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Preclinical and first-in-human evaluation of AL002, a novel TREM2 agonistic antibody for Alzheimer's disease

Hua Long[1†], Adam Simmons[1*†], Arthur Mayorga[1], Brady Burgess[1], Tuan Nguyen[1], Balasubrahmanyam Budda[1], Anna Rychkova[1], Herve Rhinn[1,2], Ilaria Tassi[1,3], Michael Ward[1,4], Felix Yeh[1,5], Tina Schwabe[1,6], Robert Paul[1,6], Sara Kenkare-Mitra[1] and Arnon Rosenthal[1]

Abstract

Background Variants of the gene triggering receptor expressed on myeloid cells-2 (TREM2) increase the risk of Alzheimer's disease (AD) and other neurodegenerative disorders. Signaling by TREM2, an innate immune receptor expressed by microglia, is thought to enhance phagocytosis of amyloid beta (Aβ) and other damaged proteins, promote microglial proliferation, migration, and survival, and regulate inflammatory signaling. Thus, TREM2 activation has potential to alter the progression of AD. AL002 is an investigational, engineered, humanized monoclonal immunoglobulin G1 (IgG1) antibody designed to target TREM2. In AD mouse models, an AL002 murine variant has been previously shown to induce microglial proliferation and reduce filamentous Aβ plaques and neurite dystrophy.

Methods Preclinical studies assessed the safety, tolerability, pharmacokinetics, and pharmacodynamics of AL002 in cynomolgus monkeys. INVOKE-1 (NCT03635047) was a first-in-human phase 1, randomized, placebo-controlled, double-blind study assessing the safety, tolerability, PK, and PD of AL002 administered as single ascending doses (SAD) in healthy volunteers.

Results In cynomolgus monkeys, weekly intravenous injections of AL002 for 4 weeks were well tolerated, dose-dependently decreased soluble TREM2 (sTREM2) in cerebrospinal fluid (CSF) and total TREM2 in hippocampus and frontal cortex, and increased biomarkers of TREM2 signaling in CSF and brain. In the phase 1 study of 64 healthy volunteers, a single intravenous infusion of AL002 demonstrated brain target engagement based on a dose-dependent reduction of sTREM2 in CSF and parallel increases in biomarkers of TREM2 signaling and microglia recruitment. Single-dose AL002 showed central nervous system penetrance and was well tolerated, with no treatment-related serious adverse events over 12 weeks.

Conclusions These findings support the continued clinical development of AL002 for AD and other neurodegenerative diseases in which TREM2 activation may be beneficial. AL002 is currently being tested in a phase 2, randomized, double-blind, placebo-controlled study in early AD.

[†]Hua Long and Adam Simmons are co-first authors.

*Correspondence:
Adam Simmons
Adam.Simmons@alector.com

Full list of author information is available at the end of the article

Trial registration Clinicaltrials.gov, NCT03635047. Registered on August 15, 2018, https://www.clinicaltrials.gov/study/NCT03635047.

Keywords TREM2, Alzheimer's disease, Microglia, Phase 1 clinical trial, Biomarkers

Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide, and there is an urgent need for treatments to halt or slow disease progression. The neuropathological hallmarks of AD consist of extracellular amyloid-β (Aβ) aggregation in the form of neuritic plaques and intracellular accumulation of phosphorylated tau as neurofibrillary tangles, which correlate with neuron and synapse loss [1, 2]. The longstanding and prevailing model of AD progression is that Aβ accumulation occurs first, followed by the emergence of tau pathology, synaptic and neuronal damage and death, and the onset of cognitive deficits [2, 3]. With an improved understanding of the disease, the innate immune system has emerged as having a crucial role in AD pathogenesis and as a target for therapeutic intervention [4]. Microglia, the brain's innate immune cells, are thought to be responsible for containment and removal of misfolded proteins including Aβ, for the replacement of damaged synaptic connections and myelin, for regulating protective functions of other brain support cells including astrocytes and oligodendrocytes, and for regulating healthy neuronal transmission [2, 5, 6].

The critical role of microglia in AD pathogenesis came from genome-wide association studies (GWAS) identifying mutations in multiple genes that regulate microglial survival, proliferation, migration, and function as risk genes for AD [7–10]. The gene triggering receptor expressed on myeloid cells-2 (*TREM2*) was found to be a prominent risk factor for AD [11, 12] and other neurodegenerative diseases [13, 14]. Variants in *TREM2*, including heterozygous missense mutation R47H, increase the risk of developing late-onset AD by 2- to 4-fold [11, 12] and are thought to confer a partial loss of function by decreasing TREM2 ligand-binding capacity [15, 16]. Homozygous loss-of-function mutations in *TREM2*, or its signaling co-receptor gene *TYROBP*, cause Nasu-Hakola disease, which is characterized by early-onset dementia [17]. TREM2 is a damage-sensing, innate immune receptor selectively expressed in the brain by microglia with proposed ligands including anionic and zwitterionic lipids [15, 18], apolipoproteins E (ApoE) [19, 20] and J (ApoJ) [16], and Aβ oligomers [21]. TREM2 signals through the immunoreceptor tyrosine-based activation motif (ITAM)–containing adaptor DAP12 (encoded by *TYROBP*) to enhance phagocytosis and promote microglial survival, proliferation, migration, and lysosomal function [2, 13, 22].

Since the discovery of *TREM2* mutations as a significant genetic risk factor for AD, the impact of TREM2 on AD pathogenesis has been intensely investigated in preclinical AD models. In mouse models of amyloid deposition and patients with AD carrying the R47H variant, TREM2 deficiency impairs the microglial response to and clustering around Aβ plaques, resulting in a diffuse plaque morphology and reduced plaque compaction [15, 23, 24]. TREM2 deficiency has also been shown to impair microglial survival during reactive microgliosis and to increase Aβ-mediated toxicity and neuronal damage in a 5×FAD model [15]. Further, TREM2-deficient microglia fail to acquire a transcriptional signature associated with microglial mitigation of Aβ [25]. TREM2 knockout has been reported to produce variable effects on Aβ load depending on the mouse model and disease stage [15, 24, 26, 27] but consistently results in increased neuritic dystrophy [23, 27, 28]. Corroborating these findings, overexpression of human TREM2 augmented microglial phagocytic activity, upregulated genes related to increased microglial function and phagocytosis, and reduced amyloid burden and neuritic dystrophy in a 5×FAD mouse model [29]. Further supporting the beneficial role of TREM2-dependent microglial response, systemic administration of TREM2 agonistic antibodies in mouse models of β-amyloidosis has been shown to increase plaque compaction, decrease Aβ burden [30, 31], reduce neuritic dystrophy [32], and improve behavioral performance [31, 32].

The effects of TREM2 on tau pathology remain unclear. Conflicting results on the effect of TREM2 deficiency on tau pathology have been reported in pure tauopathy models [33–36] as well as in combined models of Aβ and tau pathologies. Some combined models have shown that TREM2 knockout exacerbates tau seeding and spreading as well as Aβ-driven tau pathology and degeneration [37–39]. Other studies have shown that treatment with a mouse-specific TREM2 agonistic antibody resulted in increased tau seeding and neuritic dystrophy in 5×FAD mice injected with human AD brain-derived tau [40]. These findings highlight the challenges in interpreting results across different animal models.

Collectively, findings from genetic studies and animal models suggest that TREM2 agonism is a promising therapeutic approach to evaluate in AD and possibly other neurodegenerative disorders. AL002 is an investigational, engineered, humanized monoclonal immunoglobulin G1 (IgG1) antibody designed to target and activate TREM2 alone and in conjunction with the natural TREM2 ligands. AL002c, a preclinical variant of AL002 that only differs in the Fc region, activated human

TREM2 expressed in cell lines or primary myeloid cells and enhanced survival of multiple myeloid cell populations [32]. In a 5×FAD mouse model expressing human *TREM2*, AL002c induced microglial proliferation, reduced the abundance of more toxic fibrillar plaques, and reduced neurite dystrophy [32].

Here, we present preclinical and clinical studies evaluating the pharmacology, pharmacokinetics (PK), and pharmacodynamics (PD) of AL002. AL002 binds cynomolgus monkey TREM2 with high affinity, similarly to human TREM2, but does not bind rodent TREM2. Therefore, as part of the AL002 investigational new drug (IND)-enabling toxicology studies, we investigated the in vivo pharmacology, PK, safety, and tolerability of repeat-dose AL002 in cynomolgus monkeys, which included the 4-week Good Laboratory Practice (GLP) study. After establishing an acceptable preclinical profile of AL002 in nonhuman primates (NHPs), a first-in-human phase 1 clinical trial (INVOKE-1; NCT03635047) was conducted to assess the safety, tolerability, PK, and PD of AL002 administered in single ascending doses (SAD) in healthy volunteers (HVs). While preliminary results from the first-in-human trial were reported previously [32], here we present the complete safety and PK results and an assessment of various biomarkers of TREM2 signaling and microglial function, including soluble TREM2 (sTREM2), secreted phosphoprotein 1 (SPP1) protein (i.e., osteopontin), colony-stimulating factor 1 receptor (CSF1R), and IL1RN protein (i.e., interleukin-1 receptor antagonist [IL-1RA]), in the cerebrospinal fluid (CSF) and brain of NHPs and in the CSF and plasma of HVs. The results presented here informed the dose selection for the ongoing phase 2 trial (INVOKE-2; NCT04592874) evaluating the efficacy and safety of AL002 in participants with early AD.

Methods

Antibody formulation

AL002 is a recombinant humanized monoclonal antibody (mAb) designed to target the human TREM2 receptor. AL002 binds to the stalk region in the extracellular domain of human TREM2. The constant region of AL002 is IgG1, which was further engineered to reduce complement activation and increase agonistic activity [41, 42].

In vitro cell-based assays of AL002

Human macrophages were seeded in 96-well plates, round bottom non-tissue culture, at 1×10^5 cells/well in complete medium (without macrophage colony-stimulating factor). Different concentrations of AL002 and control immunoglobulin G (IgG) were added to cells for 48 h. Cells were blocked in FACS buffer (2% FCS in PBS [phosphate-buffered saline]) with Human Fc-block (Invitrogen Product #14-9161-73) and stained with fluorescently labelled anti-TREM2 (generated at Alector) and isotype antibodies using standard flow cytometry techniques. Dead cells were stained with Live/Dead Aqua (Invitrogen). sTREM2 was measured by the methods described in NHP immunoassays.

