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Study protocol: A Double Blind, Placebo-Controlled, Randomized, Multicenter, Proof of Concept and Dose-finding Phase II Clinical Trial to Investigate the Safety, Tolerability and Efficacy of Adrecizumab in Patients with Septic Shock and Elevated Adrenomedullin concentration (AdrenOSS-2)

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Study protocol: A Double Blind, Placebo-Controlled, Randomized, Multicenter, Proof of Concept and Dose-finding Phase II Clinical Trial to Investigate the Safety, Tolerability and Efficacy of Adrecizumab in Patients with Septic Shock and Elevated Adrenomedullin concentration (AdrenOSS-2)

Authors

Christopher Geven^{1*}, Alice Blet^{2*}, Matthijs Kox¹, Oliver Hartmann³, Paul Scigalla³, Jens Zimmermann³, Gernot Marx^{4*}, Pierre-Francois Laterre^{5*}, Alexandre Mebazaa^{2*}, Peter Pickkers^{1*}

¹Department of Intensive Care Medicine, Radboud Center for Infectious Diseases (RCI), Radboud university medical center, HP: 710, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

²Department of Anesthesia, Burn and Critical Care, University Hospitals Saint-Louis – Lariboisière, AP-HP, Paris, France. UMR-S 942, Inserm, Paris, France. Paris Diderot University, Sorbonne Paris Cité, Paris, France.

³Adrenomed AG, Neuendorfstraße 15A, D-16761 Hennigsdorf, Germany.

⁴Department of Intensive Care Medicine and Intermediate Care, RWTH University Hospital Aachen, Aachen, Germany.

⁵Department of Critical Care Medicine, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain (UCL), Brussels, Belgium.

* Christopher Geven and Alice Blet contributed equally to the manuscript.

* Pierre-Francois Laterre, Gernot Marx, Alexandre Mebazaa and Peter Pickkers share senior authorship as steering committee members of the AdrenOSS-2 trial.

Corresponding author: Prof. Dr. Peter Pickkers. Radboud university medical center, Department of Intensive Care Medicine (710), PO Box 9101, 6500 HB, Nijmegen, The Netherlands. E-mail: peter.pickkers@radboudumc.nl

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10 34 antibody; phase II.
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14 36 **Abstract**

15
16 37 **Introduction:** Sepsis remains a major health problem with an increasing incidence, high morbidity
17
18 38 and high mortality. Apart from treatment with antibiotics and organ support, no approved specific
19
20 39 adjunct therapies currently exist. Adrenomedullin (ADM) is a vasoactive peptide. High plasma
21
22 40 concentrations of ADM correlate with worse outcome in sepsis patients. Preclinical work with the
23
24 41 non-neutralizing ADM-binding antibody Adrecizumab showed promising effects in animal models of
25
26 42 septic shock, including improved vascular barrier function, reduced vasopressor demand and organ
27
28 43 dysfunction, and increased survival. Therapeutic use of Adrecizumab may therefore improve outcome
29
30 44 in critically ill patients with septic shock and high ADM plasma concentrations. Phase I studies in
31
32 45 healthy volunteers did not reveal any safety concerns. In this biomarker-guided trial, the safety and
33
34 46 efficacy of Adrecizumab will be investigated in patients with septic shock.
35

36 47 **Methods and analysis:** We describe a phase II, randomized, double blind, placebo-controlled,
37
38 48 biomarker-guided, proof of concept and dose-finding clinical trial in patients with early septic shock
39
40 49 and high concentration of circulating ADM. A total of 300 patients will be enrolled at approx. 30 sites
41
42 50 within the European Union. Patients are randomized to receive active treatment (2 and 4 mg/kg
43
44 51 Adrecizumab) or placebo, in a 1:1:2 ratio. Patient selection is not only guided by clinical parameters,
45
46 52 but also biomarker-guided by measurement of circulating biologically active ADM concentration at
47
48 53 admission. Primary endpoint is safety and tolerability of Adrecizumab over a 90 day period. A key
49
50 54 secondary endpoint is the Sepsis Severity Index (SSI) over a 14-day period.
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52 55 **Ethics and dissemination:** This study is approved by relevant institutional review boards/independent
53
54 56 ethics committees and is conducted in accordance with the ethical principles of the Declaration of
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3 57 Helsinki, the European Medicines Agency guidelines of Good Clinical Practice, and all other
4 58 applicable regulations. Results of this study will be published in a peer-reviewed scientific journal.

59

60 **Trial registration number:** NCT03085758

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62 **Strengths and limitations of this study**

- 63 • Extensive preclinical work and phase I studies showed promising results and favourable safety of
64 Adrecizumab, paving the way for this phase II study.
- 65 • As a non-neutralizing antibody of adrenomedullin, Adrecizumab's mode of action is novel, as it is
66 not a traditional therapy with complete neutralization of its target. Adrecizumab is thought to
67 negate detrimental effects of interstitial ADM on vascular tone, while augmenting beneficial
68 effects of circulating ADM on the vascular endothelium.
- 69 • Patient selection is not only guided by clinical parameters, but also biomarker-guided by
70 measurement of circulating biologically active plasma adrenomedullin, allowing to select patients
71 with an impaired outcome who may benefit most from Adrecizumab therapy.
- 72 • The key secondary endpoint and primary *efficacy* endpoint is the composite Sepsis Support Index,
73 which combines all-cause mortality and organ dysfunction, aimed to be more sensitive to assess
74 the efficacy of the treatment.
- 75 • Strict in- and exclusion criteria, as well as the brief time-window for inclusion (within 12 hours
76 following the initiation of vasopressor therapy) may limit generalisation of the results for the
77 entire population of critically ill patients with sepsis, although this may facilitate detection of an
78 efficacy signal.

79 Introduction

80 Worldwide, sepsis is a major health problem, with an increasing incidence and high mortality.¹⁻³ It is
81 defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.⁴
82 Septic shock is defined as a subset of sepsis in which profound circulatory, cellular, and metabolic
83 abnormalities occur, which are associated with an increased risk of mortality.⁴ The most prominent
84 abnormalities are vasodilation and loss of vascular integrity, resulting in hypotension, and ultimately,
85 in organ dysfunction and death.⁵ Besides antibiotics and organ supportive therapies such as
86 vasopressors, mechanical ventilation and renal replacement therapy (RRT), there are currently no
87 sepsis-specific adjunctive therapies registered.

88
89 Adrenomedullin (ADM) is a vasoactive peptide hormone that plays an important role in sepsis.
90 Circulating ADM exerts endothelial barrier-stabilizing effects and maintains vascular integrity.⁶⁻¹⁰
91 ADM has vasodilatory properties in the vascular interstitium, and at high concentrations, as observed
92 during sepsis, may contribute to hypotension.¹¹⁻¹³ Elevated concentrations of plasma ADM at
93 admission have been reported in septic patients, and these were correlated with vasopressor
94 requirement, organ dysfunction and mortality.¹⁴⁻¹⁶ The cut-off value of biologically active ADM (bio-
95 ADM) of 70 pg/mL at admission was found to predict mortality for sepsis patients.¹⁴ This cut-off has
96 been validated in independent, large multicentre studies.^{15 17 18}

97
98 Based on these data, ADM may be an interesting therapeutic target for sepsis. A potential new
99 adjunctive therapy for the treatment of septic shock is Adrecizumab (previously also known as
100 HAM8101). It is a *non-neutralizing* ADM-binding antibody that has shown beneficial effects in
101 preclinical studies. Adrecizumab reduced vascular leakage, organ dysfunction and need for
102 vasopressor treatment during cecal ligation and puncture (CLP) induced sepsis in several animal
103 studies, and improved urine output and survival.¹⁹⁻²¹ Importantly, Adrecizumab administration was not
104 associated with any safety concerns in the first-in-human phase I study in healthy volunteers^{22 24} and in
105 a follow-up study in healthy volunteers which were intravenously challenged with lipopolysaccharide
106 (LPS) to induce systemic inflammation.^{23 24} Of note, in the latter study, LPS-induced flu-like

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3 107 symptoms resolved more swiftly in Adrecizumab-treated subjects compared to the placebo group.
4
5 108 Pharmacokinetic analysis of Adrecizumab showed a half-life of approximately 14 days, indicating that
6
7 109 administration of a single dose is sufficient to achieve excess of plasma concentrations of the antibody
8
9 110 over adrenomedullin for the entire sepsis period.

10
11 111
12 112 Based on these preclinical and human phase I data, it is hypothesised that therapeutic use of
13
14 113 Adrecizumab may improve endothelial dysfunction, restore and maintain vascular integrity and
15
16 114 augment hemodynamics in critically ill patients with sepsis and septic shock. In the trial described in
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18 115 the present work, the safety, tolerability and efficacy of Adrecizumab is investigated in patients with
19
20 116 early septic shock and elevated concentrations of circulating bio-ADM. This will be one of the first
21
22 117 precision medicine, biomarker-guided studies in septic patients.
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24 118

26 119 **Methods and analysis**

28 120 *Design and setting*

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30 121 AdrenOSS-2 is a phase II, randomized, double blind, placebo-controlled, biomarker-guided, proof of
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32 122 concept and dose-finding clinical trial that is currently being conducted in patients with early septic
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34 123 shock and elevated concentration of circulating bio-ADM (> 70 pg/ml). A total of 300 patients will be
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36 124 recruited in medical, surgical and mixed Intensive Care Units (ICU) at approx. 30 sites across
37
38 125 Belgium, France, Germany, the Netherlands and Italy. Patient selection is guided by clinical
39
40 126 parameters as well as by biomarker concentrations, by measuring circulating bio-ADM (sphingotest[®]
41
42 127 bio-ADM, sphingotec GmbH, Hennigsdorf, Germany).²⁵ Based upon preclinical studies, two dosages
43
44 128 of Adrecizumab will be investigated (2 and 4 mg/kg bodyweight), in addition to a placebo control arm.
45
46 129 After informed consent has been signed by the patient or his/her legal representative, circulating bio-
47
48 130 ADM concentrations will be assessed. If bio-ADM concentrations are > 70 pg/mL, the clinical
49
50 131 coordination center (CCC) will be contacted for final confirmation of patient eligibility and the patient
51
52 132 will be randomized. An interim analysis for futility is planned after 150 patients have completed day
53
54 133 28 of the study. An overview of the study design is depicted in Figure 1 and study procedures in
55
56 134 Figure 2.

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3 135 *Primary objective*

4 136 The primary objective is safety and tolerability, consisting of: mortality possibly related to
5
6 137 Adrecizumab, interruption of infusion due to suspected intolerability of Adrecizumab, new treatment-
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8 138 emergent adverse events possibly related to Adrecizumab, and changes in severity and frequency of
9
10 139 treatment-emergent adverse events. During the study, an independent Data and Safety Monitoring
11
12 140 Board (DSMB) will review safety data on at least a monthly base.
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16 142 *Secondary objectives*

17
18 143 The secondary objectives are related to the efficacy and pharmacokinetics (PK) of Adrecizumab. The
19
20 144 primary efficacy endpoint, the “Sepsis Support Index” (SSI), is a composite endpoint reflecting organ
21
22 145 dysfunction or death within the first 14 days of follow-up. More precisely: within the first 14 days of
23
24 146 follow-up, every day on which a vasopressor or mechanical ventilation is used, or renal dysfunction
25
26 147 (defined as renal SOFA = 4) is apparent, or the patient is not alive anymore, is counted as 1. The sum
27
28 148 over the 14 day follow-up period is defined as the SSI score, which can have a maximum of 14 and a
29
30 149 minimum of 1 (as vasopressor usage on day 1 is an inclusion criteria). The calculation of the SSI is
31
32 150 further illustrated in Figure 3.

33
34 151 Additional secondary objectives include: SSI at day 28 of follow-up, penalized SSI (pSSI) (patients
35
36 152 who die get penalized with the maximum score), individual SSI components, persistent organ
37
38 153 dysfunction or death at day 14 and 28 of follow-up²⁶, day 28 and day 90 mortality rate and quality of
39
40 154 life (Euro-QoL-5), change over time in SOFA and other parameters such as APACHE II score and
41
42 155 functional parameters (including, but not limited to heart rate, blood pressure, PaO₂/FiO₂, fluid
43
44 156 balance, blood lactate, creatinine, pro-enkephalin, MR-proADM, inflammatory markers, including
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46 157 PCT and IL-6), and length of stay at ICU/ hospital.

