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Phase 1b dose-escalation, safety, and pharmacokinetic study of IC14, a monoclonal antibody against CD14, for the treatment of amyotrophic lateral sclerosis

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Abstract

Background: The primary objective was to demonstrate the safety and tolerability of monoclonal antibody against CD14 (IC14) (atibuclimab) in amyotrophic lateral sclerosis patients. The secondary objectives were pharmacokinetics, pharmacodynamics, and preliminary effects on disease status and biomarkers.

Methods: In this open-label, dose-escalation trial, IC14 was administered at 2 mg/kg intravenous (IV) followed by 1 mg/kg/d IV × 3 (n = 3) and in subsequent patients at 4 mg/kg IV followed by 2 mg/kg/d IV × 3 (n = 7) (NCT03487263). Disease status was measured using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale, forced vital capacity, sniff nasal pressure, Edinburgh Cognitive and Behavioral ALS Screen, and Revised ALS-Specific Quality-of-Life Score. Disease biomarkers included cerebrospinal fluid and serum levels of neurofilament light chain (NfL) and urinary p75 neurotrophin receptor.

Results: IC14 was safe and well tolerated. No antidrug antibodies were detected. The drug target saturation of monocyte CD14 receptors was rapid and sustained through day 8. There was no significant change in Revised Amyotrophic Lateral Sclerosis Functional Rating Scale, forced vital capacity, sniff nasal pressure, or Revised ALS-Specific Quality-of-Life Score following a single cycle of treatment. Cerebrospinal fluid NfL levels decreased in 6 of 9 patients sampled with declines of 15% to 40% between baseline (not significant [ns]) and day 8 in 3 patients. Serum NfL modestly decreased in 5 of 10 patients (ns) at day 8 and was sustained in 4 (4%-37%, ns) over 33 days of follow up.

Conclusion: IC14 quickly and durably saturated its target in all patients. This study demonstrated safety and tolerability in patients with amyotrophic lateral sclerosis. Even though only a single cycle of treatment was given, there were promising beneficial trends in the neurofilament light chain, a disease biomarker. The emerging understanding of the role of systemic inflammation in neurodegenerative diseases, and the potential for IC14 to serve as a safe, potent, and broad-spectrum inhibitor of immune dysregulation merits further clinical study.

Clinical Trial Registration: NCT03487263

Abbreviations: AE = adverse events, ALS = amyotrophic lateral sclerosis, ALSFRS-R = Revised Amyotrophic Lateral Sclerosis Functional Rating Scale, ALSSQOL-R = Revised ALS-Specific Quality-of-Life Score, ALT = alanine aminotransferase, AUC = area under the curve, CD14 = cluster of differentiation 14, CSF = cerebrospinal fluid, ECAS = Edinburgh Cognitive and Behavioural ALS Screen, FVC = forced vital capacity, IC14 = monoclonal antibody against CD14, IL = interleukin, IV = intravenous, mCD14 =

Editor: Subhashchandra Naik.

This work was supported by Fight MND under Grant ID:03_TRG_2017_Henderson.

All relevant data are within the paper and its Supporting Information files.

The authors RDH, PAM, KT, SH and SH reported no conflicts of interest. JMA was a consultant for Implicit Bioscience. MWA, BWZ, DTC, and GLR were employed by Implicit Bioscience.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Henderson RD, Agosti JM, McCombe PA, Thorpe K, Heggie S, Heshmat S, Appleby MW, Ziegelaar BW, Crowe DT, Redlich GL. Phase 1b dose-escalation, safety, and pharmacokinetic study of IC14, a monoclonal antibody against CD14, for the treatment of amyotrophic lateral sclerosis. Medicine 2021;100:42(e27421).

Received: 14 May 2021 / Received in final form: 18 August 2021 / Accepted: 16 September 2021

http://dx.doi.org/10.1097/MD.0000000000027421

membrane-bound CD14, MFI = mean fluorescence intensity, NfL = neurofilament light chain, ns = not significant, PK = pharmacokinetics, RO = receptor occupancy, sCD14 = soluble CD14, SNP = sniff nasal pressure, Teff = effector T lymphocyte, TLR = toll-like receptor, Treg = regulatory T lymphocyte.

Keywords: amyotrophic lateral sclerosis, anti-CD14, atibuclimab, IC14, monoclonal antibody to CD14, motor neurone disease

1. Introduction

Amyotrophic lateral sclerosis (ALS), also called motor neurone disease, is a progressive and fatal neurodegenerative disorder. There is a growing understanding of the key role of neuroinflammation in mediating disease progression in ALS.[1]

Cluster of differentiation 14 (CD14) is a glycoprotein found on the surface of myeloid cells and as a soluble protein in plasma. It functions as an accessory molecule for several toll-like receptors (TLRs), a family of pattern recognition receptors that respond to conserved patterns in pathogens and human molecules associated with inflammation and tissue injury. It is a key organizer of response to infection and injury, notably in microglia.[2] CD14 plays a pivotal role in the signaling between innate immune cells and $CD4^+$ memory T cells that drive chronic disease and autoimmunity.

