HYPoxic Enhancement Before Major Surgery (HYPE)

A pilot randomised controlled trial of preoperative Roxadustat vs placebo in patients undergoing major surgery

Statistical Analysis Plan: Version 1.0

March 11th 2026

Posted online on March 13th 2026 (prior to locking the database)

Coordinating Chief Investigator

Associate Professor Andrew Toner

Investigators

Damien Foo (Biostatistician, Curtin University), Professor Tomas Corcoran (Royal Perth Hospital), Professor Cormac Taylor (University College Dublin, Ireland), Professor Simon Keely (University of Newcastle, NSW), RN Natalie Hird (Royal Perth Hospital).

Trial Sponsor

East Metropolitan Health Service trading as Royal Perth Hospital

ANZCTR Trial Registration Number

ACTRN12624000188538, date registered 27.02.2024.

Contents

1 Study Design and Research Questions

1.1 Summary of Design

This is a phase 2, single-centre, double-blind, placebo-controlled randomized trial to evaluate the safety and potential efficacy of preoperative roxadustat in patients undergoing elective major noncardiac surgery. Patients take three preoperative doses of roxadustat 100mg or placebo with dose 1 four days before surgery, dose 2 two days before surgery and dose 3 on the morning of surgery.

1.2 Randomisation and Blinding

The Clinical Trials Pharmacy at Royal Perth Hospital prepare randomization tables allocating participants in a 1:1 ratio to either the intervention (roxadustat) or the control (placebo) group. Randomisation tables use random permuted blocks of 2 and 4 and are stratified according to 4 surgery types: major joint replacement; open abdominal surgery; laparoscopic abdominal surgery; other major surgery. A clinical trial supply company prepare study drug using over encapsulation, leaving contents only identifiable by a kit code on the packaging. Study drug transferred directly to the Clinical Trials Pharmacy and dispensed appropriately on enrolment of participants. Study investigators, patients, data collectors and statisticians writing analysis code are fully blinded to the treatment allocation. After the last enrolment, data is cleaned and statistical code is finalized. Treatment allocation is then obtained from the Clinical Trials Pharmacy and the final statistical code is executed.

1.3 Research Questions

1. Is a short course of preoperative roxadustat well tolerated before major non-cardiac surgery and are there any other safety concerns that inform progression to higher phase trials?
2. Is a short course of preoperative roxadustat feasible?
3. Does preoperative roxadustat protect against perioperative systemic inflammation, organ injury or anaemia?
4. Does preoperative roxadustat alter the white blood cell and platelet composition of blood that could impact immunity and clotting respectively?
5. Does preoperative roxadustat have potential to improve surgical recovery within 30 days and beyond?

2 Outcomes

Outcomes are listed below, with the research question being addressed by each specific outcome noted in square brackets e.g. Research Question 1, [Q1].

2.1 Primary Outcomes

Preoperative Primary outcome: The occurrence of any patient-reported preoperative adverse event during the study drug treatment. [Q1]

Postoperative Primary outcome: The occurrence of the following postoperative complications meeting the standardized endpoints in perioperative medicine (StEP) initiative criteria within 30 days of surgery: atrial fibrillation; myocardial injury; myocardial infarction; non-fatal cardiac arrest; cardiac death; coronary revascularization; major adverse cardiac event; deep vein thrombosis; pulmonary embolus; pneumonia; respiratory failure; mechanical ventilation; acute kidney injury*; stroke; surgical site infection; urinary system infection; sepsis; septic shock. [Q1]

2.3 Secondary Outcomes

1 The occurrence of patient-reported preoperative adverse events during the study drug treatment, sub-classified according to blinded investigator assessment of causality, seriousness and expectedness [Q1]
2 The occurrence of specific patient-reported preoperative adverse events during the study drug treatment e.g. headache [Q1]
3 Day of surgery pH from venous blood prior to anaesthesia [Q1]
4 Day of surgery base excess from venous blood prior to anaesthesia [Q1]
5 Day of surgery lactate from venous blood prior to anaesthesia [Q1]
6 Day of surgery glucose from venous blood prior to anaesthesia [Q1]
7 Perioperative potassium values measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q1]
8 Perioperative bicarbonate values measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q1]
9 Number of prescribed drug doses successfully taken [Q2]
10 Overall drug compliance defined as follows: Full, all indicated doses taken on correct days; Partial, some indicated doses taken on correct days; None, no indicated doses taken on correct days [Q2]
11 Postoperative C Reactive Protein values and trajectories measured daily from postoperative day 1 to 3 [Q3]
12 Postoperative high sensitivity troponin I values and trajectories measured daily from postoperative day 1 to 3 [Q3]
13 Perioperative creatinine values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q3]

