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Safety and Efficacy of Human Chorionic Gonadotropin Hormone-Derivative EA-230 in Cardiac Surgery Patients: A Randomized Double-Blind Placebo-Controlled Study

Roger van Groenendael, MD[1–4]
Remi Beunders, MD[1–3]
Pleun Hemelaar, MSc[1,2]
Jan Hofland, MD, PhD[4]
Wim J. Morshuis, MD, PhD[5]
Johannes G. van der Hoeven, MD, PhD[1–3]
Jelle Gerretsen, BSc[1,2]
Gert Wensvoort, DVM, PhD[6]
Emma J. Kooistra, MD[1–3]
Wout J. Claassen, MD[1]
Denise Waanders, BSc[1]
Maud G. A. Lamberts, BSc[1]
Leonie S. E. Buijsse, BSc[1]
Matthijs Kox, PhD[1–3]
Lucas T. van Eijk, MD, PhD[2–4]
Peter Pickkers, MD, PhD[1–3]

OBJECTIVES: To determine the safety and efficacy of human chorionic gonadotropin hormone-derivative EA-230 in cardiac surgery patients. Cardiac surgery induces systemic inflammation and may impair renal function, affecting patient outcome. EA-230 exerted immunomodulatory and renoprotective effects in preclinical models and was safe and showed efficacy in phase I and II human studies.

DESIGN: Double-blinded, placebo-controlled, randomized study.

SETTING: Collaboration of the Cardiothoracic Surgery, Anesthesiology, and the Intensive Care departments of a tertiary hospital in the Netherlands.

PATIENTS: One hundred eighty patients undergoing an on-pump coronary artery bypass procedure with or without concomitant valve surgery.

INTERVENTIONS: Ninety mg/kg/hr EA-230 or placebo administered during surgery.

MEASUREMENTS AND MAIN RESULTS: During the study, no safety concerns emerged. EA-230 did not modulate interleukin-6 plasma concentrations (area under the curve 2,730 pg/mL × hr [1,968–3,760] vs 2,680 pg/mL × hr [2,090–3,570] for EA-230 and placebo group, respectively; $p = 0.80$). Glomerular filtration rate increased following surgery (mean ± SEM increase in the EA-230 vs placebo groups: glomerular filtration rate$_{iohexol}$ measured using iohexol plasma clearance: 19 ± 2 vs 16 ± 2 mL/min/1.73 m^2; $p = 0.13$ and estimated glomerular filtration rate with the Modification of Diet in Renal Disease equation using creatinine: 6 ± 1 vs 2 ± 1 mL/min/1.73 m^2; $p = 0.01$). The "injury" stage of the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease criteria for acute kidney injury was 7% in the EA-230 group versus 18% in the placebo group ($p = 0.07$). In addition, EA-230-treated patients had a less positive fluid balance compared with placebo-treated patients (217 ± 108 vs 605 ± 103 mL; $p = 0.01$), while the use of vasoactive agents was similar in both groups ($p = 0.39$). Finally, hospital length of stay was shorter in EA-230 treated patients (8 d [7–11] vs 10 d [8–12]; $p = 0.001$). Efficacy results were more pronounced in patients that had longer duration of surgery and thus longer duration of study drug infusion.

CONCLUSIONS: EA-230 was safe in patients undergoing on-pump cardiac surgery. It did not modulate interleukin-6 plasma concentrations but appeared to exert beneficial renal and cardiovascular effects and shortened in-hospital length of stay.

KEY WORDS: acute kidney injury; cardiac surgery; EA-230; immunomodulation; safety; systemic inflammation

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Systemic activation of the immune system, as a result of pronounced (surgery-induced) tissue injury or infection, affects most critically ill patients admitted to the ICU (1–4). It is well recognized that a dysregulated systemic inflammatory response is associated with organ dysfunction (5, 6), as exemplified by the relationship between cytokine levels and the occurrence and severity of acute kidney injury (AKI) (7–9). To date, no pharmacologic interventions are licensed to limit systemic inflammation or protect renal function (10–13).

Open-heart surgery is performed in over 2 million patients annually worldwide (14). This procedure induces a systemic inflammatory response (3) and is associated with an approximate 20% risk of developing AKI (11, 15, 16). Activation of blood components by the use of cardiopulmonary bypass (CPB), surgically induced tissue damage, ischemia-reperfusion injury following aortic cross-clamping and endotoxemia due to translocation of gut bacteria play a role in the activation of the immune system, resulting in increased capillary permeability, loss of vascular tone, and organ tissue injury (3, 17–19).

Immunotolerance during pregnancy allows for the improbable symbiosis of two major histocompatibility complex-incompatible individuals (20, 21). Remarkably, several autoimmune diseases improve in pregnant women without increasing susceptibility to develop infections (22–25). In addition, the glomerular filtration rate (GFR) increases (26). This favorable immune-tolerant phenotype and increase in GFR observed during pregnancy has been attributed to the human chorionic gonadotropin (hCG) hormone and has led to the discovery of EA-230, a hCG-derived linear tetrapeptide. Treatment with EA-230 attenuated pro-inflammatory cytokine release, prevented organ dysfunction and renal injury, and improved survival in several animal models of systemic inflammation (27–33). Furthermore, EA-230 mitigated renal injury and improved survival in murine models of renal transplantation and renal ischemia-reperfusion injury (34–36). Finally, EA-230 was shown to be well tolerated in phase 1 studies (37) and attenuated the release of inflammatory mediators during experimental human endotoxemia (38).

The purpose of the current phase 2 clinical study was to determine the safety and immunomodulatory and renoprotective effects of EA-230 in cardiac surgery patients.

MATERIALS AND METHODS

Study Design and Participants

This first inpatient randomized, double-blind, placebo-controlled phase 2 study was conducted in patients undergoing elective on-pump cardiac surgery. The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki and in compliance with the International Conference on Harmonisation E6 Guideline for Good Clinical Practice (CPMP/ICH/135/95), the Dutch Medical Research Involving Human Subjects Act, and the European Directive (2001/20/CE). The study protocol, including a detailed statistical analysis plan, was published previously (39) (full protocol in **Methods S5**, http://links.lww.com/CCM/G113).

