AIDA

Clinical Study Protocol

EudraCT 2014-001276-56

Multicentre randomised controlled clinical trial to compare minocycline plus rifampicin with linezolid against MRSA in cSSSI

A prospective, open label, randomised controlled clinical trial, with pharmacokinetic-pharmacodynamic validation, to compare antimicrobial treatment with oral minocycline plus rifampicin to treatment with oral linezolid for complicated skin and skin structure infections (cSSSI) caused by Methicillin resistant Staphylococcus aureus *(MRSA).*

Sponsor

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Date of Issue: **21 March 2014**

Background to European 7th Framework Programme

This protocol is part of the European 7th Framework cooperation programme, FP7, (Project number 278348), which aims to promote transnational cooperation across a number of fields, including Health. The objective of the FP7 Health programme is to improve the health of European citizens, increase the competitiveness and boost the innovative capacity of European health-related industries and businesses, whilst addressing global health issues including emerging epidemics.

The AIDA project (Preserving old **a**nt**i**biotics for the future: assessment of clinical efficacy by a pharmacokinetic/pharmaco**d**ynamic **a**pproach to optimise effectiveness and reduce resistance for off-patent antibiotics), is part of this FP7 Health programme. It aims to promote transnational research in major infectious diseases and confront major threats to public health.

Its strategic objective is to confront the increasing emergence and spread of antimicrobial drug resistant pathogens in a multi-disciplinary approach through the development of effective infection prevention and control strategies. The project aims to answer the question of clinical effectiveness and optimal dosing of 5 off-patent antibiotics for infections caused by multiple drug resistant (MDR) bacteria in three randomised controlled clinical trials. This protocol is one of the above three trials, and should allow for the identification of optimal treatment regimens for off-patent antibiotics of infections caused by multi-drug resistant bacterial pathogens, as well as improved standardisation of such treatments.

Protocol Review and Approval

Co-ordinating Investigator: Alasdair MacGowan
Alasdair MacGowan (Apr 28, 2014)

Statistician: Vaughan Reed
Vaughan Reed (Apr 2, 2014)

Date of Issue: **21 March 2014**

Individual Investigator's Signature Page

Sponsor:	European 7th Framework Programme
Study Medication Name:	Minocycline and rifampicin, linezolid
Protocol Title:	Multicentre randomised controlled clinical trial to compare minocycline plus rifampicin with linezolid against MRSA in cSSSI
Date of Issue:	21 March 2014

All documentation that has been supplied to me by the Sponsor, concerning this study, and that has not been previously published, will be kept in the strictest confidence. This documentation includes the study protocol and Case Report Forms.

The study will not commence without the prior written approval of a properly constituted Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of the Coordinating Investigator and the IEC, except where necessary to eliminate an immediate hazard to the patients. Furthermore, signed informed consent will be obtained from each patient before entry into the study.

I have read and understood and agree to abide by all conditions and instructions contained in this protocol.

_____ _____

Investigator's Signature Date (dd/mmm/yyyy)

_____

Printed Name

Synopsis of AIDA Protocol

TITLE	A prospective, open label, randomized controlled clinical trial, with pharmacokinetic-pharmacodynamic validation, to compare antimicrobial treatment with oral minocycline plus rifampicin to treatment with oral linezolid for complicated skin and skin structure infections (cSSSI) caused by Methicillin resistant *Staphylococcus aureus* (MRSA).
SPONSOR	European 7th Framework Program - FP7
INDICATION	Complicated Skin and Skin Structure Infections caused by MRSA
OBJECTIVES	**Primary objective**: To demonstrate non-inferiority between patients treated with oral minocycline plus rifampicin and those patients treated with gold standard therapy of linezolid in terms of clinical cure at Test of Cure (TOC). **Secondary objectives**: • to assess the safety profile between the two treatment groups • to assess microbiological eradication of MRSA from the site of infection between the treatment groups • to assess the risk of emergence of resistance through sensitivity testing • to demonstrate a relationship between minocycline plus rifampicin drug exposure and outcome, including emergence of resistance, using state of the art pharmacokinetic-pharmacodynamic tools • to demonstrate a reduction in health care costs and resource utilization associated with the use of minocycline plus rifampicin compared with linezolid
TRIAL DESIGN	Prospective, open label, randomized, controlled multicentre clinical trial with a full PK/PD evaluation
NUMBER OF SUBJECTS	195 evaluable patients, 130 patients in minocycline/rifampicin treatment group, 65 patients in linezolid treatment group plus 15%.
TARGET POPULATION	Adult patients, with complicated skin and skin structure infection caused by MRSA, may be enrolled on the basis of clinical criteria and fulfilment of the inclusion and exclusion criteria.
Length of study	30 months enrolment including 12 month PK/PD study
End of study enrolment	Dec 2015
Investigational Medical Products - Dose/route/ regimen	Minocycline 100mg 12hourly po, rifampicin 600mg 24hourly po, linezolid 600mg 12 hourly po, OR as local national Summary of Product Characteristics (SmPC) guidelines.
Efficacy assessments	Clinical evaluation by a blinded Clinical Investigator supported by photographic data. Clinical response of signs and symptoms of infection and microbiological assessment.
Safety assessments	Clinical review and laboratory monitoring
Pharmacokinetic/ Pharmacodynamic assessments	4 plasma blood draws (over 24 hours) are required on Day 1, and 4 plasma blood draws (over 24 hours) are require on Day 5. Each sample will be a minimum of 5mls and will be separated as soon as possible, and then frozen between -20°C and -80°C.
Health Economic Evaluation	Costs associated with the use of minocycline plus rifampicin compared with treatment with linezolid in 4 categories; visits to providers, medications, interventions and testing.
Statistical Methods	**Sample size** For non-inferiority testing with a 5% significance level, 87% clinical cure rate for the comparator, linezolid at a 2:1 ratio and non inferiority limit of 15 percentage points, a sample size of 130 patients in the minocycline/rifampicin treatment group and 65 in the linezolid treatment group would be required

for 90% power.

Analysis Populations

Four different populations will be analysed; the intention-to-treat (ITT), the per protocol (PP), and the microbiologically evaluable subsets of the ITT and PP populations (mITT and mePP respectively). The ITT population will include all patients who were allocated a treatment by the minimization website regardless of how much treatment was actually taken. Patients will be considered clinically evaluable (PP) if they meet the inclusion criteria, have a clinical outcome of either Cure or Failure at TOC (Day 14), receive drug for at least 5 days and have not departed in any significant way from the protocol. If medication is stopped early by the physician, either because of adverse events or because the infection has completely cleared and further treatment is not deemed necessary, this will not be regarded as a protocol violation.

