Efficacy and safety of low-dose IL-2 as an add-on therapy to riluzole (MIROCALS): a phase 2b, double-blind, randomised, placebo-controlled trial

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Summary

Background Amyotrophic lateral sclerosis (ALS) is a life-threatening disease characterised by progressive loss of motor neurons with few therapeutic options. The MIROCALS study tested the hypothesis that low-dose interleukin-2 ($IL\text{-}2_{LD}$) improves survival and function in ALS.

Methods In this randomised, double-blind, placebo-controlled trial, male and female riluzole-naive participants, with either a possible, laboratory-supported probable, probable, or definite ALS diagnosis (revised El Escorial criteria), aged 18–76 years, with symptom duration of 24 months or fewer, and slow vital capacity of 70% or more, underwent a riluzole-only 12–18 week run-in period before randomisation in a 1:1 ratio to either 2 million international units (MIU) $IL\text{-}2_{LD}$ or placebo by subcutaneous injection daily for 5 days every 28 days over 18 months. The primary endpoint was survival at 640 days (21 months). Secondary outcomes included safety, ALS Functional Rating Scale-Revised (ALSFRS-R) score, and biomarker measurements including regulatory T-cells (Tregs), cerebrospinal fluid (CSF)-phosphorylated-neurofilament heavy-chain (CSF-pNFH), and plasma and CSF-chemokine ligand 2 (CCL2). The primary endpoint analysis used unadjusted log-rank and Cox's model adjusted analyses using pre-defined prognostic covariates to control for the disease and treatment response heterogeneity. The study was 80% powered to detect a two-fold decrease in the risk of death by the log-rank test in the intention-to-treat (ITT) population, including all randomly allocated participants. MIROCALS is registered with ClinicalTrials.gov (NCT03039673) and is complete.

Findings From June 19, 2017, to Oct 16, 2019, 304 participants were screened, of whom 220 (72%) met all criteria for random allocation after the 12-to-18-week run-in period on riluzole. 136 (62%) of participants were male and 84 participants (38%) were female. 25 (11%) of the 220 randomly allocated participants were defined as having possible ALS under El Escorial criteria. At the cutoff date there was no loss to follow-up, and all 220 patients who were randomly allocated were documented as either deceased (90 [41%]) or alive (130 [59%]), so all participants were included in the ITT and safety populations. The primary endpoint unadjusted analysis showed a non-significant 19% decrease in risk of death with $IL\text{-}2_{LD}$ (hazard ratio 0·81 [95% CI 0·54–1·22], p=0·33), failing to demonstrate the expected two-fold decrease in risk of death. The analysis of the primary endpoint adjusted on prognostic covariates, all measured at time of random allocation, showed a significant decrease of the risk of death with $IL\text{-}2_{LD}$ (0·32 [0·14–0·73], p=0·007), with a significant treatment by CSF-pNFH interaction (1·0003 [1·0001–1·0005], p=0·001). $IL\text{-}2_{LD}$ was safe, and significantly increased Tregs and decreased plasma-CCL2 at all timepoints. Stratification on CSF-pNFH levels measured at random allocation showed that $IL\text{-}2_{LD}$ was associated with a significant 48% decrease in risk of death (0·52 [0·30–0·89], p=0·016) in the 70% of the population with low (750–3700 pg/mL) CSF-pNFH levels, while in the 21% with high levels (>3700 pg/mL), there was no significant difference (1·37 [0·68–2·75], p=0·38).

Interpretation With this treatment schedule, $IL\text{-}2_{LD}$ resulted in a non-significant reduction in mortality in the primary unadjusted analysis. However, the difference between the results of unadjusted and adjusted analyses of the primary endpoint emphasises the importance of controlling for disease heterogeneity in ALS randomised controlled trials. The decrease in risk of death achieved by $IL\text{-}2_{LD}$ therapy in the trial population with low CSF-pNFH levels requires further investigation of the potential benefit of this therapy in ALS.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a rare disease of the voluntary motor system, associated with loss of motor neurons in the brain and spinal cord, leading to relentlessly progressive disability and death, with a median survival of 2–3 years from symptom onset.[1] While riluzole[2,3] has a modest effect on survival, there is still a pressing need for more effective disease-modifying treatments. However, identification of more effective treatments for ALS has been hampered by poor understanding of relevant targets, the clinical and pathogenic heterogeneity of the ALS syndrome,[4,5] and failure to assess drug target response.

Neuroinflammation was shown to be involved in a broad spectrum of ALS phenotypes, thus providing a promising therapeutic target.[6] We developed a methodology to test the hypothesis that modifying the inflammatory components of ALS pathogenesis could improve survival and slow the functional deterioration in ALS.

$CD4^+FOXP3^+$ regulatory T-cells (Tregs) are a T-cell subset with wide immunoregulatory properties in which numerical and functional impairments have been linked to ALS severity, progression and survival.[7–10] Tregs require cytokine interleukin-2 (IL-2) for their generation, activation, and survival,[11] presenting a feasible and measurable therapeutic route to enhance immune tolerance and dampen neuroinflammation.

In a previous phase 2a trial of low-dose Il-2 ($IL\text{-}2_{LD}$), we showed that 1 million international units (MIU) or 2 MIU was safe, well tolerated, and led to a dose-dependent increase in Treg number and function in people with ALS.[12] Moreover, chemokine ligand 2 (CCL2, also known as monocyte chemoattractant protein-1 [MCP-1]), a marker of microglial activation shown to reflect disease severity,[13,14] was significantly reduced in the plasma of treated participants.

Here we report the main outcomes of a phase 2b, randomised, double-blind, placebo-controlled, clinical trial with a primary objective of evaluating the clinical efficacy and safety of $IL\text{-}2_{LD}$ over 18 months treatment. A key element in our trial design was the incorporation of prespecified biomarkers: Tregs for drug target engagement, cerebrospinal fluid (CSF)-CCL2 (CSF-CCL2)

Research in context

Evidence before this study

For the background in preparing the protocol (submitted to regulators on April 11, 2016) we searched MEDLINE, PubMed, ClinicalTrials.gov, EudraCT, Embase, the COCHRANE Central Register of Clinical trials, and WHO Clinical Trials Registry Platform from Jan 1, 1990, to April 1, 2016, using the keywords "ALS", "amyotrophic lateral sclerosis", "motor neuron disease", "motor neurone disease", "Lou Gehrig's disease", "randomised clinical trials", "interleukin-2", AND "interleukin2" without language restrictions. We also hand-searched reviews with these search terms between Jan 1, 1990, and April 1, 2016. All searches were updated to Aug 31, 2024, which revealed one phase 2a randomised, double-blind, placebo-controlled trial of interleukin-2 low dose ($IL\text{-}2_{LD}$) in amyotrophic lateral sclerosis (ALS). There were no other reports of randomised, double-blind, placebo-controlled trials of $IL\text{-}2_{LD}$ in ALS, motor neurone disease, or Lou Gehrig's disease. We identified three small, non-randomised, open-label trials of $IL\text{-}2_{LD}$ in ALS—either alone or in combination with other agents—but no reliable conclusions could be drawn on the efficacy or safety of $IL\text{-}2_{LD}$ in ALS due to the absence of adequate masking, lack of placebo controls, and inadequate power due to small numbers of participants.