For safety reasons, the study was conducted in two phases. After inclusion of 60 (low-risk) patients in the first phase (criteria listed in **Methods S1**, http://links.lww.com/CCM/G113), enrollment was interrupted for an independent interim safety analysis by the Data Safety Management Board (DSMB). Enrollment of both low- and high-risk patients was continued in the second phase of the study. Following inclusion of 90 patients, the DSMB conducted a second safety analysis and an early efficacy analysis and recalculated the sample size with intrinsic study data according to the adaptive trial design (summary in **Methods S2**, http://links.lww.com/CCM/G113).

Patients older than 18 years scheduled for elective coronary artery bypass grafting (CABG) procedure, with or without valve surgery, with use of CPB were eligible for participation. A standardized protocol was used for the surgical procedure and anesthetic management. Patients that were immune-compromised were excluded (procedural protocol details and full in- and exclusion criteria in Methods S1, http://links.lww.com/CCM/G113).

Randomization and Intervention

Patients were randomized in a 1:1 ratio to receive either EA-230 (90 mg/kg/hr throughout the surgical procedure) or placebo (sodium chloride with identical osmolality, appearance, and texture as the EA-230 solution), both manufactured by HALIX BV (Leiden, The Netherlands) and provided by the study sponsor. Study drug was administered from surgical incision until the end of CPB with a maximum of 4 hours of infusion aimed to cover the period during which the inflammatory insults originated. This dosage exerted significant

immunomodulatory effects during experimental human endotoxemia (38). A stratified randomization procedure was conducted using the GCP-approved data management software Castor EDC (Amsterdam, The Netherlands) to equally distribute patients according to: 1) a CABG procedure with or without valve surgery; 2) preoperative renal function with an estimated GFR of less than or equal to 30, 31–90, or greater than 90 mL/min/1.73 m^2; and 3) a EuroSCORE II of less than 4 or greater than or equal to 4 (40). Apart from the intervention, all patients received clinical care as usual. All attending physicians, study personnel, and patients were blinded to treatment allocation (detailed study and blinding procedures are described in Methods S5, http://links.lww.com/CCM/G113).

Safety

The primary endpoint was safety and tolerability of EA-230, assessed by monitoring of: 1) vital signs: heart rate and blood pressure during postoperative ICU admission, 2) routine laboratory variables until the first postoperative day, and 3) frequency of (serious) adverse events ([S]AEs) and suspected unexpected serious adverse reactions until day 90 post-surgery. Only treatment-emergent SAEs are presented. Furthermore, major clinical adverse events (MCAEs) related to cardiac surgery were registered: cerebrovascular accidents, myocardial infarction, rethoracotomy, hospital readmission, and pleural and/or pericardial puncture.

Efficacy—Inflammation

Immunomodulatory effects of EA-230 were quantified by comparing the area under the plasma concentration-time effect curves (AUECs) of interleukin (IL)-6 from pre-surgery until the first postoperative day. In addition, other inflammatory mediators (IL-8, IL-10, IL-17, tumor necrosis factor-α, monocyte chemoattractant protein-1, IL-1 receptor antagonist, macrophage inflammatory protein [MIP]-1α, MIP-1β, intercellular adhesion molecule-1, and vascular cell adhesion protein-1), leukocyte counts, body temperature, and insulin sensitivity were determined (detailed in **Methods S3**, http://links.lww.com/CCM/G113).

Efficacy—Renal

Effects of EA-230 on renal function were assessed by determining the GFR using plasma clearance of iohexol ($GFR_{iohexol}$) (41), plasma concentrations of creatinine and the Modification of Diet in Renal Disease (MDRD) equation to estimate GFR (eGFRGFR$_{MDRD}$), and creatinine clearance to calculate the endogenous creatinine clearance (GFR_{ECC}) (42). Furthermore, the occurrence rate of different stages of AKI was registered during hospital admission according to the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) criteria (43). Finally, urinary excretion of creatinine, urea, and tubular injury markers was determined serially (detailed in Methods S3, http://links.lww.com/CCM/G113).

Efficacy—Cardiovascular

Cardiopulmonary variables (vasoactive or inotropic agent requirement, expressed as the "inotropic score" during ICU admission [44], fluid balance, and the pulmonary alveolar-arterial [A-a] gradient) were determined.

Efficacy—General Clinical Effects

General patient outcome measures were disease severity with the Acute Physiology and Chronic Health Evaluation (APACHE) IV and Sequential Organ Failure Assessment (SOFA) score, the ICU and hospital length of stay, and 90-day mortality.

Sample Size Calculation and Statistical Analysis

Efficacy outcome variable IL-6 was used for the sample size calculations using data of a previous study (38) (detailed in **Methods S4**, http://links.lww.com/CCM/G113). Early efficacy analysis and a final sample size recalculation were performed halfway the study by the DSMB, according to the adaptive study design (detailed in Methods S2, http://links.lww.com/CCM/G113). The study team was blinded to these interim analyses.

Continuous variables are presented as mean with SEM, or median with interquartile range (IQR), depending on their distribution (determined by Shapiro-Wilk tests). Geometric mean and 95% CI are used for the *inotropic score*. Measurements over time were analyzed using repeated measures two-way analysis of variance (on log-transformed data in case of non-normally distributed data). Other data were tested using Student *t* tests or Mann-Whitney *U* tests, for parametric and

nonparametric data, respectively. Categorical data are presented as frequencies and percentages and were analyzed using Pearson chi-squared tests or Fisher exact tests. Time to event data were analyzed using log-rank tests.

The intention-to-treat population consisted of all patients who received study medication and their safety analyses were reported. The per-protocol population consisted of all patients without any major protocol deviation during the study and their efficacy analyses were reported. The subgroups created by stratified randomization were analyzed separately.

