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Extended drotrecogin alfa (activated) treatment in patients with prolonged septic shock

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Abstract *Objective:* To determine the efficacy and safety of extended drotrecogin alfa (activated) (DAA) therapy. *Design:* Multicentre, randomised, double-blind, placebo-controlled study. *Setting:* Sixty-four intensive care units in nine countries. *Patients:* Adults with severe sepsis and vasopressor-dependent hypotension after a 96-h infusion of standard DAA. *Interventions:* A total of 193 patients received an intravenous infusion of extended DAA 24 μg/kg/h or sodium chloride placebo for a maximum of 72 h. *Measurements and results:* At extended therapy initiation (baseline), DAA-group patients had lower protein C levels ($P = 0.23$) and higher vasopressor requirements, particularly for the primary vasopressor used, norepinephrine ($P = 0.03$), compared with placebo-group patients. DAA treatment did not result in a difference in the primary outcome of time to resolution of vasopressor-dependent hypotension versus placebo ($P = 0.419$). However, few patients reached resolution (DAA 34%, placebo 40%) as most continued to require vasopressor support after 72 additional hours of treatment. Treatment did not reduce 28-day all-cause mortality and in-hospital mortality or improve organ function compared with placebo, although there was a lower percentage change in D-dimers ($P < 0.001$) and increases in protein C levels were numerically greater on extended infusion. There was no difference in serious adverse events including bleeding events. *Conclusions:* Extended DAA treatment did not result in more rapid resolution of vasopressor-dependent hypotension, despite demonstrating anticipated biological effects on D-dimer and protein C levels. A reduced planned sample size combined with baseline imbalances in protein C levels and vasopressor requirements may have limited the ability to demonstrate a clinical benefit.

Keywords Drotrecogin alfa (activated) · Xigris · Sepsis · Shock · Vasopressor · Protein C

Introduction

Drotrecogin alfa (activated) (DAA) (recombinant human activated protein C) is indicated in the European Union for the treatment of adults with severe sepsis with multiple organ failure when added to best standard care. The use of DAA should be considered mainly in situations when therapy can be started within 24 h after the onset of organ failure [1]. DAA is the first specific pharmacological intervention to reduce mortality in severe sepsis, given as a 96-h intravenous infusion at a dose of 24 μg/kg/h [2, 3]. DAA has also been found to ameliorate cardiovascular and respiratory organ dysfunction [4], reduce markers of thrombin generation (D-dimer, prothrombin F1.2) and inflammation (interleukin-6), and result in a more rapid normalisation of protein C and plasminogen levels (important components of the antithrombotic and fibrinolytic systems), compared with placebo [1, 5]. The main safety concern associated with DAA treatment is bleeding [2, 3, 6, 7]. Studies have found that approximately 22% of patients remain vasopressor-dependent at the end of the standard 96-h infusion of DAA [4], suggesting that a longer treatment period could be beneficial for some patients. D-dimer levels decrease during DAA treatment but rapidly increase immediately after the end of the infusion [5], suggesting ongoing coagulopathy, although one study of 12 patients failed to show such an increase [8]. Therefore, this study investigated the effect of an extended infusion of DAA for up to 72 h on vasopressor requirements in adults with persistent septic shock on completion of a 96-h infusion of standard therapy [9].

Materials and methods

The study was approved by the site's ethics committee and conducted in accordance with the Declaration of Helsinki. All patients or their legal representative gave written informed consent.

Design

This was a multicentre, randomised, double-blind, placebo-controlled study in which adults in intensive care units (ICUs) with persistent septic shock, after treatment with a 96-h infusion of standard DAA, were randomised to receive placebo or DAA for up to 72 additional hours. This resulted in a maximal length of infusion of up to 7 days compared to the standard treatment of approximately 4 days.

Patients were screened up to 12 h before the end of the 96-h standard DAA infusion. Within 12 h before the end of the standard infusion, eligible patients or their legal representative provided informed consent and were randomised to treatment (stratified by the investigator site). Patients were followed up for 24 days after initiation of the study drug (28 days after standard drug initiation). Patients still hospitalised at day 24 were followed up until they left hospital up until day 86.

