BLOCKade of calcium channels and beta adrenergic receptors for the treatment of hypertension in Heart Failure with Preserved Ejection Fraction (BLOCK HFpEF)

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Statistical Analysis Plan

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1. Trial Summary

Trial Title/Acronym	BLOCKade of calcium channels and beta adrenergic receptors for the treatment of hypertension in Heart Failure with Preserved Ejection Fraction (BLOCK HFpEF)
Number of Subjects	50
Study Site	University of Pennsylvania
Randomized Intervention	Amlodipine besylate 5mg to 10mg daily for approximately four weeks versus metoprolol succinate 100mg to 200mg daily for approximately four weeks
Primary Aim	To compare the BP-lowering effect of amlodipine besylate and metoprolol succinate therapy by home BP monitoring in HFpEF
Secondary Aims	1. To compare the effect of amlodipine besylate and metoprolol succinate therapy on aerobic capacity 2. To compare the effect of amlodipine besylate and metoprolol succinate therapy on quality of life 3. To compare the effect of amlodipine besylate versus metoprolol succinate therapy on LV diastolic function and arterial load
Exploratory Aims	1. To assess if amlodipine besylate and metoprolol succinate have differential effects in men versus women, African Americans versus non-African Americans, and diabetic versus non-diabetic subjects 2. To compare the effect of amlodipine besylate versus metoprolol succinate therapy on: A) Non-dipping measured using home BP monitoring (novel) B) BP variability using home BP monitoring C) Ventilatory threshold and VO_2 kinetics
Primary Endpoint	Difference in mean home systolic BP after four weeks of amlodipine besylate versus metoprolol succinate
Secondary Endpoints	1. Difference in mean office systolic BP 2. Difference in mean home diastolic and office diastolic BP 2. Difference in mean home pulse pressure and office pulse pressure 3. Total work performed and peak oxygen uptake (VO_2) during a symptom-limited maximal effort exercise test 4. Quality of life score, assessed using the Kansas City Cardiomyopathy Questionnaire 5. Measures of LV diastolic function, including E/e' and brain natriuretic peptide 6. Measures of arterial load, including arterial wave reflection, central systolic blood pressure, augmentation index, pulse pressure amplification, and forward and backward wave amplitudes

2. Study Design

1) This is a randomized double-blind crossover trial in which fifty (50) subjects with HFpEF were assigned to treatment with:
    A) Amlodipine besylate 5mg to 10mg by mouth daily for approximately four weeks;
    B) Metoprolol succinate 100mg to 200mg by mouth daily for approximately four weeks.
2) The order of the interventions (AB-BA design) was randomized, with an approximately one-week washout period separating each intervention.
3) The crossover design exposed each subject to both treatments, reducing inter-subject variability and maximizing statistical power to evaluate the comparative effectiveness of amlodipine versus metoprolol in this patient population.

3. Statistical considerations

3.1 Power calculations

We randomized 50 subjects to one of 2 sequences, each of which consists of 2 periods (AB/BA design). We considered a mean within-subject difference in daytime systolic BP of 5 mmHg between the therapies to be the minimum clinically significant difference.[1] A previously published double-blind crossover trial randomized subjects with a diagnosis of hypertension, who were otherwise healthy, to treatment with amlodipine besylate 5-10mg daily versus metoprolol succinate 100-200mg daily.[2] The trial demonstrated a slightly greater decline in awake ambulatory BP with metoprolol succinate compared to amlodipine, which did not reach statistical significance. Using standard deviations from this study (the most conservative standard deviation was 10.6 mmHg for the mean baseline ambulatory SBP of 148 mmHg) and assuming a retention rate of 85% and a within-subject correlation of 0.8, we have 90% power at an alpha of 0.05 to detect a within-subject difference in mean daytime systolic BP of 5 mmHg between the different therapies and to assess for effect modification by sex.[3] Based on the preliminary findings presented using TOPCAT data, we also have 90% power to detect a 5 point difference in KCCQ score between therapies, which is considered clinically significant.[4] Power calculations were performed using PASS16.[5]

3.2. Data Analysis Plan

3.2.1. Primary Outcome

The primary outcome variable will be the within-subject difference in mean daytime home SBP between the two therapies.

3.2.2. Secondary Outcomes

All secondary outcome measures are continuous variables:

Secondary Endpoints

1. Difference in mean office systolic BP
2. Difference in mean home diastolic and office diastolic BP
2. Difference in mean home pulse pressure and office pulse pressure

3. Total work performed and peak oxygen uptake (VO_2) during a symptom-limited maximal effort exercise test
4. Quality of life score, assessed using the Kansas City Cardiomyopathy Questionnaire
5. Measures of LV diastolic function, including E/e' and brain natriuretic peptide
6. Measures of arterial load, including arterial wave reflection, central systolic blood pressure, augmentation index, pulse pressure amplification, and forward and backward wave amplitudes

3.2.3. Primary Intervention

The predictor of interest for all aims will be the intervention (amlodipine vs. metoprolol), with analyses based upon the total number of subjects randomized.