Added value of this study

MIROCALS represents the first large, long-term, randomised, double-blind, placebo-controlled trial of treatment with subcutaneous $IL\text{-}2_{LD}$ as add-on to riluzole in people with ALS, powered for efficacy. The primary endpoint measure was survival, and there was no loss to follow-up at the cutoff of 21 months from random allocation. By design, we recruited an incident population more representative of the real-world ALS population than previous ALS trial samples. We also applied a strategy to control for disease heterogeneity—a major confounding factor in previous ALS trials—using systematic measures of a biomarker (cerebrospinal fluid [CSF] phosphorylated neurofilament heavy chain [CSF-pNFH]) to adjust for disease activity and rate of progression. $IL\text{-}2_{LD}$ was safe and well tolerated, suggesting that it is suitable for larger trials assessing its therapeutic value in this vulnerable population.

Implications of all the available evidence

At present, the only drug approved in the USA and Europe with a proven effect on survival is riluzole. Despite our primary outcome not reaching statistical significance, our findings indicate that $IL\text{-}2_{LD}$ offers the possibility of a much-needed, safe, and well-tolerated agent conferring useful survival benefit in addition to riluzole across the ALS spectrum. Nonetheless, further trials are required to support our observations and to explore different treatment schedules. The integration of biomarkers of disease activity and target response into our trial design offers a new approach to control for disease heterogeneity, an important and poorly understood confounding factor in ALS trials. Our findings also indicate that targeting Tregs to boost immune tolerance is a worthwhile strategy for developing new therapies in ALS.

for microglial activation, and CSF-phosphorylated neurofilament heavy chain (CSF-pNFH) for neuronal damage, each reported as a prognostic factor for survival in ALS.[9,14–16]

Methods

Study design and participants

MIROCALS was a randomised, double-blind, placebo-controlled, parallel group trial of IL-2_{LD} as add-on therapy to riluzole in people with ALS recruited from ten ALS centres in France and from seven centres in the UK. Randomisation was stratified on country (France or UK) and on site of symptom onset (bulbar or limb).

Eligible participants were aged between 18 and 75 years, had symptom onset within the past 24 months (defined as weakness, or in the case of bulbar onset, dysarthria) in order to recruit at the earliest opportunity in the disease course, and should not have previously used riluzole or to have used it for less than 4 weeks, subject to a 4-week washout period. To achieve a sample representative of the real-world ALS population, we widened participation to include patients with possible or laboratory-supported probable ALS.[17] Diagnosis was systematically reassessed at the end of the treatment period. Tolerance of riluzole over 12–18 weeks (the run-in period) was required for random allocation. Before recruitment, all participants were required to provide signed informed consent for their participation in the study. Full inclusion and exclusion criteria are shown in the appendix (p 3). In compliance with the French Commission Nationale de l'Informatique et des Libertés, data concerning race or ethnicity were not collected.

The study was conducted in compliance with Good Clinical Practice (ICH guidelines) and in accordance with the ethical principles of the Declaration of Helsinki, with approvals from the relevant Institutional Ethical Review Bodies in the UK (Integrated Research application System, #207544) and France (Comité de Protection des Personnes Ile de France-VI, #37–16). An independent Data Safety Monitoring Board (DSMB) reviewed the unblinded data for safety only at agreed stages of the study. The study sponsor was the Centre Hospitalier-Universitaire de Nîmes (CHU-Nîmes), France.

Randomisation and masking

Participants successfully completing the 12–18 week run-in period were randomly assigned to receive either IL-2_{LD} or placebo in a 1:1 ratio within each stratum via a web-based application (Telemedicine Technologies, Boulogne, France). Randomisation lists consisting of randomly drawn blocks of four were established per stratum by an independent statistician. All participants and investigators of the study remained masked to treatment allocation during the whole study until unmasking, following freezing of the database. Treatment allocation was provided to the central pharmacy responsible for treatment manufacturing, the pharmacovigilance department responsible for declaring suspected unexpected serious adverse reactions (SUSARs), and the DSMB for unmasked review of safety.

All data generation for core biomarkers was performed by investigators who were masked to treatment status and visit number using random barcode labelling of samples before banking and subsequent analysis.

Procedures

Recombinant human IL-2 was purchased as aldesleukin (Proleukin, 22 MIU per vial, Novartis, Basel, Switzerland and Clinigen, London, UK). For the placebo, 5% dextrose in water for injection was obtained from Baxter Healthcare (Baxter UK, Newbury, UK).

Compounding, preparation, and labelling of the final product were performed by a licensed aseptic manufacturing facility under Good Manufacturing Practices and managed by WGK Clinical Services (London, UK). The final product was in the form of 1 mL syringes containing 0·6 mL of either a solution of 2 MIU of aldesleukin (IL-2_{LD}) or 5% dextrose (placebo). Each treatment cycle lasted 5 days as one subcutaneous injection per day. The cycle was repeated every 4 weeks for 19 cycles, after which time the study treatment was discontinued. The schedule, labelling, presentation, and volume of injection for active IL-2_{LD} and placebo were identical. Dose flexibility according to tolerance was allowed through a volume adjustment of prepared syringes to 0·3 mL or 0·15 mL as deemed appropriate.

Randomly allocated participants were followed up every 2 months for 4 months (short term treatment period), then at month 6, and then every 3 months thereafter until the end of the trial period (long-term treatment period; appendix pp 4–6).

CSF was sampled in volumes of 15–20 mL via lumbar puncture using a negative pressure procedure[18] for biomarker assessments at inclusion (before the first riluzole dosing), random allocation (after 12–18 weeks on riluzole and before first investigational medicinal product [IMP] dosing), and at day 112 before commencing the fifth cycle (trough level of fourth cycle of IL-2_{LD}). Blood samples for core biomarkers were paired with CSF samples with two additional timepoints to estimate the maximum effect of IL-2_{LD} following one cycle (day 8) and five cycles (day 120) of treatment, respectively (appendix p 7).

Plasma, peripheral blood mononuclear cells (PBMCs), and CSF samples collected at clinical centres were banked in a central biobank facility (Généthon, France) until further analysis. Fresh blood samples for cytometry were couriered to the central laboratory for immediate analysis. All pre-analytical procedures were performed in the spirit of Good Clinical Laboratory Practice, and core biomarker measurements (flow cytometry, pNFH, and CCL2) were performed in the central laboratory, which is a Good Laboratory Practice-compliant facility (Biocytex, Marseille, France).

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See Online for appendix

Plasma-pNFH and CSF-pNFH were measured by ELISA (BioVendor, Brno, Czech Republic) following the manufacturer instructions. Initial validation of assay performance demonstrated a large matrix effect[19] impacting both between and within patient determinations of plasma-pNFH levels (data not shown). As such, plasma-pNFH measures were considered as exploratory measures. Plasma-CCL2 and CSF-CCL2 were measured by ELISA (R&D Systems) following the manufacturer instructions.

Clinical flow cytometry was performed on fresh blood at the central laboratory, and used two panels of fluorochrome-conjugated antibodies to determine the frequency and absolute number of lymphocyte subpopulations including $CD4^+CD25^+CD127^{lo/-ve}FOXP3^+$ Tregs. Additional details for biomarker methods are shown in the appendix (pp 8–11).

Outcomes

Data collection for clinical outcomes was performed via an electronic case report form provided and managed by Telemedicine Technologies (Boulogne-Billancourt, France).

The primary endpoint for clinical efficacy was survival over a 92 week (21 month) follow-up period. Before unmasking, the cutoff for surviving patients was set at 640 days for censoring (3 months after the end of the last study treatment cycle). The outcome was the date of death from any cause based on death certificates or date of last status from medical documentation in case of censoring. Supportive endpoints were time to composite outcomes including time to tracheostomy or non-invasive ventilation ([NIV] ≥6 h per day) or death, time to use of gastrostomy or death, or time to any of these outcomes.

