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Drotrecogin alfa (activated) in the treatment of severe sepsis patients with multiple-organ dysfunction: data from the PROWESS trial

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Abstract *Objective:* Based on the results of the PROWESS trial the European Agency for the Evaluation of Medicinal Products has recently approved drotrecogin alfa (activated) for treatment of adult patients with severe sepsis and multiple-organ failure. We report study's data on efficacy and safety in patients with multiple-organ dysfunction. *Design and setting:* Randomized, double-blind, placebo-controlled, multicenter trial in 164 medical centers. *Patients:* 1271 patients (75.2% of the intention-to-treat population, *n*=1690) with multiple-organ dysfunction at study entry. *Interventions:* Drotrecogin alfa (activated) *n*=634, 24 μg/kg per hour for 96 h or placebo (*n*=637).
Results Observed 28-day mortality was significantly lower with drug treatment than with placebo (26.5%vs. 33.9%), cardiovascular and respiratory organ dysfunction resolved more rapidly over the first 7 days, and serious bleeding events were more frequent (2.4% vs. 1.3%).
Conclusions: Treatment with drotrecogin alfa (activated) significantly reduced 28-day mortality and more quickly resolved cardiovascular and respiratory organ dysfunction. The difference in serious bleeding event rates may be clinically significant; however, the overall benefit-risk profile appears favorable.

Keywords Drotrecogin alfa (activated) · Activated protein C · Xigris · Recombinant proteins · Sepsis · Septic shock

Introduction

Severe sepsis, defined as sepsis associated with acute organ dysfunction [1], remains a major cause of morbidity and mortality [2, 3]. Over 2,000 persons develop severe sepsis in the United States each day, and one-third of these patients die [3]. Reported prevalence rate of severe sepsis in European intensive care units ranges from 9% to 25%, with mortality rates estimated at 30–40% [4, 5, 6, 7]. The societal burden of severe sepsis is thought to be associated with the loss of up to 135,000 lives every year and to cost as much as € 7.6 billion in patient health care expenses [7] (A. Davies, unpublished data).

Severe sepsis arises from activation of the innate immune response [8]. This multifaceted response [8, 9] triggered by diverse pathogens leads to secretion of pro- and anti-inflammatory cytokines, activation and mobilization of leukocytes, activation of coagulation and inhibition of fibrinolysis [10, 11], and increased apoptosis [12]. As a result of this activation in coagulation the thrombin generated not only promotes fibrin deposition in the microvasculature but also exacerbates ongoing inflammation via both direct and indirect mechanisms, which leads to multiple-organ dysfunction [13]. Activated protein C functions as an important, endogenous regulator of hemostatic stress and immune response via its antithrombotic [14] and profibrinolytic [15] properties. In animal models of sepsis activated protein C has been shown to both reduce mortality and to limit or prevent development of various markers of organ dysfunction [16, 17, 18, 19].

The multinational, phase 3 Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial evaluating drotrecogin alfa (activated), a recombinant human activated protein C, for the treatment of severe sepsis demonstrated a significant reduction in 28-day all-cause mortality (19.4% relative risk reduction, p=0.005) [20] and morbidity [21]. The European Agency for the Evaluation of Medicinal Products recently approved drotrecogin alfa (activated) for treatment of adult patients with severe sepsis with multiple-organ failure (two or more organ dysfunctions based on PROWESS entry criteria) when added to best standard of care, concluding that the benefit-risk profile in multiple-organ dysfunction (MOD) patients was more favorable than the profile observed in patients with single-organ dysfunction. This contribution presents additional information on the characteristics of the subpopulations with MOD and single-organ dysfunction as well as specific safety and efficacy information on drotrecogin alfa (activated) in these subgroups that goes beyond that reported in the original PROWESS publication [20].

Methods and materials

PROWESS Trial

PROWESS was a randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of drotrecogin alfa (activated) (Xigris®, Eli Lilly, Indianapolis, Ind., USA) in adults with severe sepsis [20]. After obtaining their informed consent patients were randomly assigned to receive either drug at a dose of 24 μg/kg per hour or placebo for a total of 96 h and were followed for 28 days from the start of study drug administration or until death. Both groups also received standard supportive care. The intention-to-treat population, defined as those patients who received study drug for any length of time, totaled 1690 patients. Patients at high risk of bleeding and those likely to die from other serious comorbid conditions other than sepsis within the 28-day follow-up period were excluded from the trial. A protocol amendment implemented early in the PROWESS trial is described in further detail at the publisher's website (http://dx.doi.org/10.1007/s00134-003-1731-1) and is discussed in recent literature [22, 23, 24]. Use of steroids was neither specified in the protocol nor contraindicated. Heparin for deep vein thrombosis prophylaxis (<15,000 U/day) was permitted. Although use was recorded, exact dose and type of heparin or steroids administered were not collected.

