High-dose albumin treatment for acute ischaemic stroke (ALIAS) part 2: a randomised, double-blind, phase 3, placebo-controlled trial

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Summary

Background In animal models of ischaemic stroke, 25% albumin reduced brain infarction and improved neurobehavioural outcome. In a pilot clinical trial, albumin doses as high as 2 g/kg were safely tolerated. We aimed to assess whether albumin given within 5 h of the onset of acute ischaemic stroke increased the proportion of patients with a favourable outcome.

Methods We did a randomised, double-blind, parallel-group, phase 3, placebo-controlled trial between Feb 27, 2009, and Sept 10, 2012, at 69 sites in the USA, 13 sites in Canada, two sites in Finland, and five sites in Israel. Patients aged 18–83 years with ischaemic (ie, non-haemorrhagic) stroke with a baseline National Institutes of Health stroke scale (NIHSS) score of 6 or more who could be treated within 5 h of onset were randomly assigned (1:1), via a central web-based randomisation process with a biased coin minimisation approach, to receive 25% albumin (2 g [8 mL] per kg; maximum dose 750 mL) or the equivalent volume of isotonic saline. All study personnel and participants were masked to the identity of the study drug. The primary endpoint was favourable outcome, defined as either a modified Rankin scale score of 0 or 1, or an NIHSS score of 0 or 1, or both, at 90 days. Analysis was by intention to treat. Thrombolytic therapies were permitted. This trial is registered with ClinicalTrials.gov, number NCT00235495.

Findings 422 participants were randomly assigned to receive albumin and 419 to receive saline. On Sept 12, 2012, the trial was stopped early for futility (n=841). The primary outcome did not differ between patients in the albumin group and those in the saline group (186 [44%] *vs* 185 [44%]; risk ratio 0·96, 95% CI 0·84–1·10, adjusted for baseline NIHSS score and thrombolysis stratum). Mild-to-moderate pulmonary oedema was more common in patients given albumin than in those given saline (54 [13%] of 412 *vs* 5 [1%] of 412 patients); symptomatic intracranial haemorrhage within 24 h was also more common in patients in the albumin group than in the placebo group (17 [4%] of 415 *vs* 7 [2%] of 414 patients). Although the rate of favourable outcome in patients given albumin remained consistent at 44–45% over the course of the trial, the cumulative rate of favourable outcome in patients given saline rose steadily from 31% to 44%.

Interpretation Our findings show no clinical benefit of 25% albumin in patients with ischaemic stroke; however, they should not discourage further efforts to identify effective strategies to protect the ischaemic brain, especially because of preclinical literature showing convincing proof-of-principle for the possibility of this outcome.

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Introduction

Stroke is the fourth leading cause of death in North America, the second leading cause of death globally, and a major cause of disability.[1] By 2030, stroke prevalence in the USA is projected to reach 4%, and stroke-related disability is estimated to become the fourth most important cause of disability-adjusted life years in developed countries.[2] In developing countries, stroke incidence has increased in the past few decades, and rates of disability and mortality in medically underserved regions are at least ten times greater than those in developed countries.[3] Roughly 80% of all strokes are ischaemic.[4] The only specific treatment shown to improve outcome in patients with acute ischaemic stroke is intravenous alteplase (tissue plasminogen activator, tPA), but the therapeutic benefit of this treatment declines sharply in the first few hours after stroke onset.[5]

Preclinical studies[6] have implicated the contribution of various injurious biochemical, molecular, and vascular events in ischaemic brain injury, and have shown that the ischaemic brain could be protected by strategies designed to counteract these injury mechanisms. However, so far, translation of these approaches into treatment methods for patients with ischaemic stroke has been disappointing.[7,8] In experimental studies[9,10] of acute ischaemic stroke, high doses of 25% human albumin have been consistently neuroprotective by reducing the volume of brain infarction, diminishing cerebral oedema, and improving behavioural function, with a therapeutic window of efficacy of at least 4 h after

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*The appendix shows a full list of study committees and investigators

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See Online for appendix

stroke onset.[9,10] Albumin improved perfusion to the ischaemic penumbra,[11] normalised the apparent diffusion coefficient within the residual infarct,[9] reversed stagnation in post-ischaemic cortical venules,[12] and improved microvascular haemodynamics distal to an arterial thrombosis.[13-15] In a pilot safety trial[10] in 82 individuals with acute ischaemic stroke, 25% albumin given in doses encompassing the neuroprotective range shown in experimental studies was tolerated with no major complications, and an exploratory analysis suggested possible efficacy.[16,17]

For **study protocol** see https://dcu.musc.edu/Projects/MajorProjects.asp

In July, 2006, we began the randomised, multicentre Albumin in Acute Stroke (ALIAS) efficacy trial[18] to assess whether 25% albumin treatment given within 5 h of the onset of acute ischaemic stroke would confer neurological and functional benefit. After 434 participants had been enrolled, the Data and Safety Monitoring Board (DSMB) suspended further enrolment for safety reasons. An unmasked safety analysis of that trial (termed ALIAS part 1) showed that more patients died in the first 30 days in the albumin group than in the placebo group, with patients older than 83 years mostly affected. Deaths were also increased in patients in the albumin group who had received excessive intravenous fluids. We modified the study design on the basis of these safety findings and, with frequent supervision from the DSMB, we restarted the trial as ALIAS part 2 to identify whether a weight-adjusted intravenous infusion of 25% albumin within 5 h of stroke onset would increase the proportion of patients with a favourable outcome at 90 days compared with those given isotonic saline. Here we report the results of ALIAS part 2.

