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Epirubicin for the Treatment of Sepsis & Septic Shock (EPOS-1): study protocol for a randomized, placebo-controlled Phase IIa dose escalation trial
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Running Head
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ABSTRACT

Introduction: Sepsis remains the major cause of death among hospitalized patients in intensive care. While targeting sepsis-causing pathogens with source control or antimicrobials had a dramatic impact on morbidity and mortality of sepsis patients, it remains insufficient for about one-third of the affected individuals that still succumb. Pharmacological targeting of mechanisms that reduce sepsis-defining organ dysfunction should be beneficial. When given in low doses, the anthracycline epirubicin promoted tissue damage control and lessen severity of sepsis acting independently of the host pathogen load, conferring disease tolerance to infection. Since epirubicin at higher doses can be myelotoxic, a first-dose response trial is necessary to assess potential harm by this drug in this indication.

Methods and analysis: EPOS-1 is a randomized, double-blind, placebo-controlled phase 2 dose-escalation phase IIa clinical trial to assess the safety of a single low dose of epirubicin as an adjunctive in patients with sepsis. The primary endpoint is the 14-day myelotoxicity. Secondary and explorative outcomes include 30- and 90-day mortality. organ dysfunction, PK/PD, cytokine release. Patients will be randomized in three consecutive phases. For each study phase patients are randomized to one of the two study arms (epirubicin or placebo) in a 4:1 ratio. Patients in the epirubicin group will receive a single dose of epirubicin (3.75 mg/m^2, 7.5 mg/m^2 or 15 mg/m^2 depending on study phase. After each study phase, a DSMB will recommend continuation or premature stopping of the trial. The primary analyses for each dose level will report the proportion of myelotoxicity together with a 95% confidence interval. A potential dose-toxicity association will be analyzed using a logistic regression model with dose as covariate. All further analyses for this study will be descriptive.

Ethics and dissemination: The protocol is approved by the German Federal Institute for Drugs and Medical Devices. The results will be submitted for publication in peer-reviewed journals.

Trial registration: Clinicaltrials.gov NCT05033808

STRENGTHS AND LIMITATIONS OF THIS STUDY

- EPOS-1 is a randomized, placebo-controlled, double-blind, single dose escalation phase IIa trial.
- This is the first clinical trial that pharmacologically targets a disease tolerance mechanism.
- Epirubicin will be repurposed with another concentration in a new indication.
- This trial is not powered to assess an effect on mortality.

INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1] and despite improvement in outcomes mortality still ranges from 15-25% and can be as high as 50% in case of septic shock [1] [2]. Treatment relies on infection control by antibiotics and source control and supportive therapy, *e.g.* by fluid resuscitation, vasopressors, respiratory support, or dialysis. Sepsis mortality rates have not decreased substantially over the last years and new treatment strategies are scarce. Targeting the immune system has mainly failed [3], potentially due to the syndromic nature of sepsis and the wide variety of clinical presentations. Immunophenotyping [4] and subsequent personalized immunotherapy are currently deployed in clinical trials that include patients presenting only with extreme phenotypes such as immunosuppression or hyperinflammation [5]. Yet, for the common sepsis denominator, *i.e.* organ dysfunction [6], no effective sepsis-specific treatments are established in clinical practice [2, 7]. Noteworthy, the strategies that have been deployed to decrease specifically infectious disease mortality all share the same mode of action, *i.e.* the reduction of pathogen burden. This strategy is essentially also used by the immune system and in this context referred to as “resistance to infection” [8-10]. Another defense strategy termed “disease tolerance to infection” has not been explored pharmacologically in medicine. In experimental models, this defense strategy has been shown to decrease disease severity by supporting host homeostasis via limiting the extent of tissue damage associated with infection and promoting its repair [8, 9, 11]. It is achieved using genetically encoded and evolutionarily conserved stress and damage response mechanisms [11]. Anthracyclines, a class of drugs used in chemotherapy for over 30 years, have been shown to enhance disease tolerance when given in low doses.

Notably, epirubicin has been shown to increase survival in animal models of sepsis. This effect was not associated with a decrease in pathogen loads of the infected organism [12]. This indicates that application of epirubicin would act in a way to enforce disease tolerance mechanisms. Further data shows that epirubicin activated the DNA damage response pathways in cells, rendering them less susceptible to infection-associated stress [12]. Survival benefits prevailed when epirubicin was administered 24 hours after sepsis induction [12]. This makes epirubicin a potential candidate for a new therapeutic option in sepsis. We are not aware of any studies or case reports that applied anthracyclines for this indication. Epirubicin has been used at doses up to 30 mg/m^2 without toxicity in earlier studies with cancer patients. This is a higher dosage then what is intended in the EPOS-1 trial. Based on the existing preclinical evidence, we designed the EPOS-1 trial to test the hypothesis that low-dose epirubicin in safe in patients with sepsis.

METHODS AND ANALYSIS

EPOS-1 is a randomized, placebo-controlled dose escalation phase IIa trial to assess the safety of a single low dose of epirubicin as an adjunctive therapy for patients with sepsis. The primary endpoint of the study is myelotoxicity at day 14 after application of epirubicin. Secondary and exploratory endpoints are the rate and level of organ dysfunction, the pharmacokinetic/pharmacodynamic of epirubicin, concentration of cytokines in plasma and the DNA damage in leukocytes and mortality.

Study design and setting

The trial will recruit sepsis patients admitted to intensive care (ICU) and intermediate care units (IMC) in German university hospitals. Patients will be randomized subsequently to three study phases with increasing doses of epirubicin or placebo in a 4:1 ratio. After each study phase a safety analysis will take place before the trial with new patients proceeds to the next higher dose.

Study population

The study population consists of adult patients ≥18 years of age admitted to the intensive care units (ICU) or intermediate care units (IMC) with sepsis or septic shock in one of the five

participating centers. There are no gender restrictions or preferences. Screening will be performed daily at the respective trial centers to assess whether eligible subjects are present in the ICUs or IMC. Pregnant or breastfeeding women are not eligible for participation in this clinical trial. All inclusion and exclusion criteria are listed in *Box 1*.

Trial management

The trial is led by the sponsor representative and coordinating investigator (SW) and his deputy (DTR). They are supported by the Center for Clinical Studies of Jena University Hospital (ZKS) (project manager CH), which is responsible for trial management and monitoring the source data. Biosamples are analyzed at the laboratory of the coordinating investigator and in the laboratories of cooperating partners.

The data and safety monitoring board (DSMB) is composed of three external experts (an intensive care physician, an oncologist, and a statistician). The DSMB is regulated by a standardized operating procedure. The main function of the DSMB is to monitor the safety of the study. All Suspected Unexpected Serious Adverse Reaction (SUSARs) and all cases fulfilling the primary endpoint definition of myelotoxicity will be reported to the data safety monitoring board (DSMB). Data for interim analysis will be cleaned and prepared for presentation and reported to the DSMB if at least two patients in the placebo group and at least eight patients in the respective epirubicin group have completed the 14-day follow-up. The DSMB will convene meetings in context of interim analysis and additional ad hoc meetings if necessary. Following each meeting, the DMSB will recommend continuation, modification, or discontinuation of the study based on observed toxicities.