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48 158 For the PK sub-study (n=80 patients), endpoints are key PK parameters, including peak plasma
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50 159 concentrations [C_{max}], systemic exposure [AUC], volume of distribution [V], systemic clearance [CL]
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52 160 and elimination half-life [t_{1/2}] of Adrecizumab.
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56 162 *Patient selection*

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3 163 A total of 300 adult patients with early septic shock and elevated bio-ADM concentration will be
4
5 164 randomized. Early septic shock is defined as sepsis with hypotension (MAP < 65 mmHg) refractory to
6
7 165 fluid resuscitation and requiring vasopressor therapy.⁴ Patients with a measurement of circulating bio-
8
9 166 ADM > 70 pg/mL will be eligible to be randomized. The window for inclusion and infusion of study
10
11 167 medication is 12 hours following initiation of vasopressor therapy. A lactate concentration > 2 mmol/L
12
13 168 is not an inclusion criteria, as concentrations may change quickly in response to initial therapy.
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15 169 Patients will be screened for clinical inclusion and exclusion criteria (Table 1). Eligibility will be
16
17 170 confirmed by the CCC in Brussels, Belgium. Patients that fulfil all inclusion criteria and none of the
18
19 171 exclusion criteria will be eligible to be randomized.

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21 17222 173 **Table 1.** In- and exclusion criteria.23
24 174 **Inclusion criteria**

- 25 175 1. Written informed consent by patient or legal representative (according to country – specific regulations)
- 26 176 2. Male and female patient, age ≥ 18 years
- 27 177 3. Body weight 50 – 120 kg
- 28 178 4. Bio-ADM concentration > 70 pg/mL
- 29 179 5. Patient with early septic shock (start of vasopressor therapy < 12 hours)
- 30 180 6. Women of childbearing potential must have a negative serum or urine pregnancy test before randomization
- 31 181 and have to use a highly effective method of contraception

32 182
33 183 **Exclusion criteria**

- 34 184 1. Moribund
- 35 185 2. Pre-existing unstable condition (e.g. a recent cerebral hemorrhage or infarct, a recent acute unstable
- 36 186 myocardial infarction (all < 3 months), congestive heart failure - New York Heart Association (NYHA) Class IV
- 37 187 3. Patients that required cardiopulmonary resuscitation in the last 4 weeks prior to evaluation for enrollment
- 38 188 4. Severe Chronic Obstructive Pulmonary Disease (COPD) with chronic oxygen need at home (GOLD IV)
- 39 189 5. Any organ or bone marrow transplant within the past 24 weeks
- 40 190 6. Uncontrolled serious hemorrhage (≥ 2 units of blood / platelets in the previous 24 hrs.). Patients may be
- 41 191 considered for enrollment if bleeding has stopped and patient is otherwise qualified
- 42 192 7. Uncontrolled hematological / oncological malignancies
- 43 193 8. Absolute neutropenia < 500 per μ L
- 44 194 9. Severe chronic liver disease (Child-Pugh C)
- 45 195 10. Systemic fungal infection or active tuberculosis
- 46 196 11. Neuromuscular disorders that impact breathing / spontaneous ventilation
- 47 197 12. Burns > 30% of body surface
- 48 198 13. Plasmapheresis
- 49 199 14. Women who are pregnant or nursing
- 50 200 15. Participation in a clinical trial involving another investigational drug within 4 weeks prior to inclusion
- 51 201 16. Unwilling or unable to be fully evaluated for all follow-up visits

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3 206 *Randomization*

4 207 Patients are randomly assigned to receive active treatment (2 mg/kg Adrecizumab, 4 mg/kg
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6 208 Adrecizumab) or placebo, using a block randomization scheme (1:1:2 treatment allocation ratio). A
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8 209 randomization code list will be generated by an independent statistician not involved in the study. For
9
10 210 each center, study medication is provided in boxes containing 4 pairs of vials according to the 4-block-
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12 211 randomization list, allowing stratification by center.
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16 212
17 213 *Informed consent*

18 214 Prior to any study-related procedures, patients must provide informed consent in accordance with the
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20 215 EU Clinical Trial Directive, the Declaration of Helsinki and ICH-GCP requirements. For patients
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22 216 unable to provide consent themselves due to their medical condition written informed consent is to be
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24 217 obtained by the patient's legal representative or by other accepted procedures according to applicable
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26 218 national law and local regulations, e.g. consent by relatives or family members. In addition,
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28 219 retrospective patient consent to voluntarily continue the study will be obtained once the patient has
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30 220 sufficiently recovered. Patient and/or the patient's legal representatives can withdraw their consent on
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32 221 study participation at any time without providing an explanation.
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35 222
36 223 *Blinding*

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38 224 The study will be performed in a double-blinded fashion. All study personnel, including the
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40 225 investigator and site staff, patients, monitors, sponsor and CRO staff will be blinded to treatment
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42 226 assignment until study closure. The randomization list is kept strictly confidential and accessible only
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44 227 to authorized persons who are not involved in the conduct of the study. In case of emergency, blinding
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46 228 will only be broken if specific emergency treatment would be indicated by knowing the treatment
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48 229 status of the patient. Specific emergency envelopes will be available at each site. The investigator is
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50 230 required to notify the sponsor within 24 hours following the code break reporting the reason for
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52 231 unblinding. The investigational drug and its matching placebo are indistinguishable and all study drug
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54 232 kits will be packed in the same way. Unblinding will be authorised by the sponsor after completion of
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56 233 the study, locking of the database and performance of a blinded data review.
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3 234 *Study intervention*

4 235 A single dose of the study drug (2 or 4 mg/kg Adrecizumab, or placebo) is administered over a 1 hour
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6 236 period by continuous intravenous infusion, as soon as possible, but at the latest, within 12 hours
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8 237 following start of vasopressor therapy. Study drug is administered separately from any concomitant
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10 238 drugs using a dedicated lumen of a central venous catheter or a separate peripheral line. Study
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12 239 medication is provided in boxes according to the 4-block-randomization list. Each box contains 4 pairs
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14 240 of vials for a 1:1:2 treatment allocation ratio. The following pairs of vials are supplied in the box, in a
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16 241 blinded fashion: a set of 2 vials of Adrecizumab (for reconstitution of the 4 mg/kg dose), a set of 1 vial
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18 242 of Adrecizumab and 1 vial of placebo (for reconstitution of the 2 mg/kg dose) and two sets of two
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20 243 placebo vials. All vials are indistinguishable from each other, containing the same volume of solution,
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22 244 the same aqueous buffer and identical packaging. The study drug, adjusted to the patient's body
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24 245 weight, has to be reconstituted from a pair of vials. All study drug are stored in a secure and
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26 246 adequately temperature-monitored pharmacy storage facility at 2 – 8°C.

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30 248 *Concomitant medication*

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32 249 There are no specific restrictions regarding use of concomitant medication or other therapies. All
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34 250 patients will be treated according to “International Guidelines for Management of Severe Sepsis and
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36 251 Septic Shock”.²⁷ All concomitant medical treatments and medication will be recorded from inclusion
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38 252 until day 28 or ICU discharge (whichever comes first).

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42 254 *Patient and public involvement*

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44 255 Patients and the public were not involved in elaboration of the study protocol. There is no plan to
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46 256 disseminate the results directly to the study participants. Results will be published in a peer-reviewed
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48 257 journal and presented on conferences.

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52 259 **Statistical and analytical plan**

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54 260 *Sample size calculations*

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3 261 The sample size was calculated for the primary efficacy endpoint (SSI up to day 14). A sample size of
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5 262 n=150 patients is planned for the combined treatment groups receiving 2 and 4 mg/kg Adrecizumab.
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7 263 As both dosages result in an excess of antibody over the target peptide ADM, no difference in
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9 264 treatment effect is expected between the dosage groups. Therefore, the two dosage groups are pooled
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11 265 together for the final analysis, unless either dose is insufficient or safety and tolerability analysis
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13 266 indicate that one dose is not safe or tolerable. Power calculation was based on simulation analyses.
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15 267 The distribution of the SSI was based on real patient data from the ALBIOS study (n=539)¹⁵ and
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17 268 underlying assumptions were re-evaluated using results from the AdrenOSS-1 observational study.¹⁸
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19 269 For the simulations, a sample size of n=150 per group (treatment or placebo), and an effect size of
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21 270 10% decrease in SSI in the treatment group resulted in a power of the study of more than 80% to
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23 271 demonstrate an improvement of SSI of > 0 with at least 80% probability.
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25 272

273

273 *Statistical analyses*

274 Continuous variables will be summarized by the number of patients, mean, SD or median, quartile and
275 range, as appropriate. Categorical variables will be summarized using number and percentage by
276 category. Demographic and medical background data, secondary endpoints and safety variables will
277 be analyzed by means of descriptive and exploratory methods.

278 The primary analysis for efficacy will be performed as an intention-to-treat analysis based on the
279 combined dosage groups of Adrecizumab (n=150 patients total) versus placebo. A secondary analysis
280 will compare the two doses for differences in efficacy. In case patients did not receive the treatment
281 they were randomized to, an analysis based on the actual treatment will also be performed (as-treated-
282 analysis). For efficacy, a first analysis will determine whether the improvement in SSI due to
283 treatment is > 0 with at least 80% probability. Only if this is achieved, the classical p-value using
284 appropriate methods will be calculated. The primary efficacy endpoint, 14-day SSI, will be analyzed
285 using the non-parametric Wilcoxon test, to estimate the treatment effects (based on the ‘pseudo-
286 median’) as well as its confidence interval. All-cause mortality will be evaluated using Kaplan-Meier
287 plots comparing treatment versus placebo (log-rank test) and Cox regression modelling including
288 covariates to adjust for potential confounders. In order to identify subgroups which may possibly

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3 289 benefit more from Adrecizumab treatment, interactions with other drugs, as well as exploratory
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5 290 subgroup analyses are planned in patients defined by disease severity, biomarkers, concomitant
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7 291 medication or other clinical data.

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11 293 *Interim analysis with futility stop*

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13 294 An unblinded interim analysis is planned after 50% of patients completed the study on day 28. The
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15 295 study will be terminated if the probability of a positive outcome after recruitment of all patients is
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17 296 below 40%, based on the primary efficacy endpoint 14 day SSI. In case the futility stop is reached, but
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19 297 if some of the other efficacy endpoints show a promising outcome for the full study, the futility stop
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21 298 may be suspended. Statistical consequence of applying the futility analysis was included in the power
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23 299 simulation.

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27 301 **Data quality assurance**

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29 302 All data management activities are done according to ICH-GCP as required by regulatory agencies. A
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31 303 commercial Contract Research Organisation (CRO), M.A.R.C.O. GmbH & Co. KG (M.A.R.C.O.[®]),
32
33 304 will be responsible for data management. All sites will maintain source documentation and enter
34
35 305 patient data into an electronic case report form (eCRF). Automated and manual checks will be
36
37 306 performed to ensure completeness and consistency of the data. The eCRF was designed by
38
39 307 M.A.R.C.O.[®] in the Amedon system. Validation checks are implemented in the system or programmed
40
41 308 with SAS[®], version 9.1 or higher, according to the data validation plan set up by M.A.R.C.O.[®].

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45 310 **Safety assessments**

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47 311 *Medication error*

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49 312 Adequately trained hospital staff will prepare, double-check and administer study medication. The
50
51 313 dose levels that are administered in the study have not caused any safety concerns in previous studies
52
53 314 in healthy volunteers²²⁻²⁴ or in preclinical safety and toxicological studies in animals and non-human
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55 315 primates. The risk for adverse health effects due to medication errors are thought to be minimal.