Patients and study personnel remained blinded to treatment throughout the study, apart from a pharmacist or designee who obtained treatment assignments from an interactive voice response system and prepared the drug (covered to maintain the blind).

The primary efficacy outcome was time to resolution of vasopressor-dependent hypotension (dopamine ≥5 μg/kg/min; or epinephrine, phenylephrine, vasopressin, or norepinephrine at any dose) within 72 h. The dose of vasopressor was assessed approximately every 6 h, however no specific targets of hemodynamic therapy were provided. Secondary efficacy measures included 28-day all-cause mortality, 90-day in-hospital mortality, organ function (sequential organ failure assessment, SOFA [10]), and biomarker evaluations (protein C, D-dimers, prothrombin time). Safety measurements included monitoring of serious adverse events (SAEs) and adverse events (AEs); the following were collected: SAEs not considered clinical outcomes; bleeding events reported as non-serious AEs that occurred during the study infusion and that led to or contributed to the need for a transfusion of packed red blood cells; non-serious AEs that that were considered by the investigator to be study drug related; AEs, including bleeding events, that led to permanent discontinuation of study drug.

Patients

Patients were aged ≥18 years, had severe sepsis, and continued to require vasopressor support, having been treated with at least 84 h of a planned 96-h infusion of standard DAA.

Patients were excluded if they were expected to require extensive or multiple surgical procedures within the next 3 days, had a platelet count <30,000/mm^3, were receiving therapeutic heparin [>15,000 IU/day of unfractionated heparin or larger doses of low molecular weight heparin than used for prophylaxis of deep venous thrombosis, or >15 IU/kg/h for renal replacement purposes], were not expected to survive 24 days given their pre-existing uncorrectable medical condition, had received treatment within the last 30 days with any drug that had not received regulatory approval, were pregnant or breastfeeding, were contraindicated for treatment with

DAA, had not given written informed consent, or were no longer vasopressor dependent. Patients whose family or primary physician had not committed to aggressive management of the patient were also excluded.

Drug administration

DAA 24 μg/kg/h was administered as a maximum of 72-h extended infusion. There was to be no time interval between the standard and study infusions; however, a maximum of 2 h was allowed in case of unforeseen circumstances. Interruptions were acceptable as long as infusions were restarted within 24 h and within the 72-h treatment period. If a patient resolved their need for vasopressor support for 12 continuous hours before completion of treatment, the infusion was discontinued and not restarted. Patients randomised to placebo received sterile 0.9% sodium chloride.

Statistics

A sample of 270 patients was planned, based on calculations from PROWESS (recombinant human activated protein C worldwide evaluation in severe sepsis) [2]. From these calculations, 135 patients per group would have at least 81% power to detect a difference between treatment groups using the log rank statistic with a two-sided significance level of 0.1 if the true hazard ratio was 0.63. This corresponds to a difference of about 16.5% in time to resolution of vasopressor-dependent cardiovascular organ failure.

Analyses used the intent-to-treat (ITT) population (all randomised patients who received study drug for any length of time). Time to resolution of vasopressor-dependent hypotension was estimated for each group using the product-limit (Kaplan–Meier) method and a two-sided log-rank test was used for the primary comparison. For mortality, relative risk (RR) and odds ratio (OR) estimates with associated 95% confidence intervals (CIs) were calculated. For other efficacy variables and health outcomes, groups were compared with analysis of variance (ANOVA). Baseline characteristics at the initiation of extended therapy were compared using ANOVA or a chi-square test. Safety measurements were compared using Fishers exact test.

Results

Patients

The study was conducted at 64 centres in nine countries (Austria, Belgium, France, Germany, Italy, Poland, Spain, UK, US) in 2004–2007. Recruitment was slow because fewer patients than anticipated remained vasopressor dependent after a standard DAA infusion, and despite an increase in site numbers, the planned sample size was reduced to 200 patients to ensure that the study completed in an acceptable timeframe. This resulted in the minimum statistical difference that could be detected between the groups increasing to 19.2%.