Randomization and study procedures

The local pharmacies at each trial site have access to a web-based central randomization service, which is available 24 hours / 7 days. The randomization list is prepared by an independent statistician via a computer-based algorithm and is stratified by study center. For each study phase patients are randomized to one of the two study arms (epirubicin or placebo) in a 4:1 ratio. A unique patient ID is generated for data collection throughout the trial (*Figure 1*). Patients in the epirubicin group will receive a single dose of epirubicin. The amount of epirubicin is determined by the study phase and increased from 3.75 mg/m^2 in phase 1 to 7.5 mg/m^2 in phase 2 to 15 mg/m^2 in phase 3. This corresponds to approximately 4%-16% of the epirubicin dosage applied in a single course of chemotherapy. The highest dose corresponds to the amount that had beneficial effects in mice [12].

The study medication is prepared in the hospital pharmacies of the trial sites by unblinded personal and then delivered to the ICU/IMC. Since epirubicin has a typical red color the study medication is delivered blinded in colored bags already connected to colored infusion systems. In addition, the bags will be covered by an opaque light protection pouch. At the trial site the infusion system is prefilled with NaCl solution via a side port before connection to the patient's central line. The transparent parts of the central line are then covered by an opaque towel before the application of the IMP is started. After administration of the IMP the infusion system is flushed by normal saline to remove all residues of the IMP before the towel is removed and the infusion system is disconnected from the patient's infusion line.

An overview of the study procedures and assessments is provided in *Table 2*. Acute physiology data will be documented directly before and at seven visits up to 24 hours after the Interventional Medical Product (IMP) administration. Plasma will be centrifugated and stored at -80°C for further analysis. Peripheral Blood Monocytic cells (PBMCs) will be isolated at the trial sited using a commercially available kit (MACSprep™ PBMC Isolation Kit, Miltenyi Biotec)

Primary endpoint

Safety as assessed by myelotoxicity until day 14 after epirubicin application is the primary endpoint. Myelotoxicity is the most relevant side effect of anthracycline treatment in cancer patients and can result in neutropenia. This would put patients at risk of developing severe infections. In cancer studies, myelotoxicity is a commonly used outcome parameter [13-15]. The primary endpoint myelotoxicity will be determined by automated or manual differential

blood count in the respective Departments of Clinical Chemistry. A blood count will be measured 24 hours, 2, 3, 5, 7, 10, 12 and 14 days, days after administration of verum/placebo. Assessing myelotoxicity in sepsis patients can be complicated since leukopenia, neutropenia and thrombocytopenia [16-19] are all being observed in a relevant proportion of sepsis patients. In rare cases this might be a sign of sepsis-induced myelosuppression, but in most cases, this is caused by increased consumption or sequestration. Immature platelet fraction (IPF) is a parameter reflecting megakaryocyte activity and is therefore reflecting platelet production [20]. Thrombocytopenia with a normal or elevated IPF is indicative of increased consumption and turnover with a normal bone marrow function and is a common finding in sepsis [20, 21]. In contrast, thrombocytopenia with a decreased IPF is indicative of a bone marrow depression. Leukopenia and neutropenia in sepsis are typically present early in the disease and are followed by normal or elevated leucocyte counts, while neutropenia due to myelotoxicity is prolonged. Neutropenia and thrombocytopenia in sepsis are not closely correlated with each other, as the pathophysiological processes are different, while myelosuppression normally affects all cell types.

To differentiate the best possible way between sepsis-associated alterations and "real" Epirubicin-induced myelotoxicity the primary safety endpoint of myelotoxicity is defined as follows:

Neutropenia of grade 3 or 4 (***Error! Reference source not found.***) at two consecutive study visits up to day 14 or thrombocytopenia of grade 3 or 4 (***Error! Reference source not found.***) at two consecutive study visits up to day 14 accompanied by neutropenia or thrombocytopenia of grade 2, 3 or 4 at both study visits and accompanied by an IPF below *2.5%* at one or two of the consecutive study visits (*Figure 2*).

Secondary endpoint

Secondary endpoints for safety are cardiotoxicity, assessed by transthoracic echocardiography 7 days after epirubicin application, the frequency of other typical side effects (diarrhea, mucositis, alopecia, nausea and vomiting) and the overall rate of adverse and severe adverse events. In addition, we will assess the inflammatory response measuring serum cytokines, PCT and CRP. A "success" rate defined as a decrease of procalcitonin (PCT) serum concentration by 80% or more of its intra-individual peak value or to 0.5 µg/L or lower within 72 hours after randomization (following the "Stop Antibiotics on Procalcitonin guidance Study" (SAPS) by de Jong *et al.* [22] will be assessed. For organ function, SOFA on days of assessment, mean total SOFA and SOFA changes over time in the participants will be assessed. We will further assess fluid balance, urine output, need for renal replacement therapy, paO_2/FiO_2 ratio, need for respiratory support and catecholamines and inotropes. Mortality will be assessed at day 14, 28 and 90 after randomization, quality of life will be assessed at day 90 in survivors by *Short Form 36 Health Questionnaire* (SF-36). Explorative objectives include pharmacokinetics and pharmacodynamics of epirubicin, by measuring DNA damage. Effects on inflammatory response will be further assessed by measuring additional cytokines and additional molecular markers for organ damage will be analyzed. For better characterization of immune cell composition, thrombocyte numbers and bone marrow function FACS of PBMCs, Anti-PF4 antibodies and reticulocytes will be assessed.

Sample size and Power Considerations

This is an exploratory trial to show safety of low-dose epirubicin in sepsis. It will serve as a pilot study for a subsequent a larger subsequent phase II/III trial, in case that epirubicin is safe in this indication. In each cohort, we will assign eight patients to epirubicin and two to placebo. Based on the assumption that the probability of a myelotoxicity is 18% the probability of observing at least one myelotoxicity out of eight verum-treated patients equals 79.6% based on a binomial distribution. Thus, a total of 30 (8 x verum *vs.* 2 x placebo from each phase) patients that reach the 14-day safety endpoint is required. Assuming a mortality of sepsis patients of 30%, it is anticipated, that approximately 12 patients in the epirubicin group and 3 patients in the placebo group per phase will need to be included in the study. Dropouts until day 14 will be replaced until necessary numbers are reached (see *Figure 1*).

Data collection/data management
Data will be collected on an electronic case report form (CRF) using OpenClinica® (OpenClinica, LLC, Waltham, MA, USA) by a trained investigator or study assistant at each the respective trial center. Monitoring will be performed by the ZKS Jena to its local standard operating procedures (SOPs). Monitoring in general will be performed on-site. All serious adverse events (SAEs), whether related or not related to study medication, must be reported until 90 days after administration of IMP/control. Patients or relatives are contacted on day 28 and day 90 after randomization to obtain survival status of the participants.
The recommendation will be brought to the attention of the competent higher federal authority and the leading ethics committee as part of the annual safety report or as an urgent safety measure, if necessary.

Statistical analysis
The primary analyses for each dose level will report the proportion of myelotoxicity together with a 95% confidence interval. A potential dose-toxicity association will be analyzed using a logistic regression model with dose as covariate. All further analyses for this study will be descriptive. Data analyses will be provided by treatment and overall if applicable. After first and second phase, the data safety monitoring board will meet and recommend whether the study will be stopped, or the next higher dose phase can be initiated. The DSMB will be provided with the necessary pre-analyzed and raw data. The major stopping rule of the trial will consist of increased toxicity in epirubicin groups as assessed by myelotoxicity.