Elevated levels of circulating soluble CD14 (sCD14) have been described in ALS and are associated with (and predictive of) a rapid rate of disease progression and a poor clinical prognosis.[3] Elevated sCD14 levels are positively correlated with ALS disease severity scores.[3] CD14 plays an important role in the initiation of innate and chronic inflammatory cascades, including chronic inflammation that contributes to neurodegeneration in patients with ALS.[4–7]

Monoclonal antibody against CD14 (IC14) (atibuclimab) is a chimeric monoclonal antibody directed against CD14 and is composed of murine variable and human IgG4 Fc regions. It recognizes both membrane-bound CD14 (mCD14) and sCD14 and is non-lytic. IC14 has been administered to over 150 healthy volunteers and patients with sepsis, community-acquired pneumonia, or acute lung injury at cumulative doses of up to 16 mg/kg over 4 to 5 days and has been generally well tolerated.[8–11] None of the patients developed antibodies to IC14.[9]

IC14 binds with high affinity (in the nanomolar range) to sCD14 in circulation and to mCD14, so this binding is effectively irreversible. Two pharmacodynamic assays distinguish IC14 from other therapeutic approaches and provide a useful modality for measuring biologic activity. These assays measure serum sCD14 and the percent inhibition of mCD14 on monocytes. Given the number of promising drug candidates that have failed to translate encouraging preclinical data into clinical benefit in patients with ALS, and where drug target engagement was either not measured or was unmeasurable, occupancy by IC14 of its target, mCD14 on circulating monocytes, provides a useful modality to directly measure inhibition of the target. Previous studies in critical care patients showed that high saturation of mCD14 was quickly achieved following an initial dose of 4 mg/kg.[8] This was the first time that IC14 was administered to patients with a progressive neurodegenerative disease.

2. Materials and methods

Subjects were required to meet the definition of familial or sporadic ALS by Awaji-Shima Consensus Recommendations.[12] Additional inclusion criteria were first ALS symptoms within 3 years; seated forced vital capacity >65% of predicted; not taking riluzole or on a stable dose for at least 4 weeks; adequate bone marrow reserve, renal, and liver function; adequate contraception or not of childbearing potential; and medically safe to undergo a lumbar puncture. Exclusion criteria were dependence on mechanical ventilation; treatment with experimental drug within 30 days; treatment with an immunomodulator or immunosuppressant within 12 months; recurrent infections; presence of a defined significant medical condition; or history of severe allergic or anaphylactic reaction to monoclonal antibodies.

Written informed consent was obtained from all patients. Ten patients were screened without screening failures (see Flow Diagram 1, Supplemental Digital Content, http://links.lww.com/MD2/A519, which shows patients screened). The 10 screened patients were sequentially assigned open-label dosing with IC14 for a single cycle at 1 of 2 dose levels and monitored for safety, tolerability, immunogenicity, pharmacokinetics, and pharmacodynamics (see Table S1, Supplemental Digital Content, http://links.lww.com/MD2/A518, which shows the baseline demographics of study groups). IC14 was administered at 2 mg/kg intravenous (IV) followed by 1 mg/kg/d IV for 3 days on days 3 to 5 (n = 3); or 4 mg/kg IV followed by 2 mg/kg/d IV for 3 days on days 2 to 4 (n = 7). Each dose was administered as an intravenous infusion in sterile normal saline for infusion over 2 hours. The following safety endpoints were evaluated: adverse events (AEs), changes in clinical laboratory values, changes in vital sign, physical examination findings, slit lamp ophthalmologic findings, and presence or absence of anti-IC14 antibodies. The study was designed as a safety and pharmacokinetic (PK) study, so a placebo group was not included.

Serum IC14 PK parameters (i.e., maximum concentration, time of maximum concentration, area under the curve [AUC], half-life, clearance, and apparent volume of distribution during terminal phase) were estimated based on observations taken during a 24-hour interval after dose 1 (i.e., pre-dose and post-dose at 15 min, 6 h, 12 h, and 24 h) and dose 4 (pre-dose and post-dose at 15 min, 12 h, and 24 h), and day 8. Cerebrospinal fluid IC14 concentrations were measured at baseline and on days 5 and 8. PK analyses were performed using non-compartmental analysis using WinNonlin (Phoenix version 6.4) software (Certara, Princeton, NJ). All AUC parameters were estimated using the linear trapezoidal method. PK parameters dependent on characterization of the terminal phase were only reported if at least 3 time points in the terminal phase of the concentration vs time profile were measurable, R^2 was greater than 0.8, and the $AUC_{\%extrap}$ was less than 25%.