Post hoc analyses on immunomodulating, renoprotective, and cardiovascular effects of EA-230 were performed in patients with below and above median durations of surgery, as duration of surgery induces a more pronounced inflammatory response and also implies longer duration of study drug administration.

Statistical analyses were conducted using SAS Version 9.3 (SAS, Cary, NC) and SPSS Version 25 (IBM, Armonk, NY).

RESULTS

One-hundred eighty patients were enrolled between July 2016 and November 2017. Ninety-one patients were randomized to receive EA-230 and 89 to receive placebo treatment (**Fig. 1**). In one patient, the surgeon decided to perform an off-pump procedure after sternotomy was performed. This patient was therefore considered a secondary exclusion and followed-up for safety analysis only. Stratified randomization equally distributed patients between treatment groups (**Table 1** and Fig. 1), results of these strata subgroups are summarized in **Table S1** (http://links.lww.com/CCM/G114). No major protocol deviations occurred and double-blind conditions were maintained at all times. Baseline characteristics are listed in Table 1.

Safety

A total of 500 AEs were reported. In the EA-230 group, 217 AEs and 13 SAEs occurred, compared with 283 AEs and 19 SAEs in the placebo group. There were no (S)AEs judged "probably" or "definitely" related to the study drug. Eleven patients treated with EA-230 (11%) experienced a MCAE compared with 15 patients (17%) of the placebo group. Routine laboratory variables and vital signs were similar between treatment groups. Safety results are listed in **Table 2** (primary) and **Table S2** (http://links.lww.com/CCM/G114) (detailed).

Efficacy—Inflammation

AUEC plasma IL-6 concentrations were not different between the EA-230 and placebo groups (median [IQR] AUEC of 2,730 pg/mL × hr [1,968–3,760] and 2,680 pg/mL.× hr [2,090–3,570] in the EA-230 and placebo groups, respectively; $p = 0.80$; **Fig. 2*A***). Differences between treatment groups within subgroups were not significant (EA-230 vs placebo in short [$p = 0.88$] and long duration of surgery [$p = 0.41$]; **Fig. 3**). No effect of EA-230 on other circulating inflammatory mediators was observed (**Figs. S1** and **S2**, http://links.lww.com/CCM/G114). Furthermore, no difference between treatment groups in leukocyte count, body temperature, A-a gradient, insulin dose, and glucose concentrations was observed (Table 2; and **Fig. S3**, http://links.lww.com/CCM/G114).

Efficacy—Renal

In both groups, the $GFR_{iohexol}$ was higher on the first postoperative day compared with the day before surgery; this increase was not different between treatment groups (mean ± SEM delta of 19 ± 2 mL/min/1.73 m^2 and 16 ± 2 mL/min/1.73 m^2 in the EA-230 and placebo group, respectively; $p = 0.13$; **Fig. 2*B***). Nevertheless, higher urinary creatinine and urinary urea concentrations were observed in the EA-230 group compared with the placebo group ($p = 0.03$ and 0.004, respectively) (Table 2; and **Fig. S4**, http://links.lww.com/CCM/G114), corresponding with a decrease in plasma creatinine concentrations observed in the EA-230 group, but not in the placebo group (mean ± SEM delta of –6 ± 1 μmol/L vs +1 ± 2 μmol/L in the EA-230 and placebo group, respectively; $p = 0.003$) (Fig. S4, http://links.lww.com/CCM/G114). This difference remained statistically significant over the first 7 postoperative days ($p = 0.02$) (Fig. S4, http://links.lww.com/CCM/G114). In accordance with the latter results, a significant difference between treatment groups in $eGFR_{MDRD}$ was found from baseline until the first postoperative day (mean ± SEM delta of 6 ± 1 vs 2 ± 1 mL/min/1.73 m^2 in the EA-230 and placebo group, respectively; $p = 0.01$; Fig. 2*B*). For patients with a longer duration of surgery, treatment with EA-230 augmented both