Ethics and dissemination
Sponsor of the trial is the Friedrich Schiller University. The trial was approved by the ethics committee of the Jena University Hospital on 20 December 2021 (2021-2440-AMG-ff) and the German Health Authorities (BfArM) on 08 November 2021. In addition, the local ethics committees at each site approved the study protocol and the study competence of each site. Written informed consent is obtained from all patients or their legal representatives. If this is not possible before enrolment in due time, the ethics committees have approved a deferred consent process where the inability to provide consent is confirmed by an independent physician, and the patient is enrolled without informed consent. As soon as the legal representative of the patient is available, written informed consent is immediately obtained; otherwise, the patient is withdrawn from the study and all study procedures are ended.
The trial is governed by the international standards for Good Clinical Practice (GCP) developed by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the Directive 2001/20/EC for clinical trials and General Data Protection Regulation (GDPR) 679/2016 (EC). Results of the trial will be published in a peer reviewed journal and reported on clinicaltrials.org. All publication will be available in open access.

Patient and public involvement
Patients or the public were not involved in the design of the EPOS-1 trial. The trial design was endorsed by the Deutsche Sepsis Gesellschaft.

DISCUSSION

Despite tremendous research efforts during the last decades, no specific therapy for sepsis exists that targets sepsis-associated organ dysfunction [2]. Instead, treatment relies on the timely administration of broad-spectrum antibiotics, mechanical organ support, along with focus sanitation and if necessary organ replacement therapy. Increasing rates of antimicrobial resistance and lack of innovation of new antimicrobials further add to the problem [2, 7]. Therefore, new therapeutic approaches are urgently needed. In this study for the first time, we will pharmacologically intent to manipulate disease tolerance to infection, a molecular mechanism that lessens disease severity by enforcing tissue damage control [23-25]. Presumably, manipulation of tissue damage control mechanisms will not impose selection pressure on the pathogens and therefore should not cause anti-microbial resistance to the applied drugs [11, 26]. The primary aim of this study is to demonstrate safety and tolerability

of a low dose of epirubicin in sepsis patients. This drug has recently been shown to induce disease tolerance and tissue damage control in animal models of sepsis [12, 27].

With this randomized-controlled, multicenter trial, we aim to investigate whether the administration of low-dose epirubicin is safe in patients with sepsis and septic shock. If this approach proves to be successful, we would be able to provide a sepsis-specific therapy for the 1st time; *i.e.* targeting the deleterious organ failure. This might ultimately also decrease the rate of antibiotic consumption in the critically ill and improve the anti-microbial resistance rates. In addition, if epirubicin proves to be safe and beneficial for patients with sepsis, it might also extend treatment options for patients living in areas with limited resources and high antimicrobial resistance rates such as in African countries or the Indian subcontinent, amongst others in which assessment of causing pathogens, determination of antimicrobial resistance patterns is not available for the majority of patients and in which expensive antibiotics cannot be applied.
The overall treatment algorithm of patients participating in the clinical trial follows the standard practice for this condition and is in accordance with current guidelines for the treatment of such patients

A drug licensed for chemotherapy will be applied to a highly vulnerable group, *i.e.* sepsis patient. Intuitively, this seems to be contraindicated. However, our approach is not intended to use its chemotherapeutic potency. Instead, its potential to induce damage response mechanisms will be applied [12]. Drug dosages are significantly lower than applied in a single chemotherapeutic cycle. Therefore, relevant toxicity is not expected. Close safety monitoring will be performed and the major stopping rule of the trial will consist of increased toxicity in the groups that receive epirubicin. As such, in our opinion, the benefits substantially outweigh the potential risks in this trial.

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FIGURES AND TABLES

Box 1: Inclusion and exclusion criteria of the EPOS-1 trial.

Inclusion Criteria
Patients ≥18 years admitted to the ICU/IMC with sepsis or septic shock
Sepsis diagnosis within 24 hours prior to screening regardless of site of infection
Informed consent of patient or their legal representative or if not possible a statement by an independent physician
Exclusion criteria
Leukopenia/Neutropenia/Thrombocytopenia-prior or upon inclusion (Leukocyte Count <4,000/μL; Neutrophil/Thrombocyte Count below Lower Limit of Normal)
Weight >135 kg/BMI >45.
Active neoplasia.
History of chemotherapy.
Hypersensitivity to epirubicin.
History of bone marrow or solid organ transplantation.
Immunosuppressive therapy.
Acute severe infection within 4 weeks prior to admission (Hospitalization for an infection or in case of hospital acquired infection transfer to a higher level of care due to the infection)
Chronic infection.
Cardiomyopathy with a documented ejection fraction <30% or ICD implantation.
Acute liver failure following the European Association for the Study of the Liver definition as International Normalized Ratio (INR) >1.5 and elevation of transaminases > 3 times of the upper normal limit.
Pregnancy during all trimester/breast-feeding.
Chronic mechanical ventilation dependency.
Cystic fibrosis.
Concomitant medication with Verapamil or Cimetidine.
Prior enrollment in this study.
Participation in another clinical intervention trial.

Figure 1: Study design of the EPOS-1 trial. Black bordered circles indicate minimal participants for the safety analysis. Red bordered circles indicate patients that were randomized and received the study drug or placebo are expected not to reach the 14-day safety endpoint considering sepsis-related mortality of up to 30%. Assessment indicates a safety assessment of the DSMB and a study continuation or stop following their recommendation.

Abbreviation: Epi.. epirubicin, hrs.. hours.