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3 317 *Overdose risks*

4 318 No drug specific antidote for Adrecizumab is available. An overdose is defined as any dose higher
5
6 319 than the assigned treatment dose. However, if by accident, the maximum volume would be withdrawn
7
8 320 from a pair of Adrecizumab vials during preparation of study medication, this would not exceed the
9
10 321 tested maximum dose of 8 mg/kg Adrecizumab in healthy volunteers, which did not result in any
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12 322 safety concerns.²²⁻²⁴

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16 324 *AE reporting*

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18 325 All patients are monitored for adverse events (AEs). AEs are defined as any untoward medical
19
20 326 occurrence in a patient administered a product and which does not necessarily have a causal
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22 327 relationship with this treatment. Investigators must document all AEs (whether serious or non-serious
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24 328 and judged related or unrelated to the study drug) that occur during the study period extending from
25
26 329 day 1 (inclusion) until 90 days after study drug administration in the eCRF. If the AE is serious, a
27
28 330 ‘serious adverse event report form’ must also be sent to the safety contact of the sponsor (spm², Safety
29
30 331 Projects & more GmbH, Hirschberg an der Bergstraße, Germany) within 24 hours of becoming aware
31
32 332 of the SAE. The severity of the AE will be rated as “mild”, “moderate”, “severe”, “life-threatening”,
33
34 333 “disabling” or “death related to event”. Investigators will use medical judgement to determine whether
35
36 334 there is evidence for a causal relationship and will describe this causality using terms such as
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38 335 “certain”, “probably/likely”, “possible”, “unlikely” or “unrelated”. All AEs will be followed-up until
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40 336 they have abated, or until a stable situation has been reached, and will be reported as such.

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44 338 *External data monitoring committee*

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46 339 An independent DSMB has been established to monthly review safety data including SAEs and,
47
48 340 overall safety data, and will judge the relevance of events for patient safety. DSMB members will have
49
50 341 no direct relationship to the study or to the study sponsor. The DSMB, composed by two clinical
51
52 342 experts in the field of sepsis, a biostatistician and a pharmacovigilance representative, will operate
53
54 343 independently. The DSMB is empowered to recommend changes in the design of the study to ensure
55
56 344 the safety of the patients and scientific integrity of the study.

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3 345 **Withdrawal**

4 346 Participation is strictly voluntary and a patient or their legal representative may withdraw the patient
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6 347 from the study at any time without providing an explanation. This will not affect his/her right for
7
8 348 future medical care. If a patient would withdraw from the study, the date, circumstances and any
9
10 349 reason provided will be documented on the withdrawal page of the eCRF. No data obtained after
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12 350 withdrawal of consent will be recorded on eCRFs - with the exception of any case of death until 90
13
14 351 days for safety reasons - nor will they be evaluated as part of the study, unless the patient gives
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16 352 specific permission.
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20 354 **Study period**

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22 355 The study started enrolling patients in December 2017. The estimated study enrolment completion
23
24 356 date is anticipated in the first half of 2019. Please note that this manuscript was finalized prior to the
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26 357 interim analysis.
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30 359 **Ethics and dissemination**

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32 360 *Ethics*

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34 361 The study was started after approval of the study protocol and all other relevant study documents by
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36 362 the relevant institutional review boards / independent ethics committees. The study is performed in
37
38 363 accordance with the Declaration of Helsinki, ICH, Code of Federal regulations and all other applicable
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40 364 regulations. Collection of personal data is performed according to country-specific regulations.
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44 366 *Confidentiality*

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46 367 After written informed consent has been obtained, patients will be assigned a unique 6-digit patient
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48 368 identification number. This allows identification of patients, while maintaining patient confidentiality.
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50 369 Confidentiality of all patient identities will be maintained, except during source data verification when
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52 370 monitors, auditors and other authorized agents of the sponsor or its designee, the ethics committee or
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54 371 any other applicable regulatory authorities are granted direct access to the study patient's original
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56 372 medical records. No material bearing a patient's name will be kept on file by the CRO or Sponsor.
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3 373 *Study monitoring*

4 374 The study is monitored by a clinical monitor, who will visit the investigator and study sites at periodic
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6 375 intervals in addition to phone, letter and e-mail contact. The monitor will follow the study closely
7
8 376 through reviewing of study records and source documents, and will discuss the conduct of the study
9
10 377 with the investigator and other site personnel.

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14 379 *Dissemination policy*

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16 380 The data of the study will be reported at scientific meetings and published in a peer-reviewed scientific
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18 381 journal, regardless of the results on outcome.

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3 387 **Discussion**

4 388 The development of new therapies for the treatment of sepsis and septic shock has proven to be a
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6 389 challenging task over the last decades. Many trials have investigated potential adjunctive therapies,
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8 390 predominantly focussing on anti-inflammatory agents. Unfortunately, this enormous effort put into
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10 391 dozens of clinical trials has not yielded compounds with clinically relevant beneficial effects. This can
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12 392 be explained by many factors, such as heterogeneous study populations and difficulties in selecting
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14 393 patients who may best benefit from an intervention. Also, the timing of the intervention, inappropriate
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16 394 outcome measures and the complexity of the disease with multiple pathways of injury hamper clinical
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18 395 research in sepsis patients.^{5 28}

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20 396 Importantly, when antibodies were used, most interventions were based on complete neutralization of
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22 397 the target. However, physiology probably is more balanced as some targets can exert both beneficial
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24 398 and detrimental effects, often even simultaneously. This may also represent a major contributing factor
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26 399 to the failure of many therapies to improve outcome witnessed in the last decades. Along these lines, it
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28 400 might be argued that a partially neutralizing therapy is more effective than total neutralization. The
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30 401 AdrenOSS-2 trial is an innovative, biomarker driven trial with a novel, supposedly clinically relevant
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32 402 efficacy endpoint.

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36 404 Patient heterogeneity is a substantial contributor to the difficulties in identifying effective therapies for
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38 405 sepsis. Patient selection is innovative in this study for two reasons. First, a more homogeneous
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40 406 subgroup of sepsis patients is selected, based on the combination of presence of early signs of shock,
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42 407 i.e. requiring vasopressor support, as well as elevated concentration of the biomarker bio-ADM.
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44 408 Selecting patients in the early phase of septic shock should select patients with preventable organ
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46 409 dysfunction compared to patients for whom septic shock and need of vasopressors lasted more than 12
47
48 410 hours. Furthermore, as previously described, measuring bio-ADM at baseline correlates strongly with
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50 411 the need for organ supporting therapy and mortality.^{14 15 17 18} Therefore, including bio-ADM as an
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52 412 inclusion criteria likely allows for better selection of patients who not only need vasopressor but also
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54 413 with a poor outcome. Combining need of vasopressor and high bio-ADM may contribute to obtaining

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3 414 a more homogeneous population of patients whom may benefit most from this adjunctive sepsis
4 415 therapy. To our knowledge, this is one of the first precision medicine study in sepsis patients.²⁹

6 416

8 417 ADM is a key vasoactive peptide involved in several important pathways in sepsis, which makes it an
9 418 attractive therapeutic target in sepsis.¹⁰ It has previously been described as a double-edged sword in
10 419 sepsis.³⁰ On vascular smooth muscle cells, ADM exerts vasodilatory effects and thereby induces
11 420 vasodilation and hypotension.¹¹⁻¹³ This effect of interstitial ADM may exacerbate the severity of shock
12 421 and may lead to organ hypoperfusion and organ dysfunction. In contrast, ADM present in the
13 422 circulation exerts potent endothelial barrier stabilizing effects, reducing vascular leakage that may
14 423 improve survival, as was demonstrated in *in vitro*^{6 7 31 32} and *in vivo* in animal models of sepsis and
15 424 systemic inflammation.^{8 9 33 34} However, direct administration of ADM during sepsis poses several
16 425 limitations. Because of a short half-life,¹¹ continuous infusion of ADM would be required. In addition,
17 426 due to ADM's potent vasodilative effects, ADM-induced hypotension might be an issue, which might
18 427 further aggravate shock in septic patients. A non-neutralizing antibody might attenuate ADM's
19 428 vasodilatory effects on VSMCs and potentiate ADM's effects on endothelial cells.

21 429

22 430 Adrecizumab, a *non-neutralizing* ADM-binding antibody, is one of the first therapies specifically
23 431 aimed at improving vascular endothelial barrier function, and represents a new candidate drug for the
24 432 treatment of septic shock. A detailed description of Adrecizumab's supposed mode of action is
25 433 described elsewhere.³⁵ Briefly, during sepsis, increased concentrations of ADM in the interstitial
26 434 compartment are thought to contribute to hypotension. Adrecizumab, which is confined to the blood
27 435 compartment, shifts the distribution of ADM away from the interstitium towards the blood, by
28 436 preventing diffusion of bound ADM.³⁵ This results in a strong increase of (bound) ADM
29 437 concentrations in the blood,^{22 23 24} where it, being bound to a non-neutralizing antibody, interacts with
30 438 receptors on endothelial cells and reduces vascular leakage and tissue edema. At the same time,
31 439 concentrations in the interstitium are reduced through this mechanism, leading to less vasodilation and
32 440 subsequent hypotension. This increase in plasma ADM concentration was observed in a rapid and
33 441 dose dependent manner upon i.v. administration of Adrecizumab, both in animals and in humans.²¹⁻²⁴

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3 442 Through reducing vascular leakage, tissue edema and hypotension, Adrecizumab could increase tissue
4 443 perfusion and improve the prognosis of sepsis patients, whereas it might also reduce the use of
5 444 vasopressors, thereby limiting potential adverse effects of vasopressors.^{36,37}

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8 445 Adrecizumab, administered as a single intravenous dose (due to its long half-life of 14 days), showed
9 446 promising results in preclinical studies of systemic inflammation and septic shock, including
10 447 attenuation of vascular leakage, lower vasopressor infusion rates and less organ dysfunction, related to
11 448 improved survival.¹⁹⁻²¹

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17
18 450 Substantial effort has been directed at reducing mortality in sepsis patients. Nevertheless, all major
19 451 sepsis trials have failed to improve survival. Although survival is a clear and relevant end-point, it may
20 452 be too insensitive to demonstrate a beneficial effect of a novel intervention. Therefore, novel endpoints
21 453 beyond all-cause mortality should be considered.³⁸ A composite endpoint, the “Sepsis Support Index”
22 454 (SSI), is used in the present study as the primary efficacy endpoint. The SSI is a composite index
23 455 reflecting days on organ supportive therapy (hemodynamics, pulmonary), days with organ dysfunction
24 456 (renal), as well as all-cause mortality. These organ systems were improved by Adrecizumab
25 457 administration in preclinical models, and support of these organ systems defines ICU care, indicating
26 458 that a therapeutic effect is of clinical relevance. The SSI is thought to allow for earlier and more
27 459 sensitive observations of possible clinically relevant beneficial effects of Adrecizumab compared to
28 460 more traditional primary efficacy endpoints.

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34 462 Potential limitations of the study include strict in- and exclusion criteria and a short window for patient
35 463 inclusion (within 12 hours following vasopressor therapy). These limitations result in a more
36 464 homogenous study population, but they may make recruitment more difficult and limit the
37 465 generalizability of the results.

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42 467 In conclusion, despite the exponential increase of knowledge gathered in the last decades pertaining
43 468 the pathophysiology of septic shock, this has not translated to effective therapeutic interventions and
44 469 as a consequence, this condition remains to have an unacceptable high morbidity and mortality. The

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3 470 AdrenOSS-2 trial is one of the first personalized medicine trial in septic shock patients, aimed at
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5 471 characterizing the safety and efficacy of the ADM-binding antibody Adrecizumab in septic shock
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7 472 patients with elevated concentrations of bio-ADM. The trial incorporates a number of innovative
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9 473 features such as biomarker guided patient selection and a novel efficacy endpoint in its design to avoid
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11 474 pitfalls of previous sepsis trials. Adrecizumab represents a promising approach to treat this lethal
12
13 475 syndrome. The results of this proof-of-concept and dose-finding phase II trial are eagerly awaited, and
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15 476 will importantly aid the design of future trials with this drug.

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21
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23
24 481

25 26 482 **Contributors**

27
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29
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37
38 488

39 40 489 **Competing interests**

41
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43
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55
56 497 manuscript. Adrenomed AG holds patent rights on anti-ADM antibodies.