All patients by definition must have a positive culture of MRSA at baseline so the microbiologically evaluable patients will include all patients who have undergone 14 days follow-up with evaluation of eradication or relapse. Where the infection has completely cleared at the TOC visit (Day 14) and no sample can be obtained for culture the infection will be regarded as eradicated.

Table of Contents

Glossary of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
bid	Twice daily
BMI	Body Mass Index
BSAC	British Society of Antimicrobial Chemotherapy
CI	Confidence Interval
CDC	Center for Disease Control
CRF	Case Report Form(s)
CRP	C-reactive protein
cSSSI	complicated Skin and Skin Structure Infection
DIC	Ductal *in situ* carcinoma
DOB	Date of Birth
(e)CRF	(electronic) Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Agency
GCP	Good Clinical Practice
Hb	haemoglobin
hCG	human Chorionic Gonadotrophin
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
ITT	Intent to Treat
iv	intravenous
LFTs	Liver Function Tests
MAH	Marketing Authorisation Holder
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin susceptible *Staphylococcus aureus*
OR	Odds ratio
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious Adverse Event
SD	Standard Deviation
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedures
TOC	Test of Cure
WBC	White blood cell
WMA	World Medical Association

Investigators and Study Administrative Structure

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1 Study design and conduct

1.1 Background and Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) remains an important human pathogen being responsible for a range of infections in both hospitalised and community based patients. A large proportion of these infections require antimicrobial chemotherapy but only a small number have severe and life threatening disease.

MRSA incidence is increasing across the European continent, adding to the number of infections caused by methicillin susceptible *S. aureus* (MSSA). Thirteen European countries reported MRSA rates of ≥25% in 2008. This included all the Mediterranean countries, Romania, UK and Ireland. Four countries had MRSA rates of >40% - all in the Mediterranean. MRSA rates are 5-10% in the Baltic States but 10-25% in Eastern Europe and >25% in Western Europe. Some European countries with low rates of MRSA are reporting increasing numbers of isolates while others with endemic MRSA infection (France, Slovenia, UK) are on a decreasing trend. Overall, MRSA is still an increasing problem all over Europe with 22% of *S. aureus* isolates being MRSA on average (EARSS Annual Report, 2008).

The present therapy for MRSA is limited, especially in terms of oral therapies where the only drug that has undergone large scale clinical evaluation in its oral formulation is linezolid (Falagas *et al*, 2008). The use of linezolid is limited by its cost and by adverse events associated with long term therapy. This leaves a clear unmet clinical need for the evaluation of other oral anti MRSA therapies.

Tetracyclines, such as minocycline and doxycycline, are well absorbed, with long half lives and good tissue penetration (Agwuh & MacGowan, 2006). *In vitro* data suggests minocycline has the better anti *Staphylococcal* activity (Minth *et al*, 1974). However, robust clinical data are absent in supporting their use.

Two small clinical studies have been conducted in skin and skin structure infections. Cenizal *et al*, 2007, evaluated doxycycline in 15 patients with MRSA. Telephone follow-up at 4-5 weeks indicated a 17.6% relapse rate with doxycycline. In a larger study, 90 patients with MRSA SSTI were treated with tetracyclines, 4 patients failed treatment (Ruhe and Menon, 2007). Although, there is little information in the medical literature on the use of tetracyclines to treat MRSA, the CDC in the USA and the BSAC in the UK have recommended their use to treat MRSA skin infections.

The recent emergence of community-acquired MRSA (CA-MRSA) in North America has further added to the burden of MRSA infection, making the need for oral agents with proven efficacy a greater medical need.

Minocycline

Minocycline is a semi synthetic tetracycline which first became available in 1972. Its main mechanism of action is on protein synthesis. Minocycline is primarily bacteriostatic but is more active than other tetracyclines against *S. aureus*. Minocycline is completely absorbed producing peak concentration of 1.5-2.5mg/L after a 100mg oral dose with a half life of 12-16 hr. It is widely distributed in the body with a protein binding of 76%. Minocycline is associated with a number of adverse reactions, including auto immune disorders, benign intracranial hypertension, and gut, skin, and vestibular disturbance. Minocycline is contra-indicated in children and pregnant women.

Rifampicin

Rifampicin is a semi synthetic derivative of a natural antibiotic rifampicin B, which belongs to the naphthalenic rifampicin class. The mode of action is inhibition of DNA dependent RNA polymerase and it is bactericidal against *S. aureus* with minimum inhibitory concentrations (MICs) in the range 0.002-0.005mg/L. Rifampicin is well absorbed, producing peak concentrations of 7-10mg/L after a 600mg dose. Rifampicin diffuses into most organs and has a protein binding of 60-80%. Rifampicin is associated with a number of adverse reactions, including hepatotoxicity, gut effects and hypersensitivity.

Minocycline plus rifampicin

Minocycline plus rifampicin is a widely used combination of antibiotics to treat MRSA in Europe (Dryden *et al*, 2010). However, the evidence base for its use is limited. The combination is synergistic *in vitro* against MRSA and MSSA (Chow *et al*, 1991) and rifampicin adds to the activity of minocycline in pre clinical pharmacodynamic models (Bowker *et al*, 2009). The combination has been used clinically to eradicate MRSA carriage (Darouiche *et al*, 1991). In a combination study, minocycline plus rifampicin was more effective at eradicating MRSA carriage at one week than minocycline alone but not compared with rifampicin alone (Muder *et al*, 1994). A systematic review of comparative trials using rifampicin as an adjuvant for the treatment of Gram positive infections indicated eight randomised controlled trials (RCTs) were performed, five involving infection by *S. aureus*. No statistical difference in cure rates in *Staphylococcal* infection was found (OR 0.57 95% CI 0.27-1.17) or rate of adverse events. More controlled trials were felt to be needed on rifampicin combinations (Bliziotis *et al*, 2007). Since both minocycline and rifampicin are available as generics, they have substantial cost advantages over linezolid as oral therapy. However, there is no randomised comparative clinical study to assess their effectiveness in treating MRSA infection.

