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Statistical Analysis Plan

Comments inserted reflect corresponding sections from the Table in Gamble et al JAMA with regards to creating a SAP.

Inhaled Selective Pulmonary Vasodilators for Lung Transplantation Outcomes (INSPIRE-FLO)

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SAP Version: 4.0

SAP Date: 6/3/2021

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Author and Statistician: Mary Cooter

Primary Investigator: Kamrouz Ghadimi, MD

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Duke Health Department of Anesthesiology

ClinicalTrials.gov Identifier: NCT03081052

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IRB Pro00078035 version 1.8

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SAP Revisions

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Original SAP date: 4/17/2020

Revision 1: 9/23/2020

- Removed references to heart/LVAD cohort analysis and transferred to separate SAP for that cohort's analysis
- Revised variable list for table summaries per meeting/discussion
- Clarified primary PGD 3 outcome will be according to physician score (ISHLT 2016 definition)

Revision 2: 3/15/2021

- Format for JAMA submission per Gamble 2017 paper, transfer of elements from IRB protocol into SAP document
  - Added trial registration number and IRB information to cover page
  - Add SAP revision summary page
  - Added study methods information to study population or dataset section previously only listed in IRB
  - Added to the statistical methods section to specify level of significance, type of confidence intervals, analysis programs used, approach to missing data and multiple comparison correction
- Fix typo for equivalence margin in statistical methods section (incorrectly listed the heart/LVAD cohort margin rather than the lung transplant cohort margin).
- Addition of 90-day mortality to secondary outcomes
- Addition of AKI staging to AKI outcome analysis
- Addition of 90-day Adverse Events to secondary outcomes and statistical methods harms analysis section
- Description of post-hoc PGD3 at alterative time point analyses added

Revision 3: 6/3/2021 (Response to Reviewers)

- Add robustness analysis for 95% CI and 2-sided p-values for primary equivalency outcome comparison
- Modify secondary duration of mechanical ventilation analysis to time-to-event censoring follow-up time at time of tracheostomy when patients went from intubation straight to tracheostomy
- Add P:F ratio of Donor from UNOS data source, and randomization strata to cohort summary table
- Add descriptive primary outcome summary by randomization strata to Supplement 3

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Study Objective: Inhaled Nitric Oxide (iNO) is a selective pulmonary vasodilator (PVD) with FDA-approval in the neonatal population alone. In adult patients, iNO is used off-label to treat pulmonary hypertension, right ventricular (RV) failure, and ventilation-to-perfusion mismatch. Adult patients who undergo durable LVAD implantation (e.g. Heartware®, Heartmate 2®, or Heartmate 3®) or cardiac transplantation for end-stage heart failure or those that have endured lung transplantation as a result of end-stage lung disease, compose the largest subpopulation which receives PVD therapy at Duke University Hospital. Intravenous Epoprostenol is FDA approved for adult patients with pulmonary hypertension and is the only agent which has displayed mortality benefit in these patients. The inhaled formulation of Epoprostenol (iEPO) was developed in order to maintain efficacy and avoid the systemic side effects of vasodilation and thrombocytopenia. Inhaled iEPO is used off-label in our cardiothoracic surgical patients for new-onset perioperative pulmonary arterial hypertension (PAH), known preoperative PAH, RV dysfunction with LVEF > 35-40%, and promotion of ventilation to perfusion matching through alveolar deposition of the prostanoid compound and vasodilation of the intimately associated intra-acinar pulmonary arteries. This vasodilation can decrease pulmonary vascular resistance and can improve oxygenation while avoiding systemic effects commonly seen in the intravenous formulation. iEPO has been introduced in the cardiothoracic operating rooms (OR) and ICU as a cost-conscious alternative medication to iNO.

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1. Hypotheses:
   iEPO displays an equivalent efficacy profile to iNO for pulmonary vasodilation and oxygenation and have a similar impact on clinical outcomes.