Methods

Study design and participants

We undertook this multicentre, randomised, double-blind, placebo-controlled, phase 3 trial between Feb 27, 2009, and Sept 10, 2012, at 69 sites in the USA, 13 sites in Canada, two sites in Finland, and five sites in Israel. Panel 1 lists the inclusion and exclusion criteria. A non-contrast CT or an MRI scan was required to exclude intracranial haemorrhage. Standard-of-care thrombolytic therapy was permitted and consisted of intravenous alteplase, intra-arterial alteplase, or endovascular thrombolysis with approved devices and catheters, on the basis of local clinical judgment. Endovascular procedures, if used, had to begin within 5 h and be completed within 7 h of stroke onset.

Human albumin 25% solution was manufactured for the trial and prepared in glass bottles by Baxter Healthcare (Westlake Village, CA, USA). Isotonic saline in glass bottles was purchased commercially. Study-drug kits were assembled and distributed to the sites by the US Department of Health and Human Services, Supply Service Center (Perry Point, MD, USA). Approval was obtained from an Institutional Review Board or Research Ethics Board at each study site. A DSMB appointed by the National Institute of Neurological Disorders and Stroke (NINDS) oversaw the safety and performance of the trial. Safety events were adjudicated by two independent medical monitors. We acquired an investigational new drug application with the US Food and Drug Administration and a Clinical Trial Application at Health Canada. Written informed consent was obtained from the patient or a legal representative before enrolment. The study protocol is available online.

Randomisation and masking

Patients were randomly assigned in a 1:1 ratio via a centralised web-based randomisation process to either 25% albumin solution (2 g/kg estimated bodyweight) or saline, with a biased coin minimisation approach that accounted for the status of treatment group balance within and across sites.[19] The time of randomisation was defined as the time at which the study-drug kit was unsealed. In patients who received intravenous alteplase, infusion of study drug needed to begin within 90 min after the start of alteplase. All study personnel and patients were masked to the identity of the study drug. Each sealed kit contained two bottles (initially of 500 mL and 250 mL, then, for manufacturing reasons, both of 500 mL) of the same substance (either albumin or saline) encased in cardboard blinding boxes similar to those used in the Saline versus Albumin Fluid Evaluation (SAFE) Trial,[20] in addition to filters and opaque sheathing to conceal the intravenous tubing.

Procedures

The primary endpoint was favourable outcome at 90 days after randomisation, defined as either a modified Rankin scale (mRS) score of 0 or 1 or a National Institutes of Health stroke scale (NIHSS) score of 0 or 1, or both. The mRS ranges from 0 to 6, with higher scores denoting greater disability; scores of 0 or 1 denote absence of any functional disability. The NIHSS score ranges from 0 to 42; a score of 0 suggests a normal neurological examination and a score of 1 suggests negligible abnormality. A bedside nurse or other personnel not associated with the trial administered the study drug (8 mL/kg estimated bodyweight) by constant intravenous infusion over 2 h (plus or minus 15 min). Participants weighing 94 kg or more were given 750 mL. Vital signs were monitored frequently. Serum chemistry was collected at 24 h and 48 h. Intravenous fluid intake was recorded at 24 h and 48 h. A follow-up brain CT or MRI scan was obtained at 24 h. An electrocardiogram (ECG) was repeated at 24–48 h. We assessed neurological and cardiac status, including NIHSS score, at 24 h and 48 h, and at 7 days or discharge, whichever came first.

Intravenous fluid management and diuretic administration were mandated. Patients were not to receive total intravenous fluids in excess of 4200 mL during the first 48 h, including the volumes of study drug and thrombolytic drug (if used). For patients exceeding

***Panel 1*: Inclusion and exclusion criteria**

Inclusion criteria

- Acute ischaemic stroke.
- Age 18–83 years (participants must not have had their 84th birthday).
- National Institutes of Health stroke scale score of 6 or greater as assessed immediately before thrombolysis treatment if the patient was eligible for thrombolysis, or before randomisation for patients not eligible.
- Start of albumin or placebo within 5 h of stroke onset and within 90 min of the start of thrombolysis with intravenous alteplase if that treatment was used.
- Signed and dated informed consent obtained.

Exclusion criteria

- An episode or exacerbation of congestive heart failure from any cause in the past 6 months. An episode of congestive heart failure was any heart failure that required a change in drug or diet, or hospital admission.
- Known valvular heart disease with congestive heart failure in the past 6 months.
- Known (or in the investigator's clinical judgment) existence of severe aortic stenosis or mitral stenosis.
- Cardiac surgery with thoracotomy (eg, coronary artery bypass graft or valve replacement surgery) in the past 6 months.
- Acute myocardial infarction in the past 6 months.
- Signs or symptoms of acute myocardial infarction, including electrocardiogram findings, on admission.
- Elevated serum troponin concentration on admission (>0·1 μg/L).
- Suspicion of aortic dissection on admission.
- Acute arrhythmia (including any tachycardia or bradycardia) with haemodynamic instability on admission (systolic blood pressure <100 mm Hg).
- Findings on physical examination of any of the following abnormalities: jugular venous distention (jugular venous pressure >4 cm above the sternal angle); third heart sound; resting tachycardia (heart rate >100 beats per min) attributable to congestive heart failure; lower-extremity pitting oedema attributable to congestive heart failure; bilateral rales; definite evidence of pulmonary oedema, bilateral pleural effusion, or pulmonary vascular redistribution on chest x-ray, if done.
- Acute or chronic lung disease needing supplemental chronic or intermittent oxygen therapy.
- Historical modified Rankin scale score greater than 2. Patients who lived in a nursing home or who were not fully independent for activities of daily living (eg, toileting, dressing, eating, cooking and preparing meals) immediately before the stroke were not eligible.
- In-patient stroke.
- Profound dehydration.
- Fever, defined as a core body temperature of more than 38·0°C.
- Serum creatinine concentrations of greater than 180 μmol/L.
- Severe chronic anaemia (haemoglobin <75 g/L).
- Evidence of intracranial haemorrhage (ie, intracerebral haematoma, intraventricular haemorrhage, subarachnoid haemorrhage, epidural haemorrhage, acute or chronic subdural haematoma) on the baseline CT or MRI scan.
- History of or known allergy to albumin.
- History of or known allergy to natural rubber latex.
- Pregnancy, breastfeeding, or positive pregnancy test. Women of childbearing age should have had a negative pregnancy test before study drug was given.
- Concurrent participation in any other therapeutic clinical trial.
- Evidence of any other major life-threatening or serious medical disorder that would prevent completion of the study protocol, impair the assessment of outcome, or in which albumin treatment would be contraindicated or might cause harm to the patient.