Organ dysfunction criteria

All patients were assessed for the presence or absence of prospectively defined acute sepsis-induced organ dysfunction at baseline [20] among the following systems: cardiovascular, respiratory, renal, hematological (platelet count), and metabolic. The criteria for dysfunctional organs or systems were:

- Cardiovascular system dysfunction—either of the following:
  - Arterial systolic blood pressure ≤90 mmHg or mean arterial pressure ≤70 mmHg for at least 1 h despite adequate fluid resuscitation or adequate intravascular volume status
  - The need for vasopressors to maintain systolic blood pressure a ≥90 mmHg or mean arterial pressure ≥70 mmHg

- Respiratory dysfunction—either of the following:
  - PaO_2/FIO_2 ≤250 in the presence of other dysfunctional organs or systems (cause of sepsis is not pneumonia)
  - PaO_2/FIO_2 ≤200 if the lung was the only dysfunctional organ (lung is site of infection)

- Hematological dysfunction—either of the following:
  - Platelet count <80,000/mm^3
  - A decline of 50% in platelet count from highest value recorded over the 3 days preceding enrollment

- Renal dysfunction:
  - Urine output <0.5 ml/kg per hour for 1 h, despite adequate fluid resuscitation

- Metabolic dysfunction: unexplained metabolic acidosis, which was defined as:
  - pH≤7.30 or base deficit ≥5.0 mEq/l
  - A plasma lactate level > 1.5 times the upper limit of normal for the lab.

Patients had to meet at least one of the above five criteria for enrollment in the study. The first sepsis-induced organ or system dysfunction had to develop within 24 h before study enrollment. At least one organ dysfunction had to be accompanied by infection criteria and modified criteria of systemic inflammatory response

syndrome as described by Bernard et al. [20] for enrollment in the trial.

In addition, prospectively defined sequential evaluations of organ dysfunction at baseline and daily through the 28-day study were performed using Sequential Organ Failure Assessment (SOFA) [25] scores based on local laboratory data, vasopressor doses, and mechanical ventilation status. For SOFA assessments only cardiovascular assessment and ventilator status over the entire 28 days and platelet count during infusion were obligatory. Other local laboratory and vital sign parameters were collected if available. In the study as a whole 2.4% of cardiovascular, 19.5% of respiratory, 22.2% of renal, 23.2% of hematological, and 53.2% of hepatic SOFA score values were not collected on days when patients were alive [21]. Data collection for SOFA scores was most frequent in the ICU (41.5–99.9% collected) and least frequent in the general ward (25.2–92.9% collected). Presence of overt disseminated intravascular coagulation (DIC) was assessed post hoc based on an adaptation of the definition recently proposed by the International Society of Thrombosis and Hemostasis using the degree of decrease in platelet count, elevation in fibrin-related marker, and prolongation of prothrombin time but not decrease in fibrinogen level because this was not measured in the trial [26]. Acute Physiology and Chronic Health Evaluation (APACHE) II [27] scores were recorded as the most extreme values in the 24 h prior to administration of drug.

Biomarkers

Blood samples for measurement of D-dimer (Liatest D-DI, latex immunoassay, Diagnostica Stago, Asnieres, France), protein C (Staclot Protein C, Diagnostica Stago), and interleukin-6 (IL-6; Quantikine HS enzyme immunoassay, R&D Systems, Minneapolis, Minn., USA) levels were obtained at baseline and on days 1–7. A central laboratory performed all biomarker analyses.

Definition of bleeding and thrombotic events

We determined the subset of bleeding events within all study adverse events by applying a prospective *bleeding event* ("any bleeding") definition. Serious bleeding events were defined prospectively as any intracranial hemorrhage, any life-threatening bleed, a requirement of 3 U packed red blood cells or more per day for 2 consecutive days, or any bleeding event meeting any of the other criteria defining a serious adverse event [20]. A *serious thrombotic event* was defined post hoc as any of the following serious adverse events: cerebral arterial thrombosis, cerebral infarct, cerebrovascular accident (excluding hemorrhage), myocardial infarction, peripheral arterial thrombosis, deep venous thrombosis, or pulmonary thromboembolism.

Statistical analyses

Efficacy and safety analyses were performed on the intention-to-treat population of the trial. Patients were analyzed according to the treatment group to which they were randomly assigned. Survival curves were compared using Cox regression. Mortality rates and 95% confidence intervals for relative risk at 28-days were calculated for various subgroups of patients, and treatment-by-subgroup interactions were evaluated using the Breslow-Day test for homogeneity of odds ratios across strata [28]. Logistic regression was used to compare 28-day mortality rates between treatment groups adjusted for possible imbalances at baseline with regard to preexisting conditions and severity indices.

Comparisons between baseline characteristics of MOD and single-organ dysfunction populations used the χ^2 for categorical data and Wilcoxon rank-sum tests for continuous data. Biomarker levels over time were evaluated in patients with at least one non-missing baseline and subsequent measurement using analysis of variance of rank-transformed data with last observation carried forward imputation for missing data. Time to resolution of organ dysfunction was defined as the time patients with a baseline SOFA score higher than 0 took to reach a score of 0. Time to development of organ dysfunction was defined as the time patients with a baseline SOFA score of 0 took to reach a score higher than 0. These times-to-event data were analyzed over days 1–7 using Cox regression with censoring at death or discharge. All statistical tests were two-tailed and used the 5% significance level. All analyses were performed using SAS (Release 8.02 for Windows, SAS Institute, Cary, N.C., USA) or S-Plus 2000 software (Professional Release 3 for PC, Insightful Corporation, Seattle, Wash., USA).

