any adverse event	18 (75)	20 (87)
Patients with any serious adverse event	0	2 (9)
Patients with any adverse event leading to discontinuation of study drug	2 (8)	1 (4)
Patients with severe adverse events	0	2 (9)
Patients with moderate adverse events	3 (13)	7 (30)
Patients with mild adverse events	15 (63)	11 (48)

^aAdverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Preferred Term and grouped by System Organ Class.

dose group had more improvement on the ADCS-ADL₂₃, but by the end of the study had worsened more (−1.3 points; 95% CI, −3.4 to 0.7) than the 100 mL group (−0.7 points; 95% CI, −4.3 to 3.0), with an LS mean between-group difference of −0.7 (95% CI, −4.7 to 3.4). Additional efficacy results are available in Appendix G in supporting information.

3.4 | Exploratory imaging

The vMRI results identified no decrease in right or left hippocampal volume or temporal lobe cortical thickness after 24 weeks (Appendix C in supporting information). The rsfMRI results identified no differences in resting state connectivity between scans (Appendix D in supporting information). The ASL results identified no differences in cerebral blood flow between scans (Appendix E in supporting information).

4 | DISCUSSION

This trial of GRF6019 in patients with mild-to-moderate AD met its primary endpoint by demonstrating that daily dosing for 5 consecutive days with 100 mL or 250 mL of GRF6019 at Weeks 1 and 13 was safe, feasible, and well tolerated. The majority of AEs were mild. There was no clear difference in the AE profile (frequency, intensity) between the 100 and 250 mL doses. In the PLASMA study using FFP,¹¹ there was a greater number of AEs per patient within a shorter study duration than with GRF6019. Given a better safety profile, room-temperature stability, and no requirement for cross-matching, PFs such as GRF6019 are a source of plasma proteins that are better suited than FFP for clinical application.

The lack of a placebo control precludes any definitive conclusions about efficacy; however, a comparison to historical controls is helpful in the interpretation of the data. Patients treated with GRF6019 showed a lack of cognitive decline and limited functional decline over the 24-week study period. In published trials comprising more than 3200 patients with mild-to-moderate AD and a mean baseline MMSE score of 20.6,^{27–32} the mean progression over 6 months in placebo-treated patients was −1.1 points on the MMSE (vs +0.3 in the two dose groups combined in this study), +2.2 points on the ADAS-Cog11 (vs −0.6), +0.7 points on the CDR-SB (vs +0.1), and −3.1 points on the ADCS-ADL (vs −1.0). Although comparisons to historical data must be interpreted with caution and the sample size was small, these preliminary efficacy signals, in combination with the safety and tolerability data, are supportive of further clinical development of PF for the treatment of mild-to-moderate AD.

Although the study was not statistically powered to detect differences in efficacy endpoints between the two dose levels, the data observed are nonetheless informative. At the end of the study, a 2.5-point difference in the change from baseline on the MMSE and a 0.5-point difference on the ADAS-Cog11 were observed between the 100 and 250 mL dose levels. Because the dose level was blinded to patients, trial partners, raters, and investigators, this potential dose response on cognition is intriguing and cannot be attributed to learning or placebo effects. There was less worsening on the ADCS-ADL₂₃ in the 100 mL group compared to the 250 mL group at the end of the study, which is not consistent with the difference in cognitive effects. A similar apparent discrepancy between cognition and function was observed in a Phase 2 study of total plasma exchange, in which the active arm outperformed placebo on cognition, while the opposite was observed on function.³³

