Statistical Analysis Plan

Inorganic Nitrite Delivery to Improve Exercise Capacity in HFpEF

INDIE-HFpEF

A randomized, double-blind, placebo-controlled crossover study to assess the effect of inorganic nitrite (NO_2) on aerobic capacity (peak VO_2).

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Sponsor
National Heart, Lung and Blood Institute

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1. Overview

1.1 Synopsis

The *Inorganic Nitrite Delivery to Improve Exercise Capacity in HFpEF (INDIE-HFpEF)* trial is a placebo-controlled 2*2 crossover trial to assess the effect of inorganic nitrite (NO_2) with dose up-titration on exercise capacity as assessed by cardiopulmonary exercise test.

1.2 Study Treatments

Given the crossover nature of the study, each patient will receive both inorganic nitrite and matching placebo, with the treatment order determined during the randomization process. Each study phase will consist of a 2 week washout period, 1 week at the 46 mg dose (TID), and 3 weeks at the 80 mg dose (TID). Study medication is administered three times per day, at least four hours apart in a blinded fashion during each study phase.

2. Study Design

2.1 Overview

The INDIE-HFpEF study is a randomized, double-blind, placebo-controlled crossover study in heart failure patients with preserved ejection fraction. A total of 100 patients will be enrolled in the trial.

The treatments in this study are blinded. Each study phase will last for 6 weeks. The first 2 weeks of each study phase will be the washout period with study treatment received during the last 4 weeks.

The over-arching hypothesis is that, compared to placebo, treatment with inorganic nitrite will lead to improved exercise capacity as assessed by cardiopulmonary exercise testing.

2.2 Randomization

Patients are randomized in a 1:1 ratio to a specific treatment receipt order: inorganic nitrite in study phase 1 and Placebo in study phase 2 OR Placebo in study phase 1 and inorganic nitrite in study phase 2. The randomization scheme consists of a permuted block design with stratification by clinical site.

2.3 Data Sources

A database of case report form and biomarker core lab data will be created in Inform, and the data then transferred to SAS for analysis. The randomized treatment assignment will be provided through data provided by the Webez system, an Almac Clinical Services web-based randomization system. The accelerometer data was provided by the Accelerometer Core Lab located at Mayo Clinic. The core lab provided both cleaned and raw data for download. The data is stored on Solaris server uxctstp01 in the folder /hf_net/INDIE/data.

3. Analysis Population and Missing Data

All randomized patients will be included in the analysis population for assessing the primary, secondary and tertiary endpoints. Given the crossover design of the study, each patient will have endpoints measured at two times; the first at the conclusion of Phase 1 (Visit 2) treatment and the second at the conclusion of Phase 2 treatment (Visit 3). All endpoints will be analyzed on an intent-to-treat basis.

However, as described in subsequent sections of this document, some patients may be excluded from certain analyses if key data elements are missing. With the extensive efforts being made in collaboration with the clinical sites to ensure data quality and completeness, it is expected that exclusion of patients for any endpoint analysis will be minimal. The specific endpoint descriptions in Sections 8 through 10 describe the circumstances that would lead to a patient being excluded from a specific analysis.

4. General Methodology

Medians, 25^{th} and 75^{th} percentiles will be presented for continuous variables; the number and percentage of patients in each category will be presented for categorical variables. For all endpoints a p-value ≤0.05 will be considered statistically significant and all tests will be 2-sided. Analyses will be performed using validated SAS software (SAS Institute, Inc., Cary, NC). Appropriate statistical models will be used to examine the effect of treatment on both the primary, secondary, and tertiary outcomes in the study.

The majority of the endpoints will be analyzed using a linear mixed model. Within the context of the mixed models, the period effect will be examined to determine whether both treatment effects are consistently higher or lower when comparing Phases 1 and 2 of the study (e.g., Peak VO_2 is increased in Phase 2 for both treatments vs Phase 1). The sequence effect will be examined to determine if the treatment effect differs based on the treatment order (inorganic nitrite then Placebo or Placebo then inorganic nitrite). Alternative modeling may also be performed using paired t-tests or appropriately structured general linear models.

5. Primary Endpoint

Primary Endpoint
#1: Within patient comparison of exercise capacity as assessed by peak VO_2 (ml/kg/min), comparing inorganic nitrite to placebo

See Section 8 for a detailed description of the primary endpoint, including rules that will be followed for handling incomplete data.

