Statistical Analysis Plan

CHIPS: Controlled Human Infection for Penicillin against Streptococcus pyogenes

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Version: 2.0

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Statistician — 9/Oct/2023 — Date

CPI — 09/Oct/2023 — Date

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Revision History

Revision	Date	Author(s)	Description
1	04/09/2022	JM	Version 1.0 created for protocol version 1.0 16/04/2021.
2	9/10/2023	JM	Version 2.0 updated for final analysis, which accounts for additional sensitivity analyses and post-interim clarification of the intended decision rules to make trial adaptations. It is acknowledged that these were confusing in the protocol and SAP version 1.0.

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1 Introduction

This Statistical Analysis Plan (SAP) describes the planned analyses, evaluation of decision criteria and reporting for the CHIPS trial. The SAP was written by an appropriately qualified statistician and version 1.0 was written without access to the trial database and was reviewed by clinical investigators who were blinded to individual treatment allocation and treatment-related study results from the interim analyses. The planned analyses identified in this SAP will be included in future manuscripts and trial report(s). Exploratory analyses not necessarily identified in this SAP may be performed to further address the objectives of the trial. Any post-hoc or unplanned analyses not specified in this SAP will be clearly identified as such in the final trial report and any resulting manuscripts for publication.

1.1 Background and Rationale

There is renewed focus on RHD control globally and nationally, highlighted by the unanimous adoption of the Global Resolution on Rheumatic Fever and RHD by the World Health Assembly in 2018 and recent agreement by Australian Health Ministers to progress a roadmap towards RHD elimination. The Global Resolution calls for new technological approaches to improving RHD control including the "development of a safe and effective Group A Streptococcal vaccine and development of a long-acting formulation of penicillin that might improve secondary prophylactic regimens". Benzathine benzyl-penicillin G (BPG) has been recommended as the first-line antibiotic for ARF secondary prophylaxis since the 1950s and has remained unchanged for over 60 years. The discordance between the efficacy of current long-acting penicillin formulations and the fact that most patients do not attain target exposure profiles to penicillin implies a key knowledge gap relating to the triangular relationship between the laboratory-derived MIC of Strep A, long term penicillin exposure and clinical infections. Identification of the target penicillin concentrations required to prevent Strep A infections will guide the Target Product Profile of a reformulated long-acting penicillin and allow for licensure based on pharmaco-equivalence with BPG trough levels. The intervention, benzyl-penicillin G, will hereafter be known as penicillin and the terms concentration and dose are used interchangeable.

1.2 Objectives & Estimands

1.2.1 Primary objective, endpoint, population summary and handling of intercurrent events

Estimand 1: ***The primary objective is to determine the minimum plasma penicillin concentration required to prevent experimental human Strep A pharyngitis.*** The primary endpoint is clinical pharyngitis between the oropharyngeal challenge and 5 days post challenge, represented by a binary variable, with 0 indicating pharyngitis and 1 indicating absence (i.e. no break through Strep A infection). Development of pharyngitis is according to pre-defined clinical prediction rules consisting of clinical and microbiological diagnostic criteria consisting of: (i) local signs and symptoms, (ii) measure of tonsil size; and (iii) microbiological confirmation in real-time from a throat swab using the ABBOTT ID Now Strep A 2 test. The primary population summary is the minimum plasma penicillin concentration required to prevent experimental human Strep A pharyngitis from the appropriate Bayesian Emax model. The proportion of participants without Strep A clinical pharyngitis will also be reported for each penicillin dose.

Rare but anticipated intercurrent events that may occur between oropharyngeal challenge and 5 days post challenge and either preclude or effect the assessment of pharyngitis are: (i) development of probable or proven invasive, or severe Streptococcal infection; (ii) serious adverse events; (iii) non-adherence to allocated intervention, including cessation due to an allergic reaction; and (iv) participant withdrawal unrelated to items (i)-(iii). In these rare cases, pharyngitis by the fifth study day after challenge will be assumed for participants who have intercurrent events outlined in (i), i.e. a compsite strategy, and participants who experience intercurrent events (ii)-(iv) will have missing data recorded (and be excluded from the analysis), unless there is prior evidence of Strep A pharyngitis after the challenge.

Sensitivity analyses: Will be performed using pre-specified Emax and logistic models over a range of priors and including dose as either allocated by randomisation or individual mean penicillin concentrations (at steady state) over the challenge period, obtained from laboratory assays of the 12-hourly blood samples for each participant.

1.2.2 Secondary objectives, endpoints, population summaries and handling of intercurrent events

Estimand 2: ***To identify the target plasma penicillin concentration required to prevent Strep A colonisation of the pharynx.*** The endpoint is sustained colonization, defined as Strep A isolated (profuse, heavy or medium) on 2 consecutive days between oropharyngeal challenge and 5 days post challenge, represented by a binary variable, with 0 indicating colonization and 1 indicating absence. The population sum-

mary is the minimum plasma penicillin concentration (dose) required to prevent Strep A colonisation of the pharynx from the appropriate Bayesian Emax model. The proportion of participants without sustained Strep A colonization will also be reported for each penicillin dose.

