Statistical Analysis Plan (SAP)

Trial: Tranexamic acid to Reduce Infection after Gastrointestinal Surgery: the TRIGS Trial

Contents

Section 1. Administrative Information

1. Title

Tranexamic acid to Reduce Infection after Gastrointestinal Surgery: the TRIGS Trial

2. Trial registration

Prospectively registered (ClinicalTrials.gov identifier NCT04192435)

3. SAP version

Version: 1.0 Date: 07/11/2025

4. Protocol Version

This document has been written based on information contained in the TRIGS study protocol Version 1.4 dated 30 June 2022.

5. SAP Revisions

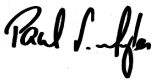
This SAP provides additional details and clarifies analysis approaches outlined in the TRIGS study protocol.

6. Names and affiliations

Document prepared by Professor Jessica Kasza, Monash University; and Professor Paul Myles, Alfred Hospital

Signatures:

Signature of senior statistician responsible (Prof Jessica Kasza):  *Date: 13/11/2025*

Signature of chief investigator (Prof Paul Myles):  *Date: 13/11/2025*

Section 2: Introduction

7. Brief background and rationale

Refer to the protocol for a detailed background to this trial, version 1.4, dated 30 June 2022.

8. Objectives

Research hypothesis:

We hypothesise that among adult patients schedule for elective or semi-elective open or lap-assisted gastrointestinal surgery (oesophageal, gastric, hepatobiliary, pancreatic, colorectal) with one or more risk factors for complications that a bolus of study drug (0.15 ml/kg TxA 15mg/kg before surgical incision, and then infusion at 0.05 ml/kg/h until end of surgery) will reduce the incidence of surgical site infection compared to matched placebo.

Study objective:
Primary objective: To determine whether TxA, compared to matched placebo, decreases the risk of surgical site infection.

Section 3: Trial Methods

9. Trial design

International, multicentre, pragmatic, double-blind, placebo-controlled randomised clinical trial.

10. Randomisation

Patients will be randomly assigned using permuted blocks of sizes 6 to 12 from a computer-generated list to either receive IV TxA or placebo on the day of surgery, but after confirmation of scheduling. Random assignment will be done using a computer-generated code, accessed via a web-based service. Group assignment will be stratified by site and diabetes status.

11. Sample size

In our recently completed RELIEF trial,[1] in which we enrolled near-identical patients to that proposed in this trial, the pooled incidence of SSI was 14% (consistent with global data[2]) and red cell transfusions were used in 12% of patients. An incidence of SSI of up to 25% has been reported in other at-risk major gastrointestinal surgical cohorts.43, 44 Assuming an incidence of 12%, and using a type I error of 0.05, we require 1,484 per group in order to have 90% power to detect a 30% reduction in SSI to 8.4% (i.e. relative risk 0.7). Were the control group incidence 14%, this sample size provides 94% power to detect a 30% reduction to 9.8%. We plan to enrol 3,300 patients. This will also provide ≥90% power to detect a 30% reduction in blood transfusions.

Assuming 30% of patients are diabetic and an incidence of SSI in non-diabetic patients in the placebo arm of 10%, this sample size provides ≈70% power to detect a difference in treatment relative risks of 0.55 in non-diabetic patients and 0.94 in diabetic patients on the multiplicative relative risks scale.45

12. Framework

This trial uses a superiority hypothesis testing framework between groups for the primary outcomes. Results will be reported in accordance with the CONSORT 2025 statement.

13. Statistical interim analyses and stopping guidance

Interim analyses will consider the defined study and safety endpoints after enrolment of 50% and 75% of patients, adjusted according to the O'Brien and Fleming method. Results will be made available to the DSMC, consisting of experts in the fields of surgery, infectious diseases and anaesthesia, and an independent statistician (Chair: Prof Monty Mythen, UK).

Section 4: Statistical Principles

14. Level of statistical significance

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level.

