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RESEARCH**

APPLICATION NUMBER:

219083Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	NDA
Application number(s)	219083
Priority or standard	Standard
Submit date(s)	9/26/2024
Received date(s)	9/26/2024
PDUFA goal date	12/26/2025
Division/office	Division of Cardiology and Nephrology (DCN)
Review completion date	12/18/2025
Established/proper name	Aficamten
(Proposed) proprietary name	MYQORZO
Pharmacologic class	Cardiac myosin inhibitor
Other product name(s)	CK-3773274
Applicant	Cytokinetics Inc
Dosage form(s)/formulation(s)	Tablet, film-coated
Dosing regimen	5 mg, 10 mg, 15 mg, and 20 mg
Applicant-proposed indication(s)/population(s)	Treatment of adults with symptomatic obstructive hypertrophic cardiomyopathy (oHCM)
SNOMED CT code for proposed indication disease term(s)¹	45227007 Hypertrophic obstructive cardiomyopathy (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	5 mg, 10 mg, 15 mg, and 20 mg
Approved indication(s)/population(s) (if applicable)	Treatment of adults with symptomatic obstructive hypertrophic cardiomyopathy (oHCM) to improve functional capacity and symptoms
SNOMED CT code for approved indication disease term(s)¹	45227007 Hypertrophic obstructive cardiomyopathy

¹ For internal tracking purposes only.

Abbreviations: PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

Table of Contents

Table of Tables	vii
Table of Figures	xiv
Glossary	1
I. Executive Summary	3
1. Overview	3
1.1. Summary of Regulatory Action	3
1.2. Conclusions on Substantial Evidence of Effectiveness	3
2. Benefit-Risk Assessment	5
2.1. Benefit-Risk Framework	5
2.2. Conclusions Regarding Benefit-Risk	9
II. Interdisciplinary Assessment	10
3. Introduction	10
3.1. Review Issue List	11
3.1.1. Key Efficacy Review Issues	11
3.1.1.1. Is the Observed Treatment Effect of Aficamten on pVO ₂ Clinically Meaningful?	11
3.1.1.2. Impact of Imputation of Missing Data for Continuous Variable Endpoints on Assessment of Efficacy	11
3.1.1.3. Impact of Imputation of Missing Data for Categorical Variable Endpoints on Assessment of Efficacy	11
3.1.1. Key Safety Review Issues	11
3.1.1.1. Risk of Heart Failure Due to Systolic Dysfunction	11
3.2. Approach to the Clinical Review	11
3.3. Approach To Establishing Substantial Evidence of Effectiveness	12
4. Patient Experience Data	16
5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology	17
5.1. Nonclinical Assessment of Potential Effectiveness	17
5.1.1. Mechanism of Action	17
5.1.2. In Vitro and In Vivo Pharmacology	18
5.1.3. Established Pharmacologic Class and Rationale	19
5.2. Clinical Pharmacology/Pharmacokinetics	20
6. Efficacy (Evaluation of Benefit)	23

6.1. Assessment of Dose and Potential Effectiveness	23
6.2. Clinical Studies/Trials Intended To Demonstrate Efficacy	31
6.2.1. CY 6031 (SEQUOIA-HCM)	31
6.2.1.1. Study Design, CY 6031	31
6.2.1.2. Eligibility Criteria, CY 6031	33
6.2.1.3. Statistical Analysis Plan, CY 6031.....	34
6.2.1.4. Results of Analyses, CY 6031.....	38
6.2.2. CY 6021 (REDWOOD-HCM).....	49
6.3. Key Efficacy Review Issues	50
6.3.1. Is the Observed Treatment Effect of Aficamten on pVO ₂ Clinically Meaningful?	50
6.3.2. Impact of Imputation of Missing Data for Continuous Variable Endpoints on Assessment of Efficacy.....	53
6.3.3. Impact of Imputation of Missing Data for Categorical Variable Endpoints on Assessment of Efficacy.....	54
7. Safety (Risk and Risk Management).....	54
7.1. Potential Risks or Safety Concerns Based on Nonclinical Data.....	54
7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug- Specific Factors	56
7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience	56
7.4. FDA Approach to the Safety Review	56
7.5. Adequacy of the Clinical Safety Database	57
7.6. Safety Results	58
7.6.1. Safety Results.....	58
7.6.1.1. Overview of Treatment-Emergent Adverse Events	58
7.6.1.2. Deaths	58
7.6.1.3. Serious Treatment-Emergent Adverse Events.....	59
7.6.1.4. Adverse Events Leading to Treatment Discontinuation.....	60
7.6.1.5. Clinical Events of Special Interest	61
7.6.1.6. Treatment-Emergent Adverse Events.....	61
7.6.1.6.1. Hypertension	62
7.6.1.7. Echocardiograms: LVEF	63
7.6.1.8. Laboratory Findings	64

7.6.1.9. Assessment of Drug-Induced Liver Injury	67
7.6.1.10. Vital Signs	68
7.7. Key Safety Review Issues	71
7.7.1. Risk of Heart Failure Due to Systolic Dysfunction	71
8. Therapeutic Individualization	73
8.1. Intrinsic Factors	73
8.2. Extrinsic Factors	77
8.3. Plans for Pediatric Drug Development	82
8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential	83
9. Product Quality	84
9.1. Device or Combination Product Considerations	85
10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review	85
11. Advisory Committee Summary	85
III. Additional Analyses and Information	86
12. Summary of Regulatory History	86
13. Pharmacology Toxicology	87
13.1. Summary Review of Studies Submitted With the Investigational New Drug Application	87
13.1.1. Safety Pharmacology	89
13.1.2. Pharmacokinetics	90
13.1.2.1. Absorption and Distribution	90
13.1.2.2. Metabolism and Excretion	90
13.1.2.3. Drug-Drug Interactions	93
13.1.3. General Toxicology	93
13.1.3.1. Pivotal General Toxicology Studies	95
13.1.4. Genetic Toxicology	97
13.1.4.1. Carcinogenesis, Mutagenesis, and Impairment of Fertility	98
13.1.4.1.1. Carcinogenicity	99
13.1.4.2. Reproductive Toxicology	99
13.1.4.2.1. Summaries of Pivotal Developmental and Reproductive Toxicology Studies	102
13.1.5. Other Toxicology/Specialized Studies	112

13.1.5.1. Metabolites	112
13.1.5.2. Skin and Eye Irritation Studies.....	114
13.1.5.2.1. Impurities/Degradants	114
13.2. Individual Reviews of Studies Submitted With the New Drug Application	117
14. Clinical Pharmacology	119
14.1. In Vitro Studies.....	119
14.2. In Vivo Studies	122
14.2.1. Study CY 6011: Single- and Multiple-Dose Pharmacokinetics in Healthy Subjects and Single-Dose Pharmacokinetics in CYP2D6 Poor Metabolizers.....	122
14.2.2. Study CY 6012: Food Effect of the To-Be-Marketed Formulation in Healthy Volunteers.....	129
14.2.3. Study CY 6014: DDI Potential With CYP3A Inhibitors, CYP Inducers, and P-gp Substrates in Healthy Volunteers.....	131
14.2.4. Study CY 601-10: DDI Potential With CYP3A, CYP2C9, CYP2C19, and CYP2D6 Inhibitors in Healthy Volunteers	137
14.2.5. Study CY 6017: Effect of Hepatic Impairment on Aficamten Pharmacokinetics	142
14.2.6. Study CY 6013: Mass Balance	144
14.2.7. Study CY 6019: Single-Dose Pharmacokinetics and Effect on QTc Interval in Healthy Subjects	146
14.2.8. Study CY 6021: Dose Finding	146
14.2.9. Trial CY 6031: Dose Proportionality and Phase 3 Trial Formulation	148
14.3. Bioanalytical Method Validation and Performance	149
14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety...	177
14.5. Pharmacometrics Assessment.....	177
14.5.1. Summary of Applicant’s Population PK Analysis.....	177
14.5.2. Exposure-Response Analysis	181
14.5.3. Physiologically-Based PK Modeling	192
14.5.3.1. Executive Summary.....	192
14.5.3.2. Background.....	192
14.5.3.3. Methods	192
14.5.3.4. Results	200
14.6. Pharmacogenetics	208

15. Study/Trial Design	210
16. Efficacy	211
16.1. Additional Efficacy Results in CY 6031 (SEQUOIA-HCM).....	211
16.2. Key Efficacy Results in CY 6021 (REDWOOD-HCM)	216
17. Clinical Safety	218
17.1. Narratives.....	218
18. Clinical Virology	223
19. Clinical Microbiology	223
20. Mechanism of Action/Drug Resistance.....	223
21. Other Drug Development Considerations	223
22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)	224
23. Labeling: Key Changes	224
23.1. Approved Labeling Types	226
24. Postmarketing Requirements and Commitments	226
25. Financial Disclosure	227
26. References	228
27. Review Team.....	230
27.1. Reviewer Signatures	230

Table of Tables

Table 1. Application Information	i
Table 2. Benefit-Risk Framework.....	5
Table 3. Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations ¹ for Aficamten	13
Table 4. Patient Experience Data Submitted or Considered.....	16
Table 5. Summary of Clinical Pharmacology and Pharmacokinetics.....	20
Table 6. Steady-State Plasma PK Parameters of Aficamten Following Once Daily Dosing of 5 and 10 mg Aficamten in Healthy Participants	20
Table 7. Summary of Statistical Assessment for Food Effect on Plasma Aficamten Exposure Following a Single Oral Dose of Aficamten 20 mg as Proposed Commercial Tablet Formulation, Study CY 6012	21
Table 8. Highest Doses Achieved in Study CY 6021 (Cohorts 1 and 2).....	24
Table 9. Echocardiogram Criteria for Scheduled Dose Titrations in Weeks 2, 4, and 6, Trial CY 6031	26
Table 10. Tabulated Proposed Aficamten Echocardiography-Based Dose Titration Criteria With Instructions for Dose Titration, Timing of Next Potential Dose Titration, and Management of Potential Events of LVEF <50%	27
Table 11. Proposed Commercial Dosing Regimen: Clinical Trial Simulation of Percent of Site-Read LVEF <50% or Site-Read Post-Valsalva LVOT-G <30 mm Hg Over 3 Years of Daily Aficamten Administration.....	29
Table 12. Echocardiogram Criteria for Scheduled Dose Titrations, Trial CY 6031	32
Table 13. Subject Screening and Enrollment, Trial CY 6031	38
Table 14. Subject Disposition, All -Randomized Set, Trial CY 6031	39
Table 15. Summary of Important Protocol Deviations Based on Recategorization of Severity Postdatabase Lock, Trial CY 6031	39
Table 16. Baseline Demographics, Full Analysis Set, Trial CY 6031	40
Table 17. Clinical Characteristics, Full Analysis Set, Trial CY 6031	41
Table 18. Change in pVO ₂ on CPET From Baseline to Week 24, Full Analysis Set, Trial CY 6031	42
Table 19. Summary of Missing pVO ₂ on CPET at Week 24, Full Analysis Set, Trial CY 6031	42
Table 20. Change in KCCQ-CSS From Baseline at Weeks 12 and 24, Full Analysis Set, Trial CY 6031	44
Table 21. Proportion of Subjects With ≥1 Class Improvement in NYHA Functional Classification From Baseline to Weeks 12 and 24, Full Analysis Set, Trial CY 6031.....	45

Table 22. Change in Valsalva Core-Laboratory-Read LVOT-G From Baseline to Weeks 12 and 24, Full Analysis Set, Trial CY 6031	46
Table 23. Proportion of Subjects With Valsalva LVOT-G <30 mm Hg Per Core Laboratory Determination at Weeks 12 and 24, Full Analysis Set, Trial CY 6031	47
Table 24. Total Duration of SRT Eligibility During the 24-Week Treatment Period in Subjects Who Were SRT Eligible at Baseline, Full Analysis Set, Trial CY 6031	48
Table 25. Change in Total Workload During CPET From Baseline to Week 24, Full Analysis Set, Trial CY 6031	49
Table 26. Proportion of Subjects Achieving Improvement Defined by Both pVO ₂ and NYHA Functional Class Criteria at Week 24, Full Analysis Set, Trial CY 6031	52
Table 27. Duration of Exposure, Safety Population, Trial CY 6031	57
Table 28. Duration of Exposure, Safety Population, Study CY 6022	57
Table 29. Overview of Treatment-Emergent Adverse Events, Safety Population, Trial CY 6031	58
Table 30. Subjects With Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term, Safety Population, Trial CY 6031	59
Table 31. Subjects With Adverse Events ¹ Leading to Treatment Discontinuation by System Organ Class and Preferred Term, Safety Population, Trial CY 6031	60
Table 32. Summary of Clinical Events of Special Interest, Safety Population, Trial CY 6031	61
Table 33. Subjects With Treatment-Emergent Adverse Events ¹ by System Organ Class and OND Custom Medical Queries (Narrow) Occurring at ≥2% Higher Frequency in Aficamten Group Than Placebo Group, Safety Population, Trial CY 6031	62
Table 34. Subjects With One or More Chemistry Analyte Values With Elevated or Low Values Meeting Specified Levels, Safety Population, Trial CY 6031	64
Table 35. Subjects With One or More Kidney Function Analyte Values Exceeding Specified Levels, Safety Population, Trial CY 6031	66
Table 36. Subjects With One or More Liver Biochemistry Analyte Values Exceeding Specified Levels, Safety Population, Trial CY 6031	68
Table 37. Percentage of Subjects With Maximum Systolic Blood Pressure by Category of Blood Pressure Postbaseline, Safety Population, Trial CY 6031	70
Table 38. Percentage of Subjects With Maximum Diastolic Blood Pressure by Category of Blood Pressure Postbaseline, Safety Population, Trial CY 6031	70
Table 39. Shift Table by Category of Systolic Blood Pressure at Baseline vs. Category of Maximum Postbaseline Systolic Blood Pressure, Safety Population, Trial CY 6031	70

Table 40. Shift Table by Category of Diastolic Blood Pressure at Baseline vs. Category of Maximum Postbaseline Diastolic Blood Pressure, Safety Population, Trial CY 6031	71
Table 41. Incidence and Event Rate of Serious Heart Failure in the Aficamten Clinical Program	72
Table 42. MDRD: Summary of Aficamten Population PK-Predicted Exposures at Steady State Following Once Daily Dosing of 15-mg Aficamten in Subjects With oHCM by Renal Function, Studies CY 6021 and CY 6031; N=183	75
Table 43. Observed Effect of Coadministered Drugs on the Pharmacokinetics of Aficamten in Healthy Subjects, Studies CY 6014 and CY 601-10	78
Table 44. Aficamten Mean AUC Increase Predictions Based on Different PBPK Models.....	80
Table 45. Aficamten Mean AUC Increase Predictions Based on Different PBPK Models and the Clinical Pharmacology Review Team’s Dose Recommendations	80
Table 46. Nonclinical Data Supporting Labeling on Fertility, Pregnancy, and Lactation	84
Table 47. Summary of Key Regulatory History, SEQUOIA-HCM	87
Table 48. Primary Pharmacology Studies.....	87
Table 49. Secondary Pharmacology Studies.....	89
Table 50. Safety Pharmacology Studies	89
Table 51. Metabolite Exposures in Nonclinical Studies	92
Table 52. Study Information: Nonclin-0054 Study	95
Table 53. Observations and Results: Nonclin-0054 Study	95
Table 54. Toxicokinetics: Nonclin-0054 Study	96
Table 55. Study Information: Nonclin-0056 Study	96
Table 56. Observations and Results: Nonclin-0056/CK-3773274 Study	97
Table 57. Toxicokinetics: Nonclin-0056Study	97
Table 58. Genetic Toxicology.....	98
Table 59. Reproductive Toxicity Summary.....	100
Table 60. Study Information: Nonclin-0264 Study	102
Table 61. Observations and Results: Nonclin-0264 Study	102
Table 62. Study Information: Nonclin-0161 Study	103
Table 63. Observations and Results: Nonclin-0161 Study	104
Table 64. Notable Findings From Rat EFD Study Nonclin-0161	105
Table 65. Toxicokinetics: Nonclin-0161 Study	106

Table 66. Study Information: Nonclin-0199Study	107
Table 67. Observations and Results: Nonclin-0199Study	107
Table 68. Notable Findings From Study Nonclin-0199.....	108
Table 69. Toxicokinetics: Nonclin-0199 Study	109
Table 70. Study Information: Nonclin-0360 Study	109
Table 71. Observations and Results: Nonclin-0360 Study	110
Table 72. Notable Findings From Study Nonclin-0360.....	111
Table 73. Toxicokinetics: Nonclin-0360 Study	112
Table 74. Summary of Skin and Eye Irritation Studies	114
Table 75. Impurities and Dose Multiples: CK-4030838 Study	116
Table 76. Summary: Nonclin-0545 Study	117
Table 77. Main Test, Plate Incorporation Assay.....	119
Table 78. Summary of Plasma Aficamten PK Parameters Following a Single Oral Dose of Aficamten at Ascending Dose Levels in Healthy Subjects, Study CY 6011, Part 1	124
Table 79. Summary of Plasma and Urine Aficamten PK Parameters Following Multiple Oral Doses of Aficamten at Ascending Dose Levels in Healthy Subjects, Study CY 6011, Part 2	126
Table 80. Summary of Plasma Aficamten PK Parameters Following a Single Oral Dose of 10-mg Aficamten in CYP2D6 PMs, Study CY 6011, Part 3	127
Table 81. Summary of Statistical Assessment for Effect of CYP2D6 Phenotype (Normal vs. Poor Metabolizer) on Plasma Aficamten Exposure Following a Single Oral Dose of 10-mg Aficamten Under Fasted State, Study CY 6011	127
Table 82. Summary of Plasma Aficamten PK Parameters Following a Single Oral Dose of 10-mg Aficamten as Encapsulated Granules and Phase 2 Tablet Formulation, Study CY 6011, Part 5	128
Table 83. Summary of Statistical Assessment for Relative Bioavailability of Plasma Aficamten Between Encapsulated Granules and Phase 2 Tablet Formulation of 10-mg Aficamten, Study CY 6011, Part 5.....	129
Table 84. Summary of Plasma PK Parameters of Aficamten Following Fasted- and Fed-State Administration of a Single Oral Dose of Aficamten 20 mg as Proposed TBM Tablet Formulation Study CY 6012	130
Table 85. Summary of Statistical Assessment for Food Effect on Plasma Aficamten Exposure Following a Single Oral Dose of Aficamten 20 mg as Proposed TBM Tablet Formulation, Study CY 6012.....	130
Table 86. Summary of Plasma PK Parameters of Aficamten, CK-3834282, and CK- 3834283 Following a Single Oral Dose of 10-mg Aficamten Alone and Upon Coadministration With 200-mg Itraconazole QD, Study CY 6014, Cohort 1	132

Table 87. Summary of Statistical Assessment for the Effect of Itraconazole Coadministration on Aficamten Plasma Exposure, Study CY 6014, Cohort 1	133
Table 88. Summary of Plasma PK Parameters of Aficamten, CK-3834282, and CK-3834283 Following a Single Oral Dose of 20-mg Aficamten Alone and Upon Coadministration With 300-mg Carbamazepine BID, Study CY 6014, Cohort 2.....	134
Table 89. Summary of Statistical Assessment for the Effect of Carbamazepine Coadministration on Aficamten Plasma Exposure, Study CY 6014, Cohort 2	135
Table 90. Summary of Plasma PK Parameters of Free and Total Dabigatran Following a Single Oral Dose of 75-mg Dabigatran Etexilate Alone and Upon Coadministration With 20-mg Aficamten, Study CY 6014, Cohort 3	136
Table 91. Summary of Statistical Assessment for the Effect of Aficamten Coadministration on Total and Free Dabigatran Plasma Exposure, Study CY 6014, Cohort 3	137
Table 92. Summary of Plasma Aficamten Pharmacokinetics Following 10-mg Aficamten on Day 1 and 10-mg Aficamten Coadministered With 400-mg QD Fluconazole on Day 19, PK Analysis Population, Trial 601-10, Cohort 1.....	139
Table 93. Summary of Statistical Comparisons of Plasma Aficamten PK Parameters Following 10-mg Aficamten Coadministered With 400-mg QD Fluconazole on Day 19 vs. 10-mg Aficamten on Day 1, PK Analysis Population, Trial 601-10, Cohort 1	139
Table 94. Summary of Plasma Aficamten Pharmacokinetics Following 10-mg Aficamten on Day 1 and 10-mg Aficamten Coadministered With 40-mg QD Paroxetine on Day 26, PK Analysis Population, Trial 601-10, Cohort 2	140
Table 95. Summary of Statistical Comparisons of Plasma Aficamten PK Parameters Following 10-mg Aficamten Coadministered With 40-mg QD Paroxetine on Day 26 vs. 10-mg Aficamten on Day 1, PK Analysis Population, Trial 601-10, Cohort 2.....	140
Table 96. Summary of Plasma Aficamten Pharmacokinetics Following 10-mg Aficamten on Day 1 and 10-mg Aficamten Coadministered With 40-mg Fluoxetine QD on Day 28, PK Analysis Population, Trial 601-10, Cohort 3	141
Table 97. Summary of Statistical Comparisons of Plasma Aficamten PK Parameters Following 10-mg Aficamten Coadministered With 40-mg Fluoxetine QD on Day 28 vs. 10-mg Aficamten on Day 1, PK Analysis Population, Trial 601-10, Cohort 3.....	142
Table 98. Summary of Plasma PK Parameters of Aficamten Following a Single Oral Dose of 20-mg Aficamten in Subjects With Moderate Hepatic Impairment and Normal Hepatic Function, Study CY 6017	143
Table 99. Summary of Statistical Assessment for the Effect of Moderate Hepatic Impairment on Plasma Exposure of Aficamten Following a Single Oral Dose of 20-mg Aficamten, Study CY 6017	144

Table 100. Summary of Plasma PK Parameters for Aficamten, M1a (CK-3834282), M1b (CK-3834283), and Total Radioactivity Following a Single Oral Dose of 20 mg ¹⁴ C-Aficamten in Healthy Male Subjects, Study CY 6013	145
Table 101. Echocardiogram Criteria for Scheduled Dose Titrations, Study CY 6021, Cohorts 1 and 2	147
Table 102. Simulated Steady-State Exposure by Dose Level in Subjects With oHCM, Trial CY 6031	149
Table 103. Bioanalytical Methods Supporting the Clinical Pharmacology Assessment of Aficamten and Its Metabolites CK-3834282 and CK-3834283	150
Table 104. Summary of Method Performance for Determination of Aficamten in Human Plasma (CA26485-01).....	151
Table 105. Summary of Method Performance for Determination of Aficamten in Human Urine (CA27882-01)	159
Table 106. Summary of Method Performance for Determination of CK-3834282 and CK-3834283 in Human Plasma (CA29736-01).....	162
Table 107. Summary of Method Performance for Determination of Moxifloxacin in Human Plasma (ZZ00945-02)	172
Table 108. Summary of Method Performance for Determination of Free Dabigatran in Human Plasma (ZZ55164-01)	175
Table 109. Summary of the Number of Subjects and PK Samples	179
Table 110. Population Parameter Estimates for the Final PPK Model	179
Table 111. PK/PD Subjects and Data Summary.....	183
Table 112. Post-Valsalva LVOT-G PK/PD Final Model Parameters.....	185
Table 113. LVEF PK/PD Final Model Parameters.....	185
Table 114. Summary of Safety Endpoints by Treatment Group and by C _{max,ss} Quartiles in the Aficamten-Treated Group for the Exposure-Safety Dataset	191
Table 115. Final Input Parameters in the Aficamten Model.....	195
Table 116. Observed and Predicted Effects of Carbamazepine on Various CYP Substrates	196
Table 117. Observed and Predicted Median Clearance Ratios for Coadministration of Carbamazepine With S-Warfarin.....	196
Table 118. Observed and Predicted Seladelpar Exposure and Effects of CYP Precipitants on Seladelpar Exposure.....	197
Table 119. Simulated Effects of Rifampin 15 mg Once Daily on the Exposure of CYP Substrates	198
Table 120. Predicted and Observed Fluconazole Interactions With Substrates of CYP3A, CYP2C9, and CYP2C19	200

Table 121. Predicted and Observed Effects of Paroxetine on CYP3A Substrates	200
Table 122. Simulated and Observed PK Parameters Following Oral Administration of Aficamten in Healthy Subjects	201
Table 123. Simulated and Observed PK Parameters Following Oral Administration of a Single Dose of 10-mg Aficamten in Healthy Subjects With Different CYP2D6 Phenotypes	203
Table 124. Predicted Drug Interactions of a Single Dose Aficamten as a Victim of CYP Inhibitors and Inducers in Healthy Subjects	204
Table 125. Summary of Model Development and Verification of the Aficamten PBPK Model With or Without CYP2D6-Mediated Metabolism	207
Table 126. Simulated Changes in CYP2C9 Expression and Aficamten AUC _{inf} in CYP2C9 NM and PM Populations	208
Table 127. Simulated Changes in CYP2C19 Expression and Aficamten AUC _{inf} in CYP2C19 NM and PM Populations	208
Table 128. Clinical Pharmacogenetics Implementation Consortium Standard for CYP2C9 Genotype-to-Phenotype Translation Using Activity Scores	209
Table 129. Frequency of the CYP2C9 Phenotypes Across Racial/Ethnic Populations...	209
Table 130. Summary of Changes to the Protocol for SEQUOIA-HCM.....	210
Table 131. Forest Plot of Change from Baseline to Week 24 in pVO ₂ on CPET by Demographics Subgroups, Full Analysis Set, Trial CY 6031	213
Table 132. Forest Plot of Change From Baseline to Week 24 in pVO ₂ on CPET by Baseline Characteristics Subgroups, Full Analysis Set, Trial CY 6031	214
Table 133. Tipping Point Analysis: Change in pVO ₂ on CPET From Baseline at Week 24, Full Analysis Set, Trial CY 6031	215
Table 134. Change From Baseline in Peak Resting LVOT-G, PD Analysis Set, Study CY 6021	216
Table 135. Change From Baseline in Peak Post-Valsalva LVOT-G, PD Analysis Set, Study CY 6021.....	217
Table 136. Summary of Change in NYHA Class From Baseline to Week 10, PD Analysis Set, Study CY 6021.....	217
Table 137. Key Labeling Changes and Considerations	224
Table 138. Covered Clinical Studies: Trial CY 6031	227
Table 139. Reviewers of Integrated Assessment	230
Table 140. Additional Reviewers of Application	230
Table 27-1 Signatures of Reviewers	230

Table of Figures

Figure 1. Schematic Illustration of the Mechanism of Action of Aficamten in Obstructive Hypertrophic Cardiomyopathy.....	17
Figure 2. Proposed Commercial Dosing Regimen: Clinical Trial Simulation of Aficamten Exposure and Percent of Subjects With Site-Read Post-Valsalva LVOT-G <30 mm Hg or Site-Read LVEF <50% Over First 52 Weeks of Daily Administration	28
Figure 3. Plasma Levels of Aficamten During the Maintenance Phase, Trial CY 6031 ...	30
Figure 4. Correlation Analyses Between Maintenance Phase LVEF and Prior LVEF or Peak Aficamten Concentrations in Aficamten-Treated Subjects With oHCM, Trial CY 6031	30
Figure 5. Correlation Analyses Between LVEF at Week 24 (LVEF _{Week24}) and Preceding LVEF at Various Timepoints Including Week 20 (4-Week Preceding LVEF _{Week24}), Week 16 (8-Week Preceding LVEF _{Week24}), Week 12 (12-Week Preceding LVEF _{Week24}), and Week 8 (16-Week Preceding LVEF _{Week24}) in Aficamten-Treated Subjects With oHCM, Trial CY 6031	31
Figure 6. Trial Design, Trial CY 6031.....	32
Figure 7. Cumulative Distribution of Change From Baseline to Week 24 in pVO ₂ , Full Analysis Set, Trial CY 6031	51
Figure 8. Mean LVEF Across Time, Safety Population, Trial CY 6031.....	63
Figure 9. Hepatocellular Drug-Induced Liver Injury Screening Plot, Safety Population, Trial CY 6031	67
Figure 10. Mean Systolic Blood Pressure Change From Baseline Over Time by Treatment Group, Safety Population, Trial CY 6031	69
Figure 11. Mean Diastolic Blood Pressure Change From Baseline Over Time by Treatment Group, Safety Population, Trial CY 6031	69
Figure 12. Pharmacokinetic Comparison by Renal Function at Steady-State	76
Figure 13. CK-394113 Amidoxime Intermediate and Related Impurities	118
Figure 14. Geometric Mean (90% CIs) Plasma Aficamten Concentration-Time Profiles Following the Administration of Single Oral Doses of Aficamten (0 to 24 Hours) (Part 1, SAD) (Semi-Log Scale)	123
Figure 15. Geometric Mean (90% CIs) Plasma Aficamten Concentration-Time Profiles Following the Administration of Single Oral Doses of Aficamten (Day 1) and Multiple Once Daily Oral Doses of Aficamten (Day 14/17) (0 to 12 Hours) (Part 2, MAD) (Semi-Log Scale).....	125
Figure 16. Biotransformation Pathways of Aficamten in Human	146
Figure 17. Goodness-of-Fit Plots for Final PPK Model	180
Figure 18. pcVPC vs. Time in Subjects With oHCM for Final PPK Model	180

Figure 19. Impact of Covariates on Steady-State AUC _{tau} in Subjects With oHCM.....	181
Figure 20. Post-Valsalva LVOT-G and LVEF vs. Exposure Stratified by Measurement Type.....	184
Figure 21. Visual Predictive Check for LVOT-G and LVEF PK/PD Models.....	186
Figure 22. Relationship Between Aficamten PK Exposures (C _{avg,ss}) and Efficacy Endpoints Change From Baseline to Week 24 Using All Data (Placebo and Treatment Group, Left Side Figures) and Data from Treatment Group Only (Right Side Figures) Based on Trial CY 6031	187
Figure 23. Relationship Between Aficamten C _{avg,ss} and (A) Probability of Being a Responder (≥1 NYHA Class Improvement From Baseline to Week 24) and (B) Change in hs-cardiac-TnI From Baseline for Placebo and Aficamten-Treated Subjects (Left Panel) and Aficamten-Treated Subjects Alone (Right Panel).....	189
Figure 24. Boxplots of Aficamten C _{max,ss} Grouped by Presence or Absence of Event for Safety Endpoints	190
Figure 25. Modeling and Simulation Strategy	194
Figure 26. Simulated and Observed Mean Plasma Concentration-Time Profiles of a Single Oral Dose of 10-mg Seladelpar in Healthy Subjects	197
Figure 27. Simulated vs. Observed AUC for CYP2D6 Substrates and AUC Ratios for CYP2D6 Phenotypes Relative to EM	198
Figure 28. Simulated and Observed Plasma Concentration-Time Profiles of Tolbutamide and S-Warfarin Following a Single Oral Dose in CYP2C9 NMs and PMs	199
Figure 29. Simulated and Observed PK Profiles Following Oral Administration of 10- mg Aficamten Once Daily for 14 days in Healthy Subjects	201
Figure 30. Simulated and Observed Plasma Concentration-Time Profiles of a Single Oral Dose of 10-mg Aficamten in Healthy CYP2D6 NMs and IMs	202
Figure 31. Simulated and Observed Plasma Concentration-Time Profiles of a Single Oral Dose of 10-mg Aficamten in Healthy CYP2D6 PMs.....	202
Figure 32. Depletion of Aficamten and M1 Formation Following Incubation of Aficamten With Human Liver Microsomes and Recombinant CYP Enzymes (A) or Isoform Selective Chemical Inhibitors (B).....	207
Figure 33. Subject CY-6022-005001-002: LVEF and NT-proBNP Over Time, Study CY 6022	218
Figure 34. Subject CY-6022-005001-003: LVEF and NT-proBNP Over Time, Study CY 6022	219
Figure 35. Subject CY-6022-001002-006: LVEF and NT-proBNP Over Time, Study CY 6022	220
Figure 36. Subject CY-6031-001009-002: Liver Laboratory Over Time, Trial CY 6031.....	221

Figure 37. Subject CY-6031-001022-001: Liver Laboratory Over Time, Trial CY 6031.....	221
Figure 38. Subject CY-6031-008006-008: Liver Laboratory Over Time, Trial CY 6031.....	222
Figure 39. Subject CY-6031-012002-005: Liver Laboratory Over Time, Trial CY 6031.....	223

Glossary

ADME	absorption, distribution, metabolism, and excretion
AE	adverse event
AF	atrial fibrillation
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the concentration time curve
BP	blood pressure
CG	Cockcroft-Gault
CI	confidence interval
C _{max}	maximum plasma concentration
COA	Clinical Outcome Assessment
CPET	cardiopulmonary exercise testing
CPK	creatine phosphokinase
C _{tau}	trough plasma concentration
CV	coefficient of variation
CYP	cytochrome P450
DBP	diastolic blood pressure
DDI	drug-drug interaction
ECAC	Executive Carcinogenicity Assessment Committee
EFD	embryofetal development
eGFR	estimated glomerular filtration rate
E-R	exposure-response
ETASU	elements to assure safe use
FAS	full analysis set
FDA	Food and Drug Administration
GLP	good laboratory practice
HCM	hypertrophic cardiomyopathy
hERG	human ether-à-go-go related gene
HF	heart failure
HI	hepatic impairment
IC ₅₀	half maximal inhibitory concentration
ICD	implantable cardioverter-defibrillator
ICH	International Council for Harmonisation
IND	investigational new drug
IP	investigational product
KCCQ-CSS	Kansas City Cardiomyopathy Questionnaire-Clinical Summary Score
LS	least-squares
LV	left ventricular
LVEF	left ventricular ejection fraction
LVFS	reduced left ventricular fractional shortening
LVOT	left ventricular outflow tract

NDA 219083
MYQORZO (aficamten)

LVOT-G	left ventricular outflow tract gradient
MAD	multiple-ascending dose
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	mixed model for repeated measures
mRNA	messenger ribonucleic acid
NDA	new drug application
nHCM	non-obstructive hypertrophic cardiomyopathy
NOAEL	no-observed-adverse-effect level
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
oHCM	obstructive hypertrophic cardiomyopathy
OLE	open-label extension
OSI	Office of Scientific Investigations
PBPK	physiologically based PK
PD	pharmacodynamic
PDUFA	Prescription Drug User Fee Act
P-gp	P-glycoprotein
PI	prescribing information
PK	pharmacokinetic
PM	poor metabolizer
pVO ₂	peak oxygen consumption
QD	once daily
QXW	every X weeks
REMS	risk evaluation and mitigation strategy
RI	renal impairment
SAD	single-ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
SEE	substantial evidence of effectiveness
SRT	septal reduction therapy
TBM	to-be-marketed
TEAE	treatment-emergent adverse event
T _{max}	time to maximum concentration
ULN	upper limit of normal

I. Executive Summary

1. Overview

1.1. Summary of Regulatory Action

Aficamten is an allosteric and reversible inhibitor of cardiac myosin. Aficamten reduces the force generated by myosin at the cardiac sarcomere which reduces cardiac contractility. The Applicant, Cytokinetics, submitted NDA 219083 for aficamten to support the proposed indication, “treatment of adults with symptomatic obstructive hypertrophic cardiopathy (oHCM).” NDA 219083 relies on data from a single adequate and well-controlled trial, SEQUOIA-HCM, to support the proposed indication along with confirmatory evidence from the phase 2 REDWOOD-HCM study.

All review disciplines, the Cross Disciplinary Team Leader, the deputy director of the Division of Cardiology and Nephrology, and the signatory concur that the evidence submitted to NDA 219083 satisfy the statutory requirement for substantial evidence of effectiveness to support the approval of aficamten for the indication to improve exercise capacity and reduce symptoms in patients with symptomatic oHCM, and that these benefits outweigh aficamten’s risks.

1.2. Conclusions on Substantial Evidence of Effectiveness

Substantial evidence of effectiveness (SEE) was established with one adequate and well-controlled clinical investigation and confirmatory evidence.

SEQUOIA-HCM Trial

SEQUOIA-HCM was a Phase 3, multicenter, randomized, double-blind, and placebo-controlled trial that evaluated the efficacy and safety of aficamten in subjects with symptomatic oHCM. The trial included a screening period of up to 6 weeks, a double-blind, placebo-controlled treatment period of 24 weeks, and a 4-week safety follow-up period. A total of 282 eligible subjects were randomized 1:1 to aficamten or placebo at 82 sites in 14 countries. The starting and maximum doses of aficamten were 5 mg and 20 mg orally once daily with or without food, respectively.

The primary efficacy endpoint was the change from baseline in peak oxygen consumption (pVO_2) at Week 24 (significance level: 2-sided alpha 0.05). Secondary efficacy endpoints, listed as per the statistical testing hierarchy all at the significance level of 2-sided alpha 0.05 were:

1. Kansas City Cardiomyopathy Questionnaire-Clinical Summary Score (KCCQ-CSS) change from baseline at Week 24.
2. Proportion of subjects with >1 New York Heart Association (NYHA) functional class improvement at Week 24.
3. Valsalva left ventricular outflow tract gradient (LVOT-G) change from baseline at Week 24.

4. Proportion of subjects with valsalva LVOT-G <30 mm Hg at Week 24.
5. Duration of septal reduction therapy (SRT) eligibility up to 24 weeks of treatment for those who were SRT at baseline.
6. KCCQ-CSS change from baseline at Week 12.
7. Proportion of subjects with >1 NYHA functional class improvement at Week 12.
8. Valsalva LVOT-G change from baseline at Week 12.
9. Proportion of subjects with Valsalva LVOT-G <30 mm Hg at Week 12.
10. Change from baseline in total workload during cardiopulmonary exercise testing (CPET).

Baseline characteristics were similar between the treatment groups.

The pVO₂ primary endpoint result showed a least-squares (LS) mean difference (95% confidence interval) of 1.7 (1.0, 2.4) for aficamten compared to placebo, p-value<0.0001. The treatment effect on the primary endpoint was generally consistent across various predefined subgroups based on demographic characteristics, baseline disease characteristics, and concomitant medications.

The results of all the secondary efficacy endpoints listed above were also statistically significant.

The clinical utility of pVO₂ is the ability to assess functional capacity and the effectiveness of an intervention in cardiomyopathy trials, and thus, it is an accepted endpoint for a regulatory decision. The observed treatment effect on pVO₂ was interpreted to be clinically meaningful (see Section [6.3.1](#)). This finding was accompanied by a large treatment effect of aficamten on LVOT-G as well as clinically meaningful improvement on functional capacity and symptoms assessed with the KCCQ-CSS and NYHA functional class.

In summary, the SEQUOIA-HCM trial provided evidence from an adequate and well controlled clinical investigation that aficamten improves functional capacity and symptoms in subjects with symptomatic oHCM as measured by pVO₂, KCCQ-CSS and NYHA class.

REDWOOD-HCM Study

REDWOOD-HCM was a Phase 2, multi-center, randomized, placebo-controlled, dose-finding study that evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of aficamten in adults with symptomatic oHCM. The study was performed at 23 centers in Italy, Netherlands, Spain, and the United States. Subjects with oHCM and LVOT-G ≥30 mm Hg at rest or ≥50 mm Hg with Valsalva were randomized 2:1 to receive aficamten (n=28) or placebo (n=13) in 2 dose-finding cohorts (i.e., 14 subjects per dose-finding cohort). Doses were titrated, up to 3 escalating doses, based on LVOT-G and left ventricular ejection fraction (LVEF).

Efficacy was evaluated by echocardiography (i.e., LVOT-G, LVEF), NYHA class, and patient-reported outcome quality of life assessment. Results showed a clinically meaningful reduction in LVOT-G in the combined aficamten cohorts compared to placebo, at rest (-25 mm Hg) and post-Valsalva (-41 mm Hg) at Week 10, providing confirmatory evidence of effectiveness of aficamten to reduce LVOT-G in symptomatic oHCM subjects.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	- Hypertrophic cardiomyopathy (HCM) is a rare genetic myocardial disorder characterized by left ventricular (LV) hypertrophy, hyperdynamic contraction, and impaired relaxation. - The prevalence of asymptomatic HCM among young adults in the United States has been estimated at 1 in 200 to 1 in 500, with symptomatic HCM being less common based on medical claims data (estimated at lower than 1 in 3,000). - About 70% of individuals diagnosed with HCM have obstructive HCM (oHCM) with a prevalence of 2-5 per 100,000 people. The presence of left ventricular outflow tract (LVOT) obstruction is an important prognostic factor in HCM. - Complications of oHCM include arrhythmias, dilated cardiomyopathy, mitral regurgitation, heart failure (HF), stroke, and sudden death from ventricular tachycardia/ventricular fibrillation. The incidence of sudden death is approximately 1% per year, often in people <30 years of age. The cause of death from oHCM is age-dependent (sudden death in the younger population, HF in middle age, and stroke in the elderly). - Patients with oHCM have a wide range of symptoms and disease courses. The typical symptoms of oHCM include dyspnea, reduced exercise tolerance, angina, palpitations, and syncope. Some patients suffer from high symptom burden causing significant debilitating consequences, thus profoundly affecting their quality of life.	oHCM is a rare, chronic, progressive disease characterized by cardiomyocyte disarray, hypercontractile myocardium, and impaired LV relaxation with variable clinical presentations and clinical course. oHCM is associated with significant cardiovascular-related morbidity and mortality, including functional limitation and risk of sudden cardiac death.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current treatment options	- First-line, guideline-directed pharmacologic management of symptomatic patients with oHCM includes beta-blockers and non-dihydropyridine calcium channel blockers (off-label use) - Propranolol is the only beta-blocker approved for improving NYHA functional class in patients with oHCM. - Patients with oHCM who do not respond to first-line therapy are candidates for advanced pharmacologic therapy including disopyramide and cardiac myosin inhibitors - The first-in-class cardiac myosin inhibitor, mavacamten, was approved by the FDA in 2022 for treatment of adults with symptomatic oHCM to improve functional capacity and symptoms. - Mavacamten has substantial drug-drug interactions (DDIs) and prolonged half-life that increase the risk of systolic dysfunction and HF. It is only available through a risk evaluation and mitigation strategy (REMS) program. - Septal reduction therapy (SRT) with either surgical myectomy or alcohol septal ablation is generally reserved for drug-refractory symptoms or intolerant adverse effects from pharmacologic therapy - Although these procedures are generally effective, they have important risks and access to the procedures may be limited.	There remains an unmet need for additional effective and safe treatments for patients with symptomatic oHCM to address the impact of the disease.
Benefit	- The effectiveness of aficamten for the treatment of adults with oHCM was evaluated in the SEQUOIA-HCM trial, a randomized, double-blind, placebo-controlled, multicenter, international, parallel-group study in patients with symptomatic oHCM. - The primary efficacy endpoint was change from baseline in pVO₂ at Week 24. At baseline, mean pVO₂ was 18.5 mL/kg/min. The study met its primary endpoint showing greater increase in pVO₂ with aficamten compared to placebo with a LS mean difference (95% confidence interval) of 1.7 (1.0, 2.4), p-value<0.0001. - The clinical meaningfulness of the observed treatment effect on pVO₂ of 1.7 mL/min/kg between groups was supported by the following exploratory analyses: - The empirical cumulative density function curves of changes in pVO₂ by NYHA class groups	SEQUOIA-HCM demonstrated statistically persuasive and clinically meaningful treatment effect of aficamten versus placebo on pVO ₂ , KCCQ-CSS, NYHA functional class, LVOT-G, and days eligible for SRT. These data support effectiveness of aficamten to improve functional capacity and symptoms in patients with symptomatic oHCM.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	– A greater proportion of subjects achieving a composite endpoint of pVO₂ and NYHA class improvement in the aficamten versus placebo group (42% versus 14%) – Statistically significant and clinically meaningful treatment effects on all secondary endpoints, including those that assessed symptoms and functional capacity (KCCQ-CSS, NYHA functional class), LVOT-G and days eligible for SRT. • Subgroup analyses of the primary efficacy endpoint by baseline characteristics including beta-blocker use at baseline and stratification factors showed a consistent effect.	
Risk and risk management	• Safety evaluation of aficamten was primarily based on the data obtained in the SEQUOIA-HCM trial (N=282 receiving at least one dose of study drug; n=142 in the aficamten group and n=140 in the placebo group). This study provided controlled data with a median exposure of 6 months. Long-term safety is supported by the open-label extension Study CY 6022. With the updated safety data through August 31, 2024, the median exposure for all aficamten-treated patients was about 11 months. • Safety evaluation focused on the on-target effects of aficamten, whereby reduction of contractility could increase the risk of HF with reduced ejection fraction. – There was a small decrease in LVEF (~4 to 5% with an SD of 7%) during 24 weeks of aficamten treatment compared with placebo. LVEF decline appeared reversible with drug discontinuation. – The incidence of LVEF <50% was low (4 to 5% assessed by core laboratory or site-read), and none of the subjects who experienced LVEF <50% had associated signs and symptoms of HF except one with reported adverse event (AE) of HF 4 weeks prior to the reduction of LVEF. – No aficamten-treated oHCM patients experienced a serious HF event or severe systolic dysfunction (LVEF <30%) in SEQUOIA-HCM and the extension study. – Hypertension was the only AE that occurred at a frequency of ≥5% higher in the aficamten group compared to the placebo group (8% versus 2%). – There was a greater mean change from baseline in both systolic and diastolic blood pressure in the aficamten group versus placebo group at	The principal safety concern of aficamten is the risk of HF due to systolic dysfunction resulting from an excessive pharmacologic effect of aficamten. In SEQUOIA-HCM, in which regular pharmacodynamic (PD) monitoring via echocardiogram was implemented, the risk of HF and/or systolic dysfunction was low during 28 weeks of treatment. The risk remained low in the ongoing extension study in patients with oHCM. We determined that an elements to assure safe use (ETASU) REMS is required to assure safe use of aficamten for the following reasons: (1) an iatrogenic risk of HF with aficamten exists based on the mechanism of action of the drug; (2) the total exposure to aficamten to date remains relatively low (~300 oHCM patients); and (3) the risk is likely to be higher in the postmarket compared to trial setting (in the postmarket setting aficamten is likely to be used in a wider range of patients with higher comorbidities and where clinical monitoring is less rigorous).

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	the end of the 24-week treatment period (2/3 mm Hg versus -3/-1 mm Hg, respectively). - Concomitant use of potent inhibitors of multiple CYP enzymes, including CYP2C9, CYP2C19, and CYP3A4, such as fluconazole and fluvoxamine, with aficamten may increase aficamten exposure and increase the risk of HF and/or systolic dysfunction. Dose modification is required when concurrent use of these drugs occurs. - There are insufficient human pregnancy data in the aficamten-exposed subjects to inform the safety of aficamten use in pregnancy. The animal reproductive toxicity data do not suggest a serious risk of developmental toxicity during pregnancy.	The only observed adverse reaction with aficamten was hypertension. Aficamten-associated increases in blood pressure are consistent with the hemodynamic consequence of attenuating the LVOT obstruction. This risk can be managed through labeling. To further assess safety of aficamten during pregnancy, we will issue a postmarketing requirement for a descriptive pregnancy safety study and lactation study to collect prospective and retrospective data in women exposed to aficamten during pregnancy and/or lactation.

Abbreviations: CYP, cytochrome P450; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire-Clinical Summary Score; N, number of subjects; n, number of subjects in specific group; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; pVO₂, peak oxygen consumption

2.2. Conclusions Regarding Benefit-Risk

In the phase 3 trial, aficamten improved peak oxygen consumption (an assessment of functional capacity) over 24 weeks compared to placebo in adults with oHCM. While the mean treatment effect size appears modest (i.e., treatment difference of 1.7 mL/kg/min at Week 24 from a baseline mean of 18.5 mL/kg/min), these changes are accompanied by a large reduction in LVOT-G (mean treatment effect of -48 mmHg on Valsalva LVOT-G at Week 24 from a baseline mean of 83 mmHg) as well as benefits on patient symptoms and functional capacity assessed using KCCQ-CSS (mean treatment effect of 7 from a baseline mean of ~75) and NYHA functional class (59% improvement with aficamten at Week 24 compared to 24% improvement with placebo). We conclude that aficamten clearly improves symptoms and functional capacity in adults with oHCM.

Due to aficamten's mechanism of action the main concern is a risk of precipitating HF, although the risk appears low when echocardiography is used to monitor LVEF and guide dose titration. There is also drug-drug interaction potential, which can be managed by adjusting the aficamten dose and/or avoiding coadministration.

Aficamten's benefits on patient symptoms and functional capacity outweigh the risk of HF when the risk is mitigated with an ETASU REMS, which will help ensure patients on aficamten have regular echocardiograms to monitor for reduced LVEF and guide dose titration. The drug-drug interaction potential can be adequately mitigated with labeling, that describes the scenarios for dose adjustment and/or avoidance of aficamten. There is a risk of hypertension, consistent with the reduced LVOT, that can also be adequately managed with labeling, informing prescribers of this risk, which can be assessed with periodic blood pressure monitoring (which is routine in clinical care) and treated, as needed. Patients will also be informed of these risks through a Medication Guide, which is required to be dispensed with each new prescription and refill.

Based on these considerations, we conclude that the overall benefit-risk assessment is favorable for aficamten for the treatment of adults with symptomatic oHCM to improve functional capacity and symptoms and that aficamten should be approved.

II. Interdisciplinary Assessment

3. Introduction

NDA 219083 relies on a single, Phase 3 trial, SEQUOIA-HCM (CY 6031) and a Phase 2, dose-finding study, REDWOOD-HCM to support the following proposed indication for aficamten:

“AFICAMTEN is a cardiac myosin inhibitor indicated for the treatment of adults with symptomatic obstructive hypertrophic cardiomyopathy (oHCM)”

The Applicant also has an ongoing long-term, single-arm, open-label extension study to assess the safety and durability of aficamten in patients with hypertrophic cardiomyopathy (HCM).

Disease Background ([Autore et al. 2005](#); [Maron et al. 2006](#); [Ommen et al. 2024](#))

Hypertrophic cardiomyopathy is an autosomal dominant genetic heart disease that affects men and women globally and is characterized by left ventricular (LV) hypertrophy, hyperdynamic contraction and impaired relaxation. The prevalence of asymptomatic HCM among young adults in the United States has been estimated to be approximately 1 in 500, with symptomatic HCM based on medical claims data being less common (estimated at <1 in 3000) (1). A large percentage of patients are asymptomatic and/or undiagnosed.

There are two HCM phenotypes based on the presence or absence of obstruction of the LVOT, oHCM and non-obstructive hypertrophic cardiomyopathy (nHCM), where obstruction is defined as a peak LVOT gradient ≥ 30 mm Hg at rest or ≥ 50 mm Hg with provocation (e.g., Valsalva maneuver). Approximately 70% of individuals diagnosed with HCM have oHCM (2). The health impacts of HCM are variable with a wide range of symptoms and disease courses.

Many patients experience minimal or few symptoms and minor or no limitations in daily activities; however, others suffer significant debilitating consequences and high symptom burden, most frequently dyspnea, chest pain and palpitations with profound effects on their lives. The presence of LVOT obstruction is an important prognostic factor in HCM (3). HCM-related adverse outcomes include sudden death, progressively limiting symptoms caused by diastolic dysfunction and/or LVOT obstruction, heart failure (HF) caused by systolic dysfunction, and thromboembolic complications.

First-line, guideline-directed pharmacologic management of oHCM includes beta-blockers and non-dihydropyridine calcium channel blockers. Patients with oHCM who do not respond to first-line therapy are candidates for advanced pharmacologic therapy including disopyramide and cardiac myosin inhibitors. The first-in-class and only approved cardiac myosin inhibitor is mavacamten, approved by the FDA in 2022 for treatment of adults with symptomatic oHCM to improve functional capacity and symptoms. However, mavacamten has substantial drug-drug interactions (DDIs) and a long half-life that increase the risk of systolic dysfunction and heart failure. Mavacamten is available only through a REMS program. Septal reduction therapy with either surgical myectomy or alcohol septal ablation is generally reserved for drug-refractory symptoms or intolerant adverse effects from pharmacologic therapy. Although SRT is effective, it is only available in experienced HCM centers and there are potential procedural complications, such as spill-over (alcohol infusion into other arteries causing an expanded myocardial

infarction), disruption of electrical conduction causing blocks and potential pacemaker requirements, and resurgence of LVOT obstruction requiring a repeat procedure. There continues to be an unmet need for a disease-specific drug to treat symptomatic oHCM.

Drug Class

Aficamten (also referred to as CK-3773274), is a small molecule, allosteric and reversible inhibitor of cardiac myosin. Aficamten reduces the force generated by myosin at the cardiac sarcomere. In oHCM patients, myosin inhibition with aficamten reduces cardiac sarcomere contractility and LVOT obstruction.

3.1. Review Issue List

3.1.1. Key Efficacy Review Issues

3.1.1.1. Is the Observed Treatment Effect of Aficamten on pVO₂ Clinically Meaningful?

See Section [6.3.1](#) for an assessment of this efficacy review issue.

3.1.1.2. Impact of Imputation of Missing Data for Continuous Variable Endpoints on Assessment of Efficacy

See Section [6.3.1](#) for an assessment of this efficacy review issue.

3.1.1.3. Impact of Imputation of Missing Data for Categorical Variable Endpoints on Assessment of Efficacy

See Section [6.3.1](#) for an assessment of this efficacy review issue.

3.1.1. Key Safety Review Issues

3.1.1.1. Risk of Heart Failure Due to Systolic Dysfunction

See Section [7.7.1](#) for an assessment of this safety review issue.

3.2. Approach to the Clinical Review

The clinical review team assessed the data related to efficacy and safety, and the biometric review team assessed the data related to efficacy.

3.3. Approach To Establishing Substantial Evidence of Effectiveness

1. Verbatim indication:

Treatment of adults with symptomatic obstructive hypertrophic cardiomyopathy (oHCM) to improve functional capacity and symptoms.

11. SEE was established with:

- a. Adequate and well-controlled clinical investigation(s):
 - i. ☐ Two or more adequate and well-controlled clinical investigations, **OR**
 - ii. ☐ One adequate and well-controlled clinical investigation with highly persuasive results that is considered to be the scientific equivalent of two clinical investigations
- OR**
- b. ☒ One adequate and well-controlled clinical investigation and confirmatory evidence,¹²³
- OR**
- c. ☐ Evidence that supported SEE from a prior approval (e.g., 505(b)(2) application relying only on a previous determination of effectiveness; extrapolation; over-the-counter switch)⁴

12. Complete response, if applicable

- a. ☐ SEE was established
- b. ☐ SEE was not established

¹ FDA draft guidance for industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products* ([December 2019](#))

² FDA guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products* ([May 1998](#))

³ FDA guidance for industry *Demonstrating Substantial Evidence of Effectiveness Based on One Adequate and Well-Controlled Clinical Investigation and Confirmatory Evidence* ([September 2023](#))

⁴ See footnote 2

Table 3. Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations¹ for Aficamten

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Subjects Planned; Actual Randomized	No. of Centers and Countries
CY 6031 (SEQUOIA-HCM)	Adult subjects with oHCM	Control Type: Placebo concurrent Randomization: Stratified randomization Randomization Ratio: 1:1 Blinding: Double-blind Biomarkers: Biomarkers as exploratory endpoint Innovative design features: None	Drug (established name): Aficamten Dose: 5, 10, 15, or 20 mg administered QD. Subjects started at 5-mg QD and were evaluated for stepwise up-titration based on echocardiography-guided criteria. Number treated: • Aficamten: N=142 • Placebo: N=140 Duration (quantity and units): Up to 24 weeks	Primary: Change in pVO ₂ by CPET from baseline to Week 24 Secondary: See footnotes ²	Planned: 270 Actual: 282	Centers: 82 Countries: 14

NDA 219083
MYQORZO (aficamten)

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Subjects Planned; Actual Randomized	No. of Centers and Countries
CY 6021 (REDWOOD-HCM)	Adult subjects with oHCM and nHCM	Cohort 1 and 2: - Control type: Placebo concurrent - Randomization: Randomization - Randomization ratio: 2:1 to aficamten or placebo - Blinding: Double-blind	Drug (established name): Aficamten Dose: Stepwise up-titration based on echocardiography-guided criteria. - Cohort 1: 5, 10, or 15 mg/placebo - Cohort 2: 10, 20, or 30 mg/placebo - Cohort 3 & 4: 5, 10, or 15 mg	Primary: Subject incidence of reported AEs, subject incidence of reported SAEs, and subject incidence of LVEF <50% Secondary: See footnote ³	Planned: 94 Actual: 95	Centers: 23 Countries: 4
	Cohort 1 and 2: oHCM subjects excluding use of disopyramide					
	Cohort 3: subjects with oHCM receiving disopyramide	Cohort 3 and 4: Control Type: No control, single arm, open label				
	Cohort 4: Subjects with nHCM excluding use of disopyramide		Number treated: - Cohort 1: N=14/7 (aficamten/placebo) - Cohort 2: N=14/6 (aficamten/placebo) - Cohort 3: N=13 - Cohort 4: N=41			
			Duration (quantity and units): Up to 10 weeks			

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Subjects Planned; Actual Randomized	No. of Centers and Countries
CY 6022	Adult subjects with oHCM or nHCM who participated in CY 6031 or CY 6021	Control type: No treatment concurrent (single-arm) Randomization: No randomization Randomization ratio: No randomization Blinding: Open label Biomarkers: Biomarkers as exploratory endpoint Innovative design features: None	Drug (established name): Aficamten Dose: 5, 10, 15, or 20 mg administered QD. Subjects started at 5-mg QD and were evaluated for stepwise up-titration based on echocardiography-guided criteria. Number treated: Aficamten • Subjects with oHCM N=213 • Subjects with nHCM N=34 Duration (quantity and units): Up to 125 weeks as of data cutoff	Primary: Subject incidence of reported AEs and subject incidence of reported SAEs, subject incidence of LVEF <50% Secondary: See footnotes ⁴	Planned: 600 Actual: 213 as of data cutoff	Centers: 59 Countries: 11

Source: Clinical Study Report and adsl.xpt

¹ Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and PK studies.