Results

Baseline patient characteristics

Of the 1690 intention-to-treat population 1271 (75.2%) had MOD at study entry [drotrecogin alfa (activated), n=634; placebo, n=637]. The remaining 419 patients had single-organ dysfunction at entry [drotrecogin alfa (activated), n=216; placebo, n=203]. Patients with MOD were significantly older, more severely ill as measured by APACHE II scores, and were more likely to have chronic obstructive pulmonary disease (COPD), recent surgery, and overt DIC, and tended to have more cancer than patients with single-organ dysfunction (Table 1). Patients with MOD had significantly higher APACHE II age points and acute physiology points than those with single-organ dysfunction patients (age points, 3.6 vs. 3.1; acute physiology points, 21.3 vs. 17.6; both $p \leq 0.001$).

Lungs and abdomen were the most common sites of infection, occurring in 51.7% and 22.3% of patients with MOD. MOD patients were more likely to have intra-abdominal infections (22.3% vs. 12.7%; p=0.001), and bacteremia (34.5% vs. 27.0%; p=0.005) than single-organ dysfunction patients. Baseline levels of markers of coagulopathy (D-dimer, protein C) and inflammation (IL-6) in the drotrecogin alfa (activated) group and the placebo group with MOD are shown in Table 2. Protein C levels were lower at baseline, and D-dimer and IL-6 levels higher in patients with MOD than in those with single-organ dysfunction. Among patients with MOD at baseline steroid use was more common in patients with APACHE II scores of 25 or higher (n=685) than in those with scores lower than 25 (34.5% vs. 20.7%, p<0.0001). Baseline steroid use was also more common in MOD patients with COPD (n=161, 48.8%) than in those patients without COPD (n=196, 20.8%; p<0.0001). At baseline in the MOD group there were numerically higher rates of myocardial infarction, congestive cardiomyopathy, diabetes, pancreatitis, liver disease, COPD, cancer, recent trauma, recent surgery, shock, and use of mechanical ventilation and vasopressors in placebo patients than in drug-treated patients (Table 1). However, there were numerically higher rates of hypertension, overt DIC, pro-

Table 1 Baseline characteristics of patients receiving drotrecogin alfa (activated) (*treatment*) and placebo in the PROWESS trial: overall and in subgroups with single-organ dysfunction (one organ dysfunction at study entry) and with multiple-organ dysfunction (two or more organ dysfunctions at study entry) and statistical significance of differences between the subgroups (*COPD* chronic obstructive pulmonary disease, *APACHE II* Acute Physiology Age and Chronic Health Evaluation [27], *DIC* disseminated intravascular coagulation)

	Single-organ dysfunction			Multiple organ dysfunction			*p*
	Overall (*n*=419)	Treatment (*n*=216)	Placebo (*n*=203)	Overall (*n*=1271)	Treatment (*n*=634)	Placebo (*n*=637)	
Age (years)	57.6±17.7	58.4±18.3	56.8±17.0	61.5±16.4	61.2±16.8	61.8±16.1	0.0001
Males	245 (58.5%)	124 (57.4%)	121 (59.6%)	719 (56.6%)	353 (55.7%)	366 (57.5%)	0.531
Whites	331 (79.0%)	173 (80.1%)	158 (77.8%)	1053 (82.8%)	522 (82.3%)	531 (83.4%)	0.079
Prior or preexisting conditions							
Hypertension	146 (34.8%)	80 (37.0%)	66 (32.5%)	473 (37.2%)	245 (38.6%)	228 (35.8%)	0.413
Myocardial infarction	57 (13.6%)	28 (13.0%)	29 (14.3%)	167 (13.1%)	75 (11.8%)	92 (14.4%)	0.804
Congestive cardiomyopathy	34 (8.1%)	19 (8.8%)	15 (7.4%)	96 (7.6%)	35 (5.5%)	61 (9.6%)	0.751
Diabetes	95 (22.7%)	49 (22.7%)	46 (22.7%)	269 (21.2%)	127 (20.0%)	142 (22.3%)	0.538
Pancreatitis	24 (5.7%)	11 (5.1%)	13 (6.4%)	38 (3.0%)	18 (2.8%)	20 (3.1%)	0.016
Liver disease	10 (2.4%)	6 (2.8%)	4 (2.0%)	30 (2.4%)	12 (1.9%)	18 (2.8%)	1.00
COPD	78 (18.6%)	40 (18.5%)	38 (18.7%)	330 (26.0%)	149 (23.5%)	181 (28.4%)	0.002
Cancer	62 (14.8%)	32 (14.8%)	30 (14.8%)	241 (19.0%)	113 (17.8%)	128 (20.1%)	0.056
Recent trauma	20 (4.8%)	7 (3.2%)	13 (6.4%)	51 (4.0%)	21 (3.3%)	30 (4.7%)	0.485
Recent surgery	96 (22.9%)	49 (22.7%)	47 (23.2%)	406 (31.9%)	196 (30.9%)	210 (33.0%)	0.0005
APACHE II score	21.7±7.2	21.4±7.1	22.0±7.2	25.8±7.6	25.7±7.5	25.9±7.8	0.0001
Mechanical ventilation	241 (57.5%)	117 (54.2%)	124 (61.1%)	1034 (81.4%)	506 (79.8%)	528 (82.9%)	<0.0001
Shock	153 (36.5%)	83 (34.4%)	70 (34.5%)	1047 (82.4%)	515 (81.2%)	532 (83.5%)	<0.0001
Use of any vasopressor	133 (31.7%)	63 (29.2%)	70 (34.5%)	924 (72.7%)	453 (71.5%)	471 (73.9%)	<0.0001
DIC							
Overt [26]	52 (12.4%)	23 (10.7%)	29 (14.3%)	326 (25.6%)	171 (27.0%)	155 (24.3%)	<0.0001
Nonovert	367 (87.6%)	193 (89.4%)	174 (85.7%)	945 (74.4%)	463 (73.0%)	482 (75.7%)	