   Primary: Incidence of Grade 3 Primary Graft Dysfunction (PGD) within 72 hrs

   Secondary
   a. 30-day, 90-day, and in-hospital mortality
   b. Duration of postoperative mechanical ventilation
   c. ICU length of stay
   d. Hospital length of stay
   e. Incidence of Acute Kidney Injury
   f. In-hospital, 30-day, and 90-day Adverse Events

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2. Outcomes: All outcomes analyzed collectively for primary reporting

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Outcome	Specifications	Variables and Source
PGD 3	defined by the International Society of Heart and Lung Transplantation (ISHLT) within 72 hours Grade 3 Primary Graft Dysfunction (PGD): Defined as physician indicated PGD with either a) PaO2-to-FiO2 ratio < 200 or b) VV ECMO within 72 hours	1. Physician indicated PGD Score (ABG form) 2. All PaO2 and FiO2's (ABG form) 3. VV_ECMO and date (adverse events form)
30-day, 90-day, and in-hospital mortality	From day of transplant	(in hospital events form or adverse events has dates)
Duration of postoperative mechanical ventilation	Use time of first extubation/trach placement as endpoint ICU arrival as start point Track time of re-intubation or trach start to see if within 12 hrs of extubation	Information Until Discharge
ICU length of stay	Days till first ICU discharge	Information Until Discharge
Hospital length of stay	Days till hospital discharge or in hospital death	Information Until Discharge
AKI	Modified KDIGO criteria: raise of 50% or 0.3 over any 48 hr period during first 10 postop days. Any AKI as well as staging	Daily postoperative creatinine (hepatic and renal function form)
90-day Adverse Events	Incidence and timing of in-hospital and post-discharge events collected in CRF	RedCap Adverse Events, 30-day follow-up and 90-day follow-up forms

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1. **Study population or dataset:**

This will be a triple-blinded parallel group 1:1 randomized clinical trial to study the equivalence of iNO and iEPO for the primary outcome in the lung transplant cohort of PGD3 within 72-hours.

Inhaled PVD therapy is administered to every patient undergoing thoracic transplantation and LVAD implantation at our institution and each patient is eligible for enrollment. Over a 3-year period (1 year for follow-up) we will prospectively enroll 200 lung transplant subjects and 224 heart transplant or LVAD implantation patients who will be informed and consented prior to their scheduled procedure

The study was designed based on the following assumptions and power calculations for the lung transplant cohort:

We estimated sample size based on equivalence test of the incidence rates of a binary outcome (e.g. PGD grade 3 (PGD-3)) of two treatment groups as an illustration. Assuming the incidence rate of PGD-3 under iEPO treatment is 0.30 and acceptable margin of the equivalence is ± 0.19, we will need 200 lung transplant patients to have 80% power to detect an actual difference at α=0.05 between two treatment group under this margin. This implies that the acceptable range of incidence rate for iNO treatment is from 0.11 to 0.49.

Randomization was generated using nQuery Advisor® version 7 (Statsols, Cork, Ireland). For lung transplant patients a 5 level stratified 1:1 block randomization schedule was created. A fixed block size of 4 was used and the 5 strata were defined by a combination of transplant type and indication and were defined as follows: SOLT (Restrictive), BOLT (Obstructive), BOLT (Restrictive), BOLT (PPH), BOLT (Other).

Patients are randomized at time of consent which may be within months or hours of transplant. Patients who are withdrawn prior to transplant due to eligibility changes or physician decisions will not be included in the analysis population. Any patient who receives transplant following study consent will be analyzed and included in the ITT population.

2. **Covariates for multivariate models**

VARIABLE	**FORM LOCATION**	**IF REDCAP VARNAME**
RANDOMIZATION GROUP	Blinded randomization csv file prepared by independent statistician	
DONOR CHARACTERISTICS	UNOS data	
DEMOGRAPHICS		
AGE	patient information	age
SEX	patient information	Gender (1=Male, 2=Female)
DONOR P:F	UNOS	
RACE	patient information	Race 1 American Indian 2 Asian 3 Pacific Islander 4 African American 5 Caucasian/ White 6 2 or more races 777 Unknown
BMI	history/physical	Bmi
MEDICAL HISTORY		
EF PREOP	history/physical	ef_preop
PREVIOUS STERNOTOMY	history/physical	previous_sternotomy_yn
HISTORY OF HTN	history/physical	hypertension_hx
HISTORY OF PULMONARY HTN	history/physical	pulmonary_hypertension
TAKING PULMONARY HTN MEDICATIONS	history/physical	pre_op_ph_meds