A total of 201 patients entered the study and 199 were randomised. Six patients discontinued before receiving treatment; four DAA patients owing to entry criteria exclusion and two placebo patients owing to entry criteria exclusion and death. A total of 193 patients (94 DAA, 99 placebo) received study drug for any length of time and were included in the ITT and safety populations. Two ITT placebo patients discontinued from the study, one lost to follow-up and one patient decision. Fifty-three (27.5%) patients had protocol violations, most commonly study drug discontinuations.

Demographic and baseline characteristics are presented in Table 1. Protein C levels were lower in the DAA group. More DAA patients were admitted from acute care hospitals (20.2 vs. 15.2%, $P = 0.422$) and had a history of cancer (26.1 vs. 16.0%, $P = 0.089$), and significantly more placebo patients had a past history of myocardial infarction (18.3 vs. 6.5%, $P = 0.015$). According to SOFA scores, the greatest level of dysfunction was evident in the cardiovascular system with 84.2% and 71.4% of patients rated as grade 4 before the 96-h infusion of standard treatment and at baseline, respectively. There were no other significant differences between the groups except for markers of cardiovascular dysfunction, where more DAA patients had the highest cardiovascular SOFA score ($P = 0.026$). There was also a trend ($P = 0.062$) for more DAA patients to have the highest score before the 96-h infusion of standard treatment. The greater level of cardiovascular dysfunction in the DAA group was supported by higher vasopressor doses (CVI, $P = 0.006$), particularly in the primary vasopressor used, norepinephrine ($P = 0.034$, Table 1).

96-h standard drug treatment

The mean number of hours of standard treatment was 99.0 (median 96, range 89.5–124.0). There was a mean of 0.5 (median 0, range −0.3 to 17.0) hours between the end of standard treatment and the start of study treatment.

Efficacy

Primary outcome

Vasopressor-dependent hypotension resolved in 32 DAA patients (34.0%) and 40 placebo patients (40.4%). The difference between the groups was not statistically

Table 1 Patient demographics and disease severity at baseline (ITT patients)

Variable	Treatment group			
	DAA $N = 94$	Placebo $N = 99$	Total $N = 193$	P value
Mean (SD) age (years)	62.0 (13.4)	62.7 (13.0)	62.4 (13.2)	0.758
Sex, *n* (%)				
Male	58 (61.7)	59 (59.6)	117 (60.6)	0.764
Female	36 (38.3)	40 (40.4)	76 (39.4)	
Origin, *n* (%)				
Caucasian	85 (90.4)	93 (93.9)	178 (92.2)	0.532
African	4 (4.3)	5 (5.1)	9 (4.7)	
East Asian	3 (3.2)	1 (1.0)	4 (2.1)	
Other[a]	2 (2.1)	0 (0.0)	2 (1.0)	
Mean (SD) weight (kg)	80.2 (17.9)	81.4 (20.8)	80.8 (19.4)	0.668
Location before hospital admission, *n* (%)				
Skilled nursing facility	0 (0.0)	1 (1.0)	1 (0.5)	0.422
Home	74 (78.7)	83 (83.8)	157 (81.3)	
Acute care hospital	19 (20.2)	15 (15.2)	34 (17.6)	
Other	1 (1.1)	0 (0.0)	1 (0.5)	
Source of infection, *n* (%)				
Community	68 (72.3)	62 (62.6)	130 (67.4)	0.150
Nosocomial	26 (27.7)	37 (37.4)	63 (32.6)	
Mean (SD) organ failures	2.8 (1.0)	2.9 (0.9)	2.9 (1.0)	0.31
APACHE II score				
Mean (SD)	28.4 (7.4)	27.7 (8.9)	28.1 (8.1)	0.553
Range	7.0–50.0	5.0–51.0	5.0–51.0	
McCabe–Jackson co-morbidity score, *n* (%)				
Non fatal	73 (80.2)	70 (73.7)	143 (76.9)	0.379
Ultimately fatal	16 (17.6)	19 (20.0)	35 (18.8)	
Rapidly fatal	2 (2.2)	6 (6.3)	8 (4.3)	
Protein C level				
Mean (SD)	66.8 (28.4)	72.9 (32.9)	69.8 (30.8)	0.23
Range	13.0–129.0	12.0–185.0	12.0–185.0	
CVI				
Mean (SD)	4.2 (1.4)	3.8 (1.3)	4.0 (1.4)	0.006
Range	1.0–8.0	1.0–8.0	1.0–8.0	
Norepinephrine dose, μg/kg/min				
Number of patients	82	91	173	0.034
Mean (median) dose	1.24 (0.26)	0.96 (0.16)	1.09 (0.20)	
Steroid use during 96-h standard treatment				
Number of patients (%)	79 (84.0)	78 (78.8)	157 (81.3)	0.35