The secondary endpoint for clinical efficacy was the slope of change of the ALS Functional Rating Scale-Revised (ALFRS-R) functional score, assessed monthly from random allocation to week 78.

Treatment target engagement was evaluated by changes from random allocation in Tregs (expressed as absolute numbers per µL or as percentage of $CD4^+$ T-cells).

CCL2 levels were evaluated in CSF as a proxy for microglial activation, and in plasma as a surrogate for CSF levels and as a marker of systemic inflammation, expressed as change from random allocation (appendix p 8). CSF-pNFH levels were evaluated as a marker of active neuronal damage and expressed as change from random allocation (appendix p 9). CSF-pNFH was selected as a stable, reproducible and strong predictor of survival having a reliable available assay.[16]

Safety was assessed by adverse events reporting throughout the study, including systematic check of expected $IL\text{-}2_{LD}$ related adverse events (eg, flu-like symptoms, injection site reactions) following each cycle, and at study visits through physical examination and routine laboratory tests (appendix p 12). Pharmacovigilance was managed by the trial management team at CHU-Nîmes; onsite monitoring was overseen by ICON (Dublin, Ireland).

Owing to the COVID-19 epidemic, outcomes planned to be measured at study visits had to be discarded, such as secondary outcomes including slow vital capacity and health-related quality of life (measured by EQ-5D), as a result of a substantial number of participants without measures under treatment. When study visits were not allowed, ALS Functional Rating Scale-Revised (ALSFRS-R) measurements were performed through telephone contact with participants or participants' carers, while adverse events were reported via telephone calls with participants or participants' carers and general practitioners, and routine laboratory tests for safety were requested at local laboratories.

Statistical analysis

The total number of patients in the power calculation was calculated for the primary survival endpoint. For detecting a 17% absolute difference in survival between groups at 21 months (expected survival of 0·65 for placebo *vs* 0·82 for $IL\text{-}2_{LD}$ and a hazard ratio [HR] of 0·46 for $IL\text{-}2_{LD}$ *vs* placebo) by the log-rank test, 108 patients per group were needed to achieve 80% power at a two-sided α of 0·05.

As there were no previous estimates of a treatment-by-biomarker prognostic factor interactions in ALS on which to base power calculations, the power of the interaction tests between treatment and these factors could not be formally estimated.

The threshold for statistical inference was set at a two-sided $p<0·05$. Entry parameters were described by treatment groups and comparisons were calculated using Wilcoxon's rank sum test for continuous variables and a χ^2 test or Fisher's exact test as appropriate for categorical variables (appendix pp 13–14).

The primary analysis was performed on the intention-to-treat population (ITT) which included all patients who were randomly allocated who had received at least one trial medication at the time of random allocation. The treatment effect on the primary endpoint (survival) was assessed through an unadjusted analysis, and also through predefined adjusted analysis (appendix pp 13–14). The unadjusted analysis was performed using a stratified log-rank test, and checking for treatment by strata interactions (limb *vs* bulbar onset and UK *vs* French centres) performed using Cox's model; in case of a statistically significant interaction, a separate analysis within each stratum of interest was planned.

Adjusted analyses were performed using predefined candidate covariates via stepwise multivariate Cox's model regression to select a robust set of prognostic factors. Following prognostic factor selection, treatment and interactions of treatment with each selected factor were subsequently tested and goodness-of-fit was compared through a likelihood ratio test. In the case of

significant treatment by factor interaction with continuous variables, these were broken down in classes and a separate analysis performed within each stratum. The threshold used to categorise the prognostic factors interacting with treatment was defined independently from the survival data and based on the distribution characteristics of the parameter.

To ensure unbiased estimates of treatment effect in the primary outcome analysis, prespecified supportive analyses were performed using composite outcomes (eg, time to NIV or death) to test the primary analysis Cox's model for its robustness and consistency across these outcomes.

Treatment effect on the secondary endpoint (slope of change of ALSFRS-R) was assessed through unadjusted and predefined adjusted analysis. For each patient, ALSFRS-R measures were summarised by slope of change from random allocation to week 78 using simple linear regression. Treatment effect was assessed through joint-rank analysis performed with the WinRatio test,[20] combining slope of change and time-to-event data. Treatment effect adjusted for prognostic factors selected in the Cox's model and treatment by factor interaction was performed by calculating a combined assessment of function and survival (CAFS) score for each patient, subsequently subjected to covariance analysis.[21] Upon significance of the treatment by factor interaction, separate WinRatio tests were carried out as per the above defined strata.

For core biomarker measures (CSF-pNFH, plasma-CCL2 and CSF-CCL2, and Tregs), changes from random allocation to week 17 for CSF, and to weeks 2, 17, and 18 for blood, were used to assess the treatment effects through the Wilcoxon rank sum test.

Comparisons of serious and non-serious adverse events (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] version 19.0) between treatment groups were performed with the Pearson χ^2 test or Fisher's exact probability test.

All statistical analyses were performed using R-software version 4.4.0.

An independent audit body, Qualilab (Olivet, France), has performed audits and review of the trial at all stages: implementation of the study, tools, and standard operating procedures, of clinical sites and site pharmacies, the central pharmacy contract manufacturing organisation for treatment manufacturing, the central laboratory for core biomarker assays, the monitoring partner (ICON), and the biobanking facility for sample storage (Généthon). Qualilab made a comprehensive audit of the primary outcome and core biomarker data and their source documentations, statistical analysis, and study reporting.

Role of the funding source

None of the funding organisations took any part in the study conception, design, data collection, analysis, interpretation, or writing of the report.

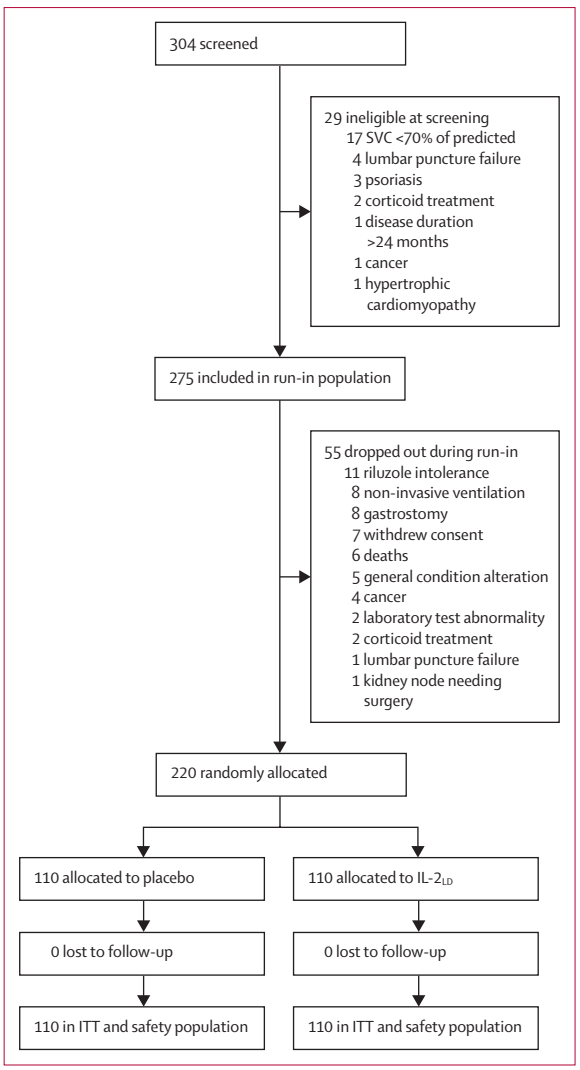

Figure 1: **Trial profile**
IL-2_{LD}=low-dose interleukin-2. ITT=intention-to-treat. SVC=slow vital capacity.