Rare but anticipated intercurrent events are similar to those outlined in Estimand 1. In these rare cases, colonisation by the fifth study day after challenge will be assumed for participants who have probable or proven invasive, or severe Streptococcal infection and participants who experience a SAE, non-adherence to allocated intervention or withdraw within 5 days of challenge will have missing data recorded (and be excluded from the analysis), unless there is prior evidence of Strep A colonization after the challenge.

Sensitivity analyses: As for estimand 1.

To characterise plasma humoral and cellular immunological profiles of immune response to experimental challenge with Strep A in healthy participants. No estimands are defined for this objective. The quantitative data will be summarised and reported by penicillin dose.

Estimand 3: ***To identify the target salivary penicillin concentration requiredto prevent Strep A pharyngitis or colonisation.*** Will not be analysed or reported.

Estimand 4: ***To characterise plasma inflammatory (CRP and procalcitonin) and metabolomic profiles of Strep A pharyngitis.*** The endpoint is a C-reactive protein (CRP) level >20 mg/L at any point between oropharyngeal challenge and 5 days post challenge, represented by a binary variable, with 0 indicating any levels >20 mg/L and 1 indicating any levels $\leq$ 20 mg/L. The population summary is the minimum plasma penicillin concentration (dose) required to prevent CRP levels >20 mg/L from the appropriate Bayesian Emax model. The proportion of participants that have a C-reactive protein $\leq$ 20 mg/L will also be reported for each penicillin dose.

Rare but anticipated intercurrent events are similar to those outlined in Estimand 1. In these rare cases, a CRP level >20 mg/L will be assumed for participants who have probable or proven invasive, or severe Streptococcal infection, and missing data outcomes will be recorded for participants that experience SAE, non-adherence to allocated intervention and withdrawal within 5 days of challenge, unless there is prior evidence of a CRP level >20 mg/L after the challenge.

Sensitivity analyses: As for estimand 1.

To characterise the 'local' immunological, inflammatory and phenomic profiles of Strep A pharyngitis. No estimands are defined for this objective. The quantitative data will be summarised and reported by penicillin dose.

The objective is to identify whether Cystatin C- based markers of renal function improve estimates of penicillin G renal clearance compared with creatinine-based measures. No estimands are defined for this objective. The quantitative data will be summarised and reported by penicillin dose.

Estimand 5: ***To explore microbiological and local factors associated with Strep A adhesion to tonsillar mucosa.*** The endpoint is any positive cultures from microbological testing of tonsillar mucosa between oropharyngeal challenge and 5 days post challenge, represented by a binary variable, with 0 indicating at least one positive culture and 1 indicating no positive cultures. The population summary is the minimum plasma penicillin concentration (dose) required to prevent positive cultures from microbiological testing of tonsillar mucosa, from the appropriate Bayesian Emax model. The proportion of participants that have no evidence of a positive culture will also be reported for each penicillin dose.

Rare but anticipated intercurrent events are similar to those outlined in Estimand 1. In these rare cases, a positive culture will be assumed for participants who have probable or proven invasive, or severe Streptococcal infection and missing data outcomes will be recorded for participants that experience SAE, non-adherence to allocated intervention and withdrawal within 5 days of challenge, unless there is prior evidence of a positive culture after the challenge.

No further estimands are defined for this objective. All quantitative data will be summarised and reported by penicillin dose.

To explore Strep A transcriptomic changes in response to penicillin exposure in Strep A pharyngitis. No estimands are defined for this objective. The quantitative data will be summarised and reported by penicillin dose.

To investigate potential environmental contamination of Strep A via large respiratory droplets, airborne small respiratory droplets, and surface contact. No estimands are defined for this objective. The quantitative data will be summarised and reported by penicillin dose.

To explore motivations, attitudes, and experiences of CHIPS study participants. No estimands are defined for this objective. The qualitative data regarding participants' motivations, engagement and experiences will be summarised and reported.

2 Study Design

2.1 Type

Double-blind, placebo-controlled, randomised, dose-ranging trial designed to determine the minimum effective steady-state plasma penicillin concentration required to prevent pharyngitis following direct oropharyngeal inoculation of Streptococcus pyogenes – Strain emm75 (Strep A; M75). Participants will be equally randomised (1:1:1:1:1) to each of five interventions, including placebo, in each cohort. Participants will be recruited in 4 consecutive cohorts, with 15 participants in each (a total of 60 particpants).

2.2 Interventions

Benzyl-penicillin G (abbreviated to penicillin) given as a bolus dose followed by continuous infusion at initial doses:

- 0ng/mL (placebo)
- 3ng/mL
- 5ng/mL
- 12ng/mL
- 20ng/mL

Intervention arms may be substituted, according to pre-specified trial adaptations, with doses up to 100ng/mL to increase the precision of the estimated minimum effective dose (MED) after completion of the second (interim 2, n=30) or third cohort (interim 3, n=45), and if pre-specified decision thresholds to stop recuitment have not been met.

Trial adaptation: After the third scheduled analysis, when 45 participants had been recruited and completed the study procedures, the allocation to the treatment arms was adapted to 6:0:0:9:0:0 (fourth cohort n=15) for penicillin doses 0:3:6:9:12:20. In addition, blood samples were obtained twice daily (steady state) during the study period to obtain up to a maximum of 10 concentrations of penicillin per participant. If a participant met the primary endpoint prior to Day 5 then dosing of penicillin was ceased and no further samples were obtained for pharmacokinetic outcomes.