PK/PD of AL002 in NHPs

All experimental procedures were conducted according to the approved protocols from the relevant institutions: 4-week GLP study, Covance Laboratories, Inc #8372868; dose range-finding (DRF) study, Covance Laboratories, Inc #8372866; non-GLP interval study, Charles River Laboratories, Inc. # 2946-001. All procedures complied with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

4-week GLP toxicology study design and sample collection

Cynomolgus monkeys (*Macaca fascicularis*, origin Cambodia) from Covance Research Products, Inc (now Labcorp Drug Development) were 24 to 50 months of age and housed in stainless steel cages. AL002 was administered once weekly via intravenous (IV) slow bolus injection to male and female monkeys ($N=32$; $n=16$ female; $n=6$–10 per dose) at a dose of 0 (vehicle), 20, 80, or 250 mg/kg for a total of 5 doses (Days 1, 8, 15, 22, 29). Blood samples were collected from the femoral vein and CSF samples were collected from the cisterna magna at various timepoints for toxicokinetic (TK) and PD analyses. Toxicology endpoints included body weight, food intake, clinical observations, injection site observation, ophthalmic and respiratory examinations, neurologic evaluations, ECG, immunophenotyping, cytokine release, clinical pathology (e.g., hematology, coagulation, serum chemistry, urinalysis), and anatomical pathology. Twenty-four animals (6 per dose group, half female) were sacrificed and necropsied on Day 31 (2 days after the last dose). Four animals (2 female) in the vehicle group and 4 animals (2 female) in the 250 mg/kg dose group underwent a 4-week recovery period following the last dose (Day 29) and were sacrificed and necropsied on Day 59. A comprehensive list of tissues (including the injection site) was examined microscopically by a board-certified veterinary pathologist and subjected to pathology peer review. Brain samples were collected from the right frontal cortex and right hippocampus.

Non-GLP dose range–finding (DRF) study in cannulated NHPs

Cynomolgus monkeys from Covance Research Products, Inc were 24 to 50 months of age and housed in stainless steel cages. AL002 was administered IV (slow bolus) once weekly over a 20-day period (Days 1, 8, 15) in 16 cannulated monkeys at 20, 80, or 250 mg/kg ($n=2$ per sex per

group) for a total of 3 doses. CSF samples were collected at various time points through a catheter and access port, which was inserted by pre-study hemilaminectomy.

Non-GLP interval study

Naïve female cynomolgus monkeys ($N=20$; body weight 2–3 kg; Charles River Laboratories, Inc. originally sourced from Worldwide Primates, Inc.) were randomized by weight and assigned to receive monthly IV (slow bolus) injections of vehicle control, 80 mg/kg AL002, or 250 mg/kg AL002 ($n=5$ per group) for a total of 3 doses over a 3-month period (Days 1, 29, 58). The week prior to dosing, animals were surgically implanted with intrathecal lumbar catheters for CSF collection. Due to a surgical complication unrelated to study drug, one animal from the 80 mg/kg AL002 monthly dose group was euthanized in extremis on Day 22. CSF samples were collected from the cisterna magna via intrathecal catheter from all animals predose and at various timepoints postdose on Days 1, 29 and 58, and analyzed for PK and PD biomarkers.

Brain sample processing

Frozen brain samples were lysed with N-Per Neuronal Protein Extraction Reagent (cat #87792, Thermo Scientific) and Halt Protease inhibitor (cat# 1861278) on ice for 20 min according to manufacturer instructions. Supernatants were transferred to a new tube and stored at -80 °C until further analysis. The total protein concentration in each sample was measured by BCA protein analysis kit (cat# 23225, Thermo Scientific) according to manufacturer instructions. The protein concentration values were used to normalize analyte concentrations measured in brain tissues.

Immunoassays in NHPs

The quantification of AL002 concentrations in cynomolgus monkey serum and CSF samples from the 4-week GLP study was performed using a validated electrochemiluminescence assay (ECLA) on the Meso Scale Discovery (MSD®) platform. AL002 was captured by a biotinylated anti-ID1-6E3 antibody and detected by a ruthenylated anti-hIgG (CH2 domain) antibody. After incubation with the detection antibody, the plate was washed and 2X read buffer was added to the wells. The plate was read immediately in the MSD Sector Imager S600.

Custom cynomolgus monkey TREM2 MSD-based assays were developed to measure TREM2 expression in tissue or sTREM2 in CSF. Briefly, single spot MSD plates (Meso Scale Diagnostics, Rockville, MD) were coated with capture antibody 8F11 (generated at Alector) in PBS at 4 °C overnight. Monkey CSF samples or tissue lysates were diluted in binding buffer and added to the wells for 1 h at room temperature. Biotinylated goat anti-human TREM2 polyclonal antibody (R&D Systems) was added as detection antibody and incubated at room temperature, followed by detection with SULFO-TAG™ labeled strepdavidin (Meso Scale Diagnostics, Rockville, MD). Plates were analyzed on a Sector Imager (Meso Scale Diagnostics, Rockville, MD).

A human Colony Stimulating Factor 1 Receptor (CSF1R) enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Cat # DY329) was qualified for the measurement of cynomolgus monkey CSF1R and utilized to measure CSF1R in the brain samples per manufacturer instruction. The determination of the concentration of SPP1 protein (i.e., osteopontin) in cynomolgus monkey CSF from the interval study was based on an osteopontin ELISA kit from R&D Systems (Catalog No. DOST00).

SomaScan® proteomics assay was used to determine the concentrations of IL1RN protein (i.e., IL-1RA) and SPP1 protein in cynomolgus monkey CSF from the 4-week GLP study. CSF samples were collected at various time points and shipped to SomaLogic, who provided SomaScan data from multiple standardization stages.

RNA-seq was used to quantify gene expression in cynomolgus monkey brain tissue from the 4-week GLP study. Brain tissue was dissociated as previously described [43]. RNA was extracted using Qiagen Plus Micro kit, and libraries were prepared using Lexogen QuantSeq 3'FWD kit according to manufacturer's recommendation for low input RNA samples. All samples were quality checked at each stage for RNA and library quality and quantity prior to sequencing. Raw sequencing data were processed according to the following protocol: STAR aligner has been used to align reads to the macaque genome (*Macaca_fascicularis*_5.0.93 assembly).

Statistical analysis for preclinical studies

Statistical analysis to compare the mean values for multiple groups in the 4-week GLP study was performed using one-way ANOVA with Tukey's multiple comparisons test (total TREM2, frontal cortex *SPP1* and *CSF1R* mRNA, frontal cortex CSF1R protein). CSF SPP1 and IL1RN protein data (SomaScan) were analyzed using two-way ANOVA with Bonferroni's test. Two-way ANOVA with Tukey's multiple comparisons test was used to analyze the longitudinal CSF SPP1 protein data. Unless otherwise noted, data were analyzed with GraphPad Prism, and a p-value of <0.05 was considered statistically significant.

SomaScan utilized median normalized data (medNormSMP), where normalization of an analyte Relative Fluorescence Units (RFU) was applied to samples against the median RFU of that analyte from all the samples (non-controls) on the plate. The SomaDataIO R package was used to transform the expression data from the medNormSMP file, as well as to extract covariates of interest and probes features. Expression data was further

log2 transformed, and a linear model was used to evaluate differentially expressed proteins in treated and control samples. Two types of models were used – linear dose-dependent across all doses, and for each individual dose comparing treated and control samples. Bonferroni correction was used to correct for multiple testing, and adjusted *p*-value cutoffs of 0.05 and 0.1 were used as significant and suggestive, respectively.

For RNAseq data analysis, raw read counts were normalized into Reads Per Million (RPM) and log2 transformed. Differentially expressed genes were evaluated using a linear model by comparing treated and control samples at each dose. Differential expression analysis was done separately for hippocampus and cortex samples accounting for sex as a covariate in the model. Signed *p*-values were converted into Z-scores as previously described [44]. Bonferroni multiple testing correction was used. Adjusted *p*-value cutoffs of 0.05 and 0.1 were used as significant and suggestive, respectively.

Clinical study design

INVOKE-1 (NCT03635047) was a two-part, phase 1, randomized, double-blind, placebo-controlled study designed to evaluate the safety, tolerability, immunogenicity, PK, and PD of AL002 administered as SAD in HVs (Fig. 1) and multiple doses (MD) in participants with mild-to-moderate AD (data not shown for discontinued MD cohort). In the SAD portion of the study, Cohorts A through C sequentially enrolled 3 HVs into 3 single-participant cohorts treated with AL002 0.003 mg/kg, 0.03 mg/kg, or 0.2 mg/kg. For Cohorts D through I, 8 HVs (6 active: 2 placebo) were sequentially enrolled and treated with single doses of AL002 or placebo, at dose levels ranging from 0.6 to 60 mg/kg. In addition, 2 open-label single-dose cohorts were enrolled: Cohort K enrolled 6 participants treated at 45 mg/kg; Cohort N enrolled 8 participants treated at 60 mg/kg with CSF sampled at later timepoints than the corresponding double-blind cohort (Cohort I).

After receiving single-dose AL002 or placebo on Day 1, all HVs were followed for safety for 12 weeks. During the follow-up period, participants returned for scheduled visits on Days 1, 2, 3, 5, 8, 13, 30, 43, 57, and 85. Serum PK samples and plasma samples were collected at each onsite study visit. Lumbar puncture to obtain CSF samples was performed at predose baseline, Day 3 (i.e., 48 h after infusion), and Day 13 in the 6 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg cohorts (Cohorts F–I), and on Days 30, 43, and 57 in the open-label 60 mg/kg cohort (Cohort N).

In the MD portion of the study, participants with mild to moderate AD received multiple IV infusions of AL002. Cohort J received 15 mg/kg AL002 on Days 1, 8, 15, and 22, and returned for scheduled visits on Day 29, 36, 50, 64, 78, 106, and 134. Cohort L received 60 mg/kg AL002 on Days 1 and 29 and returned for scheduled visits on Days 31, 36, 43, 57, 71, 85, 113, and 141. Cohort M was a planned open-label cohort of AD patients who carried at least one of the two *TREM2* mutations (R47H or R62H), but the MD portion of the study was discontinued due to the COVID-19 pandemic before Cohort M opened for enrollment.

Participants

HVs (SAD cohort) were eligible for inclusion if they met the following key criteria: males or nonpregnant females

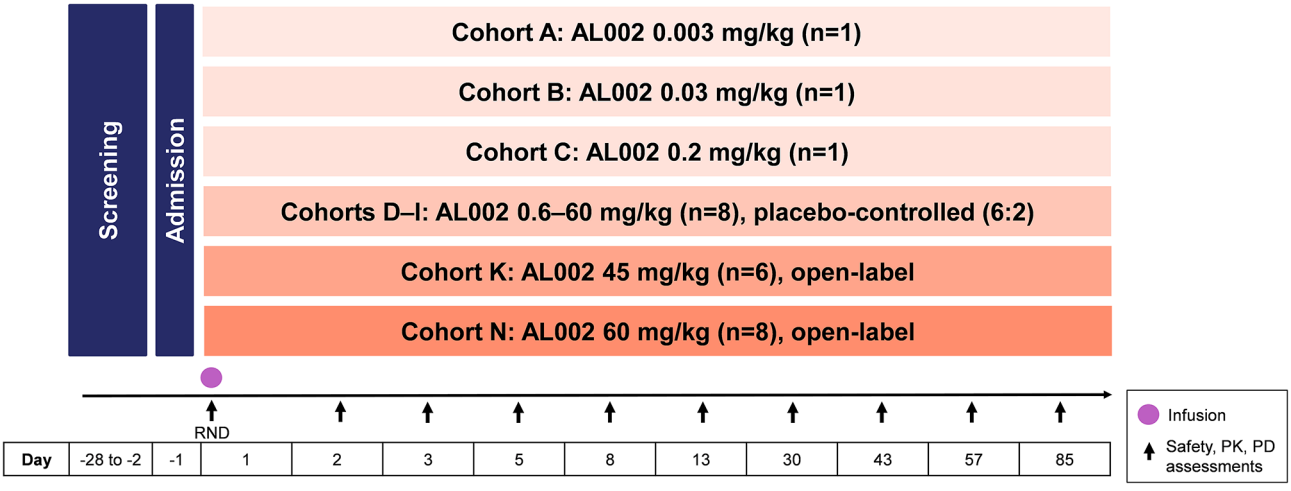

Fig. 1 AL002 phase 1 study design. Cohorts A through C sequentially enrolled 3 HVs into 3 single-participant cohorts treated with AL002 0.003 mg/kg, 0.03 mg/kg, or 0.2 mg/kg. For Cohorts D through I, 8 HVs (6 active: 2 placebo) were sequentially enrolled and treated with single doses of AL002 or placebo, at the following dose levels: 0.6 mg/kg, 2 mg/kg, 6 mg/kg, 15 mg/kg, 30 mg/kg, and 60 mg/kg. In addition, 2 open-label single-dose cohorts were enrolled: Cohort K enrolled 6 participants treated at 45 mg/kg, and Cohort N enrolled 8 participants treated at 60 mg/kg with CSF sampled at later timepoints. CSF, cerebrospinal fluid; HV, healthy volunteer; PD, pharmacodynamic; PK, pharmacokinetic; RND, randomization

aged 18 to 65 years with body weight between 50 and 120 kg; in good physical health based on no clinically significant findings.