15. Description of any planned adjustment for multiplicity, and if so, including how the type 1 error is to be controlled

No adjustment for multiplicity is planned.

16. Confidence intervals to be reported

All confidence intervals will be two-sided 95% confidence intervals.

17. Analysis Populations and Estimand

Except where stated otherwise, all analyses will be conducted under the principle of modified intention-to-treat, whereby all participants are included in their randomised groups, but only those participants who underwent a surgical incision will be included in the analysis.

The primary estimand of interest is described in the table below.

<table>
<tr><td colspan="2">Objective: To determine whether TxA, compared to matched placebo, decreases the risk of surgical site infection.</td></tr>
<tr><td colspan="2">Estimand: Relative risk of incidence of any surgical site infection (superficial, deep, or organ space) among adult patients undergoing elective or semi-elective open or lap-assisted gastrointestinal surgery (oesophageal, gastric, hepatobiliary, pancreatic, colorectal) with one or more risk factors for complications assigned to receive treatment, relative to assignment to receive a matched placebo.</td></tr>
<tr><td colspan="2">Treatment: 0.15 ml/kg TxA 15mg/kg before surgical incision, and then infusion at 0.05 ml/kg/h until end of surgery</td></tr>
<tr><td>ESTIMAND</td><td>ANALYSIS</td></tr>
<tr><td>Target population: adult patients undergoing elective or semi-elective open or lap-assisted gastrointestinal surgery (oesophageal, gastric, hepatobiliary, pancreatic, colorectal) with one or more risk factors</td><td>Analysis set: All randomized participants who undergo surgery. Patients assigned to receive TxA through randomization will be the treatment group, patients assigned to receive the placebo the comparator group.</td></tr>
<tr><td>Variable: SSI (superficial, deep, and organ space), as defined by the US Centers for Disease Control (CDC), 14 up to 30 days after surgery. This includes all five separate categories of SSI, (Superficial Incisional Primary, Superficial Incisional Secondary, Deep Incisional Primary, Deep Incisional Secondary and Organ Space infection).</td><td>Outcome measure: Surgical site infection. Trial patients will be seen and have their medical records (and any cultures done) reviewed on a daily basis whilst in hospital, and a formal wound inspection done on Day 3 and day of discharge, plus a 30-day medical record/pathology review done. Any late (>30 postoperative days) notifications of SSI up to 90 days after surgery will be included in the outcome.</td></tr>
<tr><td>Handling of intercurrent events:
- Treatment as randomized not received: patients included in their randomized groups (treatment policy strategy)
- Death prior to identification of SSI within the relevant window: if a patient dies prior to the identification of an SSI, their outcome will be “no SSI” (“while alive” strategy)</td><td>Handling of missing data: Multiple imputation will be applied if >5% of the participants in the analysis set have a missing primary outcome. Results of a complete case analysis will also be provided.
Supplementary analysis: The analysis of the primary outcome will be repeated in As-treated and Per Protocol analysis sets.</td></tr>
<tr><td>Population-level summary measure: Relative risk</td><td>Analysis approach: The relative risk will be</td></tr>
</table>

of SSI if treated with TxA compared with no TxA.	estimated using a log-binomial model adjusted for the stratification variable of diabetes, with random effects for the stratifying variable of site.

Section 5: Trial Population

18. Screening Data

Screening data will be collected and a CONSORT flow diagram will be used to summarise this data.

19. Eligibility

Trial inclusion criteria are described in the trial protocol. Reasons for exclusion will be summarised in the CONSORT flow diagram.

20. Recruitment

A CONSORT flow diagram will be used to describe the number of people consented to participate, randomised, allocated to each treatment group, those lost to follow up (including reasons) and analysed.

21. Withdrawal/follow-up

All participants who are enrolled and randomly assigned to a group, and undergo surgery, must be followed for the duration of the study (unless they withdraw consent) even if they are withdrawn from the active phase of the trial. Losses to follow-up (including reasons) will be summarised in the CONSORT flow diagram by treatment group.

