Impact of Bromocriptine on Clinical Outcomes for Peripartum Cardiomyopathy (REBIRTH)

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REBIRTH Statistical Analysis Plan.

Randomization Scheme. Patients will be allocated 1:1 to bromocriptine or standard therapy using a web-based system to maintain allocation concealment with randomly permuted blocks of size 2, 4, and 6. Randomization will be stratified by race (African American versus Non-African American) as PPCM outcomes are thought to be worse in African American women, and by NYHA class (class IV versus classes I-III). Study site will not be a stratification factor due to the resulting small sample size within each combination of treatment arm, study site, and race group.

Sample Size Calculation. The primary outcome for this RCT will be left ventricular ejection fraction (LVEF) assessed 6 months post-randomization. We will analyze the difference in post-randomization LVEF between groups using analysis of covariance (ANCOVA) with the 6-month LVEF assessment as the outcome, a fixed effect for the treatment assignment, and the baseline LVEF included as a covariate as well as race and number of months post-partum at study enrollment. This analytic strategy matches the salient clinical question: "For two patients with the same baseline LVEF, what is the expected difference in their follow-up LVEF if one is treated with bromocriptine while the other is treated with standard therapy?" In addition, the approach of using ANCOVA to compare the post-treatment values with a covariate adjustment for baseline values is known to improve efficiency versus analysis that fail to adjust for baseline, or comparisons of change scores.[1]

Power calculations were performed by simulation in R (see programming code below for transparency). The REBIRTH trial will enroll post-partum women with a baseline LVEF <0.35. We assume that this draws from a patient population with mean LVEF of 0.27 and SD of 0.1 (similar to baseline distribution LVEF in previous trials in this population; the largest available trial of bromocriptine in a comparable population, by Hilfiker-Kleiner et al, reported mean LVEF=0.27-0.28 and SD of 0.1 at study enrollment).[2] Therefore, we will design REBIRTH assuming the population is recruited from patients with mean LVEF=0.27 (SD=0.1) at study enrollment. The Hilfiker-Kleiner et al trial, in which all n=63 participants were treated with bromocriptine (randomized to 2 different dose regimens), showed recovery to mean LVEF of 0.49-0.51 (SD=0.10-0.12) at 6 months after treatment with bromocriptine.[2] Therefore, we will assume a population mean recovery to LVEF=0.5 (SD=0.1) in patients treated with bromocriptine. We have powered the trial based on a minimum clinically important difference of 0.05 (e.g. if the population experiences recovery to mean LVEF=0.45 with standard care versus recovery to mean LVEF=0.50 when treated with bromocriptine, translating a treatment effect of +0.05 with bromocriptine). We intend to recruit 200 women into the randomized trial; assuming up to 10% attrition, we expect to retain a total sample size of 180 women (90 per treatment group) with available data for the primary outcome analysis. Based on 100,000 simulations, this sample size would have 90.7% power to detect a treatment effect using the 0.05 significance level. If attrition is as high as 20% (leaving an available sample size of 160 for the final outcome analysis), the study design retains 87.2% power. As a result, we are well-positioned to detect a clinically meaningful treatment effect even if the study falls slightly short of the target sample size and/or experiences higher-than-expected attrition, or if the variability in follow-up LVEF measures is greater than anticipated. Note that we have chosen LVEF as primary outcome rather than harder clinical endpoint such as event-free survival because it would be impossible to recruit a large enough sample to test the secondary outcome with sufficient power, so we acknowledge that the power to detect clinically significant differences will be limited.

In addition, there will be a planned sample-size re-estimation that occurs at the same time as the interim efficacy analysis with N=100 patients, described in more detail in the Interim and Final Analyses section below. This will potentially adjust the final target sample size, if needed, to preserve the overall power to detect the minimum clinically important effect size (an absolute difference of 0.05 in 6-month LVEF for bromocriptine versus control) in the event of a larger-than-expected standard deviation in one or both of the treatment arms.