Linezolid

Linezolid is a synthetic oxazolidinone which has been available for clinical use for about ten years. Its main mechanism of action is on protein synthesis. Linezolid is bacteriostatic. It is almost completely absorbed, producing peak concentrations of 18-21mg/L after an oral dose of 600mg. The half life is 5.5 hours. It is well distributed through the body with protein binding of 30%. Adverse events occurring at a frequency of >1% are diarrhoea, nausea, headache, candidiasis, taste alteration, vomiting and abnormal liver function tests. Bone marrow suppression and optic neuritis have been reported more rarely. Linezolid has been approved by

the EMA for use in complicated skin and skin structure infection (cSSSI), hospital acquired pneumonia (HAP) and community acquired pneumonia (CAP).

Pharmacokinetics and Pharmacodynamics (pK-pD) allows rational antibiotic doses and combinations to be used in clinical practice and allows the potential for greater understanding of the risks of emergence of resistance. Pre clinical pK-pD evaluations define the dominant pD index, and its size for antibacterial effect or risk of emergence of resistance. In addition, microbiological interactions can be studied – for example, using Grasso interface plots. Such findings are translated into clinical practice using Monte Carlo simulation, population pK model building, and comparing drug exposure in infected patients to clinical or microbiological outcomes using logistic regression and CART analysis.

1.2 Rationale for the Study

MRSA infections occur often and represent a significant healthcare burden that is increasing across Europe. Currently available oral therapies proven in well conducted clinical trials are effective but expensive and may be toxic in long term use (linezolid). Alternatives tested in randomised clinical trials are all intravenous (daptomycin, vancomycin, teicoplanin, tigecycline, telavancin, ceftaroline). In contrast, inexpensive oral therapy with generic agents may be equally effective to these current *i.v.* or oral treatments with linezolid, but this has not been established in credible randomised controlled trials. The purpose of this study is to compare the combination of two such generic agents (minocycline plus rifampicin) with the current gold standard therapy (linezolid) in a non-inferiority study using complicated skin and skin structure infection as our exemplar. Complicated skin and skin structure infection was selected as it is a common disease syndrome, is easily recognised as clinically relevant by practitioners yet sufficiently homogenous for robust clinical trial design and analysis. A nested pK-pD study will confirm that the drug exposures, translated from pre clinical studies and mathematical modelling, are appropriate for man to produce microbiological cures and to minimise the risk of resistance.

2 Study Objectives

2.1 Primary Objective

The primary objective is to:

- demonstrate non inferiority between patients treated with minocycline plus rifampicin and those patients with gold standard therapy of linezolid in terms of clinical cure at Test of Cure.

The secondary objectives are to:

- assess the safety profile between the two treatment groups
- assess microbiological eradication of MRSA from the site of infection between the treatment groups
- assess the risk of emergence of resistance through sensitivity testing
- demonstrate a relationship between drug exposure and outcome, including emergence of resistance, using state of the art pharmacokinetic-pharmacodynamic tools

3 Study Design and Dosing Regimen

This is an Investigator-initiated open label, randomised clinical trial to compare a combination of minocycline plus rifampicin with the present gold standard therapy, linezolid, in patients requiring oral therapy for cSSSI due to MRSA. Patients satisfying the entry criteria will be assigned, in a 2:1 ratio and by minimization to their respective treatment groups. Before study inclusion, no therapy for >24hr with antibacterials active against MRSA will be allowed, unless baseline culture is positive for MRSA and previous therapy is considered to have failed. Assessments of bacteriology, clinical signs and symptoms of infection plus clinical and laboratory safety evaluations will be made at Baseline/Day 1, during study therapy at Day 5 (+/-1) and at the Test of cure (TOC)/Day 14 (+/-2).

Clinical efficacy will be assessed in terms of resolution of signs and symptoms, present at baseline/day 1.

Bacteriological efficacy will be assessed in terms of eradication of MRSA from the site of infection.

Safety will be assessed by review of reported adverse events and by changes in laboratory parameters and patient vital signs.

Risk of emergence of resistance will be assessed on the basis of sensitivity testing of isolates from the infection site at the end of treatment and from a nose swab taken at the final assessment.

Patients will have infection with signs and symptoms of complicated skin and skin structure infection with MRSA. Approximately 200 cases are projected to be collected during the study. See section 7.3 for further information on sample size calculation.

Table 1 Schedule of Assessments

	Baseline (Day 1)	Day 5 (+/-1)	Test of Cure Day 14 (+/-2)
Inclusion/Exclusion Evaluation	X		
Demographics (Gender, DoB)	X		
Informed consent/assent	X		
Randomisation	X		
Medical/Surgical History	X		
Concomitant medicine	X	X	X
Physical Examination (weight, height)	X		
Vital signs (BP, pulse)	X	X	X
Signs/symptoms of cSSSI	X	X	X
Swab of infection site and/or blood culture	X		X
Nose and throat swab for MRSA	X		X
Laboratory tests; * Haematology: e.g. WBC, neutrophil, Hb, platelets, Creatinine clearance Chemistry: e.g. Na+, creatine, LFT, CRP	X	X	X
Pregnancy test	X		
First dose administered	X		
PK/PD Blood draw (4 samples over 24hrs) 5mls **	X	X	
Adverse Events		X	X
Clinical Efficacy Assessment			X
*** approximate volume per draw– 5mls ** approximate volume per draw – 5mls, total amount per 24 hours= 20mls**			

3.1 Dosing Regimen

Minocycline and Rifampicin will be dosed together.

Minocycline
The recommended daily dose, as prescribed in local national guidelines or in the Summary of Product Characteristics (SmPC), will be used.

Potential side effects include; nausea, fever, diarrhoea, vomiting, photosensitivity, dyspepsia, altered taste, dizziness, fatigue, skin itch, oesophagitis, oesophageal ulcers, Candida infection, discolouration of teeth in children, pancreatitis, hepatotoxicity, renal toxicity, especially in those with pre existing renal disease, haemolytic anaemia, thrombocytopenia, increases or decreases in white blood cell count (WBC), neutropaenia, systemic lupus and erythematous like syndrome.

Rifampicin
The recommended daily dose, as prescribed in local guidelines or in the Summary of Product Characteristics (SmPC), will be used.

