CAMERA2

Statistical Analysis Plan

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A1. INTRODUCTION

This document details the presentation and analysis for the main paper(s) reporting results from the CAMERA2 trial. Main details of the study are provided in the protocol [1]. It is intended that the results reported in the main paper(s) arising from this study will not divert from the strategy set out here; subsequent papers of a more exploratory nature will not be bound by this strategy though they are expected to follow the broad principles laid down for the main paper(s).

A1.1. OVERVIEW DESIGN

The CAMERA2 trial is an open-label, parallel-group, randomised (1:1) controlled trial at 27 sites across Australia, New Zealand, Singapore, and Israel. Adults (>18 years) with Methicillin-resistant *Staphylococcus aureus* (MRSA) grown from at least one blood culture and able to be randomised within 72 hours of the index blood culture are eligible for inclusion. Participants are randomised to vancomycin or daptomycin (i.e., "standard therapy") given intravenously, or to standard therapy plus 7 days of an anti-staphylococcal β-lactam.

A1.2. OBJECTIVES:

The primary objective of the **C**ombination **A**ntibiotic therapy for **ME**thicillin-**R**esistant *Staphylococcus **a**ureus* infection (CAMERA2) trial is to estimate the effect of the combination standard antibiotic and β-lactam therapy on a composite "complication-free 90-day survival", compared to the standard antibiotic treatment. Secondary objectives are to evaluate the effect of the combination treatment on a number of indicators of treatment success, and direct healthcare costs. Further details of each outcome are provided in the protocol [1].

A2. STUDY OUTCOMES

A2.1. PRIMARY OUTCOME:

The primary outcome measure – Complication-free 90-day survival – is a composite of four criteria, assessed 90 days after randomisation:

1. All-cause mortality
2. Persistent bacteraemia at day 5 or beyond
3. Microbiological relapse – positive blood culture for MRSA at least 72 hours after a preceding negative culture
4. Microbiological treatment failure. Positive sterile site culture for MRSA at least 14 days after randomisation. This includes pus from deep tissue or organ abscesses, synovial fluid, blood, or other normally sterile sites. It does not include urine, sputum, or superficial swabs

A2.2. SECONDARY OUTCOMES:

Secondary outcome measures are the following, with reference to day of randomisation to day 90:

1. All-cause mortality at days 14, 42 and 90
2. Persistent bacteraemia at day 2
3. Persistent bacteraemia at day 5
4. Acute kidney injury (AKI) defined as at least stage 1 modified RIFLE criteria (1.5-fold increase in the serum creatinine, or glomerular filtration rate (GFR) decrease by 25%) at any time within the first 7 days, or, new need for renal replacement therapy at any time from days 1 to 90. This endpoint does not apply to participants who were already on haemodialysis at randomisation.
5. Microbiological relapse – positive blood culture for MRSA at least 72 hours after a preceding negative culture
6. Microbiological treatment failure – positive sterile site culture for MRSA at least 14 days after randomisation
7. Duration of intravenously administered antibiotic treatment

The final secondary outcome highlighted in the protocol, "Direct healthcare costs", will be subject to a future sub-study.

A3. ANALYTICAL APPROACH

A3.1. Primary analysis: modified intention-to-treat analysis

Primary analysis of the primary and secondary outcomes will be according to a modified intention-to-treat (ITT) principle. Specifically, the modified ITT population is defined as:

1. If in the composite treatment arm: all those who received at least one dose of study drug
2. If in the standard treatment arm: at least one day without β-lactams
3. Have available day-90 data (primary endpoint)
4. Meet all eligibility criteria (i.e., have not been excluded based on post-randomisation data)

A3.2. Secondary analysis: per-protocol analysis

In order to assess the efficacy of the combination therapy, a secondary, per-protocol analysis will be conducted. The per-protocol population is defined as:

1. For the combination group: received at least 75% of β-lactam doses,
2. For the standard treatment group: received at most one defined daily dose of β-lactam
3. Have available day-90 data (primary endpoint)

A3.3. Survival Analysis of all-cause mortality

As per the protocol, we will evaluate the hazard associated with the treatment group, according to all-cause mortality (and corresponding figures).