² CY 6031 secondary endpoints: Change in KCCQ-CSS from baseline to Week 12 and Week 24; proportion of subjects with ≥1 class improvement in NYHA Functional Class from baseline to Week 12 and Week 24; change in Valsalva LVOT-G from baseline to Week 12 and Week 24; proportion of subjects with Valsalva LVOT-G <30 mm Hg at Week 12 and Week 24; duration of eligibility for SRT during the 24-week treatment period in subjects who were eligible for SRT at baseline; and change in total workload during CPET from baseline to Week 24

³ CY 6021 secondary endpoints: Change in LVOT-G over 10 weeks (Cohort 1, 2, and 3 only); NYHA Functional class; health status and health-related quality of life; other PD markers via echocardiography; the pharmacokinetics of aficamten

⁴ CY 6022 secondary endpoints: Change from baseline values at 12-week intervals through end of participation in the following: Peak LVOT-G at rest and with Valsalva provocation, proportion of subjects with resting LVOT-G <50 mm Hg, proportion of subjects with resting LVOT-G <30 mm Hg, proportion of subjects with post-Valsalva LVOT-G <50 mm Hg, proportion of subjects with post-Valsalva LVOT-G <30 mm Hg, proportion of subjects with LVEF ≥50%, resting LVOT-G <30 mm Hg, and post-Valsalva LVOT-G <50 mm Hg. Time to the following event through last follow-up: first resting LVOT-G <50 mm Hg, first resting LVOT-G <30 mm Hg, first post-Valsalva LVOT-G <50 mm Hg, first post-Valsalva LVOT-G <30 mm Hg, first LVEF ≥50%, resting LVOT-G <30 mm Hg, and post-Valsalva LVOT-G <50 mm Hg.

Abbreviations: AE, adverse event; AF, atrial fibrillation; CPET, cardiopulmonary exercise testing; CV, cardiovascular; ICD, implantable cardiac defibrillator; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire – Clinical Summary Score; LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; nHCM, non-obstructive hypertrophic cardiomyopathy; No., number; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; oHCM, obstructive hypertrophic cardiomyopathy; PD, pharmacodynamic; PK, pharmacokinetic; pVO₂, peak oxygen consumption; QD, once daily; SAE, serious adverse event; SRT, septal reduction therapy; TEAE, treatment-emergent adverse event; ULN, upper limit of normal

4. Patient Experience Data

The SEQUOIA-HCM trial collected data on (1) patient's perception of their symptoms and health status at baseline and various timepoints using the KCCQ-CSS; and (2) NYHA Functional Class, which is a clinician-reported outcome.

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	Type of Data	Section Where Discussed, if Applicable
Clinical Outcome Assessment Data Submitted in the Application		Section 6.2.1.4
☒	Patient-reported outcome	
☐	Observer-reported outcome	
☒	Clinician-reported outcome	
☐	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	Type of Data	Section Where Discussed, if Applicable
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

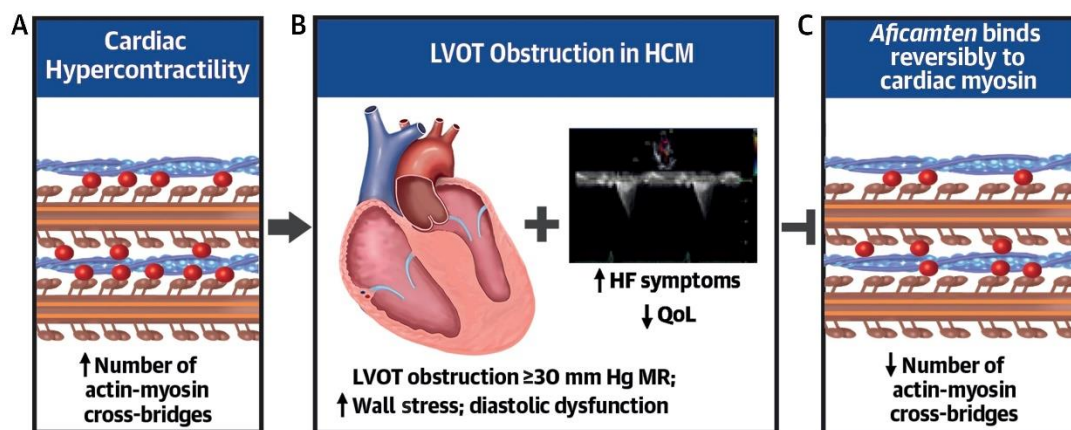
5.1. Nonclinical Assessment of Potential Effectiveness

5.1.1. Mechanism of Action

Aficamten is a small molecule inhibitor of cardiac myosin intended for the treatment of adults with symptomatic oHCM. oHCM is characterized by left ventricular hypertrophy and hypercontractility, with impaired relaxation and filling during diastole. This causes abnormal hemodynamics and clinical symptoms such as chest pain, fatigue, and syncope. By specifically inhibiting cardiac myosin, a sarcomeric molecular motor in cardiomyocytes, aficamten reduces the excessive contractility that contributes to the pathophysiology of oHCM.

At a molecular level, cardiomyocyte contractility is driven by the cyclic interaction between myosin and actin at the cardiac sarcomere. Myosin and actin interactions are regulated by calcium, the presence of which allows myosin's motor head domain to bind actin. Subsequent adenosine triphosphate (ATP) hydrolysis powers sarcomere shortening and cardiomyocyte contraction ([Figure 1](#)). The myosin-actin complex dissociates after each power stroke, which primes the system for another round of contraction. Aficamten binds directly to the head domain of cardiac myosin and is more selective for the cardiac β -myosin heavy chain isoform ([Moussavi-Harami and Regnier 2024](#)), which is predominantly expressed in the ventricles. Aficamten binding stabilizes myosin in an off-actin conformation, decreasing the number of functional myosin heads entering a force-generating state, resulting in reduced cardiomyocyte contractility ([Figure 1](#)).

Figure 1. Schematic Illustration of the Mechanism of Action of Aficamten in Obstructive Hypertrophic Cardiomyopathy



Source: Adopted from ([Maron et al. 2023](#))

Abbreviations: HCM, hypertrophic cardiomyopathy; HF, heart failure; LVOT, left ventricular outflow tract; QoL, quality of life

5.1.2. In Vitro and In Vivo Pharmacology

Experiments in vitro with myofibrils from various muscle types across species and cultured cardiomyocytes interrogated the primary pharmacology and selectivity of aficamten. Detailed summaries of individual studies are provided in Section [13.1](#) of this review. Aficamten decreased the ATPase activity of bovine cardiac and slow muscle myofibrils with similar half maximal inhibitory concentration (IC_{50}) values, due to the same myosin isoform (MYH7) being expressed in each muscle type but was a weak inhibitor of both rabbit fast muscle myofibrils and chicken gizzard smooth muscle (see [Table 48](#)). These results show that aficamten spares smooth muscle and fast-twitch skeletal muscle contractility at concentrations that inhibit cardiac and slow-twitch skeletal muscle contractility in vitro. Notably, no signals related to skeletal muscle weakness were observed in in vivo nonclinical studies or in clinical trials. Cardiac myosin proteins are highly evolutionarily conserved, allowing comparison of data across myofibril preparations from different species, which allows for clinically relevant nonclinical pharmacology studies in animals. Aficamten inhibits contractility without impacting intracellular cardiomyocyte calcium concentrations, demonstrated in vitro in both rat ventricular cardiomyocytes and human cardiomyocytes derived from induced pluripotent stem cells, suggesting low risk for arrhythmias with clinical use. Aficamten potency was similar in engineered heart tissues derived from induced pluripotent stem cells from a normal donor ($IC_{50}=1.08\mu M$) and those from a donor heterozygous for MYH7 R403Q ($IC_{50}=1.65\mu M$), demonstrating its efficacy against a cardiac myosin mutation associated with hypertrophic cardiomyopathy and its potential to treat HCM of multiple etiologies.

Prospect of clinical benefit was shown using heterozygous transgenic MYH7 R403Q mice with HCM, and a cat model of hereditary oHCM (A31P MYBPC3 mutation), using aficamten doses comparable to those used in toxicology studies. Notably, the transgenic mouse develops LV thickening without LVOT obstruction, while the cat model reliably reflects human oHCM symptoms including LVOT obstruction. Aficamten dose-dependently reduced left ventricular fractional shortening (LVFS) compared to baseline after a single dose in both disease models. In vivo pharmacology studies were also conducted in healthy rats and dogs without HCM. In healthy rats, IC_{10} (the plasma concentration to result in a 10% reduction in LVFS) was observed at 270 ng/mL and IC_{50} at 2,662 ng/mL. In healthy dogs, the IC_{10} was observed at 60 ng/mL and IC_{50} at 430 ng/mL, with a slightly steeper dose response than rats.

There was no major additional magnitude of the pharmacodynamic (PD) effect in healthy rats between a single dose or repeated dosing with aficamten, and the effects reversed 24 hours after the final dose. Reduced LVFS caused by aficamten was rapidly rescued in rats by administration of dobutamine, a β -adrenergic agonist, demonstrating a pharmacological method to reverse its action. The pharmacodynamics of aficamten was not significantly impacted in a heterozygous MYH7 R403Q mouse model of hypertrophic cardiomyopathy, as the dose response relationship was similar to that observed in wild-type mice. Aficamten decreased the LVOT pressure gradient in a cat model of oHCM after a single dose, indicating improvement of mitral valve obstruction coinciding with reduced LVFS.

Taken together, these data provided a therapeutic rationale for relieving LVOT obstruction in oHCM subjects treated with aficamten. Aficamten was shown to reversibly (non-covalently) inhibit the contractility of purified myofibrils, intact cultured cardiomyocytes, and hearts in vivo. Aficamten acts at the sarcomere, independently of electrophysiology. Aficamten was similarly

potent between cardiomyocytes with wild type myosin and R403Q mutants, one of the most common clinical mutations associated with HCM. Aficamten had no appreciable activity against smooth muscle or skeletal fast-twitch muscle at clinically relevant concentrations but may theoretically have unintended effects on skeletal slow-twitch muscle myofibrils, although no safety signal has been identified. Aficamten reduced LVFS and LVOT pressure gradient in a cat model of oHCM, providing rationale for its development as a therapeutic.

Overall, the nonclinical data submitted by the Applicant support the proposed therapeutic rationale for the development of aficamten for oHCM and provide adequate information to label its mechanism of action (Section 12.1 of the product prescribing information [PI]).

5.1.3. Established Pharmacologic Class and Rationale

For Established Pharmacologic Class of aficamten, the Applicant proposed “cardiac myosin inhibitor,” which was used for Camzyos (mavacamten).

Several lines of in vitro and in vivo evidence suggest that aficamten binds to the cardiac myosin motor and decreases its ATPase activity, providing a molecular basis of reducing cardiac contractility to treat hypertrophic cardiomyopathy. Thus, the use of “cardiac myosin inhibitor” as the Established Pharmacologic Class is supported. Aficamten would be the second-in-class cardiac myosin inhibitor, should it be approved. Mavacamten, the first-in-class, was approved for hypertrophic cardiomyopathy in 2023.

5.2. Clinical Pharmacology/Pharmacokinetics

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information																					
Pharmacologic Activity																						
Established pharmacologic class (EPC)	Cardiac myosin inhibitor																					
Mechanism of action	Aficamten is an allosteric and reversible inhibitor of cardiac myosin motor activity. Aficamten reduces the force generated by myosin at the cardiac sarcomere, which contributes to the pathophysiology of hypertrophic cardiomyopathy (HCM). In HCM subjects, myosin inhibition with aficamten may reduce cardiac sarcomere contractility and left ventricular outflow tract (LVOT) obstruction.																					
Active moieties	Aficamten																					
QT prolongation	A lack of QTc prolongation across the therapeutic concentration range of aficamten was demonstrated (Study CY 6019). At a single dose of 50 mg (i.e., similar exposure as 20 mg once daily [QD] dosing to steady-state), the upper limit of the predicted placebo-corrected change from baseline in QT interval corrected by Fridericia ($\Delta\Delta\text{QTcF}$) 90% confidence interval (CI) for aficamten was <10 msec.																					
General Information																						
Bioanalysis	Bioanalysis of aficamten, its metabolites (i.e., CK-3834282 and CK-3834283), and other analytes in plasma and urine were conducted using validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods. Overall, the bioanalytical method validation and performance are acceptable.																					
Healthy subjects versus patients	Healthy subjects had slightly (~24%) lower aficamten AUC _{tau} , C _{max} , and C _{tau} relative to subjects with oHCM. Aficamten t _{1/2} and corresponding time to steady-state were similar between the 2 populations (Population PK analysis CYT-AFI-PMX-001).																					
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	<table><tr><th colspan="5">Table 6. Steady-State Plasma PK Parameters of Aficamten Following Once Daily Dosing of 5 and 10 mg Aficamten in Healthy Participants</th></tr><tr><th>Parameter</th><th>Dosage Regimen</th><th>Arithmetic Mean ± %CV</th><th>Dosage Regimen</th><th>Arithmetic Mean ± %CV</th></tr><tr><td>AUC_{tau} (ng·hr/mL)</td><td rowspan="3">5-mg QD</td><td>1,348 (22.4)</td><td rowspan="3">10-mg QD</td><td>2,690 (23.7)</td></tr><tr><td>C_{max} (ng/mL)</td><td>70.4 (21.1)</td><td>143.5 (20.4)</td></tr><tr><td>Median t_{1/2} (hr) (min, max)</td><td>82.3 (75.5, 104)</td><td>81.7 (53.2, 94.4)</td></tr></table> Source: Study CY 6011, Part 2	Table 6. Steady-State Plasma PK Parameters of Aficamten Following Once Daily Dosing of 5 and 10 mg Aficamten in Healthy Participants					Parameter	Dosage Regimen	Arithmetic Mean ± %CV	Dosage Regimen	Arithmetic Mean ± %CV	AUC _{tau} (ng·hr/mL)	5-mg QD	1,348 (22.4)	10-mg QD	2,690 (23.7)	C _{max} (ng/mL)	70.4 (21.1)	143.5 (20.4)	Median t _{1/2} (hr) (min, max)	82.3 (75.5, 104)	81.7 (53.2, 94.4)
Table 6. Steady-State Plasma PK Parameters of Aficamten Following Once Daily Dosing of 5 and 10 mg Aficamten in Healthy Participants																						
Parameter	Dosage Regimen	Arithmetic Mean ± %CV	Dosage Regimen	Arithmetic Mean ± %CV																		
AUC _{tau} (ng·hr/mL)	5-mg QD	1,348 (22.4)	10-mg QD	2,690 (23.7)																		
C _{max} (ng/mL)		70.4 (21.1)		143.5 (20.4)																		
Median t _{1/2} (hr) (min, max)		82.3 (75.5, 104)		81.7 (53.2, 94.4)																		

NDA 219083
MYQORZO (aficamten)

Characteristic	Drug Information								
Maximally tolerated dose or exposure	75 mg single dose, healthy subject (Study CY 6011) 30-mg QD for 4 weeks, subjects with oHCM (Study CY 6021)								
Dose proportionality	Dose proportional for 5 mg to 20 mg once daily (Population PK analysis in Trial CY 6031).								
Accumulation	A 4.8-4.9-fold accumulation in AUC and a 2.5-2.7-fold accumulation in C _{max} following a QD dose of 5-10 mg was observed relative to single dose (Study CY 6011).								
Time to achieve steady state	Steady state was reached after 10-12 days of QD dosing of aficamten 5-10 mg (Study CY 6011). Steady state is predicted to be reached following approximately 17 days of QD dosing of aficamten 15 mg in subjects with oHCM (Population PK report CYT-AFI-PMX-001)								
Bridge between to-be-marketed and clinical trial/study formulations	The proposed to-be-marketed (TBM) formulation is identical to the Phase 3 formulation except for a change in the film-coating color ((b) (4)), which is nonfunctional and not expected to alter aficamten pharmacokinetics.								
Absorption									
Bioavailability	The absolute bioavailability of aficamten has not been determined.								
Median T _{max}	1.0-2.8 hours following QD multiple doses (Study CY 6011)								
Food effect (fed/fasted) Geometric least-squares mean and 90% CIs	Administration with a high-fat meal (i.e., 800-1000 calories and approximately 50% fat) altered the rate but not the extent of absorption of aficamten (Study CY 6012). The fed/fasted GLS mean and 90% CIs are as follows: Table 7. Summary of Statistical Assessment for Food Effect on Plasma Aficamten Exposure Following a Single Oral Dose of Aficamten 20 mg as Proposed Commercial Tablet Formulation, Study CY 6012 <table> <tr> <th>Parameter</th><th>Fed/Fasted Geometric Least-Square Mean (90% CIs)</th></tr> <tr> <td>AUC_{0-∞}</td><td>1.05 (1.03-1.08)</td></tr> <tr> <td>C_{max}</td><td>0.85 (0.72-1.01)</td></tr> <tr> <td>Median T_{max} (min, max)</td><td>2.19 hr (0.58, 4.08) (fed) 0.60 hr (0.26, 2.50) (fasted)</td></tr> </table> Source: Sponsor Tables 9 and 10, Module 2.7.1 (Study CY 6012)	Parameter	Fed/Fasted Geometric Least-Square Mean (90% CIs)	AUC _{0-∞}	1.05 (1.03-1.08)	C _{max}	0.85 (0.72-1.01)	Median T _{max} (min, max)	2.19 hr (0.58, 4.08) (fed) 0.60 hr (0.26, 2.50) (fasted)
Parameter	Fed/Fasted Geometric Least-Square Mean (90% CIs)								
AUC _{0-∞}	1.05 (1.03-1.08)								
C _{max}	0.85 (0.72-1.01)								
Median T _{max} (min, max)	2.19 hr (0.58, 4.08) (fed) 0.60 hr (0.26, 2.50) (fasted)								
Distribution									
Volume of distribution	313 L (Population PK report CYT-AFI-PMX-001).								
Plasma protein binding	Aficamten is approximately 94% bound to plasma proteins (Study CY 6017). The blood to plasma ratio of aficamten was 0.94 (Study Nonclin-0032).								
Drug as substrate of transporters	Aficamten is not a substrate of drug transporters (Study Nonclin-0009).								

NDA 219083
MYQORZO (aficamten)

Characteristic	Drug Information
<i>Elimination</i>	
Mass balance results	When 20 mg (100 µCi) of ¹⁴ C-aficamten was administered to healthy males, approximately 89.7% of the radioactive oral dose was recovered, with 32.0% excreted in urine and 57.7% excreted in feces. Aficamten was eliminated primarily by metabolism and, to a lesser extent, by fecal excretion, with renal excretion playing a minor role (Study CY 6013).
Clearance	The population PK-derived clearance (CL/F) estimate of aficamten in subjects with oHCM was 2.62 L/hr (Population PK report CYT AFI-PMX-001).
Half-life	Aficamten has a terminal elimination half-life of approximately 80 hours (Population PK report CYT-AFI-PMX-001).
Metabolic pathway(s)	Aficamten is primarily eliminated via CYP2C9-mediated metabolism (50%) with contributions from CYP3A (26%), CYP2D6, and CYP2C19 (Studies Nonclin-0029 and Nonclin-0451).
Primary excretion pathways (% dose)	See Mass balance study results above.
<i>Intrinsic Factors and Specific Populations</i>	
Body weight	Body weight is predicted to have a modest effect on aficamten pharmacokinetics. Aficamten plasma AUC _{tau} , C _{max} , and C _{tau} in a subject with oHCM at the fourth (i.e., highest) weight quartile were 34%, 35%, and 33% lower, respectively, relative to a subject with oHCM at the first (i.e., lowest) weight quartile (Population PK report CYT-AFI-PMX-001). No dose adjustment of aficamten is warranted based on body weight.
Age	Age was not identified as a statistically significant covariate on aficamten pharmacokinetics (Population PK report CYT-AFI-PMX-001).
Renal impairment	No impact of mild or moderate renal impairment on aficamten pharmacokinetics was identified in population PK analysis based on the Phase 2 (Study CY 6021) and Phase 3 (Trial CY 6031) data (Population PK report CYT-AFI-PMX-001).
Hepatic impairment	The exposure (i.e., C _{max} and AUC) of aficamten were similar (i.e., no meaningful difference) in subjects with normal liver function and subjects with moderate hepatic impairment (i.e., Child-Pugh Class B) (Study CY 6017). No studies were conducted in subjects with severe hepatic impairment (i.e., Child-Pugh Class C).
<i>Drug Interaction Liability (Drug as Precipitant)</i>	
Inhibition/induction of metabolism	In vitro studies suggest aficamten does not substantially inhibit any of the CYP isoforms (Studies Nonclin-0033 and Nonclin-0052). Aficamten is not predicted to be an in vivo inducer in humans (Studies Nonclin-0016 and Nonclin-0494).
Inhibition/induction of transporter systems	Aficamten is a weak P-gp inhibitor (Study CY 6014)
<i>Immunogenicity (if Applicable)</i>	
Bioanalysis	N/A
Incidence	N/A
Clinical impact	N/A

Source: Compiled by Reviewer

Abbreviations: AUC, area under the concentration-time curve; AUC_{0-∞}, area under the concentration-time curve from time 0 to infinity; AUC_{tau}, area under the concentration-time profile from time zero to time tau; C_{max}, maximum plasma concentration; C_{tau}, trough plasma concentration; CV, coefficient of variation; CYP, cytochrome P450; hr, hour; max, maximum; min, minimum; oHCM, obstructive hypertrophic cardiomyopathy; P-gp, P-glycoprotein; PK, pharmacodynamic; QD, once daily; t_{1/2}, half-life; T_{max}, time to maximum plasma concentration

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

Applicant's Dose Selection for Phase 3 Trial

The clinical development program sought to maintain a plasma concentration of aficamten required for desired efficacy, but avoiding supratherapeutic concentrations that may result in systolic dysfunction, leading to decrease in stroke volume and cardiac output, compensatory increases in heart rate, and potentially clinical heart failure. Therefore, the goal of dose selection was to find the lowest possible dose to safely achieve resting and Valsalva LVOT-G targets (i.e., resting LVOT-G of <30 mm Hg and Valsalva LVOT-G of <50 mm Hg) while maintaining normal LVEF (i.e., LVEF $\geq$ 50%).

Dose selection for the Phase 3 development program was determined from a Phase 2, multicenter, randomized, double-blind, placebo-controlled, dose-finding study in subjects with oHCM (Study CY 6021, REDWOOD-HCM). The REDWOOD-HCM study had two dosing regimen cohorts and one placebo cohort. Subjects with symptomatic oHCM were randomized in a 2:1 ratio to aficamten or placebo and received up to 3 escalating doses of aficamten (i.e., 5, 10, and 15 mg in Cohort 1 and 10, 20, and 30 mg in Cohort 2) or placebo once daily for 10 weeks. Dose escalation in the aficamten cohorts was based on echocardiographic guidance. Subjects maintained background therapy including beta-blockers and/or calcium channel blockers, either as monotherapy or combined, but excluding the use of disopyramide given its unique safety profile and the potential impacts on the studied outcomes.

Study CY 6021 was conducted with an objective to treat with the lowest possible dose to safely achieve resting and Valsalva LVOT-G targets and maintain normal LVEF. The 5 mg starting dose was demonstrated to be pharmacodynamically effective and was well-tolerated by all subjects. There were 11 out of 41 subjects from Cohorts 1 and 2 who required doses higher than 10 mg to achieve target LVOT-G. Two subjects were escalated up to 30 mg ([Table 8](#)) but one of the two subjects on the 30-mg dose was down-titrated to 20 mg due to LVEF <50%, and the other subjects did not achieve an adequate PD response. Based on these data the Applicant concluded that the 30-mg dose is not likely to add substantial clinical benefit versus risk; therefore, 20 mg was selected as the highest dose in the Phase 3 trial. The Applicant's dose selection for the Phase 3 trial was deemed reasonable.

Table 8. Highest Doses Achieved in Study CY 6021 (Cohorts 1 and 2)

	Highest Dose Achieved (n)					
	Placebo	5 mg	10 mg	15 mg	20 mg	30 mg
Cohort 1 (n = 21)	7	4	5	5	-	-
Site-read LVEF < 50%	0	0	0	0	-	-
Cohort 2 (n = 20)	6	-	8	-	4 ^a	2 ^a
Site-read LVEF < 50%	0	-	0	-	1	1
Cohort 1 and 2 (n = 41)	13	4	13	5	4 ^a	2 ^a
Site-read LVEF < 50%	0	0	0	0	1	1

Source: Table 47, Module 2.7.2

^a One subject down-titrated to lower dose based on site-read echocardiography

Abbreviations: LVEF, left ventricular ejection fraction; n, number of subjects

Evaluation of the Phase 3 Trial Dosage Regimen

The Phase 3 trial (CY 6031, SEQUOIA-HCM) dose regimen was evaluated using two complementary methods. First, exposure-response (E-R) analyses were performed to identify and quantify the relationship between steady-state aficamten exposure derived from population pharmacokinetic (PK) modeling and key primary, secondary, and exploratory efficacy and safety endpoints (see Section [14.5.2](#)). Second, PK/PD modeling of the relationship between aficamten pharmacokinetics and the primary echocardiographic measurements for dose titration, Valsalva LVOT-G, and LVEF, was performed (see Section [14.5.1](#))

The Applicant's population PK (PPK) model described the aficamten serum concentration-time profiles adequately following the administration of aficamten in subjects with oHCM. The PPK model was assessed as acceptable for simulating post hoc exposure metrics, e.g., area under the concentration-time curve (AUC)_{tau}, maximum plasma concentration (C_{max}), and trough plasma concentration (C_{tau}) of aficamten for PK/PD and E-R analyses of efficacy and safety measurements. The PK/PD model of aficamten was adequate to link aficamten exposure to LVEF and LVOT-G for aficamten-treated subjects from the REDWOOD-HCM study and Trial CY 6031. The results were used to support the proposed commercial dose regimen. The pharmacometrics reviewer conducted independent analyses to evaluate the frequency of echocardiographic assessment during titration and maintenance phases. The final PK/PD analysis is comprised of a curve with LVEF or LVOT-G declining in a slightly exponential manner with increasing aficamten exposure and is supportive of the proposed dose titration scheme. Refer to the following section below. Except for the probability of ≥1 NYHA class improvement at Week 24 and change in N-terminal pro-B-type natriuretic peptide (NT-proBNP) at Week 24, the results of exposure-efficacy analyses did not show a significant E-R relationship in the aficamten-treated group. The results of exposure-safety analyses did not show a trend between aficamten exposure (C_{avg,ss} and C_{max,ss}) and safety endpoints such as major adverse cardiovascular events, cardiovascular adverse events, or hypertension (please refer to Section [14.5](#)).

In the Phase 3 trial, CY 6031, the efficacy and safety for aficamten 5 mg - 20 mg has been adequately demonstrated. In conclusion, the Applicant's selection of the Phase 3 dosage regimen was appropriate.

Dose Titration Scheme

Phase 2 and 3 Development

In both Phase 2 and 3 studies, an individualized echocardiography-guided dose titration strategy was consistently used to maximize achievement of target LVOT-G and maintain LVEF $\geq 50\%$. For subjects with oHCM in the Phase 2 study (CY 6021), three opportunities for dose titration were provided (i.e., Weeks 2, 4, and 6). Dose titration proceeded until the reduction of the Valsalva LVOT-G to < 50 mm Hg and resting LVOT-G to < 30 mm Hg. Doses were adjusted to the maximum protocol-specified dose if LVEF $\geq 50\%$. If LVEF was $< 50\%$ at any time, the subjects was returned to the prior dose level or placed on placebo if the subjects was on the lowest dose level (i.e., 5 mg in Cohort 1 and 10 mg in Cohort 2).

The Phase 3 trial (CY 6031) followed a similar dose titration strategy as the Phase 2 study (CY 6021); however, the LVOT-G target criteria for dose titration was simplified by focusing on the Valsalva LVOT-G only. The Valsalva LVOT-G target was reduced from 50 mm Hg to 30 mm Hg to maximize the potential therapeutic benefit of aficamten. In addition, the lower limit of LVEF for dose escalation was increased from 50% to 55% to provide a safety margin from the threshold of LVEF ($< 50\%$) that triggered dose reduction in both studies. If LVEF was $< 50\%$ at any time, the subjects was returned to the prior dose level, or to placebo if the subject was on 5 mg. Subjects were able to receive up to 4 increasing dose levels (i.e., 5, 10, 15, and 20 mg) of aficamten over 3 dose titration opportunities. Doses of aficamten were individually titrated at Weeks 2, 4, and 6 using echocardiography, as outlined in [Table 9](#). Dose escalation occurred only if a subject had a Valsalva LVOT-G ≥ 30 mm Hg and an LVEF $\geq 55\%$. Week 8 represented the start of the predefined maintenance phase during which subjects with oHCM received echocardiographic assessments every 4 weeks through the end of trial at Week 24. Starting from Week 8, the dose was down-titrated if the LVEF was $< 50\%$. If LVEF was $< 50\%$ at any time, the subject was returned to the prior dose level, or placed on placebo if the subject was on 5 mg.

Dosing titration was implemented using an interactive Web Response System (IWRS) in CY 6031. A sonographer and cardiologist (both blinded to randomized treatment assignment) performed and interpreted the echocardiogram and entered the results into the IWRS for dose titrations and adjustments. Investigational product (IP) was dispensed at scheduled trial visits using IWRS. Therefore, the investigator and site staff remained blinded to the echocardiogram images and results as well as treatment group allocation and dosing.

Table 9. Echocardiogram Criteria for Scheduled Dose Titrations in Weeks 2, 4, and 6, Trial CY 6031

Biplane LVEF		Valsalva LVOT-G	Action
< 50%			Reduce Dose ^a
≥ 50% - 55%			No Dose Change
≥ 55%	and	< 30 mmHg	No Dose Change
≥ 55%	and	≥ 30 mmHg	Increase Dose

IP = investigational product; LVEF = left ventricular ejection fraction; LVOT-G = left ventricular outflow tract gradient

^a Once a participant's IP dose was down titrated, no further escalation was permitted. If LVEF was < 50% on 5 mg, the participant received placebo.

Source: Table 2, Trial CY 6031 CSR

Proposed To-Be-Marketed Dose Titration Scheme and Echocardiographic Assessment Frequency

The goal of the proposed to-be-marketed aficamten daily dosing regimen is to maintain the favorable benefit-risk profile established in the Phase 3 trial (CY 6031), while allowing for more flexible clinic visits for subjects with oHCM. Therefore, the Applicant proposed visits of every two to eight weeks during the dose titration phase and every three to six months during the maintenance phase for echocardiographic assessments based on additional modeling and simulations (See Section [14.5](#)).

[Table 10](#) provides a tabulated version of the proposed dosing algorithm. The echocardiography-based dose titration criteria (Rows 1 and 3 to 5) are identical to that employed in the Phase 3 trial ([Table 9](#)) with the exception of the recommendation for subjects on the highest aficamten dose (i.e., 20 mg) who still demonstrate LVEF ≥55% and LVOT-G ≥30 mm Hg to move to the maintenance phase ([Table 10](#), Row 2). Proposed guidance on the resumption of aficamten therapy in subjects with oHCM after dose interruption due to an LVEF <50% is also provided ([Table 10](#), Rows 6 to 8).

Table 10. Tabulated Proposed Aficamten Echocardiography-Based Dose Titration Criteria With Instructions for Dose Titration, Timing of Next Potential Dose Titration, and Management of Potential Events of LVEF <50%

Row	Current			Dose Titration	Next	
	LVEF (%)	LVOT-G (mmHg)	Dose (mg)		Phase	Visit
1	≥ 55	≥ 30	5 to 15	Increase by 5 mg	Titration	2 to 8 weeks
2	(b) (4)			No change	Maintenance	Approximately 6 months
3	≥ 55	< 30	Any			
4	≥ 50 to < 55	Any	Any	No change	Maintenance	Approximately 3 months
5	≥ 40 to < 50	Any	(b) (4)	Decrease by 5 mg	Titration	2 to 8 weeks
6	≥ 40 to < 50	Any		Interrupt for ≥ 7 days;		
7	< 40			(b) (4)		
8	< 40	Any		Interrupt for ≥ 7 days;		
				(b) (4)		

LVEF = left ventricular ejection fraction; LVOT-G = left ventricular outflow tract gradient.

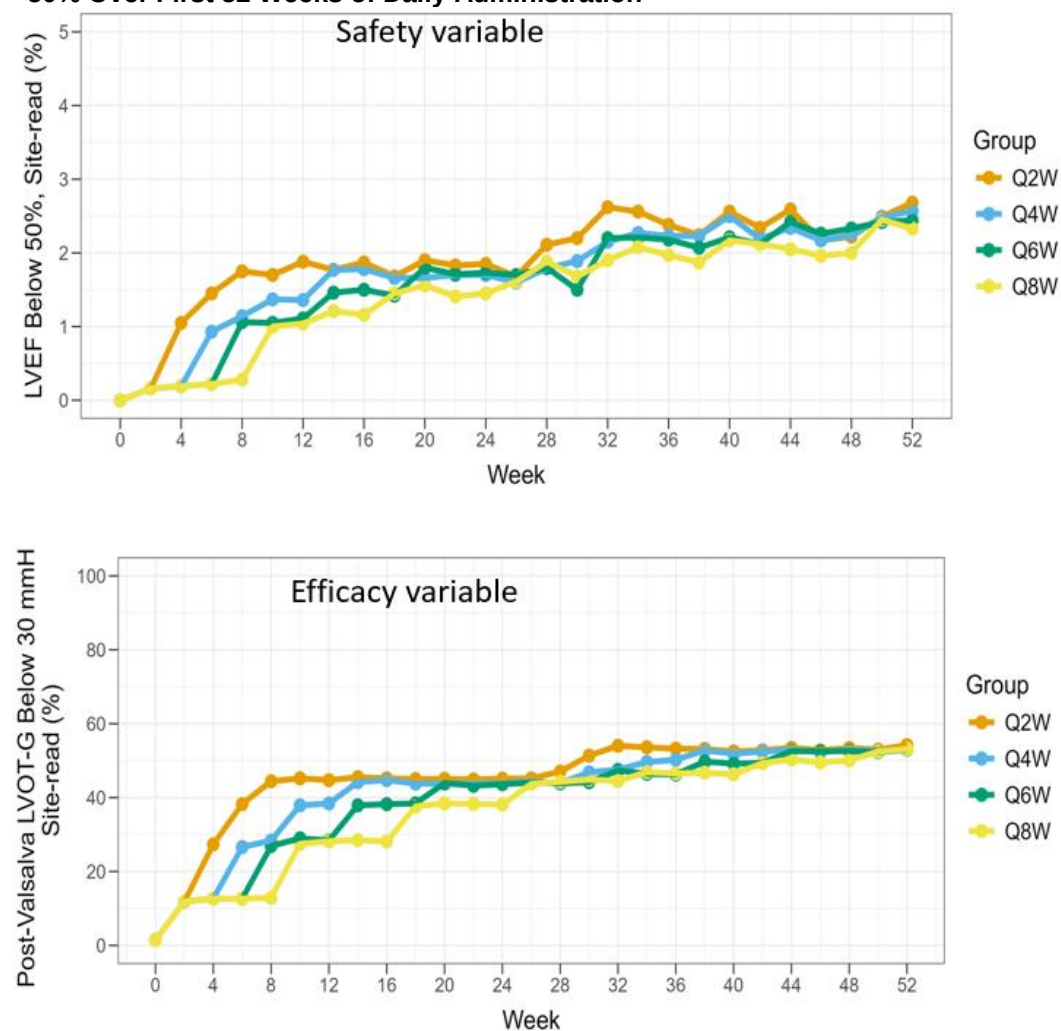
^a Dependent on intercurrent illness and patient clinical status.

Source: Table 56, Module 2.7.2

The Applicant's proposed echocardiographic assessments during titration and maintenance appeared reasonable based on the population PK/PD modeling and simulation:

1. Assessment during titration phase: The Applicant's proposed flexible echocardiographic assessment every 2 to 8 weeks (instead of at Weeks 2, 4, and 6 that was done in the Phase 3 trial) after initiation of treatment appeared reasonable. The pharmacometrics reviewer evaluated the frequency of echocardiographic assessment during the titration phase by performing model-based simulations at different titration frequencies of echocardiographic assessments ranging from every 2 weeks to every 8 weeks. [Figure 2](#) and [Table 11](#) summarize the percent of subjects with Valsalva LVOT-G <30 mm Hg and LVEF <50% based on various echocardiographic assessment frequencies during the titration phase. Briefly, from a safety perspective, every 2-week echocardiographic assessment (orange color) and every 8-week echocardiographic assessment (yellow color) provided similar percentages of subjects with LVEF <50% at 24-weeks. For efficacy, compared to every 2-week echocardiographic assessment, every 8-week echocardiographic assessment resulted in 7% fewer subjects with LVOT-G <30 mm Hg at 24 weeks. Beyond 24 weeks, a similar number of subjects achieved the titration goals for LVEF ≥50% or Valsalva LVOT-G <30 mm Hg irrespective of titration frequencies.

Figure 2. Proposed Commercial Dosing Regimen: Clinical Trial Simulation of Aficamten Exposure and Percent of Subjects With Site-Read Post-Valsalva LVOT-G <30 mm Hg or Site-Read LVEF <50% Over First 52 Weeks of Daily Administration



Source: Reviewer's analysis

Note: The solid line represents simulation results for a virtual population of 10,000 subjects

Abbreviations: LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; QXW, one monitoring visit every X weeks

Table 11. Proposed Commercial Dosing Regimen: Clinical Trial Simulation of Percent of Site-Read LVEF <50% or Site-Read Post-Valsalva LVOT-G <30 mm Hg Over 3 Years of Daily Aficamten Administration

Endpoint	Week	Q2W	Q4W	Q6W	Q8W
Subjects (%) with LVEF <50%	12	1.9 [0.0,4.2]	1.4 [0.0,3.5]	1.1 [0.0,2.8]	1.0 [0.0,2.8]
	24	1.8 [0.0,4.2]	1.7 [0.0,3.5]	1.7 [0.0,3.5]	1.5 [0.0,3.5]
	52	2.7 [0.7,4.9]	2.6 [0.7,4.9]	2.4 [0.7,4.9]	2.3 [0.7,4.2]
	104	3.2 [0.7,5.6]	3.0 [0.7,5.6]	3.0 [0.7,5.6]	3.1 [0.7,5.6]
	156	3.0 [0.7,5.6]	3.0 [0.7,5.6]	3.0 [0.7,5.6]	2.9 [0.7,5.6]
Subjects (%) with LVOT-G <30 mmHg	12	44.7 [38.0,51.4]	38.4 [32.4,45.1]	28.4 [21.8,34.5]	28.2 [21.8,34.5]
	24	45.1 [38.0,51.4]	43.9 [37.3,50.7]	43.6 [36.6,50.7]	38.1 [31.7,45.1]
	52	54.2 [47.2,61.3]	53.1 [45.8,60.6]	53.0 [45.8,59.9]	53.1 [46.5,59.9]
	104	58.7 [52.1,65.5]	58.1 [51.4,65.5]	57.9 [51.4,64.8]	57.8 [51.4,64.8]
	156	59.5 [52.8,66.9]	59.5 [52.8,66.2]	59.2 [52.8,66.2]	58.8 [52.1,65.5]

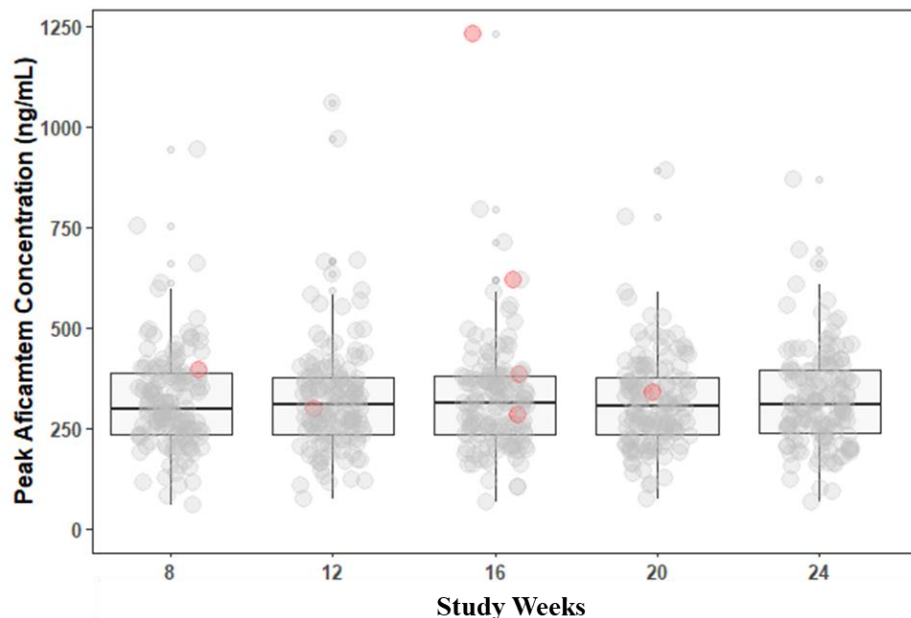
Source: Applicant's Population PK/PD and Exposure Response Report, Table S5 and S6, Page 12

Note: The numbers represent simulation results for a virtual population of 10,000 subjects. The prediction intervals indicate 5th to 95th percentiles for the percentage of subjects on a given dose for 1000 simulations, each with 142 subjects (size of Phase 3 trial). Abbreviations: LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; PD, pharmacodynamic; PK, pharmacokinetic; QXW, one monitoring visit every X weeks

- Assessment during maintenance phase: In the Phase 3 trial, echocardiographic assessments were performed every 4 weeks during the maintenance phase regardless of LVEF. For the to-be-marketed regimen, the Applicant proposed echocardiographic assessments every 3 to 6 months for subjects in the maintenance phase, and to increase the frequency of echocardiographic assessments to every 3 months for LVEF <55% to ≥50%. The Applicant's proposal of assessing LVEF and Valsalva LVOT-G every 3 to 6 months in subjects during the maintenance phase appears reasonable. [Figure 3](#) shows that the plasma levels of aficamten remained stable over 6 months with low inter- and intra-subject variability (%CV: 33.9% and 20.5% respectively) during the maintenance phase of the Phase 3 trial, CY 6031, with seven subjects having LVEF <50%. Thus, PK variability over time is modest and contributes to the low within-subject variability of LVEF in the maintenance phase. Considering there were seven subjects showing LVEF <50%, correlation analyses were performed to identify the predictor (aficamten peak concentration or prior LVEF) for LVEF <50% using the data from Trial CY 6031. The results suggested that prior LVEF has better association with subsequent LVEF as compared to peak plasma concentration during the maintenance phase ([Figure 4](#)). The risk of LVEF <50% was modestly higher (around 50%) in Trial CY 6031 if the preceding LVEF was less than 55%. Considering LVEF <55% as a predictor of subsequent LVEF <50%, correlation analyses were performed between LVEF at Week 24 (LVEF_{wk24}) and preceding LVEFs at various timepoints including Week 20 (4-week preceding LVEF_{wk24}), Week 16 (8-week preceding LVEF_{wk24}), Week 12 (12-week preceding LVEF_{wk24}), and Week 8 (16-week preceding LVEF_{wk24}) using Trial CY 6031 data to identify the optimal assessment time for subjects with LVEF <55% to ≥50% ([Figure 5](#)). The results suggested similar associations between prior LVEFs (i.e., 4-week preceding

LVEF_{wk24}, 8-week preceding LVEF_{wk24}, and 12-week preceding LVEF_{wk24}) and subsequent LVEF at Week 24, while weaker association was observed between LVEF at Week 8 (i.e., 16-week preceding LVEF_{wk24}) and LVEF at Week 24. Thus, the Applicant's proposed increased echocardiographic assessment to every 3 months is reasonable for subjects with LVEF <55% to $\geq 50\%$.

Figure 3. Plasma Levels of Aficamten During the Maintenance Phase, Trial CY 6031

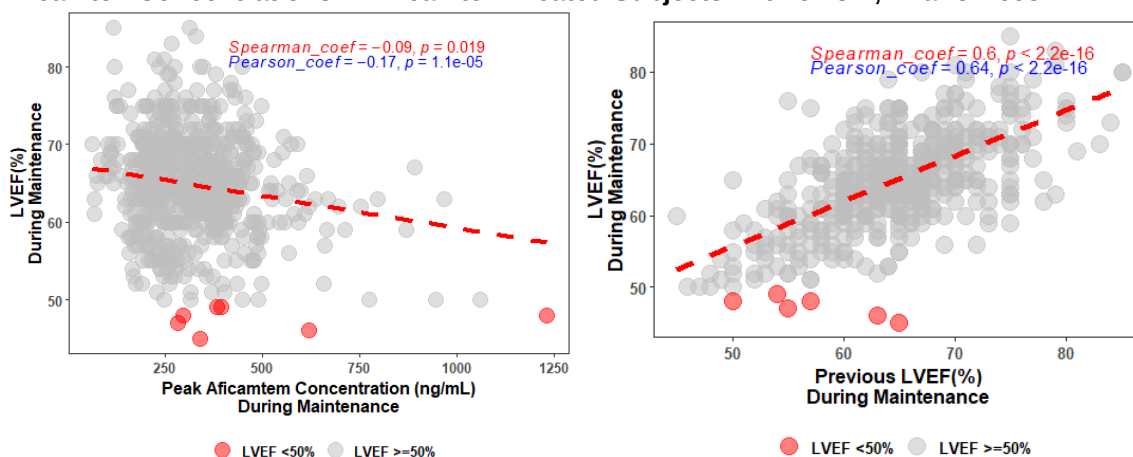


Source: Reviewer's analysis

Note: Pink closed circles represent the subjects with LVEF <50%, grey closed circles represent the subjects with LVEF $\geq 50\%$. The data were collected from subjects enrolled in Phase 3 trial CY 6031.

Abbreviation: LVEF, left ventricular ejection fraction

Figure 4. Correlation Analyses Between Maintenance Phase LVEF and Prior LVEF or Peak Aficamten Concentrations in Aficamten-Treated Subjects With oHCM, Trial CY 6031

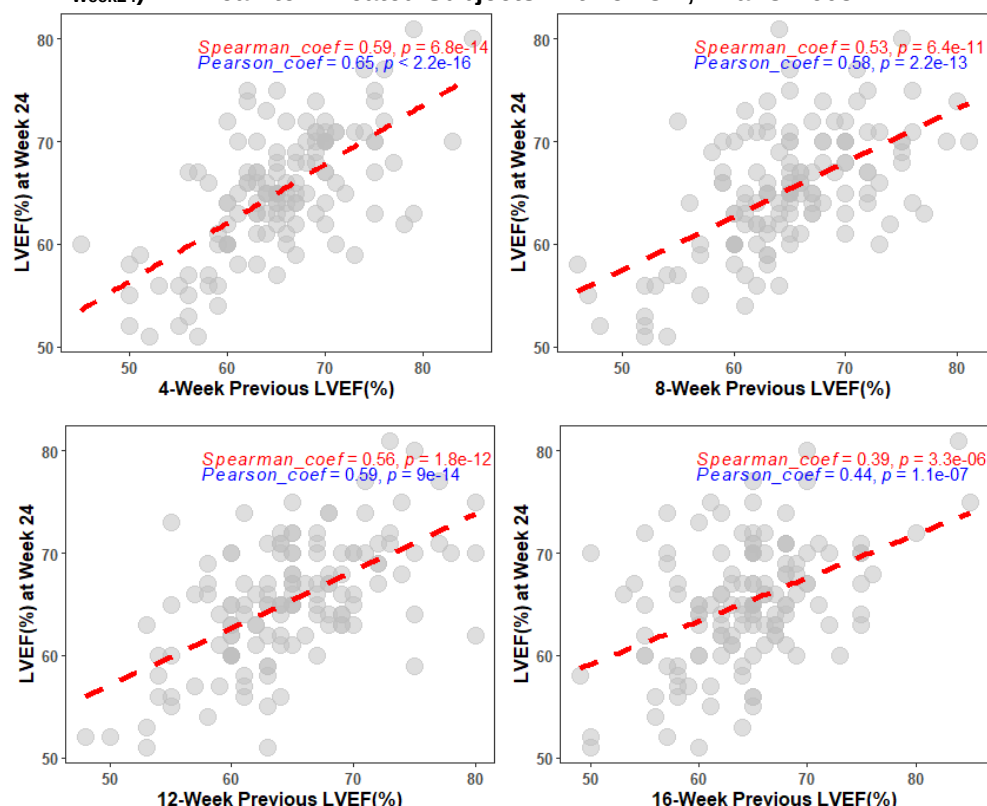


Source: Reviewer's analysis

Notes: The dashed red line represents the linear regression line. The grey and red solid circles represent subjects with LVEF $\geq 50\%$ and LVEF < 50%, respectively. The data were collected from subjects enrolled in the Phase 3 trial.

Abbreviations: coef, coefficient; LVEF, left ventricular ejection fraction; oHCM, obstructive hypertrophic cardiomyopathy

Figure 5. Correlation Analyses Between LVEF at Week 24 ($LVEF_{Week24}$) and Preceding LVEF at Various Timepoints Including Week 20 (4-Week Preceding $LVEF_{Week24}$), Week 16 (8-Week Preceding $LVEF_{Week24}$), Week 12 (12-Week Preceding $LVEF_{Week24}$), and Week 8 (16-Week Preceding $LVEF_{Week24}$) in Aficamten-Treated Subjects With oHCM, Trial CY 6031



Source: Reviewer's analysis

Notes: The dashed red line represents the linear regression line. The data were collected from subjects enrolled in the Phase 3 trial. Abbreviations: LVEF, left ventricular ejection fraction; oHCM, obstructive hypertrophic cardiomyopathy

6.2. Clinical Studies/Trials Intended To Demonstrate Efficacy

6.2.1. CY 6031 (SEQUOIA-HCM)

The primary assessment of efficacy of aficamten for the treatment of adults with symptomatic oHCM was based on the results of the pivotal Phase 3 Trial CY 6031 (SEQUOIA-HCM).

6.2.1.1. Study Design, CY 6031

SEQUOIA-HCM (CY 6031) was a multi-center, randomized, double-blind, placebo-controlled trial in subjects with symptomatic oHCM. Eligible subjects were randomized in a 1:1 ratio to receive aficamten or placebo in addition to background oHCM therapy for 24 weeks. Randomization was stratified by use of beta-blockers (capped at 70% for beta-blocker users) and CPET exercise modality (treadmill or bicycle). Other enrollment limits included disopyramide users that were capped at 10% of total enrollment and subjects with persistent atrial fibrillation (AF) at screening that were capped at 15% of total enrollment. Patients treated with

disopyramide represent a small and specific subgroup of oHCM patients who are symptomatic despite standard of care. The pre-specified enrollment caps minimize the potential confounding factors due to potential differences in patient characteristics and ensure generalizability of the trial results.

Investigational product was administered orally once daily with or without food. The starting dose of aficamten was 5 mg. Doses were individually titrated at Weeks 2, 4, and 6 based on echocardiography-guided criteria (Table 12). Dose escalation with 5-mg increment at Weeks 2, 4, and 6 occurred only if a subject had a Valsalva LVOT-G ≥ 30 mm Hg and LVEF $\geq 55\%$ (available doses: 5 mg, 10 mg 15 mg, and 20 mg). At any visit, the dose was down-titrated if the LVEF was $<50\%$ (40% to 49%). If a subject’s LVEF was $<40\%$ at any time, IP would be interrupted and held for at least 7 days. Repeat echocardiograms would be performed, and a subject could be restarted on IP after down-titration when a normal LVEF ($\geq 55\%$) was documented.

Table 12. Echocardiogram Criteria for Scheduled Dose Titrations, Trial CY 6031

Biplane LVEF		Valsalva LVOT-G	Action
$< 50\%$			Reduce Dose ^a
$\geq 50\% - 55\%$			No Dose Change
$\geq 55\%$	and	< 30 mmHg	No Dose Change
$\geq 55\%$	and	≥ 30 mmHg	Increase Dose

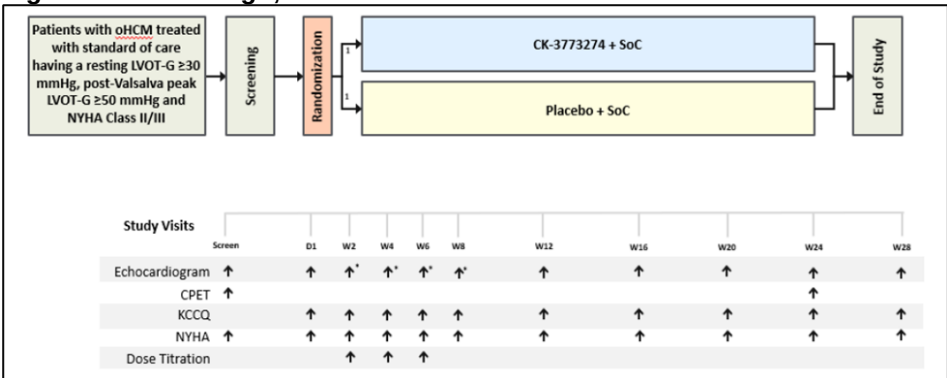
IP = investigational product; LVEF = left ventricular ejection fraction; LVOT-G = left ventricular outflow tract gradient

^a Once a participant’s IP dose was down titrated, no further escalation was permitted. If LVEF was $< 50\%$ on 5 mg, the participant received placebo.

Source: CY 6031 Clinical Study Report, Table 2

The double-blind placebo-controlled treatment period lasted 24 weeks. Following the last dose of IP, there was a 4-week safety follow-up period.

Figure 6. Trial Design, Trial CY 6031



Source: CY 6031 protocol, Figure 2

Abbreviations: CPET, cardiopulmonary exercise testing; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVOT-G, left ventricular outflow tract gradient; NYHA, New York Heart Association; oHCM, obstructive hypertrophic cardiomyopathy; SoC, standard of care

6.2.1.2. Eligibility Criteria, CY 6031

Key Inclusion Criteria

- Between 18 and 85 years old at screening.
- Body mass index $<35 \text{ kg/m}^2$ at screening.
- Acoustic windows to enable accurate transthoracic echocardiograms.
- Diagnosed with HCM per the following criteria:
 - Had LV hypertrophy with nondilated LV chambers in the absence of other cardiac causes
 - Had an end-diastolic LV wall thickness $\geq 15 \text{ mm}$ (or $\geq 13 \text{ mm}$ with a known disease-causing gene mutation or positive family history of HCM) as determined by core laboratory interpretation, **and**
 - Had resting LVOT-G $\geq 30 \text{ mm Hg}$ and Valsalva LVOT-G $\geq 50 \text{ mm Hg}$ at screening.
- LVEF $\geq 60\%$ at screening as determined by the echocardiography core laboratory.
- NYHA Functional Class II or III at screening.
- Hemoglobin $\geq 10 \text{ g/dL}$ at screening.
- Respiratory exchange ratio ≥ 1.05 and $\text{pVO}_2 \leq 90\%$ at screening.
- Subjects on beta-blockers, verapamil, diltiazem, or disopyramide should have been on a stable regimen for >6 weeks prior to randomization and anticipate remaining on the same medication regimen during the trial. Subjects treated with disopyramide must also be treated concomitantly with a beta blocker and/or calcium channel blocker.

