

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	For pre-clinical data, raw instrument data were acquired using dedicated vendor software: 480 Real-Time PCR system (Roche) for Real-Time PCR experiments, and Analyst™ Software (Thermo Fisher) for LC-MS/MS bioanalysis. Other experimental metadata were recorded by common softwares. Clinical data with all case information were managed using an electronic data capture system (eCRF).
Data analysis	In silico alignment algorithm from gggenome (https://gggenome.dbcls.jp), miRanda (Rick Masonbrink), GraphPad Prism version 8.3.0, Watson LIMS (Certara), SAS software (V.9.4, SAS Institute).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All primary data supporting the findings of this study are included in this article and its supplementary files. Individual de-identified participant data that support the

findings of this study are not publicly available due to privacy and ethical restrictions. These data are stored on the secure server of the China National Clinical Research Center for Neurological Diseases and can be accessed under controlled access to verify the research conclusions. Access to these data is granted to qualified researchers following a review and approval process managed by the center's data access committee. Researchers who provide a methodologically sound analysis proposal that complies with clinical study ethical and data integrity requirements can request access via the center's Data Management and Sharing System (<https://paper.ncrcnd.org.cn>) or by contacting the corresponding author Y.W (yilong528@aliyun.com). Requests will be evaluated within 30 days. The study protocol and statistical analysis plan are available in the Supplementary Information_Protocol and Supplementary Information_SAP.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	All participants provided informed consent prior to screening. Eligible patients (both men and women) were aged 18 to 75 years. Sex or gender information was collected based on self-reporting and recorded within the electronic case report form (eCRF). No sex/gender-based subgroup analysis was performed, because the study objectives were designed independently of sex or gender.
Reporting on race, ethnicity, or other socially relevant groupings	None of these categorization variables was used.
Population characteristics	Relevant population characteristics of the human research participants are the following: All six enrolled participants carried a confirmed pathogenic or likely pathogenic SOD1 mutation associated with ALS. Median age was 47 years (range 26–67). Four female and two male patients were included. Detailed baseline characteristics are provided in Table 1.
Recruitment	Participants were identified by review of the medical records / clinic database held within the institution, and direct physician referral. Patients with a confirmed diagnosis of amyotrophic lateral sclerosis (ALS) and carrying a pathogenic or likely pathogenic SOD1 mutation were assessed for eligibility. Complete inclusion and exclusion criteria are listed both in the main manuscript (Methods section) and in the full study protocol, provided in the Supplementary Information. All patients were recruited from clinical site in China. Potential referral bias (physicians may refer more severe phenotypes) and self-selection bias (if eligible patients declined). Single-site design limits generalisability.
Ethics oversight	This study was approved by the ethics committee of Beijing Tiantan Hospital (ethical approval No. KY2023-015-02). Written informed consent was obtained from all participants prior to any study-related procedures, in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Participants received a fixed travel allowance and a modest reimbursement for blood and cerebrospinal fluid collection procedures. No other compensation was provided for study participation.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	i) Pre-clinical study: The sample sizes for the efficacy studies in SOD1G93A mice (n=10-11/group) and rats (n=11-20/group), and the pharmacodynamic study in cynomolgus monkeys (n=10/group, balanced by sex), were determined based on standard practices in the field for such models and endpoints. These sizes were deemed sufficient to robustly assess treatment effects and safety signals to support translation to clinical trials. No statistical methods were used to determine sample size. ii) Clinical study: This was a first-in-human, single-arm, open-label study designed primarily to evaluate the safety, tolerability, pharmacokinetics (PK), and preliminary bioactivity of RAG-17 in patients with SOD1-ALS. A total of six participants were enrolled. No formal power calculation was performed for this early-phase study. This sample size was considered sufficient for an initial proof-of-concept study to inform future trial design, based on the rarity of the patient population (SOD1-ALS), clinical feasibility, and the magnitude of target engagement (SOD1 reduction).
Data exclusions	i) Pre-clinical study: Animals that developed severe health issues unrelated to the expected ALS disease progression were excluded from the final analysis. These pre-defined criteria ensured that the assessment of treatment efficacy was not confounded by unrelated morbidity. ii) Clinical study: All six enrolled participants received the intended dose of RAG-17 and were included in both the safety and efficacy analyses; no patients were excluded. However, not all planned exploratory assessments were completed for every patient due to an inability to comply with specific procedures (e.g., gait function) as a result of disease progression. All available data were analyzed and reported as specified in the protocol.
Replication	i) Pre-clinical study: Statistical analysis demonstrated significant and consistent treatment effects across key in-life (e.g., grip strength, rotarod) and post-mortem (e.g., SOD1 quantification) endpoints. Experimental findings were supported by appropriate biological and technical replicates within the study. Individual animal-level source data are not included in the manuscript but are archived by the authors and