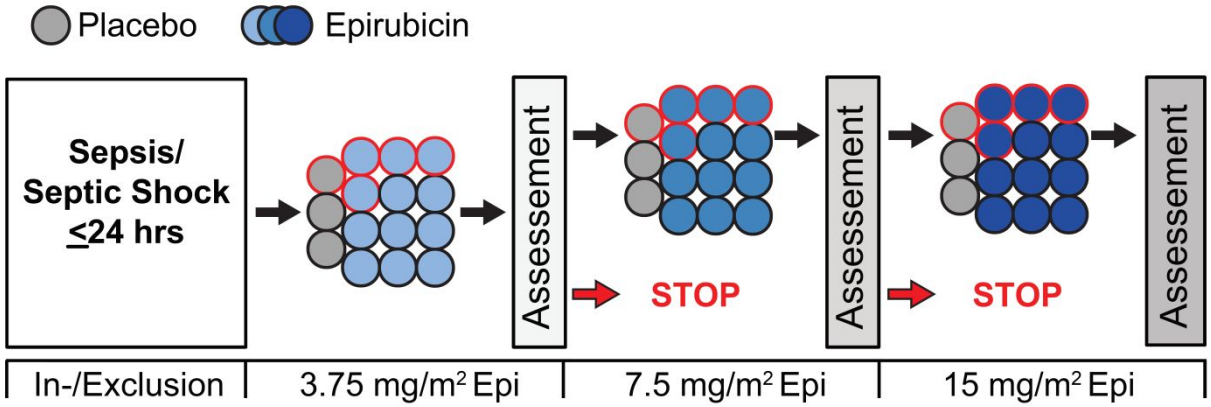
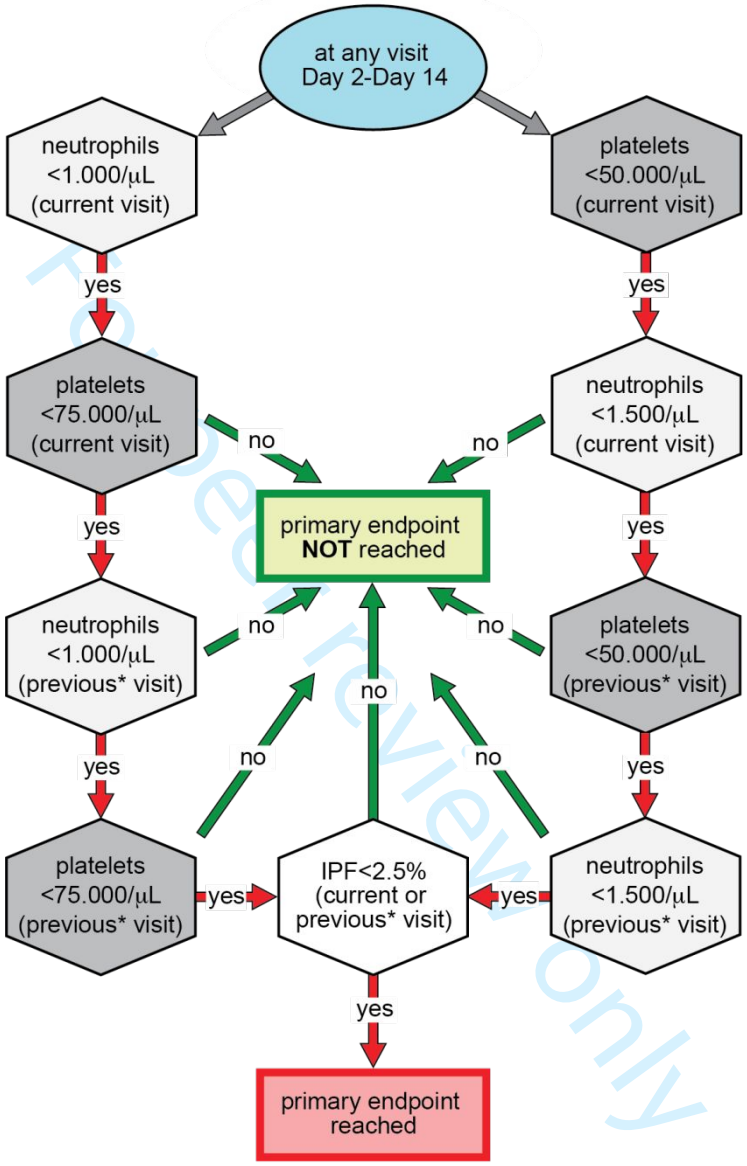


Figure 2: Flow chart that is used to determine the primary endpoint, *i.e.* myelotoxicity in the EPOS-1 trial. * for visit Day 2 the previous visit to be considered is Day 0-24 hrs.

Abbreviation: hrs.. hours



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Page 12 of 13

Table 1: Study procedures and visits.

Time (Days)	Pre		D0 First 24 hours								D1	D2	D3	D5	D7	D10	D12	D14	D28±2	D90±4
Time (Min) Time (hours)			0	15	60±5 1	120±10 2	3±0,5	6±1	12±2	24±4										
Visit		B	0	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Inclusion/Exclusion Criteria	X																			
Written Informed Consent	X																			
Pregnancy Test[1]		X																		
Randomization		X																		
Baseline Assessment		X																		
SOFA		X									X	X	X	X	X	X	X	X		
SAPS II / qSOFA		X																		
Clinical parameters		X									X	X	X	X	X	X	X	X		
Routine laboratory		X									X	X	X	X	X	X	X	X		
Treatment parameters on the ICU/IMC		X									X	X	X	X	X	X	X	X		
Application of study drug			X																	
Acute physiology			X	X	X	X	X	X	X	X										
Plasma sampling			X	X	X	X	X	X	X	X		X	X	X	X			X		
PBMC sampling			X					X		X			X		X					
Urine sampling			X							X			X							
Differential Blood Count/Neutrophils/IPF			X							X		X	X	X	X	X	X	X		
Anti-PF4			X												X			X		
AE / SAE				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Echocardiography															X					
Survival																		X	X	X
SF-36																				X

[1]*blood or urine*

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Page 13 of 13

Table 2: Grading of neutropenia and thrombocytopenia (following [28]).

	Neutropenia (acute neutrophil count)	**Thrombocytopenia** (platelets)
Grade 1	<Lower limit of normal- 1,500/µL	<Lower limit of normal-75,000/µL
Grade 2	<1,500-1,000/µL	<75,000-50,000/µL
Grade 3	<1,000-500/µL	<50,000-25,000/µL
Grade 4	< 500/µL	< 25,000/µL

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Epirubicin for the Treatment of Sepsis & Septic Shock (EPOS-1): study protocol for a randomized, placebo-controlled Phase IIa dose escalation trial

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Epirubicin for the Treatment of Sepsis & Septic Shock (EPOS-1): study protocol for a randomized, placebo-controlled Phase IIa dose escalation trial

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ABSTRACT

Introduction: Sepsis remains the major cause of death among hospitalized patients in intensive care. While targeting sepsis-causing pathogens with source control or antimicrobials has had a dramatic impact on morbidity and mortality of sepsis patients, this strategy remains insufficient for about one-third of the affected individuals that still succumb. Pharmacological targeting of mechanisms that reduce sepsis-defining organ dysfunction may be beneficial. When given at low doses, the anthracycline epirubicin promotes tissue damage control and lessened severity of sepsis independently of the host-pathogen load by conferring disease tolerance to infection. Since epirubicin at higher doses can be myelotoxic, a first dose response trial is necessary to assess the potential harm of this drug in this new indication.

Methods and analysis: EPOS-1 is a randomized, double-blind, placebo-controlled phase 2 dose-escalation phase IIa clinical trial to assess the safety of epirubicin as an adjunctive in patients with sepsis. The primary endpoint is the 14-day myelotoxicity. Secondary and explorative outcomes include 30- and 90-day mortality, organ dysfunction, PK/PD, cytokine release. Patients will be randomized in three consecutive phases. For each study phase patients are randomized to one of the two study arms (epirubicin or placebo) in a 4:1 ratio. Approximately 45 patients will be recruited. Patients in the epirubicin group will receive a single dose of epirubicin (3.75 mg/m^2, 7.5 mg/m^2 or 15 mg/m^2 depending on study phase. After each

study phase, a DSMB will recommend continuation or premature stopping of the trial. The primary analyses for each dose level will report the proportion of myelotoxicity together with a 95% confidence interval. A potential dose-toxicity association will be analyzed using a logistic regression model with dose as covariate. All further analyses will be descriptive.

Ethics and dissemination: The protocol is approved by the German Federal Institute for Drugs and Medical Devices. The results will be submitted for publication in peer-reviewed journals.

Trial registration: Clinicaltrials.gov NCT05033808, Protocol Version V6.0

STRENGTHS AND LIMITATIONS OF THIS STUDY

- EPOS-1 the first randomized, placebo-controlled, double-blind, trial that pharmacologically targets a disease tolerance mechanism.
- Epirubicin will be repurposed with another concentration in a new indication.
- This trial is not powered to assess an effect on mortality.
- Patients are included up to 48 hours after sepsis diagnosis. While this time window was long enough to decrease disease severity in mice, it is not clear whether it will be sufficient in humans.
- Protective effects were shown for bacterial sepsis in previously healthy young animals. Comorbidity and age on epirubicin metabolism in sepsis could influence the effects of epirubicin in patients with sepsis.

INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (1). Despite improvement in outcomes mortality still ranges from 15-25% and can be as high as 50% in case of septic shock (2). Treatment relies on infection control by antibiotics and source control and supportive therapy, *e.g.* by fluid resuscitation, vasopressors, respiratory support, or dialysis. Sepsis mortality rates have not decreased substantially over the recent years, and new treatment strategies are scarce. Targeting the immune system has mainly failed (3), potentially due to the syndromic nature of sepsis and the wide variety of clinical presentations. Immunophenotyping (4) and subsequent personalized immunotherapy are currently deployed in clinical trials that include patients presenting only with extreme phenotypes such as immunosuppression or hyperinflammation (5, 6, 7). Yet, for the common sepsis denominator, *i.e.* organ dysfunction (8), no effective sepsis-specific treatments are established in clinical practice (2, 9). Noteworthy, the strategies that have been deployed to decrease specifically infectious disease mortality all share the same mode of action, *i.e.* the reduction of pathogen burden. This strategy is essentially also used by the immune system and in this context referred to as “resistance to infection” (10, 11, 12). Another defense strategy termed “disease tolerance to infection” has not been explored pharmacologically in medicine (13). In experimental models, this defense strategy has been shown to decrease disease severity by supporting host homeostasis by limiting the extent of tissue damage associated with infection and promoting its repair (10, 11, 14). It is achieved using genetically encoded and evolutionarily conserved stress and damage response mechanisms (14). Anthracyclines,

a class of drugs used in chemotherapy for over 30 years (15, 16, 17, 18), have been shown to enhance disease tolerance when given in low doses (19).
Notably, it has been shown that epirubicin increases survival in animal models of sepsis. This effect was not associated with a decrease in pathogen loads of the infected organism (19). This indicates that application of epirubicin would act in a way to enforce disease tolerance mechanisms. Further data shows that epirubicin activated the DNA damage response pathways in cells, rendering them less susceptible to infection-associated stress (19). Survival benefits prevailed when epirubicin was administered 24 hours after sepsis induction (19). This makes epirubicin a potential candidate for a new therapeutic option in sepsis. We are not aware of any studies or case reports that applied anthracyclines for this indication. Epirubicin has been used at doses up to 30 mg/m^2 without toxicity in earlier studies with cancer patients (20). This is a higher dosage then what is intended in the EPOS-1 trial. Based on the existing preclinical evidence, we designed the EPOS-1 trial to test the hypothesis that low-dose epirubicin in safe in patients with sepsis.

METHODS AND ANALYSIS

EPOS-1 is a randomized, placebo-controlled dose escalation phase IIa trial to assess the safety of a single low dose of epirubicin as an adjunctive therapy for patients with sepsis. Sepsis is defined following the Sepsis-3 criteria as infection-associated organ dysfunction, represented by an increase in the Sequential Organ Failure Assessment (SOFA) score of two points or more (1). The primary endpoint of the study is myelotoxicity at day 14 after application of epirubicin. Secondary and exploratory endpoints are the rate and level of organ dysfunction, the pharmacokinetic/pharmacodynamic of epirubicin, the concentration of cytokines in plasma and the DNA damage in leukocytes and mortality.

Study design and setting

The trial will recruit sepsis patients admitted to intensive care (ICU) and intermediate care units (IMC) in German university hospitals. Patients will be randomized subsequently to three study phases with increasing doses of epirubicin or placebo in a 4:1 ratio. After each study phase a safety analysis will occur before the trial with new patients proceeds to the next higher dose.

Study population

The study population consists of adult patients ≥18 years of age with sepsis or septic shock, currently hospitalized at the intensive care unit (ICU) or intermediate care unit (IMC) regardless where the sepsis was first diagnosed in one of the five participating centers. There are no sex restrictions or bias. Screening will be performed daily at the respective trial centers to assess whether eligible subjects are present in the ICUs or IMC. Pregnant or breastfeeding women are not eligible for participation in this clinical trial. All inclusion and exclusion criteria are listed in *Box 1*. Accounting for a mortality of 30% of the study participants, we will include approximately a total of 15 participants in each phase, corresponding to 3 patients receiving placebo and 12 the study drug. This will allow for a primary endpoint assessment up to day 14 of two patients in the placebo group and eight patients in the study drug group. Patients will be recruited in five centers in order to assure adequate enrolment.

Trial management

The trial is led by the sponsor representative and coordinating investigator (SW) and his deputy (DTR). They are supported by the Center for Clinical Studies of Jena University Hospital (ZKS) (project manager CH), which is responsible for trial management and monitoring the source

data. Biosamples are analyzed at the laboratory of the coordinating investigator and in the laboratories of cooperating partners.

The data and safety monitoring board (DSMB) is composed of three external experts (an intensive care physician, an oncologist, and a statistician). The DSMB is regulated by a standardized operating procedure. The main function of the DSMB is to monitor the safety of the study. All Suspected Unexpected Serious Adverse Reaction (SUSARs) and all cases fulfilling the primary endpoint definition of myelotoxicity will be reported to the data safety monitoring board (DSMB). Data for interim analysis will be processed and prepared for presentation and reported to the DSMB if at least two patients in the placebo group and at least eight patients in the respective epirubicin group have completed the 14-day follow-up. The DSMB will convene meetings in the context of interim analysis and additional ad hoc meetings if necessary. Following each meeting, the DMSB will recommend continuation, modification, or discontinuation of the study based on observed toxicities.

Randomization and study procedures

The local pharmacies at each trial site have access to a web-based central randomization service, which is available 24 hours / 7 days. The randomization list is prepared by an independent statistician via a computer-based algorithm and is stratified by study center. For each study phase patients are randomized to one of the two study arms (epirubicin or placebo) in a 4:1 ratio. A unique patient ID is generated for data collection throughout the trial (*Figure 1*). Patients in the epirubicin group will receive a single dose of epirubicin. The amount of epirubicin is determined by the study phase. The epirubicin dosage in Phase 1 is 25% of the dosage applied in the mouse models, *i.e.* 3.75 mg/m^2 that corresponds to approximately 4% of the epirubicin dosage applied in a single course of chemotherapy. If this dose is safe, it will be escalated to 7.5 mg/m^2 and finally 15 mg/m^2 which corresponds to approximately 16% of the dosage applied in chemotherapy. It equals the dose that showed benefit in the mouse model and would be the dose to use in a future phase III trial. We expect that none of the applied dosages of epirubicin will increase toxicity in patients with sepsis. We expect that none of the applied dosages of epirubicin will increase toxicity in patients with sepsis. The highest dose corresponds to the amount that had beneficial effects in mice (19).

The study medication is prepared in the hospital pharmacies of the trial sites by unblinded personnel and then delivered to the ICU/IMC. Since epirubicin has a typical red color, the study medication is delivered blinded in colored bags already connected to colored infusion systems. In addition, the bags will be covered by an opaque light protection pouch. At the trial site, the infusion system is prefilled with NaCl solution via a side port before connection to the patient's central line. The transparent parts of the central line are then covered by an opaque towel before the application of the IMP is started. After administration of the IMP the infusion system is flushed by normal saline to remove all residues of the IMP before the towel is removed and the infusion system is disconnected from the patient's infusion line.

An overview of the study procedures and assessments is provided in *Supplementary Table 1*. Acute physiology data will be documented directly before and at seven visits up to 24 hours after the Interventional Medical Product (IMP) administration. Plasma will be centrifugated and stored at -80°C for further analysis. Peripheral Blood Monocytic cells (PBMCs) will be isolated at the trial sited using a commercially available kit (MACSprep™ PBMC Isolation Kit, Miltenyi Biotec).

