Background and Purpose—The ALIAS (Albumin in Acute Ischemic Stroke) part 1 and 2 trials evaluated whether 25% human serum albumin improves clinical outcomes after acute ischemic stroke above and beyond standard of care using similar protocols. The part 1 trial ended prematurely because of safety concerns, and the part 2 trial terminated early because of futility of finding a statistically significant effect of albumin over saline (control) administration. We combine the subject-level data of the part 1 and 2 trials to reevaluate the efficacy and safety outcomes with the larger sample size.

Methods—The combined data analyses closely follow those conducted in the part 2 trial. The primary outcome is the composite of the modified Rankin Scale and the National Institutes of Health Stroke Scale defined as a composite of the modified Rankin Scale score 0 to 1 and National Institutes of Health Stroke Scale score 0 to 1 at 90 days from randomization. The unadjusted analyses use a simple Chi-square test, and those adjusting for baseline covariates use a generalized linear model with log link (to obtain relative risks).

Results—The participant characteristics at baseline were generally similar between the treatment groups and between the trials; however, thrombolysis use was greater in part 2 (84% versus 75%), and the upper age limit imposed in part 2 resulted in a younger sample (mean age in part 1 was 69 versus 64 in part 2). In the combined sample, the proportions of good outcome in the 2 treatment groups were identical (41%). Similar results were observed in all secondary efficacy outcomes. Pulmonary edema was a consistent safety concern, with a 6-fold increase in the albumin arm (13%) compared with saline (2%; relative risk =7.76, 95% confidence interval 3.87–15.57).

Conclusions—Treatment with intravenous albumin 25% at 2 g/kg was not associated with improved outcome at 90 days and was associated with increased rates of intracerebral hemorrhage and pulmonary edema.
consistent across all predefined secondary outcomes, as well as the analysis of those treated with thrombolysis (adjusted relative risk 0.95, 99% confidence interval 0.79–1.15) and those not treated with thrombolysis (adjusted relative risk 1.09, 99% confidence interval 0.53–2.22).

The analysis of outcomes using subject-level data from the part 1 and part 2 trials reported herein was a prespecified secondary analysis of the part 2 trial.

Methods

Study Design

Both part 1 and part 2 were designed as saline-controlled, blinded multicenter trials to ascertain the effectiveness of albumin in acute ischemic stroke. The eligibility criteria were similar in the 2 trials and detailed in the respective publications. Eligible patients were randomly assigned in a 1:1 ratio to either albumin or saline via an algorithm of minimization and biased-coin accounting for clinical center in a step-forward randomization process. The primary efficacy outcome of functional independence was defined as a composite of modified Rankin Scale score 0 to 1 and National Institutes of Health Stroke Scale score 0 to 1, both assessed at 90 days from randomization.

Because of the clinical potential for interaction between albumin and thrombolysis, the ALIAS part 1 trial was designed to test the efficacy of albumin independently in each of the 2 cohorts (those who received thrombolysis and those who did not) and was effectively 2 parallel trials in a single design. For the part 1 trial, the maximum sample size of 900 subjects in each of the 2 cohorts was calculated to detect an absolute treatment difference of 10% in the proportion of subjects with favorable outcome at 90 days, assuming a proportion of 40% in the saline arm, 2-sided type I error probability of 0.05, 80% power, 1:1 randomization, and an inflation factor of 1.11 to account for ≈5% potential lost to follow-up. These calculations took into account 3 planned interim efficacy analyses conducted according to the O'Brien and Fleming-type alpha spending function for efficacy and beta spending function for futility, intended to be conducted when 225, 450, and 675 subjects had completed the primary 90-day follow-up period. The planned primary efficacy analysis was a Z test for binomial proportions with normal approximation. Because each cohort would be treated as a separate independent study group, the 2-sided alpha level of 0.05 was specified for each primary analysis. An unfavorable outcome was imputed for all subjects with the primary outcome missing or obtained outside of the specified time window.

For the ALIAS part 2 trial, several key statistical assumptions differed from part 1. ALIAS part 2 was a single trial and considered thrombolysis treatment status (defined by administration of IV tissue-type plasminogen activator, endovascular procedure, or both) as a stratifying variable for randomization. An interaction term between thrombolysis treatment status and study treatment was a prespecified covariate in the analysis model. The primary statistical model for part 2 also included adjustments for stroke severity via the National Institutes of Health Stroke Scale. The type I and type II error probabilities were revised to 0.025 (1-sided) and 0.10, respectively. This resulted in an intended sample size of 1100 subjects for an absolute treatment difference of 10% in the proportion of subjects with favorable outcome at 90 days, assuming a proportion of 40% in the saline arm, 1:1 randomization, and an inflation factor of 1.11 to account for ≈5% potential lost to follow-up. The effect size was justified based on the review of the part 1 study when new part 2 criteria were applied.