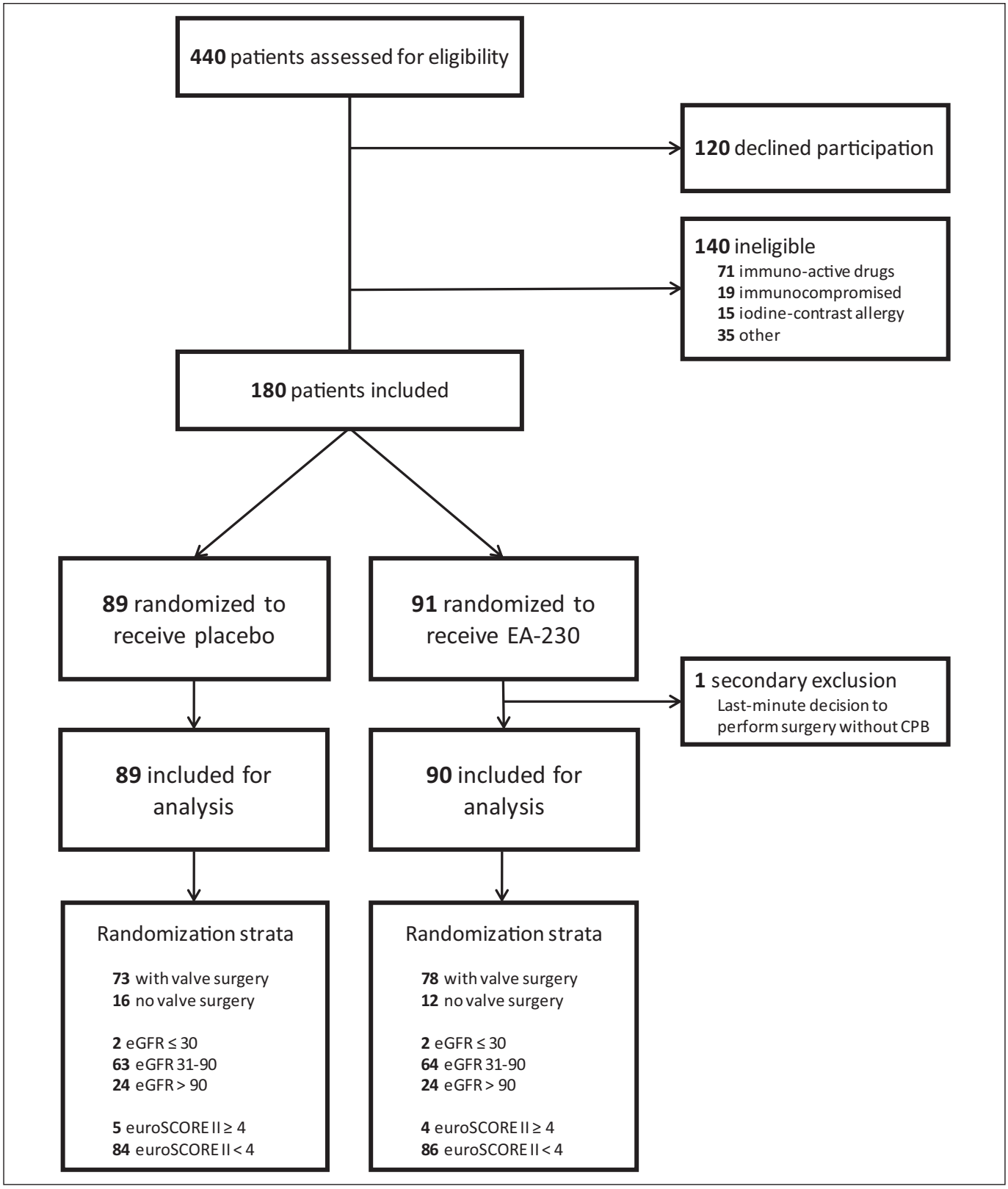

Figure 1. Study flowchart. CPB = cardiopulmonary bypass, eGFR = estimated glomerular filtration rate.

the increase in $GFR_{iohexol}$ ($p = 0.02$) and $eGFR_{MDRD}$ ($p = 0.0002$) compared with placebo, whereas no difference between treatment groups for patients with a short duration of surgery was observed ($GFR_{iohexol}$ $p = 0.47$ and $eGFR_{MDRD}$ $p = 0.27$) (Fig. 3). Urine output was similar between treatment groups ($p = 0.30$) (Table 2

TABLE 1.
Demographics, Baseline Disease Characteristics, and Stratification Groups

Characteristic	EA-230 ($n = 90$)	Placebo ($n = 89$)	p
Age (yr)	67 ± 0.9	68 ± 1.0	0.40
Gender, *n* (%)			0.63
Male	80 (51)	77 (49)	
Female	10 (46)	12 (54)	
Weight (kg)	86 ± 2	87 ± 2	0.89
Height (cm)	175 ± 1	176 ± 1	0.61
Body mass index (mass/height^2)	28 ± 0.4	28 ± 0.4	0.87
EuroSCORE II	1.6 ± 0.1	1.8 ± 0.1	0.39
Study drug treatment duration (min)	156 ± 4	153 ± 4	0.65
Below median duration (min)	126 ± 2	124 ± 2	0.46
Above median duration (min)	183 ± 4	187 ± 5	0.59
Cardiopulmonary bypass duration (min)	95 ± 3.8	95 ± 4.4	0.99
Aortic clamping duration (min)	57 ± 2.6	57 ± 3.2	0.97
Kidney function			
GFR using plasma clearance of iohexol (mL/min/1.73 m^2)	79 ± 1.9	78 ± 1.9	0.67
GFR with the Modification of Diet in Renal Disease (mL/min/1.73 m^2)	79 ± 2.3	78 ± 18	0.62
Plasma creatinine (μmol/L)	88 ± 2.9	87 ± 2.2	0.72
Subgroup stratification			
Valve surgery, *n* (%)			0.39
Yes	78 (52)	73 (48)	
No	12 (43)	16 (57)	
Estimated GFR pre-surgery, mL/min/1.73 m^2, *n* (%)			1.00
≤ 30	2 (50)	2 (50)	
31–90	64 (50)	63 (50)	
> 90	24 (50)	24 (50)	
EuroSCORE II, *n* (%)			0.72
≥ 4	4 (44)	5 (56)	
< 4	86 (51)	84 (49)	

GFR = glomerular filtration rate.
Data are presented as mean ± SD or count (%).
p values calculated using Student *t* tests or Pearson chi-squared tests.