Potential side effects include; oedema flushing, ataxia, behaviour change, impaired concentration, confusion, dizziness, drowsiness, fatigue, fever, headache, numbness, psychosis, pemphigoid, pruritis, urticaria, adrenal insufficiency, mental disorders, agranulocytosis, DIC, eosinophilia, decreased haemoglobin, leukopenia, trachocytopaenia, hepatitis, jaundice, myalgia, oesteomalacia, weakness, exudative conjunctivitis, visual change, acute renal failure, increased blood urea, haemoglobinuria, haematoma, interstitial nephritis, increased uric acid, 'flu' like syndrome, rash (1-5%), gastrointestinal (GI) upset (1-2%), anorexia, cramps, epigastric pain, heartburn, nausea, pancreatitis, diarrhoea, vomiting and abnormal Liver function tests (LFTs).

Linezolid
The recommended daily dose, as prescribed in local guidelines or in the Summary of Product Characteristics (SmPC), will be used.

Potential side effects include; candidiasis (oral or vaginal), headache, metallic taste, diarrhoea, nausea, vomiting, abnormal LFTs, increased AST, ALT or alkaline phosphatase, increased blood urea, increased LDH, creatine kinase, lipase, amylase or glucose, increased neutrophils or eosinophils, decreased haemoglobin, haematocrit or red cell count, increased or decreased platelets or white blood counts.

3.2 Concomitant Medication and Treatment

Patients receiving medication for concomitant conditions other than infections can enter into the study. Decisions regarding the continuation of medications required for the routine care of the patient will be at the discretion of the treating physician following local treatment guidelines during the patient's participation in the study. A complete listing of all concomitant medication received during the treatment phase must be recorded in the (e)CRF.

4 Study Population

Patients in this study will have an infection with MRSA, defined as either MRSA isolated from the site of infection or patients known to be colonised with MRSA that have cSSSI requiring antimicrobial therapy.

4.1 Inclusion Criteria

Patients will be enrolled in this study only if they meet all of the following numbered criteria:

1. Hospitalised with clinical evidence of at least 1 of the following MRSA infections:
    - Ulcers
    - First or second degree burns of less than 20% of body surface area with concomitant signs of cellulitis (excluding third degree burns and burns >20% of body surface area)
    - Major abscess (see exclusion criteria for qualifications)
    - Deep or extensive cellulitis, and/or
    - Wounds – trauma or post surgical

2. Presence of purulent or seropurulent drainage or at least 3 of the following signs and symptoms:
    - Drainage and/or discharge
    - Erythema (extending at least 1 cm beyond a wound edge)
    - Swelling and/or induration
    - Heat and/or localized warmth
    - Pain and/or tenderness to palpation

3. At least 1 of the following conditions considered to be pathogen-related:
    - Fever (temperature >38°C/100.4°F orally, rectally, or tympanically),
    - Elevated total peripheral white blood cells (WBCs) >10,000/mm^3, or
    - >15% immature neutrophils (bands), regardless of total peripheral WBC count

4. Accessible infection site for culture or a bacteraemia where a culture cannot be obtained from the site of infection

5. Adult at least 18 years of age

6. Written informed consent to participate in the study before any study-specific procedures are performed

7. If of childbearing potential, must be using, or be prepared to use, a mechanical method of contraception (e.g. condom) during the study.

8. If female, has a negative serum pregnancy test (serum beta-human Chorionic Gonadotropin (hCG)) result immediately prior to enrolment. If obtaining the serum pregnancy result would cause a delay in treatment, the patient can be entered on the basis of a negative urine pregnancy test result. The urine pregnancy test must be sensitive to at least 50 mU/mL of beta-hCG, pending results of the serum test. The patient must end study medication therapy if the subsequent serum pregnancy test is positive

   Patients who have received an antibiotic for a cSSSI but have not responded and are considered a failure on that treatment regimen are eligible for this study provided they have a positive MRSA baseline culture.

4.2 Exclusion Criteria

Any of the following will exclude a patient from enrolment into the study.

1. Women who are pregnant or breast-feeding

2. Pre menopausal women who refuse to substitute oral contraception during treatment by contraception using mechanical means (e.g. condom)

3. Known or suspected hypersensitivity to linezolid, minocycline or rifampicin

4. Clinical or laboratory evidence of significant impairment of hepatic function, i.e. bilirubin of >3x upper limit of normal range, AST or ALT >5x upper limit of normal range, proven histological liver changes on biopsy

5. Major abscess associated with diabetic foot conditions

6. Suspected or confirmed osteomyelitis

7. Treatment with other antimicrobials with activity against MRSA within 24hr prior to study inclusion. However, treatment failures from other therapy may be entered provided there is a positive baseline culture for MRSA.

8. In the case of a mixed infection where it is considered necessary to concomitantly treat with a Gram-negative agent, this agent must have no activity against MRSA.

9. Patients with a high probability of death within a week of study entry

10. Haemodialysis patients or those requiring other means of renal support for end stage renal disease.

4.3 Centres

Selection of centres will be based upon a number of criteria, including the following;

- Ability to provide patients and samples according to the study protocol
- Sufficient staff to enable clinical data collection and follow-up with patients in order to maximize convenience and compliance
- Availability to continue for the likely duration of the study

4.4 Randomisation

Treatment will be allocated, in a 2:1 ratio, by minimization from the Micron Research Minimization website. The Investigators will all have a centre number and individual password. Once a patient has satisfied all the Inclusion and Exclusion criteria the Investigator will enter their centre number and password to go into the minimization website. They will be asked to enter the patient's DOB, gender, type of infection (ulcer, burns, major abscess, cellulitis or wound) and smoking history (previous smoker, current smoker or never smoked). The website will return the patient number and the treatment that the patient has been allocated. Minimization will provide a good balance between the treatment groups for the factors listed above and, in addition, since the centre number is included as a factor will balance the two treatment groups at each individual centre.

4.5 Withdrawal of Subjects

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study if, in their opinion, their standard of care will be compromised by continuing in the study. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible. The rate of patient withdrawal will be monitored throughout study to ensure that the number of per protocol patients recruited is adequate to maintain the power of the study.

4.6 Expected Duration of the Study

The study enrolment period is scheduled for 30 months.

5 Schedule of Procedures and Assessments

All patients must provide written informed consent before any study specific assessments or procedures are performed. Patients will be assessed for inclusion and exclusion criteria and those who fulfil all the inclusion and none of the exclusion criteria will be recruited into the study. Once a patient has fulfilled the entry criteria, he/she will be assigned a unique identifier. The Investigator will enter the unique patient identification onto the (electronic) Case Report Form, (e)CRF.

