Original paper research protocol

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Title

Study protocol for a randomized double-blind placebo-controlled phase II study on the effects of the human chorionic gonadotropin hormone-derivative EA-230 on the systemic inflammatory response and renal function following on-pump cardiac surgery (EASI study)

Abstract

Background: The systemic inflammatory response following on-pump cardiac surgery may induce post-operative hemodynamic instability and impairment of renal function. EA-230, a linear tetrapeptide (AQGV) derived from the β-chain of the human chorionic gonadotropin pregnancy
hormone has shown immunomodulatory and renoprotective effects in several animal models of systemic inflammation. In phase I studies and a phase IIa study during human experimental endotoxaemia, these immunomodulatory effects were confirmed and an excellent safety profile was found.

**Objectives:** To examine the immunomodulatory and renoprotective effects of EA-230, as well as the safety and tolerability, in patients with systemic inflammation following on-pump cardiac surgery.

**Methods:** We describe a prospective, randomized, double-blind, placebo-controlled study in which 180 elective patients undergoing on-pump coronary artery bypass grafting with or without concomitant valve surgery will be enrolled. Patients will be randomized in a 1:1 ratio to receive either EA-230, 90 mg/kg/hour, or placebo, infused from the start of the surgical procedure until the end of the use of the cardiopulmonary bypass. The primary endpoint is the modulation of the inflammatory response by EA-230, quantified as the change in interleukin-6 plasma concentrations following surgery. Key secondary endpoints are safety and renal function.

The study will be conducted in two parts to enable an interim safety analysis by an independent data monitoring committee at n=60, and an adaptive design is used to re-assess statistical power halfway the study.

**Ethics and dissemination:** This study is approved by the independent competent authority and ethics committee and will be conducted in accordance with the ethical principles of the Declaration of Helsinki, guidelines of Good Clinical Practice and European Directive 2001/20/CE regarding the conduct of clinical trials. Results of this study will be submitted for publication in a peer-reviewed scientific journal.

**Registration:** Clinicaltrials.gov; NCT03145220 ([https://clinicaltrials.gov/ct2/show/NCT03145220](https://clinicaltrials.gov/ct2/show/NCT03145220))

**Keywords**
EA-230, Inflammation, Pregnancy, cardiac surgery, immunomodulation, renal protection, Phase II, safety

Word count

5538
INTRODUCTION

The systemic inflammatory response syndrome (SIRS) is characterized by a dysregulated inflammatory reaction in response to conditions such as a severe infection, trauma and major surgery[1, 2]. Although activation of the immune system is essential, a too pronounced systemic inflammatory response may result in failure of one or more organ systems with associated high morbidity and mortality rates up to 30%[3, 4]. Development of Acute Kidney Injury (AKI) represents an early and most frequent manifestation of inflammation-induced organ failure[5-7].

During cardiac surgery, multiple insults such as sternotomy, application of cardiopulmonary bypass (CPB) and aortic cross-clamping, are well known to contribute to a systemic inflammatory response[8-11]. The extent of this response is directly associated with impaired patient outcome, as elevated post-operative levels of interleukin (IL)-6 correlate with adverse outcomes and mortality[5, 12]. Furthermore, this inflammatory response is believed to play a central role in the pathogenesis of AKI following cardiac surgery (CS-AKI)[13, 14]. Also, renal impairment is, in turn, independently associated with adverse outcome and impaired patient survival[15, 16].

To date, no immunomodulatory treatments have proven to be effective in preventing organ injury[17-19]. Therefore, current strategies consist of supportive treatment. Novel strategies aimed to attenuate the exaggerated pro-inflammatory response are thus highly warranted.

Pregnancy is associated with an immune-tolerant adaptation of the immune system and increased glomerular filtration rate (GFR)[20, 21]. EA-230, a linear tetrapeptide (AQGV), is a novel pharmacological compound derived from the β-chain of the human chorionic gonadotropin pregnancy hormone (hCG)[22]. EA-230 was shown to exert immunomodulatory effects and to protect against organ failure and associated mortality in several experimental animal models of systemic inflammation[23-27].

Phase-I safety studies of EA-230 showed that intravenous administration is well tolerated and that it has an excellent safety profile[28]. In a phase IIa study during human experimental endotoxaemia, a
model of controlled systemic inflammation elicited by the administration of a low dose of endotoxin, no safety concerns emerged. Furthermore, subjects treated with the highest dose of EA-230 (90 mg/kg/hour) showed less flu-like symptoms, attenuated development of fever, and reduced levels of pro-inflammatory mediators (among others IL-6 and IL-8) when compared to placebo-treated endotoxaemia subjects[29].