The pharmacodynamic behavior of IC14 was characterized by measuring the concentration of sCD14 in serum, cerebrospinal fluid (CSF), and urine and the saturation of mCD14 receptors on circulating monocytes in whole blood, as measured by flow cytometry. The receptor occupancy was calculated using the

$CD45^+$ monocyte mean fluorescence intensity (MFI) flow cytometry data using the following formula:

% Receptor occupancy = ((TEST MFI−ISOTYPE CONTROL 1 MFI)/(SATURATION MFI−ISOTYPE CONTROL 2 MFI)) × 100.

The primary outcome measures were safety and tolerability of IC14 in patients with ALS. This was not designed as an efficacy study since this course of treatment involved only a single cycle. The following were measured at baseline and at day 33, with the exception of CSF biomarkers that were measured at baseline, day 5, and day 8. Disease status was measured using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R),[13] forced vital capacity (FVC), sniff nasal pressure (SNP), Edinburgh Cognitive and Behavioural ALS screen (ECAS),[14] and Revised ALS-Specific Quality-of-Life Score (ALSSQOL-R).[15,16] Disease biomarkers included CSF and serum levels of neurofilament light chain (NfL), CSF and serum cytokines (C-reactive protein, interleukin [IL]-1ß, IL-6, IL-17), and urinary p75 neurotrophin receptor. Serum NfL levels were measured using an enzyme-linked immunosorbent assay (Cloud-Clone, Katy, TX). CSF NfL levels were measured using enzyme-linked immunosorbent assay (UMAN Diagnostics AB, Umea, Sweden). Blood levels of neurofilaments, the scaffold protein released from damaged motor neuron axons, have diagnostic and prognostic value in ALS.[17–19] Higher levels of urinary p75 neurotrophin receptor predict more rapid disease progression and shorter survival; however, these findings remain to be further validated.[20]

This study was registered with ClinicalTrials.gov as NCT03487263 on April 4, 2018. The study was conducted in the Neurology Clinical Research Center at Royal Brisbane & Women's Hospital, Herston, Australia. Ethics approval was obtained from the Human Research Ethics Committee (EC00172) Royal Brisbane & Women's Hospital (reference number HREC/17/QRBW/56). Patients were enrolled between October 12, 2017 and March 1, 2018 then followed for 32 days after starting treatment.

3. Results

3.1. Safety

There were no study dropouts or discontinuations (see Flow Diagram 1, Supplemental Digital Content, http://links.lww.com/MD2/A519, which shows patient study participation). All doses of IC14 were administered according to the schedule, and there were no missed doses. IC14 was safe and well tolerated. There were no severe or serious AEs during treatment or the 28-day follow-up period. Four subjects (1 low dose, 3 high dose) had no treatment-emergent AEs. The treatment-emergent AEs consisted of post-lumbar puncture headaches (n = 6), accompanied by dizziness (n = 2), myalgia (n = 2), procedural pain (n = 2), or post-procedural discomfort (n = 1); and 1 patient each with bone pain, musculoskeletal stiffness, neck pain, upper abdominal pain, nausea, vomiting, tinnitus, catheter site bruise, dysuria, papule, or macular rash (Table 1). Two patients had falls without injury; these are not unexpected in ALS. There was no dose-relatedness of the AEs observed. All symptoms resolved without sequelae, and none led to study withdrawal. All analyses were conducted according to originally assigned dose group.

There were no significant abnormalities in hematology, chemistry, or coagulation. Two patients had mild alanine aminotransferase (ALT) elevations at baseline, and 2 developed mild ALT elevations during the study. All of them were taking the allowed stable doses of riluzole that may result in elevated ALT levels.

Table 1

Treatment-emergent adverse events regardless of causality.

Adverse event	Dose 2/1/1/1 mg/kg/d (N = 3) n (%)	Dose 4/2/2/2 mg/kg/d (N = 7) n (%)
Patients with at least 1 event	2 (67%)	4 (57%)
Headache	2 (67%)	4 (57%)
Dizziness	–	2 (29%)
Fall	1 (33%)	1 (14%)
Procedural pain	1 (33%)	1 (14%)
Post-procedural discomfort	–	1 (14%)
Myalgia	–	2 (29%)
Bone pain	–	1 (14%)
Musculoskeletal stiffness	1 (33%)	–
Neck pain	1 (33%)	–
Upper abdominal pain		1 (14%)
Nausea	1 (33%)	–
Vomiting	1 (33%)	–
Tinnitus	–	1 (14%)
Catheter site bruise	–	1 (14%)
Dysuria	1 (33%)	–
Papule	1 (33%)	–
Macular rash	1 (33%)	–

No patient formed anti-IC14 antibodies 28 days following end of treatment.