2.3 Randomisation

The randomisation process is detailed in the protocol. Separate random sequences of intervention allocations for 5 doses of Benzyl-penicillin G (including zero for placebo) were generated for each cohort using random permuted blocks by the *rpbrPar* function, in the *randomizeR* package in *R version 3.6.3 (2020-02-29)*. Different random seeds were selected for each cohort and the block size was fixed as 5. The source program to generate the sequence of allocations for each cohort is stored electronically with password protection and only the statistician can access the file.

2.4 Sample size

A total of 60 participants will be recruited in four cohorts of 15 participants each. Based on simulations performed in FACTS version 6.2 (Fixed and Adaptive Clinical Trial Simulator, Berry Consultants), a maximum of 60 participants are required (recruited in 4 cohorts of equal size; starting with 5 treatment arms) to detect the minimum effective dose (MED) between 0-20 ng/mL to prevent Strep A pharyngitis, with a power of >80% and a Type 1 error of <5%. Trial simulations were based on a simplified model with: (i) an anticipated 25% of placebo participants without evidence of clinical pharyngitis (pharyngitis-free) 5 days post challenge; (ii) a monotonic normal dynamic linear model (NDLM) with weakly informative prior distributions; (iii) equal allocation to all treatment arms; (iv) interim analyses after each cohort has completed (i.e. every 15 participants); (v) a high target of 90% pharyngitis-free and a low target of 80% pharyngitis-free in determining the MED; (vi) stopping rules for success if the p(MED>low target) is greater than 80% and for futility if the p(MED>upper target) is less than 10%. Trial operating characteristics were calculated for 8 scenarios, ranging from null efficacy to MED detected at the highest dose level and used to determine the decision criteria for trial adaptions. Further gains in statistical power are anticipated for a Bayesian sigmoidal Emax model.

3 Trial Population and reporting

3.1 Eligibility criteria & recruitment

The trial inclusion and exclusion criteria are specified in the CHIPS protocol. Screening and eligibility data will be summarised and reported using a CONSORT flow diagram, including the number of participants who completed the trial, who withdrew, and who had missing data for the primary outcome. The CONSORT flow diagram will summarise the time period from screening until completion of the oropharyngeal challenge period (5 days after challenge) in each cohort.

3.2 Withdrawal

Participant withdrawals will be recorded on the CRF and in the trial database. A listing of participant withdrawals, with participant identifier, time of withdrawal relative to oropharyngeal challenge, intervention allocated, intervention received and reason for withdrawal will be produced for the final study report. The number of withdrawals will also be included in the CONSORT flow diagram.

3.3 Protocol deviations

All protocol deviations will be recorded on the CRF and in the trial database. A listing of protocol deviations, with participant identifier, free text description of the deviation, intervention allocated and intervention received will be produced for the final study report.

3.4 Analysis set

Participants who were randomised but subsequently did not receive an oropharyngeal challenge or non-adherence to allocated intervention will be excluded from the analysis set.

3.5 Demographics and baseline characteristics

Demographics and baseline characteristics will be summarised by intervention arm and reported overall and for each cohort separately. Variables with continuous distributions will be summarised as mean, standard deviation and median; these include age, height, weight, BMI, systolic and diastolic blood pressure, pulse rate, respiratory rate and oral temperature. Categorical variables will be summarised as frequency and percentage for each category level; these include sex, ethnicity and race.

3.6 Safety outcomes

Safety outcomes, as defined in the protocol, will be reported as an individual-event listing, including participant identifier, description of the event, onset time relative to oropharyngeal challenge, duration of event, cohort number and intervention arm.

4 Statistical Methods

4.1 Baseline data

No statistical modelling or hypothesis testing will be performed on the baseline data.

4.2 Analysis of primary estimand and sensitivity analyses

A Bayesian analyses will be performed on the accumulating data after each cohort completes 5 days post oropharyngeal challenge and each participant in the cohort has been assessed for the primary endpoint (free of clinical pharyngitis). It is anticipated that between 15-25% of participants in the placebo arm will remain pharyngitis-free at the end of the challenge period. We define a upper target of 90% pharyngitis-free and a lower target of 80% pharyngitis-free to evaluate the trial decision rules. The posterior predictive distribution of probability pharyngitis-free for each allocated dose of penicillin (π_d) will be calculated from the Bayesian models outlined below.

The relationship between pharyngitis-free (response R) and penicillin dose (D) will be modelled using Bayesian Emax models (sigmoidal 4-parameter and hyperbolic 3-parameter) by either the *stan_emax* function, in the *rstanemax* package, or a *STAN* implementation of the Emax model in *RSTudio* with *R version 3.6.3 (2020-02-29)*. A range of informative and weakly informative priors are specified for these models as part of the sensitivity testing. The relationship will also be modelled using logistic regression, which assumes that given a high enough penicillin dose that the event probability of 1.0 is plausible.

The sigmoidal (4-parameter) Emax model is defined as:

$$R \in \{0, 1\}$$

$$D \in \mathbb{R}+0$$

$$R_i = E_0 + \frac{E_{max} \times {D_i}^N}{{ED_{50}}^N + {D_i}^N}$$

where E_0 is the expected response when the exposure (dose) is zero, E_{max} is the maximum effect attributed to the exposure (dose), ED_{50} is the exposure (dose) that produces half of E_{max}, and N is the slope factor (Hills coefficient) that determines the steepness of the dose-response curve. The hyperbolic (3-parameter) Emax model is similar but has a fixed Hills coefficient (N) of one.