Primary endpoint

The general toxicity profile of anthracyclines is well-known and has extensively been studied in tumor patients (15, 16, 17, 18). Epirubicin (4′-Epi-Doxorubicin) is a less toxic derivate from

doxorubicin and differs structurally only in the epimerization of the OH group in position 4 of the amino-sugar moiety (15). Myelosuppression -which is also used as the toxicity read-out in EPOS-1- is the major acute dose-limiting toxicity of epirubicin and consists predominantly of leukopenia and to a lesser extent in thrombocytopenia (15, 16). This would put patients at risk of developing severe infections. In cancer studies, myelotoxicity is a commonly used outcome parameter (21, 22, 23). In early studies, no toxicity was observed when epirubicin was administered as a single dose of 10, 20, or 30 mg/m^2 (20). The maximum tolerated single dose of epirubicin in tumor patients is suggested to be 150 mg/m^2. At lower doses of 120 mg/m^2 only Grade 2 myelotoxicities were observed (24). The nadir of myeloid toxicity occurs between 10 to 14 days after treatment. Therefore, we will closely monitor myelotoxicity (16, 17).

Safety, as assessed by myelotoxicity until day 14 after epirubicin application, is the primary endpoint. It will be determined by automated or manual differential blood count in the respective Departments of Clinical Chemistry. Blood count will be measured directly before study drug administration; 24 hours, 2, 3, 5, 7, 10, 12 and 14 days, days after administration of verum/placebo.

Assessing myelotoxicity in sepsis patients can be complicated since leukopenia, neutropenia and thrombocytopenia (25, 26, 27, 28) are all being observed in a relevant proportion of sepsis patients. In rare cases this might be a sign of sepsis-induced myelosuppression, but in most cases, this is caused by increased consumption or sequestration. Immature platelet fraction (IPF) is a parameter reflecting megakaryocyte activity and is therefore reflecting platelet production (29). Thrombocytopenia with a normal or elevated IPF is indicative of increased consumption and turnover with a normal bone marrow function and is a common finding in sepsis (29, 30). In contrast, thrombocytopenia with a decreased IPF is indicative of a bone marrow depression. Leukopenia and neutropenia in sepsis are typically present early in the disease and are followed by normal or elevated leucocyte counts, while neutropenia due to myelotoxicity is prolonged. Neutropenia and thrombocytopenia in sepsis are not closely correlated with each other, as the pathophysiological processes are different, while myelosuppression normally affects all cell types.

To differentiate the best possible way between sepsis-associated alterations and “real” Epirubicin-induced myelotoxicity the primary safety endpoint of myelotoxicity is defined as follows:

Neutropenia of grade 3 or 4 (*Table 1*) at two consecutive study visits up to day 14 or thrombocytopenia of grade 3 or 4 (*Table 1*) at two consecutive study visits up to day 14 accompanied by neutropenia or thrombocytopenia of grade 2, 3 or 4 at both study visits and accompanied by an IPF below *2.5%* at one or two of the consecutive study visits (*Figure 2*) (31).

Secondary endpoint

Secondary endpoints for safety are cardiotoxicity, assessed by transthoracic echocardiography 7 days after epirubicin application, the frequency of other typical side effects (diarrhea, mucositis, alopecia, nausea, and vomiting), and the overall rate of adverse and severe adverse events. In addition, we will assess the inflammatory response by measuring serum cytokines, PCT, and CRP. A “success” rate defined as a decrease of procalcitonin (PCT) serum concentration by 80% or more of its intra-individual peak value or to 0.5 µg/L or lower within 72 hours after randomization (following the “Stop Antibiotics on Procalcitonin guidance Study” (SAPS) by de Jong *et al.* (32) will be assessed. For organ function, SOFA on days of assessment, mean total SOFA, and SOFA changes over time in the participants will be assessed. We will further assess fluid balance, urine output, need for renal replacement therapy, paO_2/FiO_2 ratio, need for respiratory support, and catecholamines and inotropes.

Mortality will be assessed at day 14, 28, and 90 after randomization, quality of life will be assessed at day 90 in survivors by *Short Form 36 Health Questionnaire* (SF-36). Explorative objectives include pharmacokinetics and pharmacodynamics of epirubicin, by measuring DNA damage. Effects on inflammatory response will be further assessed by measuring additional cytokines and additional molecular markers for organ damage will be analyzed. For better characterization of immune cell composition, thrombocyte numbers and bone marrow function FACS of PBMCs, Anti-PF4 antibodies and reticulocytes will be assessed.

Sample size and Power Considerations

This is an exploratory trial to test safety of low-dose epirubicin in sepsis. It will serve as a pilot study for a subsequent larger phase II/III trial, in case epirubicin is safe in this indication.

Since sepsis patients are potentially more susceptible to side effects and altered drug toxicity, we base our sample size calculations on data from cancer patients. These receive four times higher doses of epirubicin (17, 33). Myelotoxicity was observed in cancer patients that received repetitive courses of epirubicin. Herait *et al.* reported grade 3 to 4 leucopenia in approximately 20% of patients that were treated every 3-4 weeks using a dose of 85 to 90 mg/m^2 epirubicin (33). In another trial, myelotoxicity grade 3 to 4 using the WHO classification was reported in 14 % of patients receiving 71-75 mg/m^2 every 3 weeks (17).

Based on the assumption that the probability of a myelotoxicity is 18% the probability of observing at least one myelotoxicity out of eight verum-treated patients equals 79.6% based on a binomial distribution. Thus, a total of 30 (8 x verum *vs.* 2 x placebo from each phase) patients that reach the 14-day safety endpoint is required. Assuming a mortality of sepsis patients of 30%, it is anticipated, that approximately 12 patients in the epirubicin group and 3 patients in the placebo group per phase will need to be included in the study. Dropouts until day 14 will be replaced until necessary numbers are reached (see *Figure 1*).

Data collection/data management

Data will be collected on an electronic case report form (CRF) using OpenClinica® (OpenClinica, LLC, Waltham, MA, USA) by a trained investigator or study assistant at each respective trial center. Monitoring will be performed by the ZKS Jena to its local standard operating procedures (SOPs). Monitoring, in general, will be performed on-site. All serious adverse events (SAEs), whether related or not related to study medication, must be reported until 90 days after administration of IMP/control. Patients or relatives are contacted on day 28 and day 90 after randomization to obtain the survival status of the participants.

The recommendation will be brought to the attention of the competent higher federal authority and the leading ethics committee as part of the annual safety report or as an urgent safety measure, if necessary.

Statistical analysis

The primary analyses for each dose level will report the proportion of myelotoxicity together with a 95% confidence interval. A potential dose-toxicity association will be analyzed using a logistic regression model with dose as covariate. All further analyses for this study will be descriptive. Data analyses will be provided by treatment and overall if applicable. After first and second phase, the data safety monitoring board will meet and recommend whether the study will be stopped, or the next higher dose phase can be initiated. The DSMB will be provided with the necessary pre-analyzed and raw data. The major stopping rule of the trial will consist of increased toxicity in epirubicin groups as assessed by myelotoxicity.

