Myocardial Ischemia and Transfusion

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The Statistical Analysis Plan for the Myocardial Ischemia and Transfusion (MINT) Trial

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INTRODUCTION

This Statistical Analysis Plan for the Myocardial Ischemia and Transfusion (MINT) trial is consistent with and expands upon the statistical methods outlined in the trial protocol. This document includes 1) a brief summary of the MINT trial design, 2) the trial aims, 3) definitions of the specified MINT trial outcomes, 4) the original sample size and power calculations, 5) the interim monitoring plan, 6) analytic approach, and 7) proposed template tables and figures.

1. TRIAL DESIGN

Study Design

The MINT trial is a randomized, open-label, multicenter clinical trial designed to determine whether a liberal transfusion strategy with a threshold of 10 g/dL reduces the composite outcome of all-cause mortality or nonfatal myocardial reinfarction through 30 days, compared with a restrictive transfusion strategy with a threshold of 7 to 8 g/dL among patients with an acute myocardial infarction and a hemoglobin concentration less than 10 g/dL. The trial is conducted in approximately 144 clinical sites in the United States, Canada, France, Brazil, New Zealand, and Australia. The two transfusion strategies are allocated with a 1:1 ratio using a permuted block design, with random variable block sizes, stratified by clinical site.

Population

The eligible study population includes adult patients with ST-segment elevation myocardial infarction or non ST-segment elevation myocardial infarction consistent with the 3rd Universal Definition of Myocardial Infarction criteria[1] that occurs on admission or during the index hospitalization, and anemia defined as a hemoglobin concentration less than 10 g/dL at the time of randomization. Patients with Type 1, Type 2,

Type 4b, and Type 4c are eligible, and the occurrence of the index MI is determined by the enrolling site study team.

Trial Transfusion Strategies

The trial compares a restrictive and a liberal approach to transfusion therapy. The transfusion strategy is followed during the index hospitalization from the time of randomization up to 30-days post-randomization, hospital discharge, or death, whichever comes first.

Restrictive Transfusion Strategy: Patients randomized to the restrictive transfusion strategy may receive a transfusion if the hemoglobin concentration falls below 8 g/dL and are strongly encouraged to receive transfusion if the hemoglobin concentration is below 7 g/dL. Transfusion is also allowed when anginal symptoms are determined by the patient's treating physician to be related to anemia and are not controlled with anti-anginal medications. Enough blood is given to increase the hemoglobin concentration to above 7 to 8 g/dL or to relieve anginal symptoms. A post transfusion hemoglobin measurement is required.

Liberal Transfusion Strategy: Patients randomly allocated to the liberal transfusion strategy receive one unit of packed RBCs following randomization and will receive enough blood to raise the hemoglobin concentration to 10 g/dL or above any time during the index hospitalization that the hemoglobin concentration is detected to be below 10 g/dL. A post transfusion hemoglobin measurement showing a hemoglobin level of at least 10 g/dL must be obtained.

For both strategies, blood is administered one unit at a time followed by a hemoglobin measurement. A patient in either group may be transfused at any time without a hemoglobin level if the patient is actively bleeding (e.g., brisk gastrointestinal bleeding) and the treating physician believes an emergency transfusion is needed. A patient in either group with history of congestive heart failure or low ejection fraction may receive diuretics prior to or after transfusion and transfusion may be delayed until the patient can safely tolerate the additional volume. Patients with end stage renal disease may receive transfusion during dialysis if requested by treating physician. The transfusion protocol is suspended for 24 hours if the patient goes to surgery.

Assessments of laboratory markers

The trial collects hemoglobin and troponin levels and electrocardiogram (ECG) readings at specified time points while the patient is in the hospital. The required collection time points for hemoglobin levels and

ECGs are within 24 hours prior to randomization, and on days 1, 2 and 3 post-randomization. The required time points for the troponin levels are within 24 hours prior to randomization and post randomization, at 12 hours, and on days 1, 2 and 3. All hemoglobin and troponin levels performed for during the index hospitalization are collected by the trial.

Participant Follow-up

At hospital discharge, death or 30 days post randomization, whichever comes first, the study staff submit data related to the hospitalization including health status, laboratory results, blood transfusions, and post randomization clinical events. Study staff contact the patient at 30 days post-randomization to ascertain vital status, administer the quality-of-life questionnaire, and determine if there has been a subsequent hospital admission or emergency room visit. Study staff obtain and review the medical records for each readmission and record relevant data and study outcomes. If an acute coronary syndrome event is suspected from the time of randomization until 30-days post-randomization, the staff submits documentation for central review. Study staff contact participants at 6 months following randomization to ascertain the patient's vital status.

Recurrent myocardial infarction is diagnosed when a suspected myocardial ischemic event is reported by the investigators at the clinical sites and confirmed by the Clinical Event Committee. In addition to adjudication of site suspected events, the trial has a surveillance process in which all cardiac troponin values are reviewed by the Clinical Event Committee to detect abnormal biomarker patterns. When criteria are met, the Clinical Event Committee requests that the site submit medical records and ECGs that are temporally related to the abnormal values. The Clinical Events Committee adjudicates occurrences of myocardial infarction, masked to assigned transfusion strategy, from randomization through 30-days post-randomization. Myocardial infarction is classified according to MI type as specified in the 3rd Universal Definition of Myocardial Infarction[1] and whether MI type was ST segment elevation MI (STEMI) non-ST segment elevation MI (NSTEMI), or cannot be determined.

2. TRIAL AIMS

Primary Aim

The MINT primary aim is to determine whether a liberal transfusion strategy with a threshold of 10 g/dL reduces the composite outcome of all-cause mortality or nonfatal myocardial reinfarction through 30 days following randomization, compared to a restrictive transfusion strategy with a threshold of 7 to 8 g/dL among patients with an acute myocardial infarction and a hemoglobin concentration less than 10 g/dL.

Secondary Aims

1) To determine whether a liberal (10g/dL) transfusion strategy reduces all-cause mortality within 30 days, compared to a restrictive transfusion strategy.

2) To determine whether a liberal (10g/dL) transfusion strategy reduces myocardial reinfarction within 30 days, compared to a restrictive transfusion strategy.