Table 2 Baseline levels of markers of coagulation and inflammation in patients receiving drotrecogin alfa (activated) (*treatment*) and placebo in the PROWESS trial: overall and in subgroups with single-organ dysfunction (one organ dysfunction at study entry) and with multiple-organ dysfunction (two or more organ dysfunctions at study entry) and statistical significance of differences between the subgroups (not all baseline blood samples obtained were suitable for measurement of biomarkers)

	Single-organ dysfunction			Multiple organ dysfunction			*p*
	Overall	Treatment	Placebo	Overall	Treatment	Placebo	
Plasma D-dimer	(*n*=375)	(*n*=193)	(*n*=182)	(*n*=1175)	(*n*=599)	(*n*=576)	0.0001
Median level (μg/ml)	3.48	3.42	3.59	4.51	4.51	4.51	
Interquartile range (μg/ml)	2.02–6.71	1.88–6.62	2.11–6.77	2.36–9.07	2.48–8.93	2.24–9.13	
Serum interleukin 6	(*n*=401)	(*n*=209)	(*n*=192)	(*n*=1234)	(*n*=618)	(*n*=616)	0.0001
Median level (μg/ml)	245.3	233.6	251.6	657.2	734.1	599.7	
Interquartile range (pg/ml)	78.3–770	89.6–591.2	71.4–1038.5	172.2–3907	190.1–3960.0	162.5–3792.0	
Plasma protein C activity	(*n*=377)	(*n*=194)	(*n*=183)	(*n*=1197)	(*n*=605)	(*n*=592)	0.0001
Median level (%)	56	54	58	45	44	46	
Interquartile range (%)	39–75	38–74	40–76	29–63	28–61	30–64	
Protein C deficiency (<81%)							<0.0001
Deficient	305 (72.8)	156 (72.2)	149 (73.4)	1074 (84.5)	553 (87.2)	521 (81.8)	
Not deficient	72 (17.2)	38 (17.6)	34 (16.8)	123 (9.7)	52 (8.2)	71 (11.2)	
Unknown	42 (10.0)	22 (10.2)	20 (9.9)	74 (5.8)	29 (4.6)	45 (7.1)	

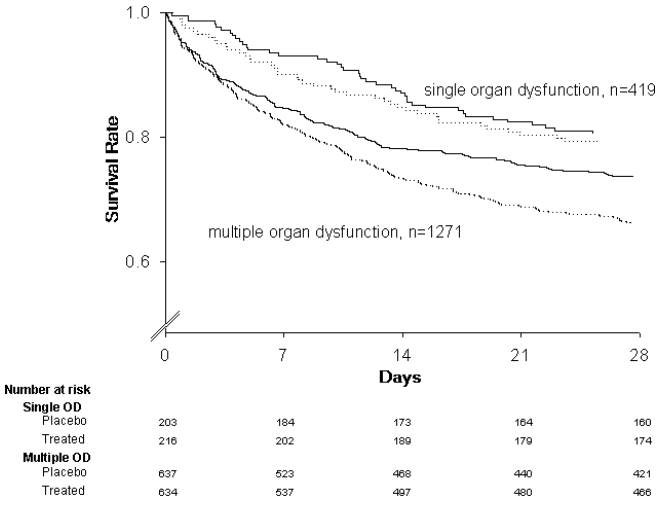

Fig. 1 Kaplan-Meier estimates of survival in patients receiving drotrecogin alfa (activated) (*solid lines*) and placebo (*dashed lines*) with single-organ dysfunction (*upper two curves*) or multiple-organ dysfunction (*lower two curves*). The *p* for interaction between therapy and organ dysfunction was 0.469. The *p* value for the effect of drotrecogin alfa (activated) on 28-day mortality in patients with multiple-organ dysfunction was 0.006 and that for patients with single-organ dysfunction was 0.61. Number at risk for all groups is indicated below the x-axis. *OD* Organ dysfunction