14 Perioperative haemoglobin values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q3]
15 Perioperative mean cell volume values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q3]
16 Perioperative red cell distribution width values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q3]
17 Perioperative total white cell count values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q4]
18 Perioperative differential white cell count values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q4]
19 Perioperative platelet count values and trajectories measured daily from day of surgery (prior to anaesthesia) to postoperative day 3 [Q4]
20 Hospital bed type required on postoperative days 1, 2 and 3, categorised as follows: Ward bed with standard monitoring; Ward bed with enhanced monitoring; critical care bed [Q5]
21 Full oral intake on postoperative days 1, 2 and 3 [Q5]
22 Independent mobilisation on postoperative days 1, 2 and 3 [Q5]
23 Surgical wound assessment on postoperative day 3 [Q5]
24 In-hospital antibiotics for suspected or actual infection [Q5]
25 In-hospital unplanned critical care admission [Q5]
26 In-hospital estimated blood loss (Day 0 to Day 7): Calculated applying the Gross' formula, which uses the maximum postoperative decrease in the level of haemoglobin adjusted for the estimated blood volume, which is in turn calculated applying the Nadler formula using sex, weight and height of the patient [Q5]
27 In-hospital red cell transfusion (yes/no) [Q5]
28 In-hospital postoperative complications meeting StEP criteria* [Q5]
29 In-hospital mortality [Q5]
30 Hospital length-of-stay: from the start (date, time) of surgery until hospital discharge (date, time) [Q5]
31 Discharge destination: Home/usual residence; Friends/relatives; Rehabilitation hospital; Another acute hospital; New residential/nursing care [Q5]
32 Postoperative day 30 location: Home/usual residence; Friends/relatives; Rehabilitation hospital; Another acute hospital; New residential/nursing care [Q5]
33 Postoperative day 30 surgical wound assessment [Q5]
34 Unplanned hospital readmission within 30 days of surgery [Q5]
35 Unplanned reoperation within 30 days of surgery [Q5]
36 Mortality within 30 days of surgery [Q5]
37 The occurrence of any postoperative complication meeting StEP criteria within 30 days of surgery* [Q5]
38 Days alive out of hospital within 30 days of surgery [Q5]
39 Days alive at home within 30 days of surgery [Q5]

40 Perioperative health related quality of life trajectory determined by administering the EQ-5D-5L Questionnaire at baseline and on postoperative day 30 including: Participant health rating from 0 (worst health imaginable) to 100 (best health imaginable); Australian index value quantitatively summarising EQ-5D-5L responses applying published sub-category weighting according to Australian society values [Q5]

41 Hierarchical composite endpoint (for win-ratio analysis) combining mortality, StEP defined complications ranked according to strength of association with mortality, and days alive out of hospital within 30 days of surgery [Q5]

42 Mortality within 3 years of surgery [Q5]

*The StEP criteria for acute kidney injury (AKI) include a sudden increase in serum creatinine. These criteria are commonly met after complete nephrectomy if the removed kidney is making a functional contribution preoperatively. Given that complete nephrectomy is an eligible operation in this study, sensitivity analyses will be performed excluding AKI after complete nephrectomy.

1 Analysis Sets

3.1 Intention-To-Treat Population

Have signed consent for the trial and have been randomized to placebo or roxadustat.

3.2 Preoperative Modified Intention-To-Treat Population

Enrolled patients who receive at least one dose of study drug. Drug tolerance and compliance outcomes will be reported in this population.