TABLE 4 Change from baseline in cognitive and functional measures

Measure, ^a mean (SD)	100 mL (n = 21)				250 mL (n = 22)				Overall (n = 43)				100 mL versus 250 mL P value ^c
	Change from Baseline				Change from Baseline				Change from Baseline				
	Baseline	Change at Day 168	95% CI	P value ^b	Baseline	Change at Day 168	95% CI	P value ^b	Baseline	Change at Day 168	95% CI	P value ^b	
MMSE ^e	20.2 (3.9)	-1.0 (4.3)	(-3.1, 1.1)	.34	19.7 (3.8)	+1.5 (3.9)	(-0.4, 3.3)	.11	20.0 (3.8)	+0.3 (4.2)	(-1.1, 1.7)	.68	.08
ADAS-Cog11 ^{d,e}	21.2 (13.3)	-0.4 (5.1)	(-2.9, 2.2)	.76	22.1 (11.1)	-0.9 (4.3)	(-3.0, 1.2)	.38	21.6 (12.1)	-0.6 (4.7)	(-2.2, 0.9)	.41	.75
CDR-SB ^f	5.6 (3.3)	-0.03 (2.3)	(-1.2, 1.1)	.96	5.6 (2.8)	+0.2 (1.7)	(-0.6, 1.0)	.56	5.6 (3.0)	+0.1 (2.0)	(-0.5, 0.7)	.74	.72
ADCS-ADL ₂₃ ^f	62.3 (13.4)	-0.7 (7.4)	(-4.3, 3.0)	.71	61.3 (14.5)	-1.3 (4.6)	(-3.4, 0.7)	.19	61.8 (13.8)	-1.0 (6.0)	(-3.0, 0.9)	.29	.74
NPI-Q Distress ^f	5.9 (5.7)	-2.9 (5.4)	(-5.6, -0.2)	.04	4.4 (5.5)	-1.1 (4.9)	(-3.4, 1.1)	.30	5.1 (5.6)	-1.9 (5.1)	(-3.6, -0.3)	.02	.75
NPI-Q Severity ^f	4.7 (4.5)	-2.3 (3.8)	(-4.2, -0.5)	.02	3.8 (3.6)	-1.2 (3.1)	(-2.6, 0.2)	.09	4.2 (4.0)	-1.7 (3.4)	(-2.8, -0.6)	.003	.68
Category Fluency Test ^f	10.9 (5.0)	-0.3 (3.8)	(-2.2, 1.6)	.72	9.1 (3.9)	+0.2 (2.4)	(-0.8, 1.3)	.65	10.0 (4.5)	-0.0 (3.1)	(-1.0, 1.0)	.96	.84
Grooved Pegboard ^e	180.7 (80.0)	-12.8 (32.9)	(-29.7, 4.1)	.13	171.6 (78.4)	-3.8 (21.1)	(-13.7, 6.1)	.43	176.0 (78.3)	-7.9 (27.1)	(-17.0, 1.1)	.08	.32

Abbreviations: ADAS-Cog11, Alzheimer's Disease Assessment Scale-Cognitive Subscale 11-item version; ADCS-ADL₂₃, Alzheimer's Disease Cooperative Study-Activities of Daily Living scale, 23-item version; ADCS-CGIC, Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; CDR-SB, Clinical Dementia Rating scale-Sum of Boxes; CI, confidence interval; MMSE, Mini-Mental State Exam; NPI-Q, Neuropsychiatric Inventory Questionnaire; SD, standard deviation.

^a Higher scores on the MMSE, ADCS-ADL₂₃, and Category Fluency Test indicate better cognition or function. Lower scores on the ADAS-Cog11, CDR-SB, NPI-Q, and Grooved Pegboard indicate better cognition or function.

^b Based on Student's statistics; P values are not corrected for multiple comparisons.

^c Based on analysis of covariance analysis including baseline and sex as the covariates.

^d At baseline, 100 mL n = 21; 250 mL n = 21; overall n = 42.

^e At Day 168, 100 mL n = 18; 250 mL n = 20; overall n = 38.

^f At Day 168, 100 mL n = 18; 250 mL n = 21; overall n = 39.