available for verification upon reasonable request.

ii) Clinical study: This single-arm, open-label, first-in-human trial was designed to evaluate safety and preliminary efficacy in a defined patient population (SOD1-ALS). As such, no applicable replication validation is provided.

Randomization	i) Pre-clinical study: SOD1 transgenic animals and their wild-type littermates were randomly assigned to experimental groups based on body weight and sex to ensure balanced distribution across all groups. ii) Clinical study: This is a single-arm, open-label study. No randomization was applied.
Blinding	i) Pre-clinical study: The following analyses were conducted in a blinded fashion: neurological scoring, grip strength test, rotarod test, RNAscope in situ hybridization, tissue distribution and quantification of RAG-17 in rats, SOD1 mRNA and protein expression in cynomolgus monkey CNS, as well as histopathological staining. For these analyses, the investigators were unaware of the treatment group assignment until after all data were collected and analyzed. ii) Clinical study: Blinding was not applied since this is a single-arm, open-label study. All participants were aware of the treatment they received, and the investigators were not blinded to treatment allocation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-Choline Acetyltransferase Antibody (Merck Millipore, Cat.No.AB144P, Lot No. 4145737), Peroxidase AffiniPure™ Donkey Anti-Goat IgG (H+L) Polyclonal Antibody (JacksonImmunoResearch, Cat.No.705-035-003, Lot No. 161760).
Validation	Anti-Choline Acetyltransferase Antibody (AB144P): Validation data provided by the manufacturer (Merck Millipore) indicates specificity for human, opossum, guinea pig, rat, mouse, zebrafish, avian, monkey, chicken Choline Acetyltransferase by WB, ICC and IHC. This antibody was also validated in our laboratory using tissue from SOD1G93A rats. Peroxidase AffiniPure™ Donkey Anti-Goat IgG (H+L) (705-035-003) Validation data provided by the manufacturer (JacksonImmunoResearch). The secondary antibodies were used according to the manufacturer's recommendations.

Eukaryotic cell lines

Policy information about [cell lines](#) and [Sex and Gender in Research](#)

Cell line source(s)	T98G cells were obtained from Cbioer (Catalog # CBP60301). COS-1 cells were obtained from ATCC (Catalog# CRL-1650). RK13 cells were obtained from ATCC (Catalog# CCL-37). MB-49 cells were obtained from Millipore Sigma (Catalog# SCC148). C6 cells were obtained from Amyjet (Catalog# BNCC100216).
Authentication	All cell lines were obtained directly from the indicated commercial suppliers. No additional cell line authentication (e.g., STR profiling) was performed by the authors.
Mycoplasma contamination	Cells were not routinely tested for mycoplasma contamination during this study.
Commonly misidentified lines (See ICLAC register)	We did not use any misidentified cell line in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Female and male cynomolgus monkeys (age, approximately 3.0-4.0 years; body weights, 2.2-3.2 kg for females and 2.4-4.4 kg for males) were obtained from Guang Dong Blooming-Spring Biological Technology Development Co., Ltd. hSOD1G93A mice (B6. Cg-Tg (SOD1*G93A)1Gur/J) (Strain ID #004435) were obtained from the Jackson Laboratory. SOD1G93A transgenic rats were obtained from
--------------------	--