The study protocol was approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) for each participating institution. All participants provided written informed consent, and the study was conducted in compliance with the ethical principles of Good Clinical Practice (GCP) as required by the major regulatory authorities and in accordance with guidelines established by the Declaration of Helsinki.

Study endpoints

Safety endpoints included the incidence, nature, and severity of adverse events (AEs), the incidence of dose-limiting adverse events (DLAEs), and the incidence of AEs that led to study discontinuation or dose reduction. Additional safety endpoints included changes from baseline or abnormalities in clinical laboratory tests and vital signs, the incidence of antidrug antibodies (ADAs), physical or neurologic abnormalities, and suicidal ideation/behavior. PK endpoints included serum and CSF concentrations of AL002 at specified time points, and PK parameters, including CSF:serum partition coefficients. PD assessments included changes in the CSF levels of sTREM2, SPP1 protein, soluble CSF1R (sCSF1R), and IL1RN protein after dosing relative to baseline and changes in plasma sTREM2 and IL1RN protein after dosing relative to baseline.

Immunoassays in humans

A bioanalytical method was developed and validated to quantify AL002 in human serum and CSF. Anti-AL002 115.45.3.2B12 was diluted in coating buffer and immobilized onto a 96-well microtiter sample plate. Samples were diluted (serum: 1:100; CSF 1:20) with Assay Buffer, dispensed onto the sample plate, and detected with biotinylated Anti-AL002 86.137.1.6E3. Streptavidin-HRP A was added, followed by a tetramethylbenzidine (TMB) peroxidase substrate solution. The reaction was stopped with Stop Solution, and plates were read on a plate reader at two wavelengths, 450 nm for detection and 630 nm for background. AL002 concentrations were determined on a standard curve obtained by plotting optical density (OD) vs. concentration using a 4-parameter logistic curve-fitting program with $1/y^2$ weighting.

As previously described [32], sTREM2 in human CSF and plasma were measured using a custom AL002-tolerant ECLA method using recombinant human TREM2 Fc Chimera (R&D Systems cat# 1828-T2-05) as a standard. sCSF1R in human CSF was measured using a commercial ELISA assay by R&D Systems. Plasma IL1RN and SPP1 protein were measured using commercially available assays on the MSD™ platform (IL-1RA U PLEX® set from cat # K151XPK, OPN R PLEX® set from cat # F21YM). Additional assays were further qualified to measure IL1RN and SPP1 protein in human CSF using the existing MSD™ Antibody Sets.

Statistical analysis for clinical studies

Sample size calculation for the study was based on the following. For a sample size of 6, there was a 95% probability that an AE with greater than or equal to 40% prevalence will be observed and an 80% probability that an AE with greater than or equal to 23.6% prevalence will be observed. All statistical analyses were performed using SAS statistical software (Version 9.4), unless otherwise noted. Continuous data were summarized using descriptive statistics. Categorical data were summarized as frequency counts and percentages. For all percentage calculations, the denominator was the number of participants in the relevant dose group or analysis population, unless otherwise stated. Baseline was defined as the last available, non-missing observation prior to first study drug administration.

Individual serum and CSF AL002 concentration data summarized by nominal sampling time point and dose level with descriptive statistics. For the estimation of PK parameters, concentrations that were below the limit of quantitation (BLQ) prior to the first quantifiable value were set equal to zero, and concentrations that were recorded as BLQ after the first quantifiable value were set to missing. PK parameters were computed from the individual serum AL002 concentrations using a non-compartmental approach using Phoenix WinNonlin version 8.2. PK parameters were summarized by AL002 dose group using descriptive statistics. Geometric mean and 95% CI were calculated for all parameters except T_{max} and $t_{½}$. Partition coefficient parameters for AL002 were calculated by dividing CSF concentrations by serum concentrations at matching timepoints.

For PD endpoints in the SAD cohort, descriptive statistics were used to summarize the percent changes from baseline for each treatment group at each specified time point. In addition, post-hoc analyses evaluating treatment effects of AL002 doses vs. pooled placebo using a restricted maximum likelihood (RMLE)-based mixed model for repeated measures (MMRM) [MIXED procedure, SAS v9.4]. Specifically, for each PD biomarker (sTREM2, IL1RN protein, SPP1 protein, and CSF1R in CSF; sTREM2 and IL1RN protein in plasma), an MMRM was fitted with the log2-transformed ratio of postbaseline to baseline biomarker values at scheduled postbaseline visits as the dependent variable and the fixed effects of log2-transformed baseline biomarker value, treatment group (pooled placebo [CSF and plasma], AL002 6, 15, 30, 45 and 60 mg/kg [CSF] and AL002 0.6, 2, 6, 15, 30, 45 and 60 mg/kg [plasma]), categorical visit, and

visit-by-treatment interaction. An unstructured covariance matrix was used to model the within-participant errors. Within-group least squares means (LSM) and their difference (LSM difference) of each AL002 dose vs. pooled placebo, and corresponding standard errors were provided. LSM and LSM difference were further converted to a percentage, within-group percent difference in geometric mean from baseline and placebo-adjusted percent difference in geometric mean ratio from baseline, respectively. *P* values comparing AL002 doses vs. pooled placebo were adjusted with a Dunnett test for each visit.

Results

Repeat-dose AL002 was well tolerated in cynomolgus monkeys

To guide human clinical dose and regimen selection, and to estimate safety margins for the first-in-human phase 1 trial of AL002, we performed a 4-week GLP toxicology study in cynomolgus monkeys. IV administration of AL002 at doses up to 250 mg/kg weekly for 4 weeks was well tolerated, with no AL002-related adverse effects on any measured safety parameter. All monkeys survived to scheduled sacrifice, and no AL002-related clinical, ophthalmic, or neurobehavioral observations were noted. There were no AL002-related changes in mean body weight, food intake, ECG parameters, respiratory rates, clinical pathology parameters, peripheral blood immunophenotyping values, cytokines, organ weight, or macroscopic observations. AL002-related microscopic findings were limited to minimal, unilateral findings of multinucleated cells in the ocular ciliary body of 4 of 10 animals in the 250 mg/kg dose group (2 animals that were sacrificed on Day 31 and 2 animals that underwent the 4-week recovery period and were sacrificed on Day 59) and were not considered adverse. The no observed adverse effect level (NOAEL) was 250 mg/kg, which was associated with a Day 22 C_{max} of 5130 µg/mL and AUC_{0-168} of 265,000 h●µg/mL (Table 1).

After each slow bolus IV injection, AL002 concentrations readily declined after reaching C_{max}, generally in a mono-exponential manner (Fig. 2A). Mean steady state volume of distribution (V_{ss}) values ranged from 63.3 to 88.2 mL/kg on Day 1 and from 37.5 to 74.3 mL/kg on Day 22. Exposure, as assessed by AL002 mean C_{max} and AUC_{0-168} values, generally increased slightly less than dose proportionally with the increase in dose level from 20 to 250 mg/kg/dose. The mean C_{max} and AUC_{0-168} values on Day 22 were slightly higher than on Day 1, indicating some accumulation of AL002 after multiple doses in monkeys. Mean accumulation ratio values ranged from 0.989 to 1.28 for C_{max} and from 1.21 to 1.33 for AUC_{0-168}.

Mean concentrations of AL002 in the CSF increased with dose level from 20 to 250 mg/kg/dose (Fig. 2B). The mean CSF/serum ratio for all AL002 dose groups was 0.0860%, indicating that AL002 administered IV was able to penetrate the central nervous system in monkeys. Overall, the safety and PK profiles of AL002 in monkeys supported further clinical development of the molecule.

Table 1 Mean toxicokinetic parameters for AL002 in cynomolgus monkeys in the 4-week GLP study

Dose	Day	C_{max}, µg/mL	Clearance, mL/h/kg	AUC_{0-168}, h●µg/mL
20 mg/kg[a]	1	401	NA	25,800
	22	504	0.615	33,700
80 mg/kg[a]	1	1590	NA	82,100
	22	1560	0.811	99,400
250 mg/kg[b]	1	4430	NA	219,000
	22	5130	0.976	265,000

[a] $n=6$ (3 male, 3 female)

[b] $n=10$ (5 male, 5 female)

AUC_{0-168}, area under the concentration-time curve from time zero to 168 h; C_{max}, maximum concentration; GLP, good laboratory practice; NA, not available

AL002 demonstrated TREM2 pathway engagement in cynomolgus monkeys

TREM2 pathway biomarkers were measured in cynomolgus monkey CSF and brain to evaluate the treatment effect of AL002. sTREM2 is generated via proteolytic cleavage and shedding of the TREM2 receptor ectodomain and is measurable in peripheral circulation and CSF [45]. In an in vitro cell-based assay, AL002 treatment of primary macrophages resulted in reduction in both membrane TREM2 and sTREM2 released in the culture media, indicating AL002 binding to TREM2 causes receptor activation and internalization, thereby reducing membrane TREM2 available for cleavage (Additional file 1; Supplementary Fig. 1). In a non-GLP DRF study conducted in cannulated monkeys over a 20-day period, AL002 dose-dependently decreased CSF sTREM2, with both the 80 and 250 mg/kg dose groups showing a more than 70% reduction compared with predose levels and compared with the control group, whereas the 20 mg/kg group showed a partial reduction (Fig. 3A).