22. Baseline descriptive measures

Baseline characteristics will be summarised as appropriate (means and standard deviations for continuous variables that appear to be distributed approximately symmetrically, medians and interquartile ranges for other continuous variables, counts and percentages for categorical variables). Tests of statistical significance will not be undertaken for comparing baseline characteristics of treatment groups; rather the clinical importance of any imbalance will be noted.

An appendix table will provide summaries of baseline characteristics and compare these characteristics between two groups: those patients for whom the primary outcome was provided, and those patients for whom the primary outcome is missing. T-tests will be used to compare continuous characteristics between these groups, and chi-squared tests will be used to compare categorical characteristics, and this will help to determine which variables should be included as auxiliary variables in models for the imputation of the outcome.

Section 6: Analysis

23. Outcome definitions

Name	Description	Scale	Outcome definition
Primary Outcome			
Surgical site infection	Binary outcome: any incidence of surgical site infection (superficial, deep, and organ space)	Binary variable: 0 = No surgical site infection 1= surgical site infection	SSI (superficial, deep, and organ space), as defined by the US Centers for Disease Control (CDC),[14] up to 30 days after surgery – see Table. This includes all five separate categories of SSI, (Superficial Incisional Primary, Superficial Incisional Secondary, Deep Incisional Primary, Deep Incisional Secondary and Organ Space infection). Trial patients will be seen and have their medical records (and any cultures done) reviewed on a daily basis whilst in hospital, and a formal wound inspection done on Day 3 and day of discharge, plus a 30-day medical record/pathology review done. Any late (>30 postoperative days) notifications of SSI up to 90 days after surgery will be included in our primary endpoint
Secondary Outcomes			
Any red cell transfusion	Binary outcome: any red cell transfusion	Binary variable: 0 = no red call transfusion; 1 = any red cell transfusion	Any red cell transfusion up to hospital discharge
Volume of red cell transfusion (units)	Continuous outcome: total no. of units transfused	Continuous variable with lower limit of 0	Total no. of red cell units transfused up to hospital discharge
Sepsis	Binary outcome: any incidence of sepsis	Binary variable 0 = no sepsis; 1 = sepsis	Definition of sepsis using CDC-guided definition occurring on or after day 3 but during initial hospitalisation
Pneumonia	Binary outcome: any incidence of pneumonia	Binary variable 0 = no pneumonia; 1 = pneumonia	Definition of pneumonia using CDC-guided definition occurring on or after day 3 but during initial hospitalisation
Other infection	Binary outcome: any incidence of urinary tract (UTI) or "other" infection	Binary variable 0 = no other infection; 1 = UTI or other infection	Any clinical documentation of UTI or other infection
Anastomotic leak	Binary outcome: any incidence of anastomotic leak of any grade	Binary variable 0 = no anastomotic leak; 1 = any anastomotic leak	A defect of the intestinal wall at the anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- an extra luminal compartments. 1. Grade A - anastomotic