Results

From June 19, 2017, to Oct 16, 2019, 304 participants were screened, of whom 220 (72%) met all criteria for random allocation after the 12-to-18-week run-in period on riluzole (figure 1). Before recruitment, all participants provided a signed informed consent for participation in the study. 136 (62%) of participants were male and 84 participants (38%) were female.

Random allocation achieved equal numbers of participants in each group (110 to $IL\text{-}2_{LD}$ and 110 to placebo), with no overt imbalance in demographic, clinical, or core biomarker characteristics (table 1). Interim analyses for safety raised no concerns, and no unmasking was required until database lock.

Participants were recruited early in the course of the disease, with median time from diagnosis to inclusion of 1·4 months, and 25 (11%) of the 220 randomly allocated participants were defined as having possible ALS

according to El Escorial criteria. A diagnosis accuracy check at the end of the trial detected only five (2%) participants in whom the diagnosis was not confirmed (two [2%] of 110 in the placebo group and three [3%] of 110 in the IL-2_{LD} group; appendix p 15).

At the cutoff date there was no loss to follow-up, and all 220 patients who were randomly allocated were documented as either deceased (90 [41%]) or alive (130 [59%]).

	Placebo (N=110)	$IL2_{LD}$ (N=110)
Patients per country		
France	69 (63%)	68 (62%)
UK	41 (37%)	42 (38%)
Sex		
Female	43 (39%)	41 (37%)
Male	67 (61%)	69 (63%)
ALS diagnosis*		
Clinically definite	30 (27%)	22 (20%)
Clinically probable	33 (30%)	43 (39%)
Clinically probable—laboratory supported	33 (30%)	34 (31%)
Clinically possible	14 (13%)	11 (10%)
Site of symptom onset		
Bulbar	23 (21%)	22 (20%)
Limb	86 (78%)	88 (80%)
Other†	1 (1%)	0
Family history‡		
Yes	13 (12%)	14 (13%)
No	91 (83%)	89 (81%)
Unknown	6 (5%)	7 (6%)
Age, years	60·4 (23·4 to 74·8)	59·0 (35·9 to 76·1)
Disease duration since first symptom, months	11·1 (2·4 to 23·9)	10·7 (2·8 to 23·5)
Time since diagnosis at inclusion, months	1·3 (0·0 to 12·0)	1·6 (0·0 to 9·5)
Percentage of predicted SVC at inclusion	93% (70 to 138)	94% (71 to 138)
ALSFRS-R at random allocation, point score 0–48	39 (16 to 48)	40 (25 to 47)
ALSFRS-R slope run-in, point score per month	−0·6 (−5·8 to 1·2)	−0·7 (−4·1 to 1·3)
Core biomarkers		
Treg percentage of CD4 at random allocation	5·1% (2·5 to 10·1)	5·2% (2·2 to 15·1)
Treg percentage of CD4 run-in change	0·56% (−2·1 to 3·1)	0·84% (−12·2 to 4·78)
Absolute number of Treg per µL at random allocation	36·4 (9·3 to 91·4)	35·8 (2·9 to 76·2)
Absolute number of Treg per µL at run-in change	2·7 (−17·1 to 46·2)	3·0 (−133·8 to 32·5)
CSF-pNFH at random allocation, pg/mL	2442 (140 to 10981)	2306 (55·3 to 8515)
CSF-pNFH run-in change, pg/mL	129 (−3785 to 3717)	97 (−2412 to 1847)
Plasma-CCL2 at random allocation, pg/mL	298 (189 to 486)	295 (170 to 497)
Plasma-CCL2 run-in change, pg/mL	−3·8 (−229·8 to 200·1)	7·8 (−283·7 to 123·7)
CSF-CCL2 at random allocation, pg/mL	571 (322 to 1168)	554 (276 to 1493)
CSF-CCL2 run-in change, pg/mL	29·5 (−276·2 to 305·3)	19·5 (−242·3 to 1145·7)

Data are n (%) or median (range). ALS=amyotrophic lateral sclerosis. ALSFRS-R=ALS Functional Rating Scale-Revised. CCL2=chemokine ligand 2. CSF=cerebrospinal fluid. $IL2_{LD}$= low-dose interleukin 2. ITT=intention-to-treat. pNFH=phosphorylated neurofilament heavy chain. SVC=slow vital capacity. Treg=regulatory T-cells. *El Escorial criteria. †Other site of onset=respiratory (randomly allocated to the limb stratum). ‡Family history as assessed in the clinic was not a criterion for exclusion.

Table 1: **Demographic and clinical parameters at baseline in the ITT population**

Overall mean compliance to IMP, estimated as the percentage of received doses to the number of intended doses at full dosage, was 89·0% (SD 20·6); compliance with placebo was 91·0% (18·8) and 87·0% for IL-2_{LD} (22·1). 174 (79%) of the 220 participants were still on the IMP at the end of the treatment period (week 78) or at time of death (90 [82%] of 110 on placebo and 84 [76%] of 110 on IL-2_{LD}; appendix pp 16–17).

At the cutoff date (640 days) there were 49 deaths (45%) in the placebo group, and 41 deaths (37%) in the verum group. Assessment of the unadjusted treatment effect on the primary survival endpoint showed a non-significant 19% decrease in the risk of death (HR 0·81 [95% CI 0·54–1·22], p=0·33) in the IL-2_{LD} treated group relative to placebo (figure 2A). Multivariate Cox's model analysis identified age, ALSFRS-R, CSF-pNFH levels, plasma-CCL2 levels, and absolute number of Tregs as significant, independent prognostic factors of survival (appendix p 18). Of all these factors, CSF-pNFH was the sole factor with a significant negative interaction with treatment (appendix pp 18–20). Assessment of the treatment effect on the primary endpoint, using a Cox's model based on these parameters, showed a significant 68% decrease in the risk of death (0·32 [0·14–0·73], p=0·0070) in the IL-2_{LD} group relative to placebo when the interaction is included (table 2). The significant HR of the CSF-pNFH by treatment interaction indicates that the magnitude of the treatment effect decreases with increasing CSF-pNFH levels.

Our sensitivity analyses support these results, while checking for eventual model overfitting, eventual random bias in sample assays, or analyses using bootstrapping methods testing for the robustness and consistency of the model with the primary analysis results, and are shown in the appendix (pp 21–22).

As planned in case of significant treatment by factor interaction, the CSF-pNFH variable was broken down into classes, and a separate analysis was performed within each stratum. Per our prespecified statistical analysis plan, three strata were defined based on the overall distribution characteristics of CSF-pNFH levels at the time of random allocation (as shown in appendix p 23): below the lower limit of quantification ([LLOQ]; 19 [9%] of 220, the overall ITT population); over the limit of quantification and lower than or equal to 3700 pg/mL ([low]; 154 [70%] of 220); and over 3700 pg/mL ([high]; 47 [21%] of 220).

Consistent with the Cox's model we observed a highly significant difference in participant survival between the three strata (LLOQ 19 [100%] of 19, low 97 [63%] of 154, and high 14 [30%] of 47; p<0·0001 by the log-rank test; figure 2B).