Consistent with the reduction of sTREM2 in monkey CSF, a dose-dependent reduction of total TREM2 protein levels was observed in monkey brain tissues following terminal sacrifice in the 4-week GLP study (Fig. 3B, C). In the 250 mg/kg group, TREM2 levels were significantly reduced in both the frontal cortex ($p<0.001$) and hippocampus ($p<0.0001$) compared with vehicle-treated controls. In the 80 mg/kg group, hippocampal TREM2 levels were significantly reduced compared with controls ($p<0.001$), while the reduction in TREM2 levels in frontal cortex did not achieve significance by one-way ANOVA. For the 20 mg/kg group, while there was a trend in reduction in both hippocampal and frontal cortex TREM2 levels, it did not reach statistical significance.

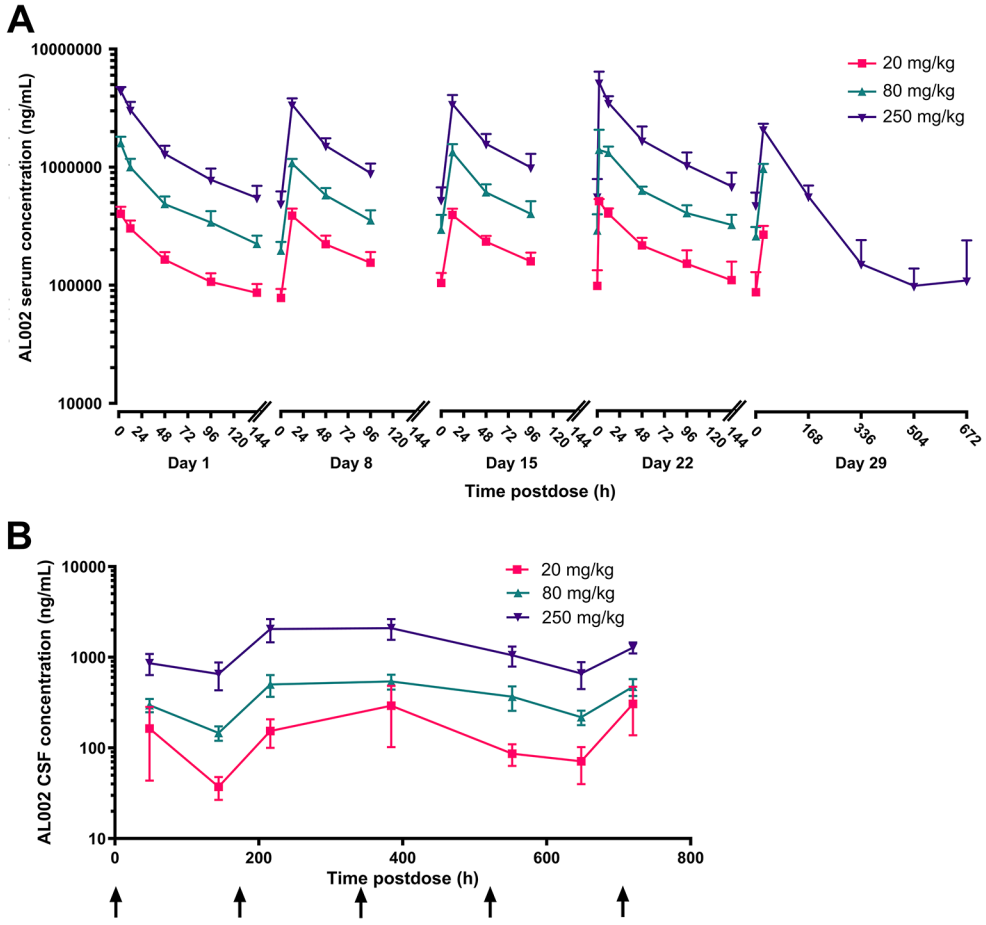

Fig. 2 Pharmacokinetics of repeat-dose AL002 in cynomolgus monkeys. (**A**) Mean + SD serum concentrations of AL002. For Days 1 to 22, $n = 6$ in 20 mg/kg and 80 mg/kg, $n = 10$ in 250 mg/kg. For Day 29, $n = 4$ in the 250 mg/kg group. (**B**) Mean ± SEM CSF concentration of AL002. LLOQ standard was 5.0 ng/mL. $n = 6$ in 20 mg/kg and 80 mg/kg, $n = 10$ in 250 mg/kg. Black arrows indicate time of dosing. CSF, cerebrospinal fluid; h, hours; LLOQ, lower limit of quantification; SD, standard deviation

To further identify TREM2 downstream signaling pathways and identify clinically measurable biomarkers, we conducted both RNAseq and proteomics screens in animals treated with AL002. For RNAseq, as *TREM2* is expressed at moderate levels in the cynomolgus monkey brain, we utilized the bacterial artificial chromosome (BAC) transgenic (Tg) mice that overexpress human *TREM2* to generate an augmented TREM2 activation signal to help identify genes downstream of *TREM2*. The human *TREM2* BAC Tg mice expressed 5- to 10-fold higher levels of *TREM2* compared to cynomolgus monkeys based on brain TREM2 levels (data not shown). Two days after administration of 50 mg/kg AL002c, gene expression in the frontal cortex of the human *TREM2* BAC Tg mice was tested by RNAseq. Strong effects on gene signatures were observed when AL002c-treated animals were compared to control IgG-treated animals. The most upregulated mouse genes were *Tyrobp* (Dap12), *Csf1r*, and *Gfap* (Supplementary Fig. 2). RNAseq was also conducted on the brain tissues of cynomolgus monkeys treated with AL002 in the GLP study, with a focus on the genes identified in the human TREM2 BAC Tg mice. The above genes (*TYROBP*, *CSF1R*, *GFAP*), as well as *SPP1*, were confirmed to be upregulated in the cynomolgus monkey brain (Supplementary Fig. 2).

To further assess soluble biomarker changes in monkey CSF following AL002 administration, we used SomaScan to measure the levels of different protein analytes in the monkey CSF before and 48 h after AL002 treatment. Proteins that significantly increased with AL002 treatment in a dose-dependent manner included CAMKK1, IL1RN protein, SPP1 protein, and TNFSF8, as shown in the volcano plot (Fig. 3D).

Orthogonal and clinically translatable immunoassays were developed to confirm signaling changes observed in RNAseq and SomaScan proteomics analysis of AL002-treated NHPs. *Spp1* expression and protein (i.e., osteopontin) levels are TREM2-dependent and are strongly reduced with TREM2 knockout [25, 29, 46]. In the 4-week GLP study, weekly AL002 treatment resulted in

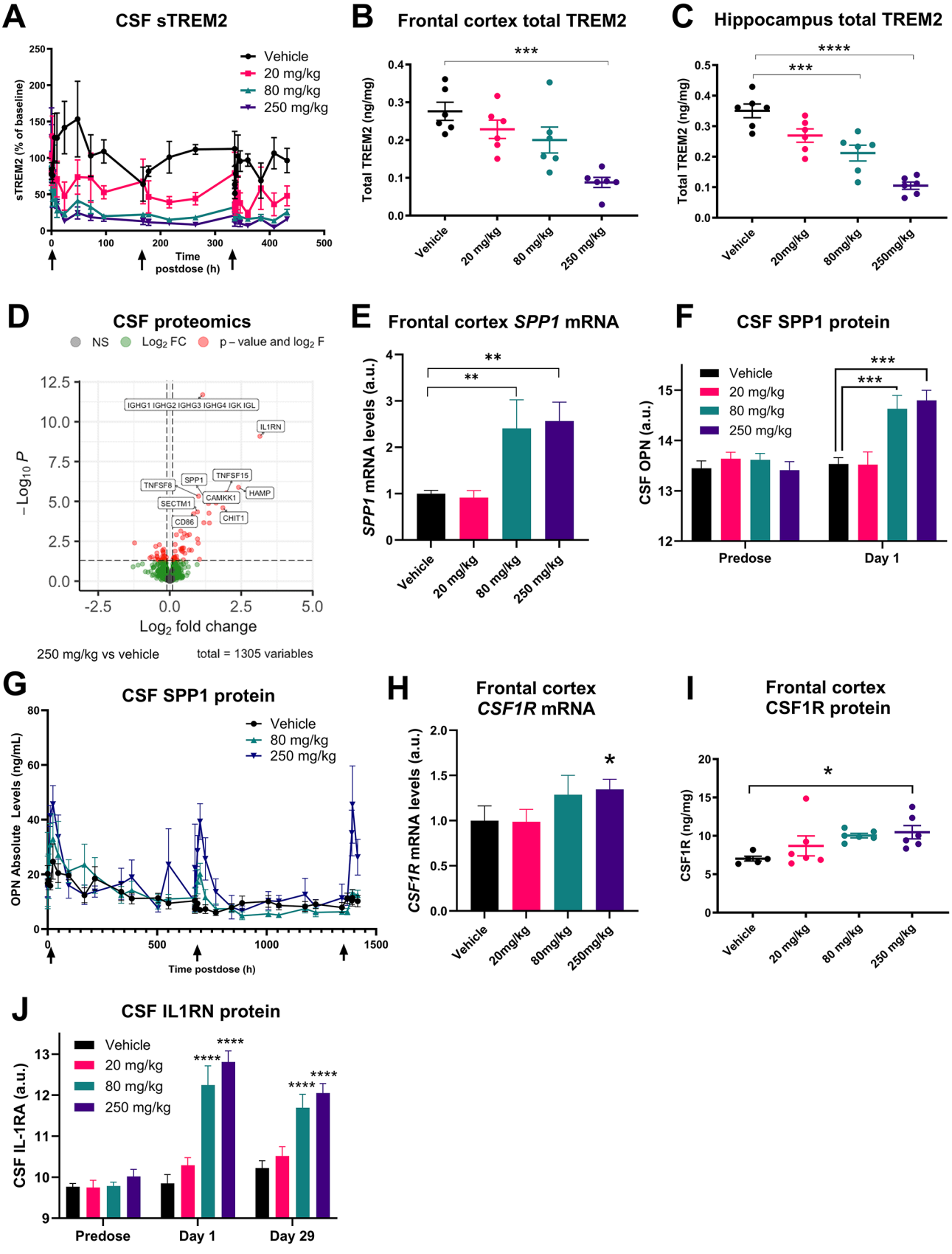

Fig. 3 (See legend on next page.)