Key Exclusion Criteria

- Significant valvular heart disease:
 - Moderate-severe valvular aortic stenosis and/or regurgitation.
 - Moderate-severe mitral regurgitation not due to systolic anterior motion of the mitral valve.
- Documented history of current obstructive coronary artery disease or myocardial infarction.
- Known or suspected infiltrative, genetic or storage disorder causing cardiac hypertrophy that mimics oHCM.
- Prior treatment with cardiotoxic agents such as doxorubicin.
- History of LV systolic dysfunction (LVEF $<45\%$) or stress cardiomyopathy at any time during their clinical course.
- Had any electrocardiogram abnormality considered by the investigator to pose a risk to subject safety (e.g., second degree atrioventricular block type II).
- Documented paroxysmal AF during the screening period.

- Paroxysmal or permanent AF is only excluded if:
 - Rhythm restoring treatment has been required ≤ 6 months prior to screening.
 - Rate control and anticoagulation has not been achieved at least 6 months prior to screening.
- History of syncope or sustained ventricular tachyarrhythmia with exercise within 6 months prior to screening.
- Implantable cardioverter-defibrillator (ICD) placement within 3 months prior to screening or planned ICD placement during the trial.
- History of appropriate ICD discharge for life-threatening ventricular arrhythmia within 6 months prior to screening.
- Had been treated with SRT or cannot postpone plans for SRT until after the trial period
- Inability to exercise on a treadmill or bicycle.
- Documented room air oxygen saturation reading $< 90\%$ at screening.
- Had received prior treatment with aficamten or mavacamten.

6.2.1.3. Statistical Analysis Plan, CY 6031

The review division provided comments on the Trial CY 6031 protocol (preliminary meeting minutes dated June 1, 2021, End-of-Phase 2 meeting minutes dated August 3, 2021) and the statistical analysis plan (SAP) (dated August 11, 2023, and November 1, 2023).

Sample Size

The Applicant specified a sample size of 270 subjects, randomized 1:1 to aficamten or placebo, providing $> 90\%$ power to detect a change from baseline in pVO_2 to Week 24 of 1.5 mL/kg/min compared to placebo (2-sided type I error of 0.05), assuming a standard deviation of 3.5 mL/kg/min, and controlling for limiting beta-blocker use (less than $\sim 70\%$), limiting exercise modality of bicycle (less than $\sim 50\%$), and 10% of subjects with missing data (i.e., change from baseline data of the primary endpoint).

During the trial, the Applicant pre-specified periodic assessments of the blinded aggregate pooled missing data rate and overall pooled SD for the change from baseline in pVO_2 at Week 24. Based on these assessments, the Applicant could increase the sample size once to maintain the intended power. Thus, there were 282 subjects, rather than 270, enrolled and randomized in the trial.

Efficacy Endpoints

Primary

The primary endpoint of the trial was Change in pVO_2 by CPET from baseline to Week 24.

Key Secondary

- Change in KCCQ-CSS from baseline to Week 12 and Week 24.
- Proportion of subjects with ≥ 1 class improvement in NYHA Functional Class from baseline to Week 12 and Week 24.
- Change in Valsalva LVOT-G from baseline to Week 12 and Week 24.
- Proportion of subjects with Valsalva LVOT-G < 30 mm Hg at Week 12 and Week 24.
- Total duration of SRT eligibility during the 24-week treatment period in subjects who were SRT eligible at baseline.
- Change in total workload during CPET from baseline to Week 24.

Primary Analysis Population

Primary efficacy analyses were performed on the full analysis set (FAS), which included all randomized subjects.

Efficacy Analyses

Efficacy analyses were performed in the FAS by the randomized treatment group. All hypothesis tests were reported as 2-sided p-values. To preserve an overall type I error rate of 0.05, testing of the primary and secondary endpoints followed a specified order using a closed testing procedure as described below.

Null and Alternative Hypotheses

The null hypothesis for the primary endpoint was that the treatment difference (aficamten minus placebo) of the mean change from baseline of pVO₂ at Week 24 was 0.

Multiple Comparisons and Multiplicity

A closed testing procedure was used to address multiplicity and preserve the overall type I error rate. The primary endpoint was tested first at a two-sided 0.05 alpha threshold. If the primary endpoint achieved statistical significance, secondary endpoints were then tested in sequential order at the two-sided 0.05 level:

- KCCQ-CSS change from baseline at Week 24.
- Proportion of subjects with ≥ 1 NYHA functional class improvement at Week 24.
- Valsalva LVOT-G change from baseline at Week 24.
- Proportion of subjects with Valsalva LVOT-G < 30 mm Hg at Week 24.
- Duration of SRT eligibility up to 24 weeks of treatment for those who were SRT eligible at baseline.
- KCCQ-CSS change from baseline at Week 12.
- Proportion of subjects with ≥ 1 NYHA functional class improvement at Week 12.
- Valsalva LVOT-G change from baseline at Week 12.

- Proportion of subjects with Valsalva LVOT-G <30 mm Hg at Week 12.
- Change from baseline in total workload at Week 24.

Primary Endpoint Analysis

The primary analysis was performed using an analysis of covariance (ANCOVA) model that included terms of treatment, randomization stratification factors (beta-blocker use status and CPET modality), baseline pVO₂ and baseline body weight as covariates in the FAS. Data analyzed include all observed Week 24 pVO₂ values from subjects who completed 24 weeks of treatment, as well as from subjects who early terminate from the treatment but remain in the trial with Week 24 pVO₂ measurements. Data were imputed for subjects who were missing Week 24 pVO₂. Missing data were imputed using multiple imputation methodology under the missing at random (MAR) assumption for the primary analysis. One hundred imputed MAR datasets were generated. Results were combined using Rubin's rule to produce an LS mean estimate of the treatment difference, its 95% confidence interval (CI), and p-value (p). Additional statistical details are provided in Section [16](#).

Secondary Endpoints Analysis

Change in KCCQ-CSS and change in Valsalva LVOT-G from baseline to Week 12 and Week 24 were analyzed using a mixed model for repeated measures (MMRM) model with baseline as a covariate, randomization stratification factors, visit, treatment group, and interaction terms of treatment by visit and baseline by visit. Since there was a >10% difference in baseline KCCQ-CSS across regions, treatment by visit and region and baseline by visit and region were also included in the model. The treatment effect at each visit week was estimated from the interaction terms of treatment by visit by region, with coefficients for each region determined by proportion of subjects evaluated in each region. An unstructured covariance matrix was specified. All data observed up to Week 24 post-randomization was included in the model. The LS mean estimates, differences, 95% CIs, and p-values were reported for the results.

The proportion of subjects with ≥ 1 class improvement in NYHA Functional Class and proportion of subjects with Valsalva LVOT-G <30 mm Hg at Week 12 and Week 24 were analyzed using the Cochran–Mantel–Haenszel test stratified by randomization factors. For the proportion of subjects with ≥ 1 class improvement in NYHA class at Week 12, Week 16 NYHA class was used if Week 12 NYHA class was not available. Week 8 NYHA class was used if Week 16 NYHA was not available essentially making this a closest observation imputation. Subject was considered as not achieving ≥ 1 improvement in NYHA class at Week 12 if no NYHA at Weeks 8, 12, and 16 was available. Similarly, Week 20 NYHA class was used if Week 24 NYHA was not available. Subjects were considered as not achieving ≥ 1 improvement in NYHA class at Week 24 if Week 20 and Week 24 NYHA were not available.

For the proportion of subjects with post-Valsalva LVOT <30 mm Hg at Week 12, Week 16 post-Valsalva LVOT was used if the Week 12 visit was performed but post-Valsalva LVOT was not available. Week 8 post-Valsalva LVOT was used if Week 16 was not available. Similarly, Week 20 post Valsalva LVOT was used if Week 24 was performed but post Valsalva LVOT was not available. The p-value and 95% CI were obtained using an exact method.

Total duration of SRT eligibility was calculated as the number of days subjects are SRT eligible, from the date of the randomization until Week 24. Because the SRT eligibility status of a subject

may change during the trial conduct, only intervals during which the subject is SRT eligible, were summed. Total duration of SRT eligibility during the 24-week of treatment period was analyzed using an ANCOVA model that includes treatment and randomization stratification factor beta blocker use/nonuse as fixed effects, and significant baseline characteristics as covariates. Significance of baseline covariates was evaluated using a stepwise model selection method with a default stay or entry level of 0.05.

Change in total workload during CPET from baseline to Week 24 was analyzed using the same analysis approach as for the primary endpoint analysis.

It should be noted that the review team did not agree with the Applicant's approach for missing data. For the primary analyses, the Applicant imputed the missing data under the missing-at-random assumption despite the advice provided by the biometrics division, dated August 11, 2023, on the CY 6031 statistical analysis plan (SAP):

We continue to disagree that an MAR assumption is appropriate given that patients with missing data are likely to be different from those who remain in the trial on treatment. It is generally not acceptable because patients who drop out of the study and are no longer on study treatment are not expected to behave in the same way as those who continue in the study on treatment, which would not be in line with a treatment policy estimand, and those who die should not be imputed with similar outcomes to those who are able to remain in the study. You can use different imputation models based on the type of intercurrent events which are the reasons for missing data. For example, retrieved dropouts (those who discontinue treatment but continue study visits) can be used to impute data for those who dropout of the study. For those who die before the primary endpoint can be assessed, we generally accept a worst-case type of imputation where the worst 10% of observed outcomes can be used for a multiple imputation of missing data due to death.

Additionally, the review team did not agree with the use of stepwise model selection approach due to well-known issues with overestimation of model fit and increase in type I error. In this case, no adjusting covariates were ultimately included in the model.

Subgroup Analysis of the Primary Endpoint

Subgroup analyses for the primary efficacy endpoint were performed using the following variables:

- Sex (male, female)
- Age group (<65, ≥65 years old)
- Baseline body mass index (<30 versus ≥30)
- Baseline NYHA Class (II, III)
- Baseline KCCQ-CSS (≤ median and > median)
- Baseline LVEF (≤ median and > median)
- Baseline NT-proBNP (≤ median and > median)
- CPET modality (treadmill, bicycle)

- Baseline pVO₂ (≤ median, > median)
- Beta Blocker (use, no use)
- Baseline resting LVOT (≤ median and > median)
- Sarcomeric gene mutation status

An information request was submitted on November 14, 2024, to perform subgroup analysis also by race and ethnicity. The Applicant provided these analyses, and these results were subsequently incorporated into the review.

Protocol Amendments

The original protocol dated July 26, 2021, was amended four times. In the NDA submission, the Applicant provided the following milestone dates:

- First subject enrolled: February 1, 2022
- Last subject completed: December 19, 2023
- Protocol version and date: Amendment 4 dated December 8, 2023
- SAP version and date: Final (Version 02) October 2023

A summary of protocol amendments is given in Section [16](#).

6.2.1.4. Results of Analyses, CY 6031

Subject Disposition

A total of 543 persons were screened for the trial. Of these, 261 failed screening, primarily for not satisfying the inclusion/exclusion criteria (98.9%) as shown in [Table 13](#).

Table 13. Subject Screening and Enrollment, Trial CY 6031

Disposition	Trial CY 6031
Subjects screened	543
Screening failures	261 (48.1%)
Inclusion/exclusion criteria not met	258 (98.9%)
Subject noncompliance	0 (0.0%)
Consent withdrawn	1 (0.38%)
Other	2 (0.77%)
Subjects enrolled	282 (51.9%)
Subjects randomized	282 (51.9%)

Source: Applicant’s CSR Table 14.1.1.3, verified by statistical reviewer

The remaining screened subjects (n=282) were enrolled and randomized to treatment (142 to aficamten and 140 to placebo). [Table 14](#) shows the disposition of all the randomized subjects. Of the subjects randomized to aficamten, five (3.5%) discontinued treatment early: two due to subject withdrawal, two due to a protocol deviation, and one due to an adverse event (AE). Of the subjects randomized to placebo, four (2.9%) discontinued treatment early: two due to an AE, one due to physician decision, and one due to other reason. All subjects in both treatment groups received at least one dose of IP, so the All-Randomized set matched the primary FAS.

Table 14. Subject Disposition, All -Randomized Set, Trial CY 6031

Subject Disposition	Aficamten N=142 n (%)	Placebo N=140 n (%)	Overall N=282 n (%)
Subjects received at least one dose	142 (100)	140 (100)	282 (100)
Subjects completed treatment ¹	137 (96.5)	136 (97.1)	273 (96.8)
Subjects early terminated from treatment ¹	5 (3.5)	4 (2.9)	9 (3.2)
Reason for early termination from treatment ²			
Physician decision	0	1 (25.0)	1 (11.1)
Adverse event	1 (20.0)	2 (50.0)	3 (33.3)
Withdrawal by subject	2 (40.0)	0	2 (22.2)
Protocol deviation	2 (40.0)	0	2 (22.2)
Other	0	1 (25.0)	1 (11.1)
Subjects completed trial ¹	139 (97.9)	137 (97.9)	276 (97.9)
Subjects early terminated from trial ¹	3 (2.1)	3 (2.1)	6 (2.1)
Reason for early termination from trial ³			
Physician decision	0	2 (66.7)	2 (33.3)
Adverse event	0	1 (33.3)	1 (16.7)
Withdrawal by subject	2 (66.7)	0	2 (33.3)
Other	1 (33.3)	0	1 (16.7)

Source: Applicant's CSR Table 14.1.1.1, verified by statistical reviewer

¹ Percentages were calculated using the number of subjects who received at least one dose as denominator.

² Percentages were calculated using the number of subjects who terminated treatment early as denominator.

³ Percentages were calculated using the number of subjects who terminated the trial early as denominator.

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic

Protocol Violations/Deviations

There were 62 protocol deviations that were classified as important protocol deviations for a total of 51 subjects (18.1%): 24 subjects (16.9%) in the aficamten group and 27 subjects (19.3%) in the placebo group. The incidence of important protocol deviations was similar between the aficamten and placebo groups for all category subtypes.

Table 15. Summary of Important Protocol Deviations Based on Recategorization of Severity Postdatabase Lock, Trial CY 6031

Protocol Deviation Type	Aficamten N=142	Placebo N=140	Overall N=282
Any important protocol deviation	24 (16.9)	27 (19.3)	51 (18.1)
IP administration/trial treatment	14 (9.9)	11 (7.9)	25 (8.9)
Informed consent	5 (3.5)	6 (4.3)	11 (3.9)
Procedures	4 (2.8)	5 (3.6)	9 (3.2)
Laboratory tests	3 (2.1)	4 (2.9)	7 (2.5)
AE/SAE	0	1 (0.7)	1 (0.4)

Source: Applicant's CSR Table 14.1.3.2

Subjects were counted once for each row if multiple protocol deviations were reported.

Abbreviations: AE, adverse event; IP, investigational product; N, number of subjects in treatment group; SAE, serious adverse event

Demographics

[Table 16](#) examines the between-group balance on demographics in the FAS population. Most subjects were male (59.2%), White (79.1%), and non-Hispanic (91.5%). Overall, approximately half of the subjects were in either North America (33.3%) or China (16.3%); the remaining

subjects were included in the “rest of world.” Demographics characteristics were generally balanced between the aficamten and placebo treatment groups.

Table 16. Baseline Demographics, Full Analysis Set, Trial CY 6031

Demographics	Aficamten N=142	Placebo N=140	Overall N=282
Sex, n (%)			
Male	86 (60.6)	81 (57.9)	167 (59.2)
Female	56 (39.4)	59 (42.1)	115 (40.8)
Age, years			
Mean (SD)	59.2 (12.55)	59.0 (13.37)	59.1 (12.94)
Median (min, max)	59.0 (18, 83)	60.0 (23, 84)	59.5 (18, 84)
Age group (years), n (%)			
≥17 to <65	85 (59.9)	84 (60.0)	169 (59.9)
≥65 to <75	45 (31.7)	37 (26.4)	82 (29.1)
≥75	12 (8.4)	19 (13.6)	31 (11.0)
Race, n (%)			
White	108 (76.1)	115 (82.1)	223 (79.1)
Black or African American	3 (2.1)	0	3 (1.1)
Asian	29 (20.4)	25 (17.9)	54 (19.1)
Other	2 (1.4)	0	2 (0.7)
Ethnicity, n (%)			
Hispanic	3 (2.1)	6 (4.3)	9 (3.2)
Non-Hispanic	128 (90.1)	130 (92.9)	258 (91.5)
Unknown	11 (7.7)	4 (2.9)	15 (5.3)
Region, n (%)			
North America	49 (34.5)	45 (32.1)	94 (33.3)
China	24 (16.9)	22 (15.7)	46 (16.3)
Rest of world	69 (48.6)	73 (52.1)	142 (50.4)

Source: Applicant's CSR Table 14.1.4, verified by statistical reviewer

Abbreviations: max, maximum; min, minimum; N, number of subjects in treatment group; n, number of subjects with given characteristic; SD, standard deviation

Baseline Characteristics

[Table 17](#) examines the between-group balance on baseline clinical characteristics in the FAS population. At baseline, subjects had a median body mass index of 28.1 kg/m². Most subjects had NYHA Class II heart failure (75.9%). The mean LVEF was 74.8%, the mean resting LVOT-G was 55.1 mm Hg, and the mean Valsalva LVOT-G was 83.1 mm Hg. A total of 61 subjects (32 aficamten, 29 placebo) were SRT eligible at baseline. The mean pVO₂ by CPET at baseline was 18.5 mL/kg/min, with 55% of subjects using treadmill as the CPET modality. The baseline clinical characteristics were generally balanced between the aficamten and placebo treatment groups.

Table 17. Clinical Characteristics, Full Analysis Set, Trial CY 6031

Baseline Assessments	Aficamten N=142	Placebo N=140	Overall N=282
BMI (kg/m ²)			
Mean (SD)	28.0 (3.8)	28.2 (3.8)	28.1 (3.8)
Median (min, max)	28.1 (18, 35)	28.2 (19, 35)	28.1 (18, 35)
NYHA class at baseline, n (%)			
Class II	108 (76.1)	106 (75.7)	214 (75.9)
Class III	34 (23.9)	33 (23.6)	67 (23.8)
Class IV	0	1 (0.7)	1 (0.4)
SRT eligibility, n (%)	32 (22.5)	29 (20.7)	61 (21.6)
CPET modality at baseline, n (%)			
Bicycle	64 (45.1)	63 (45.0)	127 (45.0)
Treadmill	78 (54.9)	77 (55.0)	155 (55.0)
Resting LVOT-G (mm Hg) ¹			
Mean (SD)	54.8 (27.0)	55.3 (32.2)	55.1 (29.6)
Median (min, max)	50.7 (12, 147)	52.1 (8, 155)	51.1 (8, 155)
Valsalva LVOT-G (mm Hg) ¹			
Mean (SD)	82.9 (32.0)	83.3 (32.7)	83.1 (32.3)
Median (min, max)	83.5 (15, 246)	79.7 (11, 194)	81.4 (11, 246)
LVEF (%) ¹			
Mean (SD)	74.8 (5.5)	74.8 (6.3)	74.8 (5.9)
Median (min, max)	75.2 (56, 87)	75.8 (51, 87)	75.6 (51, 87)
pVO ₂ at baseline CPET (mL/kg/min)			
Mean (SD)	18.4 (4.5)	18.6 (4.5)	18.5 (4.5)
Median (min, max)	18.3 (9, 31)	18.5 (9, 32)	18.4 (9, 32)
KCCQ-CSS			
Mean (SD)	75.6 (18.4)	73.7 (17.6)	74.7 (18.0)
Median (min, max)	79.9 (11, 100)	76.6 (26, 100)	78.1 (11, 100)
Systolic BP (mm Hg) at baseline			
Mean (SD)	124.6 (15.9)	125.7 (16.0)	125.2 (15.9)
Median (min, max)	123.5 (84, 166)	124.0 (84, 172)	124.0 (84, 172)
Diastolic BP (mm Hg) at baseline			
Mean (SD)	74.6 (10.7)	74.1 (10.6)	74.4 (10.6)
Median (min, max)	74.0 (48, 102)	74.0 (42, 119)	74.0 (42, 119)
Heart rate (beats/minute) at baseline			
Mean (SD)	65.5 (10.6)	65.6 (11.9)	65.6 (11.2)
Median (min, max)	64.0 (41, 92)	64.0 (43, 102)	64.0 (41, 102)

Source: Applicant's CSR Table 14.1.4, verified by statistical reviewer

¹ Per the core laboratory

Abbreviations: BMI, body mass index; BP, blood pressure; CPET, cardiopulmonary exercise testing; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire – Clinical Summary Score; LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; max, maximum; min, minimum; N, number of subjects in treatment group; n, number of subjects with given characteristic; pVO₂, peak oxygen consumption; SD, standard deviation; SRT, septal reduction therapy

Efficacy Analyses

The statistical reviewer performed the pre-specified analyses to verify the Applicant's results and performed additional analyses where missing data were imputed under missing not at random assumptions (MNAR) using placebo-based multiple imputation.

Primary Endpoint Analysis Results

Results for the primary endpoint, change in pVO₂ by CPET from baseline to Week 24, are shown in [Table 18](#). Baseline pVO₂ was approximately 18 mL/kg/min. There was a statistically significant treatment effect of 1.74 mL/kg/min (95% CI: 1.04, 2.44; p<0.0001) when comparing

change from baseline to Week 24 in the aficamten group to the placebo group. The clinical significance of these changes in pVO₂ is discussed in Section 6.3.1.

Table 18. Change in pVO₂ on CPET From Baseline to Week 24, Full Analysis Set, Trial CY 6031

pVO ₂ (mL/kg/min)	Applicant's Prespecified Analysis		Additional Analysis ¹	
	Aficamten N=142	Placebo N=140	Aficamten N=142	Placebo N=140
Baseline				
N	142	140		
Mean (SD)	18.38 (4.45)	18.59 (4.51)		
Week 24				
N	133	130		
Mean (SD)	20.21 (5.21)	18.58 (4.72)		
Change from baseline to Week 24				
Mean (SD)	1.78 (3.12)	0.01 (2.73)		
LS mean	1.76	0.02	1.69	0.01
LS mean 95% CI	(1.26, 2.26)	(-0.48, 0.52)	(1.19, 2.19)	(-0.50, 0.51)
LS mean diff vs. placebo	1.74		1.69	
LS mean diff vs. placebo 95% CI	(1.04, 2.44)		(0.99, 2.39)	
p-Value	<0.0001		<0.0001	

Source: adsl.xpt, adcv.xpt, adbase.xpt, statistical reviewer analysis

¹ Missing data for subjects who discontinued treatment were imputed using placebo-based multiple imputation, other missing data were imputed MAR.

Missing data were imputed using regression multiple imputation including treatment group, randomization stratification factors, baseline pVO₂, sex, age, baseline hemoglobin, baseline body weight, baseline KCCQ-CSS, baseline NYHA class, and the last available post randomization NYHA functional class, resting and Valsalva LVOT under missing at random assumption.

Each completed dataset was analyzed using an ANCOVA model with fixed effects of treatment, beta blocker use (Yes/No), CPET modality, and baseline pVO₂ and baseline body weight as covariates. Results from all 100 imputed datasets were combined for overall inference using Rubin's rules.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CPET, cardiopulmonary exercise testing; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire – Clinical Summary Score; LS, least-squares; LVOT, left ventricular outflow tract; MAR, missing at random; N, number of subjects in treatment group; n, number of subjects with given characteristic; NYHA, New York Heart Association; pVO₂, peak oxygen consumption; SD, standard deviation

At Week 24, 9 subjects (6.3%) in the aficamten group and 10 subjects (7.1%) in the placebo group had missing pVO₂ data. Most missing data were attributed to either early termination from the trial or invalid pVO₂ measures assessed by the CPET core laboratory at Week 24.

Table 19. Summary of Missing pVO₂ on CPET at Week 24, Full Analysis Set, Trial CY 6031

	Aficamten N=142	Placebo N=140	Overall N=282
Week 24 pVO₂			
Missing Week 24 pVO ₂	9 (6.3)	10 (7.1)	19 (6.7)
Early termination from trial	3 (2.1)	3 (2.1)	6 (2.1)
Physician decision	0	2 (1.4)	2 (0.7)
Adverse event	0	1 (0.7)	1 (0.4)
Withdrawal by subject	2 (1.4)	0	2 (0.7)
Other	1 (0.7)	0	1 (0.4)
Invalid pVO ₂	6 (4.2)	7 (5.0)	13 (4.6)
CPET MOP	2 (1.4)	1 (0.7)	3 (1.1)
Technical	4 (2.8)	6 (4.3)	10 (3.5)

Source: Applicant's CSR Table 14.2.1.1.3, verified by statistical reviewer

Abbreviations: CPET, cardiopulmonary exercise testing; MOP, Manual of Operating Procedures; N, number of subjects in treatment group; pVO₂, peak oxygen consumption

Because FDA did not agree to the MAR assumption made in the primary analysis, the review team conducted an additional analysis imputing missing data from subjects who discontinued

aficamten using placebo-based multiple imputation. There were not a sufficient number of retrieved dropouts to perform a retrieved dropouts multiple imputation. Missing data for subjects who remained on aficamten treatment were imputed assuming MAR based on the model derived from observed data within the aficamten group. Results from this analysis, 1.69 mL/kg/min (95% CI: 0.99, 2.39; $p < 0.0001$), were slightly attenuated, but similar to the primary MAR analysis as shown in [Table 18](#).

Sensitivity analyses for the primary endpoint are presented in Section [16](#). Results are generally consistent with the primary analysis.

Primary Endpoint Subgroup Analysis Results

Subgroup analyses were conducted to assess the potential for differences in the treatment effect for various demographic groups and are presented in Section [16](#). Overall, the treatment effect of aficamten when compared to placebo was generally consistent with the overall population across demographic subgroups of age, sex, race, and ethnicity. Note that ethnic and racial minorities were not well-represented in this trial.

Secondary Endpoints Analyses Results

All secondary endpoints were found to be statistically significant for both the Applicant's specified methodology, as well as the additional FDA analyses ([Table 20](#), [Table 21](#), [Table 22](#), [Table 23](#), [Table 24](#), and [Table 25](#)).

KCCQ-CSS

The mean baseline KCCQ-CSS score was approximately 75 (scores range from 0-100, with higher scores indicating better health status). The estimated KCCQ-CSS change from baseline at Week 24 was 11.6 points for aficamten and 4.3 points for placebo with an estimated LS mean difference of 7.3 points (95% CI: 4.6, 10.1; $p < 0.0001$). For change from baseline at Week 12, estimates were 11.1 points for aficamten and 4.0 points for placebo. The LS mean difference was 7.0 points (95% CI: 4.5, 9.5; $p < 0.0001$). Seven subjects had missing KCCQ-CSS (4 in aficamten and 3 in placebo) data at Week 24. Missing data were handled as MAR by the MMRM model in the prespecified analysis.

The statistical reviewer performed an additional analysis where intermittent missing data were imputed using the Markov Chain Monte Carlo method under MAR assumption. After the first step of imputation, the monotone missing data were imputed using placebo-based imputations. Imputed datasets were analyzed using an MMRM model. The LS mean difference between treatment groups at Week 12 was 7.0 points (95% CI: 4.6, 9.5; $p < 0.0001$) and at Week 24 was 7.3 points (95% CI: 4.2, 10.0; $p < 0.0001$), essentially unchanged from the primary analysis method.

Table 20. Change in KCCQ-CSS From Baseline at Weeks 12 and 24, Full Analysis Set, Trial CY 6031

KCCQ-CSS	Applicant's Prespecified Analysis		Additional Analysis ¹	
	Aficamten N=142	Placebo N=140	Aficamten N=142	Placebo N=140
Baseline				
n	142	140		
Mean (SD)	75.6 (18.4)	73.7 (17.6)		
Week 12				
n	140	136		
Mean (SD)	86.6 (14.8)	78.5 (16.7)		
Change from baseline to Week 12				
Mean (SD)	11.1 (13.2)	4.8 (10.0)		
LS mean	11.1	4.0	11.1	4.0
LS mean 95% CI	(9.3, 12.9)	(2.3, 5.8)	(9.3, 12.9)	(2.2, 5.8)
LS mean diff vs. placebo	7.0		7.0	
LS mean diff vs. placebo 95% CI	(4.5, 9.5)		(4.6, 9.5)	
p-Value	<0.0001		<.0001	
Week 24				
n	138	137		
Mean (SD)	87.0 (15.6)	78.5 (17.6)		
Change from baseline to Week 24				
Mean (SD)	11.5 (13.2)	4.8 (11.9)		
LS mean	11.6	4.3	11.6	4.3
LS mean 95% CI	(9.6, 13.6)	(2.3, 6.2)	(9.6, 13.6)	(2.3, 6.3)
LS mean diff vs. placebo	7.3		7.3	
LS mean diff vs. placebo 95% CI	(4.6, 10.1)		(4.5, 10.0)	
p-Value	<0.0001		<0.0001	

Source: adsl.xpt, adbase.xpt, adqs.xpt, statistical reviewer analysis

¹ Intermittent missing data were imputed using the MCMC method under MAR assumption. After the first step of imputation, the monotone missing data were imputed using the imputation model built from the placebo group. Data up to Week 24 were analyzed using an MMRM model with baseline as covariate, randomization stratification factors, visit, treatment group, region, and interaction terms of treatment by visit, baseline by visit, region by treatment, region by visit, baseline by region, treatment by visit and region, and baseline by visit and region as fixed effects. An unstructured covariance matrix was specified. LS means were adjusted for region.

Abbreviations: CI, confidence interval; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire – Clinical Summary Score; LS, least-squares; MAR, missing at random; MCMC, Markov Chain Monte Carlo; MMRM, mixed model for repeated measures; N, number of subjects in treatment group; n, number of subjects with given characteristic; SD, standard deviation

An information request was submitted on December 18, 2024, and December 23, 2024, regarding the clinical meaningfulness of the KCCQ-CSS instrument and datasets with variables indicating the values imputed for missing data. The Clinical Outcome Assessment (COA) team was consulted to assess clinical meaningfulness of the KCCQ-CSS results and the proposed labeling claim (COA review dated October 10, 2025). The COA team concluded that KCCQ-CSS was fit-for-purpose to support its context of use in subjects with oHCM; and the Applicant's proposed threshold of 10 points as a reflection of KCCQ-CSS within-subject clinically meaningful change from baseline to Week 24 appeared reasonable. The COA team further concluded that the evidence provided by the Applicant generally supported the proposed labeling claims.

NYHA Improvement

There was a statistically significantly greater proportion of aficamten-treated subjects with a ≥ 1 class improvement in NYHA functional class compared with placebo-treated subjects at Week

12: 48.6% versus 17.9% with an odds ratio of 4.6 (95% CI: 2.6, 8.5; $p < 0.0001$). Similarly, at Week 24, a statistically significantly greater proportion of aficamten-treated subjects had a ≥ 1 class improvement in NYHA functional class compared with placebo-treated subjects: 58.5% versus 24.3% with an odds ratio of 4.4 (95% CI: 2.6, 7.6; $p < 0.0001$). There were 6 (2.1%) subjects at Week 24 and 4 (1.4%) subjects at Week 12 for whom NYHA class value was not available.

The statistical reviewer performed additional analysis where all subjects with missing data were considered nonresponders. The common odds ratio (versus placebo) was 4.6 (95% CI: 2.6, 8.5; $p < 0.0001$) at Week 12 and 4.4 (95% CI: 2.6, 7.6; $p < 0.0001$) at Week 24 ([Table 21](#)), essentially unchanged from the primary analysis method.

Table 21. Proportion of Subjects With ≥ 1 Class Improvement in NYHA Functional Classification From Baseline to Weeks 12 and 24, Full Analysis Set, Trial CY 6031

NYHA Class	Applicant's Prespecified Analysis		Additional Analysis ¹	
	Aficamten N=142	Placebo N=140	Aficamten N=142	Placebo N=140
Week 12	142	140		
≥ 1 class improvement, n (%)				
Yes	69 (48.6)	25 (17.9)	69 (48.6)	25 (17.9)
No	71 (50.0)	113 (80.7)	71 (50.0)	112 (80.0)
Missing	2 (1.4)	2 (1.4)	2 (1.4)	3 (2.1)
Common odds ratio (vs. placebo)	4.6		4.6	
Exact 95% CI of odds ratio	(2.6, 8.5)		(2.6, 8.5)	
p-Value	<0.0001		<0.0001	
Week 24	142	140		
≥ 1 NYHA class improvement, n (%)				
Yes	83 (58.5)	34 (24.3)	83 (58.5)	34 (24.3)
No	56 (39.4)	103 (73.6)	56 (39.4)	103 (73.6)
Missing	3 (2.1)	3 (2.1)	3 (2.1)	3 (2.1)
Common odds ratio (vs. placebo)	4.4		4.4	
Exact 95% CI of odds ratio	(2.6, 7.6)		(2.6, 7.6)	
p-Value	<0.0001		<0.0001	

Source: adsl.xpt adbase.xpt, adrs.xpt, statistical reviewer analysis

¹ All subjects with missing NYHA class were treated as nonresponders.

Proportion of responders was analyzed using the CMH test stratified by beta blocker use (Yes/No) and CPET modality. Missing data were imputed per the statistical analysis plan and were summarized based on the imputed value and, therefore, were not counted in the Missing category. Missing NYHA class was treated as nonresponder in the analysis.

Abbreviations: CI, confidence interval; CMH, Cochran–Mantel–Haenszel; CPET, cardiopulmonary exercise testing; N, number of subjects in treatment group; n, number of subjects with given characteristic; NYHA, New York Heart Association

LVOT-G

At baseline, the mean Valsalva LVOT was approximately 83 mm Hg. At Week 12, the LS mean change from baseline in Valsalva LVOT showed a statistically significant improvement in the aficamten group compared with the placebo group: -44.8 mm Hg versus $+2.8$ mm Hg with a treatment difference of -48.4 mm Hg (95% CI: -55.1 , -41.7 ; $p < 0.0001$). Similarly, at Week 24, the LS mean change from baseline in Valsalva LVOT-G showed a statistically significant improvement in the aficamten group compared with the placebo group with LS mean changes of -48.1 mm Hg and $+2.2$ mm Hg, respectively, with a treatment difference in aficamten versus placebo of -50.3 mm Hg (95% CI: -56.9 , -43.7 ; $p < 0.0001$). Overall, 6 (2.1%) subjects had

missing values at Week 12 and 11 (3.9%) had missing values at Week 24. The missing values were handled as MAR by an MMRM model.

The statistical reviewer performed an additional analysis where intermittent missing data were imputed using the Markov Chain Monte Carlo method under a MAR assumption. After the first step of imputation, the monotone missing data were imputed using placebo-based imputation. Imputed datasets were analyzed using an MMRM model and results were combined using Rubin's rule. The LS mean difference between treatment groups at Week 12 was -48.0 mm Hg (95% CI: -54.8, -41.3; $p < 0.0001$) and Week 24 was -49.8 mm Hg (95% CI: -56.6, -43.0; $p < 0.0001$), essentially unchanged from the primary analysis method.

Table 22. Change in Valsalva Core-Laboratory-Read LVOT-G From Baseline to Weeks 12 and 24, Full Analysis Set, Trial CY 6031

Valsalva LVOT-G (mm Hg)	Applicant's Prespecified Analysis		Additional Analysis ¹	
	Aficamten N=142	Placebo N=140	Aficamten N=142	Placebo N=140
Baseline				
n	142	140		
Mean (SD)	83.0 (32.0)	83.3 (32.7)		
Week 12				
n	139	137		
Mean (SD)	37.8 (28.1)	86.9 (34.5)		
Week 12 change from baseline				
n	139	137		
Mean (SD)	-44.8 (36.0)	2.8 (32.0)		
LS mean	-45.7	2.6	-45.2	2.8
LS mean 95% CI	(-50.5, -41.0)	(-2.2, 7.4)	(-50.0, -40.4)	(-2.0, 7.6)
LS mean diff vs. placebo	-48.4		-48.0	
LS mean diff vs. placebo 95% CI	(-55.1, -41.7)		(-54.8, -41.3)	
p-Value	<0.0001		<0.0001	
Week 24				
n	137	134		
Mean (SD)	35.3 (25.1)	85.7 (33.3)		
Week 24 change from baseline				
n	137	134		
Mean (SD)	-47.6 (37.4)	1.8 (35.0)		
LS mean	-48.1	2.2	-47.2	2.6
LS mean 95% CI	(-52.8, -43.4)	(-2.6, 6.9)	(-52.0, -42.3)	(-2.2, 7.5)
LS mean diff vs. placebo	-50.3		-49.8	
LS mean diff vs. placebo 95% CI	(-56.9, -43.6)		(-56.6, -43.0)	
p-Value	<0.0001		<0.0001	

Source: adsl.xpt adbase.xpt, adcv.xpt, statistical reviewer analysis

¹ Intermittent missing data were imputed using the MCMC method under MAR assumption. After the first step of imputation, the monotone missing data were imputed using the imputation model built from placebo group.

Data up to Week 24 were analyzed using a MMRM model with baseline as a covariate, randomization stratification factors, visit, treatment group, and interaction terms of treatment by visit and baseline by visit. An unstructured covariance matrix was specified. Abbreviations: CI, confidence interval; LS, least-squares; LVOT-G, left ventricular outflow tract gradient; MAR, missing at random; MCMC, Markov Chain Monte Carlo; MMRM, mixed model for repeated measures; N, number of subjects in treatment group; n, number of subjects with given characteristic

The trial required subjects to have a resting LVOT-G ≥ 30 mm Hg at screening. For the binary LVOT-G < 30 mm Hg endpoint, at Week 12, a statistically significantly greater proportion of aficamten-treated subjects had a Valsalva LVOT-G < 30 mm Hg compared with placebo-treated subjects: 52.1% versus 5.7%. This yielded an odds ratio of 18.0 (95% CI: 7.8, 44.4; $p < 0.0001$).

Similar results were seen at Week 24: 49.3% versus 3.6% with an odds ratio of 25.5 (95% CI: 10.1, 88.2; $p < 0.0001$).

There were 6 subjects with missing values at Week 12, of which 2 were imputed using values from other weeks. At Week 24, 11 subjects had missing values, of which 5 (2 in the aficamten group and 3 in the placebo group) were imputed using Week 20 values. Overall, 6 (2.1%) subjects still had missing values at Week 24 and 4 (1.4%) still had missing values at Week 12. These subjects were considered as not achieving Valsalva LVOT-G < 30 mm Hg per core laboratory determination.

The statistical reviewer performed an additional analysis using the multiple imputed data sets created above for continuous Valsalva LVOT-G for additional analyses in [Table 23](#). In each imputed data set, the subjects were categorized as a responder or nonresponder based on the imputed Valsalva LVOT-G and analyzed using the Cochran-Mantel-Haenszel test. Results were similar with an odds ratio (versus placebo) at Week 12 of 17.8 (95% CI: 8.1, 39.2; $p < 0.0001$) and at Week 24 of 23.6 (95% CI: 9.1, 61.4; $p < 0.0001$).

Table 23. Proportion of Subjects With Valsalva LVOT-G < 30 mm Hg Per Core Laboratory Determination at Weeks 12 and 24, Full Analysis Set, Trial CY 6031

	Applicant's Prespecified Analysis		Additional Analysis ¹	
	Aficamten N=142	Placebo N=140	Aficamten N=142	Placebo N=140
Valsalva LVOT-G < 30 mm Hg				
Week 12	142	140		
Valsalva LVOT-G < 30 mm Hg, n (%)				
Yes	74 (52.1)	8 (5.7)	73 (51.4)	8 (5.7)
No	66 (46.5)	130 (92.9)	66 (46.5)	129 (92.1)
Missing	2 (1.4)	2 (1.4)	3 (2.1)	3 (2.1)
Common odds ratio (vs. placebo)	18.0		17.8	
Exact 95% CI of odds ratio	(7.8, 44.4)		(8.1, 39.2)	
p-Value	< 0.0001		< 0.0001	
Week 24	142	140		
Valsalva LVOT-G < 30 mm Hg, n (%)				
Yes	70 (49.3)	5 (3.6)	70 (49.3)	5 (3.6)
No	69 (48.6)	132 (94.3)	67 (47.2)	129 (92.1)
Missing	3 (2.1)	3 (2.1)	5 (3.5)	6 (4.3)
Common odds ratio (vs. placebo)	25.5		23.6	
Exact 95% CI of odds ratio	(10.1, 88.2)		(9.1, 61.4)	
p-Value	< 0.0001		< 0.0001	

Source: adsl.xpt adbase.xpt, adcv.xpt, statistical reviewer analysis

¹ Missing data were imputed using the imputation model built from the placebo group.

Proportion of responders was analyzed using CMH test stratified by beta blocker use (Yes/No) and CPET modality. Missing Weeks 12 and 24 data were imputed per the statistical analysis plan and are summarized based on the imputed value and, therefore, are not counted in the Missing category. Subjects with missing Valsalva were treated as nonresponders in the analysis.

Abbreviations: CI, confidence interval; CMH, Cochran–Mantel–Haenszel; CPET, cardiopulmonary exercise testing; LVOT-G, left ventricular outflow tract gradient; N, number of subjects in treatment group; n, number of subjects with given characteristic

The Applicant's response to an information request to perform additional analyses was submitted on March 17, 2025, regarding use of multiple imputation for handling missing data for the secondary endpoint of 'Proportion of Subjects with Valsalva LVOT-G < 30 mm Hg.' The Applicant provided the requested analyses, and their results were consistent with the review team's 'Additional Analysis' results in [Table 23](#).

Duration of SRT Eligibility

SRT eligibility was defined as a subject being classified as NYHA class ≥ 3 and with resting or Valsalva LVOT-G ≥ 50 mm Hg. At baseline, 61 (22%) subjects (32 in the aficamten group and 29 in the placebo group) were eligible for SRT. During the 24-week treatment period, aficamten treatment resulted in a significant reduction in the time spent as SRT eligible by an LS mean of -78.1 days (95% CI: $-99.8, -56.3$; $p < 0.0001$) compared with placebo. There were no missing values. The ANCOVA model included treatment and randomization stratification factor beta blocker use/nonuse as fixed effects. No baseline characteristics were included in the model.

Table 24. Total Duration of SRT Eligibility During the 24-Week Treatment Period in Subjects Who Were SRT Eligible at Baseline, Full Analysis Set, Trial CY 6031

Week 24	Aficamten N=32	Placebo N=29
Duration of SRT eligibility (days)		
Mean (SD)	36.5 (26.5)	114.2 (54.2)
Median	28.0	118.0
Q1, Q3	15.0, 42.5	84.0, 165.0
LS mean	35.3	113.4
LS mean 95% CI	(19.5, 51.1)	(97.2, 129.5)
LS mean diff vs. placebo	-78.1	
LS mean diff vs. placebo 95% CI	($-99.8, -56.3$)	
p-Value	< 0.0001	

Source: adsl.xpt adbase.xpt, adcv.xpt, statistical reviewer analysis

SRT eligibility is defined as NYHA Class III or IV and Core-Lab Read resting or Valsalva LVOT-G ≥ 50 mm Hg. Data were analyzed using an ANCOVA model which includes treatment and randomization stratification factor beta blocker use/non-use as fixed effects and significant baseline characteristics as covariates. Stepwise model selection method was used, based on the default stay or entry level of 0.05, to evaluate significant baseline covariates. No baseline covariate was selected and included in the model. Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; LS, least-squares; LVOT-G, left ventricular outflow tract gradient; N, number of subjects in treatment group; n, number of subjects with given characteristic; NYHA, New York Heart Association; SD, standard deviation; SE, standard error; SRT, septal reduction therapy; Q, quartile

CPET Workload

Mean baseline workload was approximately 120 watts. The LS mean change in workload from baseline to Week 24 was statistically significantly greater in the aficamten group than in the placebo group: 13.4 watts versus 1.2 watts. The LS mean difference was 12.2 watts (95% CI: 6.4, 18.0; $p < 0.0001$). There were 19 (6.7%) subjects (8 aficamten, 11 placebo) with missing data at Week 24. Of these, two subjects (1 in aficamten and 1 in placebo) had missing baseline data and were excluded from the analysis.

The statistical reviewer performed additional analysis where missing value were imputed using placebo-based multiple imputation, and the results are presented under additional analysis in [Table 25](#). The LS mean difference was 12.1 watts (95% CI: 6.3, 17.9; $p < 0.0001$), essentially unchanged from the primary analysis method.

Table 25. Change in Total Workload During CPET From Baseline to Week 24, Full Analysis Set, Trial CY 6031

Workload (watts)	Applicant's Prespecified Analysis		Additional Analysis ¹	
	Aficamten N=142	Placebo N=140	Aficamten N=142	Placebo N=140
Baseline				
n	141	139		
Mean (SD)	120.4 (38.9)	124.7 (42.5)		
Week 24				
n	134	129		
Mean (SD)	134.3 (49.8)	127.2 (44.4)		
Week 24 change from baseline				
n	134	129		
Mean (SD)	14.1 (26.7)	1.4 (21.4)		
LS mean	13.4	1.2	13.0	0.9
LS mean 95% CI	(9.2, 17.6)	(-3.0, 5.4)	(8.9, 17.2)	(-3.2, 5.1)
LS mean diff vs. placebo	12.2		12.1	
LS mean diff vs. placebo 95% CI	(6.4, 18.0)		(6.3, 17.9)	
p-Value	<0.0001		<0.0001	

Source: adsl.xpt adbase.xpt, adcv.xpt, statistical reviewer analysis

¹ Missing data were imputed using placebo-based multiple imputation.

Missing data were imputed using regression multiple imputation including treatment group, randomization stratification factors, baseline pVO₂, sex, age, baseline hemoglobin, baseline body weight, baseline KCCQ-CSS, baseline NYHA class and the last available postrandomization NYHA functional class, resting and Valsalva LVOT. Each completed dataset was analyzed using an ANCOVA model with fixed effects of treatment, beta blocker use (Yes/No), CPET modality, and baseline workload and baseline body weight as covariates. Results from all 100 imputed datasets are combined for overall inference using Rubin's rules.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CPET, cardiopulmonary exercise testing; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire – Clinical Summary Score; LS, least-squares; LVOT, left ventricular outflow tract; N, number of subjects in treatment group; n, number of subjects with given characteristic; NYHA, New York Heart Association; pVO₂, peak oxygen consumption; SD, standard deviation

6.2.2. CY 6021 (REDWOOD-HCM)

The REDWOOD-HCM study was a Phase 2, multi-center, randomized, placebo-controlled, dose-finding study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of aficamten in adults with symptomatic HCM. The study consisted of 4 cohorts with treatment duration of 10 weeks with a 4-week follow-up period after the last dose. Cohort 1 consisted of subjects with oHCM not receiving disopyramide who were randomized 2:1 (n=14; n=8) to receive up to 3 escalating doses of aficamten (5 mg, 10 mg, and 15 mg once daily) or placebo. Cohort 2 (n=14 aficamten, n=6 placebo) was a replica of Cohort 1 except for the 3 escalating doses of aficamten (10 mg, 20 mg, 30 mg once daily). Cohort 3 consisted of subjects with oHCM whose background HCM therapy included disopyramide. All subjects in Cohort 3 (n=13) received up to 3 escalating doses of aficamten (5, 10, and 15 mg) based on echocardiographic guidance. Cohort 4 consisted of subjects (n=41) with nHCM whose background therapy included beta-blockers and/or calcium channel blockers, either as monotherapy or in combination therapy. Subjects receiving disopyramide were excluded from Cohort 4. Subjects in Cohort 4 received up to 3 doses of aficamten (5, 10, and 15 mg).

Efficacy was evaluated by LVOT-G, NYHA classification, and subject-reported outcome quality of life assessment. There were no adjustments for multiplicity. For subjects in Cohort 1 and 2, there were statistically significant reductions in LVOT-G, both resting and post-Valsalva, from baseline compared to pooled placebo at Week 10:

- Cohort 1:
 - Resting LVOT-G: -41 mm Hg aficamten; -15 mm Hg placebo (nominal $p=0.0009$)
 - Post-Valsalva LVOT-G: -40 mm Hg aficamten; -5 mm Hg placebo (nominal $p=0.0005$)
- Cohort 2:
 - Resting LVOT-G: -40 mm Hg aficamten; -15 mm Hg pooled placebo (nominal $p=0.001$)
 - Post-Valsalva LVOT-G: -51 mm Hg aficamten; -5 mm Hg pooled placebo (nominal $p<0.0001$)

Improvements of at least one NYHA functional class from baseline to Week 10 were observed in 43% and 64% of subjects treated with aficamten in Cohorts 1 and 2, respectively, compared to 33% of the pooled placebo-treated subjects (see Section [16.2](#)).

Cohorts 3 and 4 did not have respective control groups. Further, subjects in Cohort 4 had nonobstructive hypertrophic cardiomyopathy for which an LVOT-G evaluation was not possible. Thus, data interpretation was challenging in Cohorts 3 and 4 and, therefore, not considered to be confirmatory evidence.

In summary, the REDWOOD-HCM study demonstrated sizeable PD effects on LVOT-G when added to standard-of-care. These findings reasonably confirm/support the results from the pivotal SEQUOIA-HCM trial.

6.3. Key Efficacy Review Issues

6.3.1. Is the Observed Treatment Effect of Aficamten on pVO₂ Clinically Meaningful?

Issue

Baseline pVO₂ was approximately 18 mL/kg/min. The primary endpoint of change in pVO₂ from baseline to Week 24 was statistically significant ($p<0.0001$) with a mean difference of 1.7 mL/kg/min between treatment groups. The clinical meaningfulness of the result needs to be established.

Background

In HCM, pVO₂ is associated with clinical prognosis and death ([Masri et al. 2015](#)); however, the relationship between a change in pVO₂ and clinical outcomes is not extensively studied or well defined. An increase of 1 mL/kg/min in pVO₂ was found to be predictive of reduced risk of death from HF and need for heart transplantation in patients with HCM ([Coats et al. 2015](#)). However, this study was a retrospective observational study from a single center, and the generalizability of the study findings is uncertain. In patients with HF and reduced ejection fraction, a small increase in pVO₂ (~1 mL/kg/min) was associated with a 5% lower risk of all-cause mortality or

all-cause hospitalization ($p < 0.001$), a 4% lower risk of cardiovascular mortality or cardiovascular hospitalization, and a 7% lower risk of all-cause mortality in a randomized-controlled trial (Swank et al. 2012). However, it is uncertain whether the described association of pVO₂ with cardiovascular outcomes in patients with HF with reduced ejection fraction (HFrEF) with LVEF $\leq 35\%$ can be extrapolated to the HCM population with preserved LV systolic function and patients who have a reasonably normal life expectancy overall. Current evidence is insufficient to quantify the magnitude of clinical benefit associated with a small increase in pVO₂ in with HCM. Despite the uncertainties, it should be noted that improvement with aficamten treatment was observed across a broader range of clinical endpoints including statistical and clinical significance in KCCQ-CSS and NYHA Class. Other supporting efficacy results include large, statistically significant findings in LVOT-G reduction and improvement in total workload from the CPET. The observed consistent results clearly establish that aficamten improves how patients with oHCM function and feel. Additional analyses to demonstrate the clinical significance of the observed pVO₂ changes are described below.

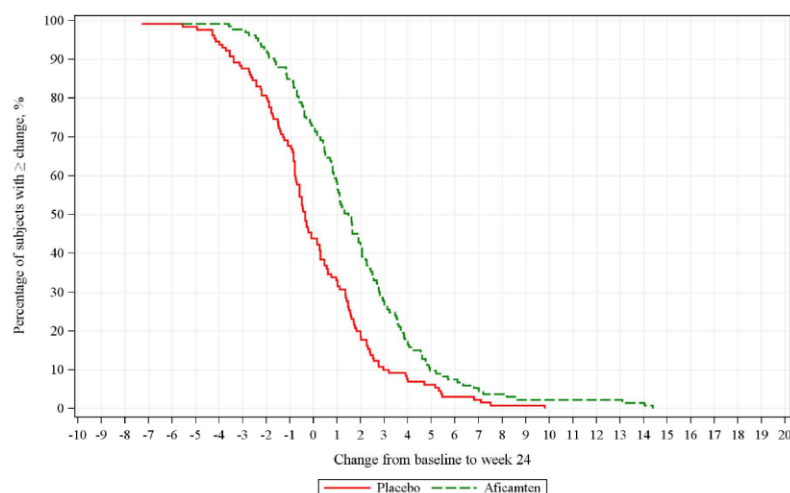
Assessment

Clinical Outcomes Assessment Consult Review

The Applicant did not propose a range of pVO₂ change that is clinically meaningful to patients. COA was consulted during the NDA review for assistance in interpreting meaningful change in pVO₂ scores. Exploratory anchor-based analyses using NYHA, KCCQ-Physical Limitation Score, and Patient Global Impressions of Change have been conducted. However, given the low correlation coefficients (i.e., $|r| < 0.3$) between pVO₂ and the anchor scales for the changes from baseline at Week 24, a suitable anchor scale to estimate pVO₂ meaningful change in CY 6031 was not identified.

While the threshold for within-subject clinically meaningful change from baseline at Week 24 in pVO₂ could not be estimated, the cumulative distribution function curves show a clear separation between groups across a wide range of pVO₂ improvement change scores in CY 6031.

Figure 7. Cumulative Distribution of Change From Baseline to Week 24 in pVO₂, Full Analysis Set, Trial CY 6031



Source: CY 6031 CSR, Figure 14.2.3

Abbreviation: pVO₂, peak oxygen consumption

Exploratory Endpoint

A composite endpoint of pVO₂ and NYHA functional class was explored to further assess functional improvement defined as:

- A pVO₂ increase of ≥ 1.5 mL/kg/min and a ≥ 1 NYHA class improvement at Week 24, **or**
- A pVO₂ increase of ≥ 3.0 mL/kg/min and no worsening of NYHA class at Week 24

A greater proportion of subjects in the aficamten group achieved this composite efficacy endpoint compared to the placebo group (42.3% versus 13.6%, respectively) (see [Table 26](#)).

Table 26. Proportion of Subjects Achieving Improvement Defined by Both pVO₂ and NYHA Functional Class Criteria at Week 24, Full Analysis Set, Trial CY 6031

	Aficamten N=142 n (%)	Placebo N=140 n (%)
Statistics		
≥ 1.5 mL/kg/min increase in pVO ₂ and ≥ 1 NYHA class improvement or ≥ 3.0 mL/kg/min increase in pVO ₂ and no worsening of NYHA class		
Yes	60 (42.3)	19 (13.6)
≥ 1.5 mL/kg/min increase in pVO ₂ and ≥ 1 NYHA class improvement	44 (31.0)	9 (6.4)
≥ 3.0 mL/kg/min increase in pVO ₂ and no worsening of NYHA class	37 (26.1)	13 (9.3)
Both ≥ 3.0 mL/kg/min increase in pVO ₂ and ≥ 1 NYHA class improvement	21 (14.8)	3 (2.1)
No	73 (51.4)	111 (79.3)
Missing	9 (6.3)	10 (7.1)
Common rate difference (vs. placebo)	28.7	
95% CI common rate difference	(18.8, 38.6)	
Common odds ratio (vs. placebo)	4.7	
Exact 95% CI of odds ratio	(2.6, 9.1)	

Source: Statistical reviewer analysis

Proportion of responders was analyzed using Cochran–Mantel–Haenszel test stratified by beta blocker use (Yes/No) and CPET modality. Responder is defined as change from baseline of ≥ 1.5 mL/kg/min in pVO₂ and ≥ 1 class improvement in NYHA functional class or change from baseline of ≥ 3 mL/kg/min in pVO₂ and no worsening of NYHA functional class. Subjects with missing pVO₂ or missing NYHA functional class are treated as nonresponders.