this amount, investigators had to provide justifications, which were centrally adjudicated on the basis of clinical reasonableness. Patients were also required to receive at least one dose of furosemide, 20 mg intravenously (or an equivalent loop diuretic), between 12 h and 24 h after administration of study drug. Physicians withholding diuretics were asked to provide a written justification. Antiplatelet therapy was recommended in all patients within 48 h of their stroke. Blood pressure was managed according to the local standard of care.

Patients were followed up for 1 year. 90 days (plus or minus 30 days) after randomisation, participants were assessed in person on the NIHSS, mRS, Barthel index, stroke-specific quality-of-life scale,[21] and trailmaking A and B tests,[22] administered by a certified and masked site investigator. Participants were also followed up by telephone contact at 1 month (plus or minus 7 days), 6 months (plus or minus 14 days), 9 months (plus or minus 14 days), and 1 year (plus or minus 14 days) after randomisation, to assess the mRS, record serious adverse events, and complete the EuroQol[23,24] (at 3 months and 1 year) and the questionnaire to validate a stroke-free status[25] (at 3, 6, 9, and 12 months).

Statistical analysis

The study cohort consisted of patients not receiving thrombolysis and those who did receive thrombolysis (defined as any intravenous or endovascular thrombolytic or thrombectomy procedures). On the basis of simulation, the total required sample size was 1100, to yield 84% power to detect a 20% interaction effect between treatment and thrombolysis status—ie, a 20% absolute difference in the treatment effect between thrombolysis and non-thrombolysis strata with a two-sided alpha of 0·10 and assuming a 4:1 ratio of thrombolysis to non-thrombolysis patients. Concurrently,

we calculated that 980 individuals would be needed to test the primary hypothesis—ie, to detect a minimum effect size of 10 absolute percentage points in the primary outcome. We assumed that the control group's favourable primary outcome would be 40%, on the basis of the NINDS rt-PA Stroke Study[26] and the ALIAS part 1 trial.[27] We estimated the sample size on the basis of 85% power, a one-sided type 1 error probability of 0·025, and allowing

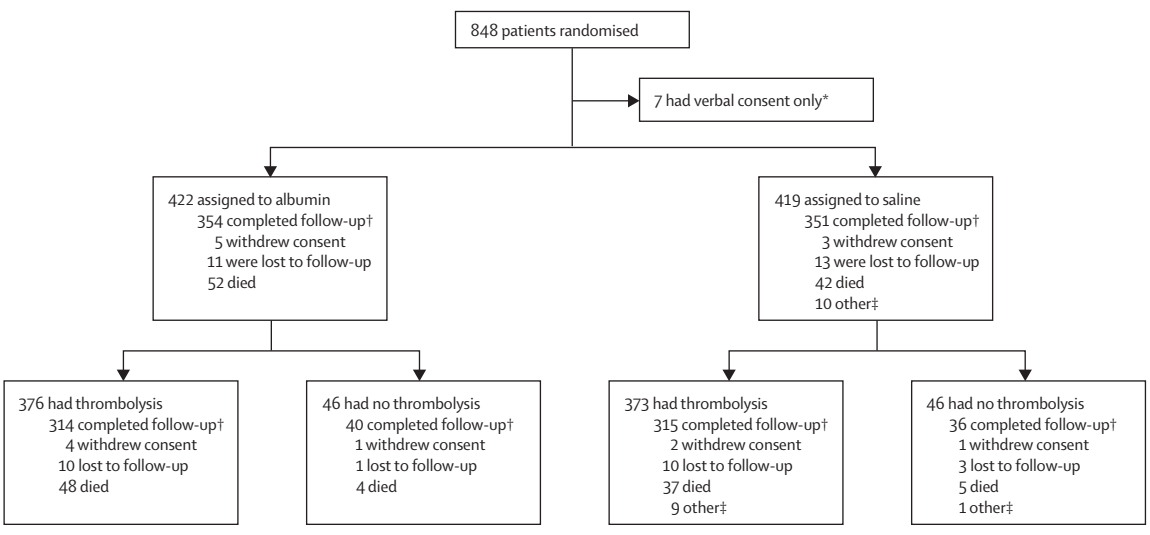

***Figure 1*: Trial profile**
*Deleted from database because written consent was required by the US Food and Drug Administration. †Participants who completed the 3-month follow-up; not all completed the 12-month follow-up because of early termination of the trial. ‡Participants whose day 90 visit was outside the plus or minus 30 day window, or whose day 90 visit or assessment was missing.