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Fig. 3 Pharmacodynamic effects of repeat-dose AL002 in cynomolgus monkeys. (**A**) CSF sTREM2 from the non-GLP DRF study ($n=4$ per dose group). From the 4-week GLP study ($n=6$ per dose group), (**B**) total TREM2 levels in the frontal cortex and (**C**) hippocampus. (**D**) Volcano plot of SomaScan data for CSF protein for 250 mg/kg group vs. vehicle 48 h after the Day 1 dose. (**E**) *SPP1* mRNA expression in frontal cortex, normalized to control values, from the 4-week GLP study. (**F**) CSF SPP1 protein levels at predose baseline and Day 1 (i.e., 48 h after dose). (**G**) Longitudinal CSF SPP1 protein levels from the non-GLP interval study ($n=5$ for the vehicle and 250 mg/kg dose group; $n=4$–5 for the 80 mg/kg dose group). From the 4-week GLP study, (**H**) *CSF1R* mRNA expression in frontal cortex, normalized to control values, (**I**) CSF1R protein levels in frontal cortex, and (**J**) IL1RN protein levels in CSF at predose baseline, 48 h after Day 1 dose, and 48 h after Day 29 dose. Black arrows indicate time of dosing. Data are means ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. a.u., arbitrary units; CSF, cerebrospinal fluid; CSF1R, soluble colony-stimulating factor 1 receptor; DRF, dose range finding; GLP, good laboratory practices; h, hours; LLOQ, lower limit of quantification; sCSF1R, soluble CSF1R; SEM, standard error of the mean; sTREM2, soluble TREM2; TREM2, triggering receptor expressed on myeloid cells-2

significantly higher *SPP1* mRNA expression in the frontal cortex in the 80 mg/kg and 250 mg/kg dose groups compared with controls ($ps<0.01$) on Day 31 (i.e., 48 h after the Day 29 dose; Fig. 3E). Consistent with the differential mRNA expression, the AL002 80 mg/kg and 250 mg/kg dose groups showed elevated CSF SPP1 protein levels on Day 1 (48 h after dose) compared with predose baseline (Fig. 3F). In the non-GLP interval study, monthly IV injections of 250 mg/kg AL002 resulted in a statistically significant increase in CSF SPP1 protein levels 12 h and 24 h after each dose compared with controls ($ps<0.01$; Fig. 3G). The 80 mg/kg monthly dose group showed partial increases in CSF SPP1 protein after the first and second dose but did not reach statistical significance by 2-way ANOVA.

CSF1R is a growth factor that is critical for microglial survival and regulates microglial homeostasis [47]. In the 4-week GLP study, weekly administration of 250 mg/kg AL002 resulted in significantly elevated *CSF1R* mRNA expression in frontal cortex (Fig. 3H). Correspondingly, the AL002 250 mg/kg dose group showed significantly higher CSF1R protein levels in the frontal cortex compared with controls ($p<0.05$), while this increase did not reach significance for the lower dose groups (Fig. 3I).

IL1RN protein (i.e., IL-1RA) is an endogenous soluble antagonist of the IL-1 receptor. In the 4-week GLP study, IL1RN protein levels in CSF were significantly elevated in the 80 mg/kg and 250 mg/kg dose groups both 48 h after the Day 1 dose and 48 h after the Day 29 dose ($ps<0.0001$; Fig. 3J).

Clinical patient population and disposition

First-in-human studies of AL002 were initiated based on PK, PD, and safety data obtained in NHPs. For the phase 1 trial, a total of 69 participants ($n=64$ HVs in part 1 SAD; $n=5$ participants with early AD in the discontinued part 2 MD) enrolled in the study between October 2018 and August 2020 from sites in North America, Australia, and Europe. Among 64 HVs who were randomized in the SAD cohorts, 53 received single-dose AL002 administered IV at dose levels ranging from 0.003 to 60 mg/kg, and 11 received placebo. The starting dose of 0.003 mg/kg is approximately 0.04% of the maximum recommended starting dose, based on the NOAEL of the 4-week GLP toxicity study. Baseline demographics for the HVs (Table 2) were generally similar across treatment groups. Overall, HVs receiving AL002 were younger, more frequently male, and less frequently white than those receiving placebo.

AL002 was well tolerated in healthy volunteers

Among HVs, AL002 was well tolerated, with no treatment-related serious adverse events (SAEs) or DLAEs up to the highest dose. Overall, 69.8% of participants in the total AL002 group experienced treatment-emergent AEs (TEAEs) compared with 81.8% of participants in the pooled placebo group (Table 3). No SAEs were observed in the total AL002 group, while 1 participant in the pooled placebo group experienced 1 SAE considered unrelated to treatment (traumatic injury). No adverse events of special interest (AESI) occurred during the study. All TEAEs in the AL002 cohorts were mild or moderate in severity, while 2 participants in the pooled placebo group experienced 3 TEAEs that were severe (injury, fatigue, nausea). Two HVs experienced TEAEs considered probably related to AL002 that led to interruption of the study drug infusion (1 in the 60 mg/kg cohort with mild nausea and 1 in the 45 mg/kg cohort with moderate parasthesias, mild nausea, and mild retching). The most frequent TEAEs reported by ≥5% of participants in the total AL002 group were headache (35.8%; 19 of 53) and nausea (17.0%; 9 of 53). There did not appear to be an increased frequency of TEAEs with increased AL002 dose, except for nausea, which was higher in the 60 mg/kg cohort. The incidence of TEAEs that were designated treatment related by the investigators was slightly lower in the total AL002 group vs. the pooled placebo group.

No clinically relevant trends or notable changes over time or between the AL002 groups and the placebo group were observed for the hematology, biochemistry, coagulation, urinalysis, or ECG parameters. The few abnormalities observed in the clinical laboratory tests of HVs were mild or moderate in severity and did not require treatment. Several participants experienced clinically significant hematology and chemistry abnormalities, with 3 clinical abnormalities deemed possibly related to AL002 treatment (neutropenia, eosinophilia, high alanine

Table 2 Baseline demographics of healthy volunteers in the phase 1 study

	AL002								Total AL002 (*n* = 53)	Pooled Placebo (*n* = 11)
	0.003-0.2 mg/kg (*n* = 3)	0.6 mg/kg (*n* = 6)	2 mg/kg (*n* = 6)	6 mg/kg (*n* = 6)	15 mg/kg (*n* = 6)	30 mg/kg (*n* = 6)	45 mg/kg (*n* = 6)	60 mg/kg (*n* = 14)		
Age, years										
Mean (SD)	41.7 (16.86)	26.0 (3.69)	31.2 (13.38)	31.8 (11.91)	32.7 (3.78)	32.7 (16.11)	32.3 (14.25)	32.7 (10.55)	32.1 (11.35)	39.7 (15.86)
Min, max	30, 61	21, 31	23, 58	23, 53	28, 38	22, 65	21, 60	20, 51	20, 65	22, 65
Sex, n (%)										
Female	0	4 (66.7)	2 (33.3)	2 (33.3)	4 (66.7)	5 (83.3)	4 (66.7)	10 (71.4)	31 (58.5)	8 (72.7)
Male	3 (100)	2 (33.3)	4 (66.7)	4 (66.7)	2 (33.3)	1 (16.7)	2 (33.3)	4 (28.6)	22 (41.5)	3 (27.3)
Race, n (%)										
White	3 (100.0)	4 (66.7)	4 (66.7)	4 (66.7)	4 (66.7)	5 (83.3)	3 (50.0)	10 (71.4)	37 (69.8)	11 (100.0)
Asian	0	2 (33.3)	1 (16.7)	0	1 (16.7)	0	2 (33.3)	0	6 (11.3)	0
Black/African American	0	0	0	0	0	0	0	3 (21.4)	3 (5.7)	0
Other	0	0	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	1 (7.1)	7 (13.2)	0
Hispanic/Latino ethnicity, n (%)	1 (33.3)	0	0	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	2 (14.3)	8 (15.1)	2 (18.2)
Screening BMI, kg/m^2, mean (SD)	24.90 (1.212)	23.53 (1.834)	24.88 (3.142)	24.32 (3.159)	24.47 (1.245)	23.97 (1.266)	28.00 (5.829)	25.41 (4.359)	25.01 (3.486)	24.45 (3.839)

BMI, body mass index; SD, standard deviation

Table 3 Summary of TEAEs in healthy volunteers in the phase 1 study

	AL002								Total AL002 (*n* = 53)	Pooled Placebo (*n* = 11)
	0.003-0.2 mg/kg (*n* = 3)	0.6 mg/kg (*n* = 6)	2 mg/kg (*n* = 6)	6 mg/kg (*n* = 6)	15 mg/kg (*n* = 6)	30 mg/kg (*n* = 6)	45 mg/kg (*n* = 6)	60 mg/kg (*n* = 14)		
Participants with ≥ 1 TEAE, n (%)	2 (66.7)	3 (50.0)	2 (33.3)	5 (83.3)	5 (83.3)	4 (66.7)	6 (100.0)	10 (71.4)	37 (69.8)	9 (81.8)
Participants with ≥1 treatment-related TEAE, n (%)	2 (66.7)	2 (33.3)	2 (33.3)	2 (33.3)	2 (33.3)	4 (66.7)	5 (83.3)	7 (50.0)	26 (49.1)	6 (54.5)
Treatment-related TEAEs in ≥ 5% of participants in the total AL002 group										
Headache	1 (33.3)	1 (16.7)	2 (33.3)	2 (33.3)	1 (16.7)	4 (66.7)	2 (33.3)	2 (14.3)	15 (28.3)	4 (36.4)
Dizziness postural	1 (33.3)	0	1 (16.7)	0	0	1 (16.7)	0	0	3 (5.7)	1 (9.1)
Nausea	0	0	1 (16.7)	1 (16.7)	0	0	1 (16.7)	6 (42.9)	9 (17.0)	1 (9.1)
Vomiting	0	0	0	0	0	0	0	3 (21.4)	3 (5.7)	2 (18.2)
Any SAE, n (%)	0	0	0	0	0	0	0	0	0	1 (9.1)
Any TEAE leading to study drug withdrawal, n (%)	0	0	0	0	0	0	1 (16.7)	1 (7.1)	2 (3.8)	0

A TEAE is defined as an AE that commenced on or after the time of first study drug administration. If a participant has multiple occurrences of a TEAE, the participant is counted only once in the count (n) column for a given System Organ Class and Preferred Term. Any AEs with a missing or unknown severity are counted as severe. A treatment-related TEAE is defined as a TEAE with a relationship to study drug of possible, probable, missing, or unknown. AEs were coded to system organ class and preferred term using MedDRA Version 21.1

AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities; SAE, serious adverse event; TEAE, treatment emergent adverse event

aminotransferase). There was no pattern of changes from baseline in vital signs which suggested that AL002 had any discernible effect on these parameters. One participant in the AL002 60 mg/kg cohort experienced mild tachycardia and mild symptomatic orthostatic hypotension that were deemed possibly related to treatment and resolved within 12 h postdose. No other vital sign TEAEs were considered treatment related.

The incidence of treatment-emergent positive antidrug antibody (TEPADAs) was 64% (34 of 53) among the total AL002 group and 9% (1 of 11) among the pooled placebo group. The majority of positive TEPADA titers were low (< 1:256) and appeared transient. A review of PK parameters showed no obvious effects of ADA on PK.