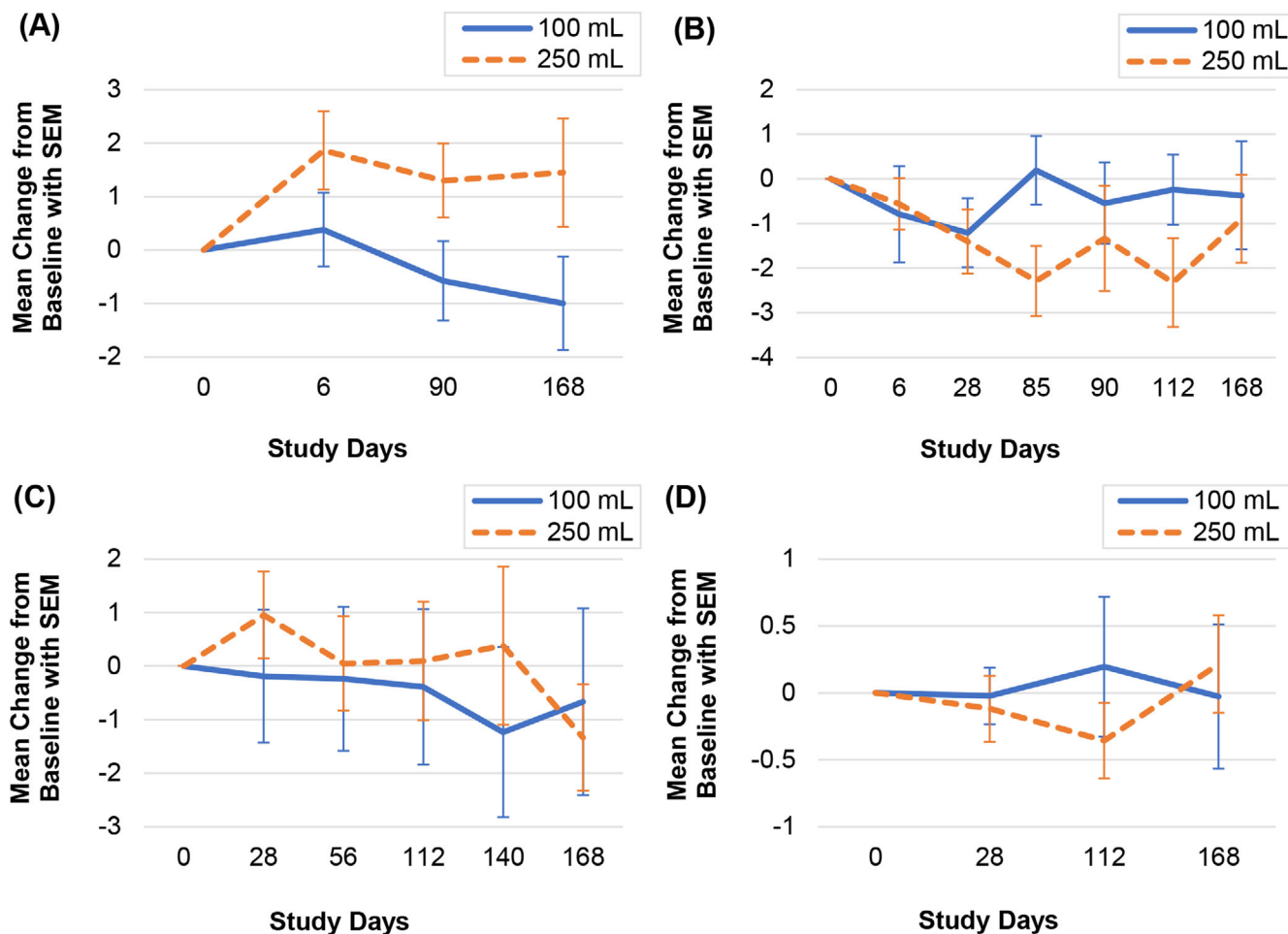


FIGURE 2 Change from baseline in cognitive and functional assessments. Mean change from baseline in total score: **A**, Mini-Mental State Exam (MMSE). **B**, Alzheimer's Disease Assessment Scale-Cognitive Subscale 11-item version (ADAS-Cog11). **C**, Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL). **D**, Clinical Dementia Rating scale (CDR)

In mice, improvements in cognition are maintained for 3 to 6 weeks after daily dosing with PFs like GRF6019 for 5 to 7 consecutive days (manuscript under review). Interestingly, the numerical superiority of 250 over 100 mL on cognition was most pronounced at earlier timepoints after dosing, which may indicate that 250 mL GRF6019 has a symptomatic cognitive benefit in AD. Whether the lack of cognitive worsening observed in this trial was due to a symptomatic effect, slowing of disease progression, or a placebo effect will need to be evaluated in future studies.