			leakage results in no change in patients' management 2. Grade B - leakage requires active therapeutic intervention but is manageable without re-laparotomy 3. Grade C - anastomotic leakage requires re-laparotomy
Peak-C reactive protein (CRP)	Continuous outcome: concentration of CRP	Continuous outcome with lower limit of 0.	Concentration of CRP on postoperative day 3
Days alive and at home up to 30 days after surgery	Number of days at home up to 30 days after surgery	Continuous outcome with range of 0 to 30	Number of days that a patient is alive and at home up to 30 days after surgery.
Postoperative diagnosis of COVID-19	Binary outcome: any diagnosis of COVID-19 in the 30 days after surgery	Binary variable 0 = no diagnosis of COVID-19; 1 = any diagnosis of COVID-19	Any diagnosis of COVID-19 in the 30-day study period
Safety Outcomes			
Thromboembolism	Binary outcome: any incidence of DVT or PE	Binary variable 0 = no no thromboembolism; 1 = thromboembolism	A clinical diagnosis of DVT or PE up to 30 days after surgery
Seizures	Binary outcome: any incidence of seizure	Binary variable 0 = no seizure; 1 = seizure	A seizure up to 30 days after surgery
Cardiovascular events (MI, stroke)	Binary outcome: any incidence of clinical MI or stroke	Binary variable 0 = no cardiovascular event; 1 = cardiovascular event	A clinical diagnosis of myocardial infarction or stroke up to 30 days after surgery
Anaphylactoid reactions	Binary outcome: any incidence of anaphylactoid reaction post-IV study drug delivery	Binary variable 0 = no anaphylactoid reaction; 1 = anaphylactoid reaction	An anaphylactoid reaction following study drug administration
Blood transfusion reactions	Binary outcome: any incidence of transfusion reaction	Binary variable 0 = no transfusion reaction; 1 = transfusion reaction	Any blood transfusion reaction following any blood product transfusion

24. Analysis methods

Analysis of primary outcome, and binary secondary/safety outcomes:
For the primary analysis of binary outcomes, we will use log-binomial models adjusting for the stratification variable of diabetes, with random effects for the stratifying variable of site. If that model fails to converge, a log-binomial model with the same link function and adjusting for the stratification variable of diabetes but with cluster-robust standard errors instead of random effects for site. Results will be expressed as risk ratios with 95% confidence intervals.

For analyses based on multiply imputed datasets: if a model fails to converge for more than one quarter of imputed datasets, the model with cluster-robust standard errors will instead be fit.

Days at home up to 30 days after surgery
DAH30 will be compared between groups using median regression adjusting for the stratification variable of diabetes, with cluster robust standard errors for site. DAH-30 is calculated using mortality and hospitalisation data from the date of the index surgery (= Day 0). For example, if a patient died on day 2 after their surgery whilst still an inpatient, they would be assigned zero (0) days at home; if a patient was discharged from hospital on Day 6 after surgery but was subsequently re-admitted for 4 days before their second hospital discharge, then they would be assigned a DAH-30 value of 20. If a patient has complications and spends 16 days in hospital, and then is transferred to a nursing facility for rehabilitation, and spend 24 days there before finally being discharged to their own home, they would be assigned a DAH-30 value of zero (0), even though 30-16-

24 = -10 because the minimum value of DAH-30 should be zero. If a patient dies within 30 days of surgery, irrespective of whether they have spent some time at home, DAH-30 will be scored as zero (0).

In addition to presenting a difference in medians between groups with respect to DAH-30, a difference in lower quartiles (i.e. 25th percentiles) will be presented.

Peak C Reactive protein and volume of red blood cell transfusion
These outcomes will be compared between groups using mixed effects linear regression models adjusting for the stratification variable of diabetes, with random effects for the stratifying variable of site.

25. Statistical Methods – adjustment for covariates

Supplementary analyses for the primary outcome and the secondary outcome of any red cell transfusion will adjust for any prognostic variables imbalanced at baseline.

For the primary outcome, variables with apparent imbalance out of the following list will be adjusted for:

- Patient age
- ASA physical status
- Diabetes
- Current smoker
- Oral steroid
- Open surgery

For the outcome of any red cell transfusion, variables with apparent imbalance out of the following list will be adjusted for:

- Patient age
- ASA physical status
- Body mass index (weight/height^2)
- Chronic renal disease
- Aspirin
- Preoperative haemoglobin
- Open surgery

26. Statistical Methods – subgroup analyses

Subgroup analyses of the primary outcome and any red cell transfusion will be conducted for the following subgroups:

i) Age strata (≤60 yr; 61–70 yr;71–75 yr;>75 yr);
ii) Patient sex;
iii) BMI strata (≤18.5; >18.5–25.0; >25.0–30.0; >30.0–35.0; >35.0);
iv) ASA physical status
v) Diabetes status (i.e. the stratification variable);
vi) Country;
vii) Type of GI surgery;
viii) Duration of surgery (approximate quartiles)

Models for these analyses will be as described in Section 27 for the primary analysis, including terms for the subgroup indicator and interactions between the subgroup and randomised group. Treatment effects in each subgroup will be reported.