3) To determine whether a liberal (10g/dL) transfusion strategy reduces the composite outcome of all-cause mortality, nonfatal myocardial reinfarction, ischemia driven unscheduled coronary revascularization (percutaneous coronary intervention or coronary artery bypass grafting), or readmission to the hospital for ischemic cardiac diagnosis within 30 days, compared to a restrictive transfusion strategy.

Tertiary Aims

1) To determine whether a liberal (10g/dL) transfusion strategy reduces all-cause mortality, nonfatal myocardial reinfarction, or unstable angina (i.e. acute coronary syndrome) within 30 days, compared to a restrictive transfusion strategy.

2) To determine whether a liberal (10g/dL) transfusion strategy reduces ischemia driven unscheduled coronary revascularization within 30-days compared to a restrictive strategy.

3) To determine whether a liberal (10g/dL) transfusion strategy reduces unscheduled readmission to hospital for ischemic cardiac diagnosis within 30 days, compared to a restrictive strategy.

4) To determine whether a liberal (10g/dL) transfusion strategy increases congestive heart failure within 30 days, compared to a restrictive transfusion strategy.

5) To determine whether a liberal (10g/dL) transfusion strategy reduces unscheduled readmission to hospital for any reason within 30 days, compared to a restrictive strategy.

6) To determine whether a liberal (10g/dL) transfusion strategy increases each of the individual thrombotic/hemorrhagic outcomes of stroke, pulmonary embolism or deep venous thrombosis, and bleeding within 30 days, compared to a restrictive strategy.

7) To determine whether a liberal (10g/dL) transfusion strategy increases each of the individual infectious outcomes of pneumonia, blood stream, and urinary tract within 30 days, compared to a restrictive strategy.

8) To determine whether a liberal (10g/dL) transfusion strategy reduces each of the individual in-hospital outcomes of length of hospital stay post randomization and number of days in intensive care unit, compared to a restrictive strategy.

9) To determine whether a liberal (10g/dL) transfusion strategy increases patient reported quality of life using the EuroQol questionnaire (EQ-5D) at 30 days compared to a restrictive strategy

10 To determine whether a liberal (10g/dL) transfusion strategy reduces all-cause mortality at 6-months following randomization, compared to a restrictive strategy.

3. DEFINITIONS OF OUTCOMES

Myocardial Reinfarction: Myocardial reinfarction will be classified by the Clinical Events Committee using 3rd Universal Definition of MI definition.[1] Patients with reinfarction will need to demonstrate a fall in the troponin value and then a subsequent rise of at least 20% with additional evidence (new ECG changes, imaging evidence, clinical history) as in the MI definition to diagnose a new event. Myocardial infarction is classified according to MI type[1] and whether MI type was STEMI, NSTEMI, or cannot be determined.

Death and Cause of Death: For each death, the cause will be determined by the site personnel into one of three categories: cardiac death (e.g., congestive heart failure, dysrhythmia), noncardiac death (e.g., infection, cancer), or undetermined cause of death. Information about the specific cause of death will also be collected.

Unscheduled Coronary Revascularization (unstaged): Ischemia driven, unscheduled coronary revascularization (coronary artery bypass surgery or PCI) within 30 days of randomization will be recorded by the sites. Prior to randomization, the site will record if a coronary revascularization is planned (staged). All coronary revascularization procedures will be recorded, but an elective planned staged procedure will not be included as an outcome. Information about the reason for the procedure will also be collected to ensure that the revascularization was done to treat ischemic heart disease.

Readmission to Hospital, Overall and for Primary Cardiac Diagnosis: All re-admissions to the hospital that had not been planned prior to randomization will be captured, and the primary diagnosis for each

hospitalization will be classified as: ischemic cardiac diagnosis (e.g., myocardial infarction, unstable angina), non-ischemic cardiac diagnosis (e.g. heart failure) or non-cardiac.

Unstable Angina: The MINT trial sites will use 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials[2] to define unstable angina. To diagnose unstable angina requires that four criteria be met: 1) worsening ischemic discomfort, 2) unscheduled hospitalization, 3) negative cardiac biomarker, 4) objective evidence of myocardial ischemia.

Congestive Heart Failure: Sites personnel will use 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials[2] to define congestive heart failure. New or worsening symptoms of congestive heart failure on presentation (increasing dyspnea, paroxysmal nocturnal dyspnea, orthopnea), has objective evidence of new or worsening heart failure, and receives initiation or intensification of treatment specifically for heart failure.

TIA or Stroke: The 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials[2] will be used to define stroke. A transient ischemic attack (TIA) is defined as "a transient episode of focal neurological dysfunction caused by brain, spinal cord, or retinal ischemia, without acute infarction." Stroke is defined on the basis of the presence of acute infarction as demonstrated by imaging or based on the persistence of symptoms more than 24 hours.

Deep Venous Thrombosis or Pulmonary Embolism: Deep vein thrombosis will be diagnosed if duplex ultrasound, magnetic resonance venogram (MRV), or venogram is definite or probable positive. Site investigators will record if location is proximal or distal. Pulmonary embolism will be diagnosed with a high probability ventilation perfusion lung scan, CT scan, or pulmonary angiogram.

Bleeding: Major bleeding will be defined as 1) fatal bleeding, and/or 2) symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra- articular or pericardial, or intramuscular with compartment syndrome, and/or 3) bleeding causing a drop in hemoglobin concentration of 2 g/dL or greater[3] from the last hemoglobin concentration prior to randomization to the nadir hemoglobin concentration during hospitalization or up to 30 days post randomization. The drop in

hemoglobin concentration will account for each unit of red blood cell transfusion transfused by subtracting 1 g/dL for each unit administered.

Infections recorded include pneumonia and blood stream infections.

Pneumonia: Pneumonia will be diagnosed using CDC criteria[4] which includes radiographic abnormalities and combination of symptoms (i.e., cough), signs (i.e., fever, tachypnea, or laboratory abnormalities (i.e., white blood cell count, hypoxemia).

Blood Stream Infection: Blood stream infection will be defined using CDC criteria[4] which includes a recognized pathogen cultured from 1 or more blood cultures and organism cultured from blood is not related to an infection at another site, at least 1 of the following signs or symptoms: fever, chills, or hypotension and signs and symptoms and positive laboratory results are not related to an infection at another site and common skin contaminant is cultured from 2 or more blood cultures drawn on separate occasions.