	Albumin (n=422)	Saline (n=419)
Age	63·4 (13·0)	64·8 (12·9)
Men	220 (52%)	235 (56%)
Race*		
White	314 (74%)	305 (73%)
Black	69 (16%)	86 (21%)
Asian	25 (6%)	20 (5%)
American Indian, Alaska Native, or First Nations People	2 (<1%)	0
Native Hawaiian or Pacific Islander	2 (<1%)	1 (<1%)
Multiple, other, or unknown	10 (2%)	7 (2%)
Ethnic group*		
Non-Hispanic or Latino	379 (90%)	372 (89%)
Hispanic or Latino	24 (6%)	26 (6%)
Unknown	19 (4%)	21 (5%)
Medical history		
Hypertension	304 (72%)	295 (70%)
Atrial fibrillation	79 (19%)	78 (19%)
Previous congestive heart failure	18 (4%)	27 (6%)
Previous myocardial infarction	45 (11%)	51 (12%)
Previous stroke	89 (21%)	79 (19%)
Previous transient ischaemic attack	52 (12%)	50 (12%)
Diabetes mellitus	78 (18%)	94 (22%)
Hyperlipidaemia	196 (46%)	213 (51%)
Peripheral vascular disease	21 (5%)	25 (6%)
Baseline NIHSS score†	11 (8–17)	11 (8–17)
Baseline ASPECTS score >7‡	325/418 (78%)	327/415 (79%)

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	Albumin (n=422)	Saline (n=419)
(Continued from previous page)		
Clinical findings		
Systolic blood pressure (mm Hg)	155·9 (28·0)	156·6 (29·9)
Plasma glucose (mmol/L)	7·1 (2·5)	7·7 (3·8)
Haemoglobin (g/L)	13·9 (1·7)	14·0 (1·8)
Creatinine (μmol/L)	86·3 (23·3)	90·5 (26·9)
Oxfordshire Community Stroke Project classification		
Total anterior circulation syndrome	102 (24%)	98 (23%)
Partial anterior circulation syndrome	232 (55%)	235 (56%)
Posterior circulation syndrome	35 (8%)	39 (9%)
Lacunar stroke	53 (13%)	47 (11%)
Stroke onset to start of study-drug infusion (min)	200 (167–249)	198 (167–243)
Characteristics of patients receiving intravenous alteplase		
n (%)	351 (83%)	361 (86%)
Stroke onset to start of intravenous alteplase (min)	126 (98–170)	130 (100–166)
Start of intravenous alteplase to start of study-drug infusion (min)	60 (49–80)	60 (48–80)
Distribution of thrombolytic procedures by type		
Intravenous alteplase only	282 (67%)	293 (70%)
Intravenous alteplase and any endovascular procedure	69 (16%)	68 (16%)
Any endovascular procedure only	25 (6%)	12 (3%)
No thrombolysis	46 (11%)	46 (11%)
Intravenous fluids		
Given within 24 h of randomisation (mL)	2101 (958, 173–7503)	2077 (986, 408–7040)
Given 24–48 h from randomisation (mL)	1178 (1000, 0–7503)	1178 (926, 0–5353)
Total fluids given within 48 h of randomisation (mL)	3279 (1671, 173–15006)	3255 (1624, 432–11235)

Data are mean (SD), n (%), n/N (%), median (IQR), or mean (SD, min–max), unless otherwise indicated. NIHSS=National Institutes of Health stroke scale. ASPECTS=Alberta stroke program early computed tomography score. *Self-reported. †A 42-point scale that quantifies neurological deficits in 11 categories, with 0 as normal function without deficits, and higher scores showing greater severities of deficit. ‡Score uses computed tomography to assess ten regions of the brain; a score of 1 shows a normal region and 0 shows a region with signs of ischaemia; total scores range from 10 (no evidence of early ischaemia) to 0 (all ten regions of the affected hemisphere show early ischaemic changes).

Table 1: **Baseline and treatment characteristics**

for a group-sequential design for three interim analyses.[28] For overwhelming efficacy, we adopted the alpha spending function approach,[29] and for futility, the beta spending function approach.[30] For both overwhelming efficacy and futility, we used O'Brien–Fleming-type stopping guidelines,[28] and the binding boundaries and corrected pooled variance estimates.[31] We did analyses by intention to treat and derived the final maximum sample size of 1092 to account for a proportion of about 5% of patients lost to follow-up with the approach of Friedman and colleagues.[32] Hence, we concluded that the 1100 individuals required to detect the interaction effect should provide sufficient power for the primary analysis of the main treatment effect.

We did analyses with SAS (version 9.3). We used the generalised linear model with log link, with adjustment for baseline NIHSS score, thrombolysis status, and treatment-by-thrombolysis status interaction term. As was prespecified in the trial's statistical analysis plan, we assessed the following baseline variables for possible confounders: age, race (black *vs* other), plasma glucose, systolic blood pressure, Alberta stroke program early computed tomography score (ASPECTS; 0–10), history of myocardial infarction, and time from symptom onset to randomisation. If the p values for these variables in the univariate analyses were less than 0·20, we included them in the secondary, covariate-adjusted analysis. We did a multivariate analysis of the treatment effect that included the following measures as covariates: baseline NIHSS, thrombolysis status, site patient volume (low, medium, or high), age, black race, plasma glucose, ASPECTS, and time from symptom onset to randomisation. The treatment effect was not changed as a result of this analysis.