Interim and Final Analyses. Interim study reports will be delivered to the DSMB every 6 months or at the DSMB discretion. Planned interim analyses for efficacy, futility, and safety are as follows:

Efficacy. There will be one planned interim analysis that allows stopping for efficacy; this will be reported at the first DSMB meeting that occurs after we have reached 50% of the anticipated information fraction on the primary outcome (n=100 patients with complete 6-month LVEF). The stopping rule will use the Haybittle-Peto boundary, recommending an efficacy stoppage if $p<0.001$ favoring benefit of bromocriptine; otherwise, the trial will continue enrollment to the intended sample size target of n=200 participants. This threshold is admittedly a conservative one, for two reasons. First, the study's ability to answer questions related to race subgroup effects will be adversely affected if the trial were stopped early; second, it is important that any decision to stop early for efficacy on the primary endpoint (LVEF) also provide adequate data to evaluate potential safety concerns. Therefore, the trial will only be permitted to stop for efficacy on the primary endpoint if the evidence is so overwhelmingly in favor of benefit that there's no possibility of a reversal by trial conclusion.

Futility. There will be no formal stoppage rules for futility based on the primary endpoint, with similar rationale as above; the ability to interrogate race subgroup effects and/or mechanistic hypotheses will be compromised if the trial is stopped before reaching the intended sample size, and it is important to accrue sufficient safety data for this agent in this population. Although there are no planned futility stopping rules, the DSMB will have latitude to recommend stopping the trial if there are concerns about safety (see below). There will also be recruitment-based futility targets set according to NHLBI guidance at each scheduled meeting.

Safety. At the recommendation of the NHBLI, we designated a primary safety composite endpoint of death/transplant/LVAD, which will be analyzed using a log-rank test at each DSMB meeting, reported every 6 months from the beginning of study enrollment to the last scheduled DSMB meeting using the O'Brien-Fleming alpha spending approach (anticipating roughly 6 interim looks, depending on length of enrollment). The DCC will provide the updated spending rules and hypothesis test results at each DSMB meeting as a function of the number of patients randomized divided by the total recruitment target; we thought it better to compute information fraction based on number recruited over time rather than a number of accrued events, as we are uncertain how many events will be accrued during the trial. The alpha-spending approach will be fairly conservative at early looks to minimize the risk of stopping early unless the evidence is clear and overwhelming, but permits flexibility to incorporate additional looks at the DSMB's request. We also note that there are other important safety outcomes, such as thromboembolic events and bleeding. While there is no pre-specified statistical analysis planned for these safety outcomes, we emphasize that the REBIRTH DSMB will have access to closed report data on all safety outcomes reported every 6 months for the duration of the trial, and may ask for additional analyses to inform any decisions to stop the study over concerns related to safety.

Sample-Size Re-Estimation. To ensure that overall statistical power is preserved, there will be a sample-size re-estimation during our planned interim analysis (occurring after we have reached

50% of the anticipated information fraction on the primary outcome). At this time, we will estimate the sample size needed to have at least 90% power to detect the originally specified minimum clinically important treatment difference (absolute difference in mean 6-month LVEF of 0.05 with bromocriptine versus control) using the observed standard deviation of 6-month LVEF in the bromocriptine and control groups. Based on the re-estimation, the final target sample size will be the *larger* of i) the original planned sample size (N=200) or ii) the sample size required to preserve at least 90% power to detect the originally specified treatment difference based on the observed standard deviation in each treatment arm after N=100 total patients have complete 6-month outcome data. For example, if the interim sample-size re-estimation says that N=250 patients are required to have 90% power based on the observed standard deviations at the N=100 patient analysis, the final target sample size will be adjusted to N=250 (since the re-estimated sample size is larger than the originally planned sample size). Conversely, if the interim sample-size re-estimation says that N=170 patients are required to have 90% power based on the observed standard deviations at the N=100 patient analysis, the final target sample size will remain the originally planned N=200 (since the re-estimated sample size was smaller than the originally planned sample size).[3,4]

The final analyses will be conducted once study follow-up is complete, after all data is cleaned, and once the study database is locked. We anticipate this will commence in the second half of Year 5.

Hypotheses. The primary study hypotheses for the REBIRTH trial are:

Aim 1) treatment with bromocriptine will improve myocardial recovery (measured by LVEF on follow-up echocardiograms) over standard therapy.

Aim 2a) Global Longitudinal Strain (GLS) and LV diastolic volumes (LVDV) at study entry will have an effect the therapeutic impact of bromocriptine.

Aim 2b) treatment with bromocriptine will improve GLS and LVDV (measured at the follow-up echocardiograms) versus standard therapy.

Aim 3a) the level of baseline biomarkers (cathepsin D, prolactin 16kD fragment and miR146a) will have an effect on the therapeutic impact of bromocriptine on the primary outcome

Aim 3b) treatment with bromocriptine will have significant effects on those biomarkers over standard therapy. This analysis will also include a comparison of treated women against a third group of patients who are not enrolled in the trial because they are breastfeeding.