Length of Stay and Intensive Care Unit Days: The trial records the number of days post randomization that the patient is in the hospital and in intensive care unit.

Quality of Life: The EuroQol questionnaire (EQ-5D),[5] a standardized instrument that measures health related quality of life in 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression, is used as a measure of patient perceived global health status 30 days after randomization. The EQ-5D utility index value[6] and heath today[5] scores will be reported.

4. SAMPLE SIZE DETERMINATION AND POWER

In the MINT pilot trial, the composite 30-day rate of death and myocardial infarction was 16.4%. Using a two-sided inequality test and a simple chi-square statistic with alpha=0.05, we determined the samples sizes required to provide 80% and 90% power to detect varying relative reductions in the 30-day event rates for death and myocardial infarction between the two assigned treatment groups. Based on these estimates, we planned to enroll a sample of 3500 patients. If the rate of missing outcome data were ≤ 5%, the trial would have 3324 patients with analyzable outcome data at 30-days. Assuming an overall event rate of 16.4%, the trial would have 80% power to detect a 20% relative reduction (i.e. 18.2% vs. 14.6%) and >90% power to detect a 25% relative reduction.

5. INTERIM MONITORING PLAN

The DSMB reviews interim analyses of the outcomes by assigned treatment group on an annual basis (from July 2018 through July 2022). The interim monitoring is designed to test for evidence of beneficial effect with either treatment strategy while maintaining the overall type I error at the pre-specified level. Stopping rules are based on the information (i.e. number of primary outcome events that have accumulated) at each inspection time and the shape of the predetermined alpha-spending function. The Lan-DeMets approach was used to allocate the type 1 error (i.e. alpha-level) at each interim time point and use of O'Brien Fleming monitoring boundaries. The DSMB will use the monitoring information to determine its recommendation to NHLBI. The DSMB may advise early termination of the trial for safety reasons or make other recommendations regarding modifications to the protocol. The final decision to stop the trial rests with the NHLBI. If the recommendation is to stop the trial, the MINT trial principal investigators are to be consulted before a final decision is made.

In order to ensure that the MINT trial has adequate power, an interim analysis to assess sample size was planned when approximately half of the participants are projected to have completed the 30-day follow-up. Revised sample size estimates were to be calculated based on the original power and effect size estimates from the trial hypothesis (80% power to detect a RR=0.80) and on the observed overall event rate (i.e. the two transfusion groups combined). The DSMB was to evaluate whether the trial sample size needs to be increased in order to restore power to maintain the ability to detect a clinically meaningful effect size. The DSMB reviewed this analysis at the January 2020 meeting. At that point, 1078 participants had finished 30-days follow-up plus an additional 6 months to ensure that all adjudication materials for the primary outcome were completed. Based on the results of this analysis, the DSMB voted to continue the MINT trial without any modification to the planned sample size (N=3500).

The original Data Safety Monitoring Plan stated that no interim futility analyses would be conducted since the MINT trial compares two established transfusion strategies with different resource and cost implications, and hence, a null result from a well-powered trial would be important for establishing treatment guidelines and policy. The study team submitted an application for additional trial funding to the NHLBI; in response, the NHLBI requested a futility analysis in February 2022. The study team submitted a conditional power plan in April 2022, and this plan was approved by a blinded statistical team at NHLBI in May 2022. The plan and results were presented to the DSMB on July 22, 2022.

The MINT interim monitoring plan included a tiered approach for determining the continuation of the MINT trial. First, the efficacy monitoring that has occurred annually since the beginning of the trial will be

performed to test if one arm is statistically superior to the other arm. Next, if superiority is not detected, then a futility analysis will occur. To determine futility, the conditional power for superiority of either arm with respect to the primary outcome will be performed assuming that the future trend is either consistent with the current trend (i.e. the maximum likelihood estimate), with the null hypothesis, with the alternative hypothesis favoring the restrictive strategy, and with the alternative hypothesis favoring the liberal strategy. If the conditional power of ANY of these tests is > 20%, then we recommend the study continue. If the conditional power of ALL of the specified tests for superiority are ≤ 20%, then we recommend moving to the non-inferiority testing. The conditional power for non-inferiority of the restrictive arm (compared to the liberal arm) will be performed assuming a relative 15% margin of non-inferiority (i.e. risk ratio for Restrictive versus Liberal < 1.15) and that the future trend is consistent with either the current trend (i.e. the maximum likelihood estimate), with the null hypothesis, with the alternative hypothesis favoring the restrictive strategy, and with the alternative hypothesis favoring the liberal strategy. If the conditional power of ANY of the non-inferiority conditional power tests is > 20%, then we recommend the study continue. If the conditional power of ALL the specified tests for non-inferiority are ≤ 20%, then termination of trial enrollment would be considered. Based on the results of this analysis, the DSMB voted that the MINT trial continue to the planned sample size (N=3500).

6. ANALYTIC APPROACH

CONSORT Chart

A CONSORT chart will be created to describe the flow of all participants who were consented to participate in the MINT trial through 180-days of follow-up. See Figure 1 as an example in the Supplement to this document.

Baseline Characteristics of the Enrolled Patient Sample

The baseline characteristics and co-interventions of the patients randomized in the trial will be described using frequencies, proportions, means and standard deviations, or medians and first and third quartiles. Characteristics will be presented for the entire sample and stratified by treatment assignment. See Table 1 in the Supplement for an example.

Adherence to Assigned Intervention

The distribution of the number of red blood cell units transfused per participant will be described for each randomized treatment group (e.g. Figure 2a). The counts and mean number of units per participant as

well as the total number of transfused units will be described, and the mean number of transfused units will be compared between assigned transfusion strategy groups using a simple Poisson test.

The mean hemoglobin concentration at Baseline, 1, 2, and 3 days post-randomization will be compared graphically between the assigned treatment groups for all randomized patients (e.g. Figure 2b). Linear mixed effect models will be used to compared the daily mean hemoglobin concentrations on day 1, 2 and 3 post-randomization by assigned treatment group adjusting for the baseline hemoglobin value and accounting for the repeated measures per participant using a random intercept.

$$\text{Post-rand Hgb}_{ij} = \beta_0 + \beta_1 \text{ Restrictive}_j + \beta_2 \text{ Day}_{jj} + \beta_3 \text{ Restrictive}_j\text{*Day}_{ij} + \beta_4 \text{ Baseline Hgb}_i + \alpha_i + \varepsilon_{ij}$$

where i=participant, j= day 1, 2 or 3,
Restrictive = 1 for assigned to Restrictive and 0 for assigned to Liberal;
Day is categorical Day1, Day 2, Day 3;
α_i is a random intercept for participant i.