The treatment effect in the low stratum showed a significant 48% decrease in the risk of death relative to placebo (HR 0·52 [95% CI 0·30–0·89]; p=0·016 by the log-rank test; figure 2C). Similarly, the treatment effect

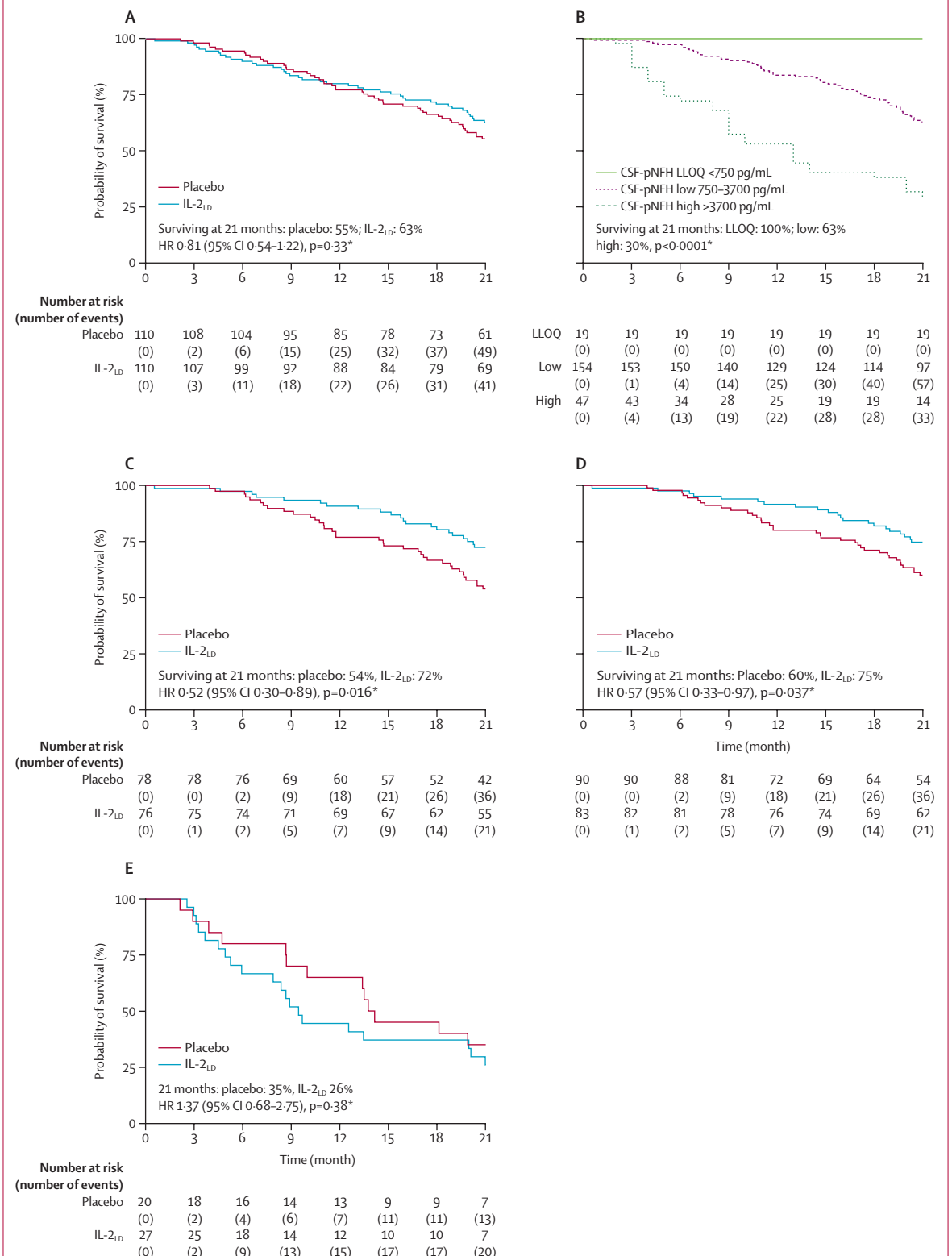

Figure 2: **Primary outcome: survival**
(A) Kaplan–Meier curves of all randomly allocated participants by treatment group. (B) Kaplan–Meier curves of all randomly allocated participants by CSF-pNFH strata. (C) Kaplan–Meier curves of participants by treatment group for the low CSF-pNFH stratum. (D) Kaplan–Meier curves of participants by treatment group, pooled LLOQ and low CSF-pNFH strata. (E) Kaplan–Meier curves of participants by treatment group, high CSF-pNFH stratum. The survival numbers are given in the appendix (p 24). CSF=cerebrospinal fluid. HR=hazard ratio. IL-2_{LD}=low-dose interleukin-2. LLOQ=lower limit of quantification. pNFH=phosphorylated neurofilament heavy chain. *p value from the log-rank test.

remained significant when pooling the LLOQ and low strata representing 173 (79%) of 220 (HR 0·57 [95% CI 0·33–0·97]; p=0·037 by the log-rank test; figure 2D). However, in the high stratum the increase in the HR for the treated group was non-significant (1·37 [0·68–2·75], p=0·38 by the log-rank test; figure 2E).

There were three participants who underwent tracheostomy (one in the placebo group and two in the IL-2_{LD} group), with two participants previously using NIV for 6 h or more per day. Among the 220 participants, 102 (46%) used NIV (53 [48%] for placebo and 49 [45%] for IL-2_{LD}), with 87 (39%) participants reaching the threshold time of 6 h or more per day to qualify as an event in the composite outcome (43 [39%] and 44 [40%], respectively; appendix p 41). There were 67 participants (30%) who underwent gastrostomy (37 [34%] for placebo and 30 [27%] for IL-2_{LD}; appendix p 37). In total, 150 (68%) participants were reported with any such event or death (80 [73%] for placebo and 70 [64%] for IL-2_{LD}; table 3).

	Hazard ratio (95% CI)	p value
ALSFRS-R at random allocation, point score 0–48	0·86 (0·83–0·89)	<0·0001
Age, years 18–75	1·029 (1·004–1·054)	0·021
CSF-pNFH at random allocation, pg/mL	1·0002 (1·00004–1·0003)	0·0091
Number of Tregs per µL at random allocation	0·982 (0·968–0·997)	0·018
Plasma-CCL2 at random allocation, pg/mL	1·0033 (0·9996–1·0069)	0·077
IL-2_{LD} treatment	0·32 (0·14–0·73)	0·0070
Treatment by CSF-pNFH interaction	1·0003 (1·0001–1·0005)	0·0011

Hazard ratios were generated by a multivariate Cox's model analysis for each of the covariates in the model. A hazard ratio greater than 1·0 denotes an increase in risk of death for each unit increase of the parameter (eg, age, CSF-pNFH, and CCL2), and a hazard ratio under 1·0 denotes a decrease in risk of death for each unit increase of the parameter (eg, ALSFRS-R score and number of Tregs). ALSFRS-R=Revised Amyotrophic Lateral Sclerosis Functional Rating Scale. CCL2=chemokine ligand 2. CSF=cerebral spinal fluid. IL-2_{LD}=low-dose interleukin-2. pNFH=phosphorylated neurofilament heavy chain.