This trial is registered with ClinicalTrials.gov, number NCT00235495.

Role of the funding source

The trial was funded by cooperative agreements from the US National Institutes of Health (National Institute of Neurological Disorders and Stroke). A representative of NINDS (CSM) participated in the study leadership committee and contributed to design decisions and to the interpretation and writing of the manuscript. Baxter Healthcare provided funds to extend the trial to Finland, but had no role in the design, undertaking, or interpretation

of the study. The sponsor of the study had no role in data collection or data analysis. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. 841 individuals were randomly assigned to receive albumin (n=422) or saline (n=419). Half (47%) the participants were from hub sites or spoke sites of the NINDS-funded Neurological Emergencies Treatment Trials (NETT) network. At 3 months, 705 (83%) participants were alive and completed the 3-month follow-up. Table 1 shows baseline and treatment characteristics. Overall, the mean age of the subjects was 64·1 years (SD 12·9), and the median baseline NIHSS score was 11 (range 6–40). For all participants, mean time from stroke onset to start of study drug was 206 min (SD 52). 749 patients (89%) received some form of thrombolytic treatment (table 1). Intravenous alteplase alone was given to 575 (68%) patients (table 1). 137 (16%) patients received both intravenous alteplase and endovascular thrombolytic therapy (table 1). 37 (4%) patients received only endovascular therapy (table 1). We did two pre-specified interim analyses for overwhelming efficacy or futility at enrolment of 275 participants and 550 participants. On Sept 10, 2012, after the DSMB requested and reviewed a third, unscheduled interim analysis (at enrolment of 732 participants), the Board recommended to the NINDS that enrolment be halted for futility and that all subjects then in the trial (n=841) be followed up only to 90 days.

For the **NETT network** see http://www.nett.umich.edu/nett/alias

	Albumin (n=422)	Saline (n=419)	RR (99% CI) or p value
Primary outcome			
NIHSS or mRS 0–1 (or both) at 90 (±30) days	186 (44%)	185 (44%)	0·96 (0·84–1·10*)
Composite outcome at 90 (±30) days			
mRS 0–1, NIHSS 0–1, or decrease in NIHSS from baseline by 10 or more points	227 (54%)	241 (58%)	0·93 (0·80–1·09)
NIHSS 0–1 at 24 h	65 (15%)	62 (15%)	0·99 (0·66–1·49)
NIHSS 0–1 at 90 days	150 (36%)	164 (39%)	0·88 (0·71–1·10)
mRS 0–1 at 90 days	155 (37%)	145 (35%)	1·03 (0·82–1·28)
mRS 0–2 at 90 days	227 (54%)	221 (53%)	0·99 (0·85–1·13)
mRS at 90 days by sliding-dichotomy analysis[33,34]	142 (34)	141 (34%)	0·98 (0·87–1·11)
Barthel index 95–100 at 90 days	227 (54%)	231 (55%)	0·95 (0·83–1·10)
EuroQol EQ-5D[24] favourable score at 90 days†	155 (37%)	152 (36%)	0·99 (0·84–1·17)
SSQOL[23] favourable score at 90 days‡	258 (61%)	268 (64%)	0·93 (0·84–1·02)
Global scale at 90 days§	..	..	0·93 (0·82–1·05)
Trailmaking A and B at 90 days			
Trails A	55 (39–105)	56 (39–90)	0·913¶
Trials B	111 (78–173)	110 (82–180)	0·923¶

Data are n (%), RR (99% CI), or median (IQR), unless otherwise indicated. Thrombolysis stratum defined as any intravenous or endovascular treatment. NIHSS=National Institutes of Health stroke scale. mRS=modified Rankin scale. SSQOL=stroke-specific quality of life. *95% CI. †Less than 0·78 *vs* otherwise. ‡3 or more *vs* otherwise. §NIHSS, mRS, Barthel index, EuroQol, SSQOL. ¶Two-sided probability, Wilcoxon rank-sum test with normal approximation.

***Table 2*: Efficacy outcomes adjusted for baseline NIHSS and thrombolysis stratum**

The proportion of individuals achieving a favourable primary outcome at 90 days (plus or minus 30 days) did not differ significantly between the albumin and saline groups (table 2). Additionally, no significant difference was shown in the primary outcome by treatment assignment in any of the predefined subgroups (table 3). Other predefined outcomes assessed at 90 days—a composite outcome (mRS 0–1, NIHSS 0–1, or decrease in NIHSS from baseline by 10 or more points), mRS assessed by sliding dichotomy, Barthel index, EuroQol, stroke-specific quality of life, and the trailmaking tests—likewise showed no significant differences by treatment assignment (table 2). Figure 2 shows the distribution of 90-day mRS scores in patients given albumin or saline.

We noted pulmonary oedema or congestive heart failure within 48 h in 13% of participants in the albumin group (table 4)—a rate that is consistent with that of patients given albumin in the ALIAS pilot trial[16] and the part 1 trial.[18] By contrast, this safety outcome arose in only 1% of patients in the saline group (table 4). This adverse event was typically of mild-to-moderate severity and was readily managed. Shortness of breath within 48 h, and atrial fibrillation within 48 h, were also more common in patients given albumin than in those given saline (table 4). Although the rate of symptomatic or large intracranial haemorrhage within 24 h in both groups was as low or lower than rates reported in other major intravenous thrombolytic trials,[26,36–38] patients given albumin nonetheless had a risk that was 2·4 times greater than patients given saline (table 4). Correspondingly, 24 h CT scans read centrally showed a 2·8-times greater risk of larger (ie, type 2[33]) parenchymal haemorrhages in the albumin group than in the saline group (table 4). Other pre-specified major safety outcomes showed no significant differences by treatment (table 4).