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For peer review only

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3 615 **Figure and table legends**
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7 617 **Figure 1.** Study design.
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11 619 **Figure 2.** Study timeline.
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15 621 **Figure 3.** Primary *efficacy* endpoint: 14-day Sepsis Support Index (SSI): example calculation.
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19 623 **Table 1.** In- and exclusion criteria.
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Screening

BMJ Open **Patients with**
Early (<12hr) septic shock and
Bio-ADM > 70 pg/mL

Randomisation

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Treatment

2 mg/kg
Adrecizumab

4 mg/kg
Adrecizumab

Placebo

n=150 patients
completed day 28

Analyses

Interim analysis for futility
Stop of study if probability of
positive outcome of efficacy
endpoint is < 40%

n=300 patients
completed follow-up

Objectives/endpoints

Final analysis

Primary objective

- *Safety and tolerability* of Adrecizumab

Secondary objectives

- *Efficacy* of Adrecizumab (intention-to-treat analysis)
 - Primary efficacy endpoint: Sepsis Support Index (SSI) within the first 14 days.
 - Other endpoints include: mortality, morbidity, vital signs, QoL and length of stay.
- *Pharmacokinetics* (C_{max} , AUC, V, CL, $t_{1/2}$)

BMJ Open Follow-up
Safety and tolerability for 90 days

- Criteria for early septic shock
- Start vasopressor

Daily assessments

(S)AEs, concomitant therapy, organ dysfunction, disease severity scores, clinical, pharmacological and biological parameters, blood sampling at day 1, 3, 5 and 7 after randomization

Time

Day₁

Day₂₈

Day₉₀

12 hour window for

- Contact CCC for randomization and to confirm:
 - Informed consent
 - Bio-ADM determination (must be > 70 pg/ml)
 - in-/exclusion criteria
- Study drug infusion (i.v. over 1-hour period)

Quality of life
questionnaire

Quality of life
questionnaire

Sepsis Support Index (SSI)

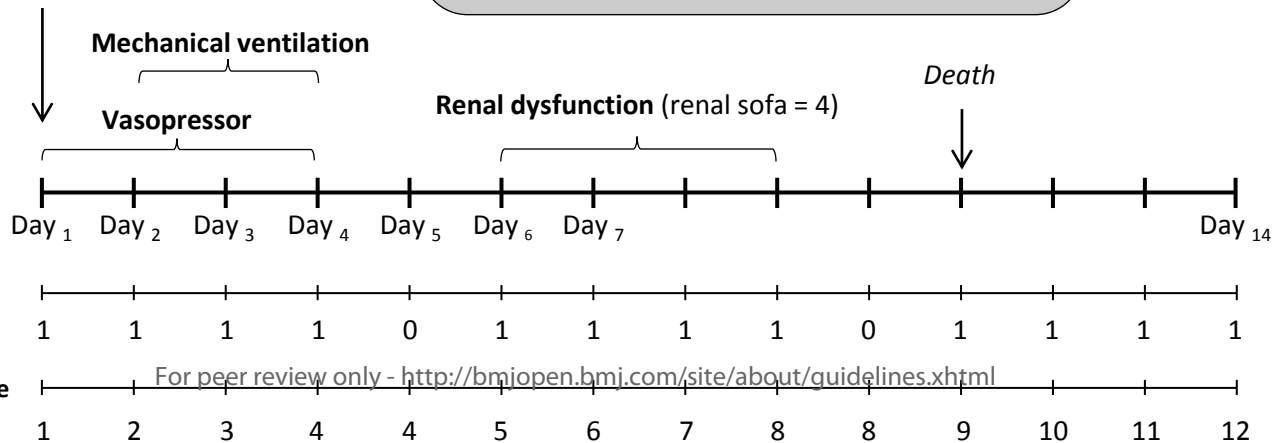
Sepsis Support Index (SSI), max. 1 point per day:

- Day on vasopressor support, or
 - Day on mechanical ventilation, or
 - Day with renal SOFA = 4, or
 - Days not alive
- } = 1 point

Example calculation of daily and cumulative Sepsis Support Index score

Patient enrolled

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BMJ Open

Study protocol: A Double Blind, Placebo-Controlled, Randomized, Multicenter, Proof of Concept and Dose-finding Phase II Clinical Trial to Investigate the Safety, Tolerability and Efficacy of Adrecizumab in Patients with Septic Shock and Elevated Adrenomedullin concentration (AdrenOSS-2)

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Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Sepsis, Adrecizumab, Adrenomedullin, Septic shock, Vascular integrity, Phase II clinical trial

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Study protocol: A Double Blind, Placebo-Controlled, Randomized, Multicenter, Proof of Concept and Dose-finding Phase II Clinical Trial to Investigate the Safety, Tolerability and Efficacy of Adrecizumab in Patients with Septic Shock and Elevated Adrenomedullin concentration (AdrenOSS-2)

Authors

Christopher Geven^{1*}, Alice Blet^{2*}, Matthijs Kox¹, Oliver Hartmann³, Paul Scigalla³, Jens Zimmermann³, Gernot Marx^{4*}, Pierre-Francois Laterre^{5*}, Alexandre Mebazaa^{2*}, Peter Pickkers^{1*}

¹Department of Intensive Care Medicine, Radboud Center for Infectious Diseases (RCI), Radboud university medical center, HP: 710, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

²Department of Anesthesia, Burn and Critical Care, University Hospitals Saint-Louis – Lariboisière, AP-HP, Paris, France. UMR-S 942, Inserm, Paris, France. Paris Diderot University, Sorbonne Paris Cité, Paris, France.

³Adrenomed AG, Neuendorfstraße 15A, D-16761 Hennigsdorf, Germany.

⁴Department of Intensive Care Medicine and Intermediate Care, RWTH University Hospital Aachen, Aachen, Germany.

⁵Department of Critical Care Medicine, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain (UCL), Brussels, Belgium.

* Christopher Geven and Alice Blet contributed equally to the manuscript.

* Pierre-Francois Laterre, Gernot Marx, Alexandre Mebazaa and Peter Pickkers share senior authorship as steering committee members of the AdrenOSS-2 trial.

Corresponding author: Prof. Dr. Peter Pickkers. Radboud university medical center, Department of Intensive Care Medicine (710), PO Box 9101, 6500 HB, Nijmegen, The Netherlands. E-mail: peter.pickkers@radboudumc.nl

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10 34 antibody; phase II.
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14 36 **Abstract**

15
16 37 **Introduction:** Sepsis remains a major health problem with an increasing incidence, high morbidity
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18 38 and high mortality. Apart from treatment with antibiotics and organ support, no approved specific
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20 39 adjunct therapies currently exist. Adrenomedullin (ADM) is a vasoactive peptide. High plasma
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22 40 concentrations of ADM correlate with worse outcome in sepsis patients. Preclinical work with the
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24 41 non-neutralizing ADM-binding antibody Adrecizumab showed promising effects in animal models of
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26 42 septic shock, including improved vascular barrier function, reduced vasopressor demand and organ
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28 43 dysfunction, and increased survival. Therapeutic use of Adrecizumab may therefore improve outcome
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30 44 in critically ill patients with septic shock and high ADM plasma concentrations. Phase I studies in
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32 45 healthy volunteers did not reveal any safety concerns. In this biomarker-guided trial, the safety and
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34 46 efficacy of Adrecizumab will be investigated in patients with septic shock.
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36 47 **Methods and analysis:** We describe a phase II, randomized, double blind, placebo-controlled,
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38 48 biomarker-guided, proof of concept and dose-finding clinical trial in patients with early septic shock
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40 49 and high concentration of circulating ADM. A total of 300 patients will be enrolled at approx. 30 sites
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42 50 within the European Union. Patients are randomized to receive active treatment (2 and 4 mg/kg
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44 51 Adrecizumab) or placebo, in a 1:1:2 ratio. Patient selection is not only guided by clinical parameters,
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46 52 but also biomarker-guided by measurement of circulating biologically active ADM concentration at
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48 53 admission. Primary endpoint is safety and tolerability of Adrecizumab over a 90 day period. A key
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50 54 secondary endpoint is the Sepsis Severity Index (SSI) over a 14-day period.
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52 55 **Ethics and dissemination:** This study is approved by relevant institutional review boards/independent
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54 56 ethics committees and is conducted in accordance with the ethical principles of the Declaration of
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3 57 Helsinki, the European Medicines Agency guidelines of Good Clinical Practice, and all other
4 58 applicable regulations. Results of this study will be published in a peer-reviewed scientific journal.
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9 60 **ClinicalTrial.gov registration number:** NCT03085758
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12 62 This manuscript is based on protocol version 4.0
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16 64 **Strengths and limitations of this study**

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18 65 • Patient selection is not only guided by clinical parameters, but also biomarker-guided by
19 66 measurement of circulating biologically active plasma adrenomedullin, allowing to select patients
20 67 with an impaired outcome who may benefit most from Adrecizumab therapy.
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22 68 • Patients will be recruited in medical, surgical and mixed Intensive Care Units at approximately 30
23 69 sites across 4 countries in Europe, promoting the studies generalizability.
24
25 70 • The study has appropriate randomization using random block sequence generation, good
26 71 allocation concealment, as well as blinding of treating and research personnel.
27
28 72 • The key secondary endpoint and primary *efficacy* endpoint is the composite Sepsis Support Index,
29 73 which combines all-cause mortality and organ dysfunction, aimed to be more sensitive to assess
30 74 the efficacy of the treatment.
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32 75 • Strict in- and exclusion criteria, as well as the brief time-window for inclusion (within 12 hours
33 76 following the initiation of vasopressor therapy) may limit generalisation of the results for the
34 77 entire population of critically ill patients with sepsis, although this may facilitate detection of an
35 78 efficacy signal.
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79 Introduction

80 Worldwide, sepsis is a major health problem, with an increasing incidence and high mortality.¹⁻³ It is
81 defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.⁴
82 Septic shock is defined as a subset of sepsis in which profound circulatory, cellular, and metabolic
83 abnormalities occur, which are associated with an increased risk of mortality.⁴ The most prominent
84 abnormalities are vasodilation and loss of vascular integrity, resulting in hypotension, and ultimately,
85 in organ dysfunction and death.⁵ Besides antibiotics and organ supportive therapies such as
86 vasopressors, mechanical ventilation and renal replacement therapy (RRT), there are currently no
87 sepsis-specific adjunctive therapies registered.

88
89 Adrenomedullin (ADM) is a vasoactive peptide hormone that plays an important role in sepsis.
90 Circulating ADM exerts endothelial barrier-stabilizing effects and maintains vascular integrity.⁶⁻¹⁰
91 ADM has vasodilatory properties in the vascular interstitium, and at high concentrations, as observed
92 during sepsis, may contribute to hypotension.¹¹⁻¹³ Elevated concentrations of plasma ADM at
93 admission have been reported in septic patients, and these were correlated with vasopressor
94 requirement, organ dysfunction and mortality.¹⁴⁻¹⁶ The cut-off value of biologically active ADM (bio-
95 ADM) of 70 pg/mL at admission was found to predict mortality for sepsis patients.¹⁴ This cut-off has
96 been validated in independent, large multicentre studies.^{15 17 18}

97
98 Based on these data, ADM may be an interesting therapeutic target for sepsis. A potential new
99 adjunctive therapy for the treatment of septic shock is Adrecizumab (previously also known as
100 HAM8101). It is a *non-neutralizing* ADM-binding antibody that has shown beneficial effects in
101 preclinical studies. Adrecizumab reduced vascular leakage, organ dysfunction and need for
102 vasopressor treatment during cecal ligation and puncture (CLP) induced sepsis in several animal
103 studies, and improved urine output and survival.¹⁹⁻²¹ Importantly, Adrecizumab administration was not
104 associated with any safety concerns in the first-in-human phase I study in healthy volunteers (n=24)²²⁻
105 ²⁴ and in a follow-up study in healthy volunteers which were intravenously challenged with
106 lipopolysaccharide (LPS) to induce systemic inflammation (also n=24).^{23 24} Of note, in the latter study,

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3 107 LPS-induced flu-like symptoms resolved more swiftly in Adrecizumab-treated subjects compared to
4
5 108 the placebo group. Pharmacokinetic analysis of Adrecizumab showed a half-life of approximately 14
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7 109 days, indicating that administration of a single dose is sufficient to achieve excess of plasma
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9 110 concentrations of the antibody over adrenomedullin for the entire sepsis period.