Abbreviations: CI, confidence interval; CPET, cardiopulmonary exercise testing; N, number of subjects in treatment group; n, number of subjects with given characteristic; NYHA, New York Heart Association; pVO₂, peak oxygen consumption

The division considers that a change in NYHA Class of 1 or more represents a clinically meaningful improvement. Adding NYHA class as a component of the efficacy endpoint strengthens the validity of clinical response. It is also important to note that some subjects can have more sizeable improvements in pVO₂, as evidenced for example, by the proportion of subjects with ≥ 3.0 mL/kg/min improvement without worsening in NYHA class. Approximately 26% of aficamten-treated subjects met this component of the exploratory efficacy endpoint compared to 9% of subjects treated with placebo. Furthermore, this composite endpoint was previously used as a primary efficacy endpoint to support approval of mavacamten, the first-in-class, cardiac myosin inhibitor (NDA 214998) in the phase 3 EXPLORER-HCM trial. The division considered the improvement defined in this composite endpoint clinically meaningful. The proportion of subjects who achieved this composite endpoint in EXPLORER-HCM was 37% versus 17% in the mavacamten and placebo group, respectively with a difference of 19.4% and odds ratio of 2.7 (p = 0.0005). Change in pVO₂ was also studied as a secondary endpoint in EXPLORER-HCM

with a mean difference of 1.4 mL/kg/min. Overall, the aficamten treatment effects on the exploratory composite endpoint of pVO₂ and NYHA functional class, as well as on the continuous pVO₂, were similar to those observed with mavacamten.

Conclusion

The totality of data indicates that the observed changes in pVO₂ in CY 6031 are both statistically and clinically significant.

6.3.2. Impact of Imputation of Missing Data for Continuous Variable Endpoints on Assessment of Efficacy

Issue

For the primary analyses of the primary and continuous secondary endpoints, the Applicant imputed all the missing data using multiple imputations under missing at random assumption regardless of reasons for the missing data.

Background

The source of missing data, particularly when linked to intercurrent events, plays a key role in determining how missing data should be handled. While missingness due to external, study-independent factors may be classified as MAR, this is uncommon. In most cases, missing data are associated with treatment adherence. It is generally not appropriate to impute missing data from non-adherent subjects using data from adherent subjects within the same treatment group, as differences in treatment exposure and behavior can lead to biased estimates of the treatment effect. We recommend that missing data from non-adherent subjects in the primary analyses be addressed using data from other non-adherent subjects within the same group who have available measurements at the specified analysis timepoint (retrieved dropouts). See Section [6.2.1.3](#).

Assessment

The statistical reviewer performed additional analyses where missing data for subjects who discontinued the treatment were imputed as missing not at random using placebo-based multiple imputation. There were not enough data to perform a retrieved dropouts' imputation. The results of these analyses are shown in [Table 18](#), [Table 20](#), [Table 22](#), and [Table 25](#).

Conclusion

The results of the additional analyses were generally similar to those seen in the Applicant's analyses and did not alter overall conclusions regarding efficacy. Results from the appropriate analyses that are generally recommended and implemented for these endpoints should be used for labeling purposes.

6.3.3. Impact of Imputation of Missing Data for Categorical Variable Endpoints on Assessment of Efficacy

Issue

For the categorical secondary endpoints, the Applicant imputed missing data using single imputation using measurements from other weeks; either last observation carried forward or next observation carried backward.

Background

The review division does not typically accept single imputation methods as this ignores uncertainty which will underestimate variability. Multiple imputation, such as a retrieved dropouts or jump-to reference, are typically preferred. Also, we do not agree with last observation carried forward or next observation carried backward imputation as it may introduce bias, misrepresent treatment effect, and underestimate variability by assuming that outcome remains constant over time. Although failure imputation is a single imputation method for responder analyses, such an analysis could be acceptable if the imputation was accounted for in the interpretation of the results with little missing data that is similar between groups (i.e., one group was not proportionally higher in missing data rates).

Assessment

The statistical reviewer performed additional analyses as shown in [Table 21](#) and [Table 23](#). For the NYHA functional class categorical endpoint, subjects with missing data were classified as non-responders in the analysis. For the categorical Valsalva LVOT-G endpoint ([Table 23](#)), missing values imputed for the analysis of the continuous Valsalva LVOT-G were categorized as responders and non-responders based on the imputed values prior to the analysis.

Conclusion

The results were generally similar to those seen in the Applicant's analyses and did not alter overall conclusions regarding efficacy. Results from the appropriate analyses that are generally recommended and implemented for these endpoints should be used for labeling purposes.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

All toxicology species were pharmacologically relevant considering the evolutionary conservation of cardiac myosin across mammals. A brief discussion of clinically relevant toxicities is included below. Detailed summaries of the nonclinical studies are provided in Section [13.1](#).

Exaggerated Pharmacology/Cardiac Toxicity

Cardiac effects were, unsurprisingly, observed in repeat-dose toxicology studies conducted in rats and dogs at or near exposure levels anticipated for clinical use, which were interpreted as direct results of the intended pharmacology in healthy animals. A similar pattern was observed in the toxicology studies with mavacamten, the first-in-class cardiac myosin inhibitor.

In healthy rats, the cardiac findings, which were interpreted as overpredicting clinical risk, consisted of dose-dependent increases in irreversible dilated cardiomyopathy, increased heart weight, ventricular wall thinning, and premature deaths at exposures ranging from approximately 4- and 11-times the clinical exposures based on free AUC and free C_{max} , respectively, at the maximum recommended human dose (MRHD). Continued treatment of healthy rats culminated in heart failure and deaths in a dose and duration-dependent fashion.

In shorter studies in healthy dogs, at exposures exceeding the anticipated clinical exposures at the MRHD, aficamten caused dose-dependent cardiac effects including increased heart rate, ventricular dilatation, increased heart weight, and cardiomyocyte degeneration with necrosis, with high-dose animals showing persistent ventricular dilatation and increased heart weight (males only) even after a recovery period. In a longer 39-week study at lower doses (up to 6-times clinical exposure), aficamten was better tolerated with only reversible increases in heart rate and QTc interval, and transient heart weight increases in males that resolved by study termination.

Effects Secondary to Cardiac Toxicity

Inhibiting cardiac contractility in otherwise healthy animals led to secondary effects on various organ systems due to altered hemodynamics and compromised blood perfusion. These effects were particularly notable in the liver and the lung. Acute liver necrosis with elevated hepatic enzymes occurred in high-dose male rats secondary to congestive heart failure caused by aficamten-induced reduced cardiac contractility, with no hepatotoxicity observed at lower doses in rats or in dog studies. Pulmonary effects in rats included increased lung weight with hemosiderin-laden alveolar macrophages and lung hemorrhage in high-dose males, consistent with secondary lung edema due to heart failure associated with cardiac dilatation. Taken together, no unanticipated off-target toxicities were observed in healthy animals with repeat aficamten dosing. The clinical relevance of the findings was considered limited for the target patient population.

Mutagenicity/Carcinogenicity

Aficamten was not mutagenic or clastogenic in a standard battery of genotoxicity studies including the Ames assay and a combined in vivo micronucleus assay and comet assay.

There were no aficamten-related tumor findings in a 26-week carcinogenicity study in transgenic rasH2 mice at doses up to 2.0 mg/kg/day or in a 2-year carcinogenicity study in Sprague Dawley rats at doses up to 3 mg/kg/day (corresponding to 4-times the exposure at the MRHD based on $AUC_{unbound}$).

Developmental Toxicity

In embryofetal and pre- and postnatal development studies, when pregnant rats were administered aficamten during the period of organogenesis, aficamten increased the incidence of

structural malformations at exposures ≥ 4 -times the clinical exposures at the MRHD of 20 mg based on $AUC_{unbound}$. No adverse effects on development were seen at 3-times the clinical exposure at the MRHD. See Sections [8.4](#) and [13.1](#) for more details.

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Aficamten is a cardiac myosin inhibitor. The primary risk associated with this class of drugs is the potential for development of signs or symptoms of HF due to an exaggerated pharmacologic effect resulting in systolic dysfunction (reduced LVEF). The risk is greater among subjects who concurrently take medications that inhibit multiple P450 pathways involved in aficamten elimination.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

Aficamten is not approved in the United States or in any foreign market.

7.4. FDA Approach to the Safety Review

FDA's safety evaluation focused on the data obtained in CY 6031 (SEQUOIA-HCM). This pivotal study provides controlled data to evaluate the safety profile of aficamten under echocardiography-based dosing and monitoring in oHCM subjects. As agreed, the Applicant provided a Summary of Clinical Safety that included safety data from the CY 6031, CY 6021 (dose-ranging, placebo-controlled Phase 2 study) and CY 6022 (ongoing open-label extension study) (see the overview of clinical trials in [Table 3](#)). Given the different dose groups and dosing strategies between studies, pooled controlled data from CY 6031 and CY 6021 were not performed. Data from CY 6021 were evaluated separately as supportive safety data. The ongoing extension Study CY 6022 provides long-term safety data in oHCM to evaluate safety topics of interest.

The overall data and submission quality are reasonable. Adverse events were analyzed by use of the Medical Dictionary for Regulatory Activities (MedDRA; Version 27.0) preferred term, and by pooling AEs with a similar medical concept (referred to as the Standardised MedDRA Query or Office of New Drugs Custom Medical Queries [OCMQs]). All AEs presented and discussed are treatment-emergent AEs unless specified otherwise. Treatment-emergent AEs are defined as any AE with an onset date on or after the first dose of study treatment, or with worsening on or after the first dose of study treatment up through 28 days after the last dose of study treatment (aficamten has a half-life of ~ 3.5 days).

FDA's safety review focused on categorizing the on-target effects of aficamten on decreasing ejection fraction and increasing the risk of systolic cardiac failure. Clinical events of special interest included major adverse cardiac events, new-onset persistent AF and ventricular arrhythmias. Other safety evaluations included the analyses of exposure, AEs, echocardiographic data, laboratory data and vital signs.

7.5. Adequacy of the Clinical Safety Database

The exposure was balanced in both treatment groups in CY 6031 with a mean exposure of 24 weeks. The ongoing extension Study CY 6022 provided an additional mean exposure of 62 weeks among 296 subjects with oHCM. A summary of exposure in CY 6031 and CY 6022 is shown in [Table 27](#) and [Table 28](#), respectively. The size of the safety database and duration of exposure are acceptable given the prevalence of the disease.

Table 27. Duration of Exposure, Safety Population, Trial CY 6031

Parameter	Aficamten N=142	Placebo N=140
Duration of treatment, weeks		
Mean (SD)	24 (2.8)	23.9 (3.2)
Median (Q1, Q3)	24.1 (23.7, 24.9)	24.3 (24, 24.8)
Min, max	3.9, 29.4	4.1, 28.6
Total exposure (person years)	65	64
Subjects treated, by duration, n (%)		
<18 weeks	4 (2.8)	4 (2.9)
≥18 to <22 weeks	1 (0.7)	1 (0.7)
≥22 to <26 weeks	128 (90.1)	127 (90.7)
≥26 to <30 weeks	9 (6.3)	8 (5.7)
≥30 weeks	0	0

Source: adex.xpt and adsl.xpt; Software: R

Duration is 24 weeks.

Abbreviations: max, maximum; min, minimum; N, number of subjects in treatment group; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

Table 28. Duration of Exposure, Safety Population, Study CY 6022

Parameter	oHCM Aficamten N=296	nHCM Aficamten N=34	Overall Aficamten N=330
Duration of treatment, weeks			
Mean (SD)	61.9 (39.1)	84 (10.4)	64.2 (37.8)
Median (Q1, Q3)	51.6 (41.5, 70.8)	80.1 (77.2, 90.7)	54.5 (43.4, 75.6)
Min, max	8.6, 170.3	71.4, 111.7	8.6, 170.3
Total exposure (person years)	351	55	406
Subjects treated, by duration, n (%)			
<12 weeks	30 (10.1)	0	30 (9.1)
≥12 to <26 weeks	4 (1.4)	0	4 (1.2)
≥26 to <50 weeks	104 (35.1)	0	104 (31.5)
≥50 to <100 weeks	115 (38.9)	31 (91.2)	146 (44.2)
≥100 to <150 weeks	25 (8.4)	3 (8.8)	28 (8.5)
≥150 to <200 weeks	18 (6.1)	0	18 (5.5)
≥200 weeks	0	0	0

Source: adex.xpt and adsl.xpt; Software: R

Exposure is calculated based on the updated dataset submitted for 120-day safety updates (seq 0013).

Abbreviations: max, maximum; min, minimum; N, number of subjects in treatment group; n, number of subjects with given treatment duration; nHCM, non-obstructive hypertrophic cardiomyopathy; oHCM, obstructive hypertrophic cardiomyopathy; Q1, first quartile; Q3, third quartile

7.6. Safety Results

7.6.1. Safety Results

7.6.1.1. Overview of Treatment-Emergent Adverse Events

The overall incidence of AEs and serious adverse events (SAEs) was generally similar between the treatment groups in CY 6031. In the aficamten group, there was one AE, compared to two in the placebo group, leading to discontinuation of study drug (see Section 7.6.1.4). Two AEs leading to treatment interruption in the aficamten group (same number in the placebo group) were cholecystitis and COVID-19; both events were transient and resolved, allowing the subjects to complete the treatment.

Table 29. Overview of Treatment-Emergent Adverse Events, Safety Population, Trial CY 6031

Event Category	Aficamten N=142 n (%)	Placebo N=140 n (%)	Risk Difference % (95% CI)
SAE	8 (5.6)	13 (9.3)	-3.7 (-10.3, 2.7)
SAEs with fatal outcome	0	0	0.0 (-2.7, 2.6)
Life-threatening SAEs	1 (0.7)	0	0.7 (-2.0, 3.9)
SAEs requiring hospitalization	7 (4.9)	10 (7.1)	-2.2 (-8.4, 3.6)
SAEs resulting in substantial disruption of normal life functions	1 (0.7)	0	0.7 (-2.0, 3.9)
Other	1 (0.7)	4 (2.9)	-2.2 (-6.5, 1.3)
AE leading to permanent discontinuation of study drug	1 (0.7)	2 (1.4)	-0.7 (-4.4, 2.6)
AE leading to dose modification of study drug	2 (1.4)	2 (1.4)	-0.0 (-3.8, 3.7)
AE leading to interruption of study drug	2 (1.4)	2 (1.4)	-0.0 (-3.8, 3.7)
AE leading to reduction of study drug	0	0	0.0 (-2.7, 2.6)
AE leading to dose delay of study drug	0	0	0.0 (-2.7, 2.6)
Any AE	105 (73.9)	99 (70.7)	3.2 (-7.2, 13.7)
Severe and worse	8 (5.6)	10 (7.1)	-1.5 (-7.7, 4.5)
Moderate	41 (28.9)	31 (22.1)	6.7 (-3.5, 16.9)
Mild	56 (39.4)	58 (41.4)	-2.0 (-13.4, 9.4)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as AEs occurring or worsening after the first dose of study drug until 28 days after the last dose of study drug.

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

Severity as assessed by the investigator.

Abbreviations: AE, adverse event; CI, confidence interval; N, number of subjects in treatment group; n, number of subjects with at least one event; SAE, serious adverse event

7.6.1.2. Deaths

No deaths occurred in either treatment group in CY 6031. With the updated safety data through August 31, 2024, there were also no deaths reported in the ongoing extension Study CY 6022. One death due to cardiac arrest occurred in the dose-ranging Study CY 6021. This death involved a patient with nHCM and was deemed unlikely to be related to aficamten. A summary of this case is provided below:

Subject CY-6021- (b) (6): A 43-year-old Asian female subject with symptomatic nHCM received escalating doses of aficamten (5 mg, 10 mg, and 15 mg) over 45 days. On Day 45, the subject suffered a cardiac arrest while showering and died despite resuscitation efforts. The subject was receiving aficamten 15 mg. The investigator assessed the cardiac arrest as severe but not related to the study drug. This assessment was based on several factors: the subject's LVEF remained within the normal range throughout the study (73% at baseline and 75% at the Week 6 visit and during hospital admission on Day 45), global longitudinal strain improved, NT-proBNP and Troponin I levels improved compared to baseline. Additionally, the subject had a history of aborted sudden cardiac death, which was considered a likely cause of the cardiac arrest.

7.6.1.3. Serious Treatment-Emergent Adverse Events

Overall, the incidence of SAEs was similar between groups in CY 6031. No subject in the aficamten group discontinued treatment due to an SAE. No SAE was considered by the investigator to be related to study treatment. Narrative review of the cases in the aficamten group did not reveal major concerns. The three SAEs of hypertrophic cardiomyopathy in the aficamten group occurred after discontinuation of treatment during the safety follow-up period (range from 8-22 days after the last aficamten treatment).

Table 30. Subjects With Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term, Safety Population, Trial CY 6031

System Organ Class Preferred Term	Aficamten N=142 n (%)	Placebo N=140 n (%)	Risk Difference % (95% CI)
Any SAE	8 (5.6)	13 (9.3)	-3.7 (-10.3, 2.7)
Blood and lymphatic system disorders (SOC)	1 (0.7)	0	0.7 (-2.0, 3.9)
Microcytic anaemia	1 (0.7)	0	0.7 (-2.0, 3.9)
Cardiac disorders (SOC)	2 (1.4)	6 (4.3)	-2.9 (-7.8, 1.2)
Arrhythmia supraventricular	1 (0.7)	0	0.7 (-2.0, 3.9)
Atrial fibrillation	1 (0.7)	1 (0.7)	-0.0 (-3.3, 3.2)
Acute coronary syndrome	0	1 (0.7)	-0.7 (-3.9, 1.9)
Acute myocardial infarction	0	1 (0.7)	-0.7 (-3.9, 1.9)
Cardiac failure congestive	0	1 (0.7)	-0.7 (-3.9, 1.9)
Sinoatrial block	0	1 (0.7)	-0.7 (-3.9, 1.9)
Ventricular fibrillation	0	1 (0.7)	-0.7 (-3.9, 1.9)
Congenital, familial and genetic disorders (SOC)	4 (2.8)	1 (0.7)	2.1 (-1.4, 6.4)
Hypertrophic cardiomyopathy	3 (2.1)	1 (0.7)	1.4 (-2.0, 5.4)
Thalassaemia	1 (0.7)	0	0.7 (-2.0, 3.9)
Gastrointestinal disorders (SOC)	0	1 (0.7)	-0.7 (-3.9, 1.9)
Upper gastrointestinal haemorrhage	0	1 (0.7)	-0.7 (-3.9, 1.9)
Hepatobiliary disorders (SOC)	1 (0.7)	0	0.7 (-2.0, 3.9)
Cholecystitis	1 (0.7)	0	0.7 (-2.0, 3.9)
Infections and infestations (SOC)	1 (0.7)	2 (1.4)	-0.7 (-4.4, 2.6)
Sepsis	1 (0.7)	0	0.7 (-2.0, 3.9)
Cellulitis	0	1 (0.7)	-0.7 (-3.9, 1.9)
Pneumonia	0	1 (0.7)	-0.7 (-3.9, 1.9)
Injury, poisoning and procedural complications (SOC)	0	1 (0.7)	-0.7 (-3.9, 1.9)
Radius fracture	0	1 (0.7)	-0.7 (-3.9, 1.9)
Ulna fracture	0	1 (0.7)	-0.7 (-3.9, 1.9)
Wrist fracture	0	1 (0.7)	-0.7 (-3.9, 1.9)

System Organ Class Preferred Term	Aficamten N=142 n (%)	Placebo N=140 n (%)	Risk Difference % (95% CI)
Neoplasms benign, malignant and unspecified (incl cysts and polyps) (SOC)	0	2 (1.4)	-1.4 (-5.1, 1.2)
Acute lymphocytic leukaemia	0	1 (0.7)	-0.7 (-3.9, 1.9)
Squamous cell carcinoma of the oral cavity	0	1 (0.7)	-0.7 (-3.9, 1.9)
Nervous system disorders (SOC)	1 (0.7)	2 (1.4)	-0.7 (-4.4, 2.6)
Carotid artery stenosis	1 (0.7)	0	0.7 (-2.0, 3.9)
Ischaemic stroke	1 (0.7)	0	0.7 (-2.0, 3.9)
Loss of consciousness	0	1 (0.7)	-0.7 (-3.9, 1.9)
Syncope	0	1 (0.7)	-0.7 (-3.9, 1.9)
Respiratory, thoracic and mediastinal disorders (SOC)	1 (0.7)	0	0.7 (-2.0, 3.9)
Respiratory failure	1 (0.7)	0	0.7 (-2.0, 3.9)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as AEs occurring or worsening after the first dose of study drug until 28 days after the last dose of study drug.

Serious adverse events defined as any untoward medical occurrence that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

Abbreviations: AE, adverse event; CI, confidence interval; incl, including; N, number of subjects in treatment group; n, number of subjects with adverse event; SAE, serious adverse event; SOC, system organ class

7.6.1.4. Adverse Events Leading to Treatment Discontinuation

Study treatment was discontinued early due to an AE for one subject (0.7%) in the aficamten group and two subjects (1.4%) in the placebo group ([Table 31](#)). In the aficamten group, the AE that led to treatment discontinuation was mild paranoia, which was considered not related to aficamten.

Table 31. Subjects With Adverse Events¹ Leading to Treatment Discontinuation by System Organ Class and Preferred Term, Safety Population, Trial CY 6031

System Organ Class Preferred Term	Aficamten N=142 n (%)	Placebo N=140 n (%)	Risk Difference % (95% CI)
Any AE leading to discontinuation	1 (0.7)	2 (1.4)	-0.7 (-4.4, 2.6)
Neoplasms benign, malignant and unspecified (including cysts and polyps) (SOC)	0	1 (0.7)	-0.7 (-3.9, 1.9)
Acute lymphocytic leukaemia	0	1 (0.7)	-0.7 (-3.9, 1.9)
Nervous system disorders (SOC)	0	1 (0.7)	-0.7 (-3.9, 1.9)
Loss of consciousness	0	1 (0.7)	-0.7 (-3.9, 1.9)
Psychiatric disorders (SOC)	1 (0.7)	0	0.7 (-2.0, 3.9)
Paranoia	1 (0.7)	0	0.7 (-2.0, 3.9)

Source: adae.xpt; Software: R

¹ Treatment-emergent adverse events defined as AEs occurring or worsening after the first dose of study drug until 28 days after the last dose of study drug.

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

Abbreviations: AE, adverse event; CI, confidence interval; N, number of subjects in treatment group; n, number of subjects with adverse event; SOC, system organ class

7.6.1.5. Clinical Events of Special Interest

Clinical events of special interest were evaluated based on customized group queries, including Standardised MedDRA Queries and OCMQs ([Table 32](#)). The incidence of these events was low and similar between groups.

Table 32. Summary of Clinical Events of Special Interest, Safety Population, Trial CY 6031

Customized Group Term	Aficamten N=142	Placebo N=140
Preferred Term	n (%)	n (%)
Incidence of MACE	3 (2.1)	5 (3.6)
CV death	0	0
CV hospitalization	3 (2.1)	4 (2.9)
Arrhythmia supraventricular	1 (0.7)	0
Atrial fibrillation	1 (0.7)	0
Carotid artery stenosis	1 (0.7)	0
Ischemic stroke	1 (0.7)	0
Acute coronary syndrome	0	1 (0.7)
Acute myocardial infarction	0	1 (0.7)
Cardiac failure congestive	0	1 (0.7)
Sinoatrial block	0	1 (0.7)
Syncope	0	1 (0.7)
Non-fatal stroke (SMQ)	1 (0.7)	1 (0.7)
Non-fatal MI (OCMQ)	0	1 (0.7)
Arrhythmia (OCMQ)	11 (7.7)	9 (6.4)
Ventricular tachyarrhythmias ¹	3 (2.1)	5 (3.6)
Requiring treatment	0	1 (0.7)
Atrial fibrillation	4 (2.8)	4 (2.9)
New onset	0	1 (0.7)

Source: adsl.spt and adae.xpt Software: Analysis Studio

Treatment-emergent adverse events defined as AEs occurring or worsening after the first dose of study drug until 28 days after the last dose of study drug.

¹The Applicant's definition includes syncope, ventricular tachycardia, loss of consciousness and ventricular fibrillation.

Abbreviations: AE, adverse event; CV, cardiovascular; MACE, major adverse cardiac events; MedDRA, Medical Dictionary of Regulatory Activities; N, number of subjects in treatment group; n, number of subjects with adverse event; OCMQ, Office of New Drugs Custom Medical Query; SMQ, Standardised MedDRA Query

7.6.1.6. Treatment-Emergent Adverse Events

Hypertension was the most common AE reported in CY 6031. It was also the only AE that occurred at a frequency of $\geq 5\%$ higher in the aficamten group compared to the placebo group (8% versus 2%) ([Table 33](#)). Evaluation of hypertension is discussed in Section [7.6.1.6.1](#).

Palpitations was another common AE reported in CY 6031, occurring more frequently in subjects on aficamten than placebo (7% versus 3%). Palpitations are subjective symptoms that are common in patients with HCM. These symptoms are nonspecific and can be attributed to a wide range of causes and underlying diseases. The palpitation events observed in the aficamten group were transient and did not recur despite continued treatment. There was no apparent pattern in the onset of these events relative to the initiation of aficamten treatment (ranging from 4 to 360 days). Importantly, reports of palpitations were not associated with any clinically significant events, such as arrhythmia. Given these observations with a low incidence, a causal relationship between these palpitation events and aficamten treatment was unlikely.

Heart failure related-events were reported in four aficamten-treated subjects (three cases reported as cardiac failure and one case reported as pulmonary edema) and one placebo-treated subject (cardiac failure congestive). Three of the events in the aficamten group occurred after discontinuation of treatment, during the safety follow-up period (range from two to eight days after last aficamten treatment). Given the long half-life of aficamten, a causal relationship between aficamten and these events cannot be excluded. The single on-treatment AE of cardiac failure (moderate intensity) was thought to be related to aficamten (see more details in Section 7.6.1.7).

Numerical imbalance of back pain, paresthesia, renal and urinary tract infection, and rash was also observed in CY 6031; however, the frequency of these AEs was overall low, and the difference between groups was small (<3%). These events were transient and generally reported to be mild in severity. Due to the nature of the events, the significance of these events in aficamten-treated subjects is unclear and these minor imbalances are likely due to chance.

Table 33. Subjects With Treatment-Emergent Adverse Events¹ by System Organ Class and OND Custom Medical Queries (Narrow) Occurring at ≥2% Higher Frequency in Aficamten Group Than Placebo Group, Safety Population, Trial CY 6031

System Organ Class OCMQ (Narrow)	Aficamten N=142 n (%)	Placebo N=140 n (%)	Risk Difference % (95% CI)
Cardiac disorders (SOC)			
Systemic hypertension	11 (7.7)	3 (2.1)	5.6 (0.6, 11.5) ²
Palpitations	10 (7.0)	4 (2.9)	4.2 (-1.0, 10.0)
Heart failure	4 (2.8)	1 (0.7)	2.1 (-1.4, 6.4)
General disorders and administration site conditions (SOC)			
Musculoskeletal and connective tissue disorders (SOC)			
Back pain	5 (3.5)	2 (1.4)	2.1 (-2.0, 6.7)
Nervous system disorders (SOC)			
Paresthesia	3 (2.1)	0	2.1 (-0.6, 6.0)
Renal and urinary disorders (SOC)			
Renal and urinary tract infection	5 (3.5)	2 (1.4)	2.1 (-2.0, 6.7)
Skin and subcutaneous tissue disorders (SOC)			
Rash	5 (3.5)	1 (0.7)	2.8 (-0.8, 7.4)

Source: adae.xpt; Software: R

¹ Treatment-emergent adverse events defined as AEs occurring or worsening after the first dose of study drug until 28 days after the last dose of study drug.

95% confidence interval excludes zero.

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

Abbreviations: AE, adverse event; CI, confidence interval; OCMQ, Office of New Drugs Custom Medical Query; incl, including; N, number of subjects in treatment group; n, number of subjects with adverse event; PT, preferred term; SOC, system organ class; TIA, transient ischemic attack

7.6.1.6.1. Hypertension

There was a small imbalance in hypertension AEs in Trial CY 6031; 11 (8%) on aficamten compared to 3 (2%) on placebo. The majority of the hypertension events in the aficamten group were mild (8 out of 11), with no severe cases or SAEs reported. None of the hypertension events led to treatment discontinuation, interruption or dose changes. Most subjects who experienced hypertension had reported a medical history of hypertension at baseline (8 out of 11). There was

no apparent pattern of event onset relative to aficamten treatment initiation (the time to onset ranged from 15 to 173 days).

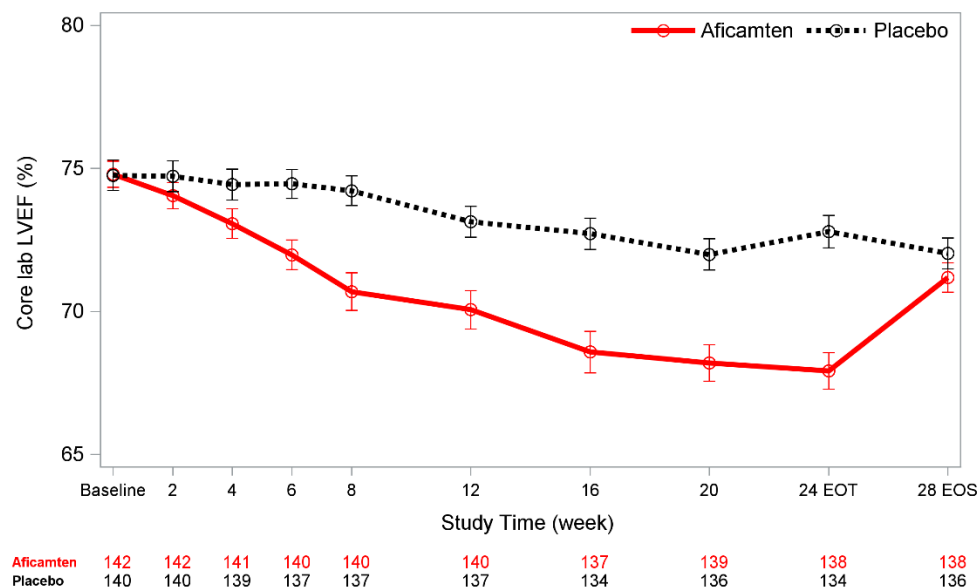
A total of 15 subjects (7%) experienced hypertension AEs in the open-label extension Study CY 6022. The nature and severity of these events were consistent with the results in CY 6031. There was one SAE of hypertensive urgency that occurred in a 57-year-old female subject with oHCM and a history of essential hypertension. Their baseline blood pressure (BP) was elevated at 174/92 mm Hg. The SAE occurred 13 days after initiating aficamten treatment. The subject presented to the emergency room after experiencing significantly elevated BP of 214/99 mm Hg at home and was subsequently hospitalized due to the SAE. Aficamten (5 mg) was interrupted, and the event resolved after 2 days. The subject restarted aficamten and was subsequently up-titrated to 10 mg and 15 mg without any recurrence of the hypertensive event.

Consistent with the AE findings, there was a small mean increase in both systolic and diastolic BP in the aficamten group compared to the placebo group (see Section 7.6.1.10). Aficamten-associated increases in BP are thought to be a consequence of relief of LVOT obstruction with improved cardiac output. The clinical review team recommends describing the hypertension AE findings and blood pressure changes in Section 6 of the labeling.

7.6.1.7. Echocardiograms: LVEF

Consistent with the aficamten mechanism of action, there was a small mean decrease in LVEF (~4 to 5% with an SD of 7%) during 24 weeks of aficamten treatment compared with placebo (Figure 8). Mean LVEF returned toward baseline at Week 28, following the 4-week safety follow-up period.

Figure 8. Mean LVEF Across Time, Safety Population, Trial CY 6031



Source: adlb.xpt; Software: SAS

Abbreviations: EOS, end of study; EOT, end of treatment; LVEF, left ventricular ejection fraction; SAS, statistical analysis software

LVEF values <50% as assessed by the core laboratory were observed for five subjects (4%) in the aficamten group and one subject (1%) in the placebo group. The median LVEF among the five subjects in the aficamten group was 47% (range from 34% to 49%). Of the five subjects in the aficamten group, none had associated signs or symptoms of heart failure, and none had LVEF <30%. There was only one subject who had concurrent LVEF <50% as assessed by both core-laboratory and site-assessed LVEF; the site assessment led to dose reduction. For the remaining 4 aficamten subjects, no dose adjustments were made because the site-assessed LVEF remained >50%. Core laboratory-assessed LVEF values returned to >50% during treatment for four subjects and by the end of the washout period for all five subjects.

Similarly, site-assessed LVEF <50% was observed for seven subjects (5%) in the aficamten group and one subject (1%) in the placebo group. All 7 aficamten-treated subjects had a subsequent dose reduction (per protocol), and all subjects had an LVEF ≥50% at the next visit. There was no treatment interruption due to LVEF <50%. There was one aficamten-treated subject with reported associated signs or symptoms of heart failure. This 41-year-old White male subject reported a nonserious AE of cardiac failure with moderate intensity at Week 12 (4 weeks before a site read LVEF of 47% at Week 16). The site-assessed LVEF at Week 12 was 55%, which was decreased from 65% at Week 8 (66% at baseline). No action was taken with aficamten due to the event. The investigator assessed this AE as related to study treatment. At Week 16, aficamten was down-titrated from 20 mg to 15 mg due to site-assessed LVEF <50%. About 10 days after down-titration, LVEF returned to ≥50% based on both site-read and core laboratory. The AE of cardiac failure was considered resolved.

There was no specific pattern regarding the onset of LVEF ≤50%, which occurred sporadically during the entire treatment period (median onset of 97 days, range from 15 days to 169 days).

7.6.1.8. Laboratory Findings

Analyses of laboratory data from the CY 6031 trial did not raise major safety concerns. Mean changes from baseline to the end of treatment were similar between the groups for most clinical chemistry and hematology parameters collected in the study. The outlier analyses did not reveal signals of concern. There was one subject in the aficamten group who had elevated creatine phosphokinase (CPK) (>10× upper limit of normal [ULN]). The considerably high level of CPK was observed during the safety follow-up period about 20 days after the last aficamten treatment. The elevated CPK was transient and returned to normal after 2 weeks.

Table 34. Subjects With One or More Chemistry Analyte Values With Elevated or Low Values Meeting Specified Levels, Safety Population, Trial CY 6031

Laboratory Parameter	Aficamten N=142 n/N _w (%)	Placebo N=140 n/N _w (%)	Risk Difference % (95% CI)
Sodium, low (mEq/L)			
Level 1 (<132)	1/140 (0.7)	3/138 (2.2)	-1.5 (-5.6, 2.0)
Level 2 (<130)	1/140 (0.7)	1/138 (0.7)	-0.0 (-3.3, 3.3)
Level 3 (<125)	0/140 (0)	1/138 (0.7)	-0.7 (-4.0, 2.0)
Sodium, high (mEq/L)			
Level 1 (>150)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 2 (>155)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (>160)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)

Laboratory Parameter	Aficamten N=142 n/N_w (%)	Placebo N=140 n/N_w (%)	Risk Difference % (95% CI)
Potassium, low (mEq/L)			
Level 1 (<3.6)	4/140 (2.9)	5/138 (3.6)	-0.8 (-5.7, 4.0)
Level 2 (<3.4)	2/140 (1.4)	2/138 (1.4)	-0.0 (-3.9, 3.8)
Level 3 (<3)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Potassium, high (mEq/L)			
Level 1 (>5.5)	0/140 (0)	1/138 (0.7)	-0.7 (-4.0, 2.0)
Level 2 (>6)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (>6.5)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Chloride, low (mEq/L)			
Level 1 (<95)	4/140 (2.9)	9/138 (6.5)	-3.7 (-9.5, 1.5)
Level 2 (<88)	0/140 (0)	1/138 (0.7)	-0.7 (-4.0, 2.0)
Level 3 (<80)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Chloride, high (mEq/L)			
Level 1 (>108)	4/140 (2.9)	6/138 (4.3)	-1.5 (-6.7, 3.3)
Level 2 (>112)	0/140 (0)	1/138 (0.7)	-0.7 (-4.0, 2.0)
Level 3 (>115)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Bicarbonate, low (mEq/L)			
Level 1 (<20)	6/140 (4.3)	6/138 (4.3)	-0.1 (-5.4, 5.3)
Level 2 (<18)	2/140 (1.4)	0/138 (0)	1.4 (-1.3, 5.1)
Level 3 (<15)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Bicarbonate, high (mEq/L)			
Level 3 (>30)	6/140 (4.3)	6/138 (4.3)	-0.1 (-5.4, 5.3)
Glucose, low (mg/dL)			
Level 1 (<70)	7/140 (5.0)	11/138 (8.0)	-3.0 (-9.4, 3.1)
Level 2 (<54)	0/140 (0)	5/138 (3.6)	-3.6 (-8.2, -0.9) ¹
Level 3 (<40)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Glucose, random, high (mg/dL)			
Level 2 (≥200)	3/140 (2.1)	4/138 (2.9)	-0.8 (-5.4, 3.6)
Level 3 (>250)	0/140 (0)	3/138 (2.2)	-2.2 (-6.2, 0.5)
Calcium, low (mg/dL)			
Level 1 (<8.4)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 2 (<8)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (<7.5)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Calcium, high (mg/dL)			
Level 1 (>10.5)	3/140 (2.1)	2/138 (1.4)	0.7 (-3.2, 4.8)
Level 2 (>11)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (>12)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Magnesium, low (mg/dL)			
Level 1 (<1.5)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 2 (<1.2)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (<0.9)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Magnesium, high (mg/dL)			
Level 1 (>2.3)	42/140 (30.0)	40/138 (29.0)	1.0 (-9.7, 11.7)
Level 2 (>4)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (>7)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Phosphate, low (mg/dL)			
Level 1 (<2.5)	5/140 (3.6)	11/138 (8.0)	-4.4 (-10.6, 1.2)
Level 2 (<2)	1/140 (0.7)	0/138 (0)	0.7 (-2.0, 3.9)
Level 3 (<1.4)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)

Laboratory Parameter	Aficamten N=142 n/N _w (%)	Placebo N=140 n/N _w (%)	Risk Difference % (95% CI)
Protein, total, low (g/dL)			
Level 1 (<6)	3/140 (2.1)	1/138 (0.7)	1.4 (-2.1, 5.5)
Level 2 (<5.4)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (<5)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Albumin, low (g/dL)			
CPK, high (U/L)			
Level 1 (>3× ULN)	1/140 (0.7)	1/138 (0.7)	-0.0 (-3.3, 3.3)
Level 2 (>5× ULN)	1/140 (0.7)	0/138 (0)	0.7 (-2.0, 3.9)
Level 3 (>10× ULN)	1/140 (0.7)	0/138 (0)	0.7 (-2.0, 3.9)
Blood urea nitrogen, high (mg/dL)			
Level 1 (>23)	17/140 (12.1)	22/138 (15.9)	-3.8 (-12.2, 4.5)
Level 2 (>27)	7/140 (5.0)	6/138 (4.3)	0.7 (-4.8, 6.2)
Level 3 (>31)	5/140 (3.6)	3/138 (2.2)	1.4 (-3.1, 6.2)

Source: adlb.xpt; Software: R

95% confidence interval excludes zero.

Note that glucose values for hyperglycemia do not follow a nested format like the other labs. Level 1 corresponds to the diagnosis of prediabetes and is not inclusive of Level 2 and Level 3. Level 2 corresponds to the diagnosis of diabetes. Level 3 represents significant hyperglycemia that may indicate need for insulin or increased risk for diabetic ketoacidosis or other complications.

Threshold levels 1, 2, and 3 as defined by the [Standard Safety Tables & Figures Integrated Guide](#).

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

Abbreviations: CI, confidence interval; CPK, creatine phosphokinase; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data; ULN, upper limit of normal

Table 35. Subjects With One or More Kidney Function Analyte Values Exceeding Specified Levels, Safety Population, Trial CY 6031

Laboratory Parameter	Aficamten N=142 n/N _w (%)	Placebo N=140 n/N _w (%)	Risk Difference % (95% CI)
Creatinine, high (mg/dL)			
Level 1 (≥1.5× baseline)	1/140 (0.7)	1/138 (0.7)	-0.0 (-3.3, 3.3)
Level 2 (≥2× baseline)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (≥3× baseline)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
eGFR, low (mL/min/1.73 m ²)			
Level 1 (≥25% decrease) ¹	2/138 (1.4)	9/138 (6.5)	-5.1 (-10.7, -0.5) ¹
Level 2 (≥50% decrease)	0/138 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (≥75% decrease)	0/138 (0)	0/138 (0)	0.0 (-2.7, 2.7)
CrCl, low (mL/min)			
Level 1 (≥25% decrease)	1/13 (7.7)	2/19 (10.5)	-2.8 (-25.9, 25.0)
Level 2 (≥50% decrease)	0/13 (0)	0/19 (0)	0.0 (-17.3, 23.4)
Level 3 (≥75% decrease)	0/13 (0)	0/19 (0)	0.0 (-17.3, 23.4)

Source: adlb.xpt; Software: R

¹ 95% confidence interval excludes zero.

Threshold levels 1, 2, and 3 as defined by the [Standard Safety Tables & Figures Integrated Guide](#).

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

eGFR values are calculated from serum creatinine using chronic kidney disease epidemiology collaboration equation.

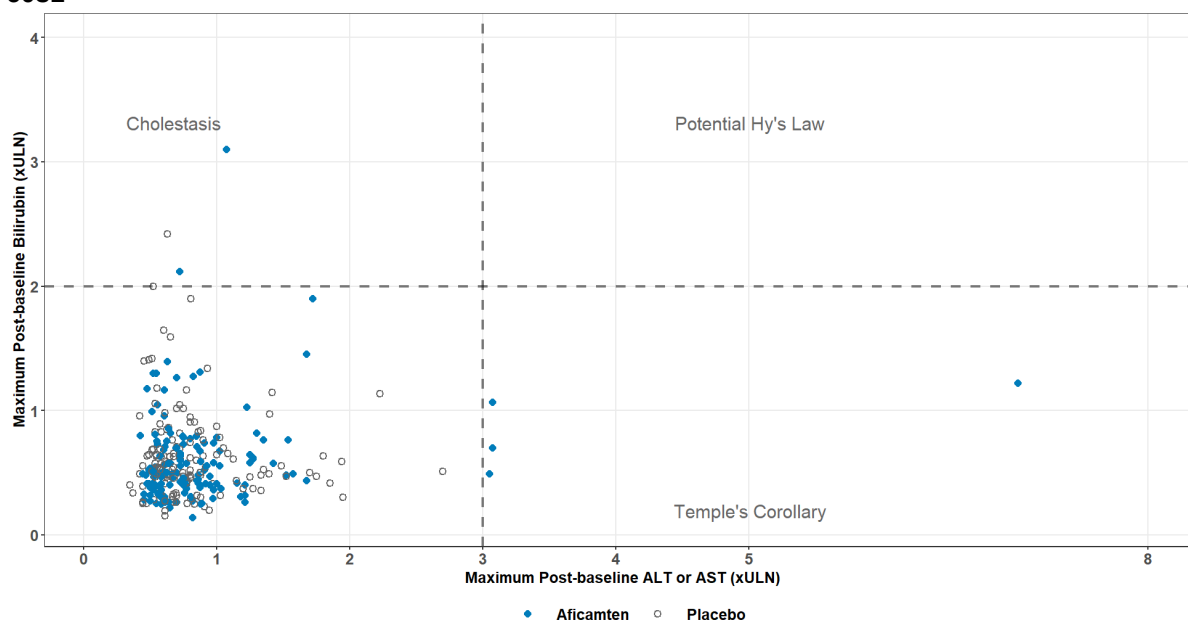
Abbreviations: CI, confidence interval; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data

7.6.1.9. Assessment of Drug-Induced Liver Injury

Review of liver enzyme tests demonstrated that no subject met the criteria for drug-induced liver injury assessed by concurrent alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $>3\times$ ULN and total bilirubin $>2\times$ ULN (i.e., possible Hy's Law cases) in either treatment group in CY 6031 (Figure 9). Four aficamten-treated subjects had ALT and/or AST $>3\times$ ULN during the study period in Trial CY 6031 (Figure 9 and Table 36). In all four cases, the transaminase elevations were mild, transient, not associated with symptoms or increased bilirubin, and did not require treatment interruption (see narratives in Section 17.1). One subject with AST $>5\times$ ULN had an AST value of 302 U/L (7x ULN) during the washout period (29 days after discontinuation of aficamten). His other liver tests were within the normal range, and his AST returned to within the normal range after 2 weeks with no intervention. Two aficamten-treated subjects had elevated bilirubin $>2\times$ ULN (one $>3\times$ ULN); both episodes were transient and occurred during the washout period about 4 weeks after discontinuation of aficamten. Overall, the liver laboratory data did not raise findings of concern.

Figure 9 shows a screening assessment for potential cases of serious drug-induced liver injury in CY 6031. There were also no subjects who met Hy's law criteria in the dose-ranging Study CY 6021 and the open-label extension Study CY 6022.

Figure 9. Hepatocellular Drug-Induced Liver Injury Screening Plot, Safety Population, Trial CY 6031



Source: adlb.xpt; Software: R

Each data point represents a subject plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period.

A potential Hy's Law case was defined as having any postbaseline total bilirubin equal to or exceeding $2\times$ ULN after a postbaseline ALT or AST equal to or exceeding $3\times$ ULN. Those subjects who meet total bilirubin equal to or exceeding $2\times$ ULN criteria within 30 days of the ALT or AST equal to or exceeding $3\times$ ULN criteria are circled in red.

The within 30 days analysis window rule does not apply to cholestasis and temple's corollary cases.

All subjects with at least one postbaseline ALT or AST, bilirubin and ULN are plotted.

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

For number of subjects in each quadrant, see the table "Subjects in Each Quadrant for Potential Hepatocellular Drug-Induced Liver Injury Screening Plot ..." and the listing "Listing of Subjects in Hepatocellular Drug-Induced Liver Injury Screening..."

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal

Table 36. Subjects With One or More Liver Biochemistry Analyte Values Exceeding Specified Levels, Safety Population, Trial CY 6031

Laboratory Parameter	Aficamten N=142 n/N _w (%)	Placebo N=140 n/N _w (%)	Risk Difference % (95% CI)
Alkaline phosphatase, high (U/L)			
Level 1 (>1.5× ULN)	0/140 (0)	2/138 (1.4)	-1.4 (-5.1, 1.3)
Level 2 (>2× ULN)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (>3× ULN)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Alanine aminotransferase, high (U/L)			
Level 1 (>3× ULN)	3/140 (2.1)	0/138 (0)	2.1 (-0.6, 6.1)
Level 2 (>5× ULN)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Level 3 (>10× ULN)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Aspartate aminotransferase, high (U/L)			
Level 1 (>3× ULN)	2/140 (1.4)	0/138 (0)	1.4 (-1.3, 5.1)
Level 2 (>5× ULN)	1/140 (0.7)	0/138 (0)	0.7 (-2.0, 3.9)
Level 3 (>10× ULN)	0/140 (0)	0/138 (0)	0.0 (-2.7, 2.7)
Bilirubin, total, high (mg/dL)			
Level 1 (>1.5× ULN)	3/140 (2.1)	5/138 (3.6)	-1.5 (-6.3, 3.0)
Level 2 (>2× ULN)	2/140 (1.4)	1/138 (0.7)	0.7 (-2.7, 4.4)
Level 3 (>3× ULN)	1/140 (0.7)	0/138 (0)	0.7 (-2.0, 3.9)

Source: adlb.xpt; Software: R

Threshold levels 1, 2, and 3 as defined by the [Standard Safety Tables & Figures Integrated Guide](#).

Duration is 24 weeks.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

For specific evaluation of DILI, see the figures "Hepatocellular Drug-Induced Liver Injury Screening Plot..." and "Cholestatic Drug-Induced Liver Injury Screening Plot..." and the tables "Patients in Each Quadrant for Potential Hepatocellular DILI Screening Plot..." and "Patients in Each Quadrant for Cholestatic DILI Screening Plot..."

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

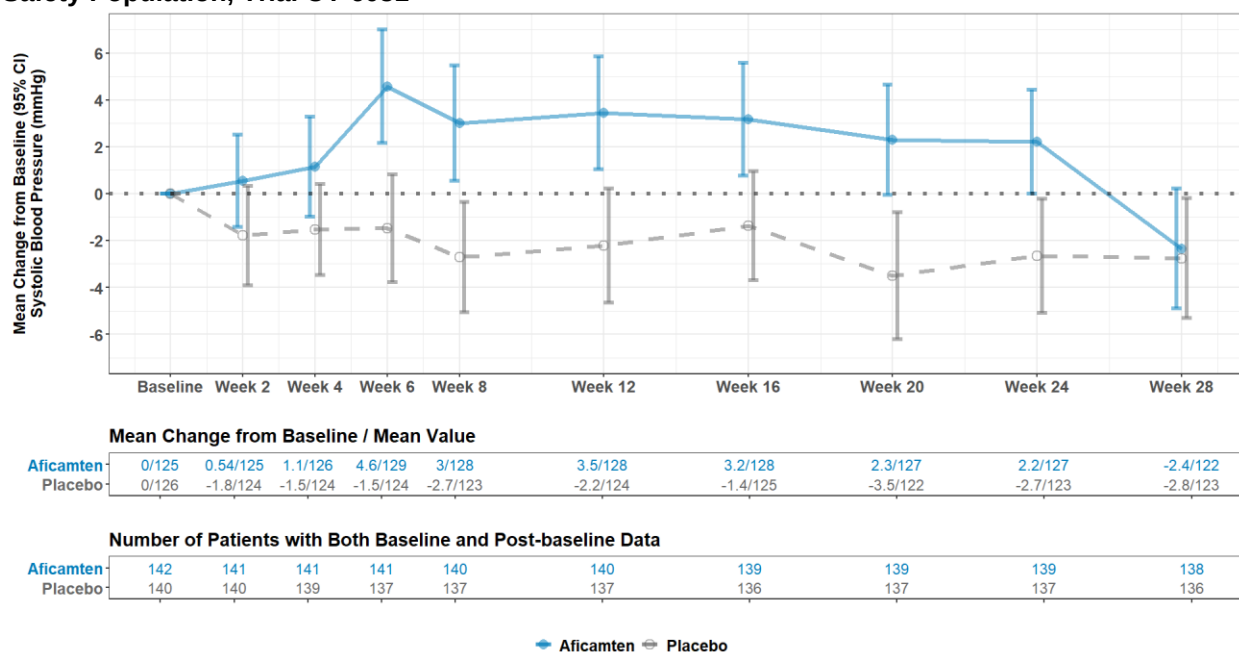
Abbreviations: CI, confidence interval; DILI, drug-induced liver injury; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data; ULN, upper limit of normal

7.6.1.10. Vital Signs

Analyses of vital signs data from CY 6031 found a greater mean change from baseline in systolic/diastolic blood pressure (SBP/DBP) in the aficamten group compared to placebo ([Figure 10](#) and [Figure 11](#)). The mean increases were sustained throughout the treatment period with ~2 to 4 mm Hg increase from baseline in the aficamten group compared to a 1 to 3 mm Hg decrease from baseline in the placebo group.

Subjects with potentially clinically significant SBP and DBP are summarized in [Table 37](#) and [Table 38](#), respectively. Elevated postbaseline SBP (≥160 mm Hg) was observed in approximately 16% of subjects in the aficamten group and 8% of subjects in the placebo group. Similarly, incidence of elevated postbaseline DBP (≥90 mm Hg) was higher in the aficamten group compared to placebo (30% versus 20%). The shift tables by baseline SBP and DBP are listed in [Table 39](#) and [Table 40](#) and do not raise additional concerns. All 11 subjects in the aficamten group who reported hypertension AEs ([Table 33](#)) had at least one post-baseline measurement of SBP ≥140 mm Hg.

Figure 10. Mean Systolic Blood Pressure Change From Baseline Over Time by Treatment Group, Safety Population, Trial CY 6031

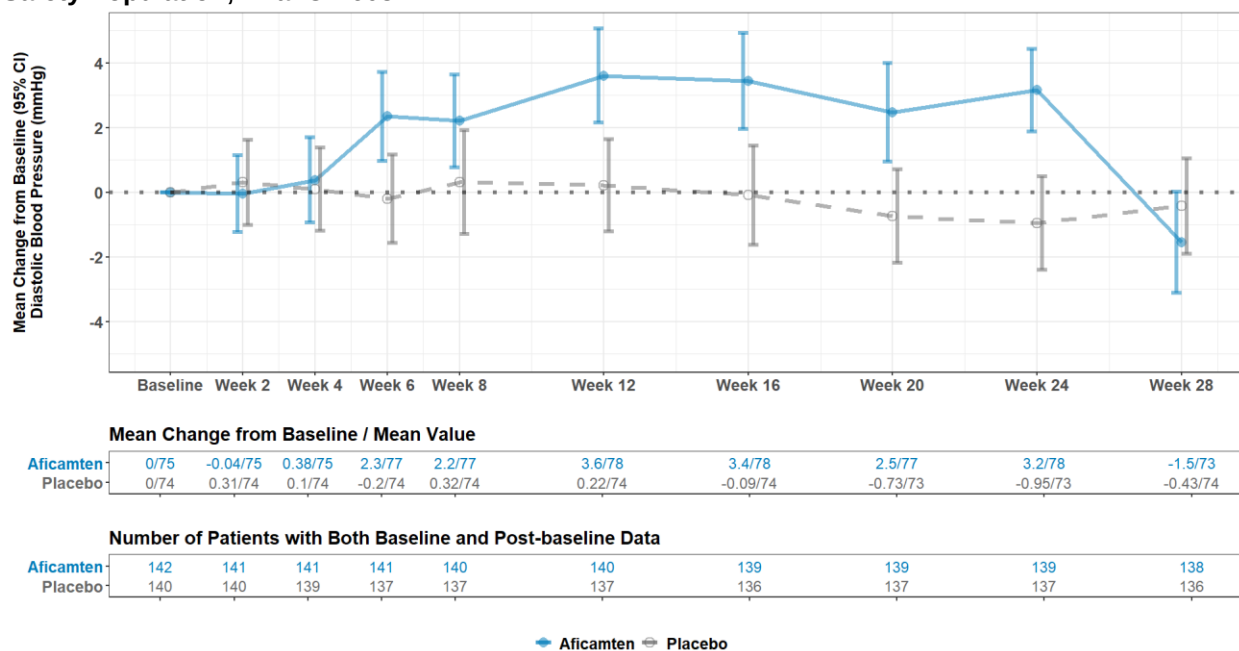


Source: advs.xpt; Software: R

Figures do not include time points with data from fewer than 10% of randomized/enrolled subjects in all treatment groups.

Abbreviation: CI, confidence interval

Figure 11. Mean Diastolic Blood Pressure Change From Baseline Over Time by Treatment Group, Safety Population, Trial CY 6031



Source: advs.xpt; Software: R

Figures do not include time points with data from fewer than 10% of randomized/enrolled subjects in all treatment groups.

Abbreviation: CI, confidence interval

Table 37. Percentage of Subjects With Maximum Systolic Blood Pressure by Category of Blood Pressure Postbaseline, Safety Population, Trial CY 6031

Systolic Blood Pressure (mm Hg)	Aficamten N=142 n/N_w (%)	Placebo N=140 n/N_w (%)	Risk Difference % (95% CI)
<90	0/142 (0)	0/140 (0)	0.0 (-2.7, 2.6)
≥90	142/142 (100)	140/140 (100)	0.0 (-2.6, 2.7)
≥120	127/142 (89.4)	124/140 (88.6)	0.9 (-6.7, 8.5)
≥140	71/142 (50.0)	56/140 (40.0)	10.0 (-1.6, 21.4)
≥160	22/142 (15.5)	11/140 (7.9)	7.6 (0.1, 15.4) ¹
≥180	1/142 (0.7)	4/140 (2.9)	-2.2 (-6.5, 1.3)

Source: advs.xpt; Software: R

¹ 95% confidence interval excludes zero.

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

Abbreviations: CI, confidence interval; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data

Table 38. Percentage of Subjects With Maximum Diastolic Blood Pressure by Category of Blood Pressure Postbaseline, Safety Population, Trial CY 6031

Diastolic Blood Pressure (mm Hg)	Aficamten N=142 n/N_w (%)	Placebo N=140 n/N_w (%)	Risk Difference % (95% CI)
<60	0/142 (0)	2/140 (1.4)	-1.4 (-5.1, 1.2)
≥60	142/142 (100)	138/140 (98.6)	1.4 (-1.2, 5.1)
≥90	42/142 (29.6)	28/140 (20.0)	9.6 (-0.5, 19.6)
≥110	1/142 (0.7)	0/140 (0)	0.7 (-2.0, 3.9)
≥120	0/142 (0)	0/140 (0)	0.0 (-2.7, 2.6)

Source: advs.xpt; Software: R

Risk difference (with 95% confidence interval) is shown between total treatment and comparator.