Table 2: **Multivariate analysis of the primary outcome (survival) in the intention-to-treat population**

The unadjusted log-rank analyses of each of the three composite outcomes in the 220 participants showed a non-significant decrease in risk of event with IL-2_{LD} (p=0·35 to 0·50; appendix p 24). However, for all three composite outcomes the adjusted Cox's model analyses revealed significant treatment effects and treatment by CSF-pNFH interactions, consistent with those observed for the primary outcome (table 3). Similarly, a significant treatment effect for the NIV–tracheostomy–death composite outcome was observed within the low CSF-pNFH stratum (p=0·04 by the log-rank) and a similar, although not significant effect in the low plus LLOQ stratum (p=0·06), but not in the high stratum (p=0·11; appendix p 24). For both the gastrostomy–death or any-event composite outcomes, similar trends were observed in the low stratum (p=0·06 and p=0·09, respectively), in the low plus LLOQ stratum (p=0·11 and p=0·10, respectively), and no treatment effect for any of these outcomes in the high stratum (p=1·0 and p=0·49, respectively).

Core biomarker measurements assessed for drug engagement (appendix p 25) showed a highly significant increase from random allocation in the absolute number and frequency of Tregs at day 8 (maximum first cycle), day 112 (trough level following four cycles), and day 120 (maximum fifth cycle) in the IL-2_{LD} group compared with placebo (p<0·0001 for all timepoints; figure 3A and B; appendix p 25). IL-2_{LD} also resulted in moderate increases in number of CD8 and NK cells and a decrease in B cells (appendix p 25).

Plasma levels of CCL2 also showed treatment-associated changes, with a significant reduction from random allocation observed at day 8, day 112, and day 120 in the IL-2_{LD} group compared with placebo (p<0·0001 for days 8 and 120, and p<0·05 for day 112; figure 3C); however, this change was not reflected in the CSF when measured at day 112 (trough level; figure 3D). Similarly, no treatment-associated change from randomisation in pNFH levels was detected in the CSF at day 112 (figure 3E).

Unadjusted analysis of the secondary clinical efficacy endpoint (ALSFRS-R slope of change) showed a 12%

	Number of events, total ($n_{placebo}$; $n_{IL\text{-}2LD}$)	Number of deaths in events, n (%)	Factors in Cox's model analysis					
			Treatment		CSF-pNFH		Treatment by CSF-pNFH interaction	
			HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Death only	90 (49; 41)	90/90 (100%)	0·27 (0·12–0·62)	0·0017	1·0002 (1·0001–1·0003)	<0·0001	1·0004 (1·0002–1·0006)	<0·0001
NIV, tracheostomy, or death	133 (70; 63)	45/133 (34%)	0·33 (0·17–0·64)	0·0009	1·0001 (1·00004–1·0002)	0·0066	1·0004 (1·0002–1·0006)	<0·0001
Gastrostomy or death	122 (65; 57)	55/122 (45%)	0·39 (0·19–0·78)	0·0089	1·0003 (1·0002–1·0004)	<0·0001	1·0003 (1·0001–1·0005)	0·0009
Any event	150 (80; 70)	30/150 (20%)	0·48 (0·25–0·90)	0·021	1·0003 (1·0002–1·0004)	<0·0001	1·0002 (1·00007–1·0004)	0·0056

CSF=cerebrospinal fluid. HR=hazard ratio. IL-2_{LD}=interleukin-2 low dose. NIV=non-invasive ventilation. pNFH=phosphorylated neurofilament heavy chain.

Table 3: **Composite event outcomes: supportive analysis using Cox's model in the intention-to-treat population**

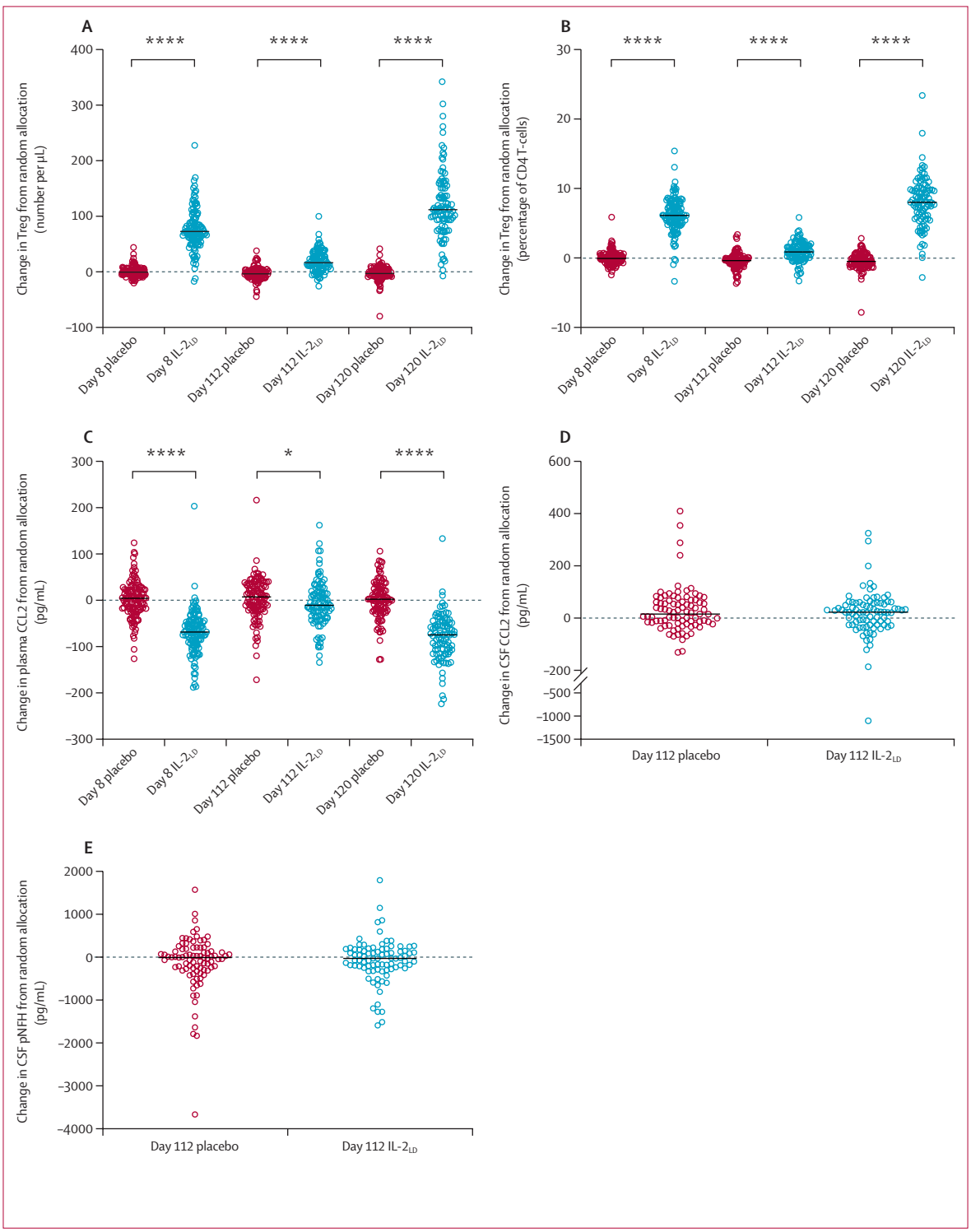

Figure 3: **Effect of IL-2_{LD} treatment on core biomarkers in blood and CSF**
Tregs changes from random allocation in (A) number and (B) frequency at day 8 (maximum of first cycle), day 112 (trough level following four cycles), and day 120 (maximum of fifth cycle). CCL2 concentration changes from random allocation in (C) plasma, measured at day 8, day 112, and day 120 and (D) CSF, measured at day 112. (E) pNFH concentration changes in CSF from random allocation at day 112. All verum to placebo comparison tests were performed by the Mann–Whitney *U* test. Black lines indicate the median of the distribution of changes from random allocation. CCL2=chemokine ligand 2. CSF=cerebrospinal fluid. IL-2_{LD}=low-dose interleukin-2. pNFH, phosphorylated neurofilament heavy chain. Tregs=regulatory T-cells. *indicates p<0·05. ****indicates p<0·0001.