3.2. Pharmacokinetics and pharmacodynamics

IC14 appears to demonstrate nonlinear kinetics, which is consistent with a monoclonal antibody that has a cellular target (Fig. 1). The IC14 exposure increased in a dose-dependent manner. The mean serum half-life following the last dose was 32.73 and 44.34 hours in the 2 dose groups, respectively (Table 2). The biological half-life is longer than the serum half-life, since IC14 binds to sCD14 and mCD14 with high affinity in the nM range and continues to have biological activity (mCD14 receptor occupancy) even when it is not present in the serum (Fig. 2). IC14 cerebrospinal fluid levels were less than 0.03% of serum levels at matched time points, showing little or no central nervous system (CNS) penetration of this protein macromolecule, as predicted (Fig. 3; Table 3).

An important target of IC14 is the mCD14. Monocyte mCD14 receptor occupancy was negligible at baseline. Both dose groups demonstrated near-complete saturation of monocyte CD14 receptors: Group A had 96% ± 1.6% immediately after a single dose and 97% ± 1.7% at the end of treatment (mean ± standard deviation [SD], $P < .0001$ for change from baseline for both values); Group B had 104% ± 3.8% immediately after a single dose and 102% ± 4.7% at the end of treatment (mean ± SD, $P < .0001$ for change from baseline for both values; Fig. 2 and Table 4). The high-dose group maintained 103% ± 2.2% saturation for 4 days after the conclusion of treatment (mean ± SD, $P = .0003$ for change from baseline). Monocyte CD14 receptor occupancy achieved saturation quickly and endured for a week, demonstrating that the IC14 exposure levels were clinically relevant, achievable, and durable.

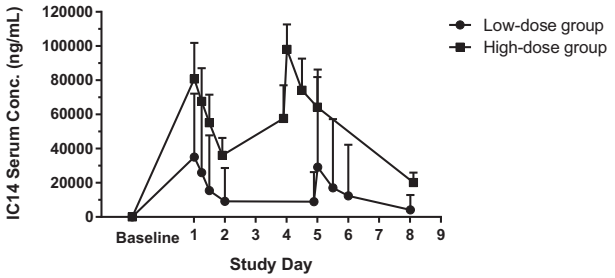

Figure 1. Mean IC14 serum concentration (ng/mL). Below-the-level-of-quantitation values were set to 0 before determining the mean. Error bars represent the 95% confidence interval. IC14 = monoclonal antibody against CD14.

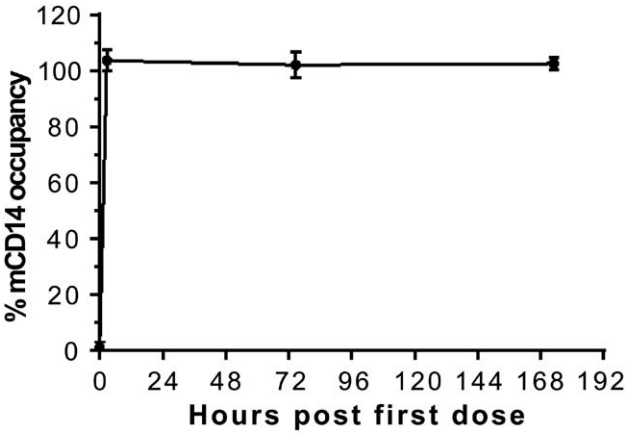

Figure 2. mCD14 monocyte receptor occupancy in ALS patients receiving IC14. Percent occupancy of membrane-associated CD14 receptors (RO) on monocytes is shown for the high-dose group. RO was 104%±3.8% immediately after a single dose and 102%±4.7% at the end of treatment (mean±SD, $P<.0001$ for change from baseline for both values). Receptor saturation of 103%±2.2% was sustained for 4 d after treatment (mean±SD, $P=.0003$ for change from baseline). Similar results were seen following administration of the low dose. ALS = amyotrophic lateral sclerosis, CD14 = cluster of differentiation 14, IC14 = monoclonal antibody against CD14, mCD14 = membrane-bound CD14, RO = receptor occupancy.

As IC14 blocks endotoxin and damage-associated molecular pattern binding to CD14, the IC14-sCD14 complexes are unlikely to function as TLR co-receptors. The assay used to measure sCD14 does not distinguish between free sCD14 and sCD14, that, is complexed with IC14. Measurements of serum sCD14 therefore significantly increased in both dose groups by the end of treatment as the total pool of sCD14 increased (Group A $P=.0076$; Group B $P<.0001$), however, this reflects the accumulation of biologically inactive IC14-sCD14 complexes and not an increase in unbound serum sCD14 (Table 4). CSF sCD14 was present at baseline in both groups and showed a significant increase at day 8 in Group B ($P=.026$), consistent with binding and retention of the IC14-sCD14 complex.