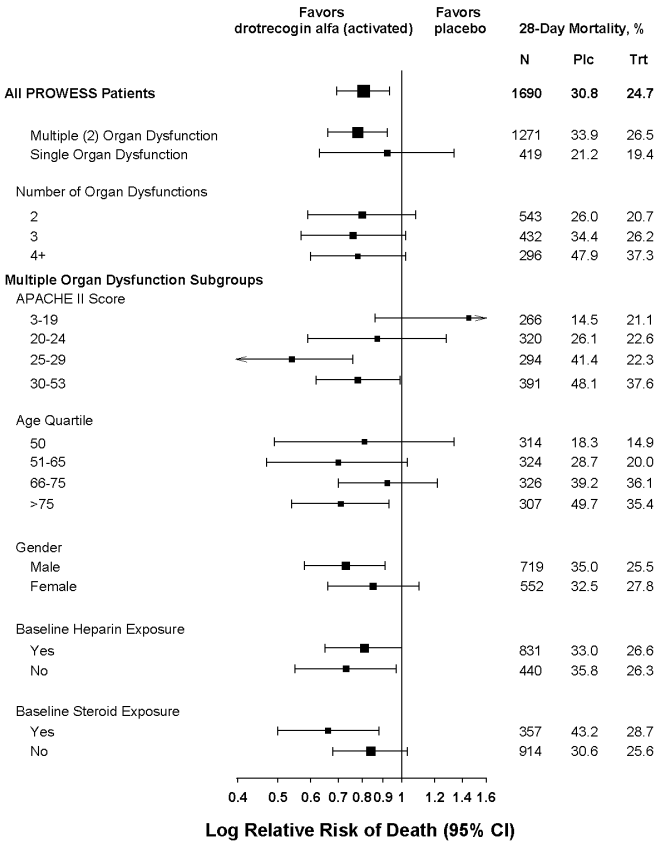

Fig. 2 The 28-day all-cause mortality in subgroups of multiple-organ dysfunction patients in the PROWESS trial. *Solid squares* Point estimates of relative risk of death in each subgroup; *horizontal lines* 95% confidence interval (*CI*). The overall PROWESS population is included for comparison. *Size of symbol* is proportional to the number of patients in each subgroup. *N* Total number of patients in the subgroup; *Plc* placebo group; *Trt* treatment group [drotrecogin alfa (activated)]

tein C deficiency, and numerically higher median baseline levels of IL-6 in drug-treated patients than in placebo patients.

Efficacy

The effect of therapy on 28-day survival in MOD patients was not significantly different from the effect of therapy on 28-day survival in single-organ dysfunction patients (p=0.469; Fig. 1). Although the within-subgroup effect of drotrecogin alfa (activated) on survival was significant in MOD patients (p=0.006) and not significant in single-organ dysfunction patients (p=0.614), the size of the subgroup with MOD was three times that of the single-organ dysfunction group, and because the trial was halted early for efficacy, the single-organ dysfunction subgroup was left relatively underpowered.

A logistic regression analysis of 28-day mortality adjusting for numerical differences in age, gender, origin, preexisting conditions (Table 1), APACHE II score, mechanical ventilation status, shock, use of vasopressor, overt DIC, baseline protein C, D-dimer, and IL-6 levels indicated that the association between drotrecogin alfa (activated) treatment and survival in MOD patients remained significant after adjusting for these factors (p=0.011).

Mortality rates at 28 days were lower in treated patients than in placebo patients regardless of the number of organ dysfunctions at study entry (26.5% vs. 33.9% with MOD, 19.4% vs. 21.2% with single-organ dysfunction; Fig. 2). The relative risk of death in MOD patients treated with drotrecogin alfa (activated) was 0.78 (95% CI 0.66–0.92) compared to 0.92 (0.63–1.34) in single-organ dysfunction patients in the treated group. The absolute risk reduction in treated patients was 1.7% in those with single-organ dysfunction, and 5.3%, 8.2%, and 10.6% in those with two, three, or four or more organ dysfunctions at baseline, respectively. There was no significant difference in the benefit of drotrecogin alfa (activated) as measured by odds ratios across these groups defined by the number of organ dysfunctions (p=0.769).

In MOD patients we observed numerically lower 28-day mortality rates with drotrecogin alfa (activated) across subgroups defined by age, gender, baseline heparin, baseline steroid exposure, underlying disease, recent surgical status, overt DIC status, ventilator and vasopres-