TABLE 2.
Safety and Efficacy Endpoints

Intention-to-Treat Population	EA-230 ($n = 91$)	Placebo ($n = 89$)	p
Safety			
TE adverse events, number of events (n [%])	217 ($n = 78$ [86])	218 ($n = 81$ [91])	–
TE serious adverse events, number of events (n [%])	13 ($n = 12$ [13])	19 ($n = 17$ [19])	–
Suspected unexpected serious adverse reactions, number of events (n [%])	0 ($n = 0$ [0])	1 ($n = 1$ [1])	–
Major clinical adverse event related to cardiac surgery, number of events (n [%])	11 ($n = 11$ [12])	15 ($n = 15$ [17])	–
Per-Protocol Population	EA-230 ($n = 90$)	Placebo ($n = 89$)	
Efficacy–inflammatory			
Interleukin-6 (peak in pg/mL)	189 [141–293]	213 [154–287]	0.99[a]
Leukocyte numbers (peak in $\times 10^9$/L)	13 [11–16]	13 [10–16]	0.25[a]
Body temperature (peak in °C)	37.1 ± 0.06	37.4 ± 0.06	0.08[a]
Insulin dose (total units during the first 24 hr of ICU admission)	3.3 [0–25]	0 [0–26]	0.77[a]
Glucose (peak in mmol/L)	9.0 ± 0.16	9.1 ± 0.16	0.58[a]
Efficacy–renal			
GFR using plasma clearance of iohexol (change from day before surgery to POM in mL/min/1.73 m^2)	19 ± 1.2	16 ± 2	0.13[a]
GFR_{MDRD} (change from day before surgery to POM in mL/min/1.73 m^2)	6 ± 1	2 ± 1	0.01[a]
Occurrence rate of acute kidney injury, Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease criteria stage "injury"	6 (7)	16 (18)	0.07[b]
Plasma creatinine (change from day before surgery to POM in µmol/L)	–5.6 ± 1.1	1.1 ± 1.9	0.003[a]
Plasma creatinine (peak during POD 1–7 in µmol/L)	82 ± 5	96 ± 11	0.02[a]
GFR_{MDRD} (lowest during POD 1–7 in mL/min/1.73 m^2)	86 ± 5	80 ± 5	0.66[a]
GFR to calculate endogenous creatinine clearance (change from day before surgery to POM in mL/min/1.73 m^2)	106 ± 4	104 ± 5	0.70[a]
Urine output (total mL during the first 24 hr in ICU)	1,815 [1,439–2,123]	1,685 [1,433–2,080]	0.30[a]
Urinary creatinine (nadir in mmol/L)	4.1 [3.2–5.7]	3.7 [2.6–5.5]	0.03[a]
Urinary urea (peak in mmol/L)	150 [112–189]	111 [81–144]	0.004[a]

(*Continued*)

TABLE 2. (*Continued*).
Safety and Efficacy Endpoints

Intention-to-Treat Population	EA-230 ($n = 91$)	Placebo ($n = 89$)	p
Efficacy—cardiovascular			
Net fluid balance (total mL during the first 24 hr of ICU admission)	787 ± 109	1,080 ± 117	0.98[a]
Fluid therapy (total mL during the first 24 hr of ICU admission)	3,272 [2,762–6,174]	3,423 [3,023–3,986]	0.84[a]
Drain production (total mL during the first 24 hr of ICU admission)	704 [558–913]	765 [578–932]	0.79[a]
Alveolar-arterial gradient (change from ICU admission to POM)	−9.7 ± 1.2	−8.6 ± 1.4	0.78[a]
Efficacy—general			
Length of stay in ICU (in hr)	21 [19–23]	22 [19–24]	0.02[c]
Length of stay in hospital (in hr)	195 [171–265]	234 [192–295]	0.001[c]
Sequential Organ Failure Assessment (change in score from ICU admission to POM)	−1 [−2 to 1]	−1 [−2.5 to 0]	0.35[a]
Acute Physiology and Chronic Health Evaluation IV (score at ICU admission)	53 ± 2	56 ± 2	0.26[d]

GFR = glomerular filtration rate, GFR_{MDRD} = glomerular filtration rate with the Modification of Diet in Renal Disease, POD = postoperative day, POM = postoperative morning, TE = treatment-emergent.

[a]*p* values calculated using repeated measures two-way analysis of variance (interaction term) on plasma concentration-time effect curves (depicted in **supplemental material**, http://links.lww.com/CCM/G114).

[b]*p* values calculated using Pearson chi-squared test.

[c]*p* values calculated using log-rank test.

[d]*p* values calculated using Mann-Whitney *U* tests or Student *t* tests, depending on the distribution of the data.

No statistical testing was performed on safety data. In Table S2 (http://links.lww.com/CCM/G114), safety data categorized on severity and organ class is reported.

Data are presented as mean ± SEM or median [interquartile range] or frequency (percentage).

and **Fig. S5**, http://links.lww.com/CCM/G114). Considering AKI, the RIFLE stage "no AKI" was present in 50 patients (56%) of the EA-230 group and 42 patients (47%) of the placebo group, while stage "injury" was observed in six patients (7%) of the EA-230 group and 16 patients (18%) of the placebo group ($p = 0.07$; Fig. 2*B*). No significant differences in urinary excretion of tubular injury markers were observed between the two groups (**Fig. S6**, http://links.lww.com/CCM/G114).

Efficacy—Cardiovascular

The net fluid balance was significantly lower in the EA-230 group compared with the placebo group on the first postoperative day (mean ± SEM of 217 ± 108 vs 605 ± 103 mL, respectively; $p = 0.01$) (**Fig. 2***C* and Fig. S5, http://links.lww.com/CCM/G114), whereas vasopressor and/or inotropic therapy requirement (*inotropic score*) was not statistically different between groups (Fig. S5, http://links.lww.com/CCM/G114). In patients with a longer duration of surgery, treatment with EA-230 resulted in a significantly lower net fluid balance on the first postoperative day ($p = 0.008$) and a significantly lower *inotropic score* ($p = 0.048$) compared with placebo (Fig. 3). These effects were not observed in patients with a shorter duration of surgery (net fluid balance: $p = 0.29$ and *inotropic score*: $p = 0.46$; Fig. 3).