Analysis Sets. The primary analyses will be performed according to the intention-to-treat (ITT) principle and will comprise all participants who have been randomized to either study arm, regardless of length of follow-up or actual intervention received. We will also report a per-protocol (PP) secondary analysis including only patients that took >80% of study medication as a sensitivity analysis. The safety analysis (SA) set will be a subset of the ITT set and include all participants who have received at least one dose of study drug or placebo.

Endpoints and Covariates. The primary efficacy endpoint is left ventricular ejection fraction (LVEF) measured 6 months post-randomization, analyzed as a continuous variable. This will address the primary hypothesis by testing whether treatment with bromocriptine results in greater myocardial recovery than standard therapy. Secondary endpoints include LVEF measured 12 months post-randomization, event-free survival through 36 months, GLS and

LVDV measured at 6 months and 12 months post-randomization. Safety outcomes include serious adverse events, specifically those that are cardiovascular related.

Handling of Missing Values. As a preventive measure, we will make every attempt to document all reasons for missing data. In addition, baseline characteristics will be compared between participants who do and do not withdraw from the study as a way to assess the impact of missing information and attrition. We will also compare the rates of lost-to-follow-up (LTF) between study arms.

Missing data will be of particular concern for the primary endpoint of LVEF. There is the potential to have informative censoring in which a participant's LVEF is missing due to death or dropout, yet the missing value that would have been observed is directly related to why it is missing in the first place. We will conduct a variety of sensitivity analyses to deal with this. First, we will use a naïve method that imputes the lowest LVEF among all participants with complete data to the patients with missing LVEF. Second, we will analyze using the win ratio approach, which offers the ability to combine censoring due to death/dropout and a continuous outcome variable into a single ordinal endpoint, as a sensitivity analysis.[5] The win ratio will be applied as follows: patients that are known to have died before the 6-month LVEF will be assigned the worst-ranked outcome; patients that are not confirmed to have died but who do not have a recorded 6-month LVEF will be assigned the second-worst-ranked outcome (as this could reflect dropout due to drug intolerance or poor health); patients that have a 6-month LVEF will be ranked according to their LVEF, with a higher LVEF representing a better outcome.

Statistical Analyses. Demographic and baseline characteristics will be presented as mean and standard deviations for continuous variables and sample proportions for categorical variables, presented overall and by treatment group according to the CONSORT guidelines. Effect sizes and corresponding 95% confidence intervals will be calculated for all primary and secondary analyses.

Hypothesis 1: We will analyze the difference in the primary endpoint (LVEF measured 6 months post-randomization) between groups using analysis of covariance (ANCOVA) with the 6-month LVEF assessment as the outcome variable, a fixed effect for the treatment assignment, and a covariate adjustment for the baseline LVEF, race, NYHA class, and the number of months post-partum. As a sensitivity analysis, we will conduct a per-protocol analysis after restricting to women within the PP analysis set.

Additionally, secondary endpoints such as LVEF measured at 12 months post-randomization will be analyzed using the same approach as the primary outcome. Event-free survival will be reported using Kaplan-Meier curves and a Cox proportional-hazards model to compare the treatment groups while adjusting for baseline LVEF, race, NYHA class, and number of months post-partum at study enrollment.

Hypothesis 2A: It is possible that treatment response to bromocriptine will vary according to the patients' degree of remodeling at baseline. We will test whether there is a significant interaction between baseline GLS and treatment effect on the primary outcome (LVEF) using the same model framework as the primary analysis: outcome variable will be 6-month LVEF, fixed effect for treatment assignment, covariate for baseline LVEF, race, NYHA class, the number of months post-partum, and in this model we will add a covariate for baseline GLS as well as an interaction term for baseline GLS * treatment assignment. If the interaction term is significant (at alpha=0.05) we will conclude that GLS is an effect modifier of the therapeutic impact of bromocriptine. The same approach will be followed for LVDV.

Hypothesis 2B: This will be tested using a similar analytic approach to Hypothesis 1: outcome variable will be the 6-month GLS / LVDV, with a fixed effect for treatment assignment and the baseline value of GLS / LVDV as a covariate as well as race, NYHA class, and number of months post-partum. If the treatment effect is significant (alpha=0.05), we will conclude that bromocriptine has a significant impact on subsequent GLS and LVDV.