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11 111
12 112 Based on these preclinical and human phase I data, it is hypothesised that therapeutic use of
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14 113 Adrecizumab may improve endothelial dysfunction, restore and maintain vascular integrity and
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16 114 augment hemodynamics in critically ill patients with sepsis and septic shock. In the trial described in
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18 115 the present work, the safety, tolerability and efficacy of Adrecizumab is investigated in patients with
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20 116 early septic shock and elevated concentrations of circulating bio-ADM. This will be one of the first
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22 117 precision medicine, biomarker-guided studies in septic patients.
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25 26 119 **Methods and analysis**

27 28 120 *Design and setting*

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30 121 AdrenOSS-2 is a phase II, randomized, double blind, placebo-controlled, biomarker-guided, proof of
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32 122 concept and dose-finding clinical trial that is currently being conducted in patients with early septic
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34 123 shock and elevated concentration of circulating bio-ADM (> 70 pg/ml). A total of 300 patients will be
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36 124 recruited in medical, surgical and mixed Intensive Care Units (ICU) at approx. 30 sites across
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38 125 Belgium, France, Germany, the Netherlands and Italy (see clinicaltrials.gov of a list of current
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40 126 centres). Patient selection is guided by clinical parameters as well as by biomarker concentrations, by
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42 127 measuring circulating bio-ADM (sphingotest[®] bio-ADM, sphingotec GmbH, Hennigsdorf,
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44 128 Germany).²⁵ Based upon preclinical studies, two dosages of Adrecizumab will be investigated (2 and 4
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46 129 mg/kg bodyweight), in addition to a placebo control arm. After informed consent has been signed by
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48 130 the patient or his/her legal representative, circulating bio-ADM concentrations will be assessed. If bio-
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50 131 ADM concentrations are > 70 pg/mL, the clinical coordination center (CCC) will be contacted for
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52 132 final confirmation of patient eligibility and the patient will be randomized. An interim analysis for
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54 133 futility is planned after 150 patients have completed day 28 of the study. An overview of the study
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56 134 design is depicted in Figure 1 and study procedures in Figure 2.

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3 135 *Primary objective*

4 136 The primary objective is safety and tolerability, consisting of: mortality possibly related to
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6 137 Adrecizumab, interruption of infusion due to suspected intolerability of Adrecizumab, new treatment-
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8 138 emergent adverse events possibly related to Adrecizumab, and changes in severity and frequency of
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10 139 treatment-emergent adverse events. During the study, an independent Data and Safety Monitoring
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12 140 Board (DSMB) will review safety data on at least a monthly base.
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16 142 *Secondary objectives*

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18 143 The secondary objectives are related to the efficacy and pharmacokinetics (PK) of Adrecizumab. The
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20 144 primary efficacy endpoint, the “Sepsis Support Index” (SSI), is a composite endpoint reflecting organ
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22 145 dysfunction or death within the first 14 days of follow-up. More precisely: within the first 14 days of
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24 146 follow-up, every day on which a vasopressor or mechanical ventilation is used, or renal dysfunction
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26 147 (defined as renal SOFA = 4) is apparent, or the patient is not alive anymore, is counted as 1. The sum
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28 148 over the 14 day follow-up period is defined as the SSI score, which can have a maximum of 14 and a
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30 149 minimum of 1 (as vasopressor usage on day 1 is an inclusion criteria). The calculation of the SSI is
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32 150 further illustrated in Figure 3.

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34 151 Additional secondary objectives include: SSI at day 28 of follow-up, penalized SSI (pSSI) (patients
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36 152 who die get penalized with the maximum score), individual SSI components, persistent organ
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38 153 dysfunction or death at day 14 and 28 of follow-up²⁶, day 28 and day 90 mortality rate and quality of
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40 154 life (Euro-QoL-5), change over time in SOFA and other parameters such as functional parameters
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42 155 (including, but not limited to heart rate, blood pressure, PaO₂/FiO₂, fluid balance, blood lactate,
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44 156 creatinine, pro-enkephalin, MR-proADM, inflammatory markers, including PCT and IL-6), total
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46 157 duration of vasopressor/catecholamine use, as well as length of stay at ICU/ hospital.

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48 158 For the PK sub-study (n=80 patients), endpoints are key PK parameters, including peak plasma
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50 159 concentrations [C_{max}], systemic exposure [AUC], volume of distribution [V], systemic clearance [CL]
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52 160 and elimination half-life [t_{1/2}] of Adrecizumab.
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56 162 *Patient selection*

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3 163 A total of 300 adult patients with early septic shock and elevated bio-ADM concentration will be
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5 164 randomized. Early septic shock is defined as sepsis with hypotension (MAP < 65 mmHg) refractory to
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7 165 fluid resuscitation and requiring vasopressor therapy.⁴ Patients with a measurement of circulating bio-
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9 166 ADM > 70 pg/mL will be eligible to be randomized. The cut-off point for bio-ADM of 70 pg/mL was
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11 167 selected based on the specific needs and purpose of this study. Per patient data available for this
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13 168 evaluation included data from the ALBIOS, Frog-ICU and AdrenOSS-1 studies, to name the largest
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15 169 and most relevant, as well as data from healthy normal individuals. Specific needs to be met for the
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17 170 study were that patients with normal bio-ADM, as well as low severity and low expected mortality
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19 171 were to be excluded, to maximise the observable treatment effect, while keeping the eligible
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21 172 population as large as possible. The window for inclusion and infusion of study medication is 12 hours
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23 173 following initiation of vasopressor therapy. A lactate concentration > 2 mmol/L is not an inclusion
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25 174 criteria, as concentrations may change quickly in response to initial therapy. Patients will be screened
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27 175 for clinical inclusion and exclusion criteria (Table 1). Screening and enrolment logs will be maintained
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29 176 for all patients. For patients not enrolled in the study, the reason for non-enrolment is documented.
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31 177 Patients will undergo various screening assessments, including recording of information on hospital
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33 178 and ICU admission (date, time, location before admission, diagnosis, origin of sepsis), documenting of
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35 179 relevant ongoing conditions, relevant medical history and comorbidities present or treated within the
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37 180 last year (cardiovascular and non-cardiovascular), concomitant medication use, age, gender, ethnic
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39 181 origin, physical examination including weight and height, blood sampling for laboratory examinations
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41 182 and bio-ADM measurement, pregnancy test (urine or serum), recording of 12-lead ECG, and
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43 183 calculation of APACHE II and SOFA score. Eligibility will be confirmed by the CCC in Brussels,
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45 184 Belgium. Patients that fulfil all inclusion criteria and none of the exclusion criteria will be eligible to
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47 185 be randomized.

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50 187 **Table 1.** In- and exclusion criteria.51
52 188 **Inclusion criteria**

- 53 189 1. Written informed consent by patient or legal representative (according to country – specific regulations)
- 54 190 2. Male and female patient, age \geq 18 years
- 55 191 3. Body weight 50 – 120 kg
- 56 192 4. Bio-ADM concentration > 70 pg/mL
- 57 193 5. Patient with early septic shock (start of vasopressor therapy < 12 hours)

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3 194 6. Women of childbearing potential must have a negative serum or urine pregnancy test before randomization
4 195 and have to use a highly effective method of contraception
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6 197 **Exclusion criteria**

- 7 198 1. Moribund
8 199 2. Pre-existing unstable condition (e.g. a recent cerebral hemorrhage or infarct, a recent acute unstable
9 200 myocardial infarction (all < 3 months), congestive heart failure - New York Heart Association (NYHA) Class IV
10 201 3. Patients that required cardiopulmonary resuscitation in the last 4 weeks prior to evaluation for enrollment
11 202 4. Severe Chronic Obstructive Pulmonary Disease (COPD) with chronic oxygen need at home (GOLD IV)
12 203 5. Any organ or bone marrow transplant within the past 24 weeks
13 204 6. Uncontrolled serious hemorrhage (≥ 2 units of blood / platelets in the previous 24 hrs.). Patients may be
14 205 considered for enrollment if bleeding has stopped and patient is otherwise qualified
15 206 7. Uncontrolled hematological / oncological malignancies
16 207 8. Absolute neutropenia < 500 per μL
17 208 9. Severe chronic liver disease (Child-Pugh C)
18 209 10. Systemic fungal infection or active tuberculosis
19 210 11. Neuromuscular disorders that impact breathing / spontaneous ventilation
20 211 12. Burns > 30% of body surface
21 212 13. Plasmapheresis
22 213 14. Women who are pregnant or nursing
23 214 15. Participation in a clinical trial involving another investigational drug within 4 weeks prior to inclusion
24 215 16. Unwilling or unable to be fully evaluated for all follow-up visits
25 216

26 217 *Measuring bio-ADM*

27 218 For measurement of bio-ADM, 5 mL EDTA blood will be collected after written informed consent is
28 219 obtained. After centrifugation (2500G, 15 minutes, 20°C), bio-ADM levels are determined using a
29 220 fully validated, CE-marked, commercially available immunoluminometric assay (sphingotest[®] bio-
30 221 ADM assay, sphingotec GmbH, Hennigsdorf, Germany). This assay is performed locally by trained
31 222 personnel. The assay is highly specific for C-terminally amidated adrenomedullin (the biologically
32 223 active form of adrenomedullin, hence named bio-ADM). Each patient sample will be measured in
33 224 duplicate, and in parallel two calibrators (one with a concentration around the decision making point
34 225 (70 pg/mL)) will be run in triplicate along with each patient sample. The functionality of the
35 226 measuring system will be checked on a monthly basis at each site. Finally, bio-ADM will be re-
36 227 measured from banked aliquots in batch at a central lab to verify locally gained results. Further details
37 228 about the assay are described elsewhere.²⁵
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41 230 *Randomization*

42 231 Patients are randomly assigned to receive active treatment (2 mg/kg Adrecizumab, 4 mg/kg
43 232 Adrecizumab) or placebo, using a block randomization scheme (1:1:2 treatment allocation ratio). A
44 233 randomization code list will be generated by an independent statistician not involved in the study. For
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3 234 each center, study medication is provided in boxes containing 4 pairs of vials according to the 4-block-
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5 235 randomization list, allowing stratification by center.

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9 237 *Informed consent*

10 238 Prior to any study-related procedures, patients must provide informed consent in accordance with the
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12 239 EU Clinical Trial Directive, the Declaration of Helsinki and ICH-GCP requirements. Informed consent
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14 240 is obtained according to local requirements in Belgium, France, Germany and the Netherlands. Written
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16 241 informed consent is obtained by trained investigators after providing adequate verbal and written
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18 242 information about the study (in order to fully understand the study and any risks it entails), and giving
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20 243 the patient opportunity to ask questions and appropriate time to decide on participation in the study.
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22 244 For patients unable to provide consent themselves due to their medical condition written informed
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24 245 consent is to be obtained by the patient's legal representative or by other accepted procedures
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26 246 according to applicable national law and local regulations, e.g. consent by relatives or family
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28 247 members. In addition, retrospective patient consent to voluntarily continue the study will be obtained
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30 248 once the patient has sufficiently recovered. Patient and/or the patient's legal representatives can
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32 249 withdraw their consent on study participation at any time without providing an explanation.

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36 251 *Blinding*

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38 252 The study will be performed in a double-blinded fashion. All study personnel, including the
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40 253 investigator and site staff, patients, monitors, sponsor and CRO staff will be blinded to treatment
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42 254 assignment until study closure. The randomization list is kept strictly confidential by the data
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44 255 management vendor and accessible only to authorized persons who are not involved in the conduct of
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46 256 the study. In case of emergency, blinding will only be broken if specific emergency treatment would
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48 257 be indicated by knowing the treatment status of the patient. Specific emergency envelopes will be
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50 258 available at each site. The investigator is required to notify the sponsor within 24 hours following the
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52 259 code break reporting the reason for unblinding. The investigational drug and its matching placebo are
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54 260 indistinguishable and all study drug kits will be packed in the same way. Unblinding will be

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3 261 authorised by the sponsor after completion of the study, locking of the database and performance of a
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5 262 blinded data review.