decrease in rate of change in the IL-2_{LD} group compared with the placebo group (median rate of change in points per month –1·11 [–13·57 to 0·21] for placebo *vs* –0·98 [–8·46 to 4·34] for IL-2_{LD}), which did not reach statistical significance by the joint rank test (Winratio 1·15 [95% CI 0·85 to 1·56], p=0·38). Although the adjusted analysis showed no significant treatment effect (CAFS ANCOVA $F_{[1,216]}$ 0·34, p=0·56), there was a strong relationship of CSF-pNFH levels on the rate of change of ALSFRS-R ($F_{[1,216]}$ 21·78, p=5·35 × 10^{-6}), and a significant treatment by CSF-pNFH interaction ($F_{[1,216]}$ 3·95, p=0·048). Stratifying by CSF-pNFH as for survival analysis showed a statistically significant decrease in rate of change (–23%) in the low CSF-pNFH stratum for the IL-2_{LD} group compared with placebo: for the low stratum (median rate of change in points per month –1·06 [–3·23 to 0·03] for placebo *vs* –0·82 [–4·20 to 4·34] for IL-2_{LD}; Winratio 1·55 [95% CI 1·07 to 2·24], p=0·021), with a similar trend (–18% decrease) in the pooled LLOQ plus low strata, (median rate of change in points per month –0·95 [–3·23 to 0·03] for placebo *vs* –0·78 [–4·20 to 4·34] for IL-2_{LD}; Winratio 1·37 [95% CI 0·97 to 1·94], p=0·074), but not in the high stratum (rate of change 6%; median rate of change in points per month –1·99 [–13·57 to 0·21] for placebo *vs* –2·11 [–8·46 to 2·17] for IL-2_{LD}; Winratio 0·72 [95% CI 0·36 to 1·43], p=0·35; appendix p 26).

The safety population contained all 220 patients. 108 (98%) participants taking placebo had at least one adverse event, compared with 110 (100%) participants taking IL-2_{LD} (appendix pp 27–39). The most common non-serious adverse events in the IL-2_{LD} group were injection site reactions (101 [92%]), flu-like symptoms (66 [60%]), gastro-intestinal symptoms (66 [60%]), and asthenia–fatigue (45 [41%]).

Serious adverse events that were non-drug-related (SAE-ND) were mainly related to disease progression in both treatment groups (70 [64%] of participants for IL-2_{LD} and 75 [68%] for placebo). Surgical and medical procedures (primarily gastrostomy, NIV, tracheostomy, and palliative care) were the most common SAE-ND in both groups, overall affecting 55 participants (50%) for placebo and 42 (38%) for IL-2_{LD}, with significantly fewer participants reported in palliative care in the IL-2_{LD} group (18 for placebo and five for IL-2_{LD}; p=0·002 by the Fisher's exact test).

Serious adverse events that were drug-related were reported in nine participants (four [4%] in placebo and five [5%] with IL-2_{LD}). In the IL-2_{LD} group, one individual presented with two episodes of pneumonia, which resolved and did not recur under half-dose treatment. Another participant with emergent asymptomatic auto-immune thyroiditis received corrective therapy with no study treatment modification. An altered state of consciousness following an injection occurred in one participant for whom treatment could be resumed at the lowest possible dose (0·5 MIU) for the remaining study period. Treatment was terminated for two participants, one for a case of hypersensitivity vasculitis and another for a case of maculopapular rash. No COVID-19 infection was reported during the study.

47 participants (21%) withdrew from treatment (23 [21%] in placebo and 24 [22%] in IL-2_{LD}), with eight participants (4%) withdrawing in relation to treatment (three [3%] in placebo and five [5%] in IL-2_{LD}; appendix p 40). Consistent with the terminal phase being the main reason for early treatment termination, median compliance to treatment was still relatively good (65·3%) compared to the overall population while survival was poor (18 [38%] of 220), with no overt imbalance between treatment groups.

No other clinically relevant adverse effects were reported in vital signs, electrocardiograms, and physical or neurological examinations. Regarding routine laboratory findings, hypereosinophilia (>1000/mL) was observed more frequently under IL-2_{LD} (21 instances in IL-2_{LD} *vs* two instances in placebo), mainly at end of a treatment cycle (four at week 2, and ten at week 18) with no clinically significant expression, except in one case of generalised rash at first cycle.

Discussion

Although the unadjusted analysis of the primary endpoint, survival, showing a 19% decrease in the risk of death did not reach statistical significance, the planned adjusted analysis showed a significant treatment effect on decreasing the risk of death and a significant treatment by pNFH interaction, supporting our strategy for controlling crucial prognostic factors in this highly heterogeneous disease. Thus, we have shown in this phase 2b, randomised, double-blind, parallel group, placebo-controlled trial, that IL-2_{LD} as an add-on to riluzole could safely be administered over a prolonged period to improve survival and reduce the rate of functional decline in people with ALS.

There was no loss to follow-up over 21 months for the primary endpoint, with full compliance for CSF and blood sampling at inclusion and random allocation, allowing robust inferences to be drawn according to our prespecified analysis strategy. Although there was some attrition in blood and CSF sampling after random allocation, there was sufficient power to detect a potential treatment effect on these biomarkers with minimal potential bias due to censoring. In keeping with our hypothesis that ALS trials require a new approach to account for disease heterogeneity, we found that the treatment efficacy is modulated by the CSF-pNFH level as measured at random allocation. The pattern of the IL-2_{LD} effect on survival being strongest in the low CSF-pNFH stratum was consistently supported by the analysis of composite outcomes and by the ALSFRS-R slope of change.

The extensive heterogeneity of ALS carries a high probability of a widely variable clinical response to therapy,[4,5] requiring appropriate adjustment on relevant prognostic factors to achieve a precise estimate of the

treatment effect and of the responder population who would most benefit from treatment.[22,23] While an incident ALS population should be more representative of the diversity of the ALS population, at the time of diagnosis the disease phenotype, such as rate of progression, might not be fully expressed, with the concomitant risk of random bias. We therefore incorporated well-documented ALS disease biomarkers in the study design [9,13–15] to overcome the effect of disease heterogeneity on the detection of a treatment effect, and to explore treatment personalisation.

For our primary objective, adjusting on CSF-pNFH and its interaction with treatment proved instrumental in revealing that IL-2_{LD} significantly reduces disease progression over 18 months of treatment for the 70% of participants with low CSF-pNFH level at random allocation. These results were consistently supported across all the clinical outcome analyses, including the composite outcomes and the secondary outcome (ALSFRS-R slope of change). In addition to CSF-pNFH, other candidate variables selected in the stepwise Cox's model included ALSFRS-R, age, Treg counts, and CCL2 as independent prognostic factors for survival. However, CSF-pNFH was the key factor for handling heterogeneity in treatment responses in this population, as it was the sole prognostic factor in the Cox's model showing a statistically significant interaction with treatment.