3.3. Clinical outcomes

An impact on clinical parameters was not anticipated in this study of a single cycle of treatment. There was no clinically significant change in ALSFRS-R, FVC, SNP, or ALSSQOL-R from baseline to day 33 (Table 5). The Edinburgh Cognitive and Behavioural ALS Screen score showed a possible beneficial trend at day 33; the change was small but statistically significant (6.0±5.25 points improvement from baseline 107.6±20.48, mean±SD, $P=.0056$) and appeared to be dose related, however, a learning effect or random variation cannot be excluded.

3.4. Biomarkers

The ALS-specific biomarker CSF NfL levels decreased over 8 days in 6 of 9 patients, with 3 showing a marked decline (15%-40%, not significant [ns]). Serum NfL showed a decrease over 5 days in 5 of 10 patients (7%-12%, ns) that was sustained to day 33 in 4 of those patients (4%-37%, ns; Fig. 4). Three patients had increased serum NfL at day 5 (0.4%-41%), but the patient with the highest NfL increase returned to baseline by day 33. There were too few patients and too short a course of treatment to make

Table 2

Serum IC14 pharmacokinetic parameters.

Statistic	C_{max} (μg/mL)	T_{max} (h)	AUC (0-last) (h*μg/mL)	Half-life (h)
Dose group 1: IC14 2 mg/kg followed by 1 mg/kg × 3 daily; PK following dose 1				
n	3	3	3	2
Mean	35.33	4.18	486.74	9.66
SD	15.11	3.31	308.44	1.60
Dose group 1: IC14 2 mg/kg followed by 1 mg/kg × 3 daily; PK following dose 4				
n	3	3	3	3
Mean	29.19	2.33	881.73	32.73
SD	23.06	0.08	787.34	5.32
Dose group 2: IC14 4 mg/kg followed by 2 mg/kg × 3 daily; PK following dose 1				
N	7	7	7	1
Mean	80.78	2.34	1266.68	10.63
SD	22.84	0.09	403.98	–
Dose group 2: IC14 4 mg/kg followed by 2 mg/kg × 3 daily; PK following dose 4				
N	7	7	7	5
Mean	98.07	2.29	4719.69	44.34
SD	15.82	0.06	1672.05	4.01

AUC = area under the curve, C_{max} = maximum concentration, IC14 = monoclonal antibody against CD14, n = number, PK = pharmacokinetics, SD = standard deviation, T_{max} = time of maximum concentration.

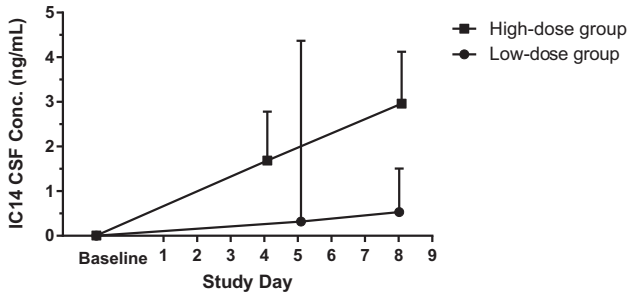

Figure 3. Mean cerebrospinal fluid concentration of IC14 (ng/mL). Lumbar puncture was performed as soon as possible after the final infusion. Below-the-level-of-quantitation values were set to 0 before determining the mean. Error bars represent the 95% confidence interval. CSF = cerebrospinal fluid, IC14 = monoclonal antibody against CD14.

conclusions about whether changes in biomarkers were dose-related (Table 6). The findings must be interpreted in the context of the multiple analyses conducted and the lack of a placebo comparator group.

Table 3

Cerebrospinal fluid concentrations of IC14 (ng/mL).

Statistic	Baseline	Following final IV dose	Day 8
Dose group 1: IC14 2 mg/kg followed by 1 mg/kg × 3 daily			
n	3	2	2
Mean	0.00	0.32	0.53
SD	0.00	0.34	0.11
Dose group 2: IC14 4 mg/kg followed by 2 mg/kg × 3 daily			
n	7	6	7
Mean	0.01	1.69	2.96
SD	0.01	1.05	1.26

IC14 = monoclonal antibody against CD14, IV = intravenous, SD = standard deviation.

Urinary p75 neurotrophin receptor (p75 NTR) appeared to approach a significant decrease in the high-dose group (baseline 4914 ± 3461 pg/mL decreased to 2808 ± 2850 pg/mL on day 4, mean ± SD, $P = .0511$; Table 6). When normalized for urinary creatinine, it did not change significantly.

There were no clinically significant changes in serum or CSF IL-1β, IL-6, IL-17, or CRP levels (see Table S2, Supplemental Digital

Table 4

IC14 pharmacodynamic measurements.