All patients with at least one non-missing in-hospital hemoglobin value on day 1, 2, or 3 will be included in the analysis. If the interaction is not statistically significant ($p>0.05$) it will be removed from the model. The estimated beta coefficients for Restrictive (versus Liberal) strategy and for the interaction between Restrictive strategy and Day will be presented along with their 95% confidence intervals and p-values. Contrasts will be created from this model to display the estimated difference in hemoglobin levels between the Restrictive and the Liberal groups on Day 1, Day 2 and Day 3.

We will also quantify adherence to the protocol by presenting the following among all randomized patients. For those assigned to Restrictive strategy, we will report the number (%) of randomized patients with at least one transfusion given when Hgb>8, and the number (%) of patients with at least one transfusion when no hemoglobin level was checked. Clinical reasons will be provided when possible. For those assigned to Liberal strategy, we will present the number (%) of randomized patients with no transfusion, and the number (%) of patients where the last hemoglobin value before hospital discharge is < 10 g/dL. Clinical reasons will be provided when possible. Among all randomized patients, we will present the number (%) of patients with one and with two or more missing required hemoglobin measures, and number (%) of patients with one and with two or more missing troponin measures.

Trial Outcome Analyses

The intention-to-treat principle will be used to test the primary, secondary and tertiary aims comparing study outcomes by transfusion strategy. Two-sided tests will be used with an alpha-level=0.05 for all

aims. No adjustment of the alpha level will be made for the secondary and tertiary analyses. Results will be interpreted based upon the observed findings, their designation as secondary or tertiary outcomes, and in comparison to the primary outcome result.

Primary Outcome Analysis

The primary endpoint (the composite of all-cause mortality and myocardial infarction (death/MI) by 30 days from randomization) will be compared by assigned transfusion strategy using an unadjusted log-binomial regression model with a fixed effect variable for assigned treatment strategy and a random effect for clinical site and accounting for missing data using multiple imputation to impute missing 30-day outcome data.

$$\text{Ln}\,(p_{ij}) = \beta_0 + \beta_1\,\text{Restrictive}_{ij} + \alpha_i + \varepsilon_{ij}$$

where p_{ij} = Prob(Primary Endpoint =1 | Participant Assigned Treatment and Site),
i=site, j= participant;
α_i is the random intercept for site i

We will present the estimated risk ratio, 95% CI and p-value for the Restrictive Strategy versus the Liberal Strategy based on this model. Markov Chain Monte Carlo (MCMC) multiple imputation methods will be used to impute the missing 30-day outcome values (yes/no) for death and MI based on all available observed data (baseline, in-hospital and 30-day variables). Ten imputed data sets will be created, a log-binomial model with random effects for site will be estimated for each imputed data set, and the results will be pooled to obtain a single estimate of treatment effect with an adjusted standard error. A log-binomial model with random effects for site will be created from patients with non-missing 30-day death/MI data as a sensitivity analysis. Also, if significant imbalances in baseline risk factors are detected between the two randomized treatment groups, a multivariable log-binomial regression model adjusting for the imbalanced factors will be created as a sensitivity analysis. If adherence to the protocol is a concern, a per protocol analysis, including only patients who undergo transfusion according to their assignment (and adjusting for baseline factors that are associated with adherence to the treatment protocol, will be conducted as a sensitivity analysis.

For each of these models, the primary hypothesis test will be a two-sided test with alpha=0.05:

H_0: RR= 1.00 versus H_A: RR ≠ 1.00 using a two-sided alpha-level=0.05

such that RR = e^{β} where β is the coefficient for the Restrictive assigned strategy (versus Liberal assigned strategy) from the log-binomial model for the primary endpoint. If superiority of one of the two transfusion

strategies is not demonstrated through the primary hypothesis test, a second hypothesis test of interest is the non-inferiority of the Restrictive Strategy compared to the Liberal strategy. Setting the non-inferior margin to a 15% relative increase, we will conduct the following one-sided non-inferiority hypothesis test with alpha=0.025:

H_0: RR ≥ 1.15 versus H_A: RR < 1.15 using a one-sided alpha-level=0.025.

With this test, rejecting the null hypothesis leads to the conclusion that the restrictive arm is non-inferior to the liberal arm. This is equivalent to the demonstrating that the entire 95% confidence interval for the estimated RR is < 1.15.

Among all patients with 30-day outcome data, the observed proportion of patients who experience the primary endpoint in each assigned group, the risk ratio (Restrictive versus Liberal) and 95% confidence interval, the risk difference (Restrictive – Liberal) and 95% confidence interval will be calculated and presented. See Table 2. We will determine whether this estimated 95% confidence interval for the RR includes 1.00 to assess superiority and whether it is <1.15 to assess non-inferiority of the Restrictive strategy. All available data from randomization through 30 days will be used to identify death and MI outcomes; for patients with incomplete follow-up, we assume that no event occurred after the time of last contact.

Kaplan-Meier methods will be used to display the cumulative risk of the primary endpoint over the 30-day post-randomization follow-up period for all randomized patients stratified by assigned treatment group (Figure 3a). For the Kaplan-Meier analyses, follow-up will be censored at the time of withdrawal, lost-to-follow-up, or 30 days, and a log-rank statistic will be used to compare the two curves. The estimated risks at 30-days and the risk difference and 95% confidence interval at 30-days will be presented.

Analysis of Secondary and Other Outcomes

All of the secondary outcomes and most of the tertiary outcomes are dichotomous endpoints (i.e. presence/absence of an event during the 30-day study period). The pre-defined secondary outcomes are: 1) all-cause mortality within 30 days; 2) myocardial reinfarction within 30 days; and 3) the composite outcome of all-cause mortality, nonfatal myocardial reinfarction, ischemia driven unscheduled coronary revascularization (percutaneous coronary intervention or coronary artery bypass grafting), or readmission to the hospital for ischemic cardiac diagnosis within 30 days. Other dichotomous outcomes include: 1) all-cause mortality, nonfatal myocardial reinfarction, and unstable angina (i.e. acute coronary syndrome) 2) ischemia driven unscheduled coronary revascularization within 30-days; 3) readmission to the hospital for ischemic cardiac diagnosis, 4) congestive heart failure within 30 days; 5) unscheduled readmission to

hospital for any reason within 30 days; 6) each of the individual cardiovascular outcomes of stroke, pulmonary embolism or deep venous thrombosis, bleeding, and cardiac death within 30 days; 7) each of the individual infectious outcomes of pneumonia and blood stream infections within 30 days.