The logistic regression model is defined as:

$$R \in \{0, 1\}$$

$$R_i \sim Binomial(n, \pi)$$

$$logit(\tfrac{\pi_i}{1-\pi_i}) = \beta_0 + \beta_1 \times D_i$$

where π is the probability of the event (absence or no break through Strep A infection) conditional on dose. We interpret β_0 the log-odds of the event when the dose is zero (placebo), and β_1 as the log-odds ratio of the event for a unit increase in dose. A further sensitivity analysis will be performed including dose as individual mean penicillin concentrations (at steady state) over the challenge period, obtained from laboratory assays of the 12-hourly blood samples for each participant.

For all models, convergence (MCMC chains) will be assessed using trace plots, Gelman-Rubin statistics, and effective sample sizes. Model fit will be assessed using graphical methods; plotting observed vs. predicted values to visualize the model fit. For Emax models, the Bayes posterior predictive test for Emax will be used to assess monotone model fit. The appropriate dose-response model will be selected using the minimum Widely Applicable (or Watanabe-Akaike) Information Criterion (WAIC).

4.3 Analysis of secondary estimands and endpoints

Estimands 2, 4 and 5 will be analysed using similar Bayesian Emax and logistic models and priors specifications below.

All other endpoints will be summarised using appropriate statistics, including mean and standard deviation for continuous variables with symmetrical distributions, and median and interquartile range for asymmetric distributions. Categorical endpoints will be summarised by category level using frequencies and percentages.

4.4 Specification of the prior distributions

A range of Normal- and t-distributions priors are defined to determine the effect of the priors on the dose-reponse models. All prior distributions for E_0 and E_{max} are expressed on the logit scale. Initially, four sets of prior distributions will be investigated for the Emax models, where (i) represents the most informative prior for E_0, and (iv) represents the least informative prior for E_0, and (i)-(iii) incorporate the information from the previous Strep Group A challenge study, where 3 out of 20 participants (15%) remained free from clinical pharyngitis in the 5 days after the challenge. The *stan_emax* function specifies priors for the projected ED_{50} (denoted as P_{50}). An informative prior is incorporated for E_{max} based on the expected placebo rate from the previous challenge study and assuming that at the higher doses all participants will be free from clinical

pharyngitis after the challenge. Other priors may be explored in the final analysis but these will be identifed as post-hoc priors in the final report.

(i) $E_0 \sim t(logit(0.15), 0.5)$

(ii) $E_0 \sim t(logit(0.15), 1.0)$

(iii) $E_0 \sim t(logit(0.15), 1.5)$

(iv) $E_0 \sim t(logit(0.01), 1.5)$

$E_{max} \sim t(logit(0.99) - logit(0.15), 0.5)$

$P_{50} \sim t(3, 1)$

$N \sim N(1, 0.5)$

$\beta_0 \sim N(log(\frac{0.15}{1-0.15}, 2)$

$\beta_1 \sim N(0, 2)$

4.5 Decision quantities and criteria

These have been redefined to provide better clarity compared to those predefined in the proctol and SAP version 1.0. They more accurately reflect the intentions of the study investigators while the trial was progressing.

We define the *upper target* for the prevention of clinical pharyngitis as 90% and the *lower target* as 80%. For each interim only the primary estimand will be analysed, and the following decision quantities will be calculated, where π_d is the probability of the event (free of clinical pharyngitis) at dose d:

$$Pr(\pi_d > \textit{lower target}) \qquad\qquad Pr(\pi_d > \textit{upper target})$$

The decision quantities are posterior predictive probabilities and are compared against thresholds derived by trial simulation to maintain suitable trial operating characteristics. These pre-specified decision criteria will be used to determine trial adaptations.

A dose may be retained as *successful* if $Pr(\pi_d > \textit{lower target}) > 0.8$ A dose may be dropped as *futile* if $Pr(\pi_d > \textit{upper target}) < 0.1$

4.6 Interim analyses

Interim analyses will be performed after each cohort of 15 participants completes the fifth study day following the oropharyngeal challenge and has been assessed for clinical pharyngitis. There will be a total of 3 interim analyses when recruitment is at least 15, 30 and 45, respectively.

4.7 Missing data

No missing data is anticipated in this human challenge study, except for the rare intercurrent events defined for the primary estimand. Missing data will not be inputed.

4.8 Trial adaptations

No trial adaptations are permitted following the first interim report (i.e. when Cohort 1 has completed 5 days post oropharyngeal challenge, n=15). At the 2nd and 3rd interim analyses, trial dose *success* and *futility* will be assessed according to the pre-specified decision criteria.

Trial success: The trial may stop recruitment early if at the 2nd or 3rd interim analysis, the posterior predicted probability that the probability-pharyngitis-free for the minimum penicillin dose (3ng/mL) is greater than the lower target is greater than 0.8, i.e. $Pr(\pi_{dose=3} > lowertarget) > 0.8$.