A proof-of-principle study is now warranted to investigate 1) whether EA-230 modulates the systemic inflammatory response in patients, 2) to explore whether this is beneficial in terms of prevention of organ (kidney) dysfunction, and 3) to confirm the safety profile of EA-230 in patients. In the present work, we describe the design of a double-blind, placebo-controlled, randomized, phase II study with EA-230 in patients undergoing elective on-pump cardiac surgery.
METHODS AND ANALYSIS

Design and setting

The present study is a single-centre, prospective, double-blind, placebo-controlled, randomized, single-dose phase II study with an adaptive design to evaluate the immunomodulatory effects and safety of EA-230 in patients undergoing on-pump cardiac surgery for coronary artery bypass grafting (CABG) with or without concomitant valve surgery. One hundred and eighty eligible patients are planned for inclusion and randomized to either receive active or placebo treatment in a 1:1 ratio. The study will be conducted in a tertiary hospital, the Radboud University Medical Center (Nijmegen, the Netherlands). The primary objective is to assess the immunomodulatory effects of EA-230. Key-secondary endpoints are safety of EA-230 and effects on renal function. With regard to safety in this first-in-patient study, the study will be conducted in two parts. In the first part, 60 patients (of which 40-50 low-risk patients, see Table 1 for details) will be included with the primary focus on safety, followed by an extensive independent interim safety analysis. Inclusion for the second part of the study will only continue if no safety concerns are raised.

Additionally, an adaptive design is used to re-evaluate the statistical power and group size of the study using patient data obtained during the first half of the study. This study is described in accordance with the Standard Protocol Items: Recommendations for Interventional Trial (SPIRIT) guidelines[30] and registered at www.clinicaltrials.gov (NCT03145220).

Table 1: in-/exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>1. Coronary artery disease, scheduled for elective on-pump CABG surgery with or without concomitant valve surgery.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2. Written informed consent to participate in this study prior to any study-mandated procedure.</td>
</tr>
<tr>
<td></td>
<td>3. Patients aged &gt;18, both male and female.</td>
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<tr>
<td></td>
<td>4. Patients have to agree to use a reliable way of contraception with their partners from study entry until 3 months after study drug administration.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional exclusion criteria to select low-risk patients</th>
<th>1. EuroSCORE II &gt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2. Renal function impairment: serum creatinine &gt;200 μmol/L</td>
</tr>
<tr>
<td></td>
<td>3. Liver function impairment: ALAT/ASAT &gt;3 times above upper level of</td>
</tr>
</tbody>
</table>
For the First 60 patients only

<table>
<thead>
<tr>
<th>Reference range</th>
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<tbody>
<tr>
<td>4. Left ventricular dysfunction: Ejection fraction &lt;35%</td>
</tr>
<tr>
<td>5. CABG procedure with concomitant valve surgery</td>
</tr>
</tbody>
</table>

Exclusion criteria

1. Immune compromised
   - Solid organ transplantation
   - Known HIV
   - Pregnancy

2. Use of immunosuppressive drugs (list provided in online supplementary material).

3. Non-elective/Emergency surgery

4. Hematological disorders
   - Known disorders from myeloid and/or lymphoid origin
   - Leucopenia

5. Known hypersensitivity to any excipients of the drug formulations used.

6. Treatment with investigational drugs or participation in any other intervention clinical study within 30 days prior to study drug administration

7. Inability to personally provide written informed consent (e.g. for linguistic or mental reasons)

8. Known or suspected of not being able to comply with the study protocol

Study objectives

**Primary objective**

- Inflammation-related: To assess the immuno-modulatory effects of EA-230 in patients with systemic inflammation following on-pump cardiac surgery.

**Key secondary objectives**

- Related to renal function: To assess the effects of EA-230 on changes in renal function (GFR).
- Related to safety: To assess the safety and tolerability of EA-230 in patients undergoing on-pump cardiac surgery.

End-points for the additional objectives are described in Table 2.