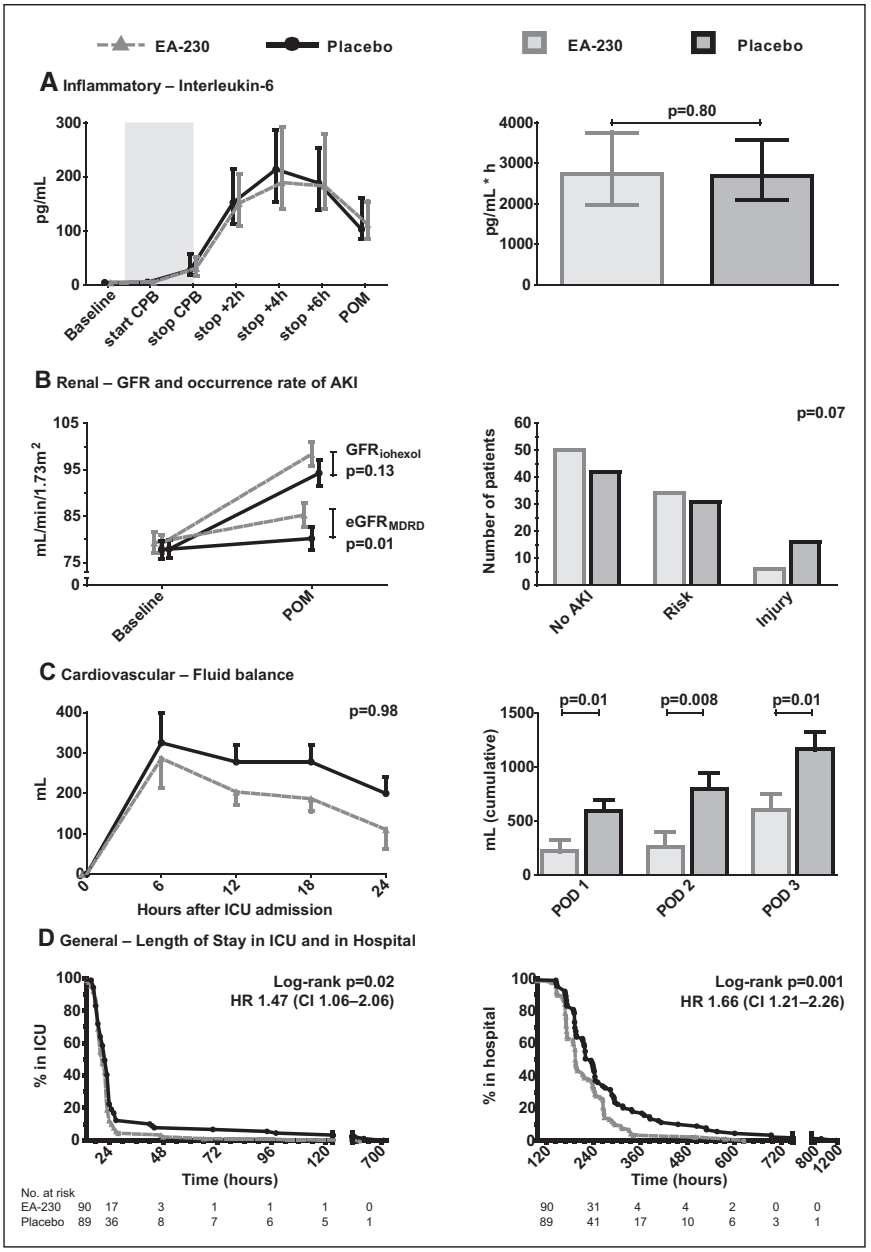

Figure 2. Efficacy endpoints. **A**, Inflammatory. *Left*: Plasma concentrations of interleukin (IL)-6 over time from preoperative time point (baseline) until the next postoperative morning (POM) ($p = 0.99$). *Blue box* indicates the period in which study drug was administered. *Right*: Area under the plasma concentration-time effect curve of IL-6. Data presented as median and interquartile range. *p* values calculated using repeated measures two-way analysis of variance (interaction term, *left*) or Mann-Whitney *U* test (*right*). **B**, Renal. *Left*: Renal function expressed as glomerular filtration rate using plasma clearance of iohexol ($GFR_{iohexol}$) and estimated glomerular filtration rate using the Modification of Diet in Renal Disease equation ($eGFR_{MDRD}$) from the day before surgery (baseline) until the next POM. Data presented as mean and SEM. *p* values calculated using repeated measures two-ay analysis of variance (interaction term). *Right*: Classification of acute kidney injury according to the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) criteria; patients were classified as "no acute kidney injury" ($n = 50$ in the EA-230 group, $n = 42$ in the placebo group), "Risk" ($n = 34$ in the EA-230 group, $n = 31$ in the placebo group), or "Injury" ($n = 6$ in the EA-230 group, $n = 16$ in the placebo group), no patients were classified as stage "Failure," "Loss of function," or "End-stage of renal disease." Data presented as percentages of patients. *p* value calculated using Pearson chi-squared test. **C**, Cardiovascular. *Left*: Net fluid balance during the first 24 hr after ICU admission ($p = 0.97$). *Right*: Cumulative postoperative net fluid balance on postoperative day (POD) 1 ($n = 90$ in the EA-230 group and $n = 89$ in the placebo group), on POD 2 ($n = 90$ in the EA-230 group and $n = 89$ in the placebo group), and on POD 3 ($n = 86$ in the EA-230 group and $n = 85$ in the placebo). POD 4–7 not depicted due to few available data. Data presented as mean and SEM. *p* values calculated using repeated measures two-way analysis of variance (interaction term, *left*) or Student *t* tests (*right*). **D**, General. *Left*: Length of stay in the ICU ($p = 0.02$). *Right*: Length of stay in the hospital ($p = 0.001$). *p* values calculated using log-rank test. CPB = cardiopulmonary bypass, HR = hazard ratio (the event is discharge).

Efficacy—General Clinical Effects

Both the ICU ($p = 0.02$) and hospital ($p = 0.001$) length of stay was shorter in patients treated with EA-230 compared with the placebo group (**Fig. 2***D*). After 24 hours, 12% of patients treated with EA-230 were still in the ICU compared with 23% of placebo-treated patients. The median [IQR] hospital length of stay was 8 days [7–11] and 10 days [8–12] in the EA-230 and placebo group, respectively. EA-230 exerted no significant effect on APACHE IV score at ICU admission ($p = 0.29$) and on SOFA scores from ICU admission to the first postoperative day ($p = 0.49$) (Table 2 and **Fig. S7**, http://links.lww.com/CCM/G114). All patients survived until end-of-study, 90 days post-surgery.

DISCUSSION

In patients following cardiac surgery with CPB, treatment with EA-230 was well tolerated and no safety issues emerged. These results are similar to those obtained in healthy volunteers in the absence (37) and presence (38) of systemic inflammation. Treatment with EA-230 did not influence the immune response, but beneficial effects on renal and cardiovascular variables, and the ICU and hospital length of stay were observed.