[a] Includes Hispanic and West Asian; APACHE II, acute physiology and chronic health evaluation II; CVI, cumulative vasopressor index (sum of the rankings for all vasopressors being used by a patient at a given time based on their potency and dose; potential scores range between 1 and 20); DAA, drotrecogin alfa (activated); SD, standard deviation

significant ($P = 0.419$; Fig. 1). Sixty-two (66.0%) of the 94 extended DAA-group patients and 59 (59.6%) of the 99 placebo patients were counted as no resolution. Patients were considered as having no resolution if they continued to require vasopressor support after 72 h of treatment or stopped the infusion for the last time during the 72 h for reasons other than because they resolved their need for vasopressor support for >12 continuous hours [i.e. death ($n = 17$), procedure ($n = 2$)], or resolved their need for vasopressor support for <12 h ($n = 2$).

Secondary outcomes

By 28 days after the start of the 96-h infusion of standard drug therapy, 37 DAA patients (39.8%) and 31 placebo patients (32.3%) had died (OR 1.39, 95% CI 0.76–2.51; RR 1.23, 95% CI 0.84–1.81; $P = 0.283$). Ninety-day in-hospital mortality was similar between groups: 51 DAA patients (54.3%) and 43 placebo patients (43.9%; OR 1.53, 95% CI 0.86 to 2.68; RR 1.24, 95% CI 0.92 to 1.65; $P = 0.15$). The main causes of death were sepsis-induced multi-organ failure and refractory septic shock.

Repeated measures analyses indicated that cardiovascular and renal SOFA scores improved over time (P values, <0.001 and 0.011, respectively), whereas respiration and liver scores worsened (P values, 0.024 and 0.050, respectively). However, there were no significant differences between the two treatment groups in any mean organ system SOFA score (Table 2). There were also no treatment-group differences in: hospital-, ICU-, catecholamine-, renal replacement therapy-, and mechanical

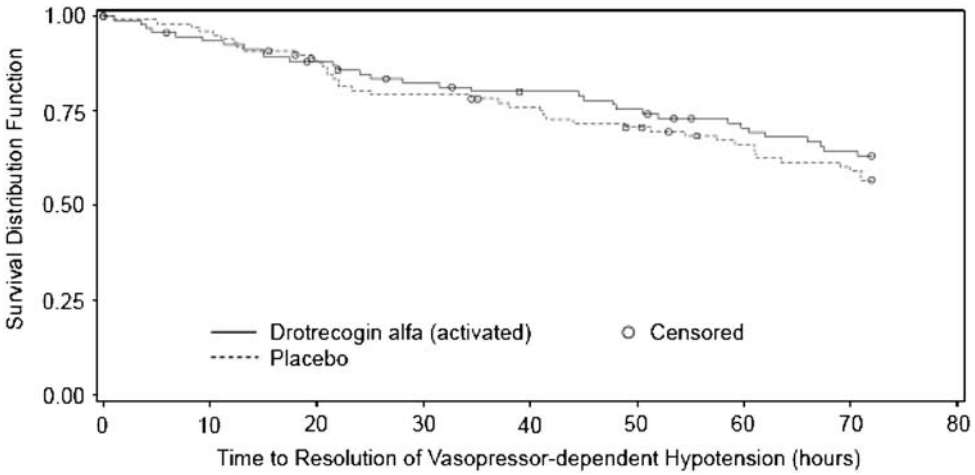

Fig. 1 Kaplan–Meier curve of analysis of time to resolution of vasopressor-dependent hypotension (hours) by treatment group (ITT patients)