Trial futility: The trial may stop recruitment early if at the 2nd or 3rd interim analysis, the posterior predicted probability that the probability-pharyngitis-free for the maximum penicillin dose (20ng/mL) is greater than the upper target is less than 0.1, i.e. $Pr(\pi_{dose=20} > highertarget) < 0.1$.

At the 2nd or 3rd interim analysis, if neither the *trial success* or *trial futility* criteria are met, then recruitment will continue to improved the dose-response model precision.

In addition, higher doses may be dropped if a lower dose meets the *successful* criteria and new penicillin doses may be added within the range 0-100ng/mL to improve the dose-response model fit.

4.9 Safety outcomes

No statistical modelling or hypothesis testing will be performed on the safety outcomes data.

5 Example tables, listings and figures

5.1 Recruitment

Table 5.1: Participant enrolment by cohort.

Site	Participation Start Date	Screened	Randomised	Completed Follow-up
Cohort 1	06-Sep-2022	00	00	00
Cohort 2	29-Nov-2022	00	00	00
Cohort 3	12-Apr-2023	00	00	00
Cohort 4	xx-xxx-2023	00	00	00

5.2 Data completeness and quality

Table 5.2: Summary of available data records.

Data form	Participants with an available record
Screened	0
Eligible	0
Completed challenge period	0
Discharged early	0
Withdrawn	0

Table 5.3: Selected demographics

Variable	0ng/mL N=10	3ng/mL N=10	6ng/mL N=10	9ng/mL N=10	12ng/mL N=10	20
Age (years)	24 (3)	24 (3)	24 (3)	24 (3)	24 (3)	
Sex (Female)	5 (50%)	5 (50%)	5 (50%)	5 (50%)	5 (50%)	
Height (cm)	173 (11)	173 (4)	175 (8)	173 (13)	170 (9)	
Weight (kg)	77 (14)	77 (14)	77 (7)	77 (6)	81 (9)	

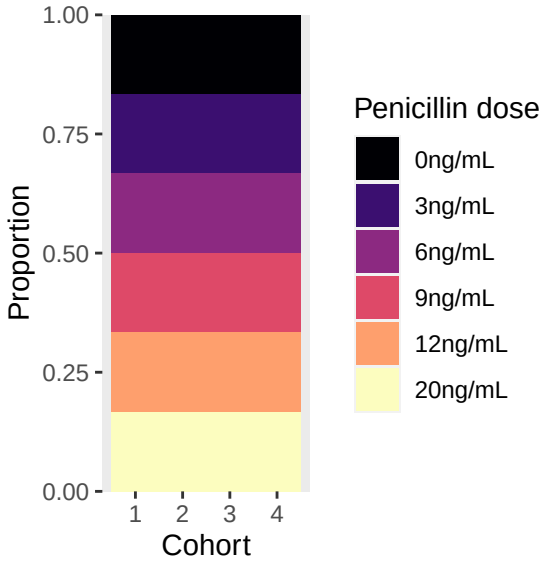

Figure 5.1: Proportion allocated to each intervention in each cohort.

5.3 Baseline characteristics

5.4 Treatment Allocations

Table 5.4: Primary and secondary estimands

Variable	0ng/mL N=10	3ng/mL N=10	6ng/mL N=10	9ng/mL N=10	12ng/mL N=10
Pharyngitis-free	1 (10%)	1 (10%)	4 (40%)	9 (90%)	8 (80%)
Colonisation-free	4 (40%)	6 (60%)	4 (40%)	3 (30%)	9 (90%)
CRP $<$ 20mg/L	7 (70%)	6 (60%)	6 (60%)	4 (40%)	5 (50%)
Positive culture	6 (60%)	7 (70%)	4 (40%)	5 (50%)	6 (60%)

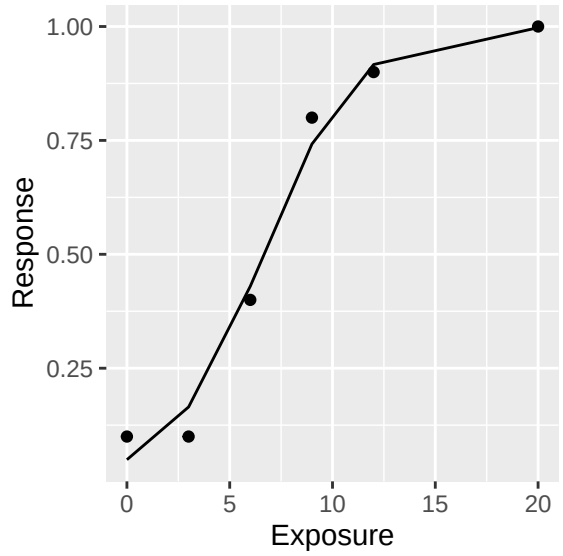

Figure 5.2: Logistic model for penicillin dose and Strep A infection-free outcome.