Table 2. Endpoints

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Endpoint</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory</td>
<td>Modulation of the inflammatory response by EA-230, quantified by the change in plasma concentrations over time of IL-6, IL-8, IL-10,</td>
<td>Surgery day – day 1</td>
</tr>
<tr>
<td>Safety and tolerability</td>
<td>Tolerability</td>
<td>Renal</td>
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<tr>
<td>------------------------</td>
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<tr>
<td>TNFa, IL-1RA, MCP-1, MIP1α, MIP1β, VCAM, ICAM and IL-17a</td>
<td>Modulation of leukocyte kinetics by EA-230, quantified by change of total cell counts over time</td>
<td>Modulation of GFR by EA-230, quantified by plasma clearance of iohexol (iGFR) and estimated by MDRD-calculation</td>
</tr>
<tr>
<td>Day -1 – Day +1</td>
<td>Modulation of changes in body temperature by EA-230, in °C over time</td>
<td>Modulation of GFR by EA-230 measured by endogenous creatinine clearance using urine and plasma creatinine</td>
</tr>
<tr>
<td>First 24 hours of ICU admission</td>
<td>Modulation of change in SOFA score by EA-230 over time</td>
<td>Modulation of plasma creatinine and proenkephalin by EA-230</td>
</tr>
<tr>
<td>First 24 hours of ICU admission</td>
<td>Modulation of needed insulin infusion rates by EA-230</td>
<td>Modulation of changes in urine output by EA-230</td>
</tr>
<tr>
<td>Signing of ICF – day 90</td>
<td>Modulation of changes in urinary renal damage markers over time of KIM-1, NGAL, L-FABP, TIMP-2*IGFBP-7, urinary IL-18, NAG by EA-230</td>
<td>Surgery – day +1</td>
</tr>
<tr>
<td>Day -1 – Day +1</td>
<td>Modulation of changes in urea, sodium, creatinine and albumin in urine over time by EA-230</td>
<td>Surgery – day +1</td>
</tr>
<tr>
<td>Surgery – day +1</td>
<td>Modulation in need for and duration of RRT by EA-230</td>
<td>Surgery – day +1</td>
</tr>
<tr>
<td>Surgery – day +1</td>
<td>Modulation in incidence of different stages of AKI by EA-230 according to the RIFLE criteria</td>
<td>Surgery – day +1</td>
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<tr>
<td>Surgery – day 90</td>
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<td>Surgery – day 90</td>
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<tr>
<td>Surgery – ICU admission</td>
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score: (dopamine dose × 1 μg/kg/min) + (dobutamine dose × 1 μg/kg/min) + (adrenaline dose × 100 μg/kg/min) + (noradrenaline dose × 100 μg/kg/min) + (phenylephrine dose × 100 μg/kg/min) + (vasopressin (mUnits/kg/min)^10000) + (milrinone x10mcg/kg/min)[31]

| Modulation of use of fluid therapy and fluid balance by EA-230 | admission |
| Modulation of CK and troponine-T plasma concentration by EA-230 | Surgery – end of hospital stay |
| Modulation of thorax drain production by EA-230 | Surgery – end of ICU admission |
| Pulmonary | Modulation of A-a O_2 gradient by EA-230 | Surgery – end of ICU admission |
| Pharmacokinetics of EA-230 | Peak blood plasma levels of EA-230 | 10 minutes prior to start and stop of CPB |
| | Blood plasma levels of EA-230, AUC, Cmax, terminal t_{1/2}, Cl, V for a limited number of patients receiving active medication (n=15) | During EA-230 administration – 6 hours after stoppage |

SOFa: Sepsis-related organ failure assessment score; ICU: Intensive Care Unit, ICF: Informed consent form; MDRD: Modification of diet in renal disease; RRT: Renal replacement therapy; AKI: acute kidney injury; RIFLE: Risk, injury, failure, loss of kidney function, and end-stage kidney disease classification; APACHE: Acute physiology and chronic health evaluation; IL: Interleukin; TNF: Tumor necrosis factor; RA: Receptor antagonist; MCP Monocyte chemoattractant protein; MIP: Macrophage inflammatory protein; KIM: Kidney injury marker; NGAL: Neutrophil gelatinase-associated lipocalin; FABP: Fatty acid-binding protein; TIMP: Tissue inhibitor metalloproteinase; IGFBP: Insulin-like growth factor binding protein-7; VCAM: Vascular cell adhesion protein; ICAM: Intercellular Adhesion Molecule.; GFR: Glomerular filtration rate; CK: Creatine Kinase; AUC: Area under the curve; Cl: Clearance; V: Volume of distribution; Cmax: Maximal concentration; t_{1/2}: Half-life; A-a: Alveolar-arterial gradient.