All patients will receive the routine care, as determined by the physician, for their presenting illness (cSSSI). Routine patient care will be conducted by tests performed locally at the study site, and local guidelines on treating cSSSI.

Baseline: Day 1
All patients will be consented prior to any study activity taking place. Patients will be screened according to the following activities:

- A brief medical history and history of current illness, according to the parameters in the (e)CRF. Patients not meeting inclusion or exclusion criteria will be excluded from the study.
- Data collected will include; patient demographics, medical/surgical history, vital signs and concomitant medications.
- Signs and symptoms of complicated skin and skin structure infections will be assessed, see Appendix 2.
- A swab of the infection site will be taken for culturing, if clinically feasible. A nasal and throat swab will also be taken to determine the presence of MRSA.
- Female patients, of childbearing potential will be given a pregnancy test and excluded from the study if the result is positive.
- Patients will be assigned to a treatment group following randomisation and appropriate study medication given.
- Patient blood samples will be taken to determine haematology (e.g. white blood cell count [WBC], haemoglobin [Hb], platelet count) and blood chemistry (e.g. C-reactive protein [CRP], sodium [Na+], creatinine).
- Four PK/PD blood draws will be taken over 24 hours post dose, from all patients, see Appendix 1.

Therapy visit: Day 5 (+/- 1 day)

- Data collected will include vital signs and concomitant medications.
- Signs and symptoms of complicated skin and skin structure infections will be assessed see Appendix 2.
- Patient blood samples will be taken to determine haematology (e.g. white blood cell count [WBC], haemoglobin [Hb], platelet count) and blood chemistry (e.g. C-reactive protein [CRP], sodium [Na+], creatinine).
- Four PK/PD blood draws will be taken over 24 hours post dose, from all patients, see Appendix 1.

- The Investigator will ask about adverse events since study entry and about any medication taken for cSSSI symptoms, or any other changes in medication. These will be documented in the (e)CRF.

Test of Cure visit (TOC): Day 14 (+/- 2 days)

- Data collected will include vital signs.
- Signs and symptoms of complicated skin and skin structure infections will be assessed see Appendix 2.
- A swab of the infection site will be taken for culturing, if clinically feasible. A nasal and throat swab will also be taken to determine the presence of MRSA.
- Patient blood samples will be taken to determine haematology (e.g. white blood cell count [WBC], haemoglobin [Hb], platelet count) and blood chemistry (e.g. C-reactive protein [CRP], sodium [Na+], creatinine).
- The Investigator will ask about adverse events since study entry and about any medication taken for cSSSI symptoms, or any other changes in medication. These will be documented in the (e)CRF.
- A clinical efficacy assessment will be made, see Appendix 3.

Follow-up call: Day 30 (+/-5 days)

- A follow up telephone call will be made to establish continued efficacy and mortality at day 30 (+/-5 days).

5.1 Microbiological Assessments

Four different populations will be analysed; (a) the intention-to-treat (ITT) patient populations, (b) the clinically evaluable population (PP), and (c) the microbiologically evaluable subsets of (c) the ITT and (d) the PP populations. The ITT population will include all patients who were allocated a treatment by the minimization website regardless of how much treatment was actually taken.

Patients will be considered clinically evaluable if they meet the inclusion criteria and receive drug for at least 4 days, have a clinical outcome of either cure or failure at the TOC visit (Day 14) and have not departed in any significant way from the protocol. Microbiologically evaluable patients will include all clinically evaluable patients who have undergone 14 days follow-up with evaluation of eradication or relapse. Clinical evaluations will be performed by a blinded clinical assessor at the centre and by photographic images (see Appendix 2).

The Primary efficacy variable will be the clinical outcome:-

- clinical cure is defined as resolution of the clinical signs and symptoms present at baseline, see Appendix 3.

Secondary outcome measures will be:-

- bacteriological cure, defined as eradication of the MRSA from the infection site

- mortality at 30 days
- length of hospital stay, or re-hospitalisation (days)
- resolution of CRP at end of treatment
- resistance to linezolid, rifampicin or minocycline in MRSA isolated from the site of infection, nasal cavities or throat cavity at end of treatment

5.2 Pharmacokinetic/Pharmacodynamic Evaluation

For a detailed description of the requirements for pharmacokinetic/pharmacodynamic (PK/PD) evaluation, please see Appendix 1.

6 Safety Instructions and Guidance

6.1 Adverse Events (AEs) and Laboratory Abnormalities

6.1.1 Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical investigation patient administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

6.1.2 Adverse Drug Reaction (ADR)

An adverse drug reaction, in contrast to an event, is characterised by the fact that a causal relationship between the drug and the occurrence is suspected. For regulatory reporting purposes, if an event is spontaneously reported, even if the relationship is unknown or unstated, it meets the definition of an adverse drug reaction.

6.1.3 Serious Adverse Events

A Serious Adverse Event is any experience at any dose that results in any of the following criteria:

- Is fatal, (results in death**; NOTE: death is an outcome, not an event)
- Is life-threatening, (NOTE: the term "life-threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe)
- Requires in-patient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above.

(**)- The term sudden death should only be used when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably.

6.1.4 Drug - Adverse event relationship

Relationship of the AE to treatment should always be assessed by the Investigator. Guidelines to determine the relationship between an AE and treatment are presented in AEs Categories for Determining Relationship to Medicinal Product (see Appendix 4).

6.1.5 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the (e)CRF. Intensity of AEs will be graded on a three-point scale (mild, moderate or severe) and reported on the (e)CRF

Mild	Discomfort noticed but no disruption of normal daily activity.
Moderate	Discomfort sufficient to reduce or affect daily activity.
Severe	Inability to work or perform normal daily activity

6.1.6 Laboratory Test Abnormalities

Any treatment-emergent abnormal laboratory result, which is clinically significant, i.e. meets one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the (e)CRF:

- Accompanied by clinical symptoms
- If applicable, leads to a change in cSSSI medication (e.g. dose modification, interruption or permanent discontinuation)
- Requires a change in concomitant therapy (e.g. the addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

Any such laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as a SAE in the (e)CRF.

6.1.7 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to medications prescribed to study participants is “related”, should be followed up until they have returned to baseline status or stabilized.

6.2 Handling of Safety Parameters

6.2.1 Reporting of AEs

All adverse events reported by the patient will be recorded in the (e)CRF.
Clinical adverse events should be always described by a single diagnosis and not by symptoms (e.g., “acute asthma attack” instead of “wheezing and breathlessness”).