A3.4. Missing data

A3.4.1. Endpoints

Whilst high degrees of loss-to-follow-up can lead to biased estimates of the intervention effect (particularly when there is differential drop out between intervention arms, which is related to the intervention) we anticipate minimal loss-to-follow-up with respect to the primary outcome and for secondary outcomes. We will perform complete-cases analyses in each case.

A3.4.2. Covariates

With respect to the covariates, we anticipate a small (<2%) amount of missing data. As the anticipated amount of missing data is small, we will analyse the data using a complete-case analysis.

A3.5. Statistical models

Primary and secondary analyses will be undertaken using nonlinear regression models for binary outcomes, as appropriate. For the primary outcome, we will fit a model to assess the absolute difference in the failure rate according to the treatment allocation. The randomisation covariates ("location" and "receipt of haemodialysis"), will be included as covariates in this model to ensure that the randomisation was successful. Unadjusted and adjusted (for randomisation covariates) results will both be reported.

Cox proportional hazards model will be used to determine the hazard associated with all-cause mortality by treatment group.

A3.6. ADDITIONAL ANALYSES

A3.6.1. SENSITIVITY ANALYSES

i. We will conduct additional "post-hoc" analyses in which we will adjust for additional pre-specified potential confounders (Table A1). The pre-specified confounders will be included in the models even when no baseline imbalance exists. We have limited the inclusion of potential confounding variables to those that we surmise to be the most important based on the investigators' assessment of clinical plausibility (Table A1). This approach has been chosen since confounder selection strategies which are based on collected data, for example selecting confounders using preliminary statistical tests, result in models with poor statistical properties such as incorrect type I error rates [2-4]. Those confounders that are highlighted as having a significant impact in the subgroup analyses will also be included in these analyses to assess the impact on the treatment effect.

ii. As a sensitivity analysis for the per protocol definition, we will assess the impact of including individuals in the per protocol population who also received non-study β-lactam doses that compensate for the study β-lactam doses that were missed (i.e., the "75% of β-lactam doses", and "no more than 1 β-lactam dose", will account for both study, and non-study antibiotics that were administered).

A3.6.2. SUBGROUP ANALYSES

We will undertake subgroup analyses for the following variables (separately) by fitting an interaction term between the intervention and variable listed below. This will allow us to assess the extent to which the effect of the intervention on the primary outcome (i.e. complication-free 90-day survival), and secondary outcomes, is influenced by these variables. Note that this study was not designed to have sufficient power to test for interaction terms in these subgroup analyses; we will interpret the results with caution.

The following variables will be considered for subgroup analyses:

i. Standard treatment (daptomycin vs. vancomycin)
ii. Vancomycin MIC of primary isolate (≥1.5 μg/ml vs. <1.5μg/ml)
iii. Received intermittent chronic hemodialysis (yes vs. no)
iv. Received >24 hours of β-lactam antibiotics within 72 hours prior to randomisation (yes vs. no)
v. Complicated Staphylococcus Aureus Bacteremia (SAB) (yes vs. no)
vi. Location of participant recruitment (Australian/New Zealand vs. Israel vs. Singapore)
vii. Baseline immunosuppression (yes vs. no)
viii. Endocarditis affecting the left side of the heart (yes vs. no)
ix. MRSA-type (community-associated vs. healthcare-associated)

A3.7. PRESENTATION OF RESULTS – TABLES AND FIGURES

A3.7.1. FLOW CHART

The CONSORT diagram for this study will be presented as described in the protocol manuscript for the study [1]. In brief, we will present the following information:

- # of participants admitted with blood cultures positive for MRSA
- # of participants excluded (according to criteria in protocol)
- # of participants enrolled (according to criteria in protocol)
- # of participants withdrawn
- # of participants for which data is collected
- # of participants included in the mITT and per-protocol populations

A3.7.2. FIGURES OF RESULTS

Figures for measures will be generated at the data cleaning stage. Figures to be included in the report will be determined on an ad-hoc basis, subject to results of the primary, secondary, subgroup analyses, and analysis of secondary endpoints.