27. Missing data reporting and assumptions/statistical methods to handle missing data

Multiple imputation of outcomes will be undertaken if the proportion of missingness of the primary outcome

is >5%.

Missing baseline characteristics will be imputed using single mean imputation. Baseline characteristics of participants who did and did not provide the primary outcome will be compared, and will be used to inform multiple imputation models. T-tests or Wilcoxon rank-sum tests will be used to compare continuous characteristics (depending on whether these had approximately symmetric distributions or not); chi-squared tests will compare categorical characteristics between participants with and without the primary outcome.

Missing outcome values will be imputed separately by treatment group. Imputation models will include treatment group, diabetes status, and baseline characteristics that appear to be different between participants who provided complete follow up data and participants who did not. Imputation models for continuous outcomes will also include terms for site. Imputation models for binary outcomes will not include terms for site due to the possibility of perfect prediction in small sites.

Initially, imputation via chained equations will be attempted for outcomes, with separate imputation for each outcome if this imputation fails. Binary outcomes will be imputed using logistic regression models, and continuous outcomes imputed via with predictive mean matching from the 5 nearest neighbours. Imputed datasets will be compared to complete data using density plots for continuous outcomes and plots of proportions for binary outcomes.

28. Additional Analyses

As-treated and per protocol analyses of the primary outcome and any red cell transfusion will also be conducted. As noted in the E9(R1) addendum, these additional analyses may be subject to severe bias and may not correspond to a relevant estimand.

Data collected prior to the trial re-start (the "contamination dataset") will also be considered. A table summarizing the baseline characteristics of patients by randomised treatment group will be presented. The primary outcome, red cell transfusion outcomes, and healthcare associated infections will be analysed as described above in this dataset.

29. Harms

Safety outcomes and adverse events will be reported by treatment group.

30. Statistical Software and code

Stata v19 or later will be used (StataCorp. Stata Statistical Software: Release 19. College Station, TX: StataCorp LLC); or R v 4.4.1 or later. Any user-written R packages used will be referenced.

The following code will be applied for the analysis of primary and secondary outcomes:

Primary outcome and binary secondary outcomes:

```
xi: gllamm outcome i.TreatmentGroup i.diabetes, i(site) fam(binomial)
link(log)
```

Primary outcome and binary secondary outcome backup code:

```
binreg outcome i.TreatmentGroup i.diabetes, rr cluster(site)
```

DAH30:

```
qreg2 outcome i.TreatmentGroup i.diabetes, cluster(site)
```

```
qreg2 outcome i.TreatmentGroup i.diabetes, cluster(site)
```

```
quantile(0.25)
```

Continuous outcomes:

```
mixed outcome i.TreatmentGroup i.diabetes || site: , reml
```

31. Modifications from the analysis outlined in the protocol

1. Estimand for the primary outcome has been defined.
2. That random effects for site will be included in regression models for the binary outcomes was clarified
3. The list of subgroups for subgroup analysis clarified as being those listed in Section 7.3 of the protocol
4. That the lower quartiles of DAH-30 will be compared has been stated.

32. References

1. Myles PS, Bellomo R, Corcoran T, et al. Restrictive versus liberal fluid therapy for major abdominal surgery. *N Engl J Med* 2018; **378**(24): 2263–74.
2. GlobalSurg C. Surgical site infection after gastrointestinal surgery in high-income, middle-income, and low-income countries: a prospective, international, multicentre cohort study. *Lancet Infect Dis* 2018.