6.2.2 Reporting of Serious Adverse Events

This study adheres to the definition and reporting requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2D. As per the details given in E2D, the Marketing Authorisation Holders (MAH) of rifampicin, minocycline and linezolid will be notified of Serious Adverse Drug Reactions by the Investigator (Ref. 4.1.1 Serious ADR’s- E2D).

Onward reporting as necessary to the Regulatory Authorities will be the responsibility of the MAH (Ref. 3.3 – E2D).

6.2.3 Pregnancy

Please refer to the guidance given in local or national Summary of Product Characteristics for linezolid, minocycline and rifampicin as appropriate.

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7 Statistical Considerations and Analytical Plan

The outcome is a binary assessment of clinical cure (cured/not cured), and the test is for non-inferiority. The analysis will therefore be by a one-sided comparison of binomial proportions. Non-inferiority will be concluded if the lower 90% confidence limit on the difference in cure rates between the study drugs and the comparator does not fall below the predetermined non-inferiority margin of minus 15%.

Spellberg *et al*, 2009, reviewed studies of outcome in cSSSI in 1900-1950, before widespread penicillin resistance, and concluded that non-inferiority margins of 7, 14 and 21 percentage points would ensure that a test drug preserved at least 50% of the efficacy of penicillin (as compared with no antibiotic treatment) for major abscess, erysipelas/cellulitis, and wounds/ulcers, respectively.

For a trial with a mixture of types of skin infection, these margins should be weighted by the prevalence of the different types. Limited pilot information indicates that for MRSA infections, approximately 62% are wounds/ulcers, 12% cellulitis/erysipelas, and 26% other infections. Considering the 'others' conservatively as abscess, the weighted non-inferiority margin appropriate to this study is approximately 17 percentage points. Allowing for some uncertainty in the prediction of case mix, a margin of 15 percentage points is appropriate. We believe that the results of the study will be used in practice to inform decisions about choice of therapy so a direct comparison between the two regimes studied will be of greater importance than an indirect comparison with no antibiotic treatment, and therefore the narrower clinically-derived margin should be used.

Spellberg *et al* 2009 gives cure rates with penicillin treatment of 96, 98, and 83% for major abscess, erysipelas/cellulitis, and wounds/ulcers, respectively. The weighted average for the expected case mix with MRSA infections is 88%. We estimate a clinical cure rate of 87% for MRSA treated with linezolid. We based our estimate on the cure rates for *S. aureus* infections treated with linezolid reported in a review by Falagas *et al*, 2008, assuming that the efficacy of linezolid would be similar for MRSA and MSSA, as there is hardly any resistance to the drug in either group and there is no difference in the minimum inhibitory concentrations required. Data from studies of respiratory infections, uncomplicated skin infections and diabetic foot infections, which are likely to involve underlying bone infection and therefore be excluded from the currently proposed trial, were excluded from the calculation.

7.1.1 Acceptable levels of error

Accepting an inferior treatment as non-inferior

This error could result in the widespread acceptance of an inferior treatment and therefore this error rate was limited to 5%. To do so, the one-sided non-inferiority test requires a significance

level of 5%, which is achieved by constructing a two-sided 90% confidence interval for comparison with the non-inferiority limit.

Dismissing an equally effective treatment as inferior
This error could result in the removal of an effective treatment from consideration. This error rate was limited to 15%. That is, when the true difference between treatments is zero, there should be a 90% probability that the confidence interval will lie entirely above the non-inferiority limit. This was achieved by an appropriate choice of sample size.

7.2 Primary and Secondary Study Objectives

The statistical methods used to analyze the primary and secondary efficacy variables are outlined below.

7.2.1 Primary Objective
The Primary Efficacy analysis will involve the PP population (the conservative approach since this is a non-inferiority study) and the main test will be whether the lower 90% confidence interval for the difference in clinical cure rate between minocycline/rifampicin and the comparator, linezolid, falls below the non-inferiority limit of minus 15 percent at the TOC visit (Day 14).

7.2.2 Secondary Objectives
The secondary objectives are:

- assessment of the safety profile between the two treatment groups
- assessment of the eradication of MRSA from the site of infection between the treatment groups at Test of Cure
- assessment of resistance to linezolid, rifampicin or minocycline in MRSA isolated from the site of infection, nasal cavities or throat cavity after therapy
- mortality at 30 days
- length of hospital stay
- CRP resolution

Bacteriological cure rates will be tested for non inferiority in exactly the same way as for the primary efficacy analysis, comparing eradicated versus all other outcomes as failure. Where there is no infectious area to take a culture from at TOC the patients will be recorded as eradicated – not presumed eradication.

Resistance levels and MIC distribution summaries will be illustrated with descriptive statistics only.

Mortality and frequency of length of hospitalizations will be compared between the treatment groups using Fisher's exact test.

The percentage of patients whose CRP has resolved at TOC will be compared using either a Chi-square test or Fisher's exact test as appropriate, taking account of the number of patients who show complete resolution.

7.3 Sample size

We require a 5% significance level in a test of non-inferiority, with 90% probability of confidence interval being above the non-inferiority limit when the treatments are equally effective. We assume a clinical cure rate with linezolid of 87%, and have set a non-inferiority limit of 15 percentage points. Since a 2:1 ratio of treatment is being used and rounding to the nearest 5 patients this requires 130 patients treated with the combination minocycline plus rifampicin and 65 treated with linezolid, 195 in total. Allowing that 15% of the patients recruited will not qualify to be in the PP population, 225 patients should be recruited.

7.4 Analysis

Baseline and demographic data will be summarised either as frequencies and percentages for class data such as gender, or as means with standard deviations (SD), medians and range (minimum to maximum) for continuous data such as age. In general data will be reported to one decimal place. Baseline data will include age (calculated from DOB) gender, weight, height, BMI, baseline temperature, area of infection (length x breadth), the type of infection, study drug dose in mg/kg, number of obese patients (BMI >30) and number of patients defined as febrile (temperature >38.5°C). These parameters will all be compared between treatment groups without statistical tests.

Medication the patients were taking at the time of entry to the study will be summarised as frequencies and percentages of the generic drug names split by major drug classes. Antimicrobials already used to treat the cSSSI will be summarised separately and will include the mean interval between the drug being withdrawn and visit one of the study where this information is available.