The DSMB recommended the trial be stopped early due to a safety signal with regards to an imbalance in incidence of AKI. Therefore, as part of the post-hoc analyses, we will generate figures of creatinine levels (baseline and over time) for individuals with and without AKI to investigate these differences (with either raw measurements, and fold-change for each individual relative to baseline).

A3.7.3. TABLES OF RESULTS

See examples tables 1-3 below. The results reported in table 3 will be the absolute difference in proportions between the standard treatment and combination therapy groups (with 95% confidence intervals). The unadjusted results will correspond to a naïve analysis of outcome by treatment group, while the adjusted analysis will account for the randomisation variables, haemodialysis, and site (as described above).

Table 1: Characteristics of participants at randomisation

	Standard Arm (n=)	Combination Arm (n=)
Country, No. (%)		
Australia/New Zealand		
Singapore		
Israel		
Age, mean (SD), years		
median (IQR), years		
Sex, No. (%)		
Female		
Male		
Nosocomial acquisition, No. (%)		
No		
Yes		
Health-care Associated Infection, No. (%)		
No		
Yes		
Charlson comorbidity index, median (IQR)		
Charlson comorbidity index ≥3, No. (%)		
Low		
High		
Indwelling vascular device, No. (%)		
No		
Yes		
Indwelling prosthetic valve or cardiac device, No. (%)		
No		
Yes		
Any antibiotic in preceding 72h, No. (%)		
No		
Yes		
Any B-lactam in preceding 72h, No. (%)		
No		
Yes		
Nephrotoxin in preceding 48h, No. (%)		
No		
Yes		
SOFA score, median (IQR)		
Baseline creatinine, mean (SD), umol/L		
Baseline creatinine, median (IQR), umol/L		
Primary Focus of Infection, No. (%)		
Infective endocarditis		
Native osteoarticular		
Pleuropulmonary infection		
Primary blood stream infection		
Skin and soft tissue infection		
Other		

Abbreviations: IQR – Inter-Quartile Range; SD – Standard Deviation.

Table 2: Antibiotic susceptibility and genotypic characteristics of bacterial strains

	Standard Arm (n=)	**Combination Arm (n=)**
Vancomycin MIC, median [IQR], μg/mL		
Vancomycin MIC ≥1.5 μg/mL, No. (%)		
Oxacillin MIC, median [IQR], μg/mL		
Multilocus sequence type, No. (%)		
STXX *(most prevalent)*		
STXX		
STXX		
STXX		
STXX *(5th most prevalent)*		
Other		

Note that the most prevalent sequences types will be reported, and the remainder will be aggregated into "Other".

Table 3: Primary and secondary outcome measures

	Standard (n=)	Combination (n=)	Estimate (95% CI) Unadjusted	Adjusted
Primary Outcome				
Complication-free 90-day survival				
Secondary Outcomes				
All-cause mortality				
day-14				
day-42				
day-90				
Persistent bacteraemia				
day 2				
day 5				
Microbiological relapse				
Microbiological treatment failure				
Acute Kidney Injury				
Duration of IV-administered antibiotic [mean (SD) /median (IQR)]				

Table A1: Potential confounders

Level	No.	Confounder
Patient	1	Age (years, continuous)
	2	Sex (Male/Female)
Site	3	Country (Australia, NZ, Singapore, Israel)*

* Note that Country is included in analyses anyway given randomisation was stratified accordingly

References

1. Tong S, Nelson J, Paterson D, Fowler V, Howden B, Cheng A, Chatfield M, Lipman J, Van Hal S, O'Sullivan M, Robinson K, Yahav D, Lye D, Davis J (2016) CAMERA2 -- combination antibiotic therapy for methicillin-resistant Staphylococcus aureus infection: study protocol for a randomised controlled trial. Trials 17:170. doi:10.1186/s13063-016-1295-3
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3. Pocock SJ, Assmann SE, Enos LE, Kasten LE (2002) Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. Stat Med 21 (19):2917-2930. doi:10.1002/sim.1296
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