These calculations took into account 3 planned interim efficacy analyses conducted according to the O'Brien and Fleming-type alpha spending function for efficacy and beta spending function for futility, intended to be conducted when 275, 550, and 825 subjects had completed the primary 90-day follow-up period. The planned primary efficacy analysis was to first test for interaction between study treatment and thrombolysis. However, because of insufficient numbers of nonthrombolysis subjects and a resulting decreased power to test the interaction effect, this interaction test was dropped from the primary efficacy analysis. An unfavorable outcome was imputed for all subjects, with the primary outcome missing or obtained outside of the specified window.

The Data Coordination Unit at the Medical University of South Carolina was the Statistics and Data Management Center for both ALIAS trials. The case report forms remained consistent between the 2 trials. The data from the 2 trials were concatenated to create the ALIAS trial combined data set with a sample size of 1275 (637 in the albumin group and 638 in the saline group). Analyses on the primary, secondary, and safety outcomes were conducted in the same manner as those for the part 2 data. The effect size estimates were adjusted for the prespecified covariates: baseline National Institutes of Health Stroke Scale (continuous), thrombolysis treatment status (defined by administration of IV tissue-type plasminogen activator, endovascular procedure, or both), and the interaction between thrombolysis treatment status and study treatment. For the unadjusted analyses comparing baseline characteristics, a simple 2 test or independent t test were used as appropriate. For the adjusted analyses of the primary and secondary efficacy and safety outcomes, generalized linear models with the log link were applied to interpret the treatment effect estimate as a relative risk (or relative benefit). The safety outcome analyses used the safety population, defined a priori as all subjects who received at least 20% of the calculated dose of study drug.

Results

Baseline characteristics were similar between the treatment groups within each trial. Subjects who were randomized into part 2 were ≈5 years younger than those in part 1. Most notably, part 2 had more subjects who received thrombolysis (84% versus 75%), which was associated with a slightly longer time from tissue-type plasminogen activator initiation to study drug infusion.

Prespecified primary and secondary efficacy outcomes showed a consistent lack of treatment effect between the 2 groups, with the relative risk (RR) ranging from 0.87 to 1.07 for all outcomes. There were differences in the absolute rates of good outcome in part 1 and part 2, likely because of the change in inclusion and exclusion criteria (eg, age limits) that were implemented in the part 2 trial. The temporal trends in the trial evolution (Figure 1) show that early on in the part 1 trial with only 25% of the subjects enrolled, the albumin group fared poorly compared with the saline group, and in the part 2 trial, the albumin group fared much better compared with the saline group. In both trials, the favorable outcome rates had converged at the time the trials were halted. In hindsight, this initial planned interim analysis with only 25% of subjects enrolled...
may have been too early to make an informed decision because of the fluctuating cumulative outcome at that point in the trial.

Figure 2 presents the distribution of the full-scale modified Rankin Scale scores by treatment for each part separately and combined. No significant differences were observed between the treatment groups (Van Elteren test $P$ value = 0.5016). In general, the combined analysis confirmed that, in the ischemic stroke populations enrolled, intravenous albumin at 2 g/kg provided no added benefit over routine standard of care plus saline control.

Figure 3 presents the safety outcomes for part 1 and part 2 and the combined cohort using the safety sample (ie, those that received at least 20% of their weight-based dose). The RRs for pulmonary edema/congestive heart failure were significant for both parts and for the combined analysis, despite more vigilant monitoring of the fluids and diuretic treatment in part 2. Recurrent stroke occurred more frequently in the albumin group, with a nonsignificant RR of 1.99 and a wide 95% confidence interval. Although not statistically significant, there was an increased risk of symptomatic intracerebral hemorrhage with albumin in part 2. As demonstrated in Figure 3, the RRs for deaths within 30 days and 90 days of randomization were reduced in part 2 and were not significantly different in the combined analysis.

**Discussion**

This preplanned pooled analysis of the ALIAS part 1 and 2 trials confirms the lack of benefit and shows greater adverse events with 25% albumin treatment. In addition, we show interesting temporal effects in the effect size over the course of the 2 trials.