5.5 Efficacy: primary endpoint

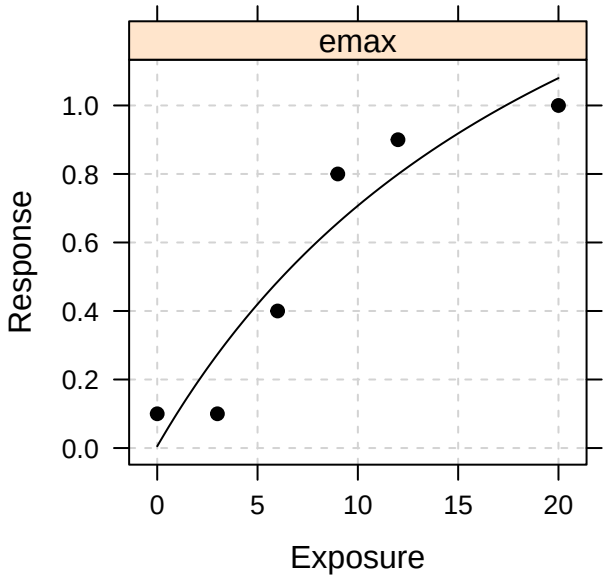

Figure 5.3: Hyperbolic model for penicillin dose and Strep A infection-free outcome.

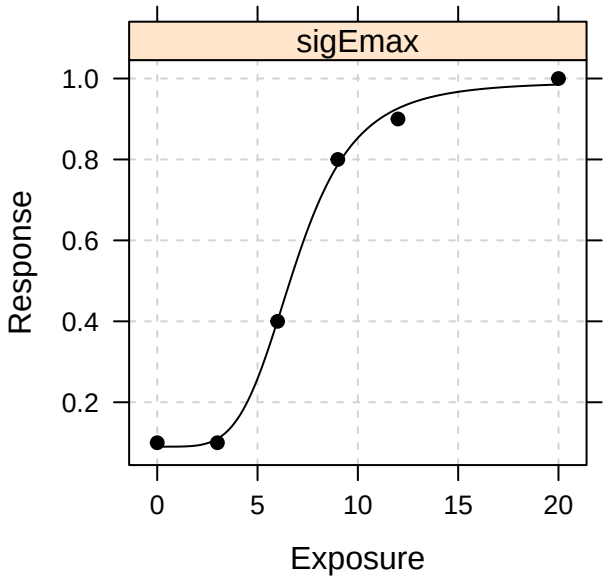

Figure 5.4: Sigmoidal model for penicillin dose and Strep A infection-free outcome.

Table 5.5: Summary of Emax model fit in STAN for pharyngitis-free endpoint (primary estimand).

```
## Inference for Stan model: mrmod.
## 4 chains, each with iter=4333; warmup=1000; thin=1;
## post-warmup draws per chain=3333, total post-warmup draws=13332.
##
##              mean se_mean        sd   2.5%    25%    50%    75%  97.5% n_eff
## led50        4.07    0.02      1.38   1.98   3.14   3.86   4.76   7.37  4744
## emax      2028.53 1394.66 116303.60   4.75   9.98  17.24  37.79 464.97  6954
## e0[1]       -1.96    0.01      0.40  -2.79  -2.23  -1.95  -1.68  -1.21  5420
## difTarget    3.50    0.01      0.67   2.26   3.05   3.47   3.93   4.89  5296
## lp__       -29.14    0.02      1.39 -32.77 -29.76 -28.78 -28.13 -27.58  4272
##           Rhat
## led50        1
## emax         1
## e0[1]        1
## difTarget    1
## lp__         1
##
## Samples were drawn using NUTS(diag_e) at Mon Oct  9 03:42:54 2023.
## For each parameter, n_eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor on split chains (at
## convergence, Rhat=1).
```

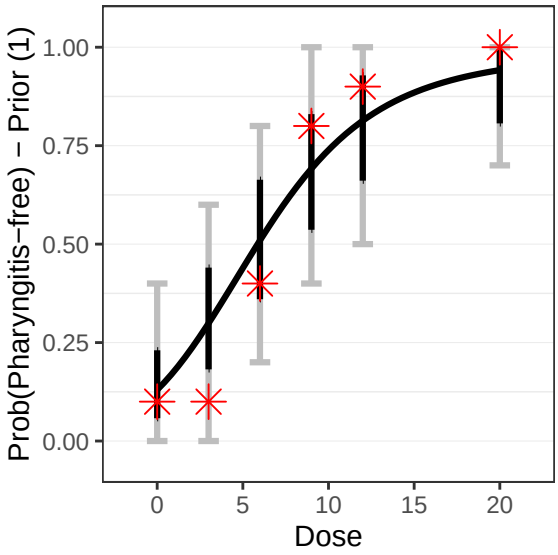

Figure 5.5: Posterior mean dose-response curve of probability pharyngitis-free with 95% CrI.

Table 5.6: Summary of Emax model fit in STAN for colonisation-free endpoint (Estimand 2).

5.6 Efficacy: secondary endpoints

Similar figures to those produced for the primary estimand will be provided for all sensitivity analyses and all estimands.

Table 5.7: Summary of Emax model fit in STAN for CRP <20mg/L endpoint (Estimand 4).