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9 264 *Study intervention*

10 265 A single dose of the study drug (2 or 4 mg/kg Adrecizumab, or placebo) is administered over a 1 hour
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12 266 period by continuous intravenous infusion, as soon as possible, but at the latest, within 12 hours
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14 267 following start of vasopressor therapy. Study drug is administered separately from any concomitant
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16 268 drugs using a dedicated lumen of a central venous catheter or a separate peripheral line. Study
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18 269 medication is provided in boxes according to the 4-block-randomization list. Each box contains 4 pairs
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20 270 of vials for a 1:1:2 treatment allocation ratio. The following pairs of vials are supplied in the box, in a
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22 271 blinded fashion: a set of 2 vials of Adrecizumab (for reconstitution of the 4 mg/kg dose), a set of 1 vial
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24 272 of Adrecizumab and 1 vial of placebo (for reconstitution of the 2 mg/kg dose) and two sets of two
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26 273 placebo vials. All vials are indistinguishable from each other, containing the same volume of solution,
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28 274 the same aqueous buffer and identical packaging. The study drug, adjusted to the patient's body
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30 275 weight, has to be reconstituted from a pair of vials. All study drug are stored in a secure and
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32 276 adequately temperature-monitored pharmacy storage facility at 2 – 8°C.

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36 278 *Concomitant medication*

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38 279 There are no specific restrictions regarding use of concomitant medication or other therapies. All
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40 280 patients will be treated according to “International Guidelines for Management of Severe Sepsis and
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42 281 Septic Shock”.²⁷ All concomitant medical treatments and medication will be recorded from inclusion
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44 282 until day 28 or ICU discharge (whichever comes first).

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48 284 *Patient and public involvement*

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50 285 Patients and the public were not involved in elaboration of the study protocol. There is no plan to
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52 286 disseminate the results directly to the study participants. Results will be published in a peer-reviewed
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54 287 journal and presented on conferences.

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289 **Statistical and analytical plan**

290 *Sample size calculations*

291 The sample size was calculated for the primary efficacy endpoint (SSI up to day 14). A sample size of
292 $n=150$ patients is planned for the combined treatment groups receiving 2 and 4 mg/kg Adrecizumab.
293 As both dosages result in an excess of antibody over the target peptide ADM, no difference in
294 treatment effect is expected between the dosage groups. Therefore, the two dosage groups are pooled
295 together for the final analysis, unless either dose is insufficient or safety and tolerability analysis
296 indicate that one dose is not safe or tolerable. Power calculation was based on simulation analyses.
297 The distribution of the SSI was based on real patient data from the ALBIOS study ($n=539$)¹⁵ and
298 underlying assumptions were re-evaluated using results from the AdrenOSS-1 observational study.¹⁸
299 Based on the previously conducted observational AdrenOSS-1 study¹⁸ performed in septic patients, we
300 anticipate a median SSI in the control group of 4 [IQR 2-11], while in the ALBIOS study¹⁵ the median
301 was 7 (IQR 4-14) (these medians reflect a selection of patients with septic shock and bio-ADM larger
302 70 pg/mL). However, due to the non-normal distribution of the SSI, the median is still highly volatile
303 (the majority of patients have either a low SSI (1-3 days, if improving and discharged early), or a high
304 SSI (14 days, as patients that die within the first 14 days are usually on organ support while alive and
305 in ICU)). For the simulations, a sample size of $n=150$ per group (treatment or placebo), and an effect
306 size resulting in an approximately 10% decrease in SSI in the Adrecizumab-treatment group
307 (compared to the simulated control group) resulted in a power of the study of more than 80% to
308 demonstrate an improvement of SSI of > 0 with at least 80% probability. The 80% probability
309 corresponds to the lower limit of the 60%-confidence interval of the effect estimate, delta SSI, which
310 is based on the estimated difference of location from the Wilcoxon test. If the simulated lower limit of
311 delta SSI was > 0 , the simulation run reached the endpoint

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313 *Statistical analyses*

314 Continuous variables will be summarized by the number of patients, mean, SD or median, quartile and
315 range, as appropriate. Categorical variables will be summarized using number and percentage by
316 category. Demographic and medical background data, secondary endpoints and safety variables will

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3 317 be analyzed by means of descriptive and exploratory methods. Regarding the primary endpoint
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5 318 (safety), all AEs will be listed. The number and percentage of patients experiencing 1 or more AEs
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7 319 will be summarized by treatment arm / control group, relationship to study drug and severity/grade.
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9 320 SAE specific listings for each patient population will be generated on reported SAEs, but not as
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11 321 SUSARs. The same will be made for related severe AEs. Mortality analysis is described below.

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13 322 The primary analysis for efficacy will be performed as an intention-to-treat analysis based on the
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15 323 combined dosage groups of Adrecizumab (n=150 patients total) versus placebo. A secondary analysis
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17 324 will compare the two doses for differences in efficacy. In case patients did not receive the treatment
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19 325 they were randomized to, an analysis based on the actual treatment will also be performed (as-treated-
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21 326 analysis). The primary efficacy endpoint, 14-day SSI, will be analyzed using the non-parametric
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23 327 Wilcoxon test, to estimate the treatment effects (based on the Wilcoxon estimate for difference in
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25 328 location) as well as its confidence interval. First, it will be determined whether the improvement in SSI
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27 329 due to treatment is > 0 with at least 80% probability (based on the lower limit of the one-sided
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29 330 confidence interval of the effect estimate of the Wilcoxon test). If this is achieved, the classical p-
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31 331 value from the Wilcoxon test will also be calculated. All-cause mortality will be evaluated using
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33 332 Kaplan-Meier plots comparing treatment (separate for each dose, as well as a comparison combining
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35 333 both doses into one group) versus placebo (log-rank test) and Cox regression modelling including
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37 334 covariates to adjust for potential confounders. Potential confounders include age, gender, MAP, HR,
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39 335 source of infection, blood culture, comorbidities and initial SOFA score, as well as variables showing
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41 336 significant between-group differences (despite randomization). In order to identify subgroups which
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43 337 may possibly benefit more from Adrecizumab treatment, interactions with other drugs, as well as
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45 338 exploratory subgroup analyses are planned in patients defined by disease severity, biomarkers,
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47 339 concomitant medication or other clinical data. The subgroup analyses is nevertheless purely
48
49 340 exploratory. Subgroups will be defined by tertiles for continuous variables. For categorical variables,
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51 341 categories will be summarized such that they best represent tertiles if more than 3 categories are
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53 342 available. Statistical analysis of secondary endpoints is exploratory, and will be specified in a separate
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55 343 statistical analysis plan, which is to be finished before conclusion of the study.

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3 345 *Interim analysis with futility stop*

4 346 An unblinded interim analysis is planned after 50% of patients completed the study on day 28. The
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6 347 study will be terminated if the probability of a positive outcome after of all patients is below 40%,
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8 348 based on the primary efficacy endpoint 14 day SSI. In case the futility stop is reached, but if some of
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10 349 the other efficacy endpoints show a promising outcome for the full study, the futility stop may be
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12 350 suspended. Statistical consequence of applying the futility analysis was included in the power
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14 351 simulation. An independent statistician is responsible for analysing the data at interim analysis, and the
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16 352 steering committee, as well as the sponsor, will remain blinded until the end of the study. Note that the
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18 353 interim analysis focuses on futility only, potential termination of the trial based on harm is based on
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20 354 the reviewing and evaluation of unblinded data on safety and mortality by the DSMB (described
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22 355 further below).
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26 357 **Data quality assurance**

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28 358 All data management activities are done according to ICH-GCP as required by regulatory agencies. A
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30 359 commercial Contract Research Organisation (CRO), M.A.R.C.O. GmbH & Co. KG (M.A.R.C.O.[®]),
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32 360 will be responsible for data management. All sites will maintain source documentation and enter
33
34 361 patient data into an electronic case report form (eCRF). The clinical center is responsible for the secure
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36 362 and restrictive archiving of source data for at least 15 years or until the written notification from the
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38 363 sponsor that the documents are no longer required. During the required period, the clinical center will
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40 364 ensure that archived data and documents will be undamaged, legible and accessible to the sponsor
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42 365 and/or for regulatory purposes, if required. The study master file, the ECRFs, code envelopes and
43
44 366 other material supplied for the performance of the study will be retained by the sponsor according to
45
46 367 applicable regulations and laws, including the new GDPR (see also the section on confidentiality).
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48 368 Regarding the eCRF, automated and manual checks will be performed to ensure completeness and
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50 369 consistency of the data, and investigator site personnel seeking access must go through training
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52 370 processes before access to the system is granted. The eCRF was designed by M.A.R.C.O.[®] in the
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54 371 Amedon system. Validation checks are implemented in the system or programmed with SAS[®], version
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56 372 9.1 or higher, according to the data validation plan set up by M.A.R.C.O.[®].

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5 374 *Missing data*

6
7 375 In general, missing data in clinical variables will not be replaced or imputed. If missing data should
8
9 376 occur in variables required for secondary efficacy endpoints (e.g. SOFA score or other secondary
10
11 377 efficacy endpoints), a sensitivity analysis will be conducted assigning missing endpoint data with the
12
13 378 worst possible value (as defined for withdrawals), in addition to the analysis based on valid data only.

14
15 379 In addition, an analysis will be conducted where missing data points will be imputed using inter- or
16
17 380 extrapolation, with the exception that missing Bilirubin will be set to normal (liver SOFA component
18
19 381 = 0). Missing follow up time information will not be replaced for mortality analysis, but rather treated
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21 382 as respective methods for survival analysis intend.

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25 384 **Safety assessments**

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27 385 *Medication error*

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29 386 Adequately trained hospital staff will prepare, double-check and administer study medication. The
30
31 387 dose levels that are administered in the study have not caused any safety concerns in previous studies
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33 388 in healthy volunteers²²⁻²⁴ or in preclinical safety and toxicological studies in animals and non-human
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35 389 primates. The risk for adverse health effects due to medication errors are thought to be minimal.

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39 391 *Overdose risks*

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41 392 No drug specific antidote for Adrecizumab is available. An overdose is defined as any dose higher
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43 393 than the assigned treatment dose. However, if by accident, the maximum volume would be withdrawn
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45 394 from a pair of Adrecizumab vials during preparation of study medication, this would not exceed the
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47 395 tested maximum dose of 8 mg/kg Adrecizumab in healthy volunteers, which did not result in any
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49 396 safety concerns.²²⁻²⁴

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53 398 *AE reporting*

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55 399 All patients are monitored for adverse events (AEs). AEs are defined as any untoward medical
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57 400 occurrence in a patient administered a product and which does not necessarily have a causal

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3 401 relationship with this treatment. Investigators must document all AEs (whether serious or non-serious
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5 402 and judged related or unrelated to the study drug) that occur during the study period extending from
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7 403 day 1 (inclusion) until 90 days after study drug administration in the eCRF. If the AE is serious, a
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9 404 ‘serious adverse event report form’ must also be sent to the safety contact of the sponsor (spm², Safety
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11 405 Projects & more GmbH, Hirschberg an der Bergstraße, Germany) within 24 hours of becoming aware
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13 406 of the SAE. The severity of the AE will be rated as “mild”, “moderate”, “severe”, “life-threatening”,
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15 407 “disabling” or “death related to event”. Investigators will use medical judgement to determine whether
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17 408 there is evidence for a causal relationship and will describe this causality using terms such as
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19 409 “certain”, “probably/likely”, “possible”, “unlikely” or “unrelated”. All AEs will be followed-up until
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21 410 they have abated, or until a stable situation has been reached, and will be reported as such.

22 411

23 412 *External data monitoring committee*

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26 413 An independent DSMB has been established to monthly review safety data including SAEs and,
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28 414 overall safety data, and will judge the relevance of events for patient safety. DSMB members will have
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30 415 no direct relationship to the study or to the study sponsor. The DSMB, composed by two clinical
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32 416 experts in the field of sepsis, a biostatistician and a pharmacovigilance representative, will operate
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34 417 independently. The DSMB is empowered to recommend changes in the design of the study to ensure
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36 418 the safety of the patients and scientific integrity of the study.