PK of AL002 in serum and CSF of healthy volunteers

Following a single IV infusion of AL002 at 0.003 to 60 mg/kg in HVs, mean serum concentrations of AL002 increased in a dose-dependent manner (Fig. 4A). Mean serum concentrations of AL002 were consistently elevated across the entire profile with each increasing dose followed by a distribution and then an elimination phase. Measurable concentrations of AL002 were seen for 84 days (the longest timepoint measured) in cohorts

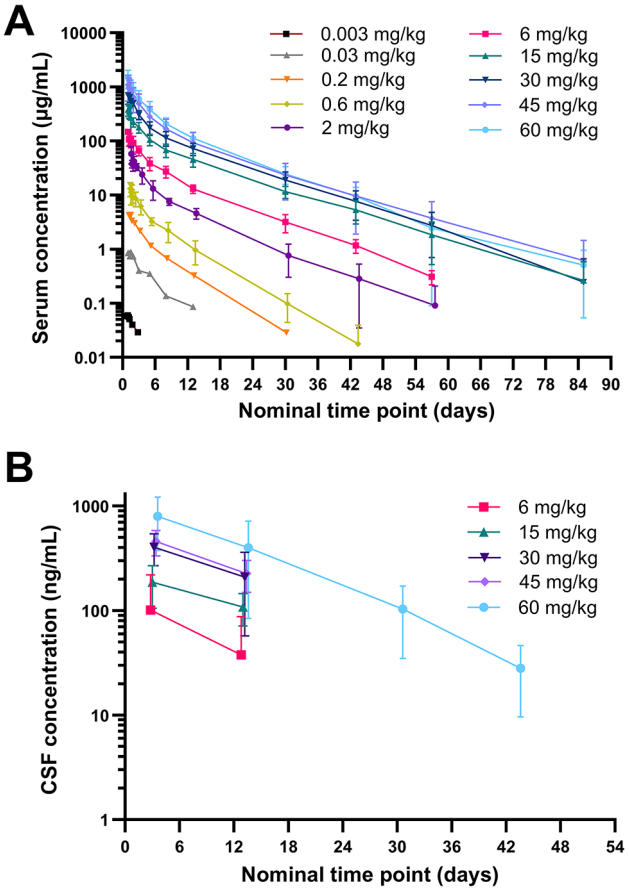

Fig. 4 Pharmacokinetics of single ascending dose AL002 in healthy volunteers. (**A**) Mean ± SD serum concentrations of AL002 (semi-logarithmic). LLOQ was 0.0200 µg/mL. (**B**) Mean ± SD CSF concentration of AL002 (semi-logarithmic). LLOQ was 5.0 ng/mL. $n=6$ for the 6 mg/kg, 15 mg/kg, and 30 mg/kg dose groups; $n=5$ for the 45 mg/kg group; $n=5$–6 for 60 mg/kg group. Data for the 60 mg/kg dose at Day 57 are not shown, as $n=1$. CSF, cerebrospinal fluid; LLOQ, lower limit of quantification; SD, standard deviation

that received AL002 at 15 mg/kg or higher. Serum PK parameters showed that mean total and peak exposures of AL002 increased with increasing dose level, with approximately dose-proportional C_{max} and AUC_{0-inf} (Table 4). Following single ascending IV infusions of AL002 at 0.6 to 60 mg/kg, maximum serum concentrations of AL002 (C_{max}) were attained at approximately 1.2 to 1.5 h postdose (median T_{max}) for all cohorts except the AL002 0.6 mg/kg and 15 mg/kg cohorts, in which C_{max} was attained at a median of 5.09 h. The mean terminal half-life of a single dose of 0.6 to 60 mg/kg AL002 in serum ranged from 121 to 216 h. The mean clearance values of AL002 (0.6 to 60 mg/kg) varied between a mean (coefficient of variation [CV]) of 22.6 (10.0) and 30.2 (23.6) mL/h, and the mean volume of distribution varied between 5.17 and 7.98 L.

Mean CSF concentration-time profiles for AL002 were similar for each cohort, with mean peak exposures on Day 3 followed by a decline in exposure as AL002 was eliminated (Fig. 4B). The mean CSF concentrations of AL002 were generally elevated across the entire profile with each increasing dose. Measurable concentrations of AL002 were seen in the CSF for 12 days (the longest timepoint measured) in cohorts that were dosed with 6, 15, 30, or 45 mg/kg AL002, and seen for 42 days in the 60 mg/kg cohort (the longest timepoint measured). CSF-to-serum partition coefficients at Day 3 ranged from 0.07 to 0.13% and at Day 13 ranged from 0.17 to 0.28% (Supplementary Table 1).

Single dose AL002 decreased CSF sTREM2 and increased markers of TREM2 signaling

Target engagement of AL002 was evaluated by measuring sTREM2 in CSF in the 6, 15, 30, 45, and 60 mg/kg dose groups. Following a single infusion of AL002 in HVs, a placebo-adjusted AL002 dose-dependent reduction was observed in CSF sTREM2 on Day 3 (i.e., 48 h after infusion) and Day 13, indicating engagement of AL002 to the TREM2 target (Fig. 5A), consistent with what was observed in the NHP toxicology studies. Dose-dependent reductions relative to placebo in CSF sTREM2 levels were observed, with a maximal 63% reduction (Day 3) at the highest dose (adjusted $p<0.0001$; Supplementary Table 2). CSF sTREM2 was still reduced from baseline (32%) at Day 30 for the AL002 60 mg/kg dose (Fig. 5B).

Pathway activation was also evaluated by measuring markers of TREM2 signaling in CSF, including SPP1 protein, soluble CSF1R (sCSF1R; a cleavage product of transmembrane CSF1R), and IL1RN protein. At least 20% increases relative to placebo in CSF SPP1 protein were observed on Day 3 for all AL002 doses, and on Day 13 for AL002 30 and 60 mg/kg doses (Fig. 5C, Supplementary Table 2). A 19% increase relative to placebo in CSF sCSF1R was observed for the 60 mg/kg dose on Day 3 (adjusted $p=0.0298$; Fig. 5D, Supplementary Table 2). Increases relative to placebo in CSF IL1RN protein of 83% to 138% were seen on Days 3 and 13, respectively, for the AL002 30, 45, and 60 mg/kg dose groups (Fig. 5E, Supplementary Table 2).

We also observed placebo-adjusted increases in plasma levels of IL1RN protein and sTREM2 following single-dose AL002. In general, a trend of placebo-adjusted AL002 dose-dependent increases in plasma IL1RN protein across visits up to Day 30, and at least 35% increases relative to placebo in AL002 15 mg/kg or higher doses from Day 2 to Day 30 were observed (Fig. 6A, Supplementary Table 3). For plasma sTREM2, placebo-adjusted increases across visits up to Day 30 of 20–91% (adjusted $p<0.1$) for the AL002 15 mg/kg dose and 18–58% (adjusted $p<0.1$ except on Days 5, 8 and 30) for the 30 mg/kg dose were observed (Fig. 6B). A trend of increases relative to placebo of 9–41% for the AL002

Table 4 Geometric mean (CV) of serum AL002 PK parameters in the phase 1 study

	AL002[a]						
Parameter	**0.6 mg/kg (*n* = 6)**	**2 mg/kg (*n* = 6)**	**6 mg/kg (*n* = 6)**	**15 mg/kg (*n* = 6)**	**30 mg/kg (*n* = 6)**	**45 mg/kg (*n* = 6)**	**60 mg/kg (*n* = 14)**
AUC_{0-t}, h●µg/mL	1280 (27.0)	5400 (16.4)	16,300 (13.6)	48,200 (6.8)	80,100 (24.3)	95,000 (129.1)	138,000 (31.9)
AUC_{0-inf}, h●µg/mL	1280 (27.0)	5430 (16.6)	16,400 (13.6)	48,200 (7.0)	80,200 (24.3)	95,200 (129.0)	138,000 (31.0)
C_{max}, µg/mL	15.1 (23.5)	60.6 (26.0)	147 (11.2)	430 (24.5)	722 (10.8)	831 (138.6)	1530 (30.3)
t_{max}, h[b]	5.09 (1.18, 9.30)	1.32 (1.18, 13.0)	1.18 (1.17, 1.38)	5.09 (1.20, 13.1)	1.32 (1.17, 9.00)	1.52 (1.17, 4.63)	1.31 (1.12, 25.0)
t½, h[c]	121 (16.0)	167 (25.2)	195 (11.3)	216 (27.7)	196 (24.2)	201 (33.0)	191 (28.2)
CL, mL/h	29.9 (25.5)	27.5 (25.0)	26.3 (14.7)	22.6 (10.0)	24.5 (25.3)	28.1 (39.9)	30.2 (23.6)
V_z, L	5.17 (12.3)	6.45 (17.9)	7.35 (14.6)	6.75 (39.5)	6.78 (32.4)	7.80 (23.8)	7.98 (39.5)

Mean exposure levels and half-life values increased with increasing doses of AL002

[a]Data from single-participant dose groups (0.003-0.2 mg/kg) are not shown

[b]For t_{max}, the median (minimum, maximum) values are presented

[c]For $t_{1/2}$, the mean (CV) values are presented

AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum concentration; NA, not available; PK, pharmacokinetics; t½, half life; T_{max}, time to reach C_{max}; V_z, apparent total volume of distribution at the terminal phase

45 mg/kg dose across visits up to Day 30 (adjusted $p < 0.05$ for visits up to Day 2), and 13–21% for the AL002 60 mg/kg dose across postdose visits on Day 1 (adjusted $p < 0.1$) and Day 2 (adjusted $p = 0.169$).

Discussion

Variants in *TREM2* are associated with greater risk of developing AD and other neurodegenerative diseases, and increasing evidence indicates that TREM2 modulates the innate immune response in AD. Thus, TREM2 activation has received widespread attention as a promising therapeutic approach to modify the course of AD and potentially other neurodegenerative diseases. We and others [30–32, 48] have shown beneficial effects of TREM2-agonistic antibodies in mouse models of AD; to our knowledge, the results presented here represent the first completed study of TREM2-agonistic antibody administration in humans. After establishing the preclinical safety and PK/PD profile of AL002 in cynomolgus monkeys, we evaluated the safety, tolerability, PK, and target engagement of AL002 in HVs in a first-in-human phase 1 clinical trial.

In the phase 1 study reported here, AL002 administered as a single IV dose was well tolerated among HVs across a wide range of doses (0.003 mg/kg to 60 mg/kg), consistent with the lack of adverse findings in monkeys. No drug-related SAEs or DLAEs occurred in HVs during the 12-week observation period. All TEAEs in the AL002 cohorts were mild or moderate in severity, with headache and nausea being the most frequently reported AEs. While positive TEPADAs were detected in 64% of HVs, TEPADAs had no obvious effect on pharmacokinetic parameters.

Consistent with the data in monkeys, PK data confirmed the distribution of AL002 into the CNS of HVs. Mean CSF concentration-time profiles for AL002 were comparable for each dose cohort, and the mean CSF concentrations of AL002 were generally elevated across the entire time profile with each increasing dose. Approximately dose-proportional increases in maximal plasma concentrations (C_{max}) and area under the concentration-time curve (AUC_{0-inf}) were observed with increasing doses of AL002. The mean terminal half-life of AL002 in serum following a single dose of 6 to 60 mg/kg ranged from 8.1 to 9.0 days.