For each of the dichotomous endpoints listed above, the observed proportion of patients who experience the endpoint in each assigned group, the risk ratio (Restrictive versus Liberal) and 95% confidence interval, the risk difference (Restrictive – Liberal) and 95% confidence intervals will be calculated and presented (Table 2). Superiority of either transfusion strategy for each endpoint will first be evaluated using a two-sided test with alpha=0.05. If superiority is not detected, the non-inferiority of the Restrictive strategy will be assessed using a 15% relative non-inferiority margin; that is, the Restrictive strategy will be deemed non-inferior for a given dichotomous endpoint when the entire 95% confidence interval for the estimated RR is < 1.15. For the 30-day dichotomous outcomes, all available data from randomization through 30 days will be used to identify outcomes. Patients with incomplete follow-up will be assumed to have no event after the time of last contact for these analyses.

The length of hospital stay from randomization to discharge and the number of days in the ICU from randomization to discharge will be described as a mean (sd) or median (first and third quartile) in each assigned group and the difference will be analyzed using non-parametric Wilcoxon rank sum tests (see for example, Table 3). The mean (sd) or median (first and third quartile) will be presented for the EQ-5D utility index value and the EQ-5D Health Today score at 30 days post-randomization, and the difference will be tested based on t-tests or the Wilcoxon rank sum test depending on the distribution of the scores. The proportion of patients who report no problems with each of the 5 individual components of the EQ-5D (Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) will be presented stratified by assigned treatment group (Table 3).

The cumulative risk of all-cause mortality through 180-day post-randomization will be estimated using Kaplan-Meier methods for all randomized patients stratified by assigned treatment group (Figure 3b). For the time-to-event analyses, follow-up will be censored at the time of withdrawal, lost-to-follow-up, or 180 days. The estimated risk at 30 days and at 180 days for each treatment group will be presented, and a log-rank statistic will be used to test the hypothesis that the mortality risk over the 180-days is equivalent in the two assigned treatment groups. The estimated risk difference (Restrictive – Liberal) and 95% confidence interval will be presented for cumulative mortality risk at 30 days and at 180 days. If neither strategy is found to be superior based on the log rank test and the 95% confidence interval for the estimated risk difference (RD) at 180-days includes 1.0, the 95% confidence interval for the 180-day mortality risk difference will be assessed using a non-inferiority margin equal to an absolute difference of

3.0%. Thus, if the entire estimated 95% confidence for the 180-day mortality RD is <3.0%, the Restrictive strategy will be considered non-inferior to the Liberal Strategy.

Missing data will not be imputed for the analyses of the secondary and tertiary hypotheses unless critical issues are noted while investigating the missing 30-day primary outcome data.

Subgroup Analyses

Subgroup analyses will be performed based on the following baseline factors: STEMI and NSTEMI, MI types 1 and 2, baseline hemoglobin level (<8, 8-8.9, ≥9 g/dL), revascularization for treatment of index MI prior to randomization (yes, no), acute anemia (acute anemia, chronic anemia), sex (male, female), age (<60, 60-69, 70-79, ≥80 years), heart failure (defined as history of congestive heart failure, congestive heart failure during the index hospitalization prior to randomization and/or left ventricular ejection fraction < 45%: yes, no), renal function (renal dialysis during index hospitalization prior to randomization, no dialysis and eGFR <30, no dialysis and eGFR 30-59, no dialysis and eGFR ≥ 60 mL/mi/1.73m^2) based on the CKD-EPI formula without race, and diabetes mellitus (diabetes mellitus treated with medication: yes, no). For participants from sites in the United States, Canada, New Zealand and Australia, we will also consider Race (White, Black, Non-White and Non-Black Race) and Hispanic ethnicity (yes, no). Participants from the European Union (EU) and Brazil are excluded from these analyses because race and ethnicity were not collected in the EU sites, and these concepts are challenging to define in Brazil.

The proportion of patients with a primary endpoint within 30 days will be reported for each assigned treatment group within each pre-defined subgroup. If subgroup categories are too small for meaningful inference, we will collapse or eliminate categories. The risk ratio (Restrictive / Liberal), 95% confidence interval and significance-level will be reported with a forest plot (Figures 4a and 4b). For each subgroup variable, a log-binomial regression model including subgroup variable, treatment assignment and the interaction between the subgroup variable and treatment assignment will be created, and the significance of the interaction term will be used to test whether the treatment effect is significantly modified by the designated subgroup variable.

$$\text{Ln}(p_i) = \beta_0 + \beta_1 \text{Subgroup}_i + \beta_2 \text{Restrictive}_i + \beta_3 \text{Subgroup}_i * \text{Restrictive}_i + \varepsilon_i$$

where p_i = Prob(Primary Endpoint=1 | Participant Assigned Treatment and Subgroup),
i= participant;

The focus of the subgroup analyses in the MINT trial is for descriptive purposes. When applicable, continuous versions of the subgroup variable will be considered for the regression models, and we will

explore the existence of inflection points for treatment effectiveness. Subgroup analyses may be examined for secondary and other outcomes utilizing similar methods. For 6-month mortality, Cox proportional hazards regression models will be created with the same covariates to test whether the effect of the randomized transfusion strategy for 6-month mortality varies significantly according to the pre-specified subgroup variables.

Planned Secondary Analyses

A number of secondary manuscripts will be undertaken to evaluate the effect of specified subgroup variables on treatment effectiveness in greater depth. These analyses will consider specified trial endpoints and other clinical outcomes that are relevant to the research question. The analyses will also explore confounders and mediators that explain the relationships between the subgroup variable, transfusion treatment strategy, and outcomes.