37 419

38 420 **Withdrawal**

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42 421 Participation is strictly voluntary and a patient or their legal representative may withdraw the patient
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44 422 from the study at any time without providing an explanation. This will not affect his/her right for
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46 423 future medical care. If a patient would withdraw from the study, the date, circumstances and any
47
48 424 reason provided will be documented on the withdrawal page of the eCRF. No study specific data or
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50 425 patient material will be collected after withdrawal of consent. No data obtained after withdrawal of
51
52 426 consent will be recorded on eCRFs, unless the patient consents to the use thereof. For safety analysis,
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54 427 the patient’s outcome status (dead or alive) at day 90 will be collected. For the main efficacy analysis,
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56 428 these patients will be excluded. In order to rule out that patient withdrawal is linked to treatment, a

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3 429 sensitivity analysis will be conducted assigning missing endpoint data with the worst possible value
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5 430 (i.e. worst possible value for patients in the treatment group, the best possible value for patients in the
6
7 431 control group). In addition, an analysis will be conducted where missing data points will be imputed
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9 432 using inter- or extrapolation, if applicable.

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12 434 **Study period**

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14 435 The study started enrolling patients in December 2017. The estimated study enrolment completion
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16 436 date is anticipated in the first half of 2019. Please note that this manuscript was finalized prior to the
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18 437 interim analysis.

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21 439 **Ethics and dissemination**

22 440 *Ethics*

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24 441 The study was started after approval of the study protocol and all other relevant study documents by
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26 442 the relevant institutional review boards / independent ethics committees. The study is performed in
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28 443 accordance with the Declaration of Helsinki, ICH, Code of Federal regulations and all other applicable
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30 444 regulations. Collection of personal data is performed according to country-specific regulations.

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32 445

33 446 *Confidentiality*

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35 447 After written informed consent has been obtained, patients will be assigned a unique 6-digit patient
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37 448 identification number. This allows identification of patients, while maintaining patient confidentiality.

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39 449 The investigators, designated CRO and sponsor and all other involved parties will preserve the
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41 450 confidentiality of all patients taking part in the study, in accordance with ICH-GCP and local
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43 451 regulations. Confidentiality of all patient identities will be maintained, except during source data
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45 452 verification when monitors, auditors and other authorized agents of the sponsor or its designee, the
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47 453 ethics committee or any other applicable regulatory authorities are granted direct access to the study
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49 454 patient's original medical records. No material bearing a patient's name will be kept on file by the
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51 455 CRO or sponsor. The code list with treatment allocations (randomization list) is stored separately from
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53 456 the Sponsor at the data management vendor (CRO) during the course of the study. These data

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3 457 management vendors will provide all relevant data (pseudonymized) to the sponsor after the end of the
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5 458 study. In addition, sets of sealed envelopes with randomization codes are kept at the site for
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7 459 emergency unblinding, with the DSMB, and with the party responsible for reporting SUSARs as
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9 460 required by regulatory agencies. Data retained from this study will be protected in accordance with all
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11 461 applicable legal requirements. Information about study patients will be kept confidential and managed
12
13 462 according to the requirements of EU-directives 2001/20/EC, 2005/28/EC and 2003/63/EC, and
14
15 463 relevant national and local legislation. All ongoing subjects signed the ICF (including the data
16
17 464 protection part) and additionally the “Information letter for ongoing Patients” regarding the new
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19 465 GDPR/(DSGVO, Germany). All patients have been informed by investigators before they signed these
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21 466 documents.

22 467

24 468 *Data access*

26 469 The following parties have access to the data: sponsor, sites and selected vendors (data management,
28 470 pharmacovigilance). Individual patient data may be used by site investigators for publication in
30 471 agreement with the sponsor. Please note that the confidentiality section also specifies some external
32 472 parties that may access data (regulatory authorities, etc.).

34 473

36 474 *Sample storage*

38 475 A biobank for biomarkers is implemented and samples are stored for potential future use.

40 476

42 477 *Study monitoring*

44 478 The study is monitored by a clinical monitor, who will visit the investigator and study sites at periodic
46 479 intervals in addition to phone, letter and e-mail contact. The monitor will follow the study closely
48 480 through reviewing of study records and source documents, and will determine if the reported data are
50 481 accurate and complete.

52 482

54 483 *Dissemination policy*

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3 484 The data of the study will be reported at scientific meetings and published in a peer-reviewed scientific
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5 485 journal, regardless of the results on outcome, in accordance with the good publication practice
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7 486 guideline of the international society for medical publication professionals. The sponsor and the
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9 487 investigator and other individuals who have expertise in the area and who are willing to interpret the
10
11 488 data and write or review articles and presentations will form a publication Steering Committee to
12
13 489 oversee the preparation of articles and presentations from this study.
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15 490

16 491 **Discussion**

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18 492 The development of new therapies for the treatment of sepsis and septic shock has proven to be a
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20 493 challenging task over the last decades. Many trials have investigated potential adjunctive therapies,
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22 494 predominantly focussing on anti-inflammatory agents. Unfortunately, this enormous effort put into
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24 495 dozens of clinical trials has not yielded compounds with clinically relevant beneficial effects. This can
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26 496 be explained by many factors, such as heterogeneous study populations and difficulties in selecting
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28 497 patients who may best benefit from an intervention. Also, the timing of the intervention, inappropriate
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30 498 outcome measures and the complexity of the disease with multiple pathways of injury hamper clinical
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32 499 research in sepsis patients.^{5 28}

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34 500 Importantly, when antibodies were used, most interventions were based on complete neutralization of
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36 501 the target. However, physiology probably is more balanced as some targets can exert both beneficial
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38 502 and detrimental effects, often even simultaneously. This may also represent a major contributing factor
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40 503 to the failure of many therapies to improve outcome witnessed in the last decades. Along these lines, it
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42 504 might be argued that a partially neutralizing therapy is more effective than total neutralization. The
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44 505 AdrenOSS-2 trial is an innovative, biomarker driven trial with a novel, supposedly clinically relevant
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46 506 efficacy endpoint.

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50 508 Patient heterogeneity is a substantial contributor to the difficulties in identifying effective therapies for
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52 509 sepsis. Patient selection is innovative in this study for two reasons. First, a more homogeneous
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54 510 subgroup of sepsis patients is selected, based on the combination of presence of early signs of shock,
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56 511 i.e. requiring vasopressor support, as well as elevated concentration of the biomarker bio-ADM.

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3 512 Selecting patients in the early phase of septic shock should select patients with preventable organ
4 513 dysfunction compared to patients for whom septic shock and need of vasopressors lasted more than 12
5 514 hours. Furthermore, as previously described, measuring bio-ADM at baseline correlates strongly with
6 515 the need for organ supporting therapy and mortality.^{14 15 17 18} Therefore, including bio-ADM as an
7 516 inclusion criteria likely allows for better selection of patients who not only need vasopressor but also
8 517 with a poor outcome. Combining need of vasopressor and high bio-ADM may contribute to obtaining
9 518 a more homogeneous population of patients whom may benefit most from this adjunctive sepsis
10 519 therapy. To our knowledge, this is one of the first precision medicine study in sepsis patients.²⁹

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20 521 ADM is a key vasoactive peptide involved in several important pathways in sepsis, which makes it an
21 522 attractive therapeutic target in sepsis.¹⁰ It has previously been described as a double-edged sword in
22 523 sepsis.³⁰ On vascular smooth muscle cells, ADM exerts vasodilatory effects and thereby induces
23 524 vasodilation and hypotension.¹¹⁻¹³ This effect of interstitial ADM may exacerbate the severity of shock
24 525 and may lead to organ hypoperfusion and organ dysfunction. In contrast, ADM present in the
25 526 circulation exerts potent endothelial barrier stabilizing effects, reducing vascular leakage that may
26 527 improve survival, as was demonstrated in *in vitro*^{6 7 31 32} and *in vivo* in animal models of sepsis and
27 528 systemic inflammation.^{8 9 33 34} However, direct administration of ADM during sepsis poses several
28 529 limitations. Because of a short half-life,¹¹ continuous infusion of ADM would be required. In addition,
29 530 due to ADM's potent vasodilative effects, ADM-induced hypotension might be an issue, which might
30 531 further aggravate shock in septic patients. A non-neutralizing antibody might attenuate ADM's
31 532 vasodilatory effects on VSMCs and potentiate ADM's effects on endothelial cells.

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46 534 Adrecizumab, a *non-neutralizing* ADM-binding antibody, is one of the first therapies specifically
47 535 aimed at improving vascular endothelial barrier function, and represents a new candidate drug for the
48 536 treatment of septic shock. A detailed description of Adrecizumab's supposed mode of action is
49 537 described elsewhere.³⁵ Briefly, during sepsis, increased concentrations of ADM in the interstitial
50 538 compartment are thought to contribute to hypotension. Adrecizumab, which is confined to the blood
51 539 compartment, shifts the distribution of ADM away from the interstitium towards the blood, by

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3 540 preventing diffusion of bound ADM.³⁵ This results in a strong increase of (bound) ADM
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5 541 concentrations in the blood,^{22 23 24} where it, being bound to a non-neutralizing antibody, interacts with
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7 542 receptors on endothelial cells and reduces vascular leakage and tissue edema. At the same time,
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9 543 concentrations in the interstitium are reduced through this mechanism, leading to less vasodilation and
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11 544 subsequent hypotension. This increase in plasma ADM concentration was observed in a rapid and
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13 545 dose dependent manner upon i.v. administration of Adrecizumab, both in animals and in humans.²¹⁻²⁴
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15 546 Through reducing vascular leakage, tissue edema and hypotension, Adrecizumab could increase tissue
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17 547 perfusion and improve the prognosis of sepsis patients, whereas it might also reduce the use of
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19 548 vasopressors, thereby limiting potential adverse effects of vasopressors.^{36 37}
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21 549 Adrecizumab, administered as a single intravenous dose (due to its long half-life of 14 days), showed
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23 550 promising results in preclinical studies of systemic inflammation and septic shock, including
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25 551 attenuation of vascular leakage, lower vasopressor infusion rates and less organ dysfunction, related to
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27 552 improved survival.¹⁹⁻²¹
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30
31 554 Substantial effort has been directed at reducing mortality in sepsis patients. Nevertheless, all major
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33 555 sepsis trials have failed to improve survival. Although survival is a clear and relevant end-point, it may
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35 556 be too insensitive to demonstrate a beneficial effect of a novel intervention. Therefore, novel endpoints
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37 557 beyond all-cause mortality should be considered.³⁸ The use of composite endpoints allows for a more
38
39 558 nuanced assessment of morbidity and mortality. A new composite endpoint, the “Sepsis Support
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41 559 Index” (SSI), is used in the present study as the primary efficacy endpoint. The SSI is a composite
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43 560 index reflecting days on organ supportive therapy (hemodynamics, pulmonary), days with organ
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45 561 dysfunction (renal), as well as all-cause mortality. These organ systems were improved by
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47 562 Adrecizumab administration in preclinical models, and support of these organ systems defines ICU
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49 563 care, indicating that a therapeutic effect is of clinical relevance. The SSI is thought to allow for earlier
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51 564 and more sensitive observations of possible clinically relevant beneficial effects of Adrecizumab
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53 565 compared to more traditional primary efficacy endpoints.
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3 567 Potential limitations of the study include strict in- and exclusion criteria and a short window for patient
4 568 inclusion (within 12 hours following vasopressor therapy). These limitations result in a more
5 569 homogenous study population, but they may make recruitment more difficult and limit the
6 570 generalizability of the results.
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12 572 In conclusion, despite the exponential increase of knowledge gathered in the last decades pertaining
13 573 the pathophysiology of septic shock, this has not translated to effective therapeutic interventions and
14 574 as a consequence, this condition remains to have an unacceptable high morbidity and mortality. The
15 575 AdrenOSS-2 trial is one of the first personalized medicine trial in septic shock patients, aimed at
16 576 characterizing the safety and efficacy of the ADM-binding antibody Adrecizumab in septic shock
17 577 patients with elevated concentrations of bio-ADM. The trial incorporates a number of innovative
18 578 features such as biomarker guided patient selection and a novel efficacy endpoint in its design to avoid
19 579 pitfalls of previous sepsis trials. Adrecizumab represents a promising approach to treat this lethal
20 580 syndrome. The results of this proof-of-concept and dose-finding phase II trial are eagerly awaited, and
21 581 will importantly aid the design of future trials with this drug.
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40 586