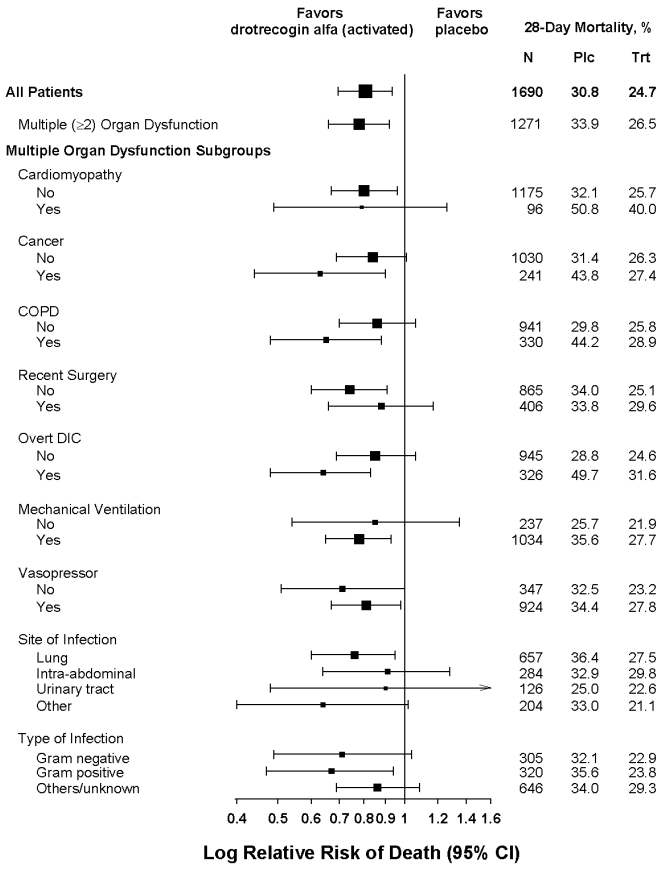

Fig. 3 The 28-day all-cause mortality in subgroups of multiple-organ dysfunction patients in the PROWESS trial. *Solid squares* Point estimate of relative risk of death in each subgroup; *horizontal lines* 95% confidence interval (*CI*). The overall PROWESS population is included for comparison. *Size of symbol* is proportional to the number of patients in each subgroup. *N* total number of patients in the subgroup: *Plc* placebo group; *Trt* treatment group [drotrecogin alfa (activated) group]

sor use, and site and type of infection (Figs. 2, and 3). Although treatment produced a smaller absolute risk reduction in mortality (3.4%) in patients aged under 50 years, this still translated into a 19% relative risk reduction in mortality when scaled by the lower risk of death these patients had at study entry (15% to 19%). There was a significant interaction between treatment and APACHE II score (p=0.009). We observed a higher 28-day mortality rate in treated patients relative to placebo in patients with APACHE II scores ranging from 3–19 (lowest severity; Fig. 2). One possible explanation for this result is that there was an age imbalance among patients with APACHE II scores of 3–19. In this subgroup there was a higher percentage of patients aged 65 years or over and those aged 75 years or over in the treatment group than in the placebo group (≥65 years 37% vs. 30%; ≥75 years 18% vs. 10%). In addition, the median age in the treated group with low APACHE scores was 6 years older than among placebo patients (58.6 vs. 52.7 years). There was a significant interaction between treatment and overt DIC status (p=0.046); however, based on the size of the subgroup concluding a greater treatment benefit in patients with overt DIC may be premature.

Resolution and development of organ dysfunction

In patients with MOD the time to resolution of organ dysfunction over the first 7 days was significantly shorter in the treated group than in the placebo group for cardiovascular and respiratory dysfunction (p=0.006 and 0.009; hazard ratios=1.2 and 1.5). At 7 days 61.2% of treated patients with cardiovascular dysfunction at baseline (n=593) had resolved compared with 54.2% of placebo patients with cardiovascular dysfunction at baseline (n=600). At 7 days 16.7% of treated patients with respiratory dysfunction at baseline (n=616) had resolved compared with 11.3% of placebo patients with respiratory dysfunction at baseline (n=609). Among MOD patients the resolution of renal, hepatic, and hematological organ dysfunction over the first 7 days was not significantly different between the groups (all p>0.3). Among patients with single-organ dysfunction the time to first resolution of organ dysfunction was not significantly different between the groups (all p>0.2).

In MOD patients without cardiovascular, respiratory, renal, hepatic, or hematological dysfunction at baseline the time to development of these organ dysfunctions was not different between treatment groups.

Biomarkers

In both MOD and single-organ dysfunction patients treated with drotrecogin alfa (activated) there was a 20% increase over baseline protein C activity by the end of study drug infusion, compared with a 10% increase in placebo patients (all days p≤0.01). Significant decreases from baseline in D-dimer levels were observed in both single (maximum decrease of 0.7 μg/ml at day 4) and MOD patients (maximum decrease of 1.0 μg/ml on day 4), compared with no change in D-dimer levels in placebo patients in each organ dysfunction group over the first 5 days (all p≤0.01). In the treated MOD patients there was a smaller decrease from baseline in D-dimer levels on day 1 (0.2 μg/ml) compared with day 2 and day 3 (0.6 μg/ml and 0.9 μg/ml). Significantly greater decreases from baseline in IL-6 levels were also observed over the first 7 days in the treated MOD patients than in placebo patients (decrease of 534 vs. 341 pg/ml at 7 days, all p≤0.01). In contrast, in single-organ dysfunction patients no difference in decrease in IL-6 levels was observed in treated and placebo patients.