	Taconic Biosciences (model 2148).
Wild animals	This study did not involve wild animals.
Reporting on sex	Female hSOD1G93A mice, female and male SOD1G93A transgenic rats as well as female and male cynomolgus monkeys were used.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All procedures and any amendments or procedures involving the care or use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Nantong University (#S20220323-001), WuXi AppTtec (#GP02-QD105-2020V1.0, H59-0027-TX), prior to the initiation of these procedures.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT05903690
Study protocol	Study protocol available as supplementary material, along with the statistical analysis plan.
Data collection	Between 22 May, 2023, and 27 November, 2023, a total of seven individuals were screened. One individual did not meet the inclusion criteria and was excluded, resulting in six being successfully enrolled. All six enrolled participants received treatment with RAG-17 and underwent clinical assessment at Beijing Tiantan Hospital, Capital Medical University. The data collection period spanned from 24 May, 2023, to 11 July, 2024. Clinical data were collected using individual case report forms (CRFs) by study physicians, with all case information managed using an electronic data capture system (eCRF).
Outcomes	i)The primary endpoints included the following safety evaluation measures: 1) incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after RAG-17 treatment; 2) changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRR-R) scores, and abnormalities in heart, lung and abdomen examinations, neurological examinations, as well as electrocardiograms within 240 days after the first injection of RAG-17. The ALSFRS-R consists of 12 items across four subdomains of bodily function (bulbar, fine motor, gross motor, and breathing). Each item is scored on an ordinal scale from 0 (total loss of function) to 4 (no loss of function), yielding total scores ranging from 0 to 48, with higher scores indicating better function. ii)The secondary endpoints included: (1) changes in CSF SOD1 protein levels at days 15, 29, 60, 120, 180 and 240 from baseline; (2) changes in plasma NfL levels at days 1, 15, 29, 60, 120, 180 and 240 from baseline; (3) pharmacokinetics of RAG-17 in plasma at days 1, 15, 29, 60, 120, 180 and 240; and (4) time to invasive mechanical ventilation or death. iii)The exploratory endpoints included changes in: (1-7) pulmonary function (including FVC), fatigue severity, muscle strength, gait function, modified Norris scale scores, quality of life and clinical staging, at days 15, 29, 90, 180 and 240; and (8-9) electromyography and 7T MRI imaging features at days 29, 90, 180 and 240. Fatigue severity was assessed using the Fatigue Severity Scale (FSS)40. Evaluation of gait function included 3D gait analysis, short physical performance battery, tandem walking, and the 6-meter walk test. Quality of life was estimated using the Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSAQ-40), a Five-level version of EuroQol Five Dimensions Questionnaire (EQ-5D-5L), Hamilton Anxiety Scale (HAMA) and Hamilton Depression Scale (HAMD). Clinical staging was evaluated using the London ALS Stage and Milano-Torino staging. The electromyographic features evaluated in this study included the composite muscle action potentials (CMAP), motor unit number index (MUNIX), and motor unit size index (MUSIX).

Plants

Seed stocks	Not applicable.
Novel plant genotypes	Not applicable.
Authentication	Not applicable.

Magnetic resonance imaging

Experimental design

Design type	Structural MRI of the human brain in vivo.
Design specifications	Whole-brain three-dimensional T1-weighted imaging (T1WI) and three-dimensional SWI.

Behavioral performance measures

Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☒ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See [Eklund et al. 2016](#))

Correction

Models & analysis

n/a | Involved in the study

☒ ☐ Functional and/or effective connectivity

☒ ☐ Graph analysis

☒ ☐ Multivariate modeling or predictive analysis