Table 2 Average SOFA scores during the study (ITT patients)

SOFA organ system	Treatment group			
	DAA ($N = 94$) Mean (SD)	Placebo ($N = 99$) Mean (SD)	*P* value for between group comparison[a]	Day *P* value[b]
Cardiovascular				
Baseline	3.7 (0.7)	3.5 (0.9)	0.768	<0.001
Average over 10 days	2.4 (1.3)	2.2 (1.2)		
Change from baseline	−1.3 (1.2)	−1.3 (1.2)		
Respiration				
Baseline	2.3 (1.1)	2.2 (1.0)	0.565	0.024
Average over 10 days	2.5 (0.9)	2.4 (0.9)		
Change from baseline	0.2 (0.9)	0.2 (0.8)		
Coagulation				
Baseline	1.2 (1.1)	1.2 (1.1)	0.817	0.133
Average over 10 days	1.1 (1.2)	1.0 (1.1)		
Change from baseline	−0.1 (1.2)	−0.1 (1.2)		
Liver				
Baseline	1.0 (1.1)	1.0 (1.1)	0.635	0.050
Average over 10 days	1.2 (1.3)	1.1 (1.2)		
Change from baseline	0.3 (0.9)	0.3 (0.9)		
Renal				
Baseline	2.0 (1.7)	2.1 (1.7)	0.304	0.011
Average over 10 days	1.8 (1.6)	2.0 (1.6)		
Change from baseline	−0.2 (1.0)	−0.1 (0.9)		

DAA drotrecogin alfa (activated), *SD* standard deviation, *SOFA* sequential organ failure assessment
[a] Difference between groups analysed using analysis of variance (ANOVA)
[b] Day *P* value analysed by repeated measures analysis of mean daily SOFA scores

ventilation-free days; time to discharge from hospital and ICU; time to discontinuation of catecholamines, renal replacement therapy, and mechanical ventilation (data not shown).

Mean thrombin generation (D-dimers) did not fall significantly from baseline to end point (5.2–5.1) in DAA patients, but increased in placebo patients (4.6–6.3; difference in least squares means −1.78, 95% CI −3.34 to −0.23; $P < 0.001$). There were no statistically significant differences for change in prothrombin time or protein C; however, DAA patients had lower protein C levels at baseline compared with placebo patients. At study end point, levels were roughly equivalent and therefore protein C had increased by a greater extent in the drug group compared with placebo, although this change did not reach statistical significance ($P = 0.216$) (Table 3).

Exploratory analyses suggested that baseline protein C levels predicted outcome; patients with levels >40% had better estimated survival at 28 days compared with those with levels ≤40% ($P = 0.03$). A patient was a non-responder if their protein C decreased or remained stable from baseline to end point; a patient was a responder if

Table 3 Protein C results (ITT patients)

Time point	Treatment group		
	DAA $N = 94$	Placebo $N = 99$	Difference between groups
Baseline, mean (95% CI)	66.8 (60.1, 73.4)	72.9 (65.2, 80.5)	−6.1
End point, mean (95% CI)	81.7 (73.5, 90.0)	79.4 (70.7, 88.0)	+2.3
Change from baseline	+14.9	+6.5	+8.4

CI confidence interval, *DAA* drotrecogin alfa (activated)