Abbreviations: CI, confidence interval; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data

Table 39. Shift Table by Category of Systolic Blood Pressure at Baseline vs. Category of Maximum Postbaseline Systolic Blood Pressure, Safety Population, Trial CY 6031

Grouping Variable	Baseline Systolic Blood Pressure	Maximum Postbaseline Systolic Blood Pressure		
		<120 mm Hg	120-159 mm Hg	≥160 mm Hg
Aficamten (N=142)	<120 mm Hg	13 (9.2%)	40 (28.2%)	4 (2.8%)
	120-159 mm Hg	2 (1.4%)	65 (45.8%)	16 (11.3%)
	≥160 mm Hg	0 (0%)	0 (0%)	2 (1.4%)
Placebo (N=140)	<120 mm Hg	15 (10.7%)	37 (26.4%)	0 (0%)
	120-159 mm Hg	1 (0.7%)	75 (53.6%)	8 (5.7%)
	≥160 mm Hg	0 (0%)	1 (0.7%)	3 (2.1%)

Source: OCS Analysis Studio, Shift Table.

Abbreviation: N, number of subjects in treatment group

Table 40. Shift Table by Category of Diastolic Blood Pressure at Baseline vs. Category of Maximum Postbaseline Diastolic Blood Pressure, Safety Population, Trial CY 6031

Grouping Variable	Baseline Diastolic Blood Pressure	Maximum Postbaseline Diastolic Blood Pressure		
		<60 mm Hg	60-89 mm Hg	≥90 mm Hg
Aficamten (N=142)	<60 mm Hg	0 (0%)	10 (7%)	0 (0%)
	60-89 mm Hg	0 (0%)	88 (62%)	29 (20.4%)
	≥90 mm Hg	0 (0%)	2 (1.4%)	13 (9.2%)
Placebo (N=140)	<60 mm Hg	2 (1.4%)	10 (7.1%)	0 (0%)
	60-89 mm Hg	0 (0%)	99 (70.7%)	22 (15.7%)
	≥90 mm Hg	0 (0%)	1 (0.7%)	6 (4.3%)

Source: OCS Analysis Studio, Shift Table.

Abbreviation: N, number of subjects in treatment group

7.7. Key Safety Review Issues

7.7.1. Risk of Heart Failure Due to Systolic Dysfunction

Issue

The Applicant proposed to manage the iatrogenic risk of HF due to systolic dysfunction via labeling and voluntary education materials without a REMS program at the time of the NDA submission. Whether the proposed strategy is sufficient to ensure the safe use of aficamten was a review issue.

Background

Systolic dysfunction is the identified risk for cardiac myosin inhibitors based on the mechanism of action. The first-in-class cardiac myosin inhibitor was approved in 2022 with a REMS to mitigate the risk of HF due to systolic dysfunction. The objectives of the REMS were to ensure periodic echocardiograms for dosing and monitoring and to screen for drug interactions that would increase exposure, thus increasing the risk of systolic dysfunction. The Applicant stated that aficamten has a PK and PD profile that minimizes the risk of iatrogenic systolic HF. The available clinical data to date also demonstrate a low risk of HF and/or systolic dysfunction. In light of these findings and the burden associated with a REMS, the Applicant proposed to mitigate the risk of HF due to systolic dysfunction without a REMS for aficamten.

Assessment

Clinical Pharmacologic Features of Aficamten

Due to its complex pharmacologic profile, the first-in-class cardiac myosin inhibitor requires implementation of a complicated and structured dosing titration algorithm in conjunction with the REMS. Aficamten has a terminal half-life of ~80 hours, with steady-state concentrations achieved after 10-12 days of treatment. Aficamten undergoes elimination primarily through CYP2C9-mediated metabolism (50%) with additional contributions from CYP3A (26%), CYP2D6, and CYP2C19 pathways. The involvement of multiple CYP enzymes in aficamten elimination reduces the likelihood of clinically significant concentration increases resulting from potent inhibition of any single metabolic pathway. This suggests that large changes in aficamten concentrations are less likely to emerge from strong inhibition of any individual pathway.

Currently, there are no strong CYP2C9 inhibitors commercially available in the United States. Aficamten exposure may be elevated with coadministration of strong inhibitors affecting multiple CYPs (such as fluvoxamine, fluconazole, and voriconazole, see Section 14). Based on these pharmacologic characteristics, aficamten demonstrates a moderate risk of increased exposure, potentially supporting implementation of a less restrictive dosing strategy and monitoring program.

Risk of Clinical Heart Failure/Systolic Dysfunction Features of Aficamten

There were no aficamten-treated oHCM subjects experiencing serious HF or severe systolic dysfunction (LVEF <30%) in CY 6031 and in the open-label extension (OLE) CY 6022 study. Three SAEs of HF/ventricular dysfunction have been reported in aficamten-treated subjects with nHCM (see Narratives in Section 17.1). Table 41 summarizes the incidence and event rate of SAEs of HF/systolic dysfunction among subjects with oHCM and nHCM in the aficamten clinical programs at the time of the NDA submission for aficamten.

Table 41. Incidence and Event Rate of Serious Heart Failure in the Aficamten Clinical Program

Event	Aficamten (oHCM+nHCM) N=376	
	n (%)	ER (100 Patient -Years)
SAE of heart failure/LVEF <30%	3 ¹ (0.8)	0.6

Source: Reviewer's table

¹ All in patients with nHCM; none with LVEF <30%.

Abbreviation: ER, event rate; N, number of subjects in the integrated summary of safety; n, number of subjects having an event; LVEF, left ventricular ejection fraction; nHCM, non-obstructive hypertrophic cardiomyopathy; oHCM, obstructive hypertrophic cardiomyopathy; SAE, serious adverse event

Safety Updates

The division requested that the Applicant report any case of SAE of HF and/or LVEF <30% in all ongoing clinical trials throughout the NDA review period. No occurrences of LVEF <30% have occurred in the aficamten clinical program through June 16, 2025. There were an additional six SAEs of cardiac failure in an ongoing phase 3 trial CY 6033 in subjects with nHCM (treatment assignments remain blinded) and two cases in subjects with oHCM (one on aficamten and the other on metoprolol) in the second phase 3 trial comparing the effect of aficamten versus an active control, metoprolol, in subjects with oHCM. Overall, no major concerns arose from these cases. All cases were resolved with or without treatment interruptions.

Uncertainty

While the risk of serious HF or severe systolic dysfunction appears to be low in aficamten-treated oHCM patients, an iatrogenic risk of HF with aficamten still exists based on the mechanism of action of the drug. The total exposure to date remains relatively low with approximately 300 patients with oHCM. There is a concern that the risk would be higher in the post-market setting where aficamten is used in a wider range of patients with higher comorbidities. It should be noted that CY 6031 excluded patients with any history of LV systolic dysfunction (LVEF <45%) or stress cardiomyopathy at any time during their clinical course. It is not unreasonable to speculate that these excluded patients may be prescribed aficamten in the real world. Further, this class of drug is relatively new, and prescribers are still learning about

this class and would benefit from mandated training. Careful monitoring in the real world is not expected to be as rigorous as in the clinical trials, and labeling alone will not ensure that prescribers appropriately titrate aficamten and obtain echocardiograms at appropriate time points.

Conclusion

The review team determined that a REMS is necessary to ensure the benefits outweigh the potential risk of HF due to systolic dysfunction. However, the review team concluded that the PK profile of aficamten does not appear to warrant the inclusion of a drug interaction monitoring component in a REMS. Dose modification related to certain strong inhibitors of multiple CYPs can be addressed via labeling. The REMS Oversight Committee concurred with this decision and recommended that prescriber education and documentation of echocardiogram monitoring must be included as part of a REMS. Hence, a REMS with ETASU consisting of ETASU A (prescriber certification), ETASU B (pharmacy certification), ETASU D (documentation of safe-use conditions), and ETASU E (patient monitoring) is required for aficamten. This decision on REMS was conveyed to the Applicant at the Mid-Cycle Communication meeting on March 4, 2025, and the Applicant subsequently submitted their proposed REMS on March 28, 2025, in response to the requirement (see the Division of Risk Management review), which was classified as a major amendment to the application and extended the PDUFA goal date by 3 months.

8. Therapeutic Individualization

8.1. Intrinsic Factors

Hepatic Impairment

The effect of moderate hepatic impairment (HI) on the pharmacokinetics of aficamten was evaluated in a dedicated Phase 1 HI study (CY 6017). Aficamten exposures following a single dose of aficamten 20 mg were similar in subjects with normal hepatic function and subjects with moderate HI (i.e., Child-Pugh Class B) (i.e., 38% increase in mean C_{max} and 5% decrease in mean AUC_{∞} with moderate HI). Based on the outcome of this study, the Applicant concluded that no adjustment of the aficamten dose is required for subjects with mild or moderate HI (i.e., Child-Pugh A or B). Details about this study can be found in Section [14.2](#) of this review. No studies have been conducted in subjects with severe HI (i.e., Child-Pugh C).

While the Clinical Pharmacology and Clinical review team believes that there would not be safety concerns with severe HI subjects as the aficamten dose will be titrated with safety monitoring, the statement of “the effects of severe hepatic (Child-Pugh Class C) impairment is unknown” has been included in the product label.

Renal Impairment

Based on the results of the mass balance study (CY 6013) that showed a minor role of renal function in aficamten elimination (i.e., renal clearance <0.1% of total clearance), the Applicant believes that clinically significant increases in aficamten exposure due to renal impairment (RI)

are unlikely and therefore, the effect of renal impairment on aficamten exposure was not evaluated in a dedicated clinical study.

The Applicant evaluated the effect of mild or moderate RI on aficamten pharmacokinetics using PPK analyses based on Phase 2 and 3 study data (link to Section [14.5](#)). The aficamten Phase 2 and 3 studies (CY 6021 and CY 6031) included subjects with oHCM that had mild (i.e., creatinine clearance [CrCL; Cockcroft-Gault equation] of 60 to 89 mL/min; N=54) or moderate (i.e., CrCL of 30 to 59 mL/min; N=13) RI. However, individuals with severe RI (i.e., estimated glomerular filtration rate [eGFR] <30 mL/min/1.73 m²) were excluded from the Phase 2 and 3 studies. While evaluating the effect of renal impairment on aficamten pharmacokinetics, the Clinical Pharmacology review team noted a discrepancy between how the Applicant classified renal impairment in the inclusion/exclusion criteria for the clinical studies and how their PPK analysis was conducted. As a result, the following IR was sent to the Applicant on April 14, 2025:

We note that inclusion/exclusion criteria for renal impairment in your clinical studies (e.g., Studies CY 6021 and CY 6031) were based on estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease (MDRD) equation while you conducted population PK analysis using renal impairment classification based on creatinine clearance (CLCr) derived from the Cockcroft-Gault (C-G) equation and used that for labeling. Clarify whether using different equations to estimate renal function would result in any difference in: (1) classification of renal function in the studied population; and (2) the effect of renal impairment on aficamten pharmacokinetics based on your population PK analysis.

Subsequently, the Applicant submitted their response as follows on April 18, 2025 (SDN: 030): “Cytokinetics has re-assessed the impact of renal function (eGFR estimated using MDRD equation) on aficamten pharmacokinetics. When compared to the C-G-based assessment, estimation of renal function using MDRD resulted in higher number of subjects being classified as having mild or moderate renal impairment (i.e., N=54 with mild RI and N=13 with moderate RI based on C-G equation). As expected for a drug that is eliminated primarily by metabolism, aficamten pharmacokinetics was comparable among subjects with normal renal function and those with mild or moderate renal impairment ([Table 42](#)). These results agree with those using the C-G classification; thereby, supporting the use of aficamten in subjects with oHCM with mild to moderate renal impairment.” The Applicant’s response was found to be reasonable from a Clinical Pharmacology perspective.

Table 42. MDRD: Summary of Aficamten Population PK-Predicted Exposures at Steady State Following Once Daily Dosing of 15-mg Aficamten in Subjects With oHCM by Renal Function, Studies CY 6021 and CY 6031; N=183

Parameter	Renal Function			Geometric Mean Ratio (90% CI)	
	Normal Function (N = 72)	Mild Impairment (N = 92)	Moderate Impairment (N = 19)	Mild Renal Impairment/ Normal Function	Moderate Renal Impairment/ Normal Function
eGFR (mL/min/1.73m ²) ^a	103 [90.0, 162]	75.0 [60.0, 89.0]	52.0 [33.0, 58.0]	NA	NA
AUC _τ (ng·h/mL) ^b	5910 (35.8)	6750 (42.9)	6820 (35.9)	1.13 (1.03-1.24)	1.16 (1.00-1.33)
C _{max} (ng/mL) ^b	255 (36.4)	292 (42.4)	296 (37.0)	1.14 (1.04-1.25)	1.17 (1.01-1.35)
C _τ (ng/mL) ^b	220 (39.5)	255 (46.3)	257 (38.9)	1.15 (1.04-1.27)	1.18 (1.01-1.38)
Body Weight (kg) ^a	82.0 [47.0, 116]	80.0 [49.0, 156]	82.0 [59.0, 123]	NA	NA
Female, N [%]	21 (29.2)	43 (46.7)	14 (73.7)	NA	NA

CI = confidence interval; NA = not applicable.

Note: Exposures were simulated using posthoc PK parameters of participants with oHCM given aficamten 15 mg tablet once daily (median last stable dose in CY 6031) with or without food.

^a Presented as median [minimum, maximum].

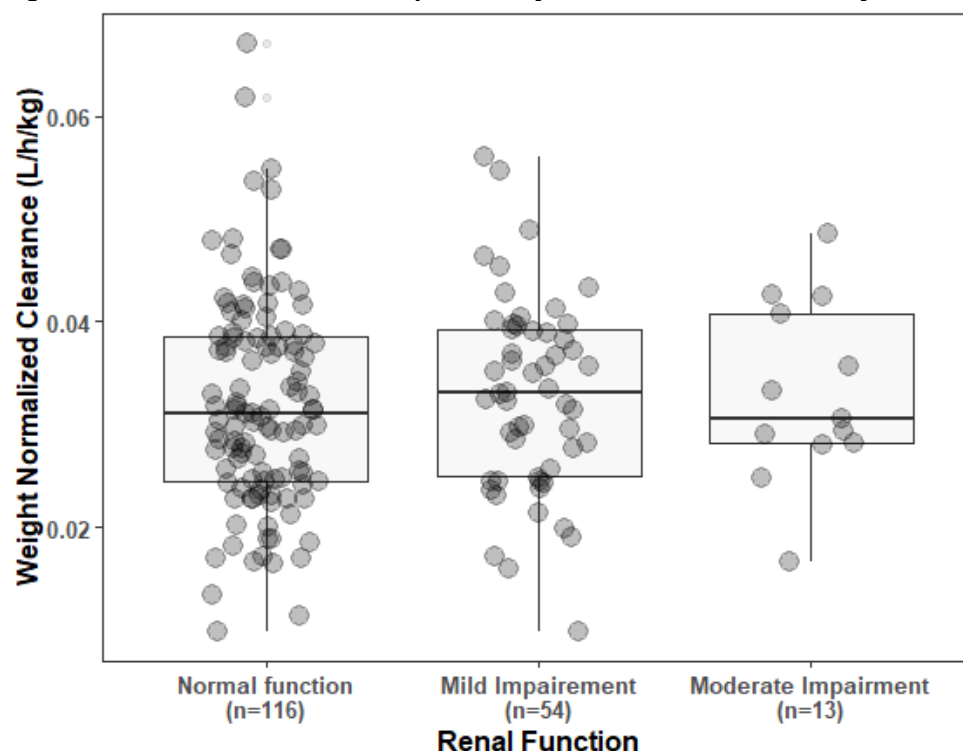
^b Presented as mean (%CV).

Source Table 2, NDA 219083, SDN: 030 (Submitted on April 18, 2025)

Abbreviations: MDRD, Modification of Diet in Renal Disease; oHCM, obstructive hypertrophic cardiomyopathy

The Pharmacometrics review team assessed the Applicant's PPK analyses for the effect of mild or moderate RI on aficamten pharmacokinetics. The PPK dataset is comprised of 54 subjects with mild RI and 13 subjects with moderate RI. Based on the PPK analysis, creatinine clearance was not identified as a significant covariate affecting aficamten pharmacokinetics. Similar weight normalized clearance was observed in subjects with normal renal function and subjects with mild and moderate RI ([Figure 12](#)). The steady-state PK comparison across renal function suggested numerical increase in aficamten exposure with increasing degree of RI, which is likely due to the decreased weight and more female subjects with increasing RI in the dataset. Overall, the Applicant's proposed dosing recommendations for subjects with mild and moderate RI is reasonable.

Figure 12. Pharmacokinetic Comparison by Renal Function at Steady-State



Source: Reviewer's analysis

Notes: The closed circle presents the data collected from subjects enrolled in the Phase 3 study; the horizontal black lines indicate the median clearance, the boxes indicate the 25th and 75th percentiles, and the upper/lower whiskers extend from the box to the largest/smallest value no further than 1.5 * IQR (inter-quartile range) from the box.

While the Clinical Pharmacology and Clinical review teams believe that there would not be safety concerns with RI subjects as aficamten dose will be titrated with safety monitoring, the statement of “the effects of severe renal (eGFR <30 mL/min based on MDRD equation) impairment is unknown” has been included in the product label.

Age

Based on PPK-estimated aficamten exposures at steady-state following once daily (QD) dosing of 15-mg aficamten (i.e., median last stable dose in Trial CY 6031) in subjects with oHCM stratified by age group (i.e., <65 years, 65 to 75 years, and >75 years), a general numerical increase in aficamten exposure was observed with increasing age (i.e., age: 18 to 83 years). The Applicant believes that this result is likely due to the decreased body weight and increased proportion of female subjects with increasing age group in the dataset. The Applicant concluded that no dose adjustment of aficamten is warranted based on age. The Pharmacometrics review team found this to be reasonable.

Body Weight

Body weight was identified as a significant covariate on aficamten pharmacokinetics in the Applicant's PPK analysis. Population PK-estimated steady-state exposures (i.e., AUC_{tau} , C_{max} , and C_{tau}) for aficamten in subjects with oHCM were compared across body weight quartiles. Body weight was predicted to have a modest effect on aficamten pharmacokinetics. Aficamten plasma AUC_{tau} , C_{max} , and C_{tau} in a subject with oHCM at the fourth (i.e., highest) weight quartile

(i.e., 95 to 156 kg) were 34%, 35%, and 33% lower, respectively, compared to a subject with oHCM at the first (i.e., lowest) weight quartile (i.e., 47 to 70 kg). The Applicant believes that these differences are expected from simple allometry and the span of median weight between the fourth and first weight quartiles. The Applicant concludes that no dose adjustment of aficamten is warranted based on body weight. The Applicant believes that the proposed individualized aficamten dose titration strategy based on achievement of target LVOT-G and maintenance of normal LVEF is expected to mitigate any effect of body weight on aficamten pharmacokinetics. The Pharmacometrics review team found this to be reasonable.

Sex

Sex was identified as a significant covariate on aficamten pharmacokinetics in the Applicant's PPK analysis. Population PK-estimated steady-state exposures (i.e., AUC_{τ} , $C_{\max}$, and C_{τ}) for aficamten in subjects with oHCM were compared by male versus female (i.e., 105 males and 78 females). Sex was predicted to have a modest effect on aficamten pharmacokinetics. Aficamten plasma AUC_{τ} , $C_{\max}$, and C_{τ} in female subjects with oHCM were 31%, 34%, and 30% higher, respectively, relative to male subjects with oHCM. The Applicant concludes that no dose adjustment of aficamten is warranted based on sex considering that the general practice of echocardiography-based aficamten dose titration is expected to mitigate any effect of sex on aficamten pharmacokinetics. The Pharmacometrics review team found this to be reasonable.

Ethnicity

The impact of ethnicity on the pharmacokinetics of aficamten was evaluated via the Applicant's PPK analyses using the Phase 2 (Study CY 6021) and Phase 3 (Trial CY 6031) data. No impact of ethnicity on aficamten pharmacokinetics was identified in the PPK analysis. Therefore, the Applicant concludes that no dose adjustment of aficamten is warranted based on ethnicity. The Pharmacometrics review team found this to be reasonable.

Overall Recommendation

The Clinical Pharmacology review team agrees that no dose adjustment for aficamten is warranted for intrinsic factors such as HI, RI, age, body weight, sex, and ethnicity that are described above.

8.2. Extrinsic Factors

Food

The effect of food on aficamten pharmacokinetics was assessed in a dedicated food effect study (CY 6012) conducted with the to-be-marketed (TBM) tablet formulation to support that aficamten may be administered without regard to food. No clinically significant differences in aficamten exposure (i.e., AUC and $C_{\max}$) were observed following its administration with a high-fat, high-calorie meal. Details of the study can be found in Section [14.2](#). Aficamten was administered without regard to food in the pivotal Phase 3 trial, CY 6031.

Drug-Drug Interactions

Effect of Other Drugs on Aficamten Pharmacokinetics

The DDI potential of aficamten was evaluated in vitro and in clinical studies.

In Vitro DDI Data

Based on the in vitro studies, aficamten is metabolized primarily through CYP2C9 with contributions by CYP3A4, CYP2C19, and CYP2D6. Aficamten is not a substrate of drug transporters.

Clinical DDI Data

Based on the in vitro findings, several clinical DDI studies were conducted including those evaluating the effects of strong CYP3A inhibition (i.e., itraconazole), CYP3A and CYP2C9 induction (i.e., carbamazepine) (Study CY 6014); strong inhibition of CYP2C19 and moderate inhibition of CYP2C9 and CYP3A (i.e., fluconazole), strong inhibition of CYP2D6 (i.e., paroxetine), and strong inhibition of CYP2C19 and CYP2D6 (i.e., fluoxetine) (Study CY 601-10) on the pharmacokinetics of aficamten ([Table 43](#)). Results from in vitro and clinical DDI assessments indicate that aficamten is primarily eliminated via CYP2C9-mediated metabolism (50%) with contributions from CYP3A (26%), CYP2C19, and CYP2D6.

Table 43. Observed Effect of Coadministered Drugs on the Pharmacokinetics of Aficamten in Healthy Subjects, Studies CY 6014 and CY 601-10

Coadministered Drug	Dose Regimen of Coadministered Drug	% Change; GMR of Aficamten PK Parameter (90% CI) ^a	
		C _{max}	AUC _∞
Strong CYP2D6 Inhibitor: Paroxetine (N = 17)	20 mg QD × 7 days + 40 mg QD × 18 days	↑ 20%; 1.20 (1.03, 1.41)	↑ 27%; 1.27 (1.19, 1.35)
Strong CYP3A Inhibitor: Itraconazole (N = 16) ^b	200 mg QD × 12 days	↔; 0.93 (0.79, 1.08)	↑ 26%; 1.26 (1.19, 1.34)
Strong CYP2C19, Moderate CYP2C9, and Moderate CYP3A Inhibitor: Fluconazole (N = 16)	400 mg QD × 18 days	↔; 0.99 (0.87, 1.13)	↑ 278%; 3.78 (3.47, 4.11)
Strong CYP2C19 and Strong CYP2D6 Inhibitor: Fluoxetine (N = 16)	20 mg QD × 7 days + 40 mg QD × 20 days	↑ 55%; 1.55 (1.28, 1.88)	↑ 32%; 1.32 (1.25, 1.40)
Moderate-to-Strong CYP3A and Weak CYP2C9 Inducer: Carbamazepine (N = 15 to 17)	100 mg BID × 3 days + 200 mg BID × 4 days + 300 mg BID × 20 days	↓ 31%; 0.69 (0.50, 0.95)	↓ 51%; 0.49 (0.36, 0.66)

↔ = no change; ↑ = increase; ↓ = decrease; AUC_∞ = area under the plasma concentration-time curve from time 0 extrapolated to infinity; BID = twice daily; CI = confidence interval; C_{max} = maximum observed plasma concentration; CYP = cytochrome P450; GMR = geometric least-squares mean ratio; N = number with estimable parameter; PK = pharmacokinetics; QD = once daily.

^a Ratios for C_{max} and AUC compare coadministered drug + aficamten/aficamten alone.

^b Itraconazole was administered as the capsule formulation under fed conditions.

Source: Table 43, Module 2.7.2

Physiologically Based PK Modeling

To further understand the effects of CYP2C9, CYP2C19, CYP2D6, and CYP3A inhibition and CYP2C9, CYP2C19, and CYP3A induction on aficamten pharmacokinetics, a physiologically

based PK (PBPK) model was developed by the Applicant to supplement the clinical DDI studies and provide a comprehensive evaluation of DDI effects on aficamten's pharmacokinetics (see Section [14.5.3](#)).

In their PBPK report, CYT/11/B, the Applicant stated that their primary model f_m values for CYP2C9 and CYP3A4 can be considered verified, but there is still some uncertainty for the f_m values for CYP2C19 and CYP2D6. The over-prediction of aficamten pharmacokinetics in CYP2D6 poor metabolizers (PMs) and the underprediction of the fluconazole DDI may both indicate a potential greater role of CYP2C19 (i.e., estimated maximum f_m =24%) and lesser role for CYP2D6 (i.e., f_m =0%). A sensitivity analysis performed using an exploratory model with CYP2D6 mediated metabolism removed and replaced by CYP2C19 mediated metabolism was also presented in their PBPK report (CYT/11/B). As expected, this model more accurately describes the pharmacokinetics in CYP2D6 PMs (i.e., no change from non-PMs) and the fluconazole DDI (i.e., predicted within 1.1-fold of the observed data) but no longer captures the effect of paroxetine DDI. As such, the Applicant relied on a model (i.e., primary model) that includes a significant contribution by CYP2D6 to align with in vitro results and accurately capture the results of the dedicated DDI study with paroxetine for prospective DDI prediction.

While evaluating the Applicant's PBPK modeling, the Clinical Pharmacology PBPK reviewer further modified the Applicant's exploratory model by assigning $f_{mCYP2D6}$ to 3% and $f_{mCYP2C19}$ to 21% and making further necessary adjustments (see Section [14.5.3](#)). As a result, the reviewer's model successfully predicted all changes in aficamten AUCs observed in clinical studies including paroxetine. The mean AUC changes predicted by the aforementioned three different PBPK models are summarized in [Table 44](#). In addition, different phenotypes of CYP2C9 (normal metabolizers [NMs] and PMs) were considered to account for the worst-case scenario for aficamten exposure increase. For example, we compared aficamten AUC in CYP2C9 PMs in the presence of an inhibitor to aficamten AUC in CYP2C9 NMs in the absence of the inhibitor. The combined inhibition effect (i.e., aficamten AUC increase in PMs in the presence of an inhibitor) will be a product of multiplying the baseline difference between PMs and NMs with the exposure increase resulting from the coadministration with an inhibitor. For CYP2C9, the effect of sulfaphenazole (i.e., strong CYP2C9 inhibitor) on aficamten AUC in CYP2C9 NMs was considered as the baseline difference in AUC between CYP2C9 NMs and PMs (i.e., 2.13-fold). For example, aficamten AUC in CYP2C9 PMs following coadministration with fluvoxamine 150-mg BID for 15 days is predicted to be approximately 4.9-fold higher compared to CYP2C9 NMs not taking fluvoxamine when using the reviewer's model in [Table 44](#) (i.e., 2.13 [NM versus PM baseline difference]×2.28 [aficamten AUC increase with fluvoxamine]).

Table 44. Aficamten Mean AUC Increase Predictions Based on Different PBPK Models

CYP precipitants	Precipitant dosing regimen	Observed	AUC ratio (PBPK model)			CYP2C9/ CYP2C19 phenotype
			Primary	Exploratory	Reviewer	
Strong CYP2C9 inhibitor	Sulfaphenazole 2000 mg QD 15d	-	2.13			CYP2C9 NM
				2.58	2.26	CYP2C9 NM/ CYP2C19 PM
Strong CYP2C19, weak CYP3A, 2C9 and 2D6 inhibitor	Fluvoxamine 100 mg QD 15d 150 mg BID 15d 100 mg QD 15d 150 mg BID 15d	-	1.47	1.72		CYP2C9 NM
			2.16	2.51		
			1.39	1.72	1.66	
			1.91	2.35	2.28	CYP2C9 PM
Strong CYP2C19, moderate CYP3A & CYP2C9 inhibitor	Fluconazole 400 mg QD 18d	3.78	2.49	3.96	3.63	CYP2C9 NM
			-	4.10	3.58	CYP2C9 PM
Strong CYP3A, moderate CYP2C19 & weak CYP2C9 inhibitor	Voriconazole 400 mg BID D1 & 200 mg BID D2-D15	-	1.94	2.66	2.95	CYP2C9 NM
			2.24	3.88	3.54	CYP2C9 PM

Primary model: $F_{m,CYP2D6}$ 21%, $F_{m,CYP2C19}$ 3%
Exploratory model: $F_{m,CYP2D6}$ 0%, $F_{m,CYP2C19}$ 24%
Reviewer's model: $F_{m,CYP2D6}$ 3%, $F_{m,CYP2C19}$ 21%

Source: Reviewer's analysis
Abbreviations: AUC, area under the concentration-time-curve; BID, twice daily; CYP, cytochrome P450; PBPK, physiologically based pharmacokinetic; QD, once daily

~3.5
~4.9
(2.28 x 2.13)
~7.5
~7.6

Dosing Recommendations Based on DDI Potential Affecting Aficamten Pharmacokinetics

This section focuses on dosing recommendations for aficamten when dose adjustment is needed based on DDI potential with other drugs given concomitantly. While drug interaction potential may exist for other drugs when given with aficamten, aficamten dose adjustments are not warranted unless discussed in this section.

[Table 45](#) summarizes predictions of aficamten mean AUC fold increase when coadministered with various CYP enzyme inhibitors together with the Applicant's proposed dose recommendations and the Clinical Pharmacology review team's dose recommendations.

Table 45. Aficamten Mean AUC Increase Predictions Based on Different PBPK Models and the Clinical Pharmacology Review Team's Dose Recommendations

CYP precipitants	Precipitant dosing regimen	Observed	AUC ratio (PBPK model)			CYP2C9/ CYP2C19 phenotype	Applicant's Proposed Dose	Review Team's Proposed Dose
			Primary	Exploratory	Reviewer			
Strong CYP2C9 inhibitor	Sulfaphenazole 2000 mg QD 15d	-	2.13			CYP2C9 NM	Adding inhibitors on aficamten Reduce dose (20→10 mg; 15→5 mg; 10→5 mg) or stay at 5 mg + monitor LVEF/LVOT-G	Agree with the Applicant
				2.58	2.26	CYP2C9 NM/ CYP2C19 PM		
Strong CYP2C19, weak CYP3A, 2C9 and 2D6 inhibitor	Fluvoxamine 100 mg QD 15d 150 mg BID 15d 100 mg QD 15d 150 mg BID 15d	-	1.47	1.72		CYP2C9 NM	Adding aficamten on inhibitors Begin at 5 mg + monitor LVEF/LVOT-G	Adding inhibitors to aficamten: • Aficamten dose > 10 mg: reduce dose to 5 mg • Aficamten dose ≤ 10 mg: avoid concomitant use Adding aficamten to inhibitors: Begin at 5 mg + monitor LVEF/LVOT-G
			2.16	2.51				
			1.39	1.72	1.66 (3.5)*			
			1.91	2.35	2.28 (4.9)*	CYP2C9 PM		
Strong CYP3A, moderate CYP2C19 & weak CYP2C9 inhibitor	Voriconazole 400 mg BID D1 & 200 mg BID D2-D15	-	1.94	2.66	2.95	CYP2C9 NM	Contraindicate if used for > 3 days	
			2.24	3.88	3.54 (7.5)*	CYP2C9 PM		
Strong CYP2C19, moderate CYP3A & CYP2C9 inhibitor	Fluconazole 400 mg QD 18d	3.78	2.49	3.96	3.63	CYP2C9 NM		
			-	4.10	3.58 (7.6)*	CYP2C9 PM		

Primary model: $F_{m,CYP2D6}$ 21%, $F_{m,CYP2C19}$ 3%
Exploratory model: $F_{m,CYP2D6}$ 0%, $F_{m,CYP2C19}$ 24%
Reviewer's model: $F_{m,CYP2D6}$ 3%, $F_{m,CYP2C19}$ 21%
* Combined effect calculation: Total aficamten AUC increase in PMs with inhibitors = predicted inhibitor effect × baseline difference between PMs and NMs (2.13-fold) → Number in the parenthesis represents the predicted combined effect (i.e., worst case scenario)

Source: FDA reviewer's analysis

Abbreviations: AUC, area under the concentration-time-curve; BID, twice daily; CYP, cytochrome P450; LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; PBPK, physiologically based pharmacokinetic; QD, once daily

The Applicant proposed to contraindicate aficamten administration with more than 3 days of fluconazole administration, regardless of CYP2C9 phenotype. This is based on the observation that fluconazole 400-mg QD increased aficamten exposure by 3.8-fold in CYP2C9 and CYP2C19 extensive, rapid, or ultra-rapid metabolizers (Study CY 601-10). As shown in [Table 45](#) above, the combined effects of multiple CYP enzyme inhibition and CYP2C9 PM status for voriconazole and fluconazole are predicted to result in a 7.5-fold and 7.6-fold higher AUC, respectively. Therefore, when initiating voriconazole or fluconazole in subjects already taking aficamten, the aficamten dose should be reduced to 5 mg (i.e., 3 to 4-fold dose decrease from 15 mg or 20-mg dose) considering approximately 3.5-fold AUC increase expected by initiating these inhibitors. Aficamten is expected to have more than a 3-fold therapeutic window based on the Phase 3 trial (CY 6031) outcome. The proposed 3 to 4-fold aficamten dose reduction is expected to appropriately mitigate any resulting additional aficamten exposure increases due to the initiation of these inhibitors. However, those on 5 mg or 10-mg aficamten should avoid concomitant use of voriconazole or fluconazole as reduction of the aficamten dose may not appropriately mitigate the risk resulting from aficamten exposure increase.

At the same time, the Applicant proposes to allow ≤ 3 days of concomitant fluconazole to enable the common usage of fluconazole in the outpatient setting for the treatment of uncomplicated vulvovaginal candidiasis. In this setting, fluconazole is administered as a single 150-mg dose (Day 1). If the infection does not resolve within 48 hours, a second 150-mg dose (Day 3) can be given. The effect of 2×150-mg fluconazole dose, separated by 48 hours, on stable aficamten therapy (i.e., steady state 15-mg QD) was evaluated via PBPK modeling; and it was predicted that this regimen will only elicit a minimal effect on aficamten exposure (i.e., maximum increase of 10% AUC over the dosing interval). The Clinical Pharmacology review team found the Applicant's proposal to be reasonable.

The aficamten dose should be reduced 2 to 3-fold when initiating fluvoxamine or sulfaphenazole. In the same manner as when initiating inhibitors such as voriconazole or fluconazole, a 2 to 3-fold aficamten dose reduction is expected to appropriately mitigate any resulting additional aficamten exposure increase due to the initiation of these inhibitors in subjects already taking aficamten considering that less than a 2.3-fold mean AUC increase is expected by initiating these inhibitors.

When initiating aficamten in subjects already taking the above-mentioned inhibitors, aficamten should be initiated at 5 mg with LVEF and LVOT-G monitoring and the dose should be increased further if needed based on treatment response. A starting dose of 5 mg is expected to be safe for aficamten despite an approximately 2-fold decrease expected in aficamten clearance due to inhibitor presence as there would not be any additional exposure increase due to the initiation of aficamten therapy as subjects are already on stable therapy with the above-mentioned inhibitors. Any subsequent aficamten dose up-titration while on inhibitor therapy can be safely guided by echocardiographic assessment to maintain LVEF >50%.

To assess the potential effect of CYP inducers on aficamten pharmacokinetics, the Applicant conducted a PBPK analysis showing that 600-mg QD dosing of rifampin (i.e., strong CYP3A inducer, strong CYP2C19 inducer, and moderate CYP2C9 inducer) was predicted to reduce the mean aficamten AUC by 79%. Given the extent of predicted drug interaction with rifampin, the Applicant is proposing to contraindicate the concomitant use of aficamten with rifampin as the reduced aficamten exposure may substantially reduce efficacy.

A clinical DDI study (CY 6014) was conducted to evaluate the effect of steady state carbamazepine (i.e., moderate to strong CYP3A inducer and weak CYP2C9 inducer) exposure on aficamten single dose (i.e., 20 mg) pharmacokinetics. This study showed a 51% decrease in aficamten mean AUC. The Applicant is proposing to reduce the dose of aficamten (i.e., 20 mg → 10 mg, 15 mg → 5 mg, 10 mg → 5 mg, or maintain the dose of 5 mg if currently on 5 mg and monitor LVEF and LVOT-G) when discontinuing a moderate to strong inducer of CYP2C9 or CYP3A.

The Applicant's proposal for both inducer drugs mentioned above is reasonable from a Clinical Pharmacology perspective.

After careful consideration, the Clinical Pharmacology review team has concluded that the listing of CYP2C9 inducers in Section 7 (i.e., Drug Interactions) of the product label is not necessary as there are no strong CYP2C9 inducers currently on the United States market (i.e., hypothetical) and other known moderate CYP2C9 inducers would have overlap with moderate to strong CYP3A inducers.

It should be noted that no significant exposure-safety relationship was identified between aficamten exposures and assessed safety endpoints such as major adverse cardiovascular events, cardiovascular adverse events, or hypertension at the evaluated dose range of 5 to 20-mg QD in the Phase 3 trial (CY 6031) and the aficamten therapeutic window is expected to be >3-fold.

Effect of Aficamten on Other Drugs' Pharmacokinetics

The Applicant concludes that in vitro studies suggest aficamten does not substantially inhibit any of the CYP isoforms tested (i.e., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4; IC₅₀ values >100μM), and it is predicted not to be an in vivo inducer in humans. The in vitro assessment of the inhibition potential for P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic cation transporter 2 (OCT2), organic cation transporter 3 (OCT3), organic anion transporter 1 (OAT1), organic anion transporter 3 (OAT3), organic anion transporting polypeptide 1B1 (OATP1B1), organic anion transporting polypeptide 1B3 (OATP1B3), multidrug and toxin extrusion protein 1 (MATE1), and multidrug and toxin extrusion protein 2-K (MATE2-K) suggested that aficamten may inhibit P-gp in vivo, leading to evaluation in a dedicated clinical DDI study (Study CY 6014). Concomitant administration of aficamten increased total dabigatran (i.e., a sensitive P-gp probe) AUC_∞ and C_{max} by 26% and 27%, respectively (Section [14.2](#)). Based on these results, the review team agrees with the Applicant's conclusion that aficamten may be coadministered with P-gp substrates.

8.3. Plans for Pediatric Drug Development

Aficamten was granted orphan designation for the treatment of HCM on January 5, 2021. Based on the orphan designation for aficamten and in accordance with Section 505B(k)(1) of the Food, Drug, and Cosmetic (FD&C) Act, a formal pediatric study plan for the population of birth to less than 18 years of age is not required. This exemption from the Pediatric Research Equity Act requirements was confirmed at the pre-NDA meeting held on February 26, 2024.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

Refer to the Division of Pediatrics and Maternal Health review dated June 4, 2025, and the summary of key nonclinical data ([Table 46](#)) supporting the labeling sections on fertility, pregnancy and lactation.

A rat embryofetal development (EFD) study demonstrated an increase in external malformations (kinked tail) at aficamten exposures higher than clinical exposures at the MRHD. This finding is of uncertain clinical relevance.

In a rabbit EFD study, a numerical imbalance in cardiac findings was observed between the mid-dose and vehicle control groups (two litters versus one, respectively). The findings consisted of increased cardiac malformations (persistent truncus arteriosus, misshapen heart, and absent ventricular septum) that were slightly above the historical control incidence. The findings were not observed with a clear dose-response (even when accounting for the loss of some litters at the highest dose). Furthermore, all three malformations also occurred in control litters, which indicates the findings are not rare (i.e., they occurred at 5 to 6% in the historical control database, a level exceeding a threshold of >1 to 2%). Furthermore, imbalances in cardiac malformations were not observed in fetuses in the rat EFD study. Taken together, while a direct relationship between aficamten and cardiac malformations cannot be entirely excluded (due primarily to aficamten's mechanism of action), the available data are inadequate to establish a causal relationship. Given the signal cannot be distinguished from a chance finding, we recommend that residual concern be mitigated through disclosure of the findings in product labeling.

Animal fertility studies do not demonstrate any adverse effect at clinical exposures, therefore, aficamten is not predicted to impair human fertility.

Given the limited human data on aficamten use in pregnancy and the absence of data regarding aficamten presence in human breast milk, the Division of Pediatrics and Maternal Health recommends issuing two post marketing requirements to conduct studies that collect pregnancy outcome data in humans and determine aficamten concentrations in human breast milk (see Section [24](#)).

Table 46. Nonclinical Data Supporting Labeling on Fertility, Pregnancy, and Lactation

Labeling Section	Nonclinical Data
Pregnancy	Administration of aficamten during organogenesis in rats was associated with increased early and late fetal resorptions, decreased mean fetal body weight, and an increased incidence of fetuses with kinked tails at 9 mg/kg/day, approximately 5-times the clinical exposure at MRHD based on free AUC. Maternal toxicity characterized by decreased body weight gain during gestation, decreased food consumption, and increased heart weight, was observed at the same dose. Administration of aficamten during organogenesis in rabbits was associated with a numerical increase in postimplantation loss (late resorptions) and fetuses with various cardiac malformations at the mid-dose (10 mg/kg) only. The high dose (20/15 mg/kg/day) caused maternal toxicity and mortalities at a clinically relevant exposure level. Administration of aficamten to pregnant rats during embryofetal organogenesis through offspring weaning in a pre- and postnatal development study caused reduced offspring viability and reduced pup birth weight at the highest dose (6 mg/kg/day) in the presence of maternal toxicities (decreased maternal body weight gain and increased maternal heart weight), at about 4-times the clinical exposure at the MRHD. An increased number of offspring with bent tails was also observed at the same dose. No adverse maternal toxicity or developmental effects occurred at the mid-dose at clinical exposure level. Aficamten did not affect neurobehavioral parameters, sexual maturation, or reproductive function of the offspring at any dose.
8.2 Lactation	In a rat PPND study, aficamten was detected in nursing offspring when administered to lactating mothers from gestation through postnatal day 4 at ≥ 0.5 mg/kg/day.
13.1 Impairment of Fertility	Aficamten exposure did not impact fertility, mating frequency or index, estrus, sperm quality, or histopathology of reproductive organs in both male and female in rats, at up to 6 mg/kg/day in females and 3 mg/kg/day in males.

Source: PharmTox reviewer table
A more detailed review of study outcomes can be found in Section [13](#).
Abbreviations: AUC, area under the concentration-time curve; MRHD, maximum recommended human dose; PPND, pre- and postnatal development

9. Product Quality

Approval

The Office of Pharmaceutical Quality review team has assessed the application with respect to chemistry, manufacturing, and controls and has determined that it meets all applicable standards to support the identity, strength, quality, and purity of each presentation of the drug product. As such the Office of Pharmaceutical Quality recommends approval of this NDA from a quality perspective. Based on the available long-term and accelerated stability data, a shelf life of 30 months is granted for the drug product when stored at 20°C to 25°C. For more information, refer to the Integrated Quality Assessment in DARRTS dated October 16, 2025.

9.1. Device or Combination Product Considerations

Not applicable.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

Clinical site inspection was conducted for the Phase 3 trial CY 6031, and the trial appears to have been conducted in compliance with Good Clinical Practice and United States regulations (Section [22](#)).

The Applicant has adequately disclosed financial arrangements with clinical investigators. Review of the financial disclosures did not raise any concerns about the validity or reliability of the data (Section [25](#)).

11. Advisory Committee Summary

Not applicable. We determined that an advisory committee meeting was not needed for aficamten, a second-in-class cardiac myosin inhibitor for oHCM, as this application did not raise efficacy, safety or other issues requiring input from external experts.

III. Additional Analyses and Information

12. Summary of Regulatory History

The IND opening study, CY 6011, entitled “A Phase 1, Double-blind, Randomized, Placebo-controlled, Multi-part, Single and Multiple-Ascending Dose Study of CK-3773274 in Healthy Adult Subjects” was submitted on October 3, 2018, and a study may proceed letter was issued on November 2, 2018.

Aficamten received Orphan Drug Designation on January 5, 2021, for the treatment of symptomatic hypertrophic cardiomyopathy. Aficamten also was granted breakthrough therapy designation on December 6, 2021.

Trial CY 6031 (SEQUOIA-HCM), entitled "A Phase 3, Multi-Center, Randomized, Double-blind, Placebo-Controlled Trial to Evaluate the Efficacy and Safety of CK-3773274 in Adults with Symptomatic oHCM" was conducted globally, including in the United States. The enrollment of Trial CY 6031 was completed on May 26, 2023. While there were no interim data locks, the protocol specified that the Applicant would monitor the aggregate pooled missing data and overall pooled standard deviation for the change in baseline in pVO₂ at Week 24 (the primary endpoint). The Applicant did not lock the database to perform this monitoring, nor did they adjust the sample size. The results of CY 6031 (SEQUOIA-HCM) and the additional safety data from the open-label extension Study CY 6022 (FOREST-HCM), to support the NDA submission for aficamten in patients with symptomatic oHCM were discussed with the division at the topline meeting on February 8, 2024 (minutes dated February 29, 2024), and the pre-NDA meeting held on February 26, 2024 (minutes dated March 26, 2024). The review team concluded the information outlined in the pre-NDA meeting package appeared sufficient to conduct a filing review of the NDA and accepted a rolling review with the clinical and nonclinical modules ahead of the full NDA submission.

On September 26, 2024, Cytokinetics Inc. submitted the final portion of the Rolling submission of their NDA for aficamten for the following proposed indication: “treatment of adults with symptomatic obstructive hypertrophic cardiomyopathy (oHCM)”.

Please see the key interactions with the Agency over the course of development, provided in [Table 47](#).

Table 47. Summary of Key Regulatory History, SEQUOIA-HCM

Topics	Key Regulatory History
Milestone meetings and important regulatory dates	• August 12, 2021: End-of-Phase 2 meeting • February 8, 2024: Topline meeting • February 26, 2024: Pre-NDA meeting • July 9, 2024: Rolling review granted • December 6, 2021: Breakthrough Designation granted • January 5, 2021: Orphan drug status granted • May 26, 2023: The SEQUOIA-HCM trial completed enrollment • December 21, 2023: Unblinding for evaluating the primary endpoint at the END of the trial (i.e., this was NOT an interim analysis). • September 26, 2024: The Applicant submitted the last portion of the rolling submission under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act

Source: Reviewer-generated table based on Applicant’s data
Abbreviations: NDA, new drug application

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

Aficamten is a small molecule inhibitor of cardiac myosin (Myh7) designed to decrease cardiomyocyte contractility and relax the cardiac muscle for the treatment of oHCM. Results from in vitro and in vivo primary pharmacology studies support the proposed mechanism of action, the results of which are summarized in [Table 48](#). The selectivity and dose-response profiles of aficamten and its metabolites were investigated using purified myofibrils, cultured cardiac cells, in engineered cardiac tissue, and in both healthy animals and animal models of oHCM.

Table 48. Primary Pharmacology Studies

Study	Findings
In vitro PD studies of aficamten and its major metabolites	Aficamten inhibited ATPase activity in bovine cardiac (IC ₅₀ =1.26µM) and slow muscle myofibrils (IC ₅₀ =1.23µM), but its major metabolites did not ¹ (Nonclin-0019, Nonclin-0460). Aficamten was 5-fold less active against rabbit fast muscle myofibrils (IC ₅₀ =6.5µM) and inactive ¹ against chicken smooth muscle (Nonclin-0019). Rat ventricular cardiomyocyte contraction was inhibited when cultured with aficamten (Nonclin-0019). Aficamten reduced the contractility of engineered heart tissues composed of induced human pluripotent stem cell-derived cardiomyocytes from wild-type (IC ₅₀ =1µM) and MYH7 R403Q heterozygous donors (IC ₅₀ =1.65µM) (Nonclin-0456).

Study	Findings
<i>In vivo</i> PD studies of aficamten in healthy animals	Rat: - A single oral dose to healthy rats (0.5, 1, 2, 4 mg/kg), resulted in a dose-dependent reduction in LVFS (ranging from 20% to 70% reduction from baseline, at the mean plasma concentration of approximately 1.1 to 13µM) [IC₅₀=8µM] which reversed 24 hours after dosing (Nonclin-0037). - Daily oral doses to healthy rats for 10 days (1, 3, 6 mg/kg/day) resulted in dose-dependent reduction in LVFS (ranging from 13 to 25% reduction in the low dose group to 75 to 79% reduction in the high dose group, relative to the baseline, at the mean plasma C_{max} of approximately 2.5µM to 16.1µM). There was no significant difference in LVFS change between Day 4 and Day 10, and the LVFS of all dose groups returned to baseline 24 hours after the last dose (Nonclin-0038). Dog: - A single oral dose of aficamten (0.75, 2, 3 mg/kg) reduced LVFS dose-dependently, from a 15% reduction relative to baseline at 0.75 mg/kg (0.3µM) to a 50% reduction at 3 mg/kg (IC₅₀=1.27µM), which partially recovered (to ~85% of pre-dosing value) by 48 hours (Nonclin-0007).
Study to assess the effect of IV administration of dobutamine on cardiac contractility in healthy rats administered an oral single dose of aficamten	Intravenous infusion of dobutamine dose-dependently (3.3-10 µg/kg/min) reversed the LVFS reduction induced by a single 2 mg/kg dose of aficamten after 5 minutes in rats (Nonclin-0049).
<i>In vivo</i> PD studies of aficamten in HCM models.	Mouse model: - Aficamten dose-dependently reduced LVFS in wild type and a MYH7 R403Q transgenic mouse model of hypertrophic cardiomyopathy, after a single dose (0.25, 0.5, 1, 1.25 and 1.5 mg/kg). The interpolated plasma concentration at 10% reduction of LVFS relative to baseline (IC₁₀) for WT and R403Q mice was 0.12µM and 0.11µM, respectively. The 50% reduction in LVFS (IC₅₀) for WT and R403Q mice was 0.90µM and 0.78µM, respectively. (Nonclin-0057). Cat model: - Aficamten dose-dependently reduced LVFS and improved left ventricular outflow tract pressure gradient by up to >90% at a dose of 2 mg/kg in a cat genetic model of oHCM (Nonclin-0141).

Source: Pharm/Tox reviewer's table

¹IC₅₀ values were >39µM, the highest dose used in each study.

Abbreviations: C_{max}, maximum plasma concentration; IC₁₀, inhibitory concentration 10%; IC₅₀, half-maximal inhibitory concentration; HCM, hypertrophic cardiomyopathy; IV, intravenous; LVFS, left ventricular fractional shortening; oHCM, obstructive hypertrophic cardiomyopathy; PD, pharmacodynamic; WT, wild-type

Secondary pharmacology studies that investigated the off-target potential of aficamten are summarized in [Table 49](#) below. All off-target interactions occurred only at very high concentrations and are unlikely to be clinically relevant.

Table 49. Secondary Pharmacology Studies

Study	Findings
Off target receptor panel screen	At 100µM (33,700 ng/mL, >100× clinical C _{max}), aficamten decreased ligand binding to cannabinoid CB1 receptor, Dopamine D4.2 receptor, and Serotonin 5HT-2b receptors by 52, 52, and 79% respectively (Nonclin-0040).
Off target cell-based assays	In a follow-up study (Nonclin-0055), aficamten inhibited (IC ₅₀ =82µM) serotonin-induced proliferation of CHO-K1 cells overexpressing the 5HT-2b receptor, indicating low risk of antagonizing the 5HT-2b receptor. No significant inhibition (IC ₅₀ >100µM) of CB1 or D4.2 was detected in cell-based assays in the same study.

Source: Pharm/tox reviewer's table

Abbreviations: CHO-K1, Chinese hamster ovary cell line; C_{max}, maximum plasma concentration; IC₅₀, half-maximal inhibitory concentration

13.1.1. Safety Pharmacology

A complete battery of safety pharmacology studies was conducted to characterize any effects of aficamten and its metabolites on the human ether-à-go-go related gene (hERG) channel, cardiovascular, respiratory, and central nervous system. Effects observed on electrocardiogram readings after repeated aficamten dosing are typical of cardiac compensation due to the intended pharmacologic action of aficamten. Aficamten and its metabolites did not have inhibitory effects on the hERG channel at clinically relevant concentrations. The safety pharmacology studies are summarized in [Table 50](#) below.

Table 50. Safety Pharmacology Studies

Study	Findings
In vitro hERG channel inhibition assay for aficamten and its major metabolites	The in vitro IC ₅₀ for aficamten against hERG was not determined due to insolubility of the drug above 10µM (3,400 ng/mL). At 3µM and 10µM aficamten, hERG was inhibited by 3% and 3.7% respectively (Nonclin-0003). IC ₅₀ values for the major metabolites of aficamten were estimated to be >30M (10,600 ng/mL) for M1a and 51µM (18,068 ng/mL) for M1b. At 10µM, M1b inhibited hERG by 18% (Nonclin-0292, Nonclin-0298).
Repeat-dose telemetry study to assess the effect of aficamten on cardiovascular function	Daily administration of aficamten at doses up to 2 mg/kg for 1-week in telemetered dogs resulted in transient cardiovascular effects (Nonclin-0013). These included: • Increased heart rate (up to 44% increase) • Decreased systolic blood pressure (up to 30% decrease) • Decreased dP/dt (up to 25% decrease) • Increased left ventricular end-diastolic pressure (up to 37% increase) These effects were expected and reflect the pharmacological action of aficamten or cardiac compensation in response to the drug's pharmacologic effects. All observed changes resolved within 6 hours after dosing.
Single-dose study to assess the effect of aficamten on respiratory system	Aficamten caused an increase in respiratory rate accompanied by a slight decrease in tidal volume lasting at least 4 hours in rats at 9 mg/kg, which reversed by 24 hours after dosing (Nonclin-0012).

Study	Findings
Single-dose study to assess the effect of aficamten on central nervous system function	Decreased body temperature and decreased alertness were observed, which reversed by 24 hours after dosing at 9 mg/kg in male rats (Nonclin-0011).

Source: Pharm/tox reviewer's table

Abbreviations: dP/dt; rate of change of pressure over time; hERG, human ether-à-go-go related gene; IC₅₀, half-maximal inhibitory concentration

13.1.2. Pharmacokinetics

The pharmacokinetics and absorption, distribution, metabolism, and excretion (ADME) of aficamten were characterized in in vitro assays and in vivo studies using pharmacologically relevant species that included the dog, rat, rabbit, mouse, and monkey. Toxicokinetics were assessed in toxicology studies in rats, dogs, rabbits, and mice. Overall, these data confirm the clinical relevance of these nonclinical models for human risk assessment for aficamten.

13.1.2.1. Absorption and Distribution

Absorption of aficamten after oral dosing was comparable between rats, dogs, and primates. Absolute oral bioavailability was ~60% for rats, ~40% for dogs and non-human primates, and was not determined for humans. Time to maximum concentration (T_{max}) in humans after dosing with the clinical formulation was 1.5 to 2 hours, which was slightly longer than T_{max} in rats and dogs dosed by oral gavage (~0.5 to 1 hour). Steady-state volume of distribution between species varied widely, from 0.5 L/kg in rats to 11 L/kg in dogs and 8 L/kg in non-human primates. No significant differences in aficamten exposure between males and females for any species were observed.

Plasma protein binding for aficamten and its major metabolites (M1a and M1b) were determined for several species in vitro. Aficamten plasma protein binding was 98%, 90%, and 75% in rats, rabbits, and dogs, respectively. M1a and M1b protein binding was ~86% and ~89% in rats, and ~45% and 43% in dogs, respectively. Human plasma protein binding in vitro for aficamten, M1a, and M1b were ~90%, ~83%, and ~93% respectively. To account for the differences in plasma protein binding between species, exposure margins for aficamten were calculated based on unbound (free) C_{max} or AUC in addition to total C_{max} and total AUC.