6. Secondary Endpoints

Secondary Endpoints
#1: Within patient comparison of arbitrary accelerometer units (AAU) during the maximally tolerated dose of inorganic nitrite vs placebo
#2: Within patient comparison of Kansas City Cardiomyopathy Questionnaire (KCCQ) symptom scores – both clinical and overall, comparing inorganic nitrite vs placebo
#3: Within patient comparison of New York Heart Association (NYHA) functional class, comparing inorganic nitrite vs placebo

#4: Patient preference for study phase (inorganic nitrite vs. placebo)
#5: Within patient comparison of medial E/e' ratio as assessed on echocardiography, comparing inorganic nitrite vs placebo
#6: Within patient comparison of left atrial volume index as assessed on echocardiography, comparing inorganic nitrite vs placebo
#7: Within patient comparison of pulmonary artery systolic pressure (PASP) as assessed on echocardiography, comparing inorganic nitrite vs placebo
#8: Within patient comparison of core lab NT Pro BNP, comparing inorganic nitrite vs placebo
#9: Within patient comparison of V_E/VCO_2 slope (ventilator efficiency) as assessed on cardiopulmonary exercise testing, comparing inorganic nitrite vs placebo
#10: Within patient comparison of VO_2 at ventilatory threshold (submaximal exercise capacity) as assessed on cardiopulmonary exercise testing, comparing inorganic nitrite vs placebo
#11: Within patient comparison of exercise duration as assessed during cardiopulmonary exercise testing, comparing inorganic nitrite vs placebo

See Section 9 for a detailed description of each secondary endpoint, including rules that will be followed for handling incomplete data.

7. Tertiary Endpoints

Tertiary Endpoints
#1: Primary endpoint comparison within the following subgroups:

a. Baseline core lab NT Pro BNP > 400 pg/mL vs ≤ 400 pg/mL
b. Baseline systolic blood pressure above and below the median
c. Age above and below the median
d. Male vs female
e. Baseline estimated glomerular filtration rate (GFR) by MDRD above and below the median
f. On vs off study drug at time of visit based on active study drug phase
g. Atrial fibrillation/flutter vs not
h. Diabetes vs no diabetes
i. BMI above and below the median
j. Baseline activity level above and below the median

#2: Within patient comparison of core lab cystatin C, comparing inorganic nitrite vs placebo
#3: Within patient comparison of core lab cyclic guanosine monophosphate (cGMP), comparing inorganic nitrite vs placebo

Note: The protocol states that we will also examine novel accelerometry endpoints, guided by NEAT-HFpEF ancillary paper analyses (in progress). As of yet, there are no novel endpoints under consideration for this study.

See Section 10 for a detailed description of each tertiary endpoint, including rules that will be followed for handling incomplete data.

8. Endpoint Descriptions

8.1 – Primary Endpoint

Endpoint Description: Exercise capacity as assessed by peak VO_2 (ml/kg/min) measured during cardiopulmonary exercise testing

Response Variable Definition: Visit 2 and Visit 3 peak VO_2, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline peak VO_2 score, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Two sensitivity analyses will be performed.

1) Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the cardiopulmonary exercise test was performed.
2) To address a potential question seen in some journal reviews, the original mixed model will be run again, adding a new covariate for random effect of enrolling site.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on peak VO_2. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A higher peak VO_2 indicates an improved exercise capacity in the inorganic nitrite period vs placebo.

9. Secondary Endpoint Descriptions

9.1 – Secondary Endpoint #1

Endpoint Description: Arbitrary accelerometry units (AAU) during maximally tolerated dose of inorganic nitrite vs placebo

Response Variable Definition: For each study phase, the average daily AAU will be calculated for the period of time that patient was taking 80 mg daily (or maximally tolerated dose if 80 mg not tolerated), approximate time periods are study weeks 4-6 and 10-12. Only complete days are used and there should be 21 days in the dosing period.

Additional Covariates: period effect, sequence effect, baseline daily average AAU, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: For this sensitivity analysis, each average will be divided by the study phase 1 14 day baseline period average daily AAU (approximately study weeks 1-2) to create a ratio. Values greater than 1 indicate increased activity.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on daily activity level. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: Higher AAU indicates more activity in the inorganic nitrite period vs placebo.

9.2 – Secondary Endpoint #2

Endpoint Description: Kansas City Cardiomyopathy Questionnaire Overall and Clinical Summary Scores

Response Variable Definition: Visit 2 and Visit 3 KCCQ scores, classified as to treatment received in prior treatment period. Each summary score will be an individual endpoint and have its own model.

Additional Covariates: Baseline KCCQ score (as appropriate), period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the KCCQ questionnaire was completed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on each KCCQ summary score. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A higher KCCQ score indicates an improved KCCQ score in the inorganic nitrite period vs placebo.

9.3 – Secondary Endpoint #3

Endpoint Description: NYHA class

Response Variable Definition: Visit 2 and Visit 3 NYHA class, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline NYHA class, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the NYHA class was assessed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on NYHA class. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower NYHA class indicates an improved heart failure symptoms in the inorganic nitrite period vs placebo.

9.4 – Secondary Endpoint #4

Endpoint Description: Patient preference for study phase (inorganic nitrite vs. placebo)

Response Variable Definition: Patient preferred study phase (active, placebo, no preference).

Statistical Tests: No formal testing will be done.

Interpretation of Results: For patients with a preference, a higher percentage of patients favoring the inorganic nitrite period vs placebo indicate a positive treatment experience.

9.5 – Secondary Endpoint #5

Endpoint Description: Medial E/e'

Response Variable Definition: Visit 2 and Visit 3 medial E/e', classified as to treatment received in prior treatment period.

Additional Covariates: Period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the echocardiogram was performed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on medial E/e'. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower medial E/e' indicates improvement in the inorganic nitrite period vs placebo.