		Measured value			Change from baseline		
Group	Timepoint	n	Mean	SD	Mean	SD	*P* value
CSF soluble CD14 (ng/mL)							
Dose regimen 1	Baseline	3	125.27	61.35	–	–	–
	Day 5	2	144.62	90.51	1.59	15.44	.908
	Day 8	2	147.01	81.35	4.00	6.28	.535
Dose regimen 2	Baseline	7	106.60	32.01	–	–	–
	Day 4	6	162.75	95.36	58.01	121.24	.294
	Day 8	7	168.02	51.46	61.42	55.32	.026
All patients	Baseline	10	122.20	40.01	–	–	–
	Day 4	6	162.75	95.36	58.01	121.24	.294
	Day 5	2	144.62	90.51	1.59	15.44	.908
	Day 8	9	163.35	53.84	48.65	54.24	.027
Serum soluble CD14 (ng/mL)							
Dose regimen 1	Baseline	3	1365.85	198.39	–	–	–
	Day 1	3	1715.14	162.50	349.29	195.51	.090
	Day 5	3	8584.48	921.10	7218.63	1094.48	.008
Dose regimen 2	Baseline	7	1376.05	293.02	–	–	–
	Day 1	7	1462.12	309.56	86.06	188.52	.273
	Day 4	7	10253.28	1510.15	8877.23	9223.10	<.0001
All patients	Baseline	10	1372.99	256.93	–	–	–
	Day 1	10	1538.02	291.02	165.03	219.90	.042
	Day 4	7	10253.28	1510.15	8877.23	1418.13	<.0001
	Day 5	3	8584.48	921.10	7218.63	1094.48	.008
Circulating monocyte CD14 receptor occupancy (%)							
Dose regimen 1	Baseline	3	−0.62	0.13	–	–	–
	Day 1	3	96.03	1.65	96.66	1.52	<.0001
	Day 5	3	97.37	1.72	97.99	1.80	.001
Dose regimen 2	Baseline	7	1.27	2.03	–	–	–
	Day 1	7	103.61	3.85	102.34	2.30	<.0001
	Day 4	7	102.04	4.71	100.77	4.65	<.0001
	Day 8	3	105.53	2.17	102.01	3.21	.0003
All patients	Baseline	10	0.70	1.90	–	–	–
	Day 1	10	101.33	4.88	100.63	3.40	<.0001
	Day 4	7	102.04	4.71	100.77	4.65	<.0001
	Day 5	3	97.37	1.72	97.99	1.80	.0001
	Day 8	3	102.53	2.17	102.01	3.21	.0003

CD14 = cluster of differentiation 14, CSF = cerebrospinal fluid, IC14 = monoclonal antibody against CD14, SD = standard deviation.

Table 5

ALS clinical outcome measurements.

		Measured value			Change from baseline		
Group	**Timepoint**	**n**	**Mean**	**SD**	**Mean**	**SD**	***P* value**
Revised ALS Functional Rating Scale (ALSFRS-R; scale 0-48)							
Dose regimen 1	Baseline	3	35.3	2.5			
	End of study	3	37.0	2.6	1.7	4.73	.60
Dose regimen 2	Baseline	7	38.3	7.2			
	End of study	7	36.9	9.7	−1.4	4.35	.42
All patients	Baseline	10	37.4	6.2			
	End of study	10	36.9	8.0	−0.5	4.45	.73
Forced vital capacity (L)							
Dose regimen 1	Baseline	3	3.9	1.2			
	Day 8	3	4.2	0.6	0.3	0.7	.59
	End of study	3	3.7	1.1	−0.2	0.2	.32
Dose regimen 2	Baseline	7	3.9	0.8			
	Day 8	7	3.9	0.9	−0.0	0.3	.69
	End of study	7	3.7	0.9	−0.2	0.3	.14
All patients	Baseline	10	3.9	0.9			
	Day 8	10	4.0	0.8	0.0	0.4	.74
	End of study	10	3.7	0.9	−0.2	0.2	.05
Sniff nasal pressure (cm H_2O)							
Dose regimen 1	Baseline	3	55.2	19.4			
	Day 8	3	69.6	27.8	14.4	8.6	.10
	End of study	3	67.7	37.8	12.5	18.4	.36
Dose regimen 2	Baseline	7	65.2	17.7			
	Day 8	7	64.6	11.7	−0.1	−0.6	.89
	End of study	7	69.1	15.6	4.0	4.0	.44
All patients	Baseline	10	62.2	17.8			
	Day 8	10	66.1	16.4	3.9	4.0	.33
	End of study	10	68.7	21.9		6.5	.18
ALS-Specific Quality of Life-Revised (ALSSQOL-R; scale 0-460)							
Dose regimen 1	Baseline	3	278.0	2.6			
	End of study	3	281.7	39.0	3.7	37.1	.88
Dose regimen 2	Baseline	7	270.1	39.1			
	End of study	7	266.0	50.2	−4.1	31.5	.74
All patients	Baseline	10					
	End of study	10					
Edinburgh Cognitive and Behavioural ALS Screen (ECAS; 0-136)							
Dose regimen 1	Baseline	3	108.7	9.07	–	–	–
	End of study	3	115.7	7.57	7.0	4.58	.1181
Dose regimen 2	Baseline	7	107.1	24.51	–	–	–
	End of study	7	112.7	24.57	5.6	5.80	.0439
All patients	Baseline	10	107.6	20.48	–	–	–
	End of study	10	113.6	20.43	6.0	5.25	.0056

ALS = amyotrophic lateral sclerosis, ALSFRS-R = Revised Amyotrophic Lateral Sclerosis Functional Rating Scale, ALSSQOL-R = Revised ALS-Specific Quality-of-Life Score, ECAS = Edinburgh Cognitive and Behavioural ALS Screen, SD = standard deviation.

