STATISTICAL ANALYSIS PLAN

A Double-blind, Randomized, Placebo-controlled, Multicenter Study to Assess the Efficacy and Safety of Aficamten in Patients with Obstructive Hypertrophic Cardiomyopathy

Protocol Number: **CY 6031**

Version*: V 1.0*

Date: 14/11/2023

Authors: Gregory D. Lewis, Matthew M.Y. Lee

Does this Supplemental Statistical Analysis Plan document any analysis with the objective of claiming pre-specification?

☑ Yes

☐ No

Version Number	**Date (DDMMYYYY)**	**Summary of Changes, including rationale for changes**
Original (v1.0)	14/11/2023	
Amendment 1 (v2.0)		
Amendment 2 (v3.0)		

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Table of Abbreviations

BL	baseline
BP	Blood pressure
CPET	cardiopulmonary exercise testing
CSR	Clinical Study Report
EQ-5D-5L	EuroQol 5-dimension 5-level instrument
HCM	hypertrophic cardiomyopathy
HR	heart rate
HRR	heart rate recovery
hs-cTnI	high sensitivity cardiac troponin I
KCCQ-CSS	Kansas City Cardiomyopathy Questionnaire-Clinical Summary Score
LVEF	left ventricular ejection fraction
LVOT-G	left ventricular outflow tract gradient
METS	metabolic equivalent of task
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
$PETCO_2$	end-tidal partial pressure of carbon dioxide
RER	respiratory exchange ratio
SAP	Statistical Analysis Plan
SEQUOIA-HCM	Safety, Efficacy, and Quantitative Understanding of Obstruction Impact of Aficamten in Hypertrophic Cardiomyopathy
SSAP	Supplemental Statistical Analysis Plan
VAT	ventilatory anaerobic threshold
VCO_2	carbon dioxide output
VE	minute ventilation
VO_2	oxygen uptake
VO_2RD	VO_2 recovery delay
W24	week 24

INTRODUCTION

This Supplemental Statistical Analysis Plan (SSAP) outlines the statistical analyses to be performed beyond the analyses described within the SAP version 1.0 for data collected within the scope of Cytokinetics protocol CY 6031, titled “A Phase 3, Multi-Center, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Efficacy and Safety of CK-3773274 in Adults with Symptomatic Hypertrophic Cardiomyopathy and Left Ventricular Outflow Tract Obstruction.” The objective of this SSAP is to prespecify non-Clinical Study Report (CSR) related analyses intended to provide supportive information around the effect of Aficamten on comprehensive measures of exercise capacity on cardiopulmonary exercise testing beyond peak oxygen uptake.

OBJECTIVES

To evaluate the effect of treatment with aficamten compared with placebo on comprehensive measures of exercise capacity on cardiopulmonary exercise testing beyond peak oxygen uptake during the course of study CY 6031.

ANALYSIS SUBSET(S)

By default, all secondary analyses will include all FAS patients (i.e. those included in the primary reporting of the SEQUOIA-HCM trial).

Please include any additional inclusion/exclusion criteria required for this specific analysis. Leave blank if no additional exclusions required.

Prespecified subgroups for the analysis are listed in Section 4.2.

DEFINITIONS

All definitions of interest used in these analyses (definitions related to study endpoints, study time points, demographics and baseline related and other study related definitions) will match those described in the study CY 6031 SAP.

ENDPOINT CATEGORIES AND ANALYSES

The objective/s of these analyses are to:

- ☐ Compare treatment groups within the subgroup categories.
- ☐ Compare specific subgroups with one another regardless of the treatment arm assigned.
- ☒ Both
- ☐ Neither (objective: _____)

Endpoints

- Main Endpoint: Change in the composite of two Z-scores of CPET parameters from baseline to Week 24: - peak VO_2 (maximal exercise capacity) - VE/VCO_2 slope (sub-maximal capacity – independent of volitional effort)

- Other Endpoints:
    - Change in the following peak exercise parameters in aficamten vs placebo from BL to W24:
        - Peak VO_2
        - Percent predicted peak VO_2
        - Peak METS
        - Peak circulatory power
        - Peak exercise time
        - Peak RER
        - Peak $PETCO_2$
        - Change in heart rate (resting to peak)
    - Change in the following sub-maximal exercise parameters in aficamten vs placebo from BL to W24
        - $VE/VCO2_{pre\text{-}VATslope}$
        - $VE/VCO2_{overall\text{-}slope}$
        - Ventilatory power
        - Ventilatory anaerobic threshold
        - VO_2/workload slope
        - Oxygen uptake efficiency slope (VO_2/logVE slope)
        - VO_2 recovery kinetics
        - O_2 pulse plateau (y/n)
        - O_2 pulse plateau gradient