3.2.4. Primary Manuscript Analyses

3.2.4.1 Descriptive Statistics

Initial descriptive estimates of all measures will be generated for study participants at each time point by treatment group using baseline demographic and clinical characteristics (Table Shell 1). Statistics will include estimates of central tendency (median, mean) and measures of variability (25^{th}-75^{th} percentile, standard deviation), as appropriate. Analyses of distributional properties will be performed to determine if variance stabilizing or normalizing transformations should be applied. Outliers will be assessed via visual inspection of distributions and checked for accuracy. The intervention groups will initially be compared within each period (i.e., amlodipine first vs. metoprolol first) using the t-test or Kruskal-Wallis analyses, depending upon whether the variables are parametric, and Chi square or Fisher's Exact tests for categorical variables.

3.2.4.2 Primary and Secondary Analyses

Our primary aim will assess the effects of amlodipine versus metoprolol on the primary and secondary endpoints. An initial assessment of the treatment effect will be performed using the paired t-test and the non-parametric Wilcoxon sign-rank test on the difference between the paired within-subject outcome measures across the two therapies (Table Shell 2). This will be followed by secondary analyses using more comprehensive linear mixed effects model analyses[6] allowing for assessments of the treatment effect on each continuous outcome of interest while accounting for effects of other covariates including period, sequence, and a random subject effect nested within sequence. For non-normal distributed outcomes, we will use non-parametric methods or consider distribution-stabilizing transformations.

3.2.4.2.1 Addressing potential confounders

Randomization should balance measured and unmeasured confounders across study arms. However, we will compare the distributions across arms for potential confounders, including participant demographics and comorbidities outlined in Table Shell 1, and perform adjustments for any that are imbalanced. Significant differences between groups in these variables will result in their respective adjustment in the modeling of the outcome. The linear mixed-effects models will incorporate adjustments for any period effect or crossover effect and will include data from dropouts.[7, 8] The model will include subject-specific intercepts as random effects, and will assume independent and identically distributed random errors within-subject. Restricted maximum likelihood estimation will be used, and an appropriate covariance matrix will

be specified. Model assumptions will be examined (e.g., QQ plots to assess normally distributed residuals for valid Wald tests).

As a secondary analysis, we will adjust the linear mixed-effects models for baseline risk factors known or likely to be associated with systolic BP control:

1. Age
2. BMI
3. eGFR
4. Number of blood pressure medications

3.2.4.2.2. Stratified analyses and effect modification

In prespecified analyses, we will perform stratified analyses and will assess for effect modification for the primary endpoint and key secondary endpoints (peak oxygen uptake during exercise and NTProBNP) by:

1. Male vs. female sex
2. African American vs. non-African American race
3. Diabetes vs. no diabetes

Exploratory analyses will evaluate effect modification by the following parameters:

1. Chronotropic incompetence vs. no chronotropic incompetence (this will be determined using the heart rate data collected from the exercise testing performed while on beta-blocker therapy and will be defined as <0.62 vs. ≥0.62).
2. COPD vs. no COPD
3. New York Heart Association Class
4. Obesity vs. no obesity (BMI ≥30 vs. <30)
5. CKD vs. no CKD (eGFR <60 vs. ≥60)

3.2.5. Second Manuscript Analyses

3.2.5.1. Exploratory Mediation Analysis

Exploratory modeling will be used to evaluate associations between biologic mechanistic pathways and the clinical outcomes (Aim 3). We will evaluate whether markers of arterial load mediate the association between intervention groups (amlodipine vs. metoprolol) and the primary (systolic BP) and key secondary outcomes (aerobic capacity and LV diastolic dysfunction). We will use mediation models as described by Baron & Kenny[12] or Preacher and Rucker[13] to test if amlodipine vs. metoprolol is associated with each outcome which in turn is associated with markers of arterial load. In brief, a series of mixed models that are formed using the strategies we described above are fit, regressing: 1) outcome (systolic BP) on group (amlodipine vs. metoprolol) (Model 1); 2) mediator (markers of arterial load) on group (amlodipine vs. metoprolol) (Model 2); and 3) outcome (systolic BP) on both mediator (markers of arterial load) and group (amlodipine vs. metoprolol) (Model 3). A change in the estimate of the exposure effect from Model 1 to 3 is evidence of mediation. We will estimate if the indirect effect of amlodipine vs. metoprolol on systolic BP is mediated by a marker of arterial load and the direct effect is not explained by amlodipine/metoprolol alone. This modeling will allow us to understand the contribution of the biologic pathways that are represented by these markers on a potential medication – systolic BP association. Absence of mediation by markers of arterial load will suggest other mechanisms may be responsible for a potential medication effect.

We are comparing multiple overlapping hypotheses related to cardiac and vascular mechanisms of HFpEF progression. To minimize the likelihood of Type 1 errors in our analyses (i.e. to account for multiple comparisons), we will employ a conservative Bonferroni approach for the comparisons of interventions.[14]

3.3 Timing and Rationale for Unblinding

The statistics team has been unblinded throughout the study to be able to develop DSMB reports and to begin performing the analyses. Dr. Cohen, Dr. Chirinos, and the members of the Core Lab and clinical study team remain blinded to allow for any further data interrogations or cleaning at the onset of analysis. All team members except Dr. Chirinos will become unblinded following initial review of the variable distributions and missingness to ensure no further data input or cleaning is needed. Dr. Chirinos will be unblinded at the final stage of analysis review, to allow for any further data input or cleaning prior to that point.

4. Publication and Dissemination Plan

The analyzed results of the trial will be submitted for publication to a peer-reviewed journal within six months following the completion of the final data collection and cleaning. The project will be registered with clinicaltrials.gov, and the results will be submitted to clinicaltrials.gov no later than one year following the completion date of the project.

5. References

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