The absence of immunomodulatory effects of EA-230 in the present work is in contrast to results obtained in several animal studies (28–31, 33) and a previous experimental human endotoxemia study (38). In the latter study, treatment with EA-230 attenuated the endotoxin-induced increase of IL-6 and several other pro-inflammatory cytokines and resulted in significantly less pronounced fever and flu-like symptoms. Several differences between endotoxin- and cardiac surgery-induced inflammation can be put forward to explain this discrepancy. First, endotoxin administration results in activation of a single Toll-like receptor 4-mediated inflammatory pathway only, whereas several other inflammatory pathways are activated in cardiac surgery patients. In the latter, surgical damage and stress result in the release of a multitude of danger-associated molecular patterns, ischemia-reperfusion injury, hemorrhagic shock, and possibly translocation of (components of) microorganisms from the gut to the circulation (3, 17–19). Nevertheless, previous animal studies have demonstrated immunomodulatory effects of EA-230 in various types of inflammation, including models of ischemia-reperfusion injury (34–36), and hemorrhagic shock (31). These models recapitulate distinct aspects of the inflammatory response observed following cardiac surgery and therefore may render this explanation for the absence of immunomodulatory effects in the current study less plausible. Second, the endotoxin-induced inflammatory response is short-lived, illustrated by a sharp but swift increase in plasma IL-6 concentrations peaking at 2 hours following endotoxin administration (38), whereas cardiac surgery elicits a much more protracted and more limited inflammatory response (IL-6 peaks approximately 6 hr after the start of surgery). Typically, timing of EA-230 administration in the endotoxin study overlapped with the peak endotoxin-induced IL-6 response, where in the present study, the peak IL-6 response emerged only at around 6 hours. As dosing of EA-230 was limited to the duration of cardiac surgery (aimed to cover the period the inflammatory insult originated), peak IL-6 levels did not overlap with EA-230 administration. Also, as the peak concentration was lower compared with that observed in the endotoxemia study, the inflammatory response following open-heart surgery may not be pronounced enough to detect an immunomodulating effect of EA-230 administered early during surgery.

Renal function measured using the clearance of iohexol was augmented on the first postoperative day in both groups, an increase in GFR that was not influenced by treatment with EA-230. In contrast, EA-230 treatment increased urinary excretion of creatinine and lowered plasma creatinine levels, resulting in improvements of creatinine-based GFR estimations. It is important to realize that the GFR determined by the clearance of iohexol over 4 hours reflects the GFR during that specific period, while plasma creatinine-based estimations reflect the GFR during the 12–24 hours before plasma creatinine measurement. Therefore, the timing of the iohexol-based GFR measurement is crucial and could have either been too early or too late to detect a possible effect of EA-230 on renal function. Furthermore, analysis based on AKI staging suggests that patients treated with EA-230 may be less likely to develop AKI. Based on these data, treatment with EA-230 appears to exert beneficial effects on early changes in renal function following cardiac surgery. Of interest, in patients with more prolonged surgery, a more pronounced effect of EA-230 on renal function was observed (in both $GFR_{iohexol}$ and $eGFR_{MDRD}$), suggesting that the duration of EA-230 infusion is of importance for its therapeutic efficacy.

Treatment with EA-230 resulted in a significant reduction of the positive postoperative fluid balance, importantly without the need for higher doses of, or longer treatment with vasopressor or inotropic agents. Again, in patients with more prolonged surgery, these effects of EA-230 were more pronounced; differences in net fluid balance were more pronounced and less need for vasopressor therapy reached statistical significance compared with the control group. Increasing evidence suggests that reduction of the positive postoperative fluid balance improves patient outcome in both critically ill and surgical ICU patients (45, 46).