3.3 Postoperative Modified Intention-To-Treat Population

Enrolled patients who receive at least one dose of study drug and proceed to elective surgery as planned and on the date booked at the time of final drug dispensing i.e. patients who have their surgery cancelled, surgery downgraded to a minor procedure no longer meeting trial eligibility, surgery delayed without appropriate re-dispensing of study drug, or surgery brought forward as an emergency for reasons unrelated to study participation will not be included. This is the primary analysis set for all other outcomes.

3.4 Per-Protocol Population

Enrolled patients who are fully compliant with the study drug protocol and proceed to surgery as planned and on the date booked at the time of final drug dispensing. Analyses performed in the postoperative modified intention-to-treat population will be repeated in this population if the rate of protocol non-compliance is greater than 10%.

4 Statistical Methods

4.1 Sample Size

The trial sample size of 150 participants represents a convenience sample to provide single-centre phase 2A tolerance and safety data for preoperative roxadustat in patients having elective, major, non-cardiac surgery.

4.2 Handling of Incomplete Data

Statistical methods will generally be based on a complete case analysis, with acknowledgement of the missingness present for each outcome. The one exception will be the mixed-effects model analyses (Section 4.5), as these remain robust in the presence of missing data and significant missingness is expected due to early hospital discharge.

4.3 Primary Outcomes

Primary outcome differences in the placebo and roxadustat groups will be reported as an absolute difference (95% confidence interval) and/or a relative difference (95% confidence interval) where appropriate.

4.4 Secondary Outcomes

Secondary outcome differences in the placebo and roxadustat groups will be reported as an absolute difference (95% confidence interval) and/or a relative difference (95% confidence interval) where appropriate.

4.5 Mixed-effects Models

Mixed-effects models will be applied to serial blood test data across the first 3 postoperative days, to explore the effect of roxadustat on the perioperative trajectories of C Reactive Protein, high-sensitivity Troponin I, creatinine and haemoglobin.

All models will use the following fixed effects: Time from surgery start to blood sampling; Surgery Type received (not randomized to) including major joint replacement, open abdominal surgery, laparoscopic abdominal surgery, other major surgery; Treatment with placebo or roxadustat; Interaction between Surgery Type and Treatment.

All models will use the following random effects: Random intercept; Random slope (where possible).

Baseline measurements at time-zero: Creatinine and haemoglobin values measured on the day of surgery (before anaesthesia) will be taken as the values at time-zero for the respective models. In contrast, C Reactive Protein and Troponin are not routinely measured on the day of surgery unless a patient is acutely unwell; a situation that

should not apply to elective surgery proceeding as scheduled. Therefore day of surgery measurements will not be taken for these assays, rather they are assumed to be normal. In order to better assess changes over time from baseline, the mixed effects models for these assays will therefore assume a day of surgery value of 1 mg/L for C Reactive Protein and 1 ng/L for Troponin. Furthermore, the lower limit of detection for the high-sensitivity Troponin assay is 2 ng/L – any measurement below this will be reported by the lab as <2ng/L. Again, to better assess changes over time all such low readings will be assumed to be 1 ng/L in the mixed effect model.

Special considerations: The C Reactive Protein model is expected to be strongly influenced by the surgery undertaken. Whilst the randomization strata should provide reasonable balance of specific operations across treatment groups for major joint replacement, open abdominal surgery and laparoscopic abdominal surgery, substantial imbalance is expected in the "other major surgery" stratum. A sensitivity analysis will therefore be performed excluding other major surgery participants. Furthermore, if a day of surgery C Reactive Protein value is measured by attending teams and high (>10 mg/L), a sensitivity analysis will be performed excluding these patients.

4.6 Candidate primary endpoints for higher phase trials

Further frequentist and/or Bayesian exploratory analyses will be conducted for the endpoints considered to be candidates for the primary endpoint of higher phase trials. Such candidate endpoints include: The occurrence of *any* StEP defined complication; Days Alive Out of Hospital; Hierarchical composite endpoint (see Section 4.7). These analyses will be used to inform sample size calculations for higher phase trials and will be considered hypothesis generating only. Nevertheless, P-Values and probabilities will be cautiously reported for these exploratory frequentist and Bayesian analyses respectively.