The clinical and preclinical data reported here indicate effective brain target engagement of AL002. In monkeys, weekly administration of AL002 dose-dependently decreased both sTREM2 in CSF as well as total TREM2 in hippocampus and frontal cortex. Similarly, a single IV infusion of AL002 resulted in placebo-adjusted dose-dependent reductions of sTREM2 in the CSF of HVs. The reduction from baseline within the highest dose group (60 mg/kg) in CSF sTREM2 persisted for up to 30 days. These consistent results across monkeys and HVs indicate that systemically administered AL002 modulates TREM2 signaling in the CNS. Brain total TREM2 is thought to represent the combination of sTREM2, membrane TREM2, and intracellular TREM2. The correspondence of the AL002-mediated reduction in brain total TREM2 and CSF sTREM2 in monkeys is consistent with

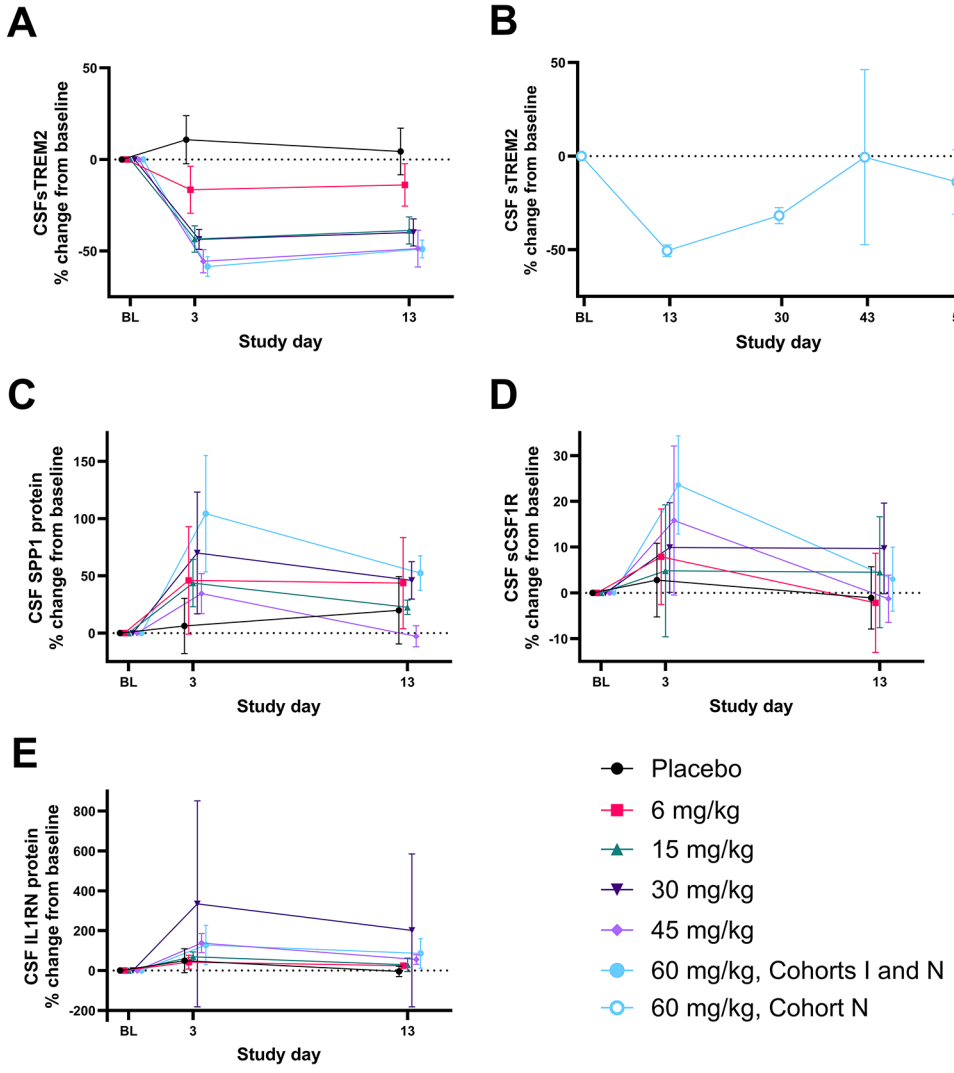

Fig. 5 Pharmacodynamics of single ascending dose AL002 in CSF of healthy volunteers. (**A**-**B**) sTREM2, (**C**) SPP1 protein, (**D**) sCSF1R, and (**E**) IL1RN protein were measured in CSF samples collected at the indicated time points. Data are presented as unadjusted mean ± SD percent change from baseline. (**A**), (**C**), (**D**), and (**E**) $n = 6$ in placebo, 6 mg/kg, 15 mg/kg, 30 mg/kg cohorts; $n = 5$ in 45 mg/kg cohort; $n = 5$–6 in 60 mg/kg cohorts I and N. (**B**) In 60 mg/kg cohort N, $n = 2$ at Day 13, $n = 5$ at Day 30 and 43, $n = 3$ at Day 57. Nudging was used to differentiate overlapping datasets. CSF, cerebrospinal fluid; CSF1R, colony-stimulating factor 1 receptor; LLOQ, lower limit of quantification; NHP, nonhuman primate; sCSF1R, soluble CSF1R; SEM, standard error of the mean; sTREM2, soluble TREM2; TREM2, triggering receptor expressed on myeloid cells-2

the use of CSF sTREM2 as a surrogate biomarker of brain TREM2 levels in the clinic.

sTREM2, the cleavage product of TREM2, is considered a biomarker of increased microglial function [49, 50]. Studies exploring CSF sTREM2 as a potential biomarker in AD disease progression have largely found that CSF sTREM2 is elevated in AD and changes during disease progression [49, 51, 52], with peak CSF sTREM2 occurring during the early symptomatic stages of AD [49]. However, in the context of AL002, the relationship between sTREM2 and increased microglial function may be decoupled. Our findings suggest that AL002 reduces CSF sTREM2 through activation of surface TREM2 receptors, resulting in receptor internalization and subsequent degradation (Fig. 7), which is represented by reduced total TREM2 levels in brain lysates.

Corroborating TREM2 target engagement, AL002 treatment in monkeys resulted in elevated markers of TREM2 signaling in the CNS. *SPP1* expression is downregulated in patients with Nasu-Hakola disease who have homozygous *TREM2* loss-of-function mutations [53], suggesting *SPP1* and its protein may serve as markers of downstream TREM2 signaling. Further, *SPP1* expression is considered a sensitive marker of increased TREM2-dependent microglial function in mouse models of AD [25, 29, 46]. Here, we observed increased brain *SPP1* expression and corresponding increased CSF SPP1 protein levels following repeat-dose AL002 in monkeys, in

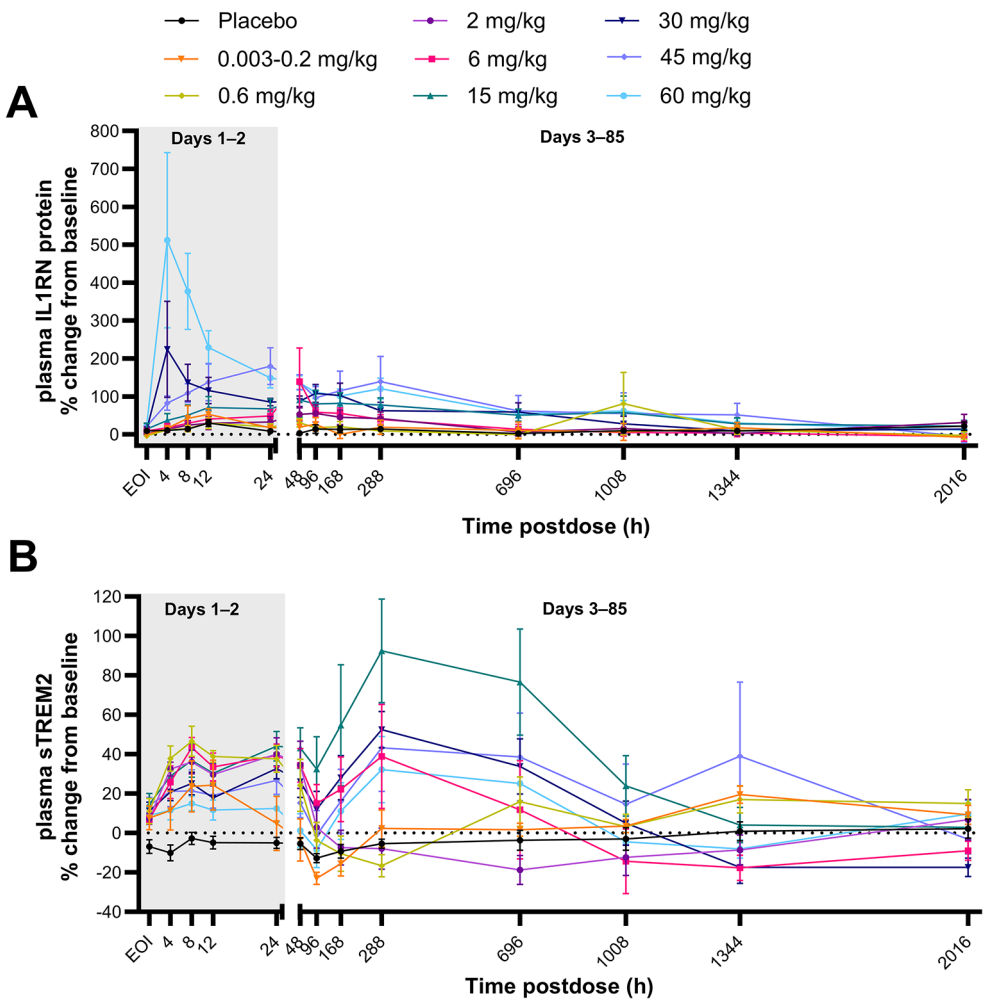

Fig. 6 Pharmacodynamics of single ascending dose AL002 in plasma of healthy volunteers. (**A**) IL1RN protein and (**B**) sTREM2 were measured in plasma samples collected at the indicated time points. Data are presented as unadjusted mean ± SEM percent change from baseline. (**A**), (**B**), $n = 11$ in placebo, $n = 3$ in 0.003-0.2 mg/kg, $n = 6$ in each of 0.6 mg/kg, 2 mg/kg, 6 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg; $n = 11$–14 in 60 mg/kg cohorts I and N. EOI, end of injection; h, hours; SEM, standard error of the mean; sTREM2, soluble TREM2; TREM2, triggering receptor expressed on myeloid cells-2

line with AL002 causing activation of TREM2. Consistent with our findings, a previous publication showed that acute AL002c treatment upregulated *Spp1* mRNA expression in a 5×FAD mouse model of AD [32]. However, chronic AL002c treatment resulted in reduced Spp1 protein levels in the brain. 5×FAD is an aggressive AD mouse model that demonstrates rapid Aβ plaque formation, significant inflammation, microgliosis, and increased *Spp1* mRNA expression levels [54, 55]. The reduced Spp1 protein level after chronic dosing is likely due to the amelioration of Aβ pathology and the resulting decrease in inflammation and microglial activity in this disease model.

We also observed increased brain mRNA expression and protein levels of CSF1R, whose signaling mediates microglial proliferation and survival [47], perhaps synergistically with TREM2 [15, 56]. In addition, increases in CSF levels of IL1RN protein were observed after each 250 mg/kg dose of AL002 in monkeys. IL1RN protein (i.e., 1L-1RA) is an antagonist that binds IL-1 receptor type 1 to block IL-1β-mediated pro-inflammatory signaling. Our finding of TREM2 activation increasing CSF IL1RN protein is consistent with previous work showing that chronic TREM2-agonistic antibody treatment increased IL-1 receptor antagonist (*Ilrn*) expression in the hippocampus of 5×FAD mice [31]. IL1RN protein has previously been implicated in TREM2-mediated neuroinflammation, with *Trem2*-deficient mice showing reduced microglial *Ilrn* expression [57].