- Relationships between main endpoint as well as both peak and non-peak exercise parameters and:
    i. Change in health status (determined by KCCQ) from BL to W24
    ii. Change in symptoms determined by NYHA from BL to W24
    iii. Change in NT-proBNP from BL to W24
    iv. Change in troponin level from BL to W24
    v. Change in Rest and Valsalva LVOT-G from BL to W24
    vi. Change in LAVI from BL to W24

- **RECOVERY MEASURES**
    a. Heart rate recovery (HRR) at 1 minute and 2 minutes
    b. VO_2 recovery kinetics (VO_2RD)
    c. $T_{1/2}$ VO_2 (time for VO_2 to decrease to 50% of peak VO_2 adjusted for resting VO_2)

- **Threshold analysis between placebo and aficamten (reference to KCCQ approach) – responder analysis - proportion achieving the following between BL to W24:**
    a. Improvement of peak VO_2 by 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 mL/kg/min
    b. Deterioration of peak VO_2 by 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 mL/kg/min
    c. Improvement of percent predicted peak VO_2 by 5, 10, 15, 20 and 25%
    d. Deterioration of percent predicted peak VO_2 by 5, 10, 15, 20 and 25%

COVARIATES AND SUBGROUPS

Baseline Covariates

By default, baseline variables shown in Table 1 will mirror those presented in the primary SEQUOIA-HCM manuscript:

Age, years

Sex, n (%)
Female
Male
Race, n (%)
Asian
White
Black or African American
etc. (race category can be sorted by descending order of % in overall
Region, n (%)
China
North America
Rest of World
Hypertrophic cardiomyopathy genetic testing performed, n (%)
Pathogenic or variant of uncertain significance
Medical history, n (%)
Family history of hypertrophic cardiomyopathy
Family history of HCM and/or known HCM-causing gene mutation
Time since HCM diagnosis, years
Myocardial infarction
Coronary artery disease
Paroxysmal atrial fibrillation
Permanent atrial fibrillation
Cardiac syncope
Sustained ventricular tachycardia
Ventricular fibrillation
Hypertension

Torsades de Pointes
Background Hypertrophic cardiomyopathy therapy, n (%)
Beta-blocker
Calcium channel blocker
Disopyramide
Implantable cardioverter-defibrillator, n (%)
Body-mass index, kg/m², mean (SD)
Resting heart rate, beats per min
Systolic blood pressure, mm Hg
Diastolic blood pressure, mm Hg
NYHA functional class, n (%)
Class II
Class III
CPET parameters
CPET modality
Peak VO_2, mL/kg per min
Peak RER
% predicted peak VO_2
Chronotropic incompetence (defined as inability to achieve 80% of predicted peak heart rate during maximal exercise)
KCCQ-CSS
NT-proBNP, geometric mean, ng/L (CV%)
High-sensitivity cardiac troponin I, geometric mean, ng/L (CV%)
Echocardiographic parameters
LVEF, %
LVOT-G, rest, mm Hg
LVOT-G, Valsalva, mm Hg

Maximum left ventricular wall thickness, mm
Left atrial volume index, mL/m²
Left ventricular mass index, g/m²

Please include any <u>additional</u> variables required for this specific analysis. Leave blank if no additional baseline variables are required.

Subgroups

Prespecified subgroups for the analysis include, but are not limited to:

Subgroups already delineated in the primary SAP include:

- *Sex*
- *Age group*
- *Baseline body mass index*
- *Baseline NYHA Class*
- *Baseline KCCQ-CSS*
- *Baseline LVEF*
- *NT-proBNP*
- *CPET modality*
- *Baseline peak VO_2*
- *Beta-blocker use*
- *Baseline resting LVOT-G (continuous)*
- *Baseline Valsalva LVOT-G (continuous)*
- *Sarcomeric gene mutation status (pathogenic or variant of uncertain significance, and non-disease causing or none)*

Additional Subgroups to be included in this analysis*:*

- Septal wall thickness

- Left atrial volume index
- Mitral regurgitant jet:left atrial area ratio (mitral regurgitation severity)
- LV end-diastolic volume
- LV end-systolic volume
- Valsalva LVOT gradient
- Left ventricular ejection fraction
- Plasma hsTroponin I concentration
- Blood pressure
- Heart rate
- Non-dihydropyridine calcium-channel blocker use
- Disopyramide use
- Chronotropic incompetence y/n (*as defined by inability to achieve 80% of predicted peak heart rate during maximal exercise)*

ENDPOINT CATEGORIES AND ANALYSES

The objective/s of these analyses are to:

☒ Compare treatment groups within the subgroup categories.

☐ Compare specific subgroups with one another regardless of the treatment arm assigned.

☐ Both

☐ Neither (objective: _____)

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