After a single oral dose in fasted rats, aficamten was detected in the heart, plasma, liver, and gastrocnemius muscle, with very low levels detected in the cerebrospinal fluid and brain. The tissue with highest exposure to aficamten was the heart, the site of aficamten's pharmacological action, with a tissue-to-plasma ratio of ~1.4, followed by the gastrocnemius with a ratio of 0.7. Notably, the heart was the primary organ of toxicity in animal toxicology studies.

Placental and milk transfer of aficamten was not directly assessed in embryofetal development study or pre- and postnatal development studies.

13.1.2.2. Metabolism and Excretion

After oral dosing, aficamten is metabolized more quickly in animals as compared to humans. Aficamten's half-life was 25 hours in rats, 79 hours in dogs, and 144 hours in human hepatocytes

in vitro. Half-life measurements across species *in vivo* are ~80 hours, ~28 hours, and ~3 hours, in humans, dogs, and rats, respectively. Drug accumulation was evident in repeat-dose studies in dogs by exposure ratios of ~2 when comparing AUC and ~1.5 when comparing C_{max} on the final day of dosing to the first day. Daily dosing in the clinic resulted in drug accumulation in humans as well, with an exposure ratio of ~5 based on AUC and ~2 based on C_{max} , consistent with longer half-life in humans.

In all species, the major circulating metabolites of aficamten were M1a and M1b (also known as CK-3834282 and CK-3834283, respectively), diastereomers of (ω -1)-hydroxylation of the ethyl group on the ethyl-1,2,4-oxadiazol-3-yl moiety of aficamten, formed by CYP enzymes. In humans, metabolite to parent ratios for M1a and M1b were 74% and 129%, respectively. These two metabolites are also major metabolites of aficamten identified in the rat *in vivo*, but at lower levels. In rats, the metabolite to parent ratios for M1a and M1b were ~9% and 29%, respectively. No unique human metabolites were identified *in vitro* using liver microsomes or *in vivo*, making the rat and dog metabolically relevant species for toxicology studies.

M1a and M1b were inactive in all assays tested. *In vitro* PD assays and 14-day repeat dose toxicology studies in rats with the major metabolites of aficamten demonstrated their relative pharmacologic inactivity. In an *in vitro* study, M1b inhibited bovine cardiac myofibril ATPase activity by 15% at 18 μ M, but no IC_{50} could be determined for M1a or M1b up to maximum concentrations of 39 μ M, demonstrating lower potency by at least 30-fold, compared with aficamten (IC_{50} =1.26 μ M), which is anticipated to be pharmacologically inactive at therapeutic exposures of aficamten. M1a or M1b singly administered to rats for 14 days at 50 mg/kg/day had no toxicologically significant effects.

M1a and M1b were measured in aficamten repeat dose studies in rats and dogs. Exposures to each metabolite relative to clinical levels are summarized in [Table 51](#).

Table 51. Metabolite Exposures in Nonclinical Studies

Nonclinical Studies	NOAEL (mg/kg)	Total M1a Exposure (AUC_{last} h*ng/mL)¹	Total M1b Exposure (AUC_{last} h*ng/mL)¹	M1a Exposure Multiple²	M1b Exposure Multiple²
Rat/13-week	3	2,830 (males)	7,200 (males)	0.6X	0.9X
Rat/embryofetal development	6	5,130	22,000	1.1X	3X
Rabbit/embryofetal development	10	18,300 (at maternal NOAEL)	10,800 (at maternal NOAEL)	4.3X	1.5X
Rat/2-year carcinogenicity	2 (M) /3 (F)	1200 (M)/1920 (F)	4340 (M)/8150(F)	0.3X (M)/0.5X (F)	0.6X (M) /1X (F)
Tg:rasH2 mouse/6-month carcinogenicity	2	969	1600	0.2X	0.2X
Rat/14-day metabolite repeat dose	50 (M1a) / 50 (M1b)	47,739	53,930	11X	7X

Source: Pharm/tox reviewer's table

¹ At aficamten NOAEL.

² After a single 20-mg aficamten dose in healthy volunteers fed state, clinical AUC for M1a and M1b were 4232 and 7316 h*ng/mL, respectively (Study CY 6012).

Abbreviations: AUC, area under the concentration-time curve; AUC_{last}, area under the concentration-time curve to the last quantifiable time-point; F, female; M, male; NOAEL, no-observed-adverse-effect level

Across the nonclinical studies included in [Table 51](#), both M1a and M1b metabolites generally demonstrated exposures exceeding 0.5-fold relative to the clinical exposure at the 20mg dose, a threshold typically warranted to achieve adequate nonclinical assessment for disproportionate metabolites. The exception was M1a exposure in male animals in carcinogenicity studies, where exposures were limited to 0.2-fold in the 6-month transgenic mouse study and 0.3-fold in the 2-year rat study. This lower exposure was attributed to two factors: cardiac toxicities associated with aficamten that limited the high dose selection, and the inherently lower M1a levels present in rodent species relative to humans, after aficamten administration. Given the higher maximum tolerated dose of aficamten in females, M1a exposure in females reached 0.5-fold relative to clinical exposure without tumor findings in the 2-year rat carcinogenicity study. Mechanistically the M1a metabolite is formed through first-pass metabolism by P450-mediated hydroxylation and is structurally very similar to aficamten, which was negative for tumor signals in 6-month and 2-year rodent carcinogenicity studies.

M1a is pharmacologically inactive, and nonclinical rodent studies achieving exposures above 0.5-fold (including the 13-week rat study) showed no proliferative or preneoplastic findings of concern. The 2-year rat study, which reached 0.5-fold exposure, similarly demonstrated no concerning signals. Furthermore, M1a was determined to be non-genotoxic based on a comprehensive battery of assays and weight-of-evidence evaluation. Collectively, these data indicate minimal carcinogenic risk for this metabolite, therefore, we conclude that a dedicated carcinogenicity study is not warranted.

13.1.2.3. Drug-Drug Interactions

Aficamten was not a substrate for OATP1B1/B3, MDR1/BCRP, or P-gp *in vitro*. In a transporter panel inhibition screen, aficamten and its major metabolites had no significant effects on the activity of OAT1, OCT2, OCT3, or OATP1B3. The IC₅₀ for aficamten against OAT3, OATP1B1, MATE1, and MATE2-K were 12, 71, 36, and 83μM, respectively. *In vitro* CYP phenotyping, induction, and inhibition studies using recombinant CYP proteins, human liver microsomes, human hepatocytes, or a human microliver model were performed. Results from the studies collectively suggest that CYPs 2C8, 2C9, 2C19, 2D6, and 3A4 contribute to metabolic clearance of aficamten in humans. Aficamten did not significantly inhibit any CYP enzyme in *in vitro* assays. Based on thresholds described in the FDA guidance for industry *Clinical Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions* ([January 2020](#)),⁵ the *in vivo* risk for inhibition by aficamten is low.

13.1.3. General Toxicology

Repeat oral dose toxicology studies up to 26 weeks in rats and 39 weeks in dogs were conducted to support the chronic oral administration of aficamten intended for clinical use. Both species are considered pharmacologically relevant given the high conservation of the cardiac myosin motor between species and based on *in vitro* and single-dose *in vivo* pharmacology studies. Exposure

⁵ Guidances are updated periodically. For the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fdaguidance-documents>.

multiples were estimated based on systemic exposure to total and unbound (free) aficamten (AUC and C_{max}) at the maximum recommended human clinical dose of 20 mg.

Repeat dosing at the clinical exposure based on free AUC (5-times greater than clinical exposure based on total AUC) for up to 6-months in rats was well tolerated. In shorter 28-day and 13-week rat studies, dosing at 2-fold greater than clinical free AUC level (8-times greater than clinical total AUC) was tolerated, while dilated cardiomyopathy and premature deaths were associated with dosing at 4-fold greater than clinical free AUC (14-times greater than clinical total AUC).

Similarly, in the 39-week dog study dosing to the clinical free AUC level caused no toxicities (0.25-times the clinical total AUC). However, a dose level 2.5-fold greater than clinical free AUC in dogs (0.6-times the clinical total AUC) had reversible increased heart weight. In a shorter 13-week dog study, dosing at 4-fold clinical free AUC (at clinical total AUC) was associated with cardiomyocyte necrosis and premature death. Taken together, the repeat-dose toxicology studies demonstrate dose and duration-dependent cardiac toxicities in healthy animals given daily aficamten.

FDA Assessment

The heart was identified as the primary organ of toxicity in both species with repeated aficamten administrations. Cardiac toxicities culminating in heart failure resulting from exaggerated pharmacology of the drug were observed in both rats and dogs in a dose- and duration-dependent fashion. Drug exposures causing cardiac toxicity for both species were >2-fold and >3-fold the maximum clinical exposures based on free AUC and free C_{max} , respectively. Cardiac toxicities in dogs were evident at clinical exposure based on total AUC and 2-times clinical exposure based on total C_{max} . In rats, cardiac toxicity occurred at 14-times clinical exposure based on total AUC and 32-times clinical exposure based on total C_{max} .

No adverse effects for either species were observed with chronic dosing at clinical exposure based on free AUC and up to 3-fold based on free C_{max} . No adverse findings occurred in dogs at exposures less than clinical total AUC and total C_{max} . For rats, no adverse findings occurred when dosing up to 5-times total AUC and 9-times total C_{max} . Hypercontractility is one of the pathological hallmarks of hypertrophic cardiomyopathy, and the indicated patient population would likely tolerate a reduction in cardiac contractility to a greater extent than animals or humans with 'normal' cardiac contractility. As such, while the nonclinical toxicity studies clearly demonstrate that aficamten is capable of inhibiting myosin/actin interactions with a narrow safety margin and, in excess, can precipitate heart failure, a broader therapeutic window may be present in the intended patient population.

13.1.3.1. Pivotal General Toxicology Studies

Study Number/Title: Nonclin-0054/A 26-Week Study of CK-3773274 by Oral Gavage in Rats With a 2-Month Recovery Period

Key Study Findings

- No adverse aficamten-related findings in this study up to the highest dose of 1.5 mg/kg/day.
- Total AUCs and total C_{max} at the no-observed-adverse-effect level (NOAEL) dose were 45,000 ng·h/mL for males and 24,900 ng·h/mL for females, and 2,780 ng/mL for males and 3,340 ng/mL for females respectively. Sex-averaged free aficamten exposure at the NOAEL dose was 559 ng·h/mL for AUC and 49 ng/mL for C_{max} .

Table 52. Study Information: Nonclin-0054 Study

Study Features and Methods	Description
GLP compliance	Yes
Doses and frequency of dosing	0, 0.25, 0.5 or 1.5 mg/kg/day
Route of administration	Oral gavage
Formulation/vehicle	0.5% (w/v) hydroxypropyl methylcellulose and 0.1% (v/v) Tween-80 in ultra-pure water.
Species/strain	Sprague-Dawley rat
Number/sex/group	15 of each sex for the vehicle control, and for each treatment group. 5 of each sex in vehicle control, and 5 of each sex in mid and high dose groups were designated for the recovery phase.
Age	9 weeks old at start of dosing
Satellite groups	3 of each sex for vehicle controls and 9 of each sex for each treatment group used for toxicokinetic analysis.

Source: Nonclin-0054 Study
Abbreviations: GLP, good laboratory practice

Table 53. Observations and Results: Nonclin-0054 Study

Endpoints	Major Findings
Endpoints with notable observations	N/A
Endpoints without notable observations	No drug-related adverse effects on mortality, clinical signs, body weight, gross and microscopic pathology, hematology, ophthalmic exam, or clinical chemistry.

Source: Nonclin-0054 Study
Abbreviations: N/A, not applicable

Table 54. Toxicokinetics: Nonclin-0054 Study

Dose (mg/kg/day)	AUC _{0-24h} ¹ (ng·h/mL)		C _{max} ¹ (ng/mL)	
	Male Total (Free)	Female Total (Free)	Male Total (Free)	Female Total (Free)
0.25	6,550 (105)	5,150 (82)	465 (7)	535 (9)
0.5	9,990 (160)	10,800 (173)	1,050 (17)	1,040 (17)
1.5 (NOAEL)	45,000 (720)	24,900 (398)	2,780 (45)	3,340 (53)

Source: Nonclin-0054 Study

¹ On study Day 182.

Abbreviations: AUC_{0-24h}, area under the curve from time 0 to 24 hours; C_{max}, maximum plasma concentration; NOAEL, no-observed-adverse-effect level

In addition to the 26-week good laboratory practice (GLP) toxicology study, sub-chronic GLP repeat dose studies including a 4-week and a 13-week study were conducted in rats. Cardiac toxicities and premature lethality were observed in these studies at doses higher than 1.5 mg/kg/day.

Study Number/Title: Nonclin-0056/CK-3773274: A 39-Week Oral Gavage Toxicity Study in the Beagle Dog Followed by a 2-Month Recovery Period

Key Study Findings

- Reversible increase in heart weight (~10% increase relative to controls) without microscopic correlates at 1 mg/kg/day, the highest dose. Dose-dependent increase in heart rate across all treated groups, and reversible increase in QTc interval at 1 mg/kg/day.
- The NOAEL for the study was 0.5 mg/kg/day, with total AUCs of 5,040 and 4,040 ng·h/mL for males and females, respectively. Total C_{max} at the NOAEL dose was 537 and 412 ng/mL for males and females, respectively. Sex-averaged exposure to free aficamten at the NOAEL dose was 1,130 ng·h/mL for AUC and 118 ng/mL for C_{max}.

Table 55. Study Information: Nonclin-0056 Study

Study Features and Methods	Description
GLP compliance	Yes
Doses and frequency of dosing	0, 0.25, 0.5 or 1.0 mg/kg/day
Route of administration	Oral gavage
Formulation/vehicle	0.5% [w/v] hydroxypropyl methylcellulose and 0.1% [v/v] Tween-80 in Ultra-Pure water
Species/strain	Beagle dog
Number/sex/group	Four of each sex for each dose group for interim sacrifice (26 weeks), four of each sex for each dose for terminal sacrifice. Two of each sex for vehicle control, mid and high dose groups for recovery.
Age	7 to 8 months old

Source: Nonclin-0056 Study

Abbreviations: GLP, good laboratory practice

Table 56. Observations and Results: Nonclin-0056/CK-3773274 Study

Endpoints	Major Findings
Endpoints with notable observations	
Organ weight	Increased mean heart weight relative to body weight in treated male dogs was statistically significant at 0.5 and 1.0 mg/kg/day at interim, but not terminal, sacrifice. Mean increased heart weights for female dogs at the same dose levels did not reach statistical significance.
ECG	Reversible increased heart rate, lengthened QTc interval, and shortened RR interval were associated with aficamten for both sexes. The only change that reached statistical significance was lengthened QTc interval in males given 1 mg/kg/day.
Clinical chemistry	At 1 mg/kg/day, mean plasma fibrinogen was decreased in males at both interim and terminal testing days (to ~0.7× relative to pre-dosing value for both timepoints). The mean fibrinogen level was only partially reversed after the recovery period.
Endpoints without notable observations	Histopathology, ophthalmology, body weight, hematology, urinalysis, coagulation, and no unscheduled mortalities

Source: Nonclin-0056 Study

Abbreviations: ECG, electrocardiogram; RR, time duration between successive heartbeats, QTc, the corrected duration of the QT interval

Table 57. Toxicokinetics: Nonclin-0056 Study

Dose (mg/kg/day)	AUC _{last} ¹ (ng·h/mL)		C _{max} ¹ (ng/mL)	
	Male Total (Free)	Female Total (Free)	Male Total (Free)	Female Total (Free)
0.25	870 (217)	540 (134)	82 (20)	57 (14)
0.5 (NOAEL)	2,060 (513)	1,700 (423)	204 (51)	177 (44)
1.0	5,040 (1,255)	4,040 (1,006)	537 (134)	412 (103)

Source: Nonclin-0056 Study

¹ On study Day 273.

Abbreviations: AUC_{last}, area under the curve from time 0 to 24 hours; C_{max}, maximum plasma concentration; NOAEL, no-observed-adverse-effect level

In addition to the GLP 39-week toxicology study in dogs, GLP repeat dose studies of 4-weeks and 13-weeks were conducted in dogs. Cardiac toxicities including lethality were observed with increasing dose at exposure levels >3-times the maximum clinical exposure level based on free AUC (at clinical exposure based on total AUC).

13.1.4. Genetic Toxicology

A GLP-compliant Ames assay and a GLP combined *in vivo* mammalian erythrocyte micronucleus test and alkaline comet assay in rats were conducted in accordance with the guidelines outlined in the International Council for Harmonisation (ICH) guidance for industry, *S1(R1) S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals* ([November 2022b](#)). Aficamten was negative for genotoxicity and clastogenicity in both systems. GLP-compliant Ames assays and *in vitro* micronucleus assays were conducted for the two major metabolites of aficamten, with a follow-up combined *in vivo* micronucleus assay and comet assay conducted for metabolite M1a. Collectively, the metabolites were also negative for genotoxicity and clastogenicity. See [Table 57](#) for details.

13.1.4.1. Carcinogenesis, Mutagenesis, and Impairment of Fertility

Aficamten was negative in the bacterial mutagenesis (Ames) assay, an *in vivo* rat micronucleus study and in an *in vivo* comet assay.

Aficamten was not associated with tumor formation in both a 6-month transgenic mouse study at clinical exposure and a 2-year rat carcinogenicity study up to 2-fold and 4-fold clinical AUC_{unbound} for males and females, respectively.

Aficamten had no effects on fertility, mating, or reproductive assessments up to 6 mg/kg/day in females and 3 mg/kg/day in males.

The genetic toxicology studies conducted for aficamten, as well as its major metabolites, are summarized in [Table 58](#).

Table 58. Genetic Toxicology

Study No/Study Title	Key Study Findings
<i>The Parent Drug (Aficamten)</i>	
Nonclin-0004/CK-3773274: Bacterial Reverse Mutation Test	Aficamten was not mutagenic in a standard and valid GLP-compliant Ames assay up to 5000 µg/plate with and without exogenous metabolic activation using S9.
Nonclin-0036/CK-3773274: Combined In Vivo Mammalian Erythrocyte Micronucleus Test and Alkaline Comet Assay in Rat	Aficamten did not cause DNA damage and was neither clastogenic or aneugenic after three consecutive days of dosing up to 10 mg/kg/day (total C _{max} =6,860 ng/mL and total AUC =95,200 ng·h/mL, free C _{max} =110 ng/mL and free AUC =1,523 ng·h/mL) in a GLP-compliant study in rats. Based on free aficamten concentrations, the exposure multiples are 6-times (C _{max} -based) and 3.5-times (AUC-based) greater than clinical exposure at the maximum recommended human dose. Compared to total aficamten at the maximum recommended human dose, the exposure multiples are 21-times (C _{max} -based) and 13-times (AUC-based) greater.
<i>Major Metabolites</i>	
Nonclin-0231/CK-3834282: Bacterial Reverse Mutation Assay	Major metabolite M1a was not mutagenic in a standard and valid GLP-compliant Ames assay up to 5000 µg/plate.
Nonclin-0234/CK-3834283: Bacterial Reverse Mutation Assay	Major metabolite M1b was not mutagenic in a standard and valid GLP-compliant Ames assay up to 5000 µg/plate.
Nonclin-0233/CK-3834282: In vitro Mammalian Cell Micronucleus Assay in TK6 Cells	Major metabolite M1a induced micronuclei formation in human lymphoblasts without metabolic activation at ≥200 µg/mL after 27 hours in a GLP-compliant and valid <i>in vitro</i> assay.
Nonclin-0236/CK-3834283: In Vitro Mammalian Cell Micronucleus Assay in TK6 Cells	Major metabolite M1b did not induce micronuclei formation in human cells with or without metabolic activation up to 353 µg/mL in a GLP-compliant and valid <i>in vitro</i> assay.
Nonclin-0276/CK-3834282: Combined in vivo Mammalian Peripheral Blood Erythrocyte Micronucleus Test and Alkaline Comet Assay in Rat	Major metabolite M1a did not induce micronuclei formation or cause hepatocellular DNA damage <i>in vivo</i> after 4 days of oral dosing up to 2000 mg/kg/day in rats. At that dose, C _{max} and AUC exposure to CK-3834282 was 131,000 ng/mL and 1,870,000 ng·h/mL, respectively. Exposure multiples were 2,900-times (C _{max}) and 440-times (AUC) greater in this study than at the maximum recommended clinical dose for aficamten. The plasma protein binding of M1a is similar between rat and human. The weight-of-evidence evaluation indicates that M1a does not exhibit genotoxic potential.

Source: Pharm/tox reviewer's table

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; GLP, good laboratory practice; M, metabolite

13.1.4.1.1. Carcinogenicity

GLP-compliant carcinogenicity studies of 6 months in transgenic rasH2 mice (Study Nonclin-0306) and 2 years in Sprague Dawley rats (Study Nonclin-0248) were conducted after Executive Carcinogenicity Assessment Committee (ECAC) concurrence regarding dose selection.

Aficamten was associated with an apparent dose-dependent trend for bronchioloalveolar adenomas in male transgenic mice in the 6-month study with dosing up to 2 mg/kg/day corresponding to a clinical exposure margin of 2-fold based on free AUC (0.6-fold based on total AUC). The trend and pairwise comparisons between vehicle treated and aficamten treated animals were not statistically significant based on ECAC statistical criteria. In a 2-year rat carcinogenicity study, low survivability at the high dose for both sexes necessitated early stoppage of dosing and study termination after ECAC concurrence. No aficamten-related hyperplastic or neoplastic microscopic findings were noted for any organ in the 2-year rat study, up to 2 mg/kg/day in males and 3 mg/kg/day in females corresponding to clinical exposure margins of 2.5-fold and 4-fold based on free AUC, respectively (8-fold and 15-fold based on male and female total AUC, respectively).

13.1.4.2. Reproductive Toxicology

GLP-compliant reproductive toxicology studies including a fertility and early embryonic development study in rats, embryofetal development studies in rats and rabbits, and a pre- and post-natal development study in rats were conducted with aficamten. Details of these studies are summarized below in [Table 59](#).

Table 59. Reproductive Toxicity Summary

Study	NOAEL (mg/kg/day)	AUC at NOAEL Total (Free) (ng•hr/mL)	C _{max} at NOAEL Total (Free) (ng/mL)	AUC Multiples at NOAEL/LOAEL ¹ Total (Free)	C _{max} Multiples at NOAEL/LOAEL ¹ Total (Free)	Basis for NOAEL
FEED rat	3 (males), 6 (females)	Male: 73,000 (1,168) Female: 99,200 (1,587)	Male: 4,700 (75) Female: 10,800 (173)	Male: 10X/NA (3X/NA) Female: 13X/NA (4X/NA)	Male: 14X/NA (4X/NA) Female: 33X/NA (10X/NA)	- No aficamten-dependent effects on mating or fertility for either sex. No significant aficamten-dependent effects on estrous, placenta, uterine or ovarian findings including implantation. - No LOAEL determined
EFD rat- maternal	6	93,900 (1,500)	10,400 (166)	12X/17X (3.5X/5X)	32X/37X (9X/10X)	- Statistically significant decrease in food consumption and body weight gain at >6 mg/kg/day. - Increased heart weight relative to controls for pregnant females at ≥6 mg/kg/day.
EFD rat- embryofetal	6	93,900 (1,500)	10,400 (166)	12X/17X (3.5X/5X)	32X/37X (9X/10X)	- Increased number of fetal resorptions (total, early resorptions for three females) at 9 mg/kg/day only, with one female having pale foci on the placenta. - Decreased fetal weight and increased incidence of short and kinked tails for fetuses at 9 mg/kg/day.
EFD rabbit- maternal	10	214(21)	110 (11)	0.03X/0.1X (0.05X/0.17X)	0.3X/1X (0.6X/2X)	Early maternal lethality at 20 mg/kg/day necessitated dose reduction to 15 mg/kg for high dose group. No adverse macroscopic findings at termination for remaining animals.

NDA 219083
MYQORZO (aficamten)

Study	NOAEL (mg/kg/day)	AUC at NOAEL Total (Free) (ng•hr/mL)	C _{max} at NOAEL Total (Free) (ng/mL)	AUC Multiples at NOAEL/LOAEL ¹ Total (Free)	C _{max} Multiples at NOAEL/LOAEL ¹ Total (Free)	Basis for NOAEL
FEED rat	3 (males), 6 (females)	Male: 73,000 (1,168) Female: 99,200 (1,587)	Male: 4,700 (75) Female: 10,800 (173)	Male: 10X/NA (3X/NA) Female: 13X/NA (4X/NA)	Male: 14X/NA (4X/NA) Female: 33X/NA (10X/NA)	- No aficamten-dependent effects on mating or fertility for either sex. No significant aficamten-dependent effects on estrous, placentae, uterine or ovarian findings including implantation. - No LOAEL determined
EFD rabbit-embryofetal	15/20	705 (71)	399 (40)	0.1X/NA (0.17X/NA)	1X/NA (2X/NA)	- An imbalance was noted for a few fetal cardiovascular malformations (aorta dilatation, truncus arteriosus, misshapen heart, absent ventricular septum, retroesophageal subclavian artery) across three litters at 10 mg/kg/dose (the mid-dose). However, the signal was not dose-related, the findings were also frequently noted in control litters, was not noted in rats, and the overall relationship to the treatment could not be assigned. - Bones of the skull (supraoccipital, intraparietal) were absent in one fetus from the high dose group (15 mg/kg/day).
PPND rat	1.5	24,900 (400)	3,340 (53)	3.3X/14X (1X/4X)	9X/32X (3X/9X)	- Decreased maternal body weight gain, increased maternal heart weight, and reduced F1 pup birth weight at 6 mg/kg/day. - Surviving F1 pups from the 6 mg/kg/day group were thin in appearance, lacked milk bands, and had increased incidence of bent tails compared to controls.

Source: Pharm/tox reviewer's table

¹ Exposure multiples based on PPK data from clinical Trial CY 6031 SEQUIOA-HCM at 20 mg (total AUC =7,570 hr•ng/mL, free AUC =424 hr•ng/mL, total C_{max}=328 ng/mL, free C_{max}=18 ng/mL).

Abbreviations: AUC, area under the concentration-time curve; AUC_{unbound}, area under the concentration-time curve unbound; C_{max}, maximum plasma concentration; C_{max unbound}, maximum plasma concentration unbound; EFD, embryofetal development; FEED, fertility and early embryonic development; LOAEL, lowest-observed-adverse-effect level; NA, not applicable; NOAEL, no-observed-adverse-effect level; PPK, population pharmacokinetic; PPND, peri- and postnatal development

13.1.4.2.1. Summaries of Pivotal Developmental and Reproductive Toxicology Studies

Study Number/Title: Nonclin-0264/A Fertility and Early Embryonic Development Study of CK-3773274 by Oral Gavage in the Male and Female Rat (GLP)

Key Study Findings

- No dose-dependent effects on mating, fertility or reproductive organs up to 6 mg/kg/day for females (F) or 3 mg/kg/day for males (M).
- The NOAEL for fertility and early embryonic development was 3 mg/kg/day for males and 6 mg/kg/day for females. Toxicokinetics were not evaluated in this study, and exposure at the NOAELs was based on values from the 13-week repeat dose study in rats at the same dose levels (Male free AUC: 1,168 ng·h/mL, total AUC: 73,000 ng·h/mL, Male free C_{max}: 75 ng/mL, total C_{max}: 4,700 ng/mL, Female free AUC: 1,587 ng·h/mL, total AUC: 99,200 ng·h/mL, Female free C_{max}: 173 ng/mL, total C_{max}: 10,800 ng/mL).

Table 60. Study Information: Nonclin-0264 Study

Parameter	Method Details
Dose and frequency of dosing	0 (untreated, females only), 0 (vehicle), 0.5, 1.5, 3 (males), 6 (females) mg/kg/day
Route of administration	Oral gavage
Formulation/vehicle	0.5% (w/v) Hydroxypropyl Methylcellulose (HPMC) and 0.1% (v/v) Tween-80 in ultra-pure water
Species/strain	Sprague Dawley Crl:CD(SD) rats
Number/sex/group	22 males for each group, 22 females for each group
Study design	Daily oral dosing for males 28 days before cohabitation, during cohabitation and continuing through the day before scheduled euthanasia (Day 59). Females were dosed daily 15 days before cohabitation, during cohabitation and continue through gestation Day 7.
Deviation from study protocol affecting interpretation of results	None

Source: Nonclin-0264 Study

Table 61. Observations and Results: Nonclin-0264 Study

Endpoints	Major Findings
Endpoints with notable observations	N/A
Endpoints without notable observations	Body weight, macroscopic pathology, ovarian and uterine exams, placental exam, reproductive organ weights, male and female fertility and mating, time to mating.

Source: Nonclin-0264 Study

Abbreviations: N/A, not applicable

Study Number/Title: Nonclin-0161/CK-3773274: An Oral Embryofetal Development Study in Rats (GLP)

Key Study Findings

- Statistically significant increased maternal mean absolute heart weight was observed at 6 and 9 mg/kg/day (up to 13% increase over controls).
- Maternal toxicity characterized by decreased body weight gain, reduced food consumption, and decreased terminal body weight at 9 mg/kg/day, which reached statistical significance at sporadic intervals throughout the study, were adverse (up to 30% reduced food consumption, 64% reduced body weight gain).
- The mean total fetuses per litter was reduced at 9 mg/kg/day, with mean postimplantation loss of ~59% and a mean early resorption rate of ~7%. Three incidences of total litter loss, and one incidence of abnormal placenta (pale foci) occurred at the same dose. See [Table 63](#).
- Surviving fetuses from the 9 mg/kg/day group had statistically significant reduction in weight compared to controls, with increased incidence of kinked and short tails. See [Table 63](#).
- The NOAEL from this study was 6 mg/kg/day for both maternal and fetal toxicity with systemic exposures of 93,900 and 1,502 ng·h/mL for total and free AUC, respectively, and 10,400 and 166 ng/mL for total and free C_{max}, respectively. Clinical safety margins based on total and free AUC are 12X and 3.5X, respectively. Clinical safety margins based on total and free C_{max} are 32X and 9X, respectively.

Table 62. Study Information: Nonclin-0161 Study

Parameter	Method Details
Dose and frequency of dosing	0, 2, 6, 9 mg/kg/day
Route of administration	Oral gavage
Formulation/vehicle	0.5% (w/v) Hydroxypropyl Methylcellulose (HPMC) and 0.1% (v/v) Tween-80 in ultra-pure water
Species/strain	Sprague Dawley Crl:CD(SD) rats
Number/sex/group	20 females for each group
Study design	Daily oral dosing from postcoital Day 6 through postcoital Day 17; animals euthanized on postcoital Day 21. The gravid uterus was examined for the number and distribution of corpora lutea, implantation sites, placentae, liver and dead fetuses, early and late resorptions. Fetal examinations include body weight, external, visceral, and skeletal abnormalities. Approximately one-half of the fetuses in each litter were examined for visceral abnormalities and the remaining one half were examined for skeletal abnormalities.
Deviation from study protocol affecting interpretation of results	None

Source: Nonclin-0161 Study

Table 63. Observations and Results: Nonclin-0161 Study

Endpoints With Notable Observations	Major Findings
Maternal toxicity	Pregnant females in the mid- and high-dose group had dose-dependent lower body weight gain compared to vehicle controls, accompanied by decreased food consumption, which were statistically significant and adverse at the high dose. There was accompanying increased absolute heart weight without microscopic correlates for both mid and high dose groups.
Offspring toxicity	Increased incidence of fetal resorptions and postimplantation losses in the high dose group resulted in fewer live fetuses per litter and lower mean fetal weight. Surviving fetuses at the high dose had increased incidence of kinked or short tails. There was an increase in incidence of incomplete zygomatic arch ossifications in the mid dose group compared to vehicle controls, but it was within historical control range.

Source: Nonclin-0161 Study, Pharm/tox reviewer's table

Table 64. Notable Findings From Rat EFD Study Nonclin-0161

Uterine Findings/Fetal Weights/Fetal Malformations	Fetus/Litter	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	9 mg/kg/day	HCD Range
Postimplantation loss (%)		5.7	9.1	9.7	59**	1.6-12.8%
Number of live fetuses		12	11	11	5**	11-16
Total fetuses		12	11	11	6**	NR
Early Resorptions		1	1	1	7**	0-2
Fetal weight (sexes combined)		6.06	6.18	5.98	5.53**	5.4-6.4
Tail, short (%)	Fetuses	0	0	0	1/0.84%	0-0.4%
	Litters	0	0	0	1/6%	0-4.8%
Tail, kinked (%)	Fetuses	0	0	0	2/4%	0-0.4%
	Litters	0	0	0	1/6%	0-4.8%

Source: Nonclin-0161 Study, Pharm/tox reviewer's table

**p<0.01

Bold values are outside of Historical Control Data (HCD) range.

Table 65. Toxicokinetics: Nonclin-0161 Study

Dose (mg/kg/day)	AUC _{last} (ng·h/mL)		C _{max} (ng/mL)	
	Day 6 PC Total (Free)	Day 17 PC Total (Free)	Day 6 PC Total (Free)	Day 17 PC Total (Free)
2.0	32,600 (522)	33,800 (541)	2,740 (44)	3,210 (51)
6.0 (Maternal and fetal NOAEL)	89,700 (1,435)	93,900 (1,502)	9,990 (160)	10,400 (166)
9.0	121,000 (1,936)	126,000 (2,016)	11,600 (186)	12,000 (192)

Source: Nonclin-0161 Study

Abbreviations: AUC_{last}, area under the curve from time 0 to 24 hours; C_{max}, maximum plasma concentration; NOAEL, no-observed-adverse-effect level; PC, postcoital

Study Number/Title: Nonclin-0199/CK-3773274: An Oral Embryofetal Development Study in Rabbits

Key Study Findings

- There were dose-dependent reductions in maternal body weight gain, which were not associated with decreased food consumption between Days 7 to 10 postcoital for all groups (7, 17, 27% reduction compared to controls), which persisted for the high dose group through Day 20 postcoital (overall body weight reduction of 11% compared to controls).
- Statistically significant increased late resorptions occurred for the mid dose only, with mean postimplantation loss occurring at a rate (10.5%) toward the high end of the historical control values (1.3 to 10.7%). See [Table 67](#).
- Fetal cardiovascular malformations including dilated aorta, absent ventricular septation, misshapen heart, retroesophageal subclavian artery, and truncus arteriosus occurred in the mid-dose group only (10 mg/kg/day) at incidences exceeding the historical control range. Truncus arteriosus was found in litters from the high dose group (20/15 mg/kg/day) at a rate (0.6%) toward the high end of the historical control range (0.61%). See [Table 67](#).
- The maternal and embryofetal NOAEL doses were 10 mg/kg/day and 15 mg/kg/day, respectively, with exposure margins below clinical exposure at the maximum recommended human dose; doses higher than 15 mg/kg/day were not tolerated in rabbits.
- The embryofetal NOAEL is based on the following:
 - A slight numerical increase in the incidence (litters) of cardiovascular malformations above concurrent and historical controls was observed in the mid-dose group.
 - The imbalance was not dose-related and findings were absent or had similar incidence between control and high-dose groups.
 - We considered whether the four litters lost (on Days 7 to 9 post-coital) at the 20/15 mg/kg high dose may have the potential to confound the analysis.
 - Importantly, the missing litters were clearly due to maternal toxicity and death and were not related to embryofetal toxicity (per se). Furthermore, postimplantation loss and total number of fetuses (per litter) in surviving females was similar to controls. These data indicate it was unlikely that maternal deaths confounded our analysis.

In conclusion, the incidence of malformations was deemed to be similar across dose groups, similar to the concurrent and/or historical control levels, lacking in any apparent dose-response, and unlikely to have been caused by lost litters (due to maternal deaths, not abortion/resorption),

and thus could not be attributed to aficamten, despite a signal reported for the first-in-class cardiac myosin inhibitor, mavacamten.

Table 66. Study Information: Nonclin-0199Study

Parameter	Method Details
Dose and frequency of dosing	0, 5, 10, 15/20 mg/kg/day
Route of administration	Oral gavage
Formulation/vehicle	0.5% (w/v) Hydroxypropyl Methylcellulose (HPMC) and 0.1% (v/v) Tween-80 in ultra-pure water
Species/strain	New Zealand White Rabbits
Number/sex/group	Twenty animals per group
Satellite groups	Three animals per aficamten dose for toxicokinetic evaluation only
Study design	Daily oral dosing from gestation Day 7 through gestation Day 19, with scheduled euthanasia on Day 29. At necropsy, ovarian/uterine examination including gravid uterine weight, placental exam, number of corpora lutea and implantation sites, number resorptions, and number live and dead fetuses were scored. All fetuses were examined for external and abnormalities, with live fetuses having an additional skeletal exam and being weighed.

Source: Nonclin-0199Study

Table 67. Observations and Results: Nonclin-0199Study

Endpoints	Major Findings
Endpoints with notable observations	
Maternal toxicity	Three animals in the high dose group (20 mg/kg/day) died by postcoital Day 8, with an additional one on postcoital Day 9 after reducing the dose to 15 mg/kg/day. One animal given 10 mg/kg/day was found dead on postcoital Day 14, with preceding signs of decreased activity, abnormal breathing, and muzzle discharge. In the low dose group, one animal was humanely euthanized on postcoital Day 19 having been found with reduced activity, thin appearance, and abdominal distension. Reductions in body weight gain were reported for all dose groups early in the study, without accompanying increased cardiac weights.
Offspring toxicity	A slight imbalance in fetal cardiovascular malformations was noted at the mid-dose at a rate slightly above the historical control range, with no apparent dose-dependent trend. The relation to aficamten is questionable given the minor increase above controls, overall low total incidence, and lack of dose-dependence.
Endpoints without notable observations	Macroscopic pathology, organ weights, pregnancy rate, uterine examination, external fetal examination, mean fetal weights

Source: Nonclin-0199 Study, Pharm/tox reviewer's table

Table 68. Notable Findings From Study Nonclin-0199

Findings	Doses and Frequency of Findings				
	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20/15 mg/kg/day*	HCD Range
Uterine findings					
Post implantation loss (%)	7.3	6.03	10.5	4.3	1.3-10.7%
Late resorption	0.2	0.3	0.7**	0.1	0.1-0.5
Total number of fetuses	11	10	10	11	
Cardiovascular malformations					
Number of fetuses/litters evaluated	209/19	183/18	186/18	166/15*	
Aorta, dilatation					
Fetuses	0	0	1/0.46%	0	0-1
Litters	0	0	1/5.6%	0	0-10.5
Truncus arteriosus, persistent					
Fetuses	1/0.4%	0	2/1.16%	1/0.61%	0-0.6
Litters	1/5.3%	0	2/11.1%	1/6.7%	0-6
Heart, misshapen					
Fetuses	1/0.4%	0	2/1.16%	0	0-0.5
Litters	1/5.3%	0	2/11%	0	0-5.3
Pericardium, blood-filled					
Fetuses	1/0.6%	0	0	1/0.56%	0-2.4
Litters	1/5.3%	0	0	1/6.7%	0-17
Ventricular septum, absent					
Fetuses	1/0.4%	0	2/1.16%	0	0-0.5
Litters	1/5.3%	0	2/11%	0	0-5.6
Subclavian artery, retroesophageal					
Fetuses	1/0.53%	0	3/1.4%	1/0.6%	0-1.2
Litters	1/5.3%	0	1/5.6%	1/6.7%	0-11.8

Source: Nonclin-0199 Study, Pharm/tox reviewer's table

*4 dams died by postcoital Day 9

**p<0.01,

Bold values outside Historical Control Data (HCD) range

Abbreviation: HCD, historical control data

Table 69. Toxicokinetics: Nonclin-0199 Study

Dose (mg/kg/day)	AUC _{last} (ng·h/mL)		C _{max} (ng/mL)	
	Day 7 PC Total (Free)	Day 19 PC Total (Free)	Day 7 PC Total (Free)	Day 19 PC Total (Free)
5	159 (16)	85 (9)	59 (6)	38 (4)
10 (Maternal NOAEL)	280 (28)	214 (21)	141 (14)	110 (11)
15/20 ¹ (Fetal NOAEL)	537 (54)	705 (71)	321 (32)	399 (40)

Source: Nonclin-0199 Study

¹ Dose adjustment made on postcoital Day 8

Abbreviations: AUC_{last}, area under the curve from time 0 to 24 hours; C_{max}, maximum plasma concentration; NOAEL, no-observed-adverse-effect level; PC, postcoital

Study Number/Title: Nonclin-0360/An Oral (Gavage) Study of the Effects of CK-3773274 on Pre- and Postnatal Development in Rats

Key Study Findings

- At the high dose, 6 mg/kg/day, mean maternal heart weight was increased relative to controls by 22%. Body weight gain during the gestation period was also reduced compared to controls at the high dose, with accompanying decreased pup viability after birth and clinical signs including absent milk band, thin appearance, and bent tail. The maternal and offspring NOAELs were at the mid dose, 1.5 mg/kg/day. See [Table 71](#).
- Aficamten was detected in plasma from nursing postnatal Day 4 pups at all doses, suggesting transfer through milk as the half-life of aficamten in rats is ~8 hours.

Table 70. Study Information: Nonclin-0360 Study

Parameter	Method Details
Dose and frequency of dosing	0, 0.5, 1.5, 6 mg/kg/day
Route of administration	Oral gavage
Formulation/vehicle	0.5% (w/v) Hydroxypropyl Methylcellulose (HPMC) and 0.1% (v/v) Tween-80 in ultra-pure water
Species/strain	Sprague Dawley CD (CrI:CD[SD]) rats
Number/sex/group	22 animals per group
Study design	Daily oral dosing of pregnant dams from gestation Day 6 through postnatal Day 20.

Source: Nonclin-0360 Study

Table 71. Observations and Results: Nonclin-0360 Study

Endpoints	Major Findings
Endpoints with notable observations	
Maternal toxicity	Increased heart weight (plus 22% compared to controls), at 6 mg/kg/day. There were accompanying minor, but statistically significant decreases in body weight gain during the early gestation period (GD 6 to 12) at the same dose.
Offspring toxicity	Decreased postnatal pup viability at 6 mg/kg/day compared to controls (70% versus 98%). Five females out of 22 in the high dose group lost all pups by postnatal Day 4.
Endpoints without notable observations	No aficamten related effects on offspring sexual maturation, mating, fertility, or on behavioral evaluations of motor activity, hearing, or learning and memory.

Source: Nonclin-0360 Study, Pharm/tox reviewer's table
Abbreviation: GD, gestation day

Table 72. Notable Findings From Study Nonclin-0360

Pup Viability/External Malformations	0 mg/kg/day	0.5 mg/kg/day	1.5 mg/kg/day	6 mg/kg/day	HCD Range
Viability index % (Birth - PND4)	98	98	98	69*	89-100%
Tail, bent (F1 male adults)					
Number affected	0	0	0	7	
% Affected	0	0	0	32	
Tail, bent (F1 female adults)					
Number affected	0	0	0	4	
% Affected	0	0	0	18	

Source: Nonclin-0360 Study, Pharm/tox reviewer's table

* p<0.05

Abbreviations: HCD, historical control data; PND, postnatal day

Table 73. Toxicokinetics: Nonclin-0360 Study

Dose (mg/kg/day)	Postnatal Day 4 Total Plasma Concentration (F₁ Sexes Combined)	Lactation Day 4 Total Plasma Concentration (F₀ Dams)
0.5	64 ng/mL	731 ng/mL
1.5 (NOAEL)	86 ng/mL	1854 ng/mL
6.0	491 ng/mL	8054 ng/mL

Source: Nonclin-0360 Study

Abbreviation: NOAEL, no-observed-adverse-effect level

13.1.5. Other Toxicology/Specialized Studies

Major metabolites (M1a and M1b) of aficamten were characterized in a non-GLP 14-day repeat dose study in rats, GLP Ames assays, and GLP *in vivo* and *in vitro* genetic toxicology studies. In addition, aficamten was evaluated in a phototoxicity assay *in vitro*, and skin sensitization and corrosion assays. In general, the major aficamten metabolites were well tolerated, not pharmacodynamically active *in vivo*, and not genotoxic or clastogenic at clinically relevant levels. Aficamten was not irritating or corrosive to the eye or skin in any study conducted. The results of these studies are summarized below:

13.1.5.1. Metabolites

Study Number/Title: CK-3834282 and CK-3834283/A 14-Day Oral Dose Tolerability Study in Sprague Dawley Rat (Nonclin-0221) (Non-GLP)

- Major aficamten metabolites were administered separately by oral gavage to rats for 14 consecutive days at 50 mg/kg/day.
- No remarkable clinical observations or effects on heart weight were found for either metabolite up to C_{max} levels of 13,933 ng/mL for males and 28,032 ng/mL for females for M1a, and C_{max} levels of 45,197 ng/mL for males and 62,663 ng/mL for females for M1b. AUC_{0-24h} values for males and females were 35,679 and 59,799 ng·h/mL for M1a, and 215,085 and 287,778 ng·h/mL for M1b, respectively.
- The major aficamten metabolites were not pharmacologically active *in vivo* up to clinical exposure multiples >100-times based on steady state C_{max}, and >10-times based on steady state AUC from clinical Study CY 6021 at an aficamten dose of 20 mg.

Study Number/Title: CK-3834282/Bacterial Reverse Mutation Assay (Study Nonclin-0231) and CK-3834283: Bacterial Reverse Mutation Assay (Study Nonclin-0234)

- Bacterial tester strains were grown with metabolite M1a or M1b up to 5000 µg/plate in the presence or absence of rat liver S9.
- Test-article precipitation was observed for both M1a and M1b at 3,333 µg/plate and 5000 µg/plate, respectively.
- M1a did not induce an increase in revertant bacterial colonies up to 3,333 µg/plate. Toxicity for M1a was noted at ≥3,333 µg/plate in the absence of S9.

- Growth of *S. typhimurium* strain TA98 with M1b at 100 µg/mL induced a 1.8X increase in revertant colonies compared to controls in the absence of S9, but there was no dose-dependent increase in revertant colonies above that dose.
- The Ames assay was negative for both M1a and M1b under the conditions of these studies.

Study Number/Title: CK-3834282/In Vitro Mammalian Cell Micronucleus Assay in TK6 Cells (Study Nonclin-0233) and CK-3834283: In Vitro Mammalian Cell Micronucleus Assay in TK6 Cells (Study Nonclin-0236)

- TK6 lymphoblasts were treated with M1a or M1b in the presence and absence of rat liver S9 up to 353 µg/mL for 4 hours, or in the absence of S9 for 27 hours.
- A statistically significant increase in micronuclei was observed at M1a concentrations ≥ 200 µg/mL in the absence of S9 after 27 hours of treatment compared to controls. The value of the micronuclei induction at the highest dose (1.55%) was only slightly above the maximum historical control value (1.5%).
- Under the conditions of the study, M1a was considered positive for inducing micronuclei without S9 activation at exposure equivalent to ≥ 4000 -times clinical $C_{\max}$ at the maximum recommended human dose.
- No statistically significant increases in micronuclei induction were found for M1b under any condition up to 353 µg/mL in the absence of S9 for 27 hours.

Study Number/Title: CK-3834282/Combined In Vivo Mammalian Peripheral Blood Erythrocyte Micronucleus Test and Alkaline Comet Assay in Rat (Study Nonclin-0276)

- Vehicle, positive controls, or M1a up to 2000 mg/kg/day was administered to rats by oral gavage for 4 consecutive days after which micronucleated reticulocytes in the periphery were counted using flow cytometry. The mean percentage of micronucleated cells from M1a treated animals at all doses was within historical negative control range.
- In the same study, hepatocytes were used for a comet assay to assess DNA damage. All M1a treated groups had Tail DNA values within historical negative control range.
- Positive controls induced increases in the mean percentage of micronucleated reticulocytes and Tail DNA in the comet assay, verifying the validity of the test system.
- M1a was not clastogenic in vivo up to 2900-times clinical $C_{\max}$ and 440-times clinical AUC at the maximum recommended clinical dose for aficamten. The *in vitro* plasma protein binding of M1a was similar between rat and human. Although the major metabolite M1a without metabolic activation was positive for inducing micronuclei in human lymphoblasts *in vitro*, it was not mutagenic in a bacterial Ames assay. M1a administered to rats *in vivo* for 4 days did not induce micronuclei in circulating reticulocytes and did not cause DNA damage in hepatocytes at a dose >1000 -times the clinical exposure based on $C_{\max}$ and >400 -times based on AUC, similar to the exposure which did induce micronuclei in lymphoblasts *in vitro*. Taken together, the weight of evidence suggests that the M1a metabolite is unlikely to be mutagenic or clastogenic at clinically relevant doses of aficamten.

13.1.5.2. Skin and Eye Irritation Studies

A tabulated summary of *in vitro*, *ex vivo*, and *in vivo* assays used to determine the irritation, corrosion, and phototoxicity potential of aficamten is provided below. All studies were valid and were conducted according to GLP and/or Organisation for Economic Co-operation and Development guidelines.

Table 74. Summary of Skin and Eye Irritation Studies

Study Number	Study Title	Results
Nonclin-0108	A GLP Neutral Red Uptake Phototoxicity Assay of CK-3773274 in BALB/c 3T3 Mouse Fibroblasts	Negative, aficamten did not induce additional cytotoxicity above the negative control when ultraviolet radiation was applied over a range of concentrations up to 32 µg/mL.
Nonclin-0283	Evaluation of the Eye Hazard Potential of CK-3773274 using the Bovine Corneal Opacity and Permeability Test (BCOP Test)	Negative, aficamten did not increase corneal permeability and only slightly increased corneal opacity when topically applied to isolated bovine corneas for 4 hours.
Nonclin-0284	CK-3773274: An <i>In Vitro</i> Evaluation of Skin Corrosion using a Human Skin Model (EpiDerm™)	Negative, topical application of aficamten to the stratum corneum of skin was not cytotoxic after 60 minutes of incubation on human-derived epidermal keratinocytes.
Nonclin-0285	CK-3773274: An <i>In Vitro</i> Evaluation of Skin Irritation using a Human Skin Model (EpiDerm™)	Negative, topical application of aficamten to the stratum corneum of skin was not cytotoxic after 60 minutes of incubation on human-derived epidermal keratinocytes.
Nonclin-0286	CK-3773274: An <i>in vitro</i> membrane barrier test for skin corrosion (Corrositex®)	Inconclusive, study deviations prevented interpretation.
Nonclin-0287	An In Chemico Determination of the Skin Sensitization Potential of CK-3773274 using the Direct Peptide Reactivity Assay (DPRA)	Inconclusive, aficamten was not compatible with assay solvents.
Nonclin-0288	Evaluation of the <i>in vitro</i> Skin Sensitization Potential of CK-3773274 with the KeratinoSens Assay	Negative, aficamten up to 250µM did not induce the Nrf2 pathway in keratinocytes (marker for oxidative stress) after 48 hours.
Nonclin-0333	Evaluation of the <i>in vitro</i> Skin Sensitization Potential of CK-3773274 with the U937 Cell Line Activation Test (U-SENS™) Assay	Positive, aficamten induced CD86 expression after 45 hours of incubation at >7 µg/mL.
Nonclin-0404	A sensitization Study of CK-3773274 by Dermal Administration in Guinea Pigs – Modified Buehler Design	Negative, aficamten did not induce erythema after 48 hours of topical application.

Source: Pharm/tox reviewer's table

Abbreviations: GLP, good laboratory practice

13.1.5.2.1. Impurities/Degradants

Impurities for aficamten included a non-mutagenic process impurity ((b) (4)) and four impurities containing structural alerts ((b) (4)) for mutagenicity. One of the mutagenic impurities was used in an Ames assay for read-through data to qualify the others. Summary reviews of toxicology studies using these impurities are below:

Study Number/Title: (b) (4): A 13-Week Oral Gavage Toxicity Study in Rats (Study Nonclin-0440)

- An aficamten process impurity (b) (4) was administered daily to rats by oral gavage for 13 weeks at 0, 0.00625, or 0.0125 mg/kg/day to qualify the impurity at a specification of not more than (b) (4)% in the aficamten drug substance.
- Mean body weight for rats administered (b) (4) was modestly reduced in males without a clear dose-dependent trend (-11% and -8% compared to controls for the low and high dose, respectively). Other clinical pathology findings during the dosing period were minor or limited to only one sex.
- At terminal necropsy, females from the high dose group had increased mean pituitary gland weight (plus 23% increase relative to brain weight compared to controls) accompanied by diffuse pars distalis hyperplasia, moderately reduced number of corpora lutea, increased vaginal mucification, and minimal mammary gland hyperplasia. The findings suggest a disruption of the endocrine system in females, however, functionally there were no accompanying disruptions to the estrus cycle.
- The findings were considered non-adverse up to (b) (4) mg/kg/day for females and up to (b) (4) mg/kg/day for males.
- Process impurity (b) (4) is being limited to not more than (b) (4)% aficamten.

Table 75. Impurities and Dose Multiples: (b) (4) Study

Table 10: Impurities and Dose Multiples									
Impurity	Specification Limits	Impurity Level %	(Nonclinical Species) Total	Human Total	Dose Multiple (mg/kg)	(Nonclinical Species) Total	Human Total	Dose Multiple (mg/m ²)	
			Daily Intake of Impurity (mg/kg)	Daily Intake of Impurity (mg/kg)		Daily Intake of Impurity (mg/m ²)	Daily Intake of Impurity (mg/m ²)		
Drug substance impurities									
(b) (4)	NMT								(b) (4)

Source: (b) (4) Study

Human: Drug total daily dose =20 mg (60 kg patient)

Abbreviation: NMT, not more than

Table 76. Summary: Nonclin-0545 Study

Study Parameters	Study Information
Study no.	Nonclin-0545: (b) (4): A Bacterial Reverse Mutation Test in Salmonella Typhimurium and Escherichia Coli
GLP compliance	Yes
Drug, lot #, and % purity	(b) (4) Lot VSR-1270-19, 99.5% purity
Study validity	
Strains/cell lines	Adequate (TA1535, TA1537, TA98, TA100, WP2 uvrA)
Concentrations in definitive study	Adequate (1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate)
Basis of concentration selection	ICH guidance
Negative control	Adequate (vehicle)
Positive control	Adequate, strain-specific positive controls used without S9 activation (b) (4)
	(b) (4)
Formulation/vehicle	Dimethyl sulfoxide
Incubation and sampling time	Adequate, using plate incorporation method and 64-hour incubation
Metabolic activation	S9 fraction (phenobarbital/ 5,6-benzoflavone induced rat liver fraction)
Comment on study validity	Acceptable

Source: Pharm/tox reviewer's table

Abbreviations: GLP, good laboratory practice; ICH, International Council for Harmonisation

Results/Conclusion

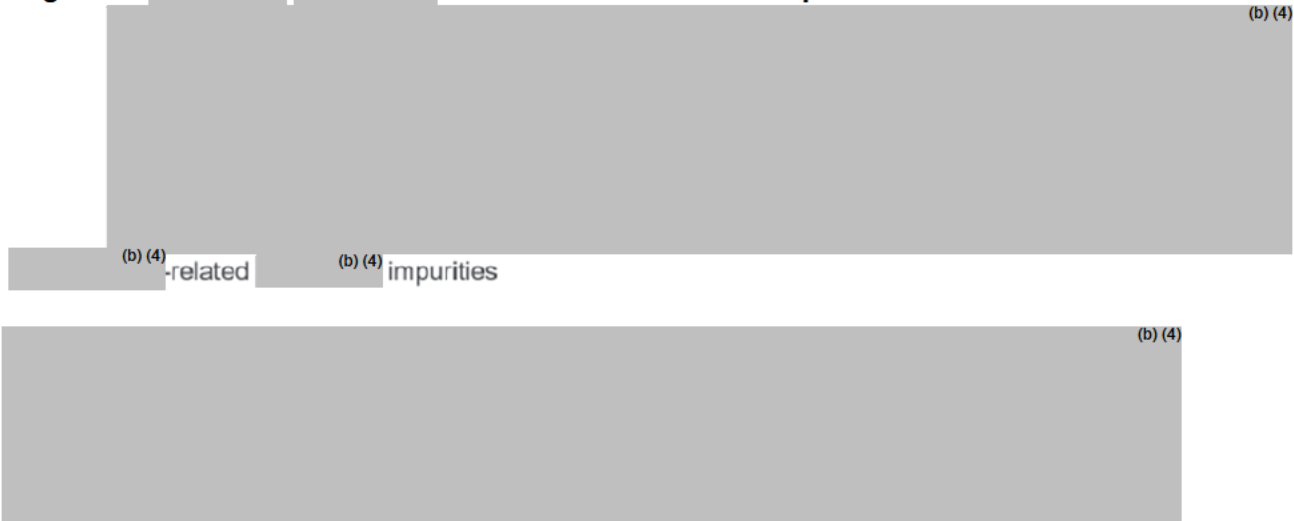
We concur with the Applicant's position that CK-3943113 was not mutagenic under any conditions used for this study. Revertant colony number slightly exceeded the 98% tolerance threshold for negative control data at 50 µg/plate and 158 µg/plate with S9 for strains TA98 and TA1537 respectively. However, revertant colony number did not exceed the ≥ 2 -fold increase cutoff above negative controls for any strain to be considered positive, and there was no dose-dependent increase in revertant colonies for any strain.