Some of the described differences between the 2 trials were expected. An upper age limit was implemented in part 2,
which resulted in a higher average age for part 1. The increased thrombolyis use in part 2 reflects the evolution of the field of thrombolytics during the course of the study. Pulmonary edema/congestive heart failure was an expected adverse event associated with albumin treatment, and thus, it was not surprising that the RRs were significant for both part 1 and part 2 and combined analyses. However, in part 2 fluid management and diuretic treatment, we demonstrated that pulmonary edema/congestive heart failure was readily remedied through careful fluid management and diuretic treatment. There were few cases where pulmonary edema/congestive heart failure was an serious adverse event resulting in, for example, intubation or an admission to intensive care unit, and none were fatal. The modifications made to the protocol for part 2 intended to address the mortality effect observed in part 1, resulted in similar death rates between the 2 treatment arms, and the combined sample analysis reflects the reduced RRs of deaths.

The ALIAS trials are the latest in a string of clinical trials of putative neuroprotection that have failed to demonstrate clinical efficacy, despite strong preclinical evidence. The phase I dose-finding study showed promising effects of albumin compared with the data from the controls in the National Institute of Neurological Disorders and Stroke r-tPA Stroke Study. Over the last several decades, there has been a decline in stroke case fatality and gradual efforts to systematically improve stroke care. This has included the use of thrombolysis, as well as improved stroke unit care, early supported discharge, and rehabilitation therapies. The use of nonconcurrent control comparison likely led to an exaggerated treatment effect because of secular and gradual improvements in stroke care; the historical controls had poorer outcomes. The small number of subjects in the pilot study, from only 2 clinical sites, enabled close monitoring of the study implementation and protocol adherence, which allowed for better signal-to-noise ratio.

In contrast, in both the ALIAS parts 1 and 2 trials, over 114 sites enrolled 1275 subjects. This order of magnitude increase in study size and number of sites likely introduced greater variability in the execution of the study protocol, which may have contributed to the dilution of the treatment effect. Nevertheless, from a practical perspective, the objective was to show that albumin would be effective across a wide variety of hospitals and clinical settings. The consistent analyses results on all efficacy outcomes suggest the clear lack of benefit of albumin treatment above and beyond the standard of care for acute ischemic stroke patients.

In the combined analysis, the 41.4% good outcome rate in the control group was on target with our design assumption. In part 2, the saline group showed a temporal trend in the cumulative good outcome rate, from ≈30% after ≈200 subjects enrolled and steadily increased to ≈60% by the end of the trial with 419 subjects. In contrast, the control group in the IMS III trial (Interventional Management of Stroke) that was implemented concurrently with ALIAS parts 1 and 2 lacked this trend; the rates of good outcome in both the treatment and control arms became steady after ≈150 subjects’ data were obtained. This stable outcome rate seen in each group in IMS III is what is generally anticipated from clinical trials.

In the case of the ALIAS part 2 Trial, this unanticipated trend in the saline group response remains unexplained. Changes in stroke care should have affected both groups. During the trial, there was a rising use of both intravenous thrombolysis and endovascular stroke treatment, and although albumin was shown to increase the risk of symptomatic intracerebral hemorrhage in combination with thrombolysis (intravenous and endovascular), the absolute risk increase was too small to account for the difference between treatment groups. Because of this temporal trend, the study came close in the early phase to crossing an overwhelming efficacy boundary. The ALIAS trial experience highlights the importance of monitoring temporal trends in effect size.

Finally, the preclinical data supporting neuroprotection remain strong, and the apparent paradox of failed translation to humans remains an extreme challenge. With the evolution of endovascular treatment for stroke since the ALIAS trials were completed, there is now an opportunity to revisit adjuvant and neuroprotective therapies. The vast majority of preclinical models of so-called neuroprotective agents have shown efficacy in models of ischemia–reperfusion. Yet, in the human case, on average, early reperfusion was achieved much <50% of the time, and commonly it has not been measured. This has now changed with the evolution of endovascular treatments, and we look forward to new studies that will reexamine the neuroprotection hypothesis in an era of proven early reperfusion.

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Disclosures
Dr Martin is currently serving on a Biogen-sponsored Phase II clinical trial Data and Safety Monitoring Board (DSMB). Dr Palesch is currently serving on a Brainsgate, Ltd, clinical trial DSMB, has
participated as a consultant for Remedy, Inc, and has served on a Biogen-sponsored Phase I clinical trial DSMB. Dr Yeatts receives consultant fees from Genentech for her role on the PRISMS Trial Steering Committee. Dr Hill reports an ownership interest (common shares) in Calgary Scientific Inc and Quikflo Health Inc. He is serving as a paid consultant outcomes adjudicator for clinical trials committee for Merck Inc. The other authors report no conflicts.

References

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