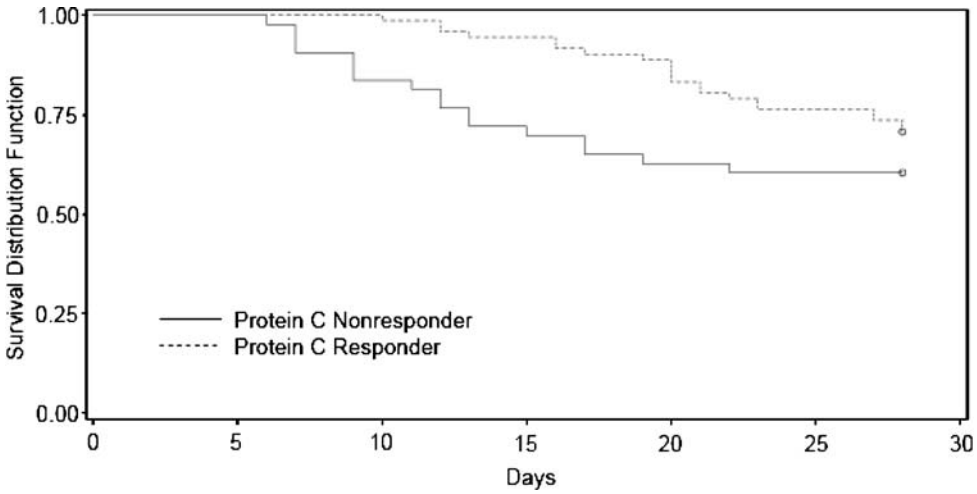

Fig. 2 28-day mortality by patient's protein C response. Responders are those showing improvement in protein C during the infusion. Non-responders are those without improvement in protein C

levels increased from baseline. There were more protein C responders after treatment with DAA compared with placebo (58.1 vs. 41.9%, $P = 0.07$). Responders had higher 28-day survival (Fig. 2), although the difference was not significant ($P = 0.10$). There was no difference between responders and non-responders in time to resolution of vasopressor-dependent hypotension ($P = 0.25$).

Safety

Overall numbers of AEs, SAEs, and bleeding events are summarised by study period in Table 4. Nine patients in each of the treatment groups reported at least one SAE, most commonly cardiac disorders. Two patients experienced SAEs that were considered related to study drug: one DAA patient with two events (colitis ischemic, retroperitoneal haemorrhage) and one placebo patient with one event (haemoptysis). Three DAA patients and four placebo patients reported non-serious AEs, mostly cardiac and vascular disorders.

Discussion

Continued administration of DAA for up to 72 additional hours after a 96-h infusion of the standard drug did not result in a more rapid resolution of vasopressor-dependent hypotension versus placebo. Few patients reached resolution as most had either died or continued to require vasopressor support at the end of follow-up. These results should be interpreted cautiously as there was a reduction in the planned sample size from 270 to 200 patients, which resulted in estimates having wide CIs (for estimates of resolution of vasopressor support, the 95% CIs were 24.4–43.6% for the extended DAA group and 30.7–50.1% for the placebo group). No standard process for withdrawal of vasopressor was implemented, which may have contributed to this variability. There were also potentially relevant baseline group differences in vasopressor requirements and protein C levels, which are important because low protein C levels have been linked to a worse outcome in severe sepsis [11]. In addition, recruitment was prolonged, which could have contributed to the heterogeneity of the sample.

Although there was no difference in the primary objective, the biological activity of extended DAA was demonstrated; thrombin generation was not significantly reduced in DAA patients, but increased in placebo patients after the withdrawal of standard DAA. In addition, there were potentially clinically relevant increases in protein C after treatment with DAA. Compared with placebo, protein C increased by a greater extent in DAA patients, but owing to lower baseline levels, final protein C levels were similar, which would predict similar clinical outcomes.

Table 4 Summary of serious and all adverse events by study period (ITT patients)

Adverse event study period	Treatment group			
	DAA[a] $N = 94$		Placebo $N = 99$	
	Number of patients (%)	Number of events	Number of patients (%)	Number of events
Serious adverse events				
Infusion period	2 (2.1)	2	3 (3.0)	4
24-day study period	9 (9.6)	12	9 (9.1)	13
Serious bleeding events				
Infusion period	1 (1.1)	1	1 (1.0)	1
24-day study period	1 (1.1)	1	2 (2.0)	2
Overall adverse events				
Infusion period	5 (5.3)	6	3 (3.0)	4
24-day study period	12 (12.8)	16	12 (12.1)	18
Overall bleeding events				
Infusion period	3 (3.2)	3	1 (1.0)	1
24-day study period	3 (3.2)	3	2 (2.0)	2

[a] Given that SAEs were not collected retrospectively from the standard DAA infusion period, these results underestimate the full risks at the start of a planned course of extended therapy