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PREOP DM	history/physical	Preop_dm
COPD	history/physical	copd
PREOP LABS	IT data pull I:\2661_Ghadimi_Transplant\ FinalLungCohort Or Redcap forms	pre_op_hgb preop_plt pt_preop inr_preop aptt_preop fun_ant_thr_preop fibrinogen_coag1 creatinine_preop ast_preop alt_preop totalbili_preop edfr_preop
SURGICAL CHARACTERISTICS		
INDICATION FOR TRANSPLANT	surgical information	randomization_strata
PRE TX HLA CLASS 1/2 %	history/physical	percent_rel_ab_class_1 percent_rel_ab_class_2 percent_rel_abs_class_1 percent_rel_abf_class_1 percent_rel_abf_class_2
LAS SCORE	history/physical	las_score
SOLT OR BOLT	Intraop	procedure
CONCOMITANT PROCEDURE	Intraop	add_procedure___13=0
ISCHEMIC TIME (LIST SEPARATE FOR BOLTS)	Intraop	lung1_ischemic_time lung2_ischemic_time2
SURGERY DURATION	IT data pull I:\2661_Ghadimi_Transplant\ FinalLungCohort	Proc_stop-proc_start
INTRAOP VA/VV ECMO	Intraop	vv_ecmo_operation or venoart_ecmo
CPB USE	Intraop	cpb_on_time
CPB TIME	Intraop	total_cpb_all
INTRAOP TRANSFUSION VOLUMES	intraop	prbc_intraop_transfusion ffp_intraop_transfusion cryo_intraop_transfusion plt_intraop_transfusion
IPVD DURATION	ABG	ipvd_stop_datetime- ipvd_start_datetime

6. Statistical Methods and results

1. Summary statistics will be computed for demographic, clinical, and outcome variables in the form of frequencies (percentage) for categorical variables and mean (standard deviation) for continuous variables for each arm. Univariate analysis will be performed to compare the difference of each variable between treatment groups by chi-square or Fisher exact tests for categorical variables, and t-tests or Wilcoxon Rank-Sum tests for continuous variables depending on data normality.
2. Level of significance set to 0.05 for all assessments. Primary outcome assessment for equivalence will be based on two-one sided tests with corresponding 90% confidence intervals. All secondary outcomes will be assessed with typical null hypotheses and 2-sided tests and 95% confidence intervals. No correction for multiple testing planned. Missing data will be summarized and if missing rate exceeds 5% multiple imputation methods will be pursued (no such missing data issues identified so not pursued)
3. For the primary outcome to assess equivalency between the two treatment groups we will calculate risk difference estimates and their 90% corresponding confidence intervals. If the confidence intervals do not contain the margin of difference then we will conclude there is sufficient evidence

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that the risk of PGD3 in each treatment group is similar. We will also report relative risk estimates and their corresponding 90% and 95% confidence intervals.

4. A robustness analysis for 95% CI and two-sided p-values for primary equivalency outcome comparison will be performed and reported.
5. For each secondary outcome we will construct univariable effect estimates and corresponding confidence intervals which will allow for assessment of difference between the treatment groups. Binary variables will be evaluated as relative risks, and numeric variables via mean differences, except for duration of mechanical ventilation analysis which will be performed as a time-to-event analysis censoring follow-up time at time of tracheostomy when patients went from intubation straight to tracheostomy.
6. Subsequently we will utilize multivariable regression analysis to further investigate the relationship between treatment group and our outcomes. All covariates meeting p< 0.15 association with treatment group will be considered for variable selection to build a multivariable regression model for the primary outcome. For each outcome of interest, we will start with a regression model (logistic regression for binary outcomes or generalized linear model for continuous outcomes) with all variables selected from univariate analysis described above. Based on stepwise variable selection, we will determine the final set of covariates to be included in the final multivariable model to test the treatment group effect.
7. In the case of patients have switched to the other arm or were weaned not per protocol due to clinical decision, we will conduct the primary analysis based on the intent to treat (ITT) without reclassifying treatment assignment. In addition, per protocol analysis, where only patients follow the protocol assignment are included will also be conducted to verify ITT results.
8. As a post-hoc analysis we will
    a. Describe the primary event rate within each randomization strata
    b. compare the incidence of PGD-3 according to the laboratory and device based definition and the rate according to physician scoring.
    c. compare the incidence of PGD-3 at the 48-78 hour time interval and at 72 hr alone
9. Harms analysis
    a. Pre-specified list of adverse events collected in-hospital, at 30-day and 90-day follow-up will be summarized and compared between groups via Chi-square or Fisher exact test for binary outcomes or t-tests or Wilcoxon rank sum tests for numeric values (See CRF for full list). As these patients are critically ill and iNO/iEPO are both standard of care no unexpected drug related events are anticipated.
    b. Any patient weaned not per protocol will be identified and case reviewed by PI for possible impact of study drug

Statistical analysis will be performed using SAS v9.4 (SAS Industries INC, Cary, NC) and R v3.6 (R Foundation for Statistical Computing). Base and STAT SAS functions were used for the majority of analyses with SAS macros NLEstimates and NLMeans used to extract risk differences from multivariable models, and R package TOSTER used for two-one sided test results.

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