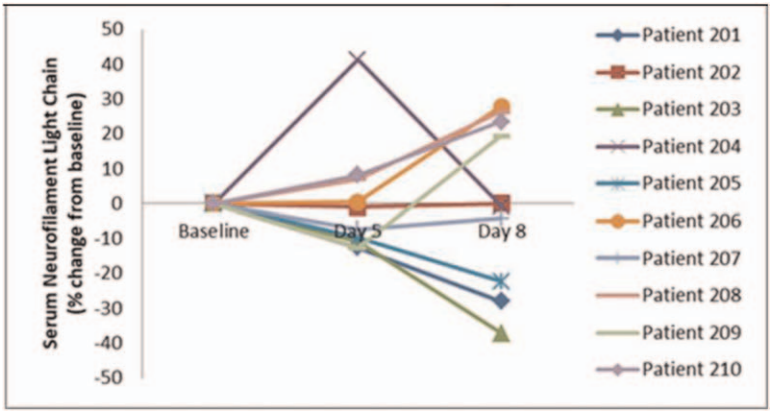

Figure 4. Biomarker serum neurofilament levels in individual subjects following a single cycle of IC14. IC14 = monoclonal antibody against CD14.

Table 6

ALS-specific biomarkers.

Group	Timepoint	Measured value			Change from baseline		
		n	Mean	SD	Mean	SD	*P* value
CSF neurofilament-light chain (pg/mL)							
Dose regimen 1	Baseline	3	15847.24	2662.94	–	–	–
	Day 5	2	15481.48	419.46	942.14	1560.17	.55
	Day 8	2	14980.19	2151.57	440.85	4131.19	.90
Dose regimen 2	Baseline	7	9661.56	5664.01	–	–	–
	Day 4	6	8725.51	5998.84	−351.87	967.50	.41
	Day 8	7	9017.24	6291.38	−644.32	1558.03	.32
All patients	Baseline	10	11517.26	5647.22	–	–	–
	Day 4	6	8725.51	5998.84	−351.87	967.50	.41
	Day 5	2	15481.48	419.46	942.14	1560.17	.55
	Day 8	9	10342.34	6097.42	−403.17	2045.22	.57
Serum neurofilament-light chain (pg/mL)							
Dose regimen 1	Baseline	3	218.25	139.14	–	–	–
	Day 5	3	196.90	120.57	−21.34	19.58	.20
	End of study	3	124.04	112.96	−94.20	43.70	.06
Dose regimen 2	Baseline	7	224.20	49.00	–	–	–
	Day 4	7	233.40	66.48	9.20	42.07	.58
	End of study	7	245.23	62.91	21.03	40.66	.22
All patients	Baseline	10	222.41	78.89	–	–	–
	Day 4	7	233.40	66.48	9.20	42.07	.58
	Day 5	3	196.90	120.57	−21.34	19.58	.20
	End of study	10	208.87	94.34	−13.54	68.01	.54
Urinary p75 neurotrophin receptor (pg/mL)							
Dose regimen 1	Baseline	3	4877.10	2929.73	–	–	–
	Day 5	3	2411.90	1487.26	−2465.20	4044.49	.40
	End of study	3	4140.23	1786.24	−736.87	4002.03	.78
Dose regimen 2	Baseline	7	4914.14	3461.23	–	–	–
	Day 4	7	2808.16	2849.97	−2105.99	2291.94	.0511
	End of study	7	6917.27	6028.87	2003.13	4388.64	.27
All patients	Baseline	10	4903.03	3145.55	–	–	–
	Day 4	7	2808.16	2849.97	−2105.99	2291.94	.0511
	Day 5	3	2411.90	1487.26	−2465.20	4044.49	.40
	End of study	3	6084.16	5171.08	1181.13	4260.40	.40

ALS = amyotrophic lateral sclerosis, CSF = cerebrospinal fluid, SD = standard deviation.

Content, http://links.lww.com/MD2/A520, which shows the inflammatory biomarkers). While these showed statistically significant changes (increase in CSF IL-6 and increases in serum IL-1β and IL-17), these changes were not of a magnitude to be clinically meaningful.