The rate of favourable primary outcome with albumin remained steady at 44–45% throughout the trial. However, our assumption that a favourable rate of 40% would be shown in the placebo group proved initially to be incorrect (figure 3); rather, the rate in patients given saline averaged 32% in the first quarter of the trial, but rose steadily thereafter, so that an interaction of treatment-by-randomisation order was evident. At the first prespecified interim analysis (n=275) presented to the DSMB, there was an absolute difference of 14·5% in the rates of favourable outcome between the groups, with a relative benefit of 1·48 favouring treatment with albumin (95% CI 1·12–1·97 adjusted for baseline NIHSS and thrombolysis status; one-sided p=0·0028). This finding suggests that the likelihood of favourable outcome was roughly 50% higher for patients in the albumin group. At the second pre-specified interim analysis (n=550), the rate of favourable outcome had increased in the placebo group, whereas the rate in the albumin group remained steady (figure 3), for a relative benefit of 1·21 favouring albumin (adjusted 95% CI

	RR (99% CI)
Thrombolysis treatment*	
Thrombolysis	0·95 (0·79–1·15)
No thrombolysis	1·09 (0·53–2·22)
Sex	
Male	1·01 (0·80–1·28)
Female	0·89 (0·67–1·19)
Race	
White	0·96 (0·79–1·18)
Black	1·09 (0·69–1·73)
Ethnic origin	
Hispanic or Latino	0·64 (0·26–1·60)
Not Hispanic or Latino	1·00 (0·83–1·20)
Stroke type	
TACS	0·98 (0·52–1·85)
Others	0·96 (0·80–1·17)
Baseline ASPECTS	
>7	0·95 (0·79–1·16)
≤7	1·04 (0·60–1·81)
Baseline NIHSS†	
≥18	1·05 (0·53–2·08)
<18	0·95 (0·79–1·15)
Baseline NIHSS quartile	
0–7	0·99 (0·72–1·35)
8–10	0·93 (0·69–1·25)
11–16	0·88 (0·59–1·32)
≥17	1·08 (0·56–2·06)
Stroke onset to randomisation (h)	
≤2	0·98 (0·49–1·95)
>2	0·96 (0·80–1·16)
Stroke onset to treatment (h)	
≤3 h	0·93 (0·71–1·22)
>3 h	0·99 (0·78–1·27)
Site volume	
1–2 participants (21 sites)	1·10 (0·37–3·30)
3–4 participants (20 sites)	1·18 (0·62–2·23)
5–11 participants (23 sites)	1·05 (0·67–1·65)
≥12 participants (25 sites)	0·92 (0·75–1·14)
Baseline glucose (mmol/L)	
<8	0·96 (0·79–1·17)
≥8	0·93 (0·61–1·42)
History of diabetes	
No	0·95 (0·78–1·16)
Yes	0·97 (0·61–1·54)
History of atrial fibrillation	
No	0·98 (0·80–1·20)
Yes	0·87 (0·56–1·35)
History of hypertension	
No	0·91 (0·66–1·24)
Yes	0·99 (0·79–1·23)

TACS=total anterior circulation syndrome. ASPECTS=Alberta stroke program early computed tomography score. NIHSS=National Institutes of Health stroke scale. *Adjusted for baseline NIHSS only. †Adjusted for thrombolysis stratum only.

***Table 3*: Subgroup efficacy analysis adjusted for baseline NIHSS and thrombolysis stratum**

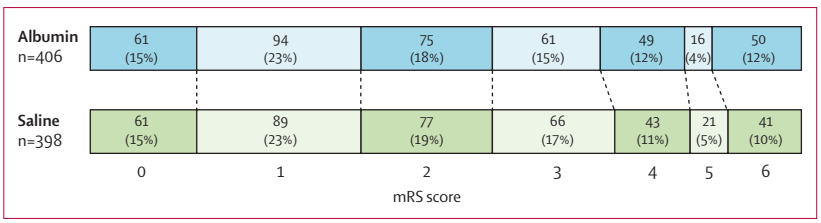

***Figure 2*: Number and proportions of patients in each mRS group**
Distribution of mRS scores (0–6) at 90 days after randomisation in patients given albumin or saline. mRS=modified Rankin scale.

	Albumin	Saline	Relative risk (95% CI)
	n/N (%)	n/N (%)	
Neurological deterioration within 48 h	47/411 (11%)	40/412 (10%)	1·18 (0·79–1·76)
Neurological death within 7 days	13/404 (3%)	13/407 (3%)	1·01 (0·47–2·15)
Recurrent stroke within 30 days	7/373 (2%)	6/370 (2%)	1·16 (0·39–3·41)
Recurrent stroke within 90 days	10/337 (3%)	12/331 (4%)	0·82 (0·36–1·87)
Atrial fibrillation within 48 h	32/410 (8%)	19/412 (5%)	1·69 (0·98–2·94)
Pulmonary oedema or congestive heart failure within 48 h	54/412 (13%)	5/412 (1%)	10·8 (4·37–26·72)
Shortness of breath within 48 h	18/410 (4%)	7/412 (2%)	2·58 (1·09–6·12)
Symptomatic ICH within 24 hours	17/415 (4%)	7/414 (2%)	2·42 (1·02–5·78)
Asymptomatic ICH within 24 h	27/415 (7%)	23/414 (6%)	1·17 (0·68–2·01)
Parenchymal haemorrhage type 1 on 24 h CT scan*†	8/407 (2%)	5/405 (1%)	1·59 (0·53–4·83)
Parenchymal haemorrhage type 2 on 24 h CT scan*‡	14/407 (3%)	5/405 (1%)	2·79 (1·01–7·66)
Death within 30 days	39/409 (10%)	37/406 (9%)	1·05 (0·68–1·61)
Death within 90 days	46/378 (12%)	41/369 (11%)	1·10 (0·74–1·63)