**Patient selection/Eligibility**

All adult patients (>18 years) scheduled for elective on-pump CABG procedure with or without concomitant valve surgery will be screened for eligibility; see Table 1 for an overview of all in- and exclusion criteria.

**Recruitment**

Figure 1 depicts a schematic flowchart of patient recruitment and randomization.

All patients scheduled for elective CABG surgery will be included in a screening log and informed using a detailed informative brochure. After screening for in- and exclusion criteria, eligible patients
will be personally consulted and a final in- and exclusion criteria check will be performed. After obtaining written informed consent, patients will be enrolled into the study.

Figure 1. Study flowchart

Overview of patient recruitment, randomization and population analysis procedures from screening to follow-up. CABG: Coronary Artery Bypass Grafting

Randomization and stratification

On the morning of surgery patients will be randomized by non-blinded independent study personnel for active or placebo treatment using GCP-approved data management software (Castor EDC, Amsterdam, The Netherlands). The Castor system applies a stratified randomization to ensure equal distribution between active and placebo treatment of patients with known risk factors for adverse outcomes. Three strata will be included: 1) a CABG procedure with or without concomitant valve surgery, 2) pre-operative renal function with an estimated GFR of ≤30, 31-90 and >90 ml/min/1.73 m², and 3) an EuroSCORE II of <4 or ≥4[32].
Blinding

Double-blind conditions will be maintained for all patients, the attending physicians and the medical study team personnel involved in all blinded study procedures, data collection and/or data analyses. Preparation of study medication will be performed by non-blinded study personnel not involved in any other study procedures. Infusion systems and solutions for active and placebo treatment are identical in appearance and texture. Unblinding will be authorised by the sponsor after completion of the study, performance of a blinded data review, and locking of the database. A sealed code break envelope is present in case emergency unblinding should be necessary.

Study Intervention

Intravenous infusion of EA-230, 90 mg/kg/hour, or placebo, will be initiated at the moment of first surgical incision using an automated infusion pump. Infusion rate is 250 mL/hour, and infusion will be continued until cessation of the CPB, or after 4 hours of continuous infusion, whichever comes first.

EA-230 formulation is packed in sterile 5 mL glass vials, containing 1500 mg/vial, dissolved in water for injection at a final concentration of 300 mg/mL with an osmolality of 800 to 1000 mOsm/kg. The placebo formulation consists of sodium chloride diluted in water for injection in identical sterile 5 mL glass vials of, containing 29 mg/mL to reach a solution with an identical osmolality. EA-230 and placebo will be prepared for continuous intravenous infusion with an osmolality of <400 mOsm/Kg by adding the appropriate amount of EA-230 or placebo to 1000 mL normal saline under aseptic conditions. Both placebo and active treatment vials will be provided by the sponsor, manufactured by HALIX BV (Leiden, The Netherlands).

Outcome measures

An overview of the study procedures from inclusion until end of follow-up is depicted in Figure 2, and a detailed overview of all outcome measures is provided in Table 2.
Primary endpoints

The primary endpoint of this study is the modulation of the inflammatory response by EA-230, quantified by the difference in the Area Under the Curve (AUC) plasma IL-6 levels over time from start of the cardiac procedure until the first post-operative day, compared to placebo. Plasma samples will be collected pre-incision (baseline), at the start of CPB, at 0, 2, 4, 6 hours after cessation of the CPB, and on the morning of the first post-operative day.

Key-secondary endpoints

- Safety and tolerability of EA-230, defined by the incidence and severity of (serious) adverse events [(S)AEs] and serious unexpected adverse reactions (SUSARs), changes in vital signs (heart rate and blood pressure) and changes in routine laboratory parameters. Vital signs and routine laboratory parameters will be registered during the first post-operative day when patients are admitted to the ICU. Safety data will be collected from inclusion into the study until 90 days after study drug administration.
- Modulation of changes in renal function by EA-230, defined by changes in the GFR measured prior to surgery and on the morning of the first post-operative day. For the determination of the GFR, an intravenous bolus of 5 mL iohexol will be administered and plasma samples will be collected in the following 4 hours (on the day prior to surgery: 90 and 240 minutes after iohexol administration and on the post-operative day 90, 180 and 240 minutes after iohexol administration) to construct a plasma disappearance curve of iohexol and to calculate the iohexol GFR (iGFR) according to the methods described by Delanaye et al[33].
**Sample size**