Ethics and dissemination

The sponsor of the trial is Friedrich Schiller University; Jena, Germany. The trial was approved by the ethics committee of the Jena University Hospital on 20 December 2021 (2021-2440-AMG-ff) and the German Health Authorities (BfArM) on 08 November 2021. In addition, the local ethics committees at each site approved the study protocol and the study competence of each site. Written informed consent is obtained from all patients or their legal representatives. If this is not possible before enrolment in due time, the ethics committees have approved a deferred consent process where the inability to provide consent is confirmed by an independent physician, and the patient is enrolled without informed consent. As soon as the legal representative of the patient is available, written informed consent is immediately obtained; otherwise, the patient is withdrawn from the study and all study procedures are ended.
The trial is governed by the international standards for Good Clinical Practice (GCP) developed by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the Directive 2001/20/EC for clinical trials and General Data Protection Regulation (GDPR) 679/2016 (EC). Results of the trial will be published in a peer reviewed journal and reported on clinicaltrials.org. All publication will be available in open access.

Patient and public involvement
Patients or the public were not involved in the design of the EPOS-1 trial. The trial design was endorsed by the Deutsche Sepsis Gesellschaft.

DISCUSSION

Despite tremendous research efforts during the last decades, no specific therapy for sepsis exists that targets sepsis-associated organ dysfunction (2). Instead, treatment relies on the timely administration of broad-spectrum antibiotics, mechanical organ support, along with source control and if necessary organ replacement therapy. Increasing rates of antimicrobial resistance and lack of innovation of new antimicrobials further add to the problem (2, 9). Therefore, new therapeutic approaches are urgently needed. In this study for the first time, we will pharmacologically intend to manipulate disease tolerance to infection, a molecular mechanism that lessens disease severity by enforcing tissue damage control (34, 35, 36). Presumably, manipulation of tissue damage control mechanisms will not impose selection pressure on the pathogens and therefore should not cause anti-microbial resistance to the applied drugs (14, 37). The primary aim of this study is to demonstrate safety and tolerability of a low dose of epirubicin in sepsis patients. This drug has recently been shown to induce disease tolerance and tissue damage control in animal models of sepsis (19, 38).

With this randomized-controlled, multicenter trial, we aim to investigate whether the administration of low-dose epirubicin is safe in patients with sepsis and septic shock. If this approach proves to be successful, we would be able to provide a sepsis-specific therapy for the 1st time; *i.e.* targeting the deleterious organ failure. This might ultimately also decrease the rate of antibiotic consumption in the critically ill and improve the anti-microbial resistance rates. In addition, if epirubicin proves to be safe and beneficial for patients with sepsis, it might also extend treatment options for patients living in areas with limited resources and high antimicrobial resistance rates such as in African countries or the Indian subcontinent, amongst others in which assessment of causing pathogens, determination of antimicrobial resistance patterns is not available for the majority of patients and in which expensive antibiotics cannot be applied. The overall treatment algorithm of patients participating in the clinical trial follows

the standard practice for this condition and is in accordance with current guidelines for the treatment of such patients.

A drug licensed for chemotherapy will be applied to a highly vulnerable group, *i.e.* patients with sepsis. Intuitively, this seems to be contraindicated. However, our approach is not intended to use its chemotherapeutic potency. Instead, its potential to induce damage response mechanisms will be applied (19). Drug dosages are significantly lower than when applied in a single chemotherapeutic cycle. Therefore, relevant toxicity is not expected. Close safety monitoring will be performed, and the major stopping rule of the trial will consist of increased toxicity in the groups that receive epirubicin. As such, in our opinion, the benefits substantially outweigh the potential risks in this trial. The first study site was initiated in June 2022 and the first patient was randomized October 19.2022. Five centers can recruit patients since June 2023. Recruitment is planned to finish by the end of 2024.

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Contributors: All authors fulfill the ICMJE recommendations for authorships. The roles and contributions of the individual authors are as follows: Sponsor Representative/Principal and coordinating investigator: **SW**; Deputy Coordinating Investigator: **DTR**; Project Manager: **CH**; Protocol writing and planning of the study: **SW, DTR, FB, MB, PS, CH, AH, LFM**; Acquisition of Funding: **SW, FB, MB**; Formulation of initial hypothesis: **SW, LFM**; Trial statistician, sample size calculation and statistical analysis plan: **PS**; Molecular analysis: **LFM, MGräl, TK**; Study Center Coordinators and recruitment of patients**: JE, TR, PM, MG, HS**. All authors contributed to, read and approved the final manuscript and contributed to the writing.

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Competing interests: **LFM** is an inventor on an international patent (WO 2013/036153) related to the use of anthacyclines for sepsis treatment. All other authors report no competing interests

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FIGURES AND TABLES

Box 1: Inclusion and exclusion criteria of the EPOS-1 trial.

Inclusion Criteria
Patients ≥18 years with sepsis or septic shock, currently hospitalized at the ICU or IMC regardless where the sepsis was first diagnosed.
Sepsis diagnosis within 48 hours prior to screening regardless of site of infection (defined as increase SOFA score of ≥ 2 points)
Informed consent of patient or their legal representative or if not possible a statement by an independent physician
Exclusion criteria
Leukopenia/Neutropenia/Thrombocytopenia-prior or upon inclusion (Leukocyte Count <4,000/μL; Neutrophil/Thrombocyte Count below Lower Limit of Normal)
Weight >135 kg/BMI >45.
Ongoing or History of chemotherapy.
Hypersensitivity to epirubicin.
History of bone marrow or solid organ transplantation.
Immunosuppressive therapy.
Acute severe infection within 4 weeks prior to admission (Hospitalization for an infection or in case of hospital acquired infection transfer to a higher level of care due to the infection)
Chronic infection.
Cardiomyopathy with a documented ejection fraction <30% or ICD implantation.
Acute liver failure following the European Association for the Study of the Liver definition as International Normalized Ratio (INR) >1.5 and elevation of transaminases > 3 times of the upper normal limit.
Pregnancy during all trimester/breast-feeding.
Chronic mechanical ventilation dependency.
Cystic fibrosis.
Concomitant medication with Verapamil or Cimetidine.
Prior enrollment in this study.
Participation in another clinical intervention trial.

Figure 1: Study design of the EPOS-1 trial. Black bordered circles indicate minimal participants for the safety analysis. Red bordered circles indicate patients that were randomized and received the study drug or placebo are expected not to reach the 14-day safety endpoint considering sepsis-related mortality of up to 30%. Assessment indicates a safety assessment of the DSMB and a study continuation or stop following their recommendation.