The main populations for analysis are the ITT population and the clinically evaluable or per protocol population (PP). All patients by definition must have had an infection with MRSA identified at baseline giving two microbiologically evaluable populations mITT and mePP where there is a microbiological outcome at the TOC visit (Day 14). Where no sample of infected tissue is available at the end of treatment because the infection has totally cleared this will be taken as a microbiological outcome of 'eradicated'.

The ITT population is defined as all patients for whom a treatment was allocated regardless of how much medication was actually taken. The PP population will be those members of the ITT population who completed the study to the TOC visit at Day 14 follow-up visit and who did not depart in any major way from the protocol. Where patients failed to take the full course of treatment (5 days minimum) this will be regarded as a major protocol violation unless either

they stopped treatment due to an adverse event considered to be caused by the study drug or they stopped treatment because the infection had totally cleared and the Investigator considered it unnecessary to complete the course. All patients in the PP population must have a clinical outcome of cured or failed. Any other outcome excludes them from the PP population.

The Primary Efficacy analysis will involve the per protocol population (the conservative approach since this is a non-inferiority study) and the main test will be whether the lower 90% confidence interval for the difference between the clinical cure rate of minocycline/rifampicin and the comparator, linezolid, falls below the non inferiority limit set at minus 15 percent.
Secondary comparisons will be by the same method for the ITT population and for the percent of eradicated infections in the mITT and mePP populations.

The percentage cure and MRSA eradication rates will be tabulated separately for each of the main infection types: wounds, burns, ulcers, major abscess and cellulitis. It is not however appropriate to test for non-inferiority on these subsets as the numbers are inevitably too small to give a reliable result.

The area of infection will be compared at each assessment together with the change from baseline. These data will be tabulated for the whole population and split by the main infection types: wounds, burns, ulcers, major abscess and cellulitis. Where the infection has completely cleared the area of infection will be taken as zero. At the Test of Cure (TOC) assessment, the area of infection will be compared between treatment groups by analysis of covariance with the area at baseline as covariate.

Vital signs and laboratory parameters will be compared between treatment groups at each assessment with change from baseline where appropriate. No statistical tests are planned for the vital signs or laboratory data. In the case of LFTs the number and percentage of values more than three times the upper limit of normal will be tabulated for each assessment.

Minimum Inhibitory Concentration (MIC) values from baseline and follow up cultures of MRSA will be tabulated as distributions with summary statistics (MIC 50, MIC 90, Range, Geometric Mean and Mode) and resistance rates to the antimicrobials tested.

7.4.1 Exploratory Objectives

Since this study is to some extent exploratory it may be appropriate to examine cure and eradication rates in subsets of the population. Any analyses added to the report after the planned comparisons have been carried out should only be regarded as exploratory and where positive can do no more than generate a new hypothesis for future study.

7.4.2 Safety

All adverse events will be documented. The intensity of adverse events will be graded as mild, moderate or severe, and described in detail along with the investigation assessment of the

relationship to drug therapy. SAEs will be reported via the usual reporting mechanism in the countries where the study is conducted, for regulated marketed products.

Safety will be evaluated in those patients receiving any treatment by means of AE reports. All patients who received at least one dose of any treatment and had a safety assessment performed will be included in the safety evaluation. Adverse events (AE) will be summarised by body system, comparing patient events between the treatment groups. Where a patient reports the same AE more than once this will be regarded as a single patient event. Further tables of Severe AEs and AEs considered by the Investigator to be caused by the study drug will also be produced. No statistical tests will be performed on the AE data.

Part II: Ethics and General Study Administration

8 Quality Control and Quality Assurance

The overall procedures for quality control and assurance of clinical study data are described in the Sponsor or Designee Standard Operational Procedures (SOPs). Accurate and reliable data collection will be assured by verification and cross-check of the (e)CRFs against the Investigator's records by the study monitor (source document verification, SDV). The data collected will be entered into a study database electronically. A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the Investigator. Throughout the study the AIDA Work Package 3 Management Team will evaluate recruitment and the progress of the project.

9 Ethical Aspects

9.1 Local Regulations/Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformance with the principles of the 'Declaration of Helsinki' (Appendix 5) or with the laws and regulations of the country in which the research is conducted; whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline (January 1997) or with local law if it affords greater protection to the patient. For studies conducted in the EU/EEA countries, the Investigator will ensure compliance with the EU Clinical Trial Directive (2001/20/EC). In other countries where "Guideline for Good Clinical Practice" exists, Investigators will strictly ensure adherence to the stated provisions.

9.2 Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from each patient participating in this study, after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. After the patient and representative have orally consented to participation in the study, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The Investigator or Designee must also explain that the patients are free to refuse to enter the study or to withdraw from it at any time, for any reason, and this will not affect their normal care. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

9.3 Independent Ethics Committees/Institutional Review Board

This protocol and any accompanying material provided to the patient (such as patient information sheets or descriptions of the study used to obtain informed consent) as well as any advertising or compensation given to the patient, will be submitted by the Investigator to an Independent Ethics Committee. Written approval from the committee will be obtained before starting the study.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the Investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the Investigator is expected to submit the protocol to a regional committee. If no regional committee exists, the Sponsor or Designee will assist the Investigator in submitting the protocol to the European Ethics Review Committee.

10 Conditions for Modifying the Protocol

Protocol modifications to ongoing studies must be made only after consultation between the Sponsor or Designee and the Investigator (Investigator representative(s) in the case of a multicenter study). Protocol modifications must be prepared by a representative of Sponsor or Designee and initially reviewed by the AIDA Work Package 3 Management Team. All protocol modifications must be submitted to the appropriate Independent Ethics Committee for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to study patients, or when the change(s) involves only logistical or administrative aspects of the study (e.g. change in monitor(s), change of telephone number(s).

11 Conditions for Terminating the Study

If the Investigator becomes aware of conditions or events that suggest a possible hazard to patients if the study continues, they must notify Micron and the Co-ordinating Investigator, who will determine whether termination of the study is necessary. The study may also be terminated early at the Sponsor's discretion in the absence of such a finding. Conditions that warrant termination include, but are not limited to:

- discovery of an unexpected, significant or unacceptable risk to enrolled patients
- Failure of the Investigator to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements

12 Study Documentation, (e)CRF and Record Keeping

12.1 Investigator's Files/Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories (1) Investigator's Study File, and (2) patient clinical source documents.