The AUC plasma IL-6 levels over time was used for the power calculation. In the preceding clinical phase IIa study with EA-230 during experimental endotoxaemia in healthy volunteers, EA-230 (90 mg/kg/hour) attenuated AUC plasma IL-6 levels by 48% compared to placebo. The present first-in-patient proof-of-principle study is powered on a 30% reduction in AUC IL-6, which is deemed a relevant immunomodulatory effect. For the statistical dispersion, AUC IL-6 data from a previous CABG surgery study conducted in the same institute were used (mean 816 pg/mL.h, standard deviation (SD) of 520 pg/mL.h)[34]. To correct for the non-parametric distribution of these data, the calculated sample size is increased by 15%[35]. With a two-sided $\alpha$ of 0.05 and a power of 80% ($\beta$ of 0.2), a group size of 82 patients per treatment arm is required. However, selection of low risk patients in the first part of the study with an expected less pronounced inflammatory response, may result in an increased SD of AUC IL-6 in the overall study, and therefore loss of study power. Hence, the sample size should be adjusted accordingly. In consultation with an independent statistician, a sample size of 90 patients per treatment arm was deemed sufficient to compensate for this loss in power. An adaptive design is used, in which sample size will be re-evaluated and possible early efficacy will be assessed halfway the study, when 90 patients have been included. These analyses will be performed
by the partially unblinded independent statistician member of the Data Monitoring Committee (DMC).

For the re-evaluation of the sample size, the pooled SD of the AUC plasma IL-6 levels of the first 90 patients enrolled will be calculated and used to compare with the original sample size according to the method of Proschan[36]. To demonstrate possible early efficacy, the approach for α-spending according to O’Brien-Fleming will be used [37]; a t-test will be performed on the collected data with the following alpha (α1(t*)):

$$\alpha_1(t^*) = 2 - 2\varphi(Z \alpha/2/\sqrt{t^*})$$

Where $t^*$ represents the information fraction ($t^* = 0.5 \times$ original sample size/new sample size). If $P<\alpha_1(t^*)$, the study will be stopped because of early demonstrated efficacy. When no significant differences are found during this interim analysis, the study will continue. For final analysis, an adjusted α will be used, corrected for the already spent α.

**Statistical analysis**

The statistical analysis plan (SAP) will be signed before database lock. The SAP is provided as online supplementary material. Data will be presented as mean and SD or standard error of the mean (SEM), or median and interquartile range, and analyses performed depending on their distribution.

The primary endpoint, difference in sIL-6 plasma concentrations over time (AUC IL-6 plasma levels) between treatment groups, will be analyzed using an unpaired Student’s t-test or Mann-Whitney U test, the latter if data are not normally distributed. In a secondary analysis, the AUC IL-6 plasma levels between treatment groups will also be compared using two-way analysis of variance (2-way ANOVA, interaction term, on log-transformed data if data are not normally distributed).

Differences in the key secondary efficacy endpoint iGFR between treatment groups over time will be analyzed using 2-way ANOVA, as described above. Safety data will be listed by patient number and summarized descriptively according to treatment.
All other data will be analyzed using unpaired Student’s t-tests or Mann-Whitney U tests for continuous data, 2-way ANOVA for continuous data over time as described above, and Chi-Square tests for categorical data.

A two-sided $P$ value $<0.05$ is considered significant. For the primary endpoint, a $P$ value corrected for alpha spending will be used as described earlier. Statistical analyses will be performed using IBM SPSS (IBM, Armonk, NY, USA) and GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Withdrawal of study patients

Patients may leave the study at any time for any reason if they wish to do so, without any consequences. The investigator can decide to withdraw a patient from the study for urgent medical reasons or in case of inability to comply with the study protocol.

Because of the likely possibility that cardiac surgery of patients enrolled in the study will be rescheduled because of intervening urgent surgeries, and because of the possibility that patients might meet an exclusion criterion shortly before the start of surgery, patients who are withdrawn from the study prior to IMP administration will be replaced and thus will not be included in any analysis.

Different populations that will be analyzed

**Intention-to treat (ITT) population**

The intention-to treat (ITT) population includes all patients who were randomized and received study treatment, irrespective of satisfying other endpoint criteria. This population will be used for the analysis of safety and tolerability and all other primary and secondary endpoints.