Multivariable regression models (linear, logistic and Cox regression) will be used to elucidate the roles of demographic and clinical factors as predictors of defined clinical and patient reported outcomes in this patient population. Outcomes of interest include the trial primary endpoint, the three secondary endpoints, congestive heart failure, unstable angina or unscheduled coronary revascularization, infection, health related quality of life as measured by the EuroQol-5D at 30-days, and six-month mortality.

We will also take a personalized medicine approach to characterize patients who benefit from a liberal transfusion strategy and patients who do as well, or better, with a restrictive transfusion strategy, with respect to the primary outcome and a few key secondary endpoints. For a given endpoint, we will use machine learning methods to identify baseline characteristics that lead to better outcomes, and we will use generated effect modifier (GEM) models to create a function of the identified risk factors that interacts with assigned treatment. This function may allow us to predict the treatment strategy with which each patient would benefit based on their individual clinical and demographic profile.

We will employ causal inference principles to assess the impact of various hemoglobin thresholds for transfusion on the primary outcome and 30-day death in the MINT trial. Using the observed trial data, we will emulate a parallel target trial with several different hemoglobin transfusion thresholds (< 10 g/dL, < 9 g/dL, < 8 g/dL and < 7 g/dL). All patients will be followed under each of the four transfusion strategies until the time they no longer adhere to that strategy based on the measured hemoglobin levels and transfusions administered. We will apply contemporary causal inference data analytic approaches aimed at minimizing biases to analyze the effect of treatment received (rather than assigned treatment) to determine the best hemoglobin threshold for this patient population.

REFERENCES

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MINT STATISTICAL ANALYSIS PLAN SUPPLEMENT: Template Tables and Figures

Figure 1: CONSORT Chart

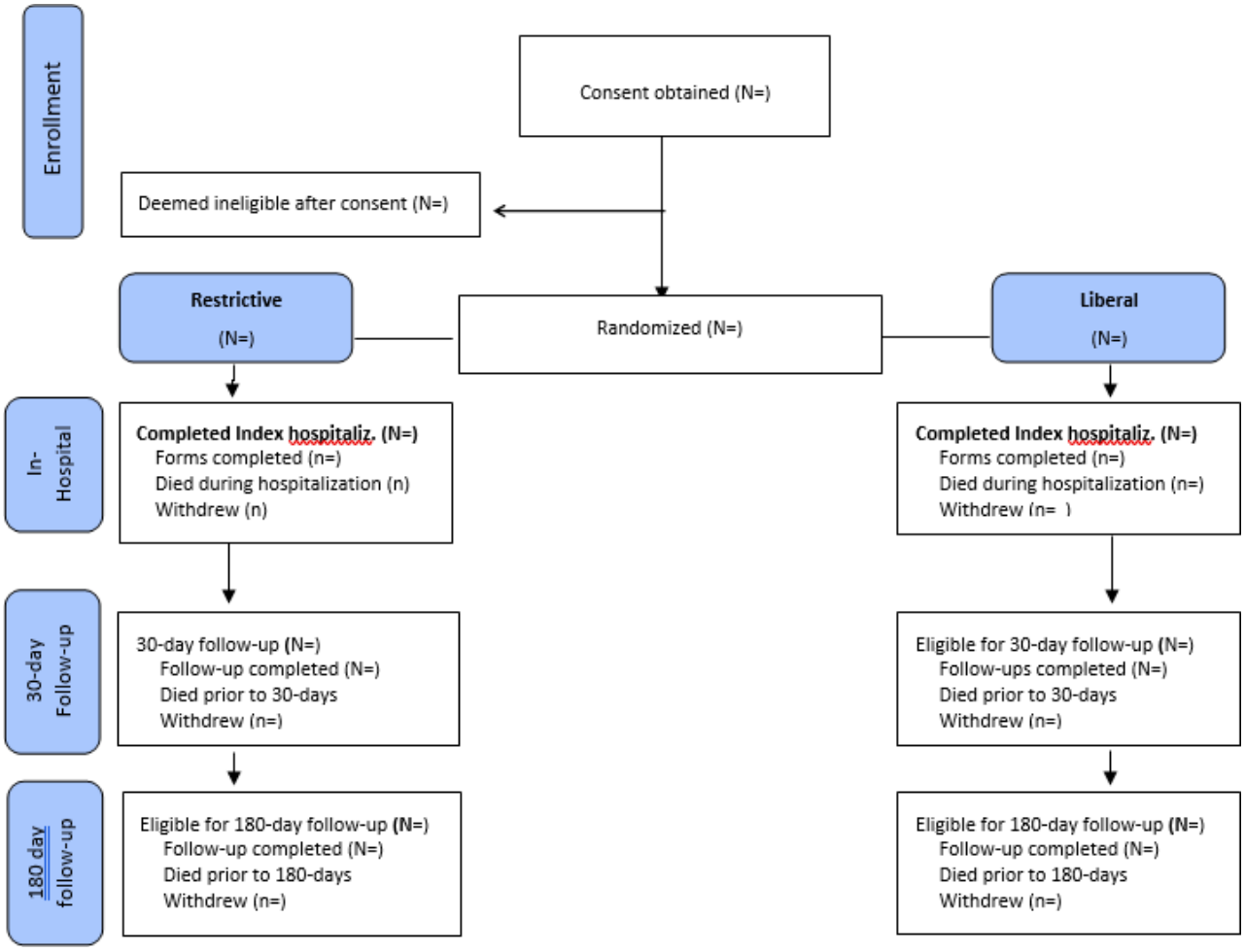

Table 1: Baseline Characteristics

Characteristic	All Participants (N=)	Restrictive (N=)	Liberal (N=)
Patient Characteristics			
Country, n (%)			
US			
Canada			
France			
Brazil			
New Zealand / Australia			
Age, years, mean (sd)			
Male, n (%)			
Hispanic Latino or Latina, n (%)			
Race			
White or Caucasian, n (%)			
Black or African-American, n (%)			
Asian, n (%)			
Other Race, n (%)			
Unknown at this time, n (%)			
BMI, kg/m^2, mean (sd)			
Tobacco smoker, n (%)			
Never			
Former			
Current			
Medical History			
MI, n (%)			
PCI, n (%)			
CABG, n (%)			
CHF, n (%)			
Stroke or TIA, n (%)			
Atrial fibrillation, n (%)			
PAD, n (%)			
Renal failure/insufficiency, n (%)			
Diabetes, n (%)			
Hypertension, n (%)			
Hypercholesterolemia/hyperlipidemia, n (%)			
COPD/asthma, n (%)			
Cancer, n (%)			
Anemia, n (%)			
Documented diagnosis of COVID-19, n (%)			
Angiogram results, n (%)			
Number of vessels with > 50% obstruction, n (%)			
0			
1			
2			
3			
Left main disease, n (%)			