12.2 Source Documents and Background Data

The Investigator shall supply the Sponsor or Designee on request with any required background data from the study documentation or clinic records. This is particularly important when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

12.3 Audits and Inspections

The Investigator should understand that source documents for this study should be made available to appropriately qualified personnel from health authority inspectors after appropriate notification. The verification of the (e)CRF data must be by direct inspection of source documents.

12.4 (Electronic) Case Report Forms (e)CRF

For each patient enrolled, a (e)CRF must be completed and signed by the Investigator or authorised delegate from the study staff. This also applies to records for those patients who fail to complete the study. If a patient withdraws from the study, the reason must be noted on the (e)CRF. The Investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor or Designee in the (e)CRFs and in all required reports.

13 Monitoring the Study

It is understood that the responsible Sponsor (or Designee) monitor will contact and visit the Investigator and will be allowed, on request, to inspect the various records of the study (eCRFs and other pertinent data) provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the (e)CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the (e)CRF. The Investigator (or his/her deputy) agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

14 Confidentiality of Study Documents and Patient Records

The Investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorised parties. On (e)CRFs or other documents submitted to the Sponsor or Designee, patients should not be identified by their names, but by an identification code. The Investigator should keep a patient enrolment log showing codes, names and addresses. The Investigator should maintain documents in strict confidence and not for submission to the Sponsor (e.g., patients' written consent forms).

15 Publication of Data

The AIDA study team recognises the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts and presentations based on the data from this trial will be described in the Clinical Study Agreement.

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Appendices

Appendix 1

PK/PD Sample handling, storage and shipping

Blood samples will be drawn from the patients into plain glass tubes and left to clot. After centrifugation, aliquots of the resultant serum will be placed into two plastic vials that have been labelled with the study identifier and these will be frozen in separate freezers at -20°C. The paired samples will be stored at the individual research centres and one of the paired samples will be sent to the central laboratory in the UK for analysis and the other stored at the research centre until the end of the trial.

Blood Sample Procedure

1. A blood draw of a minimum of 5 ml should be made at each pharmacokinetic time point into an appropriately labelled plain glass tube. If the samples are being taken through a line care must be taken to ensure that the contents of the line do not contaminate the blood draw.
2. The blood samples should be left at room temperature for 30 minutes, but not longer than 1 hour, for the clot to form
3. The sample should then be centrifuged at a minimum of 2000g for 5 min.
4. Following centrifugation, the serum obtained should be divided into two aliquots, in appropriately labelled tubes and both tubes then stored at a minimum of -20°C, in separate freezers, as soon after preparation as possible.
5. Serum samples should be retained in storage at -20°C until dispatched on dry ice to the central laboratory in the UK for analysis. One aliquot for each time point will be dispatched and one aliquot will be kept in storage, in case of sample spoilage or loss in transit.
6. The second set of samples will be dispatched to the central laboratory in the UK either on request or at the end of patient recruitment at the recruiting centre.

Sample documentation

The sample vials for storage purposes will be shipped to each of the recruiting centres prior to initiation of the study along with pre printed labels for the tubes. Each tube should be marked in marker pen with the patient identifier and time point, in case labels fall off during storage, and the corresponding label placed on the vial. The samples for individual patients should be placed in the supplied storage boxes.

Sample transportation

Detailed instructions for transfer of samples to the central laboratory will be provided in a separate document.

Appendix 2

Clinical Evaluations – Baseline/Day 1, Day 5 (+/- 1), Day 14 (+/-2)/Test of Cure (TOC)

Clinical Signs and Symptoms
Details of patients' clinical signs and symptoms will be recorded in the (e)CRF.

Width	mm
Length	mm

Main Infection Type will be recorded. The categories include: ulcer, burns, major abscess, cellulitis and wounds.

At each evaluation, the following signs and symptoms will be assessed and graded on a scale of 0 to 3 (0 = absent; 1= mild, 2 = moderate; 3 = severe);

- tenderness to palpitation
- erythema
- oedema
- purulent drainage/discharge
- induration
- ulceration
- necrotic tissue
- localised pain
- chills

Photographic Images of Infection Site

A photographic image of the site of infection will be taken at Baseline/Day 1, Day 5 (+/-) and Day 14 (+/-) /Test of Cure (TOC) for each patient.

Appendix 3

Clinical Efficacy Assessment - Day 14 (+/-2)/Test of Cure (TOC)

Clinical response will be assessed by the Investigator using the following criteria:

Cure: All signs and symptoms of cSSSI present at baseline have resolved and the patient did not receive new systemic or topical antibacterial treatment up to and including the Test of Cure Day 14

Or

Clinically relevant improvement of the local and systemic signs and symptoms of cSSSI present at baseline such that the patient would not meet study entry criteria and the patient did not receive new systemic or topical antibacterial treatment up to and including Test of Cure Day 14

And

Received at least 4 days of treatment

Failure: After >2 days of treatment

At least 1 of the following definitions is correct,

- Persistence or progression of signs and symptoms relevant to pre-treatment infection site
- Development of new clinical signs and symptoms relevant to pre-treatment infection site
- Additional antibacterial therapy for MRSA required for the treatment of the pre-treatment infection site
- Surgical procedure required as adjunct or follow up therapy due to failure of the study drug

Indeterminate:

Patients who had a clinical cure at Test of Cure/Day 14, but <4 days of treatment at Test of Cure/Day 14

Missing There are no post baseline local or systemic signs and symptoms data available to make this assessment (e.g. lost to follow up) after <2 days of treatment and no Test of Cure evaluation.

Appendix 4

AEs Categories for Determining Relationship to Medicinal Product

PROBABLE (must have first three)
This category applies to those AEs which are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., 1) bone marrow depression, 2) tardive dyskinesias.
4. It follows a known pattern of response to the suspected drug.
5. It reappears upon rechallenge.

POSSIBLE (must have first two)

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:
2. It follows a reasonable temporal sequence from administration of the drug.
3. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It follows a known pattern of response to the suspected drug.

UNRELATED
This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.) and do not meet the criteria for drug relationship listed under remote, possible, or probable.

	Probable	Possible	Unrelated
Clearly due to extraneous causes	–	–	+
Reasonable temporal association with drug administration	+	+	–
May be produced by subject clinical state, etc.	–	+	+
Known response pattern to suspected drug	+	+	–
Disappears or decreases on cessation or reduction in dose	+	+	–
Reappears on rechallenge	+	–	–

Appendix 5

WORLD MEDICAL ASSOCIATION
DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or

community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of - interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be

performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

• The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or

• Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

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