*Per-Protocol (PP) population*
Analysis on the per-protocol (PP) population will be used as a supplement to the ITT analysis and will be performed for all endpoints except safety related endpoints. The PP includes all ITT patients who have not been excluded from analysis for major protocol deviations.

**Pharmacokinetic (PK) population**

Sampling for PK analysis will be performed in 30 patients. As EA-230/placebo ratio is 1:1, the PK population will include a subset of approximately 15 patients that received EA-230. For this full PK evaluation, additional blood samples will be obtained during infusion of EA-230 until 6 hours after cessation of administration.

**Subgroup analyses**

Subgroup analyses will be performed on the predetermined pre-operative randomization strata:

- With or without concomitant valve surgery
- Pre-operative renal function with an estimated GFR of ≤30, 31-90 and >90 ml/min/1.73 m²
- EuroSCORE II of <4 or ≥4

**Safety considerations**

**Adverse events (AEs)**

All AEs will be judged by the investigators with regard to severity ('mild, moderate, or severe') according to Common Terminology Criteria for Adverse Events (CTCAE) guidelines 4.030[38], and their perceived relation to the study drug ('definitely, probably, possibly, or unrelated/unlikely to be related'). SAEs or SUSARs include death, life-threatening disease, persistent and/or significant disability and/or incapacity, and hospitalization and/or prolongation of inpatient hospitalization. The investigator will report all SAEs and SUSARs to the sponsor without undue delay after obtaining knowledge of the events. Monitoring and re-evaluation of the SAE/SUSAR and its relation to the study drug will subsequently be performed by an independent medical doctor of an independent Contract Research Organization (CRO) QPS (Groningen, the Netherlands) and reported to the Central
Committee on Research Involving Human Subjects (CCMO; The Hague, The Netherlands) within a period of maximum 15 days (or 7 days when the event is life threatening or results in death), and to the independent ethics committee of the Radboud University Nijmegen Medical Center (CMO; Arnhem-Nijmegen, The Netherlands).

**Data monitoring committee**

An independent DMC, consisting of three expert members, including one biostatistician, will assess safety of the study drug. After completion of the first part of the study (including 40-50 low-risk patients), the first DMC meeting will be held. During this interim safety analysis, inclusion will be paused and an extensive partially unblinded assessment of (S)AEs, vital signs, routine laboratory parameters will be performed. Inclusion of patients into the second part of the study will only start if no safety concerns are raised by the DMC. A second meeting will be held after 90 patients have been enrolled (also higher risk patients) to re-assess all safety parameters. Again, the DMC will advice continuation or termination of the study.

**Ethical considerations**

The study protocol, amendments, informed consent and any other written information regarding this study to be provided to the patient are approved by the CMO Arnhem-Nijmegen and CCMO, and the study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (ICH E6(R1), the Medical Research Involving Human Subjects Act (WMO), guidelines of Good Clinical Practice and European Directive (2001/20/CE). Informed consent will be obtained before any study specific procedures are performed. Substantial amendments will be provided to the CMO for approval, non-substantial amendments will be provided to the CMO for notification.

**Potential conflicts of interest, role of sponsor**
The study is funded by Exponential Biotherapies Inc (EBI, the Hague, the Netherlands). EBI is not involved in study design, randomization, data collection, (interim) data analyses, or reporting of the results.

**Data quality assurance and publication**

Data will be handled confidentially and anonymously. The study site maintains source documentation and is responsible for accurate data entry in the electronic case report form (eCRF) using the GCP certified data capture system Open Clinica (Waltham, MA, USA). Blinded study personnel are provided with an individual user name and password with complete traceability. Quality assurance, data management with full data validation and monitoring of all source documents, study procedures, study data, SAEs and SUSARs will be performed by the independent CRO QPS. The database will be locked after completion of data review, resolutions to all queries and signing of the statistical analysis plan. Following database lock, a study patient identification code list (provided by the Castor data management system, Amsterdam, the Netherlands) will be used to link the stratified interventional treatment (active or placebo) to the patient. Data and body material will be kept in secure storage at the Intensive Care research department, accessible by study personnel only. The handling of patient data in this study complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP). The principal investigator and the sub-investigators will write the manuscript which will be submitted for publication in a peer-reviewed scientific medical journal after completion of the study, regardless of the results.