13.2. Individual Reviews of Studies Submitted With the New Drug Application

Background

Four impurities were identified by the Applicant which had structural alerts for mutagenicity ((b) (4)). The impurities and the corresponding structural alert are highlighted in the Applicant's diagram below.

Figure 13. (b) (4) (b) (4) **Intermediate and Related Impurities**



Source: Applicant

In a mid-cycle communication dated 04/03/2025, the Agency suggested that the Applicant either conduct Ames testing to assess mutagenic potential or control the impurities as Class 3 per ICH M7. The Applicant agreed to conduct a GLP Ames assay for one of the four impurities (b) (4) (b) (4) and use the results as read-through data for the others. The Applicant could not synthesize and isolate sufficient (b) (4), so the Ames assay was done using (b) (4) instead, and the Agency agreed with this approach per consultation with CDER Computational Toxicology Consultation Service (CTCS). The review for the Ames study using (b) (4) is included below.

Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study Title: Study# Nonclin-0545: (b) (4): A Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*

Methods

This GLP-compliant Ames assay was conducted according to ICH-S2(R1) and OECD guidelines. The Applicant's table below summarizes the study parameters, including concentrations used, bacterial strains used, and positive and negative controls used.

Table 77. Main Test, Plate Incorporation Assay

Text Table 1
Main Test
Plate Incorporation Assay

Dose No.	Formulation Conc. (µg/mL) ^a	Dose Volume (µL/plate)	Final Conc. (µg/plate)	Number of Replicates		Strain
				-S9	+S9	
Negative Control	(b) (4)	100	(b) (4)	3	3	all
1/	(b) (4)	100	(b) (4)	3	3	all
2/	(b) (4)	100	(b) (4)	3	3	all
3/	(b) (4)	100	(b) (4)	3	3	all
4/	(b) (4)	100	(b) (4)	3	3	all
5/	(b) (4)	100	(b) (4)	3	3	all
6/	(b) (4)	100	(b) (4)	3	3	all
7/	(b) (4)	100	(b) (4)	3	3	all
8/	(b) (4)	100	(b) (4)	3	3	all
Positive Controls	(b) (4)	100	(b) (4)	3	-	TA1535, TA100
	(b) (4)	100	(b) (4)	3	-	TA1537
	(b) (4)	100	(b) (4)	3	-	TA98
	(b) (4)	100	(b) (4)	3	-	WP2 <i>invA</i>
	(b) (4)	100	(b) (4)	-	3	TA1535, TA1537
	(b) (4)	100	(b) (4)	-	3	WP2 <i>invA</i>
	(b) (4)	100	(b) (4)	-	3	TA98, TA100

^a Nominal concentrations; measured concentrations might be adjusted due to the limitations of the instruments used.

^b The OECD/ICH S2(R1) standard limit concentration (usually 5000 µg/plate).

- Not applicable.

-S9: In the absence of S9 mix; +S9: In the presence of S9 mix.

Negative Control: Dimethyl sulfoxide (DMSO).

Source: Applicant

Results

(b) (4) was not mutagenic in any conditions used for this study. Revertant colony number slightly exceeded the 98% tolerance threshold for negative control data at 50 µg/plate and 158 µg/plate with S9 for strains TA98 and TA1537 respectively. However, revertant colony number did not exceed the ≥ 2-fold increase cutoff above negative controls for any strain to be considered positive, and there was no dose-dependent increase in revertant colonies for any strain.

14. Clinical Pharmacology

14.1. In Vitro Studies

Plasma Protein Binding and Plasma Partitioning

Plasma protein binding of aficamten in humans was assessed at 0.5, 2, and 10µM aficamten and metabolites (CK-3834282 and CK-3834283) (Study Nonclin-0017, Nonclin-0249, and Nonclin-0124). The mean plasma protein binding was ≥88.8%, ≥80.5%, and ≥92.5% across concentration levels for aficamten, CK-3834282, and CK-3834283, respectively. No apparent concentration dependency of plasma protein binding was observed. No apparent differences in plasma protein

binding of aficamten was observed between clinical study samples from subjects with normal hepatic function and moderate HI (Study CY 6017).

Blood cell partitioning of aficamten and metabolites (CK-3834282 and CK-3834283) was evaluated in human whole blood at concentrations of 1 and 10 μM (Studies Nonclin-0032 and Nonclin-0201). Whole blood-to-plasma ratio was approximately 0.9, 0.8, and 0.7 across concentration levels for aficamten, CK-3834282, and CK-3834283, respectively. No apparent concentration dependency of partitioning was observed.

In Vitro Hepatic Metabolism

Incubation of aficamten (1 μM) with human liver microsomes and recombinant CYP isoenzymes showed that aficamten was metabolized by CYP2D6, CYP2C9, CYP2C19, and CYP3A4 but not by CYP1A2, CYP2B6, and CYP2C8 (Study Nonclin-0029). Formation of the metabolite CK-3834282 was completely inhibited in the presence of a pan-CYP inhibitor (i.e., 1-aminobenzotriazole) but not in the presence of individual CYP2D6, CYP2C9, CYP2C19, and CYP3A4-specific inhibitors, supporting that aficamten is metabolized by multiple CYP isoenzymes. The fraction of aficamten metabolized (f_m) by CYP2D6 was evaluated using Hurellhuman™ hepatocyte microliver model and estimated to be 52.1% in pooled hepatocytes.

In Vitro CYP Inhibition and Induction

CYP Inhibition

Aficamten (0.046 to 100 μM) exhibited no direct inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (i.e., half-maximal inhibitory concentration [IC_{50}] >100 μM) when evaluated in human liver microsomes (Study Nonclin-0033). Time-dependent inhibition was not observed for any of these CYP isoforms when incubated with 0.046 to 100 μM aficamten (Study Nonclin-0052). In a follow-up study, aficamten was potentially a weak metabolism-dependent inhibitor of CYP2C9 because minimal metabolism-dependent inhibition (i.e., approximately 22%) was observed at the highest test concentration of 50 μM ; the IC_{50} of this inhibition was >50 μM (Study Nonclin-0160). The Applicant reports that test concentration of 50 μM is approximately 580 times higher than the maximal unbound plasma concentration of aficamten at steady-state ($I_{\text{max,u}}$) of 0.054 μM based on a maximal total exposure in humans ($I_{\text{max,ss}}$) of 0.972 μM occurring with dosing aficamten 20-mg QD (Trial CY 6031) and the human unbound fraction in plasma ($f_{u,p}$) of 0.056 (Study CY 6017).

Aficamten metabolites, CK-3834282 and CK-3834283 (0.046 to 100 μM) exhibited no direct inhibition of CYP1A2, CYP2B6, CYP2D6, and CYP3A4_{midazolam} (IC_{50} >100 μM) when evaluated in human liver microsomes (Study Nonclin-0126). The IC_{50} of CK-3834282 was >100, 22.2, 88.7, and 76.8 μM for CYP2C8, CYP2C9, CYP2C19, and CYP3A4_{testosterone}, respectively. The IC_{50} of CK-3834283 was 65.3, 19.5, 96.9, and 87.3 μM for CYP2C8, CYP2C9, CYP2C19, and CYP3A4_{testosterone}, respectively. Time-dependent inhibition was not observed for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 with either metabolite.

CYP Induction

Aficamten (0.3, 1, 3, 10, 25, and 50 μM) did not exhibit potential to induce CYP2B6 or CYP3A4 but potentially induced CYP1A2 when evaluated by quantitation of messenger ribonucleic acid (mRNA) expression in human primary hepatocytes (Study Nonclin-0016). The potential of

aficamten and its metabolites (CK-3834282 and CK-3834283) for clinically relevant induction of CYP1A2 was evaluated using the “basic mRNA fold-change method” in accordance with the ICH guidance for industry *M12 Drug Interaction Studies* ([August 2024](#)). The lowest measured test concentration that resulted in a ≥ 2 -fold increase in CYP1A2 mRNA expression relative to vehicle control was approximately 926, 245, and 41 times the $I_{\max,u}$ and $C_{\max,u}$ for aficamten, CK-3834282, and CK-3834283, respectively, at the highest proposed therapeutic dose (aficamten 20-mg QD) (Study Nonclin-0494). Therefore, aficamten and its metabolites are not predicted to be potential in vivo CYP1A2 inducers in humans and a confirmatory clinical DDI study was not conducted to evaluate this potential.

In Vitro Interaction With Transporters

In vitro, aficamten (0.25 to 25 μM) was not a substrate of breast cancer resistance protein (BCRP), P-gp, organic anion transporting polypeptide (OATP) 1B1, or OATP1B3 when evaluated in Madin-Darby canine kidney-II (MDCK-II) cells (Study Nonclin-0009).

The potential for aficamten and its metabolites (CK-3834282 and CK-3834283) to inhibit efflux (BCRP and P-gp) and solute-linked carrier (i.e., organic cation transporter [OCT]2, OCT3, organic anion transporter [OAT]1, OAT3, OATP1B1, OATP1B3, multidrug and toxin extrusion proteins [MATE]1, and MATE2-K) transporters was evaluated in transporter-transfected MDCK-II cells (Studies Nonclin-0008, Nonclin-0127, and Nonclin-0169). Aficamten (50 μM) did not significantly inhibit BCRP-, OAT1-, OATP1B3-, OCT2, or OCT3-mediated transport. Clinically relevant inhibition of OATP1B1, OAT3, MATE1, and MATE2-K by aficamten is considered unlikely because the R values, calculated as $[I_{\max,u}]/IC_{50}$ for OCT2, OAT3, MATE1, and MATE2-K or as $1 + (\text{unbound fraction in plasma } [f_{u,p}] \times \text{estimated maximum plasma inhibitor concentration at the inlet to the liver } [I_{\text{in,max}}])/IC_{50}$ for OATP1B1, determined using the highest proposed therapeutic dose (aficamten 20-mg QD) were below the thresholds for potential risk of 0.1 and 1.1, respectively. The ratio for unbound hepatic inlet concentration ($C_{\max,u,\text{inlet}}/K_i$ or IC_{50} (OATP1B1 and OAT3) and $C_{\max,u}/K_i$ or IC_{50} (MATE1 and MATE2-K) determined at the highest therapeutic dose of aficamten 20-mg QD for both cases was below the threshold for potential risk of 0.1 and 0.02, respectively, per the ICH guidance for industry *M12 Drug Interaction Studies* ([August 2024](#)).

However, the potential for clinically relevant inhibition of P-gp by aficamten could not be excluded because the intestinal luminal concentration (calculated as dose/250 mL)/ IC_{50} was 48.8, which is above the threshold for potential risk of 10. Consequently, a clinical DDI study was conducted to evaluate the effect of aficamten on the pharmacokinetics of a sensitive P-gp substrate, dabigatran (Study CY 6014, Cohort 3).

In addition, the Applicant reports that aficamten metabolites, CK-3834282 and CK-383283 are not a sensitive substrate of drug transporters. CK-3834282 and CK-383283 are not expected to inhibit BCRP, OCT2, OCT3, OAT1, OAT3, OATP1B1, OATP1B3, MATE1, or MATE2-K drug transporters at clinically relevant concentrations.

14.2. In Vivo Studies

14.2.1. Study CY 6011: Single- and Multiple-Dose Pharmacokinetics in Healthy Subjects and Single-Dose Pharmacokinetics in CYP2D6 Poor Metabolizers

Study Design

This was a Phase 1, double-blind, randomized, placebo-controlled, multipart, single- and multiple-ascending dose (MAD) study to determine the safety and tolerability of aficamten in healthy subjects. This study was conducted in 5 parts.

Part 1 (SAD) and Part 2 (MAD)

Parts 1 and 2 used a double-blind, randomized, placebo-controlled design to evaluate the PK profile of aficamten following single-ascending doses (SADs) and MADs and to describe the PK/PD relationship between aficamten and cardiac function. In Part 1, a total of 57 subjects were randomized to receive a single oral dose of aficamten at ascending dose levels of 1, 3, 10, 25, 40, 50, and 75 mg capsules (n=42) or matching placebo (n=15). All 57 subjects completed Part 1. In Part 2, a total of 24 subjects were randomized to receive oral aficamten QD at ascending dose levels of 5, 7.5, and 10 mg capsules (n=18) or matching placebo (n=6) for 14 or 17 days. All 24 subjects completed Part 2.

Part 3 (CYP2D6 PM Cohort)

CYP2D6 genotypes were determined at Screening. Subjects identified as CYP2D6 PMs were excluded from the SAD (i.e., Part 1) and MAD (i.e., Part 2) cohorts and instead invited to participate in the separate CYP2D6 PM cohort (i.e., Part 3). A total of 33 subjects had CYP2D6 genotype and phenotype determined: 7 PMs (i.e., *3/*4, *4/*4, *4/*5, or *4/*12), 5 intermediate metabolizers (IMs) (i.e., *1/*4, *2A/*9, or *2A/unknown), and 21 normal metabolizers (NMs) (i.e., *1/*1, *1/*2, *1/*2A, *1/*10, *1/*41, *2/*2, *2A/*2A, *2A/*41, or *2AxN/*3) were identified. Part 3 used a double-blind, randomized, placebo-controlled, single-dose design to assess the effect of CYP2D6 genetic variants on the pharmacokinetics of aficamten. In Part 3, a total of 9 healthy CYP2D6-PMs were randomized to receive a single oral dose of aficamten 10 mg encapsulated granules capsule (n=7) or matching placebo (n=2). All 9 subjects completed Part 3.

Part 4 (Food Effect) and Part 5 (Relative Bioavailability)

Both parts 4 and 5 used an open-label, randomized, 2-way crossover design to evaluate the effect of a high-fat meal on the single-dose pharmacokinetics of aficamten (i.e., 1×10 mg encapsulated granules 5% [w/w] capsule) and the relative bioavailability between the initial clinical formulation (i.e., 2×5 mg encapsulated granules 5% [w/w] capsules) and the Phase 2 tablets (i.e., 2×5 mg 5% [w/w] blend tablets) formulation in healthy subjects, respectively. For Part 5, treatments were given in the fasted state and separated by a ≥14-day washout period. A total of 12 subjects were randomized and completed Part 5.

The food effect cohort (i.e., Part 4) in this study was assessed using a 10-mg capsule formulation while Study CY 6012 was conducted using a 20 mg TBM tablet formulation. Therefore, Study CY 6012 will be used to assess the food effect on aficamten pharmacokinetics and Part 4 of this study will not be covered in this review.

Study Results

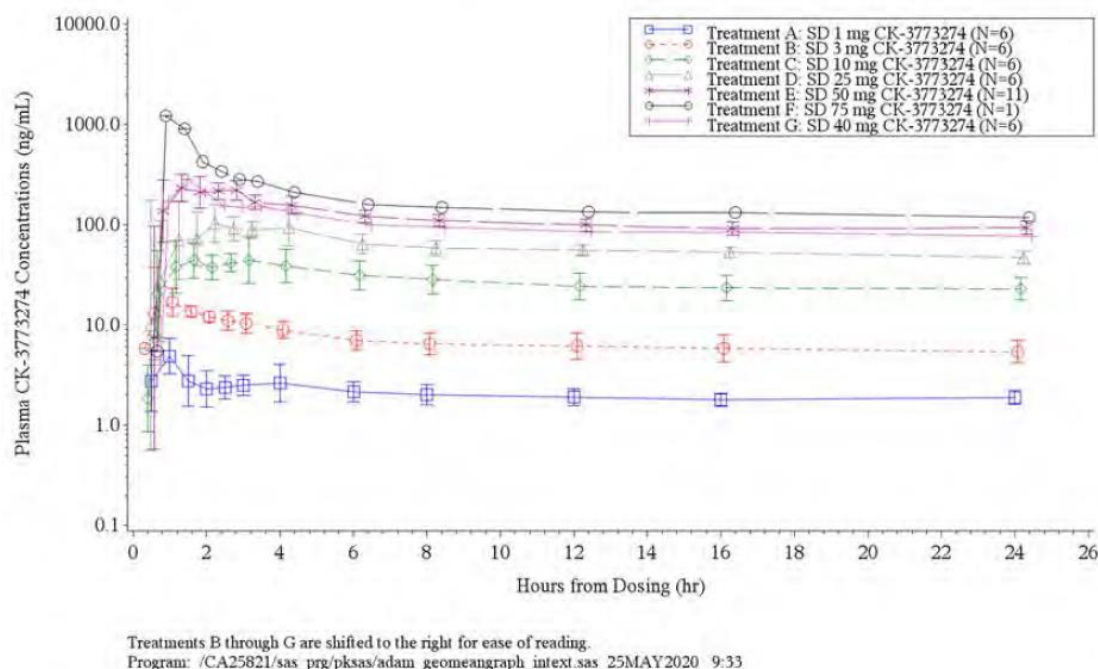
Part 1 (SAD)

Plasma aficamten AUC₂₄, AUC_∞, and C_{max} appeared to increase proportional to dose following single oral doses of 1, 3, 10, 25, 40, 50, and 75-mg aficamten ([Figure 14](#) and [Table 78](#)). Median t_{1/2} was consistent over the dose range (i.e., 75.2 to 81.6 hours).

Part 2 (MAD)

Plasma aficamten AUC₂₄ and C_{max} approximately doubled with twice the dose following single oral doses of 5 and 10-mg aficamten ([Figure 15](#) and [Table 79](#)). Similar results were observed following multiple-dose administration for 14 days. However, AUCs observed with 7.5-mg aficamten were comparable to those from 10-mg aficamten. The Applicant states that comparable exposures observed between 7.5 and 10-mg QD dosing may be due to the small sample size and variability.

Figure 14. Geometric Mean (90% CIs) Plasma Aficamten Concentration-Time Profiles Following the Administration of Single Oral Doses of Aficamten (0 to 24 Hours) (Part 1, SAD) (Semi-Log Scale)



Source: Figure 11-1, Study CY 6011 CSR

Abbreviations: CI, confidence interval; SAD, single-ascending dose

Table 78. Summary of Plasma Aficamten PK Parameters Following a Single Oral Dose of Aficamten at Ascending Dose Levels in Healthy Subjects, Study CY 6011, Part 1

Dose	Geometric Mean (Geometric CV%) [n] ^a							
	C _{max} (ng/mL)	t _{max} (h)	AUC ₂₄ (ng·h/ mL)	AUC _t (ng·h/ mL)	AUC _∞ (ng·h/ mL)	CL/F (L/h)	V _z /F (L)	t _½ (h)
1 mg	5.78 (38.4) [n = 6]	1.00 [0.50, 4.00] [n = 6]	50.4 (20.0) [n = 6]	122 (37.6) [n = 6]	NC	NC	NC	NC
3 mg	26.1 (82.4) [n = 6]	1.25 [0.50, 2.58] [n = 6]	174 (26.9) [n = 6]	699 (56.8) [n = 6]	823 (61.4) [n = 5]	3.64 (61.4) [n = 5]	378 (31.9) [n = 5]	79.6 [39.0, 98.4] [n = 5]
10 mg	70.5 (69.0) [n = 6]	2.76 [0.51, 3.00] [n = 6]	679 (34.6) [n = 6]	3088 (36.9) [n = 6]	3434 (24.3) [n = 4]	2.91 (24.3) [n = 4]	351 (29.1) [n = 4]	80.7 [68.6, 109] [n = 4]
25 mg	193 (23.3) [n = 6]	1.75 [0.50, 4.01] [n = 6]	1493 (14.5) [n = 6]	6085 (13.7) [n = 6]	7113 (15.1) [n = 6]	3.52 (15.1) [n = 6]	402 (14.1) [n = 6]	81.6 [67.5, 95.0] [n = 6]
40 mg	383 (67.9) [n = 6]	1.00 [0.50, 1.52] [n = 6]	2532 (13.0) [n = 6]	10770 (20.9) [n = 6]	11860 (20.1) [n = 5]	3.37 (20.1) [n = 5]	377 (17.6) [n = 5]	79.3 [56.0, 105] [n = 5]
50 mg	359 (38.9) [n = 11]	1.00 [0.50, 2.50] [n = 11]	2833 (26.3) [n = 11]	12260 (29.8) [n = 11]	13740 (34.8) [n = 9]	3.64 (34.8) [n = 9]	416 (29.4) [n = 9]	75.2 [64.8, 106] [n = 9]
75 mg ^b	1220 (NC) [n = 1]	0.50 [NC] [n = 1]	4556 (NC) [n = 1]	18140 (NC) [n = 1]	NC	NC	NC	NC

AUC = area under the concentration-time curve; AUC₂₄ = AUC from time 0 to 24 hours postdose; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; C_{max} = maximum concentration; CV% = percent coefficient of variation; n = number with estimable parameter; NC = not calculated; t_½ = terminal elimination half-life; t_{max} = time to maximum concentration; V_z/F = apparent volume of distribution.

^a Median [minimum, maximum] [n] presented for t_{max} and t_½.

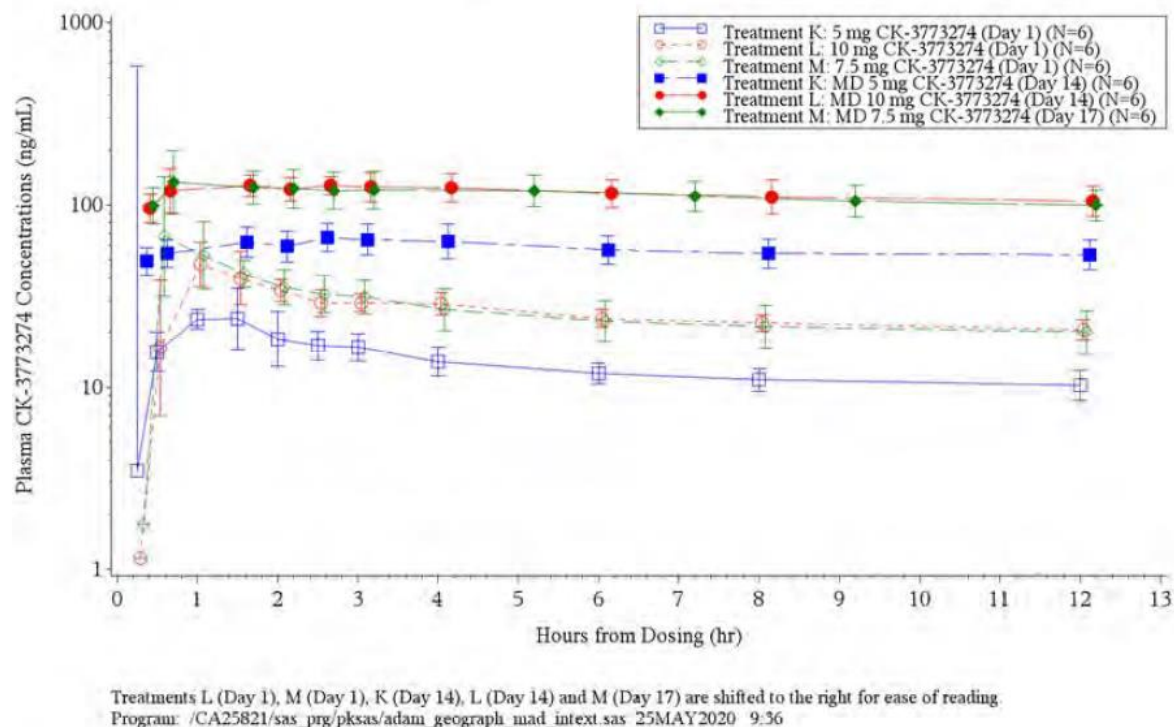
^b One participant in the 75 mg cohort had a decreased left ventricular ejection fraction < 45% on a postdose echocardiogram.

Consequently, the stopping criteria was met, and no further participants were dosed at this dose level.

Source: Table 3, Module 2.7.2

Abbreviation: PK, pharmacokinetic

Figure 15. Geometric Mean (90% CIs) Plasma Aficamten Concentration-Time Profiles Following the Administration of Single Oral Doses of Aficamten (Day 1) and Multiple Once Daily Oral Doses of Aficamten (Day 14/17) (0 to 12 Hours) (Part 2, MAD) (Semi-Log Scale)



Source: Figure 11-2, Study CY 6011 CSR

Abbreviations: CI, confidence interval; MAD, multiple-ascending dose

Table 79. Summary of Plasma and Urine Aficamten PK Parameters Following Multiple Oral Doses of Aficamten at Ascending Dose Levels in Healthy Subjects, Study CY 6011, Part 2

Day Regimen	Geometric Mean (Geometric CV%) [n] ^a							
	C _{max} ^b (ng/mL)	t _{max} ^b (h)	AUC ₂₄ ^b (ng·h/mL)	CL/F ^b (L/h)	t _{1/2} (h)	AR _{AUCtau}	fe (%)	CL _R (mL/h)
Day 1								
5 mg QD	28.0 (30.1) [n = 6]	1.25 [0.51, 2.50] [n = 6]	278 (21.0) [n = 6]	NC	NC	NC	0.030 (105) [n = 6]	5.33 (84.4) [n = 6]
7.5 mg QD	69.3 (66.5) [n = 6]	0.76 [0.49, 1.50] [n = 6]	551 (32.9) [n = 6]	NC	NC	NC	0.070 (42.6) [n = 6]	9.50 (44.0) [n = 6]
10 mg QD	54.4 (25.1) [n = 6]	1.01 [1.00, 2.51] [n = 6]	547 (10.9) [n = 6]	NC	NC	NC	0.066 (34.8) [n = 6]	12.1 (27.7) [n = 6]
Day 14								
5 mg QD	69.0 (23.2) [n = 6]	2.75 [1.50, 4.02] [n = 6]	1320 (23.0) [n = 6]	3.79 (23.0) [n = 6]	82.3 [75.5, 104] [n = 6]	4.75 (3.3) [n = 6]	NC	NC
10 mg QD	141 (19.7) [n = 6]	2.51 [0.52, 3.00] [n = 6]	2632 (22.8) [n = 6]	3.80 (22.8) [n = 6]	81.7 [53.2, 94.4] [n = 6]	4.82 (18.6) [n = 6]	NC	NC
Day 17								
7.5 mg QD	148 (39.5) [n = 6]	1.05 [0.51, 4.99] [n = 6]	2518 (25.8) [n = 6]	2.98 (25.8) [n = 6]	74.6 [61.0, 104.0] [n = 6]	4.57 (15.8) [n = 6]	NC	NC

AR_{AUCtau} = accumulation ratio based on AUC_{tau}; AUC = area under the concentration-time curve; AUC₂₄ = AUC from time 0 to 24 hours postdose; AUC_{tau} = AUC over the dosing interval; CL/F = apparent total clearance; CL_R = renal clearance; CL_{ss}/F = steady-state CL/F; C_{max} = maximum concentration; C_{max,ss} = steady-state C_{max}; CV% = percent coefficient of variation; fe = urinary excretion as % of unchanged drug; n = number with estimable parameter; NC = not calculated; QD = once daily; t_{1/2} = terminal elimination half-life; t_{max} = time to maximum concentration; t_{max,ss} = steady-state t_{max}.

^a Median [minimum, maximum] [n] presented for t_{max} and t_{1/2}.

^b C_{max,ss}, t_{max,ss}, AUC_{tau}, and CL_{ss}/F presented for Day 14 and 17.

Source: Table 4, Module 2.7.2

Abbreviation: PK, pharmacokinetic

Part 3 (CYP2D6 PM)

The t_{1/2} in CYP2D6 PM subjects ([Table 80](#)) was numerically longer than observed in non-PM subjects ([Table 78](#)) at the same dose in Part 1 (median: 84.9 versus 80.7 hours). The AUC_t and C_{max} were numerically higher (i.e., 9% and 18%, respectively) in PM phenotype relative to NM phenotype subjects, while AUC_∞ was similar between the 2 groups ([Table 81](#)). Overall, the Applicant concludes that PM subjects did not appear to have an impaired clearance that results in clinically meaningful difference in exposures.

Table 80. Summary of Plasma Aficamten PK Parameters Following a Single Oral Dose of 10-mg Aficamten in CYP2D6 PMs, Study CY 6011, Part 3

Parameter	Geometric Mean (Geometric CV%) [n] ^a
C _{max} (ng/mL)	57.3 (55.0) [n = 7]
t _{max} (h)	1.00 [0.50, 4.00] [n = 7]
AUC _t (ng·h/mL)	2604 (42.5) [n = 7]
AUC _∞ (ng·h/mL)	2966 (45.5) [n = 7]
AUC ₁₂ (ng·h/mL)	286 (25.2) [n = 7]
AUC ₂₄ (ng·h/mL)	495 (19.3) [n = 7]
CL/F (L/h)	3.37 (45.5) [n = 7]
V _z /F (L)	500 (17.9) [n = 7]
t _{1/2} (h)	84.9 [67.9, 189] [n = 7]

AUC = area under the concentration-time curve; AUC₁₂ = AUC from time 0 to 12 hours postdose; AUC₂₄ = AUC from time 0 to 24 hours postdose; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; C_{max} = maximum concentration; CV% = percent coefficient of variation; CYP = cytochrome P450; n = number with estimable parameter; t_{1/2} = terminal elimination half-life; t_{max} = time to maximum concentration; V_z/F = apparent volume of distribution.

^a Median [minimum, maximum] [n] presented for t_{max} and t_{1/2}.

Source: Table 8, Module 2.7.2

Abbreviation: PK, pharmacokinetic

Table 81. Summary of Statistical Assessment for Effect of CYP2D6 Phenotype (Normal vs. Poor Metabolizer) on Plasma Aficamten Exposure Following a Single Oral Dose of 10-mg Aficamten Under Fasted State, Study CY 6011

Parameter	CYP2D6 Phenotype	n	Geometric Mean	Poor/Normal Metabolizer Comparison	
				Geometric LS Mean Ratio (%)	90% CI (%)
AUC _t (ng·h/mL)	Poor	7	260	109	81, 147
	Normal	21	239		
AUC _∞ (ng·h/mL)	Poor	7	297	101	74, 138
	Normal	12	294		
C _{max} (ng/mL)	Poor	7	5.7	118	83, 169
	Normal	21	4.8		

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; C_{max} = maximum concentration; CYP = cytochrome P450; LS = least-squares; n = number with estimable parameter.

Source: Table 9, Module 2.7.2

Part 5 (Relative BA)

Plasma aficamten peak and total exposures were similar between encapsulated granules (i.e., Reference) and the Phase 2 tablet (i.e., Test) formulation ([Table 82](#) and [Table 83](#)). The geometric mean ratio 90% CIs of exposure for Test/Reference formulations were within 80% to 125% for all exposure metrics (AUC₁₂, AUC₂₄, AUC_t, and C_{max}) except AUC_∞, which was 78.5 to 147%.

The Phase 2 tablet formulation had an earlier $T_{\max}$ than encapsulated granules (median: 1.00 versus 2.05 hours postdose).

Table 82. Summary of Plasma Aficamten PK Parameters Following a Single Oral Dose of 10-mg Aficamten as Encapsulated Granules and Phase 2 Tablet Formulation, Study CY 6011, Part 5

Treatment Group	Geometric Mean (Geometric CV%) [n] ^a						
	$C_{\max}$ (ng/mL)	$t_{\max}$ (h)	AUC_t (ng·h/mL)	AUC_{∞} (ng·h/mL)	CL/F (L/h)	V_z/F (L)	$t_{1/2}$ (h)
Encapsulated Granules	40.2 (40.8) [n = 10]	2.05 [1.00, 3.01] [n = 10]	2130 (34.2) [n = 10]	2608 (33.9) [n = 8]	3.83 (33.9) [n = 8]	388 (32.1) [n = 8]	69.9 [50.7, 92.1] [n = 8]
Phase 2 Tablet	40.6 (37.5) [n = 11]	1.00 [0.50, 2.09] [n = 11]	2037 (37.8) [n = 11]	2798 (26.0) [n = 5]	3.57 (26.0) [n = 5]	332 (9.4) [n = 5]	67.3 [46.4, 86.4] [n = 5]

AUC = area under the concentration-time curve; AUC_{∞} = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; $C_{\max}$ = maximum concentration; CV = coefficient of variation; n = number with estimable parameter; $t_{1/2}$ = terminal elimination half-life; $t_{\max}$ = time to maximum concentration; V_z/F = apparent volume of distribution.

^a Median [minimum, maximum] [n] presented for $t_{\max}$ and $t_{1/2}$.

Source: Table 7, Module 2.7.1

Note: Subjects with %AUC_{extrap} >25% had their k_{el} dependent PK parameters excluded from the descriptive statistics (i.e., Subjects (b) (6) for the capsule formulation; Subjects (b) (6) for the tablet formulation).

Note: Subjects were excluded from summary statistics due to measurable predose concentration greater than 5% of $C_{\max}$ for that Period (i.e., Subjects (b) (6) and (b) (6) for the capsule treatment; Subject (b) (6) for the tablet formulation).

Abbreviation: PK, pharmacokinetic

Table 83. Summary of Statistical Assessment for Relative Bioavailability of Plasma Aficamten Between Encapsulated Granules and Phase 2 Tablet Formulation of 10-mg Aficamten, Study CY 6011, Part 5

Parameter	Treatment	n	Geometric LS Mean	Test/Reference Comparison	
				Geometric LS Mean Ratio (%)	90% CI (%)
AUC ₁₂ (ng·h/mL)	Phase 2 tablet (test)	11	297	99.7	95.0, 105
	Encapsulated granules (reference)	10	298		
AUC ₂₄ (ng·h/mL)	Phase 2 tablet (test)	11	530	97.4	94.7, 100
	Encapsulated granules (reference)	10	544		
AUC _t (ng·h/mL)	Phase 2 tablet (test)	11	2104	97.8	85.8, 112
	Encapsulated granules (reference)	10	2151		
AUC _∞ (ng·h/mL)	Phase 2 tablet (test)	5	2798	107	78.5, 147
	Encapsulated granules (reference)	8	2608		
C _{max} (ng/mL)	Phase 2 tablet (test)	11	41.3	103	95.8, 110
	Encapsulated granules (reference)	10	40.2		

AUC = area under the concentration-time curve; AUC₁₂ = AUC from time 0 to 12 hours postdose; AUC₂₄ = AUC from time 0 to 24 hours postdose; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CI = confidence interval; C_{max} = maximum concentration; LS = least-squares; n = number with estimable parameter.

Note: For AUC_∞, period and treatment sequence effects were removed from the model due to the lack of variability in the treatment, sequence, and period combinations.

Source: Table 8, Module 2.7.1

14.2.2. Study CY 6012: Food Effect of the To-Be-Marketed Formulation in Healthy Volunteers

Study Design

This was a Phase 1, open-label, randomized, 2-way crossover study to evaluate the effect of a high-fat meal on the single-dose pharmacokinetics of aficamten following administration as the proposed TBM tablet formulation in healthy subjects. A total of 18 subjects were randomized to treatment order, received a single oral dose of 20-mg aficamten (i.e., 1×20 mg 10% [w/w] blend tablet) under fasted and fed conditions, and completed the study. Treatments were separated by a ≥20-day washout period.

Study Results

Administration with a high-fat meal altered the rate but not the extent of absorption of aficamten, as indicated by the comparable total plasma exposure (i.e., AUC_t and AUC_∞) observed when aficamten was administered with and without food and the 15% lower C_{max} (i.e., geometric mean

ratio 90% CI: 72 to 101%) and delayed T_{max} (i.e., median: 2.19 versus 0.60 hours postdose) observed under fed condition (Table 84 and Table 85).

Table 84. Summary of Plasma PK Parameters of Aficamten Following Fasted- and Fed-State Administration of a Single Oral Dose of Aficamten 20 mg as Proposed TBM Tablet Formulation Study CY 6012

Analyte Treatment Group	Geometric Mean (Geometric CV%) [n] ^a						
	C_{max} (ng/mL)	t_{max} (h)	AUC_t (ng·h/mL)	AUC_{∞} (ng·h/mL)	CL/F (L/h)	V_z/F (L)	$t_{1/2}$ (h)
Aficamten							
Fed	101 (39.2) [n = 18]	2.19 [0.58, 4.08] [n = 18]	6008 (21.4) [n = 18]	6232 (22.2) [n = 18]	3.21 (22.2) [n = 18]	350 (20.5) [n = 18]	75.2 [51.5, 116] [n = 18]
Fasted	119 (47.0) [n = 18]	0.60 [0.26, 2.50] [n = 18]	5697 (19.4) [n = 18]	5910 (19.9) [n = 18]	3.38 (19.9) [n = 18]	378 (24.2) [n = 18]	78.5 [49.2, 117] [n = 18]

AUC = area under the concentration-time curve; AUC_{∞} = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; C_{max} = maximum concentration; CV = coefficient of variation; n = number with estimable parameter; NC = not calculated; $t_{1/2}$ = terminal elimination half-life; t_{max} = time to maximum concentration; V_z/F = apparent volume of distribution.

^a Median [minimum, maximum] [n] presented for t_{max} and $t_{1/2}$.

Source: Table 9, Module 2.7.1

Abbreviations: PK, pharmacokinetic; TBM, to-be-marketed

Table 85. Summary of Statistical Assessment for Food Effect on Plasma Aficamten Exposure Following a Single Oral Dose of Aficamten 20 mg as Proposed TBM Tablet Formulation, Study CY 6012

Analyte Parameter	Treatment	n	Geometric LS Mean	Fed/Fasted Comparison	
				Geometric LS Mean Ratio (%)	90% CI (%)
Aficamten					
AUC _t (ng·h/mL)	Fed	18	6008	105	103, 108
	Fasted	18	5697		
AUC _∞ (ng·h/mL)	Fed	18	6232	105	103, 108
	Fasted	18	5910		
C _{max} (ng/mL)	Fed	18	101	85.0	71.6, 101
	Fasted	18	119		

Source: Table 10, Module 2.7.1

Abbreviations: PK, pharmacokinetic; TBM, to-be-marketed

The Applicant's proposal to administer aficamten regardless to food intake is reasonable based on the outcome of this study.

14.2.3. Study CY 6014: DDI Potential With CYP3A Inhibitors, CYP Inducers, and P-gp Substrates in Healthy Volunteers

Study Design

This was a Phase 1, open-label, non-randomized, fixed-sequence, DDI study to assess the effect of itraconazole (i.e., strong CYP3A inhibitor) and carbamazepine (i.e., CYP inducer) on the pharmacokinetics of aficamten and to assess the effect of aficamten on the pharmacokinetics of dabigatran (i.e., administered as dabigatran etexilate, a sensitive P-gp probe) in healthy subjects. A total of 59 subjects were enrolled to receive the following treatments:

Cohort 1 (Itraconazole)

Single oral dose of aficamten 10 mg (i.e., 2 × 5-mg tablets) given 1 hour after a standardized breakfast on Day 1 and 1 hour after the itraconazole dose on Day 13. Itraconazole 200-mg QD on Days 10 to 21 given within 5 minutes of a standardized breakfast (n=17)

Cohort 2 (Carbamazepine)

Single oral dose of aficamten 20 mg (i.e., 4 × 5-mg tablets) within 5 minutes of a standardized breakfast on Day 1 and with the morning dose of carbamazepine on Day 28. Oral carbamazepine 100 mg twice daily (BID) on Days 10 to 12, 200-mg BID on Days 13 to 16, and 300-mg BID on Days 17 to 36 given within 5 minutes of a standardized meal (n=17)

Cohort 3 (Dabigatran)

Single oral dose of dabigatran etexilate 75 mg within 5 minutes of a standardized breakfast on Day 1 and a single oral dose of dabigatran etexilate 75 mg coadministered with a single oral dose of aficamten 20 mg (i.e., 4 × 5-mg tablets) given within 5 minutes of a standardized breakfast on Day 3 (n=25)

Study Results

Cohort 1

Seventeen subjects were enrolled and dosed, and all subjects completed the study in accordance with the protocol. A small increase in plasma aficamten AUC_{∞} was observed upon coadministration of aficamten with itraconazole (i.e., a strong CYP3A inhibitor) in both analysis of the entire PK population and sensitivity analysis excluding one subject with an unexplained decrease in exposures upon itraconazole coadministration ([Table 86](#)). The Applicant concludes that minimal increase in aficamten AUC_{∞} (i.e., approximately 26%) and lack of change in CK-3834282 and CK-3834283 metabolite-to-parent ratios observed upon itraconazole coadministration ([Table 87](#)) indicate that CYP3A does not contribute significantly to the formation of circulating metabolites and is responsible for only approximately 20% of aficamten elimination. As such, the Applicant believes that aficamten may be coadministered with CYP3A inhibitors without dose adjustment.

Table 86. Summary of Plasma PK Parameters of Aficamten, CK-3834282, and CK-3834283 Following a Single Oral Dose of 10-mg Aficamten Alone and Upon Coadministration With 200-mg Itraconazole QD, Study CY 6014, Cohort 1

Analyte Treatment	Geometric Mean (Geometric CV%) [n] ^a						
	C _{max} (ng/mL)	t _{max} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)	t _½ (h)	MR _{Cmax}	MR _{AUC∞}
Aficamten							
Aficamten	50.4 (51.9) [n = 17]	2.00 [1.00, 4.00] [n = 17]	2810 (22.3) [n = 17]	3410 (27.7) [n = 17]	83.4 [58.8, 140] [n = 17]	NA	NA
Aficamten + Itraconazole	42.4 (63.3) [n = 17]	2.00 [0.50, 4.00] [n = 17]	3020 (43.3) [n = 17]	3910 (45.0) [n = 17]	91.7 [64.9, 149] [n = 17]	NA	NA
Aficamten (Sensitivity Analysis^b)							
Aficamten	51.4 (53.1) [n = 16]	2.00 [1.00, 4.00] [n = 16]	2770 (22.5) [n = 16]	3320 (26.3) [n = 16]	80.6 [58.8, 111] [n = 16]	NA	NA
Aficamten + Itraconazole	47.6 (35.1) [n = 16]	2.00 [0.50, 4.00] [n = 16]	3280 (24.8) [n = 16]	4190 (34.0) [n = 16]	91.7 [64.9, 135] [n = 16]	NA	NA
CK-3834282							
Aficamten	17.8 (22.8) [n = 17]	6.00 [4.00, 36.00] [n = 17]	1890 (27.0) [n = 17]	2370 (24.0) [n = 17]	84.8 [60.7, 135] [n = 17]	0.338 (49.4) [n = 17]	0.664 (35.1) [n = 17]
Aficamten + Itraconazole	18.7 (61.7) [n = 17]	12.00 [3.00, 36.02] [n = 17]	2150 (57.7) [n = 17]	2850 (51.8) [n = 17]	107 [65.4, 161] [n = 17]	0.422 (47.2) [n = 17]	0.695 (46.0) [n = 17]
CK-3834283							
Aficamten	28.2 (24.7) [n = 17]	6.00 [4.00, 24.00] [n = 17]	2860 (23.6) [n = 17]	3670 (25.0) [n = 17]	87.9 [64.3, 176] [n = 17]	0.534 (50.7) [n = 17]	1.03 (26.7) [n = 17]
Aficamten + Itraconazole	29.8 (51.2) [n = 17]	6.00 [2.50, 36.02] [n = 17]	3160 (43.2) [n = 17]	4090 (39.0) [n = 17]	101 [64.4, 137] [n = 17]	0.67 (40.9) [n = 17]	0.998 (34.5) [n = 17]

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; C_{max} = maximum concentration; CV = coefficient of variation; MR_{AUC∞} = metabolite-to-parent ratio based on AUC_∞; MR_{Cmax} = metabolite-to-parent ratio based on C_{max}; n = number with estimable parameter; NA = not applicable; QD = once daily; t_½ = terminal elimination half-life; t_{max} = time to maximum concentration.

^a Median [minimum, maximum] [n] presented for t_{max} and t_½.

^b Sensitivity analysis excluding 1 participant who had a large unexplained decrease in aficamten exposures with itraconazole coadministration (75.0% and 82.3% decrease in AUC_∞ and C_{max}, respectively).

Source: Tables 14.2.1.2.1a, 14.2.1.2.2a, 14.2.1.4a, Study CY 6014 CSR

Abbreviations: PK, pharmacokinetic; QD, once daily

Table 87. Summary of Statistical Assessment for the Effect of Itraconazole Coadministration on Aficamten Plasma Exposure, Study CY 6014, Cohort 1

Parameter	Treatment	n	Geometric LS Mean	Aficamten + Itraconazole/ Aficamten Alone Comparison	
				Geometric LS Mean Ratio (%)	90% CI (%)
Pharmacokinetic Population Analysis					
AUC _t (ng·h/mL)	Aficamten	17	2810	108	90.9, 128
	Aficamten + itraconazole	17	3020		
AUC _∞ (ng·h/mL)	Aficamten	17	3410	115	96.2, 137
	Aficamten + itraconazole	17	3910		
C _{max} (ng/mL)	Aficamten	17	50.4	84.1	67.2, 105
	Aficamten + itraconazole	17	42.4		
Sensitivity Analysis ^a					
AUC _t (ng·h/mL)	Aficamten	16	2770	118	113, 124
	Aficamten + itraconazole	16	3280		
AUC _∞ (ng·h/mL)	Aficamten	16	3320	126	119, 134
	Aficamten + itraconazole	16	4190		
C _{max} (ng/mL)	Aficamten	16	51.4	92.7	79.3, 108
	Aficamten + itraconazole	16	47.6		

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CI = confidence interval; C_{max} = maximum concentration; LS = least-squares; n = number with estimable parameter.

^a Sensitivity analysis excluding 1 participant who had a large unexplained decrease in aficamten exposures with itraconazole coadministration (75.0% and 82.3% decrease in AUC_∞ and C_{max}, respectively).

Source: Tables 14.2.1.3.1a and 14.2.1.3.2a, Study CY 6014 CSR

One subject (i.e., Subject 001001- (b) (6)) had an unexplained decrease (75.0% and 82.3% decrease in AUC_{0-∞}, and C_{max}, respectively) in aficamten exposure in the presence of itraconazole. To evaluate whether this subject was skewing the data, sensitivity analysis in which data for this subject was excluded was performed for Cohort 1 for analysis of PK concentrations and parameters.

The Applicant's proposal to allow coadministration of aficamten with CYP3A inhibitors without dose adjustment is reasonable

Cohort 2

Seventeen subjects were enrolled and dosed of which 13 subjects completed the study in accordance with the protocol. Four subjects (i.e., Subjects 001001- (b) (6), 001001- (b) (6), 001001- (b) (6) and 001001- (b) (6)) discontinued the study due to AEs. Coadministration with carbamazepine (i.e., a CYP inducer and an agonist of constitutive androstane receptor [CAR] and constitutive androstane receptor [PXR]) decreased plasma aficamten AUC_∞ and C_{max} by approximately 50% and 30%, respectively, and increased the CK-3834282 and CK-3834283 MR_{AUC_∞} by approximately 1.3- and 1.5-fold, respectively ([Table 88](#)). Taken together with

findings from Cohort 1, which suggests that the observed induction of metabolite formation is unlikely to be only due to induction of the CYP3A pathway, Cohort 2 results indicate that aficamten elimination can be induced by other CAR- and/or PXR-mediated pathway(s). Based on these data, strong enzyme inducers (e.g., carbamazepine, phenobarbital, phenytoin, and rifampin) may need to be coadministered with caution while receiving aficamten.

Table 88. Summary of Plasma PK Parameters of Aficamten, CK-3834282, and CK-3834283 Following a Single Oral Dose of 20-mg Aficamten Alone and Upon Coadministration With 300-mg Carbamazepine BID, Study CY 6014, Cohort 2

Analyte Treatment	Geometric Mean (Geometric CV%) [n] ^a						
	C _{max} (ng/mL)	t _{max} (h)	AUC _t (ng·h/ mL)	AUC _∞ (ng·h/ mL)	t _½ (h)	MR _{Cmax}	MR _{AUC∞}
Aficamten							
Aficamten	102 (33.7) [n = 17]	1.50 [0.25, 4.00] [n = 17]	5400 (22.6) [n = 16]	6520 (27.3) [n = 16]	78.9 [60.1, 142] [n = 16]	NA	NA
Aficamten + Carbamazepine	69.5 (82.6) [n = 15]	1.50 [0.50, 4.00] [n = 15]	2960 (79.2) [n = 15]	3200 (71.8) [n = 15]	52.3 [40.6, 74.0] [n = 15]	NA	NA
CK-3834282							
Aficamten	31.0 (32.6) [n = 16]	4.00 [2.00, 24.00] [n = 16]	3030 (41.9) [n = 16]	3700 (34.0) [n = 16]	85.2 [52.8, 181] [n = 16]	0.295 (46.4) [n = 16]	0.541 (53.4) [n = 16]
Aficamten + Carbamazepine	33.8 (98.0) [n = 15]	4.00 [2.00, 23.55] [n = 15]	2040 (268.6) [n = 15]	2280 (218.1) [n = 15]	51.1 [6.2, 80.1] [n = 15]	0.465 (48.1) [n = 15]	0.681 (96.1) [n = 15]
CK-3834283							
Aficamten	57.1 (24.1) [n = 16]	10.00 [4.00, 48.00] [n = 16]	6030 (17.7) [n = 16]	7160 (17.3) [n = 16]	75.4 [46.3, 112] [n = 16]	0.544 (46.9) [n = 16]	1.05 (23.6) [n = 16]
Aficamten + Carbamazepine	64.8 (89.6) [n = 15]	8.02 [2.00, 23.55] [n = 15]	4680 (92.2) [n = 15]	5130 (83.9) [n = 15]	55.7 [38.5, 74.8] [n = 15]	0.891 (46.2) [n = 15]	1.53 (23.8) [n = 15]

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; BID = twice a day; C_{max} = maximum concentration; CV = coefficient of variation; MR_{AUC∞} = metabolite-to-parent ratio based on AUC_∞; MR_{Cmax} = metabolite-to-parent ratio based on C_{max}; n = number with estimable parameter; NA = not applicable; t_½ = terminal elimination half-life; t_{max} = time to maximum concentration.

^a Median [minimum, maximum] [n] presented for t_{max} and t_½.

Source: Tables 14.2.1.2b and 14.2.1.4c, Study CY 6014 CSR
Abbreviations: BID, twice daily; PK, pharmacokinetic

Table 89. Summary of Statistical Assessment for the Effect of Carbamazepine Coadministration on Aficamten Plasma Exposure, Study CY 6014, Cohort 2

Parameter	Treatment	n	Geometric LS Mean	Aficamten + Carbamazepine/ Aficamten Alone Comparison	
				Geometric LS Mean Ratio (%)	90% CI (%)
AUC _t (ng·h/mL)	Aficamten	16	5400	54.8	40.1, 74.9
	Aficamten + carbamazepine	15	2960		
AUC _∞ (ng·h/mL)	Aficamten	16	6520	49.1	36.4, 66.1
	Aficamten + carbamazepine	15	3200		
C _{max} (ng/mL)	Aficamten	17	102	68.9	50.0, 94.8
	Aficamten + carbamazepine	15	70.0		

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CI = confidence interval; C_{max} = maximum concentration; LS = least-squares; n = number with estimable parameter.

Source: Table 14.2.1.3b, Study CY 6014 CSR

Four subjects in Cohort 2 discontinued from the study due to AEs before full PK profiles could be generated. Two subjects (i.e., Subjects 001001-(b) (6) and 001001 to (b) (6)) were discontinued from the study on Day 33 and did not have any impact on the analysis of PK concentrations and parameters. One subject (i.e., Subject 001001-(b) (6)) was discontinued from the study on Day 28 with the last plasma sample collected on Day 28 (predose). One subject (Subject 001001-(b) (6)) was discontinued from the study on Day 2 with the last plasma sample collected on Day 1 (at 12 hours postdose). These two subjects were excluded from the statistical analysis.

Based on the results of this study with carbamazepine that showed approximately 50% decrease in AUC, the Applicant is proposing to reduce the dose of aficamten (i.e., 20 mg → 10 mg, 15 mg → 5 mg, 10 mg → 5 mg, or maintain the dose of 5 mg if currently on 5 mg and monitor LVEF and LVOT-G) when discontinuing a moderate to strong inducer of CYP2C9 or CYP3A. The Applicant's proposal is reasonable.

Cohort 3

Twenty-five subjects were enrolled and dosed, and all subjects completed the study in accordance with the protocol. A weak DDI was observed upon coadministration of aficamten with dabigatran etexilate, a sensitive P-gp probe ([Table 90](#)). Considering the small observed increase in the plasma free and total dabigatran AUC_∞ (approximately 24% and 26%, respectively) and C_{max} (approximately 25% and 27%, respectively), aficamten may be coadministered with P-gp substrates without dose adjustment ([Table 91](#)).

Table 90. Summary of Plasma PK Parameters of Free and Total Dabigatran Following a Single Oral Dose of 75-mg Dabigatran Etexilate Alone and Upon Coadministration With 20-mg Aficamten, Study CY 6014, Cohort 3

Analyte Treatment	Geometric Mean (Geometric CV%) [n] ^a						
	C _{max} (ng/mL)	t _{max} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)	CL/F (L/h)	V _z /F (L)	t _½ (h)
Free Dabigatran							
Dabigatran Etexilate	48.8 (57.4) [n = 25]	4.00 [1.00, 8.00] [n = 25]	417 (56.0) [n = 25]	428 (54.2) [n = 25]	175 (54.2) [n = 25]	1940 (58.4) [n = 25]	7.51 [5.79, 13.2] [n = 25]
Dabigatran Etexilate + Aficamten	61.1 (46.7) [n = 25]	3.00 [2.00, 8.00] [n = 25]	517 (44.8) [n = 25]	531 (44.6) [n = 25]	141 (44.6) [n = 25]	1700 (42.9) [n = 25]	8.81 [5.87, 13.4] [n = 25]
Total Dabigatran							
Dabigatran Etexilate	52.4 (56.6) [n = 25]	4.00 [1.00, 8.00] [n = 25]	448 (56.4) [n = 25]	470 (52.9) [n = 25]	160 (52.9) [n = 25]	1720 (52.6) [n = 25]	7.36 [5.74, 10.7] [n = 25]
Dabigatran Etexilate + Aficamten	66.5 (44.5) [n = 25]	4.00 [2.00, 8.00] [n = 25]	571 (43.5) [n = 25]	590 (42.3) [n = 25]	127 (42.3) [n = 25]	1480 (38.9) [n = 25]	7.93 [6.01, 12.6] [n = 25]

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; C_{max} = maximum concentration; CV = coefficient of variation; n = number with estimable parameter; NC = not calculated; t_½ = terminal elimination half-life; t_{max} = time to maximum concentration; V_z/F = apparent volume of distribution.

^a Median [minimum, maximum] [n] presented for t_{max} and t_½.

Source: Table 14.2.1.2c, Study CY 6014 CSR

Abbreviation: PK, pharmacokinetic

Table 91. Summary of Statistical Assessment for the Effect of Aficamten Coadministration on Total and Free Dabigatran Plasma Exposure, Study CY 6014, Cohort 3

Analyte Parameter	Treatment	n	Geometric LS Mean	Dabigatran Etexilate + Aficamten/ Dabigatran Etexilate Alone Comparison	
				Geometric LS Mean Ratio (%)	90% CI (%)
Free Dabigatran					
AUC _t (ng·h/mL)	Dabigatran etexilate	25	417	124	110, 140
	Dabigatran etexilate + aficamten	25	517		
AUC _∞ (ng·h/mL)	Dabigatran etexilate	25	428	124	111, 139
	Dabigatran etexilate + aficamten	25	531		
C _{max} (ng/mL)	Dabigatran etexilate	25	48.8	125	110, 143
	Dabigatran etexilate + aficamten	25	61.1		
Total Dabigatran					
AUC _t (ng·h/mL)	Dabigatran etexilate	25	448	127	113, 144
	Dabigatran etexilate + aficamten	25	571		
AUC _∞ (ng·h/mL)	Dabigatran etexilate	25	470	126	112, 141
	Dabigatran etexilate + aficamten	25	590		
C _{max} (ng/mL)	Dabigatran etexilate	25	52.4	127	111, 145
	Dabigatran etexilate + aficamten	25	66.5		

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CI = confidence interval; C_{max} = maximum concentration; LS = least-squares; n = number with estimable parameter.