To investigate the meaning of this interaction, stratification by CSF-pNFH levels identified the lower range stratum—representing 70% of the whole ITT patient population—as the responders with an approximate halving of the risk of death at 21 months in the IL-2_{LD} treated group versus placebo. Taken together, the comparison of the treatment effect according to CSF-pNFH suggests that for about 70–80% of the population below the defined CSF-pNFH threshold, IL-2_{LD} induced a significant decrease in risk of death of about 43–48% over the follow-up period; however, above the defined threshold (21% of the trial population), the treatment schedule appears to be ineffective. We interpret these findings as confirming that CSF-pNFH levels represent an aggressive process underlying the heterogeneous ALS pathology, which beyond the defined threshold overwhelms the therapeutic effect of IL-2_{LD} treatment as used in our study.

Currently, only riluzole has demonstrated a significant effect on survival in ALS. Compared to the riluzole phase 3 trial,[3] IL-2_{LD} achieved a similar decrease in risk of death by analysis unadjusted for prognostic factors (ie, HR for IL-2_{LD} of 0·81 *vs* HR for 100 mg riluzole of 0·79). However, the Il-2_{LD} effect on survival appears larger in the responder population of low CSF-pNFH stratum representing 70% of the population (HR for IL-2_{LD} of 0·52), although at the time of the riluzole trial no treatment predictors were tested as no suitable biomarkers were available for helping define responders to riluzole. Nevertheless, heterogeneity in treatment response was already evident in the riluzole trials from the striking difference in efficacy between the phase 3 trial[3] and the trial on more advanced patients.[24]

As we recruited participants at the earliest stage in the disease, including those fulfilling the criteria for El Escorial possible or laboratory-supported probable ALS, who accounted for 42% of participants, this population is likely to represent the real-world spectrum of ALS heterogeneity, strengthening the generalisability of the study results.

We also demonstrated target engagement by IL-2_{LD} via an increase in Tregs number and frequency. Moreover, the decreased plasma-CCL2 suggests this more tolerogenic environment is reducing inflammation. Although the effect on Tregs and CCL2 were still statistically significant at trough sampling time, both parameters were very close to baseline values. This observation might explain why we did not detect changes in CSF-CCL2 measured only at trough level time, and suggests that optimising the dose regimen could lead to a more sustained tolerogenic effect.

The lack of treatment response for the CSF-pNFH outcome in MIROCALS contrasts with a striking reduction in plasma and CSF neurofilaments in the tofersen studies,[21,25] which might be related to a design issue, with a sampling time too early in the treatment course to detect a significant change, a hypothesis supported by the time lag of treatment effect on survival. CSF-pNFH levels could also be insufficiently sensitive to changes in the disease pathophysiology in this time frame. For any given biomarker, the ability to detect change is a function of both its release into the sampled fluid and its clearance from the fluid. While there is no definitive understanding of the clearance pathway for pNFH in CSF or blood, its half-life is presumed to be several months,[26] making it unlikely to change substantially in response to IL-2_{LD} or riluzole over the observation period in MIROCALS. The concept of CSF-pNFH being more of a trait marker predicting the progression profile rather than a state marker defining the disease stage is supported by studies in familial ALS.[15]

It is also possible that the survival benefit of IL-2_{LD} is primarily mediated by changes in the peripheral, rather than the central, nervous immune system.[27] We have shown here, as in our previous study,[12] that IL-2_{LD} increases Treg numbers and function and decreases plasma-CCL2, changes that indicate a more tolerogenic, less inflammatory peripheral immune environment.

Taken together, the results of MIROCALS indicate that CSF-pNFH, measured to regulatory standards, provides an objective and reliable biomarker for understanding and adjusting for disease heterogeneity, hitherto a major confounding factor in ALS trials. Foremost, CSF-pNFH proved to be a powerful predictor of treatment response. Despite the challenge of using CSF-pNFH for a priori trial stratification, our result provides a strong rationale

for incorporating CSF-pNFH stratification in the design of ALS clinical trials. However, it is also important to consider all known prognostic factors in the analysis, as it is unrealistic to stratify on all relevant parameters.

There are limitations to this study. This phase 2b study is the first attempt to modify ALS disease progression with low dose IL-2, and the first study in ALS to investigate treatment modifiers as components of disease heterogeneity that need to be controlled for. Even though these were prespecified hypotheses, as for any new finding which might arise by chance alone, our findings require further clinical development.

In addition, as a phase 2b study, there was insufficient power to detect a significant therapeutic benefit of IL-2_{LD} using an unadjusted log-rank analysis. This underlines the importance of controlling for confounding factors, as random imbalance might mask the treatment effect or result in spurious positive findings. The core biomarkers were instrumental in detecting the treatment effect, but the study could not be adequately powered to achieve a satisfactory precision for detecting a treatment effect in the higher CSF-pNFH group. Although the difference in the rate of disease progression between the CSF-pNFH strata, in keeping with the biological rationale, strongly supports a pharmacodynamic interaction, adequately powered studies are needed to confirm this.

Another limitation is that we were only able to test one dosing regimen, which we based on that used in previous phase 2a trials in diabetes[28] and ALS.[12] A dose–effect response on the primary efficacy outcome would have strengthened the conclusion on the efficacy of the therapy. In light of our results, it will be important to consider options for more sustained delivery of IL-2_{LD}.

It is regrettable, but unavoidable given the impact of the COVID-19 pandemic on medical services and clinical trial units, that we were not able to provide useable data for vital capacity or quality of life, outcomes that will have to be tested in future trials.

More positively, we were able to gather complete data on ALSFRS-R, arguably the best validated and approved measure of functional change in the context of ALS clinical trials. In the adjusted analyses of ALSFRS-R slope of change with the CAFS score, compared with the Cox's analysis for the survival endpoint, the treatment-by-CSF-pNFH interaction just reached significance. However, the CAFS score has no dimension, and is therefore difficult to interpret clinically. In the low CSF-pNFH stratum, the significant 23% decrease in median slope of change with IL-2_{LD} (appendix p 26) yields an estimated difference in ALSFRS-R score of 5·04 points (out of 48) at 21 months, the end of the study.

Finally, as discussed earlier, we might have missed an appropriate time window for detecting treatment-related CSF-change with our chosen biomarkers. Ongoing work on a wide range of other plasma and CSF biomarkers may help to resolve this issue.

In conclusion, although the primary analysis showed a non-significant reduction in mortality, adjusting on CSF-pNFH, IL-2_{LD} was associated with a significant clinical benefit on survival and function in an ALS cohort representative of the real world ALS incident population. Given the satisfactory safety profile of this treatment regimen, it would be important to confirm these results in larger studies and test more intensive schedules in an attempt to improve outcomes across the full spectrum of people affected by ALS, especially for those with high CSF-pNFH levels associated with rapid disease progression, a population which poses a unique challenge for ALS therapeutics.

Based on MIROCALS results, IL-2_{LD} treatment in combination with riluzole could be considered for further clinical development as a safe and well tolerated treatment for ALS.

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Declaration of interests

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Data sharing

De-identified datasets of the data supporting the results in the manuscript and statistical code will be available upon request, 12 months following publication of the study results. Emails should be sent to the MIROCALS study at drc@chu-nimes.fr, stating the variables required and purpose of the request (objective or objectives and research plan). The study protocol and data dictionary will also be made available via the MIROCALS email. Requests will be considered on a case-by-case basis, and requestors will be asked to complete a data sharing agreement with the sponsor before data transfer.

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