**Patient and public involvement**

Patients and the public were not involved in the design and/or the conduct of the study protocol. Study outcome will be disseminated to all study participants individually. The burden of the intervention was assessed by the independent ethics committees CMO and CCMO, including laymen members.
DISCUSSION

Systemic inflammation-induced organ failure is common in critically ill patients and results in significant morbidity and mortality[3-7]. Because current patient management is limited to prevention of further deterioration and supportive treatment, there is an unmet need to develop new treatment strategies aimed at modulation of the inflammatory response. Pregnancy represents an improbable symbiosis of two major histocompatibility complex (MHC)-incompatible individuals, indicating a tolerant immune-phenotype. Also, a remarkable improvement of several immune-mediated inflammatory diseases is observed during pregnancy[39-41]. Nevertheless, pregnant women are eminently capable of combating infections, and often produce antibodies against paternal allo-antigens of the fetus, demonstrating that they are fully immunocompetent. These features are suggestive for a selective modulation of the immune system in such a way that harmful immune processes to mother and fetus are suppressed, while beneficial immune processes remain unaffected.

In this context, an array of oligopeptides related to the primary structure of hCG was designed and evaluated in experimental animal models of systemic inflammation[23, 25, 27, 42, 43]. Of the evaluated oligopeptides, AQGV, now named EA-230, showed high efficacy in reducing the severity of inflammation and mortality, both upon early and late administration[22, 23]. Since then, EA-230 has been demonstrated to be safe for administration and to modulate the systemic inflammatory response in humans[28, 29]. Following these first-in-man studies, we designed the herein described phase II study in cardiac surgery patients, which is primarily aimed at establishing proof-of-principle of EA-230’s immunomodulatory effects in patients. In addition, pregnancy has profound effects on renal function by increasing renal flow[20], which relates to an increase in GFR. Of interest, in various animal studies, including ischemia-reperfusion[24] and kidney transplant models[26], administration of EA-230 dose-dependently protected against renal injury. Therefore, evaluation of the effects of EA-230 on renal function is of specific interest in this current phase II cardiac surgery study, as a significant proportion of these patients suffer from post-operative renal injury[13, 14], which is in turn related to their clinical outcome[15, 16].
To evaluate the immunomodulatory and renoprotective effects of EA-230, we designed this single-centre, double-blind, randomized, placebo-controlled phase II study in patients undergoing on-pump cardiac surgery. In this study, we will primarily assess the immunomodulatory effects of EA-230. Our secondary key goals are to investigate whether EA-230 prevents AKI (which is possibly related to its immunomodulatory effects) and to confirm EA-230’s safety profile in this patient population. Patients undergoing on-pump cardiac surgery represent an ideal study population to evaluate the effects of EA-230 on the systemic inflammatory response in a first-in-patient proof-of-principle study. These cardiac surgery procedures are highly standardized and have a well-characterized sequence of inflammatory insults: Release of Danger Associated Molecular Pathways (DAMPS) as a result of tissue damage during incision and sternotomy, leukocyte activation induced by the use of the extracorporeal circuit, ischemia reperfusion damage following aortic cross clamping and subsequent declamping, and translocation of endotoxins due to increased gut permeability[8-11]. Due to these ‘elective’ insults, the moment of activation of the immune system is well-defined, and the following inflammatory response during and after surgery follows a relatively homogenous pattern.

Furthermore, the inflammatory response is clinically relevant, as inflammatory mediators IL-6 and IL-8 have been shown to be key orchestrators of the systemic inflammatory response following cardiac surgery, and are associated with post-operative adverse outcome, including the occurrence of AKI and long-term mortality[5, 12, 44]. Because EA-230 has demonstrated to attenuate IL-6 and IL-8 in earlier work[29], it has the potential to modulate release of these specific mediators that are associated with organ failure in cardiac surgery patients.

The in- and exclusion criteria of this study are not overly restrictive to facilitate enrolment and to increase generalizability. Although the inflammatory response is relatively comparable among cardiac surgery patients, these permissive criteria might influence study results, because patients with known pre-operative risk-factors may develop a more pronounced inflammatory response and/or have a
worse outcome. However, in a randomized study such as the present, these inter-patient differences are likely to be equally divided between treatment and placebo groups. Nevertheless, we used a stratified randomization procedure to optimize group balance. Three major pre-operative determinants for outcome, namely baseline renal function, EuroSCORE II[32] and CABG with or without valve surgery, were chosen to better ensure equal distribution between groups of patients with known risk factors.