Similar to the findings in NHPs, AL002 demonstrated effective target engagement in the CSF of HVs. A single IV infusion of AL002 resulted in placebo-adjusted dose-dependent reductions of sTREM2 in CSF as well as increased levels of SPP1 protein, sCSF1R, and IL1RN protein in CSF. In the periphery, single-dose AL002 also increased plasma levels of IL1RN protein relative

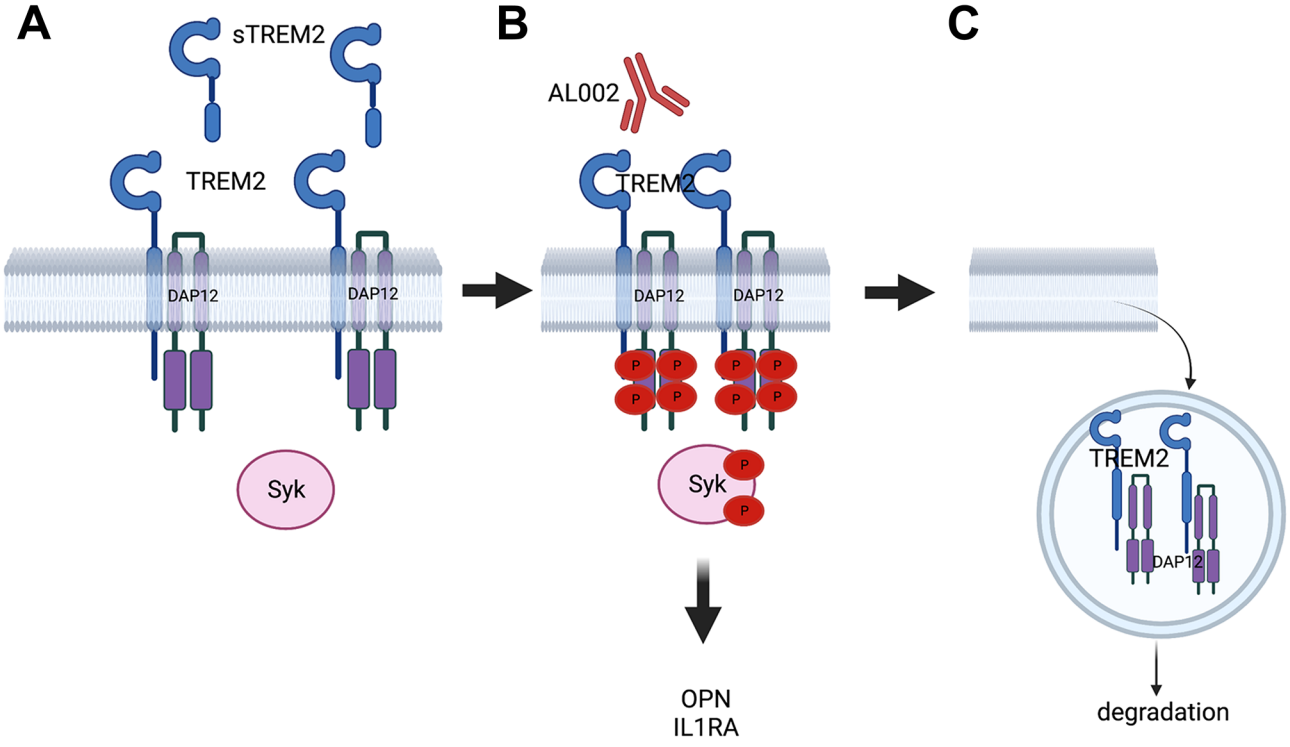

Fig. 7 Proposed mechanism of action of AL002. (**A**) TREM2 is constitutively shed into sTREM2. (**B**) AL002 binding promotes TREM2 clustering and activation via DAP12 and SYK phosphorylation, thus leading to SPP1 and IL1RN protein production. (**C**) AL002-mediated TREM2/DAP12 phosphorylation induces activation-mediated internalization of TREM2, thus leading to TREM2 degradation. This mechanism is typical of ITAM-containing receptors, thus preventing prolonged and massive stimulation of the receptor. ITAM, immunoreceptor tyrosine-based activation motif; sTREM2, soluble TREM2; TREM2, triggering receptor expressed on myeloid cells-2

to placebo; however, sTREM2 levels were increased in plasma rather than decreased as seen in CSF. The increase in plasma sTREM2 does not correlate with CSF levels and may result from decreased clearance of AL002-bound sTREM2, due to higher levels of AL002 in the periphery relative to the CNS, rather than reflect an increase in the release of sTREM2 from myeloid cells. Plasma sTREM2 and CSF sTREM2 correlations may be weak [51], and 1 study in a cohort of individuals with and without AD observed an inverse association between plasma and CSF sTREM2 levels [58]. A weak relationship between plasma and CSF sTREM2 levels supports the idea that AL002-mediated reductions in CSF sTREM2 are specific to the CNS and independent of plasma levels. Taken together, these consistent findings in HVs and NHPs indicate that AL002 engages the TREM2 signaling pathway in the brain.

The present study has several limitations. This first-in-human study of AL002 was primarily aimed at investigating the safety and tolerability of AL002 in a small number of HVs and was therefore not adequately powered to detect exploratory outcomes such as changes in biomarkers. Secondly, the MD cohort of patients with AD was terminated early due to the COVID-19 pandemic, limiting our ability to explore the effects of repeated dosing. Further, the safety of chronic TREM2 activation in humans remains to be demonstrated. Other TREM2-agonistic therapeutics are currently in phase 1 studies in HVs (NCT05450549) and patients with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP; NCT05677659), a rare neurological condition. Additional clinical studies are needed to evaluate the safety and efficacy of MD AL002 in participants with AD.

Conclusions

Previous evidence indicates that TREM2 agonism could be beneficial for AD. The safety profile and evidence of TREM2 target engagement reported here support the continued clinical development of AL002 to test this hypothesis. Based on the clear PK/PD relationship of this phase 1 study, including target engagement by sTREM2, the doses for the phase 2 trial were determined. The phase 2 trial (INVOKE-2; NCT04592874) to investigate the efficacy and safety of monthly infusions of AL002 in participants with early AD is ongoing.

Abbreviations

Aβ	Amyloid-β
AD	Alzheimer's disease
ADA	Antidrug antibody

AE	Adverse event
AESI	Adverse events of special interest
ANOVA	Analysis of variance
APOE	Apolipoprotein E
AUC	Area under the curve
AUC_{0-168}	Area under the concentration-time curve between 0 and 168 h after dosing
AUC_{0-inf}	Area under the concentration-time curve from time 0 to infinity
BAC	Bacterial artificial chromosome
BLQ	Below the limit of quantitation
C_{max}	Maximum observed concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSF1R	Colony stimulating factor 1 receptor
CV	Coefficient of variation
DLAE	Dose-limiting adverse event
DRF	Dose range finding
EC_{50}	Half-maximal effective concentration
ECG	Electrocardiogram
ECG	Electrochemiluminescence assay
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
GCP	Good clinical practice
GLP	Good laboratory practice
GWAS	Genome-wide association studies
HV	Healthy volunteer
HRP	Horseradish peroxidase
IEC	Independent ethics committee
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1
IL-1RA	Interleukin-1 receptor antagonist
IND	Investigational new drug
IRB	Institutional review board
ITAM	Immunoreceptor tyrosine-based activation motif
IV	Intravenous
LLOQ	Lower limit of quantification
LSM	Least squares means
mAb	Monoclonal antibody
MD	Multiple dose
MFI	Mean fluorescent intensity
MMRM	Mixed model for repeated measures
MRD	Minimum required dilution
MSD	Meso scale discovery
NHP	Nonhuman primate
NOAEL	No observed adverse effect level
OD	Optical density
PBS	Phosphate-buffered saline
PD	Pharmacodynamic
PK	Pharmacokinetic
RPM	Reads per million
RFU	Relative fluorescence units
RMLE	Restricted maximum likelihood
SAD	single-ascending dose
SAE	Serious adverse event
SD	Standard deviation
SEM	Standard error of the mean
sTREM2	Soluble TREM2
$t_{1/2}$	Terminal elimination half-life
TEAE	Treatment-emergent adverse event
TEPADA	Treatment-emergent positive antidrug antibody
Tg	Transgenic
TK	Toxicokinetic
T_{max}	Time to reach the maximum observed concentration
TMB	Tetramethylbenzidine
TREM2	Triggering receptor expressed on myeloid cells-2
ULOQ	Upper limit of quantification

Supplementary Information

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Supplementary Material 1

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Author contributions
H.L., H.R., I.T., M.W., F.Y., T.S., R.P. designed the studies. H.L. and I.T. conducted the experiments. H.L., A.S., A.M., Br.B., T.N., Ba.B., An.R., H.R., I.T., M.W., F.Y., T.S., and R.P. collected and analyzed the data. H.L., A.M., M.W., T.S., R.P., and Ar.R. provided supervision. H.L., A.S., Br.B., T.N., Ba.B., and An.R. wrote the manuscript. R.P. and Ar.R. acquired funding for the experiments. All authors critically reviewed the manuscript and approved the final submission.

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Data availability
Datasets described in the manuscript may be available from the corresponding author on reasonable request and at Alector's discretion.

Declarations

Ethics approval and consent to participate
The investigators agreed to conduct the human study according to the principles of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline E6: Good Clinical Practice. The investigators performed all aspects of this study in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the study protocol, and all national, state, and local laws or regulations. The original study protocol and protocol amendments were approved by appropriate institutional review boards and ethics committees at the following sites: Advarra (Columbia, MD, USA), The Alfred Hospital (Melbourne, Australia), Medical Research Ethics Committee of the Foundation BEBO (Assen, Netherlands), Skipton House (London, UK). For animal studies, all experimental procedures were conducted according to the approved protocols from the relevant institutions: 4-week GLP study, Covance Laboratories, Inc #8372868; DRF study, Covance Laboratories, Inc #8372866; non-GLP interval study, Charles River Laboratories, Inc. # 2946-001. All procedures complied with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

Consent for publication
Not applicable.

Competing interests
H.L., A.S., A.M., Br.B., T.N., Ba.B., An.R., and Ar.R. are employees of Alector, LLC and may have an equity interest in Alector, Inc. D.M., H.R., I.T., M.W., T.S., and R.P. were employees of Alector at the time of manuscript conception and may have an equity interest in Alector, Inc.

Author details
[1]Alector, Inc., South San Francisco, CA 94080, USA
[2]Leal Therapeutics, Worcester, MA, USA
[3]Deep Apple Therapeutics, Inc., San Francisco, CA, USA
[4]Independent Consultant, San Francisco, CA, USA
[5]Genentech, Inc., South San Francisco, CA, USA
[6]Nine Square Therapeutics, Inc., South San Francisco, CA, USA

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