Characteristic	All Participants (N=)	Restrictive (N=)	Liberal (N=)
LVEF results available, n (%)			
Most recent LV ejection fraction (%) within the past year, mean (sd)			
LV ejection fraction: Severity level, n (%)			
55% or greater (normal)			
45% to less than 55% (mild)			
30% to less than 45% (moderate)			
less than 30% (severe)			
Index Myocardial Infarction (MI)			
MI classification, n (%)			
Type 1			
Type 2			
Type 4b			
Type 4c			
Unknown			
Symptoms of ischemia, n (%)			
New ST-T changes or new left bundle branch block, n (%)			
Development of pathological Q waves, n (%)			
New loss of viable myocardium or regional wall motion abnormality, n (%)			
Identification of an intracoronary thrombus, n (%)			
Chest pain, n (%)			
Symptoms related to onset of index MI			
Jaw pain, n (%)			
Back pain, n (%)			
Shortness of breath, n (%)			
Epigastric pain, n (%)			
Palpitations, n (%)			
Left arm pain, n (%)			
Syncope, n (%)			
Other symptoms related to MI, n (%)			
Diagnosis of MI at time of randomization confirmed as MI at time of discharge, n (%)			
Events during index hospitalization prior to randomization			
PCI occurred prior to randomization, n (%)			
CABG occurred prior to randomization, n (%)			
Revascularization post-MI and prior to randomization, n (%)			
CHF prior to randomization, n (%)			
Renal dialysis prior to randomization, n (%)			
Intubated on ventilator prior to randomization, n (%)			
Active bleeding prior to randomization, n (%)			
Received RBC transfusion prior to randomization, n (%)			
Number of units, mean (sd)			
Laboratory Measures most recent prior to randomization			
Hemoglobin: Level (g/dL), mean (sd)			
Hemoglobin: Level (g/dL), n (%)			
<8.0			
8.0-8.9			
≥9.0			

Characteristic	All Participants (N=)	Restrictive (N=)	Liberal (N=)
Most recent creatinine value (mg/dL), mean (sd)			
eGFR (mL/mi/1.73m^2), mean (sd)			
Renal function, n (%)			
Renal dialysis during hospitalization pre-randomization			
<30 mL/mi/1.73m^2 and no dialysis			
30-59 mL/mi/1.73m^2 and no dialysis			
≥60 mL/mi/1.73m^2 and no dialysis			
Location at time of randomization			
In ICU/CCU at randomization, n (%)			

Figure 2a: Post-randomization Transfusions during Index Hospitalization

Histogram of number of units transfused as part of the protocol for all randomized patients stratified by assigned treatment.

Figure 2b: Post-randomization Hemoglobin by Assigned Strategy

Line plot of mean hemoglobin at Baseline, Day1, Day 2 and Day 3 for all randomized patients stratified by assigned treatment.

Table 2: MINT Trial Outcomes

	Cumulative Events at 30 days (Patient-Level)				
Characteristic	**Restrictive (N=)**	**Liberal (N=)**	**Risk Difference* (95% CI)**	**Risk Ratio* (95% CI)**	**p-value**
Primary Outcome (at 30 days)					
All-cause mortality/adjudicated myocardial infarction (MI), n (%)					
Secondary Outcome (at 30 days)					
All-cause mortality, n (%)					
Adjudicated myocardial infarction, n (%)					
All-cause mortality, adjudicated MI, ischemia driven unscheduled revascularization, unscheduled readmission to the hospital for ischemic cardiac diagnosis, n (%)					
Other Outcome (at 30 days)					
All-cause mortality, adjudicated non-fatal MI, unstable angina, n (%)					
Ischemia driven unscheduled revascularization, n (%)					
Unscheduled readmission to the hospital for ischemic cardiac diagnosis, n (%)					
Heart Failure, n (%)					
Unscheduled readmission to hospital for any reason, n (%)					
Stroke, n (%)					
Pulmonary embolism or deep vein thrombosis, n (%)					
Bleeding event, n (%)					
Cardiac Death, n (%)					
Pneumonia or bacteremia, n (%)					

** Risk Difference = Restrictive - Liberal for event outcomes*

Risk Ratio = Restrictive / Liberal for event outcomes

Restrictive is considered non-inferior to Liberal if the upper bound of the Risk Ratio 95% Confidence Interval is <1.15

Table 3: MINT Additional Outcomes

	Restrictive (N=)	Liberal (N=)	Mean Difference* (95% CI)	p-value		
Outcomes						
Length of hospital stay, number of days, mean (sd)						
Length of stay in the ICU, number of days, mean (sd)						
EuroQol 5D at 30-days						
EuroQol 5D results, n (%)						
EQ-5D Utility Index, mean (sd)						
EQ-5D Health Today, mean (sd)						
	Restrictive (N=)	**Liberal (N=)**	**Risk Difference* (95% CI)**	**Risk Ratio* (95% CI)**	**p-value**	
No problems identified with:						
EQ-5D Mobility, n (%)						
EQ-5D Self-Care, n (%)						
EQ-5D Usual Activities, n (%)						
EQ-5D Pain/Discomfort, n (%)						
EQ-5D Anxiety/Depression, n (%)	**Restrictive (N=)**	**Liberal (N=)**	**Restrictive K-M Estimate at 180 days**	**Liberal K-M Estimate at 180 days**	**Risk Difference** (95% CI)**	**p-value**
All-cause mortality at 180 days, n (%)						

** Mean difference = Restrictive - Liberal*

Risk Difference = Restrictive - Liberal for event outcomes

Risk Ratio = Restrictive / Liberal for event outcomes

*** For 180-day all-cause mortality Restrictive is considered non-inferior to Liberal if the upper bound of the Risk Difference 95% Confidence Interval is <3.0%*

Figure 3a: 30-day Cumulative Incidence Curves for All-Cause Death / Adjudicated MI
Cumulative risk using Kaplan Meier methods stratified by assigned treatment with estimates at 30-days presented.