Hypothesis 3A: It is possible that treatment response to bromocriptine will vary according to the patients' baseline biomarkers. We will test whether there is a significant interaction between baseline biomarker levels and treatment effect on the primary outcome (LVEF) using the same model framework as the primary analysis: outcome variable will be 6-month LVEF, fixed effect for treatment assignment, covariate for baseline LVEF, race, NYHA class, the number of months post-partum, and in this model we will add a covariate for baseline biomarker as well as an interaction term for baseline biomarker * treatment assignment. If the interaction term is significant (at alpha=0.05) we will conclude that the biomarker is an effect modifier of the therapeutic impact of bromocriptine. The same approach will be followed for each of the biomarkers (cathepsin D, prolactin 16kD fragment and miR146a).

Hypothesis 3B: In this aim, we will establish the impact of bromocriptine therapy on biomarkers in comparison to patients treated with standard therapy as well as the cohort excluded from the trial due to continued breastfeeding. Serum will be collected at entry, 1 month, 3 months, and 6 months. These data will be analyzed using a linear mixed-effects model with each respective biomarker as the outcome variable and treatment/breastfeeding group, time, and their interaction as the primary fixed effects of interest. Additionally, there will be random effect to account for the repeated observations within participants.

Subgroup and Exploratory Analyses: We will conduct two *a priori* subgroup analyses on the primary outcome of 6-month LVEF. These will be operationalized in the model by including the main effect of the subgroup and the two-way interaction with study arm, with the significance of the interaction term denoting a subgroup effect. For the *a priori* subgroup analyses, we will investigate race/ethnicity (African American versus Non-African American) and NYHA class (class IV versus classes I-III). All other subgroup analyses will be considered exploratory regardless of result. We acknowledge that we do not have sufficient power to detect subgroup effects; however, the power for the proposed clinical trial is based on our primary hypothesis in Aim 1.

R Code for Simulation Studies Demonstrating Power

```
#Load "truncnorm" package which is needed to create truncated normal data
#Note that truncated normal necessary to mimic baseline LVEF
#(e.g. patients only recruited if LVEF<=0.35)
library(truncnorm)

#Create function that simulates bivariate normal data
#(e.g. 6M LVEF correlated with baseline LVEF)
#This is necessary to avoid 'variance inflation' by
#summing baseline LVEF + change in LVEF to get 6M LVEF
#Better to generate BL and 6M using bivariate normal as follows
#Baseline LVEF truncated to values between 0 and 0.35
#Follow-Up LVEF truncated to values between 0 and 0.70
rbvn <- function(n, mu0, s0, mu6, s6, rho)
{

LVEF_BL <- rtruncnorm(n, a=0, b=0.35, mean=mu0, sd=s0)

LVEF_6M <- rtruncnorm(n, a=0, b=0.70, mean=mu6+(s6/s0)*rho*(LVEF_BL-mu0), sd=sqrt((1-
rho^2)*s6^2))

cbind(LVEF_BL, LVEF_6M)
}

#Write code that will simulate 100,000 trials using this data generating mechanism

nSims <- 100000 #number of simulated trials
nPatients <- 90 #number of patients per treatment arm available for analysis
pvalue <-numeric(nSims) #set up empty container for all simulated p-values
reject_null <-numeric(nSims) # set up empty container for final conclusions

#Set seed so results are reproducible
set.seed(73)

for(i in 1:nSims){

## Generate "Standard Therapy" Data
treatment=rep(c(1), times=nPatients)
## Generate LVEF Data for "Standard Therapy" Group
lvef <- rbvn(n=nPatients, mu0=0.27, s0=0.1, mu6=0.45, s6=0.1, rho=.2)
## Merge Into Single Data Frame
groupA=data.frame(cbind(treatment, lvef))

## Generate "Bromocriptine" Data
treatment=rep(c(2), times=nPatients)
## Generate LVEF Data for "Bromocriptine" Group
lvef<-rbvn(n=nPatients, mu0=0.27, s0=0.1, mu6=0.50, s6=0.1, rho=.2)
## Merge Into Single Data Frame
groupB=data.frame(cbind(treatment, lvef))

## Merge Placebo and Bromocriptine Groups into Single Data Frame
sampletrial=rbind(groupA, groupB)

## Analysis: ANCOVA with fixed effect for treatment and covariate for baseline LVEF
model <- lm(LVEF_6M ~ treatment + LVEF_BL, data = sampletrial)
summary(model)
pvalue[i]=summary(model)$coefficients[11]

}

reject_null[pvalue<=0.05]=1
reject_null[pvalue>0.05]=0
table(reject_null)
```

References

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