Data are for n at risk/N of events. The safety sample consisted of the 830 patients who received at least 20% of the intended dose of study drug. ICH=intracranial haemorrhage. *Central reader. †Type 1 haemorrhage describes a blood clot not exceeding 30% of the infarcted area with some mild space-occupying effect. ‡Type 2 haemorrhage describes a dense blood clot or clots exceeding 30% of the infarct volume with substantial space-occupying effect.[33]

***Table 4*: Prespecified safety events in the safety sample**

1·01–1·44; one-sided p=0·0176). However, at neither of these pre-specified analyses did the differences cross the interim-analysis boundary for overwhelming efficacy.[28] By the time the trial was stopped, we noted no difference by treatment assignment (figure 3). Table 5 shows the stopping boundaries and the statistics recorded in these interim analyses. A detailed analysis of outcome trends over time will be reported in a subsequent report.

Discussion

Despite convincing experimental evidence supporting ischaemic neuroprotection with high-dose albumin,[9-15] and despite an exploratory efficacy analysis of a target population of individuals from ALIAS part 1 who would have satisfied revised eligibility criteria of the part 2 trial that showed a trend toward a favourable outcome with albumin treatment,[27] neither the primary nor secondary outcomes of the present definitive trial differed by treatment assignment (panel 2).

A limitation of this trial was that we were unable to obtain complete 12-month assessments because of the

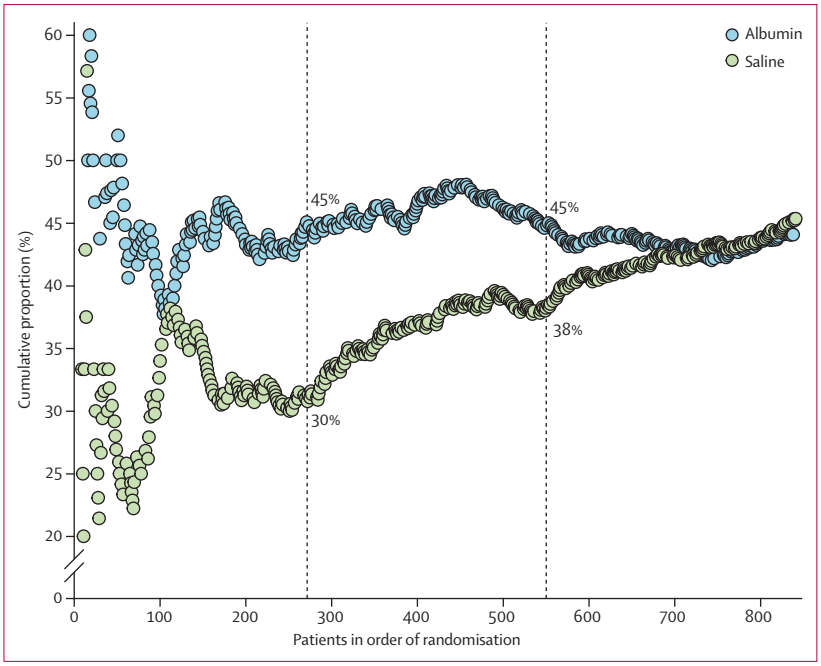

Figure 3: **Rates of cumulative primary outcome rates in patients given albumin or saline**
The two vertical lines show the points at which two prespecified interim analyses were done, at enrolment of 275 patients and 550 patients.

	N	Nominal critical point to reject H_0 for overwhelming efficacy	Nominal critical point to reject H_1 for futility	Actual test statistic
Analysis 1	275	4·3326	−1·3491	2·76
Analysis 2	550	2·9631	0·3293	2·11
Analysis 3	732	2·5410	0·9539	0·14

On the basis of one-sided α=0·025.[28-31] H_0=null hypothesis (no difference by treatment). H_1=alternate hypothesis (difference by treatment).

Table 5: **Stopping boundaries and observed statistics for overwhelming efficacy and futility**

sponsor's decision not to provide funding for follow-up beyond the 3-month primary outcome assessment. Biologically, we restricted our study design to the estimated time window of treatment effect on the basis of preclinical findings,[10] but we recognise that a 5 h window to treatment might still be too long. Furthermore, we did not control for reperfusion, either in application or timing, and because the only proven neuroprotectant strategy in human beings is the induction of prompt reperfusion, this might have affected our results.