9.6 – Secondary Endpoint #6

Endpoint Description: Left atrial (LA) volume index

Response Variable Definition: Visit 2 and Visit 3 left atrial volume index, classified as to treatment received in prior treatment period.

Additional Covariates: Period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the echocardiogram was performed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on LA volume index. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower LA volume index indicates improvement in the inorganic nitrite period vs placebo.

9.7 – Secondary Endpoint #7

Endpoint Description: Pulmonary artery systolic pressure

Response Variable Definition: Visit 2 and Visit 3 PASP, classified as to treatment received in prior treatment period.

Additional Covariates: Period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the echocardiogram was performed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on PASP. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower PASP indicates improvement in the inorganic nitrite period vs placebo.

9.8 – Secondary Endpoint #8

Endpoint Description: Core lab NT pro BNP

Response Variable Definition: Visit 2 and Visit 3 NT pro BNP, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline NT pro BNP, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the biomarkers were drawn.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on NT pro BNP. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower NT pro BNP indicates improved NT pro BNP in the inorganic nitrite period vs placebo.

9.9 – Secondary Endpoint #9

Endpoint Description: V_E/VCO_2 slope

Response Variable Definition: Visit 2 and Visit 3 V_E/VCO_2 slope, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline V_E/VCO_2 slope, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the cardiopulmonary exercise testing was performed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on V_E/VCO_2 slope. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower V_E/VCO_2 slope indicates improvement in the inorganic nitrite period vs placebo.

9.10 – Secondary Endpoint #10

Endpoint Description: VO_2 at ventilatory threshold

Response Variable Definition: Visit 2 and Visit 3 VO_2 at ventilatory threshold, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline VO_2 at ventilatory threshold, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the cardiopulmonary exercise testing was performed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on VO_2 at ventilatory threshold. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A higher VO_2 at ventilatory threshold indicates improvement in the inorganic nitrite period vs placebo.

9.11 – Secondary Endpoint #11

Endpoint Description: Exercise duration on CPET

Response Variable Definition: Visit 2 and Visit 3 exercise duration, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline exercise duration, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Sensitivity Analysis: Due to the short washout period of the study drug, a separate analysis will be performed removing those patients that discontinued active study treatment prior to the next study visit where the cardiopulmonary exercise testing was performed.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on exercise duration. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A longer exercise duration indicates improvement in the inorganic nitrite period vs placebo.

10. Tertiary Endpoint Descriptions

10.1 – Tertiary Endpoint #1

Endpoint Description: Exercise capacity as assessed by peak VO_2 (ml/kg/min) measured during cardiopulmonary exercise testing within the following subgroups:

a. Baseline core lab NT Pro BNP > 400 pg/mL vs ≤ 400 pg/mL
b. Baseline systolic blood pressure above and below the median
c. Age above and below the median
d. Male vs female
e. Baseline estimated glomerular filtration rate (GFR) by MDRD above and below the median

f. On vs off study drug at time of visit based on active study drug phase
g. Atrial fibrillation/flutter vs not
h. Diabetes vs no diabetes
i. BMI above and below the median
j. Baseline activity level above and below the median

See section 8.1 for analysis details

10.2 – Tertiary Endpoint #2

Endpoint Description: Core lab cystatin C

Response Variable Definition: Visit 2 and Visit 3 cystatin C, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline cystatin C, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on cystatin C. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A lower cystatin C indicates improvement in the inorganic nitrite period vs placebo.

10.3 – Tertiary Endpoint #3

Endpoint Description: Core lab cyclic guanosine monophosphate (cGMP)

Response Variable Definition: Visit 2 and Visit 3 cGMP, classified as to treatment received in prior treatment period.

Additional Covariates: Baseline cGMP, period effect, sequence effect, and random effect of patient

Handling of Dropouts and Missing Data: No adjustment for missing data will be made.

Statistical Tests: A mixed model (PROC MIXED in SAS) will be used to estimate and statistically compare the response to inorganic nitrite versus placebo on cGMP. Secondary analyses will be performed using paired t-tests and appropriate general linear models.

Interpretation of Results: A higher cGMP indicates an improvement in the inorganic nitrite period vs placebo.

11. Safety analysis

11.1 – Safety Endpoint #1

Safety Endpoint #1: Comparison of adverse events and serious adverse events

Response Variable Definitions: Each unique adverse event (AE) and serious adverse event (SAE) type based on preferred term within body system. The number of patients experiencing adverse events will be counted within each study phase, based on preferred term.

Additional Covariates: None

Handling of Dropouts and Missing Data:
No adjustments for missing data will be made.

Statistical Tests:
Fisher's mid-p will be calculated for each adverse event within each study phase.

Interpretation of Results: Lower event rates indicate a better outcome for inorganic nitrite vs placebo.

12. Interim Analyses

Interim data analysis for efficacy will not be conducted due to the relatively small size and short duration of this clinical trial. Safety data will be periodically assessed by the Data and Safety Monitoring Board (DSMB) based on the reporting of adverse events. There are no pre-specified guidelines for determining stopping rules due to a safety concern; the clinical opinion from the DSMB deliberations will be sole determinant.