Volumetric MRI showed no further atrophy in whole brain, temporal lobe, or hippocampus over the 24 weeks of the study, consistent with the lack of cognitive decline. In placebo-treated patients in the verubecestat trial, hippocampal volume decreased 6% in 78 weeks.²⁷ Assuming a linear rate of atrophy, this would equal 1.8% atrophy in 24 weeks. Other studies also indicate that one would expect ~2% hippocampal atrophy in 6 months³⁴⁻³⁶ and ~1% whole-brain atrophy.^{36,37}

The main limitations of this study are the absence of a concurrent placebo-control arm, the limited duration of observation, and small number of patients, which preclude deriving any conclusions regarding beneficial effects. In addition, the relatively high frequency of cognitive

assessments throughout the study may have led to a practice effect. Future plans for trials with a placebo control, longer treatment duration, and larger patient population are currently being evaluated. An additional limitation is that there was no biomarker confirmation of AD diagnosis in most patients. A clinical diagnosis of AD using the NIA-AA criteria by expert diagnosticians has a false positive rate of ~20%;³⁸ therefore, it is likely that some patients in this study did not have AD.

5 | CONCLUSIONS

Modulating the biological mechanisms underlying aging through treatments such as GRF6019 may be a viable approach to delaying the onset and/or progression of AD. This trial met its primary endpoint and demonstrated that treatment with GRF6019 is safe and well tolerated in mild-to-moderate AD. Future trials of longer duration, wider dose range, and with placebo control will determine whether GRF6019 or similar PFs confer cognitive and/or functional benefits in patients with AD.

ACKNOWLEDGMENTS/FUNDING INFORMATION

The authors wish to thank the patients and caregivers who participated in this trial, and the investigators and site staff who conducted the trial. Manuscript editing and formatting support was provided by Colleen Brown (Mark Consulting, Inc.) and Shireen Dunwoody (Dunwoody Consulting). This work was supported by Alkahest, Inc., San Carlos, CA and Grifols, S.A., Barcelona, Spain. Alkahest was responsible for study conduct, data collection, monitoring, statistical analyses, and interpretation of the data. Grifols was responsible for manufacturing investigational product. Both companies participated in the study design and reviewed the manuscript. All final content decisions were made by the authors.

CONFLICTS OF INTEREST

Jonas Hannestad, Katie Koborsi, Vicki Klutzaritz, Whitney Chao, Scott Lohr, Karoly Nikolich, and Steven Braithwaite are full-time employees of Alkahest, Inc. Dr. Paez is a full-time employee of Grifols, S.A. Dr. Jackson and Rebecca Ray are prior employees of Alkahest, Inc. Dr. Cummings has provided consultation to Acadia Pharmaceuticals, Actinogen, AgeneBio, Alkahest, Alzheon, Annovis Bio, Avanir Pharmaceuticals, Axsome Therapeutics, Biogen, BioXcel Therapeutics, Cassava Sciences, Cerecin, Cerevel Therapeutics, Cortexyme, Cytox, EIP Pharma, Eisai, Foresight Pharmaceuticals, GemVax, Genentech, Green Valley, Grifols, Karuna, Merck & Co., Novo Nordisk, Otsuka, Resverlogix, Roche, Samumed, Samus Therapeutics, Signant Health, Suven Life Sciences, Third Rock, and United Neuroscience pharmaceutical and assessment companies. Dr. Cummings also has stock options in ADAMAS, Annovis Bio, MedAvante, and BiOasis Technologies; owns the copyright of the Neuropsychiatric Inventory; and is supported by Keep Memory Alive (KMA), NIGMS grant P20GM109025, NINDS grant U01NS093334, and NIA grant R01AG053798. Dr. Kay has provided consultation to Alkahest, Allergan, Avanir Pharmaceuticals, BioXcel Therapeutics, Chase Therapeutics, Dart Neuroscience, Lilly, Merck & Co., Neurana Pharmaceuticals, Neuralstem, Novartis, Pfizer, Sage Therapeutics, and Samus Therapeutics. Dr. Kay is also co-owner of Cognitive Research Corporation, which served as the contract research organization (CRO) for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Hannestad J, Koborsi K, Klutzaritz V, et al. Safety and tolerability of GRF6019 in mild-to-moderate Alzheimer's disease dementia. *Alzheimer's Dement*. 2020;6:e12115. <https://doi.org/10.1002/trc2.12115>