Table 3 Adverse events in patients receiving drotrecogin alfa (activated) (*treatment*) and placebo in the PROWESS trial: overall and in subgroups with single-organ dysfunction (one organ dysfunction at study entry) and with multiple-organ dysfunction (two or more organ dysfunctions at study entry)

	Single-organ dysfunction					Multiple organ dysfunction					Odds ratio	95%CI
	Treatment (*n*=216)		Placebo (*n*=203)		*p*	Treatment (*n*=634)		Placebo (*n*=637)		*p*		
	n	%	*n*	%		*n*	%	*n*	%			
Bleeding events during 28-day study												
All bleeding events	51	23.6	35	17.2	0.117	161	25.4	114	17.9	0.001	1.56	1.19–2.05
Serious bleeding events	6	2.8	2	1.0	0.286	24	3.8	15	2.4	0.147	1.63	0.85–3.14
Bleeding events during infusion												
All bleeding events	42	19.4	25	12.3	0.061	118	18.6	66	10.4	0.0003	1.98	1.43–2.74
Serious bleeding events	5	2.3	0	0.0	0.062	15	2.4	8	1.3	0.147	1.91	0.80–4.53
All thrombotic events during 28-day study	27	12.5	29	14.3	0.59	69	10.8	86	13.6	0.15	0.78	0.56–1.10
Serious thrombotic events during 28-day study	4	1.9	4	2.0	1.00	13	2.1	21	3.3	0.223	0.61	0.31–1.24

Table 4 Bleeding events with baseline heparin in patients receiving drotrecogin alfa (activated) (*treatment*) and placebo in the PROWESS trial: in subgroups with and without baseline heparin

	Treatment				Placebo				p^a
	With heparin (*n*=398)		Without heparin (*n*=236)		With heparin (*n*=433)		Without heparin (*n*=204)		
	n	%	*n*	%	*n*	%	*n*	%	
Bleeding events during 28-day study									
All bleeding events	65	16.3	53	22.5	47	10.9	19	9.3	0.32
Serious bleeding events	12	3.0	12	5.1	9	2.1	6	2.9	0.78
Bleeding events during infusion									
Serious bleeding events	8	2.0	7	3.0	3	0.7	5	2.4	0.11

[a] Breslow-Day

Safety

Bleeding was the only serious adverse event (Table 3) associated with administration of drotrecogin alfa (activated). In both single-organ dysfunction and MOD patients there were more bleeding events in the treated group than in the placebo group. The majority of the bleeding events in the treated group occurred during infusion. No significant differences in serious bleeding event rates were found between the groups, although the event rates were low, thereby limiting the power for statistical inferences. A total of 831 (65%) of 1271 patients with MOD were exposed to the anticoagulant heparin at baseline [drotrecogin alfa (activated) *n*=398; placebo *n*=433]. Bleeding event rates in the presence or absence of baseline heparin are detailed in Table 4. Three fatal intracranial hemorrhages occurred in the trial [20]. All three of these events occurred in patients with MOD (two in the treated group, 0.3%; one in the placebo group, (0.16%).

In MOD patients the incidence of all thrombotic events during the 28-day study period was 10.9% in the treated group vs. 13.5% in the placebo group (p=0.15). There was also no significant difference in the incidence of serious thrombotic events during the 28-day study period between MOD patients in the two groups (2.1% vs. 3.3%, p=0.223).

Discussion

This contribution further characterizes the MOD and single-organ dysfunction subgroups of the PROWESS trial not provided in the original publication [20] or in the secondary paper devoted to morbidity [21], including the effects of drotrecogin alfa (activated) on mortality, de-

velopment and resolution of organ dysfunction, and biomarkers in these important subgroups. The European regulatory agency has approved the use of drotrecogin alfa (activated) only in patients with MOD. In addition, for the first time this contribution also provides important information about the concomitant use of heparin and steroids which could have potentially had an effect on the efficacy and safety of drotrecogin alfa (activated). A significant survival benefit at 28-days was shown for the drug in treating severe sepsis in patients in the larger subgroup of MOD patients but not in the smaller subgroup of single-organ dysfunction patients. The relative risk of death for patients with MOD treated with drotrecogin alfa (activated) was similar to that observed in the overall PROWESS trial [0.78 (0.66–0.92) vs. 0.80 (0.69–0.94)]. A 7.4% absolute risk reduction in 28-day all-cause mortality was observed in MOD patients compared with a 1.7% absolute reduction in 28-day mortality in patients with single-organ dysfunction. As was found in the overall PROWESS population [21], resolution of cardiovascular and respiratory organ dysfunction was significantly faster in the subgroup of treated MOD patients than in those receiving placebo. The only serious adverse event associated with drotrecogin alfa (activated) administration was bleeding, which occurred more often during the infusion period.

We found that patients with MOD were older, had higher APACHE II scores due to higher age and acute physiology points, and more frequent underlying diseases than patients with single-organ dysfunction. There were more patients with bacteremia, intra-abdominal infections, and recent surgery. These patients also had more severe procoagulant and inflammatory host response to infection, in agreement with other studies [29, 30, 31, 32]. Several studies have documented severity of disease, patient age above 65 years, and diagnosis of sepsis or infection at ICU admission as the three major risk factors for development of MOD [33, 34]. Our study provides data from a large cohort of severe sepsis patients with MOD that support these studies.