4. Discussion

This dose-escalation study in patients with ALS demonstrated the safety and tolerability of IC14 in this population. Post-lumbar puncture symptoms were observed as a consequence of the lumbar punctures required for CSF assessments, but there were otherwise no significant AEs or laboratory changes. Anti-monoclonal antibody formation was not observed, which is similar to observations in patients with other disorders treated with IC14.[9] Additional parameters of disease status that were measured for patient monitoring provide context for understanding inflammation in the pathogenesis of ALS. Early phase ALS clinical trials have been encouraged by patient advocates to forego placebo groups in order to allow more optimal use of a limited number of patients for clinical trials, reduce cost, and avoid delaying access to potential therapy for this life-threatening disease.[21] The lack of a placebo comparator group in this study limits the interpretation of safety, clinical outcomes, and disease biomarkers, but still allows hypothesis generation. There is potential observer or patient bias in clinical outcome data such as ALSFRS-R, FVC, SNP, ALSSQOL-R, and ECAS. The ECAS was repeated at an interval of a month; the repetition of the test may have resulted in a learning effect although this effect was not seen in other ALS patients.[22]

There is an emerging understanding of the role of inflammation in the pathogenesis of ALS. There is also an increasing understanding that activation of the peripheral immune system as well as local CNS inflammation is important.[23] The present study shows that there was little CNS penetration of IC14, so its effects occur in the periphery. The preliminary biomarker efficacy of this study suggests that the inhibition of CD14 in the periphery could potentially modulate ALS disease in the CNS.

Monocyte levels and their inflammatory phenotype have also been positively associated with increased rates of disease progression possibly as a result of activation through the Toll-like receptor signaling pathway.[24] Elevated levels of pro-inflammatory cytokines have been demonstrated in the spinal cord, cerebrospinal fluid (CSF), and in the circulation of ALS patients and in culture supernatants of peripheral blood mononuclear cells of patients.[3,25–29] In addition, pro-inflammatory TLR signaling is found in the spinal cord of patients with sporadic ALS.[30] Activated microglia and elevated numbers of

dendritic cells have been described in the spinal cord of patients with both familial and sporadic ALS and have been associated with rapid disease progression.[31,32]

In order to understand how peripheral immunomodulation might affect disease processes in the CNS, it is useful to review the emerging understanding of the role of T cell dysregulation in ALS. Of particular significance is the balance between peripheral regulatory T lymphocytes (Tregs) and inflammatory T effector cells (Teffs) which changes as the disease progresses in patients (1) and mice. Treg numbers boosted in mice resulted in delayed disease progression and prolonged survival.[33] In ALS patients, autologous Tregs were expanded ex vivo and infused back into patients, enhancing both Treg numbers and their suppressive functions.[34] A trend toward temporary slowing of disease progression was observed in treated patients in the pilot study. The balance between Treg/Teff function in the periphery could be influenced by CD14-dependent signaling pathways, since TLR signaling is implicated in the biology of pathogenic T cell populations that are activated in autoimmune and inflammatory diseases.[35]

The connection between monocyte/macrophage CD14 and lymphocytes provides a rationale for the use of IC14 in the treatment of ALS. Activated circulating macrophages play a role in direct damage of the spinal cord in ALS[36] and influence the Treg/Teff balance in patients with ALS. Circulating macrophages from healthy volunteers are also directly responsive to sCD14 in a dose-dependent manner, expressing and secreting proinflammatory cytokines.[37] The high levels of circulating sCD14 associated with rapid disease progression in ALS patients may therefore be both a reflection of disease stage as well as a source of pro-inflammatory damage-associated molecular pattern-activated macrophages, directly damaging parenchymal tissue and skewing T cells toward inflammation.

A balance between anti-inflammatory and pro-inflammatory factors in the periphery could modulate the rates of disease progression and survival in ALS. At each step of this process, CD14 plays an important role in determining this balance via the balance between anti-inflammatory Tregs and pro-inflammatory Teffs and by determining the activation state of DCs and macrophages. Tipping the balance toward anti-inflammatory mediators by targeting CD14 with IC14 therefore shows promise in slowing the progression of this devastating disease.

This study demonstrated the safety of biologically relevant doses of IC14 in patients with ALS. A single cycle of treatment with cumulative doses of 5 mg/kg or 10 mg/kg resulted in rapid and sustained blockage of CD14, a key receptor involved in inflammation signaling. The emerging understanding of the role of systemic inflammation in neurodegenerative diseases, and the potential for IC14 to serve as a safe, potent, and broad-spectrum inhibitor of immune dysregulation merits further clinical study.

Further clinical evaluation of this novel approach to treatment is merited to see if these results can be generalized. An efficacy study of IC14 for the treatment of ALS is planned.

Acknowledgments

The authors are indebted to Fight MND for support of this clinical trial. They would like to acknowledge the contributions of the patients who participated in the study and of The Motor Neurone Disease and Neurotrophic Research Laboratory, Flinders University for Up75 NTR assays.

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