The study design, focusing on safety and tolerability in the first part facilitates an extensive safety interim analysis by the DMC, therefore limiting potential risks for patients. The second part focuses on EA-230’s efficacy, in which the data of phase one are also incorporated, making for an efficient study design. Furthermore, the use of a recently recommended adaptive design [45], in which the sample size can be adjusted by comparing the original sample size with a new power calculation using the SD of obtained data halfway the study, guarantees adequate statistical power.

It needs to be acknowledged that immunomodulatory strategies have thus far failed to demonstrate clear beneficial effects in cardiac surgery patients[46]. For example, large trials on the use of high dose corticosteroids did not improve overall patient outcome in cardiac surgery patients[44, 45], although positive effects on respiratory variables in selected patient groups were found in post-hoc analyses[45]. Non-specific anti-inflammatory actions and/or the broad spectrum of detrimental side effects of these interventions may have contributed to the lack of overall beneficial effects of these compounds. The properties of EA-230 are essentially different. EA-230 was discovered as an endogenous, immunological active, breakdown product of β-hCG during pregnancy. Up till now, no side effects of EA-230 have been found, which is in sharp contrast to other immunomodulatory drugs. Also, the fact that the tolerant immune-phenotype during pregnancy is not accompanied with
complications related to immunosuppression, suggests that targeting this pathway may be of more benefit than the use of other immunomodulatory therapies.

This study is unique in terms of accurate assessment of organ function, because the key-secondary endpoint is assessment of renal function using a measured iGFR instead of an estimated GFR based on serum creatinine. Although it is well-established that serum creatinine and urine output are suboptimal parameters to assess deterioration of renal function, they remain to be the most commonly used markers to assess AKI. Creatinine is unreliable because it is a late marker, and because it is influenced by muscle mass, fluid shifts, and immobilization, and partially actively secreted by the kidneys [47-49]. The iGFR method has proven to be as reliable as the defined gold standard inulin clearance ($r^2$ of 0.96 [50]), and accurately detects even minor changes [33, 51]. Therefore, clinically significant changes in GFR can be reliably assessed in this study. These renal function measurements were not performed in any large cardiac surgery trial to date, and will substantially improve the validity of the data and quality of this study. As a potential downside, the iohexol method does require collection of multiple blood samples to create a plasma decay curve. Because of this labour-intensive process, iGFR measurements will be performed only twice in the present study: pre-operatively, representing a baseline measurement, and on the morning of the first post-operative day. As discussed earlier, conventional renal function markers are late and unreliable. Therefore, little is known about the exact post-operative course of renal function deterioration and/or decrease in GFR. As such, there is a chance that the single post-operative iGFR measurement could fail to detect a decrease in GFR following on-pump cardiac surgery.

A limitation of this study is the dose of EA-230. Only one dose is used, based on the fact that only the highest dose was effective in terms of modulating the immune response in the previous endotoxaemia study [29]. As a result, it will remain unknown whether similar efficacy in patients can
be attained using a lower dose, or higher efficacy can be achieved using a higher dose. Furthermore, the unknown results of the use of the artificial extracorporeal circulation and the more pronounced fluid balance shift in patients undergoing cardiac surgery needs mentioning, as it may alter distribution of EA-230 with non-therapeutic plasma concentrations as a possible result.

In summary, the EASI study is a double-blind, randomized, placebo-controlled phase II study with EA-230 in 180 patients undergoing on pump cardiac surgery, that applies stratification and an adaptive study design. It is designed to examine the immunomodulatory and renal protective effects of EA-230, as well as the safety and tolerability, in patients with systemic inflammation.

Acknowledgements
The authors wish to thank all research and medical personnel involved in the design and conduct of this study.

Author contributions
LvE and PP primarily designed the study. RB and RvG drafted the manuscript. MK, PP and JH helped revising the manuscript. All authors participated in the conception, design and/or coordination of the study. All authors critically reviewed and approved the final manuscript for publication.

Funding
The study was funded by Exponential Biotherapies Inc (EBI, the Hague, the Netherlands). EBI is not involved in the study design, randomization, data collection, (interim) data analyses, or reporting of the results.

Conflicts of Interest
PP received travel reimbursements and consultancy fees from EBI.

All other authors declare to have no conflicts of interest.
Data sharing statement

The full study protocol can be accessed by contacting the corresponding author PP.

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