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42 588 CG and AB drafted the manuscript. The manuscript was critically reviewed by MK, OH, PS, JZ, GM,
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48 590

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3 595 **Competing interests**

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5
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17
18 603 manuscript. Adrenomed AG holds patent rights on anti-ADM antibodies.
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For peer review only

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3 722 **Figure and table legends**
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7 724 **Figure 1.** Study design.
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11 726 **Figure 2.** Study timeline.
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15 728 **Figure 3.** Primary *efficacy* endpoint: 14-day Sepsis Support Index (SSI): example calculation.
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19 730 **Table 1.** In- and exclusion criteria.
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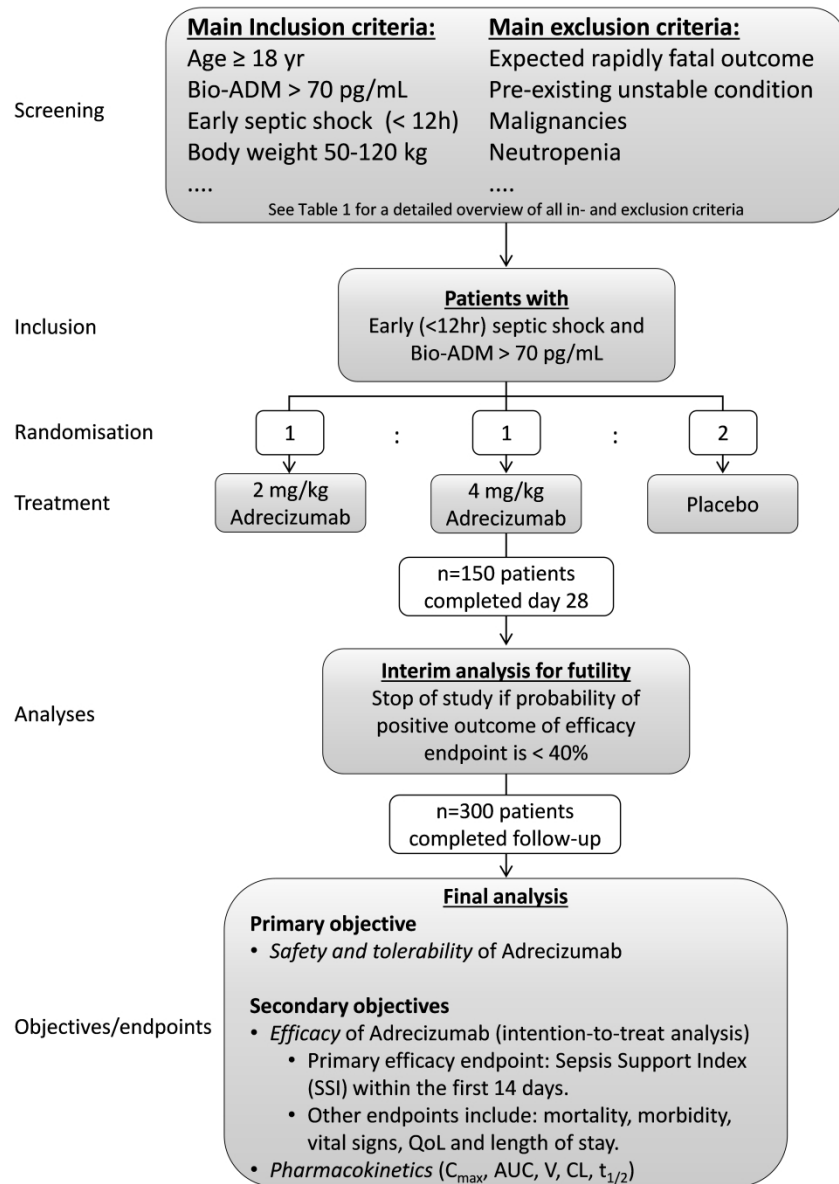


Figure 1. Study design.

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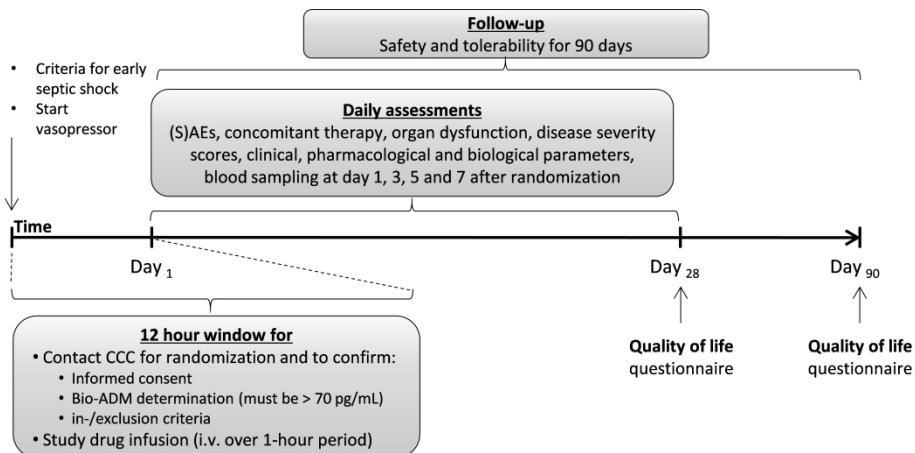


Figure 2. Study timeline.

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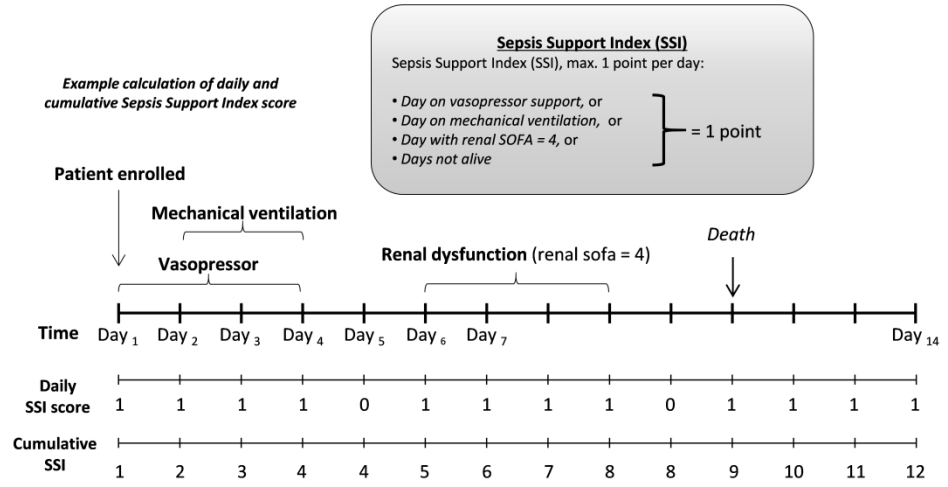


Figure 3. Primary efficacy endpoint: 14-day Sepsis Support Index (SSI): example calculation.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Page 1, lines 3-6.
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Page 3, line 60.
	2b	All items from the World Health Organization Trial Registration Data Set This information (e.g. sponsor, countries of recruitment, contacts, etc. can be found in the clinicaltrials.gov registry).
Protocol version	3	Date and version identifier P. 3, line 62.
Funding	4	Sources and types of financial, material, and other support P21, lines 591-592.
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Authors are not specified in the original study protocol. However, the steering committee and Sponsor, who were involved in writing, are listed as authors on the current manuscript.
	5b	Name and contact information for the trial sponsor P21, line 591-592.
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities P17 & P18, lines 483-489.

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- 5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
- Steering committee members, p1, lines 23-24.
Data management: p13, lines 358-361.
Interim analysis: p13, lines 345-455.
DSMB: p15, lines 413-418.
Safety contact of the sponsor: p15, lines 403-406.
Publication steering committee: p18, lines 486-489.

14 Introduction

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- Background and rationale
- 6a Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
P4-5, lines 79-117.
- 6b Explanation for choice of comparators
P5, lines 125-127.
- Objectives
- 7 Specific objectives or hypotheses
P6, lines 135-160.
- Trial design
- 8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
P5, lines 120-134.
Figure 1.

34 Methods: Participants, interventions, and outcomes

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- Study setting
- 9 Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
P5, lines 123-125.
References to list included in text.
- Eligibility criteria
- 10 Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
P6-8, lines 162-216.
Table 1.
- Interventions
- 11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
P10, lines 264-276.

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- 11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
P13, lines 345-355.
- 11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
P14, lines 385-396.
P16, lines 451-454.
- 11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
P10, lines 278-282.
- Outcomes 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
P6, lines 135-160, and P11-12, lines 313-343.
Figure 3.
- Participant timeline 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
P6-7, lines 172-175.
Figures 1 and 2 contain schematic information.
- Sample size 14 Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
P11, lines 290-311.
- Recruitment 15 Strategies for achieving adequate participant enrolment to reach target sample size
Not specified.

Methods: Assignment of interventions (for controlled trials)

Allocation:

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- Sequence generation 16a Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
P8, lines 230-235. Figure 1.

1			
2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5			assigned
6			P9, lines 251-276.
7			
8	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
9			and who will assign participants to interventions
10			P8, lines 232-233.
11			P5, lines 130-132.
12			P10, lines 268-274.
13			
14	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
15	(masking)		participants, care providers, outcome assessors, data analysts), and
16			how
17			P9, lines 251-276.
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20		17b	If blinded, circumstances under which unblinding is permissible, and
21			procedure for revealing a participant's allocated intervention during
22			the trial
23			P17, lines 458-460.
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Methods: Data collection, management, and analysis

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27	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
28	methods		trial data, including any related processes to promote data quality (eg,
29			duplicate measurements, training of assessors) and a description of
30			study instruments (eg, questionnaires, laboratory tests) along with
31			their reliability and validity, if known. Reference to where data
32			collection forms can be found, if not in the protocol
33			P13, lines 357-382.
34			P8, lines 217-228.
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37		18b	Plans to promote participant retention and complete follow-up,
38			including list of any outcome data to be collected for participants who
39			discontinue or deviate from intervention protocols
40			The first is not specified.
41			The second: P15, lines 420-432
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44	Data	19	Plans for data entry, coding, security, and storage, including any
45	management		related processes to promote data quality (eg, double data entry;
46			range checks for data values). Reference to where details of data
47			management procedures can be found, if not in the protocol
48			P13, lines 357-372.
49			P14, lines 398-410.
50			
51	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
52	methods		Reference to where other details of the statistical analysis plan can be
53			found, if not in the protocol
54			P11-13, lines 313-355
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- 20b Methods for any additional analyses (eg, subgroup and adjusted analyses)
P12, lines 336-343.
- 20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
P14, lines 374-382.

11 **Methods: Monitoring**

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- Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
P15, lines 412-418.
- 21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
P13, lines 345-355.
- Harms 22 Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
P14-15, lines 398-410.
- Auditing 23 Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Not specified

37 **Ethics and dissemination**

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- Research ethics approval 24 Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
P16, lines 440-444.
- Protocol amendments 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Not specified
- Consent or assent 26a Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
P9, lines 327-249.

1		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
2			Not specified in manuscript, however, this is specified in reviewer response (p15).
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7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
8			P16, lines 446-466.
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12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
13			P21-22, lines 594-602.
14			This is not specified for each participating study site.
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17	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
18			P17, lines 468-472.
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23	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
24			A subject insurance is arranged by the sponsor according to country-specific requirements. This is currently not mentioned in the manuscript (although the fact that the conduct of the study is done according to national legislation, etc. indicates that a mandatory insurance is arranged).
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32	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
33			P10, lines 284-287.
34			P17-18, lines 483-489.
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40		31b	Authorship eligibility guidelines and any intended use of professional writers
41			P21, lines 587-589.
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44		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
45			Not specified. Currently, the authors do not plan on sharing individual patient data after ending of the study.
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50	Appendices		
51	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
52			Not included.
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2 Biological 33 Plans for collection, laboratory evaluation, and storage of biological
3 specimens for genetic or molecular analysis in the current trial and for
4 future use in ancillary studies, if applicable
5 P8, lines 217-228.
6 P17, lines 474-475.
7

8 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013
9 Explanation & Elaboration for important clarification on the items. Amendments to the
10 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT
11 Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)"
12 license.
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