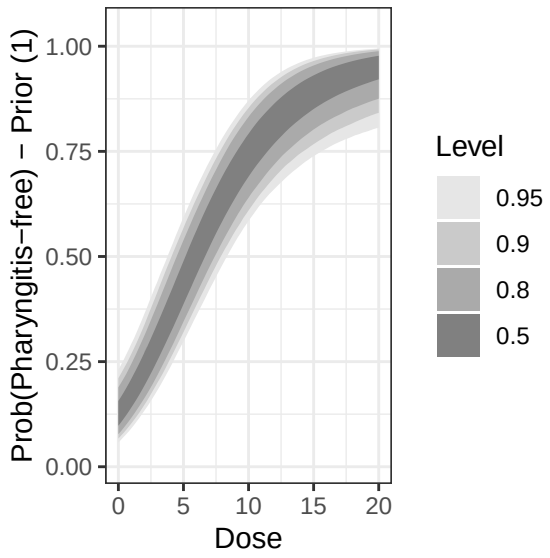

Figure 5.6: Posterior predictive distribution of probability pharyngitis-free conditional on pencillin dose with interval bounds.

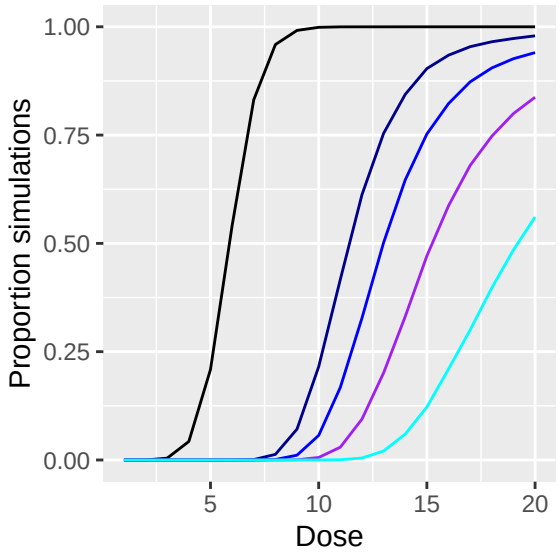

Figure 5.7: Proportion simulated trials where probability pharyngitis-free > clinical targets (black=50%, dark blue=80%, blue=85%, purple=90%, cyan=95%) by penicillin dose.

Table 5.8: Summary of Emax model fit in STAN for culture positive endpoint (Estimand 5).

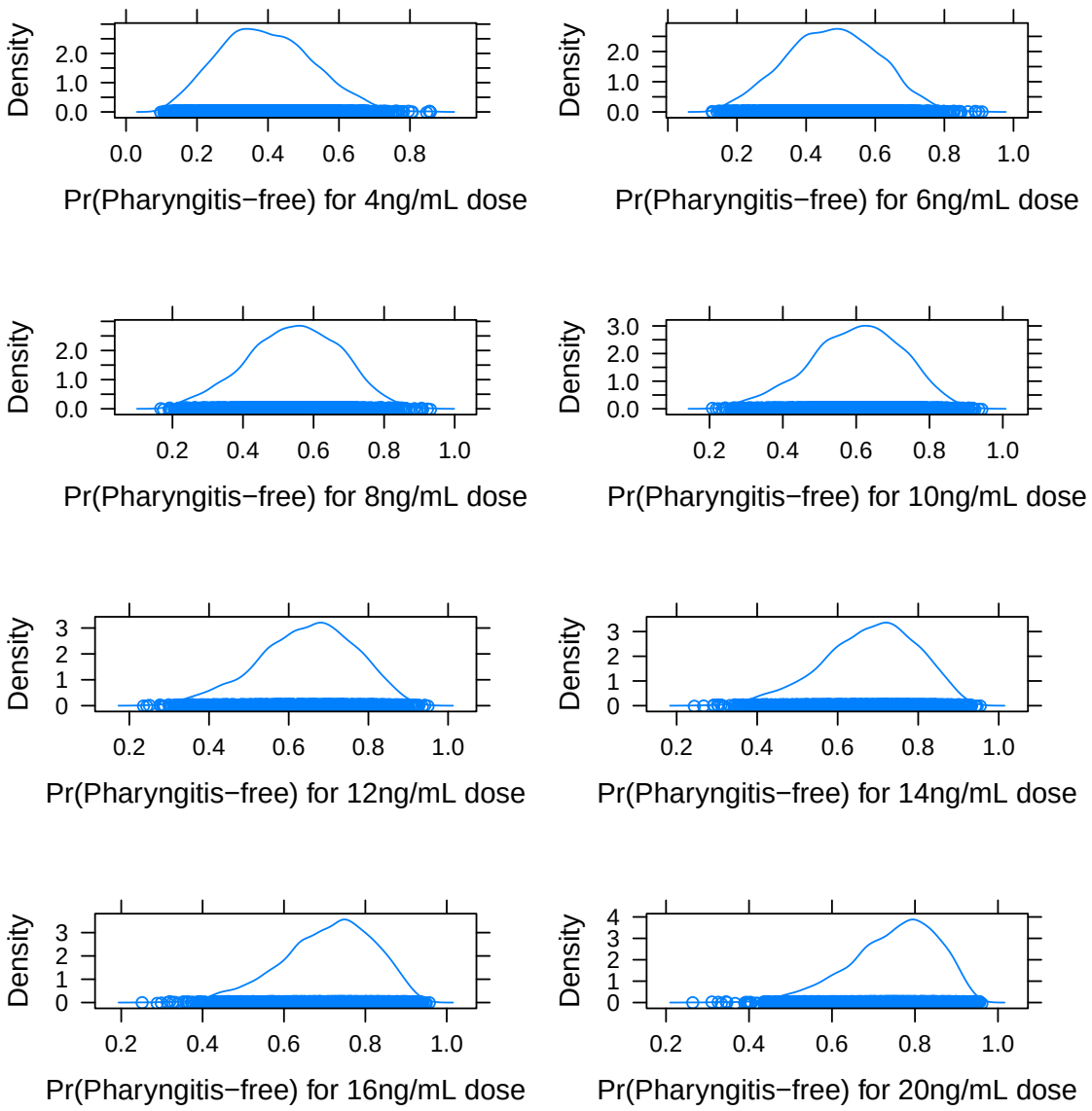

Figure 5.8: Posterior predictive distribution of probability-pharyngitis-free by penicillin dose.