The neutral outcomes of this and other major multicentre trials[41–46] of neuroprotective strategies for acute ischaemic stroke raise the concern as to whether positive results in animal models of stroke can legitimately be translated to human beings. This question has elicited extensive discussion in the scientific literature.[7,8,47–50] Preclinical studies supporting albumin in neuroprotection were done in rodents but not in primates; focal cerebral ischaemia was typically induced mechanically (by an intraluminal filament to occlude the middle cerebral artery) rather than by a thrombus or embolus; and reperfusion was induced by withdrawal of

Panel 2: Research in context

Systematic review

We searched PubMed for articles published before July 12, 2013, of the therapeutic use of albumin in stroke. We used the search string, "albumin [ti] AND brain AND (stroke OR ischemia OR hemorrhage)". The resulting reports were manually reviewed. Before our own clinical trials, custom-tailored haemodilution with albumin plus crystalloids was studied in a prospective single-centre study, and the investigators reported reduced 3-month mortality and increased functional independence.[39] Another study[40] was a retrospective review of the safety of high-dose albumin treatment in 30 participants treated within 24 h of stroke onset, and 60 controls. Albumin treatment was associated with a non-significant trend towards cardiopulmonary adverse events. The only other studies identified were our own trials—ALIAS pilot trial[16,17] and ALIAS part 1[18,27]—that formed the antecedents to the multicentre trial reported here. As of July 12, 2013, ClinicalTrials.gov listed three trials of albumin in cerebrovascular disorders: (1) a phase 2B study (NCT01684462) investigating the safety and efficacy of 20% albumin 1·25 g/kg, given within 12 h of stroke onset, presently recruiting participants in Korea; (2) an inactive phase 2 trial of albumin for intracerebral haemorrhage, with use of MRI-imaging outcome markers (NCT00990509); and (3) a phase 1 dose-escalation trial, now terminated, of albumin for subarachnoid haemorrhage (NCT01747408).

Interpretation

This trial represents the translation of the concept of high-dose albumin neuroprotection from therapeutically successful preclinical studies in rodent models of focal cerebral ischaemia, through a pilot clinical trial and an antecedent multicentre clinical trial, in which both the albumin dose and the window of treatment were selected to be similar to those shown to be therapeutically efficacious in the animal studies. The main safety event has been the consistent induction of mild-to-moderate pulmonary oedema, which can be successfully managed without serious consequences by fluid restriction and diuretic therapy. The neutral primary and secondary efficacy outcomes in the present trial were unexpected in view of suggestive signals of possible efficacy emerging from the pilot trial[17] and from a target population of the antecedent part 1 trial.[27] Furthermore, interpretation of the present results is complicated by the fact that the outcome of patients given placebo improved steadily over time, such that signals of albumin's therapeutic efficacy were present during the first half of this trial, but had disappeared by its end.

the filament rather than by thrombolysis. The animals used were young and free of medical comorbidities, contrasting with human ischaemic stroke, which typically affects middle-aged and elderly patients who often have other medical disorders. Any or all of these differences might be relevant here.

The interpretation of this trial is challenged by the fact that participants in the placebo group had a steadily improving rate of favourable outcome, which did not occur in those in the albumin group. Thus, although the first prespecified interim analysis showed an apparent relative benefit favouring albumin treatment, the effect had completely disappeared by the trial's end. Early fluctuation in cumulative effect size over time has been reported for large cardiac trials, with a conclusion that continuation to the prespecified fixed sample size is essential.[51]

We do not as yet have a satisfactory explanation for the steady improvement in rate of favourable outcome noted in the placebo, but not the albumin, group. We cannot exclude the possibility that ongoing improvements in general stroke care over the 3·5-year enrolment period might have affected the placebo group preferentially. With this hypothetical scenario, the stable rate of favourable outcome reported for the albumin group would be viewed as a so-called ceiling effect—ie, not sensitive to further improvements in stroke care—whereas the placebo group, whose favourable outcome was initially quite low, was susceptible to increases over time afforded by steady improvements in standards of care and possibly by the increasing rate of use of intravenous alteplase observed over the course of the trial. In support of the notion of an albumin-related ceiling effect, findings from an experimental study showed that albumin treatment had a significant therapeutic effect after ischaemic stroke by augmenting the brain's collateral perfusion in a strain of mice with sparse brain collaterals, whereas the effect was not shown for a mouse strain having more abundant brain collateral circulation.[13]

As in our previous trials, mild-to-moderate pulmonary oedema was the main drug-related adverse event, but was usually readily managed, and serious adverse events related to congestive heart failure were scarce. The findings of significantly greater symptomatic intracranial haemorrhage and of type 2 parenchymal haemorrhages by CT scan with albumin treatment were unanticipated and might relate to albumin's reported platelet anti-aggregatory effect.[52–54] However, this adverse event did not seem to affect the rates of neurological deterioration or death, which were similar by treatment assignment.

Although our trial did not show that high doses of albumin improve the neurological or functional outcome of patients with acute ischaemic stroke, these findings, or those of any well designed clinical trial, should not discourage future efforts to identify effective strategies to protect the ischaemic brain, especially because an abundant preclinical literature continues to provide cogent proof-of-principle that this outcome might be possible.

Contributors

MDG was the principal investigator and wrote the manuscript. MDG, YYP, MDH, and CSM designed the study. MDG, MDH, WGB, DT, and KJR supervised clinical aspects of the study. RHM and YYP did the biostatistical analyses. MDG, YYP, RHM, and MDH analysed and interpreted data. BDW directed project management and supervised site monitoring. MDH undertook central readings of CT scans and electrocardiograms. DT and KJR collected clinical data and guided the study coordinators. All authors contributed substantively to the final report.

Conflicts of interest

MDG has received consultancy fees from Aldagen. YYP was a paid member of the Data and Safety Monitoring Board for a BrainsGate trial. MDH has received funding from the Heart and Stroke Foundation of Alberta. All other authors declare that they have no conflicts of interest.

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