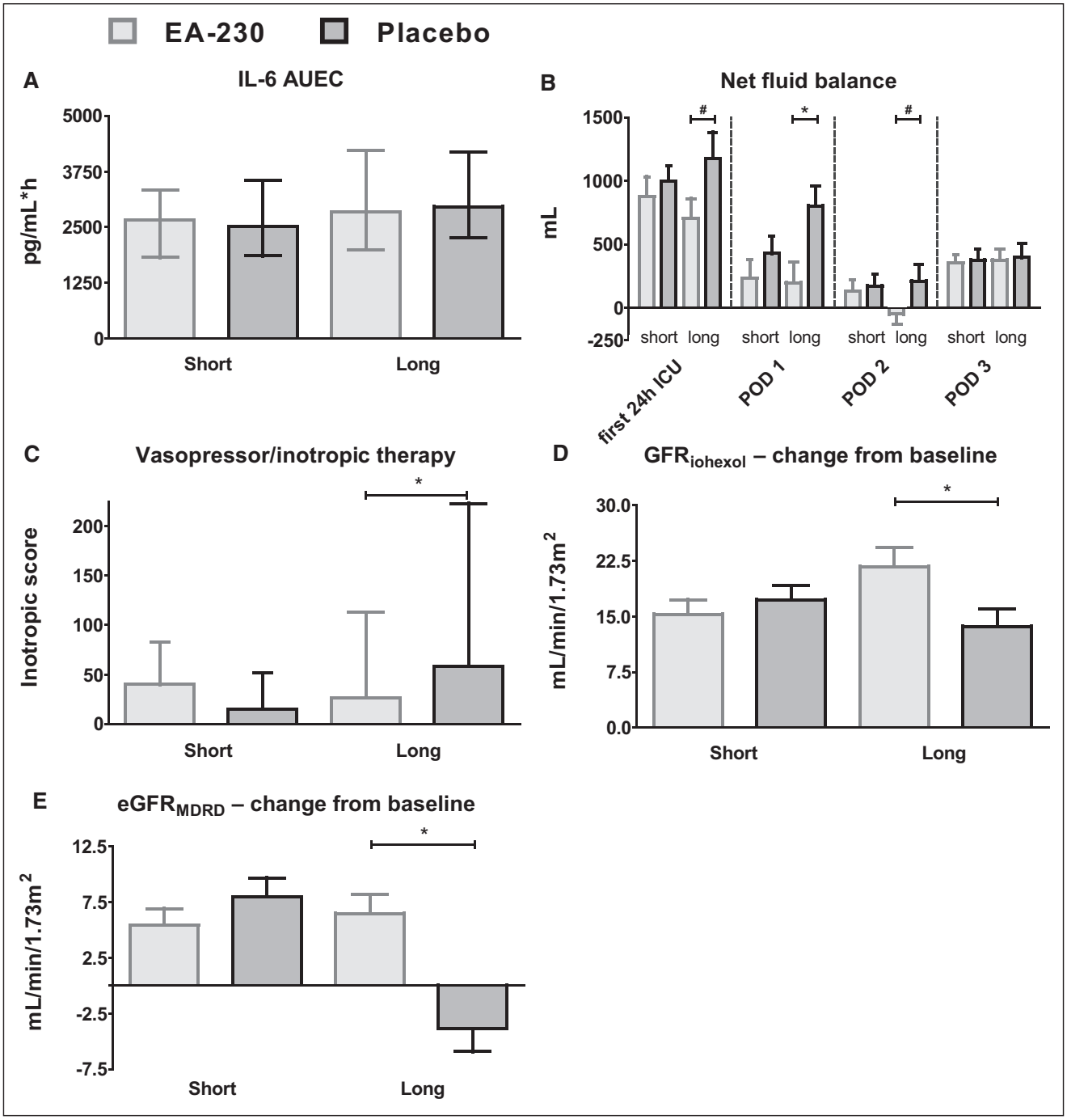

Figure 3. Post hoc analyses using the subgroups short ($n = 90$) and long ($n = 89$) surgery duration (divided using median). **A**, Area under the plasma concentration-time effect curve (AUEC) of interleukin (IL)-6 plasma concentrations tested between treatment groups (short: EA-230 vs placebo: $p = 0.88$ and long: EA-230 vs placebo: $p = 0.41$). Data presented as median and interquartile range (IQR). **B**, Net fluid balance per day (short: $p = 0.54$, $p = 0.33$, $p = 0.75$, and $p = 0.84$ for first ICU day, postoperative day [POD] 1, POD 2, and POD 3, respectively. Long: $p = 0.09$, $p = 0.008$, $p = 0.09$, and $p = 0.89$ for first ICU day, POD 1, POD 2, and POD 3, respectively). Data presented as mean and SEM. **C**, Vasoactive and inotropic agents administered during the first 24 hr of the ICU admission depicted as the *inotropic score* (short: $p = 0.28$, long: $p = 0.048$). Data presented as median and IQR. **D**, Renal function depicted as glomerular filtration rate (GFR) using plasma clearance of iohexol ($GFR_{iohexol}$) and (**E**) estimated GFR with the Modification of Diet in Renal Disease equation ($eGFR_{MDRD}$) (short: $GFR_{iohexol}$: $p = 0.47$ and $eGFR_{MDRD}$: $p = 0.27$, long: $GFR_{iohexol}$: $p = 0.02$ and $eGFR_{MDRD}$: $p < 0.0001$). Data are presented as mean and SEM. p values calculated using Student t tests. $^{*}p < 0.05$; $\#p < 0.1$.

The observed decrease in ICU and hospital length of stay in the EA-230 group compared with the placebo group may be explained by these beneficial cardiovascular and putative renoprotective effects of EA-230. These results are in line with the lower overall occurrence of (S)AEs. EA-230 may exert its putative effect through direct action on organ function (e.g., kidney and vascular system), as no effect on the inflammatory response was evident. There are several limitations of this study. First, the single-dose design, which was based on previous work (38), excludes identification of a dose-response effect. Nevertheless, our finding that the beneficial effects of EA-230 are more pronounced in patients with longer duration of surgery, and thus infusion of study drug, suggest a dose-response effect. Therefore, one may argue that in future studies, longer infusion of EA-230 should be evaluated. Second, the generalizability might be limited, as the study population in this monocenter study was restricted to patients in the Netherlands and predominantly consisted of males, although an overrepresentation of males is typical in patients undergoing cardiac surgery. Third, the considerable number of endpoints may have increased the chances of type 1 errors. Therefore, the analyses of these endpoints should be considered exploratory and hypothesis-generating and must be interpreted with caution.

CONCLUSIONS

In summary, in cardiac surgery patients treated with EA-230, no safety issues emerged. EA-230 did not influence the postoperative inflammatory response. However, beneficial trends on renal and cardiovascular variables were observed. Furthermore, length of stay was shortened after EA-230 administration. Confirmation of these findings and their relationship with timing and duration of EA-230 infusion are warranted.

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1 Department of Intensive Care Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.

2 Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, The Netherlands.

3 Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Medical Center, Nijmegen, The Netherlands.

4 Department of Anesthesiology, Pain and Palliative Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.

5 Department of Cardiothoracic Surgery, Radboud University Medical Center, Nijmegen, The Netherlands.

6 Exponential Biotherapies, Inc. (EBI), The Hague, The Netherlands.

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Address requests for reprints to: Peter Pickkers, MD, PhD, Department of Intensive Care Medicine, Radboud University Medical Center, PO Box 9101, NL 6500 HB, Nijmegen, The Netherlands. E-mail: peter.pickkers@radboudumc.nl

All protocols, including amendments, were approved by the local ethics committee (Commissie Mensgebonden Onderzoek Arnhem-Nijmegen, NL56102.091.15; 2015–2231). The study was conducted in accordance with the principles of the Declaration of Helsinki and in compliance with the International Conference on Harmonisation E6 Guideline for Good Clinical Practice (CPMP/ICH/135/95). All patients who participated in the study provided written informed consent before the start of any study-related procedures.

The study protocol and statistical analysis plan is published online. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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