Figure 3b: 180-day Cumulative Incidence Curves for All-Cause Death
Cumulative risk using Kaplan Meier methods stratified by assigned treatment with estimates at 30-days and at 180-days presented.

Figure 4a: 30-day Death/MI by Assigned Transfusion Strategy and Predefined Subgroups

All Randomized Participants: Subgroups	Subgroup Sample Size	Restrictive (N=)	Liberal (N=)	Risk Ratio	95% CI	FIGURE RR* (95% CI)	Interaction p-value
All Participants	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
MI Category							p-value
STEMI	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
non STEMI	N(%)	n (%)	n (%)	RR	LL - UL	---------X--------	
MI Type							p-value
Type 1 MI	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
Type 2 MI	N(%)	n (%)	n (%)	RR	LL - UL	---------X--------	
Baseline hemoglobin level							p-value
<8 g/dL	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
8-8.9 g/dL	N(%)	n (%)	n (%)	RR	LL – UL	---------X--------	
≥9.0 g/dL	N(%)	n (%)	n (%)	RR	LL – UL	------X-------	
Revascularization post-MI and pre-randomization*							p-value
Yes	N(%)	n (%)	n (%)	RR	LL – UL	---------X---------	
No	N(%)	n (%)	n (%)	RR	LL – UL	---------X--------	
Acute Anemia							p-value
Acute Anemia	N(%)	n (%)	n (%)	RR	LL – UL	------X-------	
Chronic Anemia	N(%)	n (%)	n (%)	RR	LL – UL	---------X---------	
Sex							
Male							
Female							
Age							
< 60 years							
60-69 years							
70-79 years							
≥ 80 years							
Heart Failure							
Yes							
No							
Renal Function							
Renal dialysis							
< 30 mL/mi/1,73m^2							
30-59 mL/mi/1,73m^2							
≥69 mL/mi/1,73m^2							
Diabetes Mellitus (treated)							
Yes							
No							

N (%) indicates the sample size of the subgroup and the proportion of the total sample in that subgroup.

n (%) indicates the number of outcome events in the specified treatment group and subgroup, and the proportion of people in the specified treatment group and subgroup who experienced the event.

RR: Rate Ratio = Restrictive / Liberal

* the No Revascularization group includes XX who received revascularization between randomization and hospital discharge YY of whom had a revascularization that was planned prior to randomization and was not based on new signs or symptoms consistent with ischemia that occurred post-randomization.

Figure 4b: 30-day Death/MI by Assigned Transfusion Strategy and Predefined Subgroups

U.S., Canada, New Zealand and Australia Participants: Subgroups	Subgroup Sample Size	Restrictive (N=)	Liberal (N=)	Risk Ratio	95% CI	FIGURE RR* (95% CI)	Interaction p-value
U.S., Canada, New Zealand and Australia Participants	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
Race							p-value
White	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
Black	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
non-White & non-Black	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
Hispanic							p-value
Yes	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	
No	N(%)	n (%)	n (%)	RR	LL - UL	---------X---------	

N (%) indicates the sample size of the subgroup and the proportion of the total sample in that subgroup.

n (%) indicates the number of outcome events in the specified treatment group and subgroup, and the proportion of people in the specified treatment group and subgroup who experienced the event.

RR: Rate Ratio = Restrictive / Liberal

Supplementary Information: Other 30-day Outcomes and Adverse Events

	Cumulative Rate of Event at 30 days (Patient-Level)	
Characteristic	**Restrictive (N=)**	**Liberal (N=)**
Outcome Events		
New suspected MI, n (%)		
Procedures: PCI, n (%)		
Ischemia driven unscheduled PCI, n (%)		
Procedures: CABG, n (%)		
Ischemia driven unscheduled CABG, n (%)		
New unstable angina, n (%)		
Transient Ischemic Attack (TIA), n (%)		
Any indication of Heart Failure, n (%)		
Any Indication of TACO, n (%)		
Strict Definition of TACO, n (%)		
Arrhythmias		
Atrial fibrillation/flutter, n (%)		
Supraventricular tachycardias other than atrial fibrillation, n (%)		
Paroxysmal supraventricular tachycardia, n (%)		
Ventricular tachycardia, n (%)		
Ventricular fibrillation, n (%)		
Cardiac arrest, n (%)		
Mobitz type 1 AV block, n (%)		
Mobitz type 2 AV block, n (%)		
Complete (3rd degree) AV block, n (%)		
Right bundle branch block, n (%)		
Left bundle branch block, n (%)		
Bifascicular block, n (%)		
Asystole, n (%)		
Multifocal atrial tachycardia, n (%)		
Nonparoxysmal junctional tachycardia, n (%)		
Thrombotic/Ischemic Events and Complications of MI		
Chest pain or stable angina, n (%)		
Mechanical complications of MI, n (%)		
Pericarditis, n (%)		
Pericardial effusion/tamponade, n (%)		
Syncope, n (%)		
Cardiomyopathy, n (%)		
Cardiogenic Shock, n (%)		
Other Adverse Events		
Acute respiratory failure, n (%)		
Acute renal failure, n (%)		
Transfusion Reactions		
Transfusion related acute lung injury, n (%)		
Acute hemolytic transfusion reaction, n (%)		
Transfusion associated sepsis, n (%)		
Anaphylactic transfusion reaction, n (%)		

Characteristic	Cumulative Rate of Event at 30 days (Patient-Level)	
	Restrictive (N=)	Liberal (N=)
Urticarial transfusion reaction, n (%)		
Febrile non-hemolytic reaction, n (%)		
Other Infections		
Urinary tract infection, n (%)		
Septic shock, n (%)		
Clostridium, n (%)		
Cellulitis, n (%)		
Endocarditis, n (%)		
Mediastinitis, n (%)		
Osteomyelitis, n (%)		
Sinusitis, n (%)		
Influenza, n (%) Gastroenteritis, n (%)		

Supplementary 30-day Information

Characteristic	Restrictive (N=)	Liberal (N=)
Number of ER visits, n (%)		
0		
1		
2		
3+		
Number of ER visits, mean (sd)		
Number of hospital readmissions, n (%)		
0		
1		
2		
3		
Number of hospital readmissions, mean (sd)		