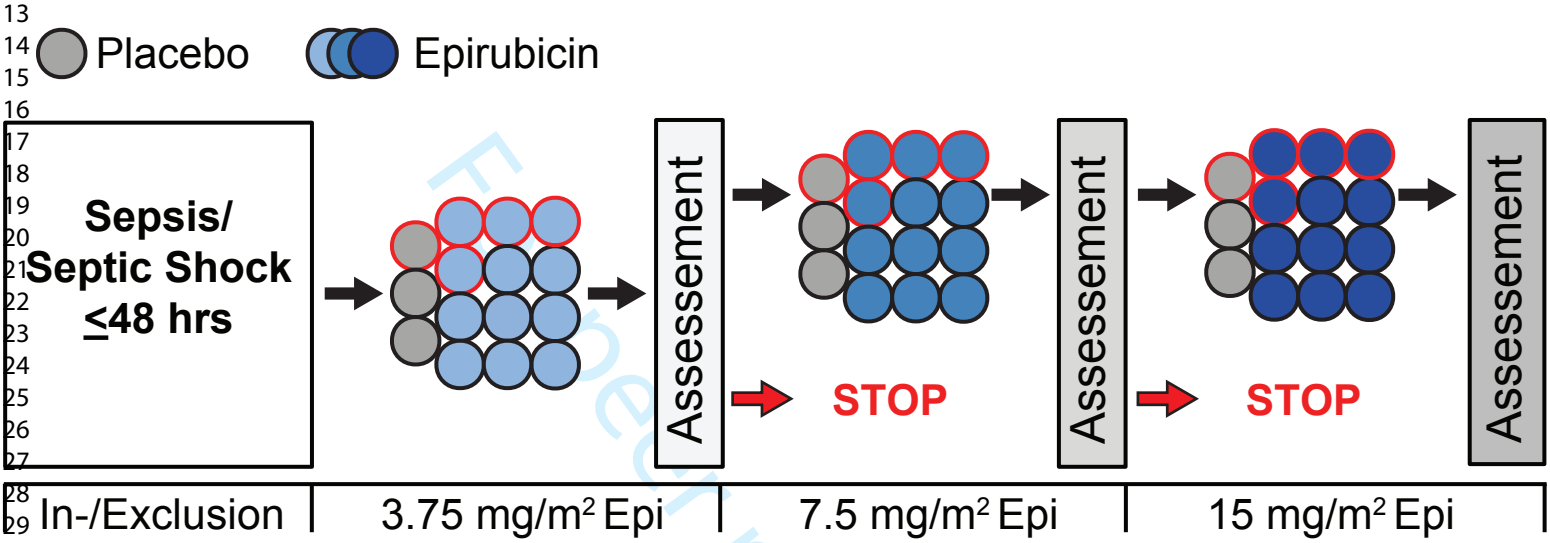
Abbreviation: Epi.. epirubicin, hrs.. hours.

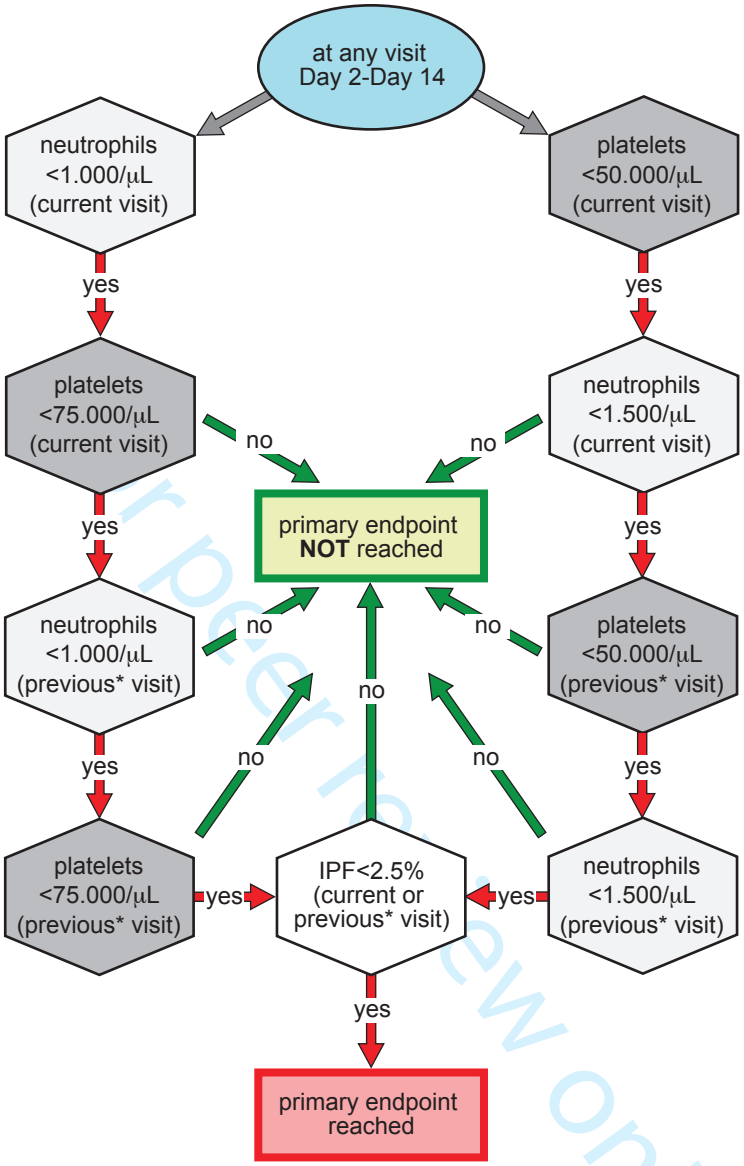
Figure 2: Flow chart that is used to determine the primary endpoint, *i.e.* myelotoxicity in the EPOS-1 trial. * for visit Day 2 the previous visit to be considered is Day 0-24 hrs.

Abbreviation: hrs.. hours

Table 1: Grading of neutropenia and thrombocytopenia.

	Neutropenia (acute neutrophil count)	**Thrombocytopenia** (platelets)
Grade 1	<Lower limit of normal- 1,500/μL	<Lower limit of normal-75,000/μL
Grade 2	<1,500-1,000/μL	<75,000-50,000/μL
Grade 3	<1,000-500/μL	<50,000-25,000/μL
Grade 4	< 500/μL	< 25,000/μL





Supplementary Table 1: Study procedures and visits.

Time (Days)	Pre		D0 First 24 hours								D1	D2	D3	D5	D7	D10	D12	D14	D28±2	D90±4
Time (Min) Time (hours)			0	15	60±5 1	120±10 2	3±0,5	6±1	12±2	24±4										
Visit		B	0	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Inclusion/Exclusion Criteria	x																			
Written Informed Consent	x																			
Pregnancy Test[1]		x																		
Randomization		x																		
Baseline Assessment		x																		
SOFA		x									x	x	x	x	x	x	x	x		
SAPS II / qSOFA		x																		
Clinical parameters		x									x	x	x	x	x	x	x	x		
Routine laboratory		x									x	x	x	x	x	x	x	x		
Treatment parameters on the ICU/IMC		x									x	x	x	x	x	x	x	x		
Application of study drug			x																	
Acute physiology			x	x	x	x	x	x	x	x										
Plasma sampling			x	x	x	x	x	x	x	x		x	x	x	x			x		
PBMC sampling			x					x		x			x		x					
Urine sampling			x							x			x							
Differential Blood Count/Neutrophils/IPF			x							x		x	x	x	x	x	x	x		
Anti-PF4			x												x			x		
AE / SAE				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Echocardiography															x					
Survival																		x	x	x
SF-36																				x

[1]*blood or urine*

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	9
	5b	Name and contact information for the trial sponsor	2
	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	9
	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)	9
Introduction			3-4
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	3
	6b	Explanation for choice of comparators	3
Objectives	7	Specific objectives or hypotheses	4
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)	4

Methods: Participants, interventions, and outcomes			**4-8**
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	
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)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4
	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)	n.a.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n.a.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13
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	5-6
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)	5-6
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	4
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	4
Methods: Assignment of interventions (for controlled trials)			**5**
Allocation:			5
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	5

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5
Methods: Data collection, management, and analysis			**4-7**
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	4,7
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7
	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)	n.a.
Methods: Monitoring			
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	7

	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	7
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	7
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	7
Ethics and dissemination			**5-8**
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	
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)	5
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	8
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
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9
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	8
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	n.a.
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	7
	31b	Authorship eligibility guidelines and any intended use of professional writers	n.a.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	x
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	x