4.7 Hierarchical composite endpoint (win ratio)

A hierarchical composite endpoint will be analyzed as a win ratio and will be tested in order of (1) mortality (yes/no), (2) StEP defined complications (yes/no), and (3) days alive out of hospital within 30 days of surgery (> 1 day).

For this endpoint, StEP defined complications are ranked from most severe to least severe, reflecting each complication's association with 30 day mortality in large surgical cohorts as follows: Non-fatal cardiac arrest[1]; Septic shock[1]; Mechanical ventilation[1]; Stroke[1]; Myocardial Infarction[1]; Pneumonia[1]; Pulmonary embolism[1]; Deep Vein Thrombosis[1]; Sepsis[1]; Organ Space Surgical Site Infection[1]; Myocardial Injury[2]; Acute Kidney Injury[3]; Atrial Fibrillation (new onset)[4]; Urinary System Infection[1]; Deep Incisional Surgical Site Infection[1]; Superficial Incisional Surgical Site Infection[1].

All complications are further graded for severity from grade 1 (least severe) to grade 4 (most severe) using the Clavien-Dindo Scale[5].

This endpoint will be analysed using an unmatched win ratio, with treatment as a fixed effect and stratified by surgery type received (major joint replacement, open abdominal surgery, laparoscopic abdominal surgery, other major surgery). With this approach, every participant in the roxadustat group will be compared with every participant in the placebo group in each stratum. All pairs are first compared for mortality within 30 days of surgery; participants surviving are declared winners over participants dying. If there is no winner, pairs are then compared for the occurrence of a StEP defined complication within 30 days of surgery; participants with no complication are considered winners over participants with a complication; participants with a lower ranked complication are considered winners over participants with a higher ranked complication; participants with a lower Clavien Dindo severity grade complication are considered winners over participants with the same complication but a higher Clavien Dindo severity grade; participants with the same complication and the same Clavien Dindo severity grade are declared ties. In the event of a tie for these first two tiers of the hierarchy, the pair is compared according to their Days Alive Out of Hospital within 30 days of surgery (DAOH30); a participant with a DAOH30 that is at least 1 day higher is considered a winner. Pairs with a DAOH30 difference less than 1 day, are considered a tie with no further comparisons.

The number of wins is divided by the number of losses for roxadustat compared with placebo to calculate the win ratio within each stratum. Stratum specific win ratios are then combined on the log scale weighted according to inverse-variance to give an overall win ratio; sensitivity analyses for the overall win ratio will include simple pooling of stratum-specific effects, weighting by the inverse of the stratum size and an unstratified analysis. The overall win ratio is tested in an exploratory fashion with a 2-sided alpha level of 0.05. Finally, to explore which component of the endpoint drives any overall effect, the win *difference* will be reported at each level of the hierarchy (total number of wins minus the total number of losses, divided by the total number of comparisons).

5 Publication Plan

The principal manuscript will analyse all endpoints apart from mortality within 3 years of surgery. This longer-term survival data will be analysed in a separate manuscript.

6 References

1 Madsen HJ, Henderson WG, Bronsert MR, et al. Associations Between Preoperative Risk, Postoperative Complications, and 30-Day Mortality. *World J Surg* 2022; **46**: 2365–76
2 Writing Committee for the VSI, Devereaux PJ, Biccard BM, et al. Association of Postoperative High-Sensitivity Troponin Levels With Myocardial Injury and 30-Day Mortality Among Patients Undergoing Noncardiac Surgery. *JAMA* 2017; **317**: 1642–51
3 French WB, Shah PR, Fatani YI, et al. Mortality and costs associated with acute kidney injury following major elective, non-cardiac surgery. *J Clin Anesth* 2022; **82**: 110933
4 AlTurki A, Marafi M, Proietti R, et al. Major Adverse Cardiovascular Events Associated With Postoperative Atrial Fibrillation After Noncardiac Surgery: A Systematic Review and Meta-Analysis. *Circ Arrhythm Electrophysiol* 2020; **13**: e007437
5 Clavien PA, Barkun J, de Oliveira ML, et al. The Clavien-Dindo classification of surgical complications: five-year experience. *Annals of surgery* 2009; **250**: 187–96