DAA drotrecogin alfa (activated)

The failure of extended DAA in weaning patients with prolonged septic shock from vasoactive support could also be related to causes other than persistent sepsis-induced cardiovascular dysfunction. It is well known that septic shock causes alterations in myocardial and vascular adrenergic receptor-mediated responses in animals [12] and humans [13]. However, two well-established lines of evidence in chronic heart failure [14] and prolonged exercise [15] suggest that a down-regulation of adrenoreceptors contributes to cardiac dysfunction due to a pronounced activation of the sympathetic system. In the same line, the reduced sensitivity to catecholamines and consequent failure of weaning from vasoactive support may, in part, be due to a desensitization of adrenergic receptor stimulation induced by the stimulatory effect of endogenous and exogenous catecholamines [16].

Exploratory analyses suggested baseline protein C predicts outcome, which agrees with other studies [17–21]. Additionally, patients were more likely to be protein C responders (levels increased from baseline) with DAA treatment compared with placebo, and responders had a more favourable outcome (Fig. 2); although non-responders in the DAA group had the overall worst outcome. Further research is needed, but improvements in protein C could be a benefit of extended treatment. However, failure of improvement in protein C despite treatment with DAA may be indicative of a poor prognosis, or that other aspects of care should be reviewed. The potential role of protein C as a biomarker to guide therapy with DAA is currently being investigated in the Research Evaluating Serial PC levels in severe sepsis patients On DrotAA (RESPOND) study [11].

Extended administration of DAA did not reduce 28-day and in-hospital mortality compared with placebo. Previous work has shown that DAA is successful in reducing mortality when infused for up to 96 h [2, 3]. Mortality in this study was higher than has been previously found, although a relatively high mortality is to be expected with a population of such critically ill patients. In PROWESS, 28-day mortality following a 96-h infusion was lower at 24.7% in the DAA group ($n = 850$) and 30.8% in the placebo group ($n = 840$) [2], compared with 39.8 and 32.3%, respectively, in this study. However, it is difficult to compare mortality between the two treatment groups in this study owing to the small sample size and baseline imbalances in underlying diseases, vasopressor use, and protein C. Despite these limitations, one might speculate that extended DAA therapy may have been associated with a higher mortality. As the only known adverse effect of DAA treatment is bleeding, it could be hypothesised that if DAA treatment was associated with harm, more bleeding events should have been expected; in the present study, this was not the case, and there were no haemorrhagic or study drug related deaths. However, these study results do not support the use of infusions of longer than 4 days in current clinical practice.

Extended DAA treatment had an acceptable safety profile, with similar numbers of SAEs between DAA- and placebo-treated patients. In addition, the incidence of bleeding-related events was low with only one patient in each group experiencing serious bleeding during the infusion period. Given the study design, SAEs were not retrospectively collected from the standard DAA infusion period, and it is unlikely that patients experiencing serious bleeding events during this period would have continued standard treatment to be eligible for inclusion in this study. Thus, these results underestimate the full risks at the start of a planned course of potentially extended

therapy; however, they do suggest that in a patient who has completed a standard 96-h infusion of DAA, the risks of continuing therapy at that stage may be relatively small; although it is difficult to make firm conclusions given the sample size and overall low event rate.

In conclusion, extended DAA treatment did not result in a difference in the primary outcome of time to resolution of vasopressor-dependent hypotension versus placebo. In addition, extended treatment did not reduce mortality or improve organ function compared with placebo, although some anticipated biological effects on D-dimer and protein C levels were found. The results of the study should be interpreted cautiously as a small sample size and baseline imbalances in protein C levels and vasopressor requirements disfavoured DAA and may have limited the ability to demonstrate a clinical benefit.

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Conflict of interest statement J.-F. D, and J. C. have served as consultants for Eli Lilly and Company; M. A., P. W., A. D., J. R. and S. L. report no conflict of interests at this time; M. B. is an employee of Eli Lilly and Company; M. C.-M., C. M, M. A. M. and J. J. are employees and stockholders of Eli Lilly and Company.

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