Table 5.9: Summary of adverse events [A-E] reported between challenge and discharge by penicillin dose received.

AE Description	0ng/mL, N=10	3ng/mL, N=10	6ng/mL, N=10	6ng/mL, N=10	12ng/m
Abdominal cramps	0	0	1	0	
Abdominal pain	0	0	1	0	
Ache	0	0	1	0	
Aching in limb	0	0	1	0	
Back pain	1	0	0	1	
Backache	0	0	0	0	
Blister	0	0	0	0	
Bloating	0	0	0	0	
Bruising of arm	0	0	0	1	
Cannula site pain	0	2	5	3	
Catheter site hematoma	0	0	0	1	
Chills	2	0	0	0	
Cold sores	0	0	0	0	
Constipation	0	0	0	0	
Contact dermatitis	0	0	0	0	
Cough	1	0	0	0	
Coughing	0	0	1	0	
COVID-19	0	1	1	0	
Cramps	0	0	1	0	
Diarrhoea	0	0	0	0	
Dry cough	1	0	1	0	
Dry throat	1	0	1	0	
Ear ache	0	0	0	0	
Epigastric pain	0	1	0	0	
External ear pain	0	0	0	0	

5.7 Safety

Allocated pencillin dose has been randomly generated to provide the templates for the safety tables and listings given below.

Table 5.10: Adverse events listing. SAE's italicised.

Record ID	Treatment	Cohort	Event Description	Since Challenge	AE Onset Date	AE Stop Date	Serious AE	Severity	Outcome	Action Taken
1	Penicillin 6ng/mL	Cohort 1	Pharyngeal Exudate	2	8/09/22 8:42	12/09/22 0:00	No	Moderate	Resolved	None
1	Penicillin 6ng/mL	Cohort 1	Folliculitis	1	7/09/22 21:50	11/09/22 0:00	No	Mild	Resolved	None
2	Penicillin 20ng/mL	Cohort 1	Pharyngitis	1	7/09/22 18:05	11/09/22 0:00	No	Mild	Resolved	Concomitant Medication
2	Penicillin 20ng/mL	Cohort 1	Joint pain - bilateral knees	1	7/09/22 11:00	9/09/22 8:00	No	Mild	Resolved	None
2	Penicillin 20ng/mL	Cohort 1	Back ache	1	7/09/22 23:00	9/09/22 8:00	No	Mild	Resolved	None
2	Penicillin 20ng/mL	Cohort 1	Contact dermatitis - R anterior upper arm	2	8/09/22 14:45	12/09/22 8:00	No	Mild	Resolved	None
3	Penicillin 6ng/mL	Cohort 1	Arm discomfort	-1	12/09/22 18:08	17/09/22 11:30	No	Mild	Resolved	None
3	Penicillin 6ng/mL	Cohort 1	Sore throat	1	14/09/22 10:45	17/09/22 12:20	No	Mild	Resolved	None
3	Penicillin 6ng/mL	Cohort 1	Generalised muscle aches	1	14/09/22 15:20	15/09/22 14:00	No	Moderate	Resolved	Concomitant Medication
3	Penicillin 6ng/mL	Cohort 1	Headache	1	14/09/22 15:10	17/09/22 14:00	No	Moderate	Resolved	Concomitant Medication
3	Penicillin 6ng/mL	Cohort 1	Left IVC tenderness	1	14/09/22 21:04	30/09/22 10:00	No	Mild	Resolved	None
3	Penicillin 6ng/mL	Cohort 1	Abdominal cramps	4	17/09/22 10:00	22/09/22 7:00	No	Moderate	Resolved	Concomitant Medication
3	Penicillin 6ng/mL	Cohort 1	Loose stools	4	17/09/22 10:00	18/09/22 11:00	No	Mild	Resolved	None
4	Placebo	Cohort 1	Sore throat	1	14/09/22 9:15	18/09/22 9:00	No	Moderate	Resolved	None
4	Placebo	Cohort 1	Multiple joint aches (knees, fingers)	1	14/09/22 14:23	15/09/22 8:18	No	Mild	Resolved	None
4	Placebo	Cohort 1	Nausea	1	14/09/22 18:22	17/09/22 12:00	No	Moderate	Resolved	Concomitant Medication
4	Placebo	Cohort 1	Vomiting	1	14/09/22 20:15	14/09/22 21:00	No	Moderate	Resolved	Concomitant Medication
4	Placebo	Cohort 1	Chills	1	14/09/22 12:20	17/09/22 7:00	No	Moderate	Resolved	Concomitant Medication
5	Placebo	Cohort 1	Chills	0	20/09/22 9:30	20/09/22 9:33	No	Mild	Resolved	None