The relative risk of mortality observed with drotrecogin alfa (activated) with increasing number of organ dysfunctions remains relatively constant while the absolute reduction in mortality increases with increasing number of organ dysfunctions (Fig. 2). The presence of MOD as a means to identify appropriate patients for treatment with drotrecogin alfa (activated) may be more clinically relevant than assessment based on APACHE II score. APACHE II scores are not validated or intended for individual patient assessment [27] and could be inaccurate if not utilizing the most extreme values during the first 24 h of admission to an ICU.

Prophylactic dose heparin was allowed in the trial to prevent deep vein thrombosis. In patients receiving heparin at baseline the bleeding event rates were actually similar or numerically lower in both the treated and the placebo patients receiving heparin compared with the patients not receiving heparin, which may reflect the nonrandomized treatment with heparin at baseline. However, given the relatively small number of serious bleeding events we cannot totally exclude the possibility that the coadministration of heparin with drotrecogin alfa (activated), also an anticoagulant, can increase the bleeding risk. Although not statistically significant, the slightly lower relative risk reduction in 28-day mortality in patients who received both drotrecogin alfa (activated) and heparin compared with those who received drotrecogin alfa (activated) alone may raise the question of potential interaction between the two drugs. There is some data from in vitro studies that suggests enhanced protein C inhibitor activity in the presence of heparin [35], and we cannot exclude the possibility that heparin potentially reduces the efficacy of drotrecogin alfa (activated). The in vitro studies are not supported by the observation from the PROWESS trial that the pharmacokinetics of drotrecogin alfa (activated) in patients receiving or not receiving prophylactic-dose heparin were similar [36]. Results in subgroups based on the use of postbaseline heparin or steroids are not reported in this contribution due to the selection biases introduced. An example of selection bias would be present if heparin treatment appeared to be saving lives only because patients who lived longer had greater likelihood of eventually receiving heparin.

The pharmacodynamic effects of drotrecogin alfa (activated) in patients with MOD include a marked decrease in D-dimer during the infusion period related to its antithrombotic properties [19, 37], a more rapid improvement in protein C concentrations probably due to a lower endogenous protein C consumption when patients received drotrecogin alfa (activated), and a more rapid decline in IL-6 levels, a global marker of inflammation, consistent with a reduction in the inflammatory response [14]. The smaller decrease in D-dimer levels observed on the first day of infusion could be related to the profibrinolytic effect of activated protein C, which tends to increase D-dimer levels [38]. These results demonstrate the ability of drotrecogin alfa (activated) to modulate the inflammation and coagulopathy associated with the sepsis cascade and the development of MOD.

As in the overall PROWESS population [20], the only serious adverse event associated with the administration of drotrecogin alfa (activated) in patients with MOD was bleeding. The serious bleeding events were more frequent during the drug infusion period than during the placebo infusion. Although this difference did not reach statistical significance in the MOD subgroup, it may indeed have clinical significance, and therefore the potential for bleeding should be weighed carefully before administration of drotrecogin alfa (activated). The rate of fatal intracranial hemorrhages in MOD patients treated with the drug was

0.3% compared to 0.16% in placebo patients. Because patients at higher risk of bleeding were excluded in PROWESS [20], consideration of these exclusion criteria when selecting patients in clinical practice for drotrecogin alfa (activated) therapy is warranted. The exclusion criteria for bleeding risk as defined in the PROWESS study have been reflected as contraindications and warning in the relevant prescribing information.

Several limitations of this study should be noted. Clinical trials such as PROWESS are typically powered to detect an overall effect and are thus underpowered to make strong inferences about particular subgroups. In addition, the PROWESS trial was stopped early due to overwhelming efficacy, thereby further limiting the statistical power [39, 40, 41]. Analyses of subgroup results from a clinical trial may be misleading due to a combination of reduced statistical power, increased variance, multiplicity, and the play of chance [42]. For instance, the present study did not demonstrate a clear treatment effect in patients with single-organ dysfunction, although this group was not statistically different from patients with MOD. This may be related to either the real absence of treatment benefit or simply a lack of statistical power in this group. An additional limitation of this study is related to classification of patients by APACHE II score. APACHE II scores were recorded as the most extreme values in the 24 h prior to administration of drug rather than the most extreme values observed on admission of the patient to the ICU. For patients enrolled in the study 2 or more days following ICU admission the APACHE II score may not have encompassed the period of resuscitation, which could result in severely ill patients with relatively low APACHE II scores. Finally, apparent differences arise between the results of the survival curve comparisons and the relative risk results because the number at risk in the survival curves decreases over time yielding time-dependent mortality estimates, while the 28-day relative risk approach assumes a single risk set which is evaluated at only one postbaseline time point.

In summary, survival benefit, improvements in markers of coagulation and inflammation, and earlier resolution of cardiovascular and respiratory organ dysfunction were observed in adults with severe sepsis with MOD treated with drotrecogin alfa (activated). The survival benefits of its administration in patients with MOD balance quite favorably against the associated bleeding risks with treatment.

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