Source: Table 14.2.1.3c, Study CY 6014 CSR

The Applicant's proposal of allowing aficamten to be coadministered with P-gp substrates without dose adjustment is reasonable.

14.2.4. Study CY 601-10: DDI Potential With CYP3A, CYP2C9, CYP2C19, and CYP2D6 Inhibitors in Healthy Volunteers

Study Design

This was a Phase 1, open-label, non-randomized, fixed-sequence, DDI study to evaluate the effect of fluconazole (i.e., strong inhibitor of CYP2C19 and moderate inhibitor of CYP3A and CYP2C9), paroxetine (i.e., strong selective inhibitor of CYP2D6), and fluoxetine (i.e., strong inhibitor of CYP2C19 and CYP2D6) on the pharmacokinetics of aficamten in healthy subjects. A total of 51 subjects were enrolled to receive the following treatments:

Cohort 1 (Fluconazole)

Single oral dose of aficamten 10 mg (2×5-mg tablets) given 1.5 hours after a standardized breakfast on Day 1 and 1 hour after fluconazole dose on Day 19. Fluconazole 400-mg QD on Days 10 to 27 given 30 minutes after a standardized breakfast (n=17).

Cohort 2 (Paroxetine)

Single oral dose of aficamten 10 mg (2×5-mg tablets) given 1.5 hours after a standardized breakfast on Day 1 and 1 hour after paroxetine dose on Day 26. Paroxetine 20-mg QD on Days 10 to 16 and 40-mg QD on Days 17 to 34 given 30 minutes after a standardized breakfast (n=17).

Cohort 3 (Fluoxetine)

Single oral dose of aficamten 10 mg (2×5-mg tablets) given 1.5 hours after a standardized breakfast on Day 1 and 1 hour after fluoxetine dose on Day 28. Fluoxetine 20-mg QD on Days 10 to 16 and 40-mg QD on Days 17 to 36 given 30 minutes after a standardized breakfast (n=17).

A washout period of 9 days separated aficamten dosing on Day 1 and the first dose of fluconazole, paroxetine, or fluoxetine.

In Cohorts 1 (fluconazole) and 3 (fluoxetine), subjects who were identified as CYP2C9 and CYP2C19 extensive (i.e., normal), rapid, or ultra-rapid metabolizers were enrolled while for Cohort 2 (paroxetine), subjects who were identified as CYP2D6 extensive (i.e., normal), rapid, or ultra-rapid metabolizers were enrolled.

The Applicant states that for Cohort 3, a 40-mg dose of fluoxetine was considered the highest clinical dose that could be administered safely in healthy subjects for 20 days. A survey of the literature indicated that maximum inhibition of CYP2D6 and CYP2C19 by fluoxetine is achieved at this dose ([Greenblatt et al. 1992](#); [Jeppesen et al. 1996](#); [Harvey and Preskorn 2001](#)). The effect of fluoxetine 60 mg, the approved maximum daily dose, was evaluated in PBPK modeling.

Study Results

Cohort 1

A total of 17 subjects entered Cohort 1 of the study and received at least one dose of study drug according to the protocol. A total of 16 subjects completed the study. One subject (i.e., 60110-001004- (b) (6)) withdrew from the study on Day 14 due to a personal reason (i.e., death in the family). Coadministration of aficamten with fluconazole led to an approximately 278% increase in plasma aficamten AUC_∞ with no change in C_{max} ([Table 93](#)). A prolongation of aficamten t_{1/2} (~3-fold) was also observed, suggesting inhibition of systemic clearance of aficamten by fluconazole, rather than an increase in aficamten bioavailability. Considering the magnitude of contribution of CYP3A to aficamten elimination observed in Study CY 6014 (i.e., increase in aficamten AUC_∞ of approximately 26% with coadministration with itraconazole), it was assumed that the increase in aficamten exposure in the presence of fluconazole was mediated primarily by inhibition of CYP2C9 and/or CYP2C19. The Applicant proposes to contraindicate the concomitant administration of aficamten with fluconazole when taken for more than 3 days. See Section [8.2](#) for the Clinical Pharmacology review team's dosing recommendations.

Table 92. Summary of Plasma Aficamten Pharmacokinetics Following 10-mg Aficamten on Day 1 and 10-mg Aficamten Coadministered With 400-mg QD Fluconazole on Day 19, PK Analysis Population, Trial 601-10, Cohort 1

Parameter (unit)	Aficamten Alone (N = 16)	Aficamten + Fluconazole (N = 16)
AUC _{0-t} (h*ng/mL)	2441.2 (15.6)	4981.4 (14.3)
AUC _{0-inf} (h*ng/mL)	2862.4 (19.0)	10873.4 (22.1)
C _{max} (ng/mL)	64.4 (30.0)	66.4 (44.4)
C _{last} (ng/mL)	3.6 (36.9)	16.5 (17.0)
t _{max} (hr)	1.53 (1.01, 2.00)	1.50 (0.99, 3.01)
t _{last} (hr)	215.92 (215.92, 215.93)	216.08 (216.05, 216.12)
t _{1/2} (hr)	76.70 (65.83, 90.40)	226.16 (209.71, 272.72)
CL/F (L/hr)	3.6 (16.7)	1.0 (20.0)
V _z /F (L)	394.7 (19.8)	327.4 (15.8)
Aficamten Alone: 10 mg aficamten on Day 1 Aficamten + Fluconazole: 400 mg fluconazole QD on Days 10-27 with 10 mg aficamten on Day 19, 1 hour post fluconazole dosing t_{1/2}, t_{max}, and t_{last} values are presented as median (Q1, Q3). All other parameters are presented as arithmetic mean (CV%). QD = Once Daily Source: Table 14.2.1.3 and Table 14.2.1.4 Program: \CA41800\sas_prg\pkas\adam_intext_pkparam.sas 19MAR2024 9:15		

Source: Table 11-1, Study CY 601-10 CSR
Abbreviations: PK, pharmacokinetic; QD, once daily

Table 93. Summary of Statistical Comparisons of Plasma Aficamten PK Parameters Following 10-mg Aficamten Coadministered With 400-mg QD Fluconazole on Day 19 vs. 10-mg Aficamten on Day 1, PK Analysis Population, Trial 601-10, Cohort 1

Parameter (unit)	Treatment B (Test)		Treatment A (Reference)				
	Geometric LSM	n	Geometric LSM	n	Geometric Mean Ratio (%)	90% Confidence Interval	Intra-participant CV%
AUC _{0-t} (h*ng/mL)	4932.61	16	2415.13	16	204.24	192.62, 216.56	9.47
AUC _{0-inf} (h*ng/mL)	10644.71	16	2818.81	16	377.63	346.79, 411.21	13.81
C _{max} (ng/mL)	61.37	16	62.06	16	98.89	86.64, 112.88	21.59
Treatment B: 400 mg fluconazole QD on Days 10-27 with 10 mg aficamten on Day 19, 1 hour post fluconazole dosing (test) Treatment A: 10 mg aficamten on Day 1 (reference) Parameters were ln-transformed prior to analysis. Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs derived from the ANOVAs. Geometric Mean Ratio (GMR) = 100 × (test/reference) Intra-participant CV% = 100 × square root (exp[MSE]-1), where MSE = Residual variance from ANOVA. QD = Once Daily Source: Table 14.2.1.6 Program: \CA41800\sas_prg\pkas\adam_intext_statsmixed.sas 19MAR2024 9:12							

Source: Table 11-2, Study CY 601-10 CSR
Abbreviation: PK, pharmacokinetic

Cohort 2

A total of 17 subjects entered Cohort 2 of the study; all 17 subjects received all doses of study drug and completed the study according to the protocol. Coadministration of aficamten with

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paroxetine led to an approximately 27% and 20% increase in plasma aficamten AUC_∞ and C_{max}, respectively ([Table 95](#)).

Table 94. Summary of Plasma Aficamten Pharmacokinetics Following 10-mg Aficamten on Day 1 and 10-mg Aficamten Coadministered With 40-mg QD Paroxetine on Day 26, PK Analysis Population, Trial 601-10, Cohort 2

Parameter (unit)	Aficamten Alone (N = 17)	Aficamten + Paroxetine (N = 17)
AUC _{0-t} (h*ng/mL)	2562.6 (21.0)	3088.5 (18.6)
AUC _{0-inf} (h*ng/mL)	3096.2 (26.0)	3910.6 (24.9)
C _{max} (ng/mL)	65.3 (47.3)	79.5 (58.9)
C _{last} (ng/mL)	4.2 (40.8)	5.8 (35.1)
t _{max} (hr)	1.55 (1.00, 1.98)	1.00 (0.75, 2.02)
t _{last} (hr)	215.92 (215.92, 215.92)	216.00 (216.00, 216.02)
t _{1/2} (hr)	86.14 (66.89, 94.79)	86.44 (81.52, 112.53)
CL/F (L/hr)	3.4 (26.8)	2.7 (24.8)
V _z /F (L)	387.4 (18.1)	348.7 (14.9)
Aficamten Alone: 10 mg aficamten on Day 1 Aficamten + Paroxetine: 20 mg paroxetine QD on Days 10-16, 40 mg paroxetine QD on Days 17-34 with 10 mg aficamten on Day 26, 1 hour post paroxetine dosing t_{1/2}, t_{max}, and t_{last} values are presented as median (Q1, Q3). All other parameters are presented as arithmetic mean (CV%). QD = Once Daily Source: Table 14.2.7.3 and Table 14.2.7.4 Program: \CA41800\sas_prg\pkas\adam_intext_pkparam.sas 19MAR2024 9:15		

Source: Table 11-10, Study CY 601-10 CSR

Abbreviations: PK, pharmacokinetic; QD, once daily

Table 95. Summary of Statistical Comparisons of Plasma Aficamten PK Parameters Following 10-mg Aficamten Coadministered With 40-mg QD Paroxetine on Day 26 vs. 10-mg Aficamten on Day 1, PK Analysis Population, Trial 601-10, Cohort 2

Parameter (unit)	Treatment D (Test)		Treatment C (Reference)				
	Geometric LSM	n	Geometric LSM	n	Geometric Mean Ratio (%)	90% Confidence Interval	Intra-participant CV%
AUC _{0-t} (h*ng/mL)	3037.79	17	2509.27	17	121.06	115.29, 127.12	8.17
AUC _{0-inf} (h*ng/mL)	3799.25	17	2997.82	17	126.73	119.21, 134.73	10.25
C _{max} (ng/mL)	72.00	17	59.78	17	120.44	102.88, 141.00	26.78
Treatment D: 20 mg paroxetine QD on Days 10-16, 40 mg paroxetine QD on Days 17-34 with 10 mg aficamten on Day 26, 1 hour post paroxetine dosing (test) Treatment C: 10 mg aficamten on Day 1 (reference) Parameters were ln-transformed prior to analysis. Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs derived from the ANOVAs. Geometric Mean Ratio (GMR) = 100 × (test/reference) Intra-participant CV% = 100 × square root (exp[MSE]-1), where MSE = Residual variance from ANOVA. QD = Once Daily Source: Table 14.2.7.6 Program: \CA41800\sas_prg\pkas\adam_intext_statsmixed.sas 19MAR2024 9:12							

Source: Table 11-11, Study CY 601-10 CSR

Abbreviations: PK, pharmacokinetic; QD, once daily

Cohort 3

A total of 17 subjects entered Cohort 3 of the study and received at least 1 dose of study drug according to the protocol. A total of 16 subjects completed the study. One subject (i.e., 60110-001004- (b) (6)) withdrew from the study on Day 1 due to a personal reason (i.e., family emergency). Coadministration of aficamten with fluoxetine led to a generally comparable increase in plasma aficamten exposure ([Table 96](#) and [Table 97](#)) as observed in Cohort 2 upon coadministration with paroxetine. Accordingly, the Applicant believes that the contributions of CYP2D6 and CYP2C19 to aficamten elimination do not meaningfully impact aficamten exposure, and aficamten may be coadministered with CYP2D6 and CYP2C19 inhibitors without dose adjustment.

Table 96. Summary of Plasma Aficamten Pharmacokinetics Following 10-mg Aficamten on Day 1 and 10-mg Aficamten Coadministered With 40-mg Fluoxetine QD on Day 28, PK Analysis Population, Trial 601-10, Cohort 3

Parameter (unit)	Aficamten Alone (N = 16)	Aficamten + Fluoxetine (N = 16)
AUC _{0-t} (h*ng/mL)	2752.1 (20.0)	3310.6 (18.7)
AUC _{0-inf} (h*ng/mL)	3222.0 (21.5)	4264.2 (22.8)
C _{max} (ng/mL)	69.4 (58.5)	103.8 (55.0)
C _{last} (ng/mL)	4.1 (33.4)	6.5 (32.0)
t _{max} (hr)	1.53 (0.99, 2.77)	1.03 (0.88, 1.53)
t _{last} (hr)	215.93 (215.92, 215.93)	216.01 (216.00, 216.03)
t _{1/2} (hr)	74.52 (64.98, 91.21)	95.36 (85.88, 103.55)
CL/F (L/hr)	3.2 (21.8)	2.5 (23.1)
V _Z /F (L)	355.0 (24.8)	339.3 (23.8)
Aficamten Alone: 10 mg aficamten on Day 1 Aficamten + Fluoxetine: 20 mg fluoxetine QD on Days 10-16, 40 mg fluoxetine QD on Days 17-36 with 10 mg aficamten on Day 28, 1 hour post fluoxetine dosing t_{1/2}, t_{max}, and t_{last} values are presented as median (Q1, Q3). All other parameters are presented as arithmetic mean (CV%). QD = Once Daily Source: Table 14.2.13.3 and Table 14.2.13.4 Program: \CA41800\sas_prg\pkas\adam_intext_pkparam.sas 19MAR2024 9:15		

Source: Table 11-19, Study CY 601-10 CSR
Abbreviation: PK, pharmacokinetic

Table 97. Summary of Statistical Comparisons of Plasma Aficamten PK Parameters Following 10-mg Aficamten Coadministered With 40-mg Fluoxetine QD on Day 28 vs. 10-mg Aficamten on Day 1, PK Analysis Population, Trial 601-10, Cohort 3

	Treatment F (Test)		Treatment E (Reference)				
Parameter (unit)	Geometric LSM	n	Geometric LSM	n	Geometric Mean Ratio (%)	90% Confidence Interval	Intra-participant CV%
AUC ₀₋₁ (h*ng/mL)	3253.83	16	2700.20	16	120.50	116.42, 124.73	5.56
AUC _{0-inf} (h*ng/mL)	4160.98	16	3153.51	16	131.95	124.62, 139.71	9.24
C _{max} (ng/mL)	94.12	16	60.72	16	155.01	127.54, 188.41	32.28
Treatment F: 20 mg fluoxetine QD on Days 10-16, 40 mg fluoxetine QD on Days 17-36 with 10 mg aficamten on Day 28, 1 hour post fluoxetine dosing (test)							
Treatment E: 10 mg aficamten on Day 1 (reference)							
Parameters were ln-transformed prior to analysis.							
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs derived from the ANOVAs.							
Geometric Mean Ratio (GMR) = 100 × (test/reference)							
Intra-participant CV% = 100 × square root (exp[MSE]-1), where MSE = Residual variance from ANOVA.							
QD = Once Daily							
Source: Table 14.2.13.6							
Program: \CA41800\sas_prg\pkas\adam_intext_statsmixed.sas 19MAR2024 9:12							

Source: Table 11-20, Study CY 601-10 CSR
Abbreviations: CV, coefficient of variation; PK, pharmacokinetic

Across all cohorts, CK-3834282 and CK-3834283 metabolite ratios (MR_{AUC∞}) decreased upon coadministration of aficamten with a CYP inhibitor. The Applicant believes that these results confirm that all evaluated DDI precipitant drugs act by altering the systemic CYP-mediated metabolism of aficamten.

Based on the study outcome of Cohort 3 in which fluoxetine increased the AUC_{0-inf} of aficamten by 32%, comparable to the increase in aficamten exposure in the paroxetine cohort (27%), the Applicant concludes that CYP2C19 plays a minor role in aficamten elimination.

Taken together, the Applicant believes that aficamten is primarily eliminated via CYP2C9-mediated metabolism with contributions from CYP3A (inferred from CY 6014), and relatively smaller contributions for metabolism via CYP2C19 and CYP2D6.

The Applicant's proposal of allowing coadministration of aficamten with CYP2D6 and CYP2C19 inhibitors is reasonable.

14.2.5. Study CY 6017: Effect of Hepatic Impairment on Aficamten Pharmacokinetics

Study Design

This was a Phase 1, open-label, non-randomized, 2-part, single-dose, parallel-design study to evaluate the pharmacokinetics of aficamten in subjects with HI compared to subjects with normal hepatic function. In Part 1, a total of 8 subjects with moderate HI (i.e., Group A) and 8 healthy subjects (i.e., matched controls; Group B) were enrolled and received a single oral dose of aficamten 20 mg (i.e., 4×5 mg Phase 3, 10% [w/w] blend tablets). The effect of mild HI was planned to be assessed in Part 2 but was not conducted based on review of the pharmacokinetics and safety assessment data from Part 1.

Study Results

Plasma aficamten AUC was similar between subjects with moderate HI (i.e., Child-Pugh score of ≥ 7 and ≤ 9) and normal hepatic function, while $C_{\max}$ was moderately (38%) higher in subjects with moderate HI relative to those with normal hepatic function (Table 98 and Table 99). There were no statistically significant differences in aficamten $t_{1/2}$ ($p=0.72$) and apparent clearance (CL/F) ($p=1.00$) between subjects with moderate HI and normal hepatic function (Study CY 6017 CSR Table 11 to 5).

Table 98. Summary of Plasma PK Parameters of Aficamten Following a Single Oral Dose of 20-mg Aficamten in Subjects With Moderate Hepatic Impairment and Normal Hepatic Function, Study CY 6017

Analyte Hepatic Function	Geometric Mean (Geometric CV%) [n] ^a							
	$C_{\max}$ (ng/mL)	$t_{\max}$ (h)	AUC_t (ng·h/ mL)	AUC_{∞} (ng·h/ mL)	$t_{1/2}$ (h)	CL/F (L/h)	$MR_{C_{\max}}$	$MR_{AUC_{\infty}}$
Aficamten								
Moderate Impairment	134 (59.7) [n = 8]	0.75 [0.50, 2.00] [n = 8]	6187 (45.3) [n = 8]	6473 (47.0) [n = 8]	106 [54.7, 131] [n = 8]	3.09 (47.0) [n = 8]	NA	NA
Normal (Matched Control)	99.0 (50.4) [n = 8]	1.50 [0.50, 6.00] [n = 8]	6666 (44.4) [n = 8]	7004 (51.0) [n = 8]	88.2 [64.0, 179] [n = 8]	2.86 (51.0) [n = 8]	NA	NA

AUC = area under the concentration-time curve; AUC_{∞} = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; $C_{\max}$ = maximum concentration; CV = coefficient of variation; $MR_{AUC_{\infty}}$ = metabolite-to-parent ratio based on AUC_{∞} ; $MR_{C_{\max}}$ = metabolite-to-parent ratio based on $C_{\max}$; n = number with estimable parameter; NA = not applicable; NC = not calculated; $t_{1/2}$ = terminal elimination half-life; $t_{\max}$ = time to maximum concentration.

^a Median [minimum, maximum] [n] presented for $t_{\max}$ and $t_{1/2}$.

Source: Table 11, Module 2.7.2

Abbreviation: PK, pharmacokinetic

Table 99. Summary of Statistical Assessment for the Effect of Moderate Hepatic Impairment on Plasma Exposure of Aficamten Following a Single Oral Dose of 20-mg Aficamten, Study CY 6017

Analyte Parameter	Hepatic Function	n	Geometric LS Mean	Moderate Hepatic Impairment/ Normal Hepatic Function	
				Geometric LS Mean Ratio (%)	90% CI (%)
Aficamten					
AUC _t (ng·h/mL)	Moderate hepatic impairment	8	6652	95.2	66.4, 136
	Normal (matched control)	8	6987		
AUC _∞ (ng·h/mL)	Moderate hepatic impairment	8	6984	94.9	63.8, 141
	Normal (matched control)	8	7363		
C _{max} (ng/mL)	Moderate hepatic impairment	8	142	138	83.5, 230
	Normal (matched control)	8	103		

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CI = confidence interval; C_{max} = maximum concentration; LS = least-squares; n = number with estimable parameter.

Source: Table 12, Module 2.7.2

Results from this study indicate no clinically relevant changes in aficamten pharmacokinetics in subjects with moderate HI versus matched control subjects with normal hepatic function. These findings can be extrapolated to subjects with mild HI, suggesting that no dose adjustment of aficamten is warranted in subjects with mild or moderate HI.

14.2.6. Study CY 6013: Mass Balance

Study Design

This was a phase 1, open-label, nonrandomized, single-dose study to determine the mass balance of ¹⁴C-aficamten following oral administration; to characterize the pharmacokinetics of aficamten and its metabolites, CK-3834282 and CK-3834283, in plasma and urine; and to investigate the metabolite profile of ¹⁴C-aficamten in plasma, urine, and feces in healthy male subjects. A total of 8 subjects were enrolled, received a single oral dose of a 20 mg capsule containing approximately 100 µCi of ¹⁴C-aficamten, and completed the study. All subjects fasted overnight for at least 10 hours and refrained from consuming water for 1 hour predose and 2 hours postdose. Subjects fasted until approximately 4 hours postdose.

Study Results

Following administration of ¹⁴C-aficamten 20 mg, absorption of aficamten was moderate and formation of metabolites, M1a (CK-3834282) and M1b (CK-3834283) was steady with C_{max} observed 3 hours later than parent (median T_{max}: 5.00 versus 2.00 hours postdose). The median t_{1/2} of aficamten was 99.6 hours and similar t_{1/2} were observed for the metabolites (i.e., 94.8 and 95.4 hours), suggesting that M1a (CK-3834282) and M1b (CK-3834283) elimination is formation rate-limited ([Table 100](#)).

Table 100. Summary of Plasma PK Parameters for Aficamten, M1a (CK-3834282), M1b (CK-3834283), and Total Radioactivity Following a Single Oral Dose of 20 mg ¹⁴C-Aficamten in Healthy Male Subjects, Study CY 6013

Analyte	Geometric Mean (CV%) [n] ^a						
	C _{max} (ng/mL)	t _{max} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)	CL/F (L/h)	V _z /F (L)	t _{1/2} (h)
Aficamten	61.8 (56.8) [n = 8]	2.00 [1.00, 6.00] [n = 8]	4120 (31.5) [n = 8]	4440 (31.8) [n = 8]	4.50 (32.1) [n = 8]	690 (35.0) [n = 8]	99.6 [81.5, 209] [n = 8]
CK-3834282	23.8 (31.1) [n = 8]	5.00 [3.00, 36.0] [n = 8]	2380 (61.7) [n = 8]	2610 (52.7) [n = 8]	NC	NC	94.8 [76.8, 208] [n = 8]
CK-3834283	42.3 (29.3) [n = 8]	5.00 [3.00, 36.0] [n = 8]	4590 (32.6) [n = 8]	4790 (31.5) [n = 8]	NC	NC	95.4 [77.6, 180] [n = 8]
Total Radioactivity ^b	111 (43.2) [n = 8]	3.50 [1.00, 6.00] [n = 8]	13700 (28.8) [n = 8]	15300 (26.0) [n = 8]	NC	NC	98.2 [80.1, 230] [n = 8]
Whole Blood Total Radioactivity ^b	66.7 (44.9) [n = 8]	4.50 [1.00, 16.0] [n = 8]	5710 (38.5) [n = 8]	8660 (23.7) [n = 8]	NC	NC	100 [86.2, 171] [n = 8]

AUC = area under the concentration-time curve; AUC_∞ = AUC from time 0 extrapolated to infinity; AUC_t = AUC from time 0 to the time of the last quantifiable concentration; CL/F = apparent total clearance; C_{max} = maximum concentration; CV% = percent coefficient of variation; n = number with estimable parameter; NC = not calculated; t_{1/2} = terminal elimination half-life; t_{max} = time to maximum concentration; V_z/F = apparent volume of distribution.

^a Median [minimum, maximum] [n] presented for t_{max} and t_{1/2}.

^b C_{max} and AUCs are presented in mass equivalents units (ngEq·h/mL and ngEq/mL, respectively).

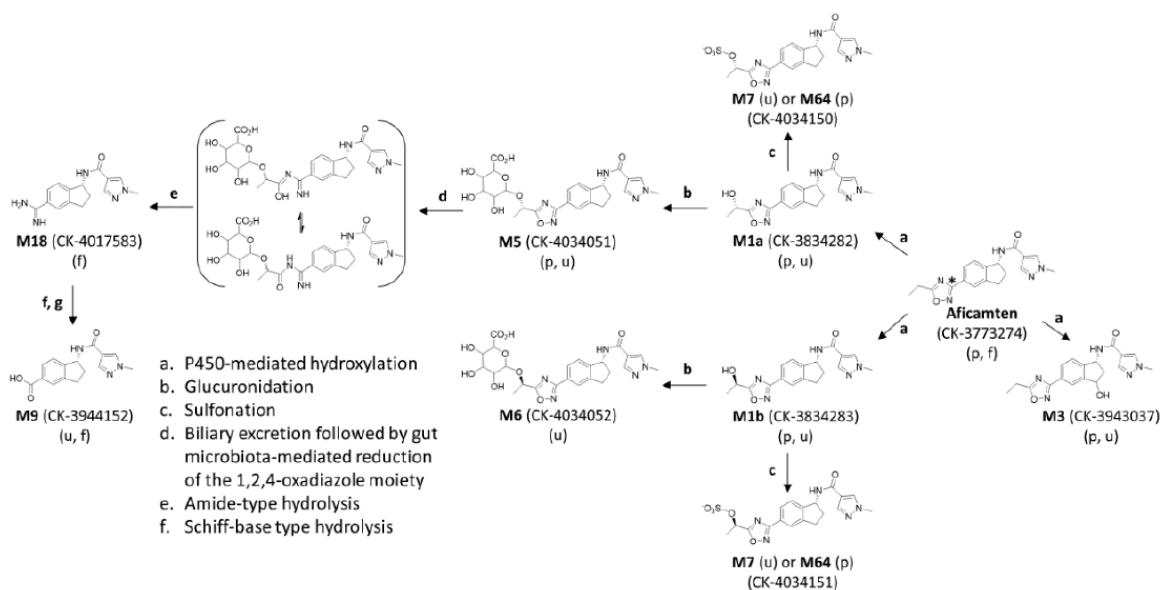
Source: Table 7, Module 2.7.2

Abbreviations: M, metabolite; PK, pharmacokinetic

At 600 hours postdose, the overall mean recovery of total radioactivity was 89.7% (range: 76.4% to 98.1%). Feces was the primary route of excretion and accounted for 57.7% of total radioactivity recovered, while urine accounted for 32.0% of total radioactivity recovered. Urine recovery of aficamten and metabolites, M1a (CK-3834282) and M1b (CK-3834283), was low, representing 0.06%, 5.3%, and 2.3%, respectively, of the administered dose. Corresponding renal clearance was 2.6, 443, and 105 mL/h, respectively.

Aficamten was primarily eliminated by metabolism and, to a lesser extent, by fecal excretion as unchanged aficamten (5.1%). Metabolism was mediated primarily by oxidation and reduction, with secondary pathways of glucuronidation, hydrolysis, and sulfonation ([Figure 16](#)). In plasma, the most abundant circulating components were aficamten, M1a (CK-3834282), M1b (CK-3834283), and M5 (CK-4034051), which accounted for 19.8%, 10.5%, 36.4%, and 10.3%, respectively, of total circulating radioactivity. Urinary metabolites were generally minor in abundance (i.e., <10% of dose) and the only major fecal metabolite identified was M18 (CK-4017583), which accounted for 44.1% of dose.

Figure 16. Biotransformation Pathways of Aficamten in Human



Note: Metabolite in brackets was not identified. * denotes the site of ¹⁴C-label in the 1,2,4-oxadiazole moiety at the 3-position carbon atom. (p), (u), and (f) indicate presence in plasma, urine, and feces, respectively.
Source: Figure 1, Module 2.7.2

14.2.7. Study CY 6019: Single-Dose Pharmacokinetics and Effect on QTc Interval in Healthy Subjects

Study Design

This was a randomized, double-blind, positive- and placebo-controlled, 3-way crossover design to evaluate effect of aficamten and its metabolites (i.e., CK-3834282 and CK-3834283) on the corrected value of the interval between the Q- and T-waves on the electrocardiogram tracing (QTc) interval. Subjects were randomized to treatment order and received a single oral dose of aficamten 50 mg (i.e., 10×5-mg aficamten tablets), placebo for aficamten, and 400-mg moxifloxacin (i.e., 1×400-mg tablet) separately in three treatment periods. Treatments were separated by a ≥21-day washout period.

Study Results

Reference is made to the QT Study Review from the Interdisciplinary Review Team for Cardiac Safety Studies dated July 22, 2024 (Reference ID: 5412605) in DARRTS. The Interdisciplinary Review Team concluded that aficamten did not cause a clinically meaningful change in the QTc interval following a single 50-mg dose.

14.2.8. Study CY 6021: Dose Finding

Study Design

This was a Phase 2, multicenter, randomized, double-blind, placebo-controlled, dose-finding study in subjects with HCM with the following key objectives: (1) to determine the safety and

tolerability of aficamten; (2) to describe the concentration- and dose-response relationship of aficamten on the resting and Valsalva LVOT-G over 10 weeks of treatment; (3) to evaluate the concentration-response relationship of aficamten on resting LVEF over 10 weeks of treatment; (4) to describe the PK/PD relationships of aficamten; and (5) to assess the effect of background HCM therapy on the PK and PD response of aficamten.

The clinical development program sought to maintain aficamten plasma concentrations required for efficacy, but with the understanding that supratherapeutic concentrations may result in systolic dysfunction, leading to decreases in stroke volume and cardiac output, compensatory increases in heart rate, and potentially clinical heart failure. Therefore, the goal of dose selection was to find the lowest possible dose to safely achieve resting and Valsalva LVOT-G targets (i.e., resting LVOT-G of <30 mm Hg and Valsalva LVOT-G of <50 mm Hg) and maintain normal LVEF (i.e., LVEF $\geq$ 50%).

Doses for the Phase 3 study were selected based on the results from subjects with oHCM enrolled in Cohorts 1 and 2 of the study and therefore, this review focuses on Cohorts 1 and 2. Cohorts 1 and 2 enrolled subjects with oHCM on background therapy including beta-blockers and/or calcium channel blockers, either as monotherapy or combined, but excluding the use of disopyramide. Subjects with symptomatic oHCM were randomized in a 2:1 ratio to aficamten or placebo and received up to 3 escalating doses of aficamten (i.e., 5, 10, and 15 mg in Cohort 1 and 10, 20, and 30 mg in Cohort 2) or placebo once daily for 10 weeks each guided by echocardiographic results. The dose titration criteria based on echocardiograms used in this study is summarized in [Table 101](#).

Table 101. Echocardiogram Criteria for Scheduled Dose Titrations, Study CY 6021, Cohorts 1 and 2

Biplane LVEF		Resting LVOT-G		Valsalva LVOT-G	Action^a
< 50%		—		—	Reduce dose ^b
$\geq$ 50%	and	< 30 mmHg	and	< 50 mmHg	No dose change
$\geq$ 50%	and	$\geq$ 30 mmHg	and/or	$\geq$ 50 mmHg	Increase dose by 5 mg

CSR = clinical study report; LVEF = left ventricular ejection fraction; LVOT-G = left ventricular outflow tract gradient.

^a If none of these conditions are met, then the dose remains unchanged.

^b If LVEF was < 50% on the lowest dose in a cohort, the participant received placebo. If a participant's dose was down-titrated to placebo, then they remained on placebo for the duration of the study.

Source: Table 4, Module 2.7.3

There was a 4-week post-treatment follow-up period following the active treatment period. Sparse sampling for pharmacokinetics was performed in this study, and the PK data collected were used to inform the Population PK (PPK) analysis.

Study Results

The 5 mg starting dose was PD effective (i.e., had responders achieving resting and Valsalva LVOT-G targets and maintaining normal LVEF) and well-tolerated by all subjects. There were 11 out of 41 subjects in Cohorts 1 and 2 who required doses higher than 10 mg to achieve target LVOT-G ([Table 8](#)). In Cohort 1, 5 subjects were escalated to the 15-mg dose. In Cohort 2, 6

subjects were escalated to the 20-mg dose; 4 subjects achieved target gradients at this dose level (although 1 subject was down-titrated to 10 mg due to LVEF <50% per site-read echocardiography), while 2 subjects were escalated further to 30 mg ([Table 8](#)). One of the 2 subjects on the 30-mg dose was down-titrated to 20 mg due to having a LVEF of 42% at Week 6 (per site-read echocardiography), and this subject also had a temporal treatment-emergent adverse event (TEAE) of peripheral edema (of moderate severity). The Applicant believes that data suggested that the 30-mg dose is not likely to add substantial clinical benefit versus risk; therefore, 20 mg was selected as the highest dose in the Phase 3 study. The Applicant's dose selection for the Phase 3 study appears to be reasonable.

14.2.9. Trial CY 6031: Dose Proportionality and Phase 3 Trial Formulation

The Phase 3 Trial (CY 6031) was conducted using multiple units of 5-mg aficamten tablets instead of the TBM formulation of 10 mg, 15 mg, and 20-mg aficamten tablets. Therefore, the establishment of dose proportionality across the proposed TBM dose range is important.

The dose proportionality assessment of multiple QD doses of aficamten was limited to 5 mg to 10 mg in healthy subjects (Study CY 6011, Part 2) due to the limit of tolerability of LVEF reduction in this population. On April 18, 2025 (SDN: 030), the Applicant submitted the information below describing their assessment of dose proportionality for doses of 15-mg QD and 20-mg QD.

The Applicant has examined dose proportionality of aficamten across the therapeutic dose range (5-mg QD to 20-mg QD) in the Phase 3 trial (CY 6031) in subjects with oHCM using PPK analysis. A two-compartment model with linear absorption and elimination was used to describe the pharmacokinetics of aficamten. The summary of the post hoc PK estimates at different dose levels where all subjects were given the same dose of aficamten is shown in [Table 102](#). Results demonstrate dose proportionality up to the highest clinical dose of 20-mg QD.

Table 102. Simulated Steady-State Exposure by Dose Level in Subjects With oHCM, Trial CY 6031

	5 mg (N=142)	10 mg (N=142)	15 mg (N=142)	20 mg (N=142)
AUC _τ (ng·h/mL)				
Mean (CV%)	2140 (41.0%)	4280 (41.0%)	6430 (41.0%)	8570 (41.0%)
Median [Min, Max]	1960 [1050, 7860]	3920 [2100, 15700]	5880 [3140, 23600]	7840 [4190, 31400]
C _{max} (ng/mL)				
Mean (CV%)	92.5 (41.1%)	185 (41.1%)	278 (41.1%)	370 (41.1%)
Median [Min, Max]	82.8 [44.8, 336]	166 [89.7, 673]	248 [135, 1010]	331 [179, 1350]
C _τ (ng/mL)				
Mean (CV%)	80.4 (44.7%)	161 (44.7%)	241 (44.7%)	322 (44.7%)
Median [Min, Max]	73.1 [33.6, 318]	146 [67.2, 636]	219 [101, 954]	292 [135, 1270]

AUC_τ = area under the concentration-time curve over the dosing interval; C_{max} = maximum concentration;

C_τ = trough concentration

Note: Exposures were simulated using posthoc PK parameters of the oHCM participants in CY 6031 assuming all given the same dose

Source: Figure 1, NDA 219083, SDN: 030 (submitted on April 18, 2025)

Abbreviation: N, number of subjects

Based on the dose proportionality established between the proposed TBM doses of 5 mg to 20-mg QD, the Biopharmaceutics review team has granted a biowaiver for the 10 mg, 15 mg, and 20-mg aficamten tablets (Refer to Integrated Quality Review in DARRTS).

14.3. Bioanalytical Method Validation and Performance

Bioanalysis of aficamten, its metabolites (i.e., CK-3834282 and CK-3834283), and other analytes in plasma and urine were conducted using validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods. Bioanalytical methods used in each study are summarized in [Table 103](#). This review focuses on studies that are relevant to regulatory decision making (highlighted in [Table 103](#) below).

Table 103. Bioanalytical Methods Supporting the Clinical Pharmacology Assessment of Aficamten and Its Metabolites CK-3834282 and CK-3834283

Analyte	Biomatrix	Bioanalytical Method; Laboratory	Clinical Studies Supported
Aficamten	Human plasma	CA26485-01; (b) (4)	CY 6011, CY 6012, CY 6013, CY 6014, CY 6017, CY 6019, CY 601-10, CY 6021, CY 6022, and CY 6031
		(b) (4)-275-CK-3773274/VR/P; (b) (4)	JX01001
		SZLCMS 9; (b) (4)	CY 6031
	Human urine	CA27882-01; (b) (4)	CY 6011 and CY 6013
		(b) (4)-275-CK-3773274/VR/U; (b) (4)	JX01001
Aficamten Metabolites (CK-3834282 and CK-3834283)	Human plasma	CA27903-01; (b) (4)	CY 6011
		CA29736-01; (b) (4)	CY 6012, CY 6013, CY 6014, CY 6017, CY 6019, CY 601-10, CY 6021, CY 6022, and CY 6031
		(b) (4)-275-CK-3773274-M/VR/P; (b) (4)	JX01001
		SZLCMS 10; (b) (4)	CY 6031
	Human urine	CA37830-01; (b) (4)	CY 6013
Moxifloxacin	Human plasma	ZZ00945-02; (b) (4)	CY 6019
Free Dabigatran (Unconjugated)	Human plasma	ZZ55164-01; (b) (4)	CY 6014
Total Dabigatran (Unconjugated and Conjugated)	Human plasma	ZZ55164-02; (b) (4)	CY 6014

(b) (4)

Source: Table 5, Module 2.7.1

Table 104. Summary of Method Performance for Determination of Aficamten in Human Plasma (CA26485-01)

Bioanalytical Method Validation Report Name, Amendments, and Hyperlinks	Validation of an LC-MS/MS Method for the Determination of CK-3773274 in Human Plasma (EDTA) Method Validation Report CA26485-01 Amendment 4		
Method Description	An aliquot of human plasma (EDTA) containing the analyte and internal standard is extracted using a protein precipitation procedure. The extracted samples are analyzed by an HPLC equipped with an AB SCIEX API 5000 triple quadrupole mass spectrometer using an ESI source. Positive ions are monitored in the MRM mode.		
Materials Used for Standard Calibration Curve and Concentration	Aficamten (CK-3773274), (b) (4) Lot 641-155 Calibration standards: 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 150, 420, and 500 ng/mL <u>Internal standard:</u> Deuterated aficamten (CK-3942912), Cytokinetics, Inc, Lot 01		
Validated Assay Range	1.00 to 500 ng/mL		
Material Used for QCs and Concentration	Aficamten (CK-3773274), (b) (4) QC concentration levels: 1.00 (LLOQ), 3.00 (LQC), 22.0 (MQC), and 375 (HQC) ng/mL <u>Internal standard:</u> Deuterated aficamten (CK-3942912), Cytokinetics, Inc, Lot 01		
Minimum Required Dilutions	NR		
Source and Lot of Reagents	Control human plasma (K ₂ EDTA), (b) (4) Ultrapure deionized water, Type-1, (b) (4) Acetonitrile, HPLC Grade (b) (4) Ammonium acetate, ACS Grade, BDH, (b) (4) N,N-dimethylformamide, (b) (4) Formic acid, 88%, AR (ACS) Grade, (b) (4) (b) (4) Methanol, ACS/HPLC Grade (b) (4) Trifluoroacetic acid, (b) (4)		
Regression Model and Weighting	Linear, weighted (1/concentration ²)		
Validation Parameters	Method Validation Summary		Source Location
Standard Calibration Curve Performance During Accuracy and Precision Runs	Number of standard calibrators from LLOQ to ULOQ	9	CA26485-01 Amendment 4 Table 4
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.0% to 2.0%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 4.8%	

Validation Parameters	Method Validation Summary		Source Location
Performance of QCs During Accuracy and Precision Runs	<u>Cumulative accuracy (%bias) in 4 QC levels</u>	-9.2% to 2.7%	CA26485-01 Amendment 4 Table 2
	<u>Interbatch %CV</u>	≤ 11.8%	
	<u>Total error</u>	NR	NA
Selectivity and Matrix Effect	No significant matrix effect was observed in any of the 10 lots of plasma that were fortified with aficamten at the LLOQ and HQC levels. Accuracy (%bias): 2.0% to 2.4% Precision (%CV): ≤ 3.3% Selectivity for aficamten in a cocktail of dabigatran, carbamazepine, and itraconazole was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -5.6% to -5.3% Precision (%CV): ≤ 3.5% Selectivity for aficamten in the presence of moxifloxacin was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -9.6% to -1.0% Precision (%CV): ≤ 2.6% Selectivity for aficamten in a cocktail of acetaminophen, atorvastatin, caffeine, cetirizine, dextromethorphan, ethinyl estradiol, famotidine, ibuprofen, levonorgestrel, metformin, omeprazole, ondansetron, pseudoephedrine, and salicylic acid was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -7.7% to 4.5% Precision (%CV): ≤ 6.3% Selectivity for aficamten in the presence of metabolites CK-3834282 and CK-3834283 was demonstrated at the LQC level. Accuracy (%bias): 3.3% Precision (%CV): 1.8%		CA26485-01 Amendment 4 Table 10, Table 13, Table 14, Table 15, and Table 16
Interference and Specificity	No significant interference (0.0%) at the retention time and mass transition of aficamten or internal standard was observed from endogenous components in any of the 10 lots of plasma screened.		CA26485-01 Amendment 4 Table 9
Hemolysis Effect	No significant effect of hemolysis in any of 3 lots of plasma at the LLOQ and HQC levels. Accuracy (%bias): -3.7% to -2.7% Precision (%CV): ≤ 4.4%		CA26485-01 Amendment 4 Table 17
Lipemic Effect	No significant effect of lipemia in any of 3 lots of plasma at the LLOQ and HQC levels. Accuracy (%bias): 2.7% to 6.0% Precision (%CV): ≤ 4.5%		CA26485-01 Amendment 4 Table 18
Dilution Linearity and Hook Effect	10-fold dilution of 2500 ng/mL 100-fold dilution of 40000 ng/mL Accuracy (%bias): 0.4% to 1.3% Precision (%CV): ≤ 4.1% Hook effect: NA		CA26485-01 Amendment 4 Table 33

Validation Parameters	Method Validation Summary	Source Location
Bench-top/Process Stability	151 hours in a polypropylene 96-well plate at 5°C for postprocessed samples 150 hours in a polypropylene 96-well plate at 5°C for processed samples 24 hours in polypropylene tubes at ambient temperature under white light 51 hours in polypropylene tubes at ambient temperature under white light (total of all thaw cycles)	CA26485-01 Amendment 4 Table 23, Table 25, and Table 34
Freeze-thaw Stability	6 cycles at -20°C and -80°C in polypropylene tubes under white light	CA26485-01 Amendment 4 Table 23 and Table 24
Long-term Storage	669 days in polypropylene tubes at -20°C 349 days in polypropylene tubes at -80°C	CA26485-01 Amendment 4 Table 21 and Table 22
Parallelism	NA	NA
Carryover	Carryover was evaluated by injecting a blank (zero sample) after high-level samples of aficamten and internal standard. There was no significant carryover (< 0.04%) evident in the carryover blanks injected at the beginning and end of 4 batches.	CA26485-01 Amendment 4 Table 8
Method Performance in CY 6011 Bioanalytical Report: CA25821-01		
Assay Passing Rate	34 of 34 runs (100%) met acceptance criteria	CA25821-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -1.6% to 1.0% Cumulative precision (%CV): ≤ 5.1%	CA25821-01 Table 3
QC Performance	Cumulative accuracy (%bias): -1.3% to 0.9% Cumulative precision (%CV): ≤ 5.6% Total error: NR	CA25821-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 222 samples, and 90.1% of samples were within ± 20.0% of the original results.	CA25821-01 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 203 days, within the established long-term stability limit of 669 days.	CA25821-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	34 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 306 datapoints) – 1 datapoint rejected	

Method Performance in CY 6012 Bioanalytical Report: CA40650-01		
Assay Passing Rate	11 of 13 runs (85%) met acceptance criteria	CA40650-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -10.0% to 7.0% Cumulative precision (%CV): ≤ 4.2%	CA40650-01 Table 4
QC Performance	Cumulative accuracy (%bias): -5.1% to 7.7% Cumulative precision (%CV): ≤ 3.8% Total error: NR	CA40650-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 104 samples, and 100% of samples were within ± 20.0% of the original results.	CA40650-01 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 67 days, within the established long-term stability limit of 669 days.	CA40650-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	11 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 99 datapoints) – no datapoints rejected	
Method Performance in CY 6013 Bioanalytical Report: CA37831-01		
Assay Passing Rate	4 of 6 runs (67%) met acceptance criteria	CA37831-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -10.4% to 5.4% Cumulative precision (%CV): ≤ 5.1%	CA37831-01 Table 4
QC Performance	Cumulative accuracy (%bias): -5.1% to 7.3% Cumulative precision (%CV): ≤ 6.9% Total error: NR	CA37831-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 32 samples, and 81.3% of samples were within ± 20.0% of the original results.	CA37831-01 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 55 days, within the established long-term stability limit of 669 days.	CA37831-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	4 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 36 datapoints) – 1 datapoint rejected	

Method Performance in CY 6014 Bioanalytical Report: CA38190-01		
Assay Passing Rate	13 of 14 runs (93%) met acceptance criteria	CA38190-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -5.6% to 3.0% Cumulative precision (%CV): ≤ 2.5%	CA38190-01 Table 4
QC Performance	Cumulative accuracy (%bias): -6.9% to -0.3% Cumulative precision (%CV): ≤ 4.9% Total error: NR	CA38190-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 128 samples, and 100% of samples were within ± 20.0% of the original results.	CA38190-01 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 112 days, within the established long-term stability limit of 669 days.	CA38190-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	13 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 117 datapoints) – no datapoints rejected	
Method Performance in CY 6017 Bioanalytical Report: CA39610-01		
Assay Passing Rate	6 of 6 runs (100%) met acceptance criteria	CA39610-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -8.6% to 6.5% Cumulative precision (%CV): ≤ 4.4%	CA39610-01 Table 3
QC Performance	Cumulative accuracy (%bias): -2.4% to 6.8% Cumulative precision (%CV): ≤ 7.4% Total error: NR	CA39610-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 54 samples, and 100% of samples were within ± 20.0% of the original results.	CA39610-01 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 94 days, within the established long-term stability limit of 669 days.	CA39610-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	6 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 54 datapoints) – 1 datapoint rejected	

Method Performance in CY 6019 Bioanalytical Report: CA39506-01		
Assay Passing Rate	9 of 9 runs (100%) met acceptance criteria	CA39506-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -7.4% to 5.0% Cumulative precision (%CV): ≤ 4.3%	CA39506-01 Table 3
QC Performance	Cumulative accuracy (%bias): -5.3% to 4.5% Cumulative precision (%CV): ≤ 6.1% Total error: NR	CA39506-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 86 samples, and 100% of samples were within ± 20.0% of the original results.	CA39506-01 Section 3.10
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 170 days, within the established long-term stability limit of 669 days.	CA39506-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	9 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 81 datapoints) – 3 datapoints rejected	
Method Performance in CY 601-10 Bioanalytical Report: CA41800-01		
Assay Passing Rate	23 of 24 runs (96%) met acceptance criteria	CA41800-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -7.2% to 3.5% Cumulative precision (%CV): ≤ 5.0%	CA41800-01 Table 4
QC Performance	Cumulative accuracy (%bias): -5.9% to 0.0% Cumulative precision (%CV): ≤ 5.5% Total error: NR	CA41800-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 160 samples, and 99.4% of samples were within ± 20.0% of the original results.	CA41800-01 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 145 days, within the established long-term stability limit of 669 days.	CA41800-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	23 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 207 datapoints) – 1 datapoint rejected	

Method Performance in CY 6021 Bioanalytical Report: CA29455-01		
Assay Passing Rate	31 of 32 runs (97%) met acceptance criteria	CA29455-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -5.8% to 3.0% Cumulative precision (%CV): ≤ 4.7%	CA29455-01 Table 4
QC Performance	Cumulative accuracy (%bias): -2.1% to 4.1% Cumulative precision (%CV): ≤ 6.4% Total error: NR	CA29455-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 180 samples, and 97.2% of samples were within ± 20.0% of the original results.	CA29455-01 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 607 days, within the established long-term stability limit of 669 days.	CA29455-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	31 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 279 datapoints) – 2 datapoints rejected	
Method Performance in CY 6022 Bioanalytical Report: CA34180-01		
Assay Passing Rate	19 of 19 runs (100%) met acceptance criteria	CA34180-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -5.0% to 4.0% Cumulative precision (%CV): ≤ 6.1%	CA34180-01 Table 3
QC Performance	Cumulative accuracy (%bias): -4.5% to 3.6% Cumulative precision (%CV): ≤ 8.7% Total error: NR	CA34180-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 55 samples, and 96.4% of samples were within ± 20.0% of the original results.	CA34180-01 Section 3.10
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 548 days, within the established long-term stability limit of 669 days.	CA34180-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	19 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 171 datapoints) – 2 datapoints rejected	

Method Performance in CY 6031 Bioanalytical Report: CA34895-01		
Assay Passing Rate	43 of 43 runs (100%) met acceptance criteria	CA34895-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -6.4% to 4.0% Cumulative precision (%CV): ≤ 4.8%	CA34895-01 Table 3
QC Performance	Cumulative accuracy (%bias): -4.8% to 13.6% Cumulative precision (%CV): ≤ 109.6% Total error: NR	CA34895-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 268 samples, and 97.8% of samples were within ± 20.0% of the original results.	CA34895-01 Section 3.10
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 639 days, within the established long-term stability limit of 669 days.	CA34895-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	43 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 387 datapoints) – 3 datapoints rejected	

ACS = American Chemical Society; CV = coefficient of variation; ESI = electrospray ionization; HPLC = high-performance liquid chromatography; HQC = high QC; EDTA = ethylenediaminetetraacetic acid; LC-MS/MS = liquid chromatography-tandem mass spectroscopy; LLOQ = lower limit of quantification; LQC = low QC; MQC = middle QC; MRM = multiple reaction monitoring; NA = not applicable; NR = not reported; QC = quality control; ULOQ = upper limit of quantification.

Source: Appendix 3a, Module 2.7.1

Out of the 146 concentration measurements for quality control B (i.e., 22.0 ng/ml) in sample analysis for Trial CY 6031, there was an outlier with a reported concentration of ~353 ng/ml (i.e., %CV =109.6) on August 17, 2023 (i.e., Run #30). This appears to be an isolated outlier and is not expected to have an impact on the study samples analyzed.

Table 105. Summary of Method Performance for Determination of Aficamten in Human Urine (CA27882-01)

Bioanalytical Method Validation Report Name, Amendments, and Hyperlinks	Validation of an LC-MS/MS Method for the Determination of CK-3773274 in Isopropanol (IPA)-Treated Human Urine Method Validation Report CA27882-01 Amendment 1
Method Description	An aliquot of IPA-treated human urine containing the analyte and internal standard are extracted using a liquid-liquid extraction procedure. The extracted samples are analyzed using Waters Acquity UPLC Binary Solvent Manager pump, Waters Acquity UPLC I-Class System (SM-FTN) autosampler, and AB SCIEX API 5000 triple quadrupole mass spectrometer using an ESI source. Positive ions are monitored in the MRM mode.
Materials Used for Standard Calibration Curve and Concentration	Aficamten (CK-3773274), Cambrex High Point, Lot 2471-1808-00416 Calibration standards: 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 150, 420, and 500 ng/mL <u>Internal standard:</u> Deuterated aficamten (CK-3942912), Cytokinetics, Inc, Lot 01
Validated Assay Range	1.00 to 500 ng/mL
Material Used for QCs and Concentration	Aficamten (CK-3773274), Cambrex High Point, Lot 2471-1808-00416 QC concentration levels: 1.00 (LLOQ), 3.00 (LQC), 22.0 (MLQC), 150 (MHQC), and 375 (HQC) ng/mL <u>Internal standard:</u> Deuterated aficamten (CK-3942912), Cytokinetics, Inc, Lot 01
Minimum Required Dilutions	NR
Source and Lot of Reagents	(b) (4)
Regression Model and Weighting	Linear, weighted (1/concentration ²)

Validation Parameters	Method Validation Summary		Source Location
Standard Calibration Curve Performance During Accuracy and Precision Runs	Number of standard calibrators from LLOQ to ULOQ	9	CA27882-01 Amendment 1 Table 4
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.8% to 4.0%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 4.2%	
Performance of QCs During Accuracy and Precision Runs	<u>Cumulative accuracy (%bias) in 5 QC levels</u>	-4.5% to 1.0%	CA27882-01 Amendment 1 Table 2
	<u>Interbatch %CV</u>	≤ 4.6%	
	<u>Total error</u>	NR	NA
Selectivity and Matrix Effect	No significant matrix effect was observed in any of the 10 lots of IPA-treated urine that were spiked with aficamten at the LQC and HQC levels. Accuracy (%bias): 0.3% to 3.3% Precision (%CV): ≤ 3.6% Selectivity for aficamten in the presence of metabolites CK-3834282 and CK-3834283 was demonstrated at the LQC and HQC levels. Accuracy (%bias): 0.5% to 3.0% Precision (%CV): ≤ 6.9%		CA27882-01 Amendment 1 Table 10 and Table 13
Interference and Specificity	No significant interference (0.0%) at the retention time and mass transition of aficamten or internal standard was observed from endogenous components in any of the 10 lots of IPA-treated urine screened.		CA27882-01 Amendment 1 Table 9
Hemolysis Effect	NA		NA
Lipemic Effect	NA		NA
Dilution Linearity and Hook Effect	10-fold dilution of 2500 ng/mL 100-fold dilution of 40000 ng/mL Accuracy (%bias): -2.0% to 2.8% Precision (%CV): ≤ 2.3% Hook effect: NA		CA27882-01 Amendment 1 Table 31
Bench-top/Process Stability	179 hours in a polypropylene 96-well plate at 5°C for processed samples 48 hours in polypropylene tubes at ambient temperature under white light		CA27882-01 Amendment 1 Table 19 and Table 20
Freeze-thaw Stability	6 cycles at -20°C in polypropylene tubes under white light		CA27882-01 Amendment 1 Table 18
Long-term Storage	501 days in polypropylene tubes at -20°C and -80°C		CA27882-01 Amendment 1 Table 16 and Table 17
Parallelism	NA		NA
Carryover	Carryover was evaluated by injecting a blank (zero sample) after high-level samples of aficamten and internal standard. There was no significant carryover (< 0.04%) evident in the carryover blanks injected at the beginning and end of 3 batches.		CA27882-01 Amendment 1 Table 8

Method Performance in CY 6011 Bioanalytical Report: CA27883-01		
Assay Passing Rate	2 of 2 runs (100%) met acceptance criteria	CA27883-01 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -6.4% to 5.0% Cumulative precision (%CV): NR	CA27883-01 Table 3
QC Performance	Cumulative accuracy (%bias): -0.3% to 1.4% Cumulative precision (%CV): ≤ 7.5% Total error: NR	CA27883-01 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 21 samples, and 90.5% of samples were within ± 20.0% of the original results.	CA27883-01 Section 3.7
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 145 days, within the established long-term stability limit of 501 days.	CA27883-01 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	2 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 18 datapoints) – 1 datapoint rejected	
Method Performance in CY 6013 Bioanalytical Report: CA37831-03		
Assay Passing Rate	3 of 3 runs (100%) met acceptance criteria	CA37831-03 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -11.0% to 5.2% Cumulative precision (%CV): ≤ 3.5%	CA37831-03 Table 3
QC Performance	Cumulative accuracy (%bias): -8.8% to 2.7% Cumulative precision (%CV): ≤ 3.9% Total error: NR	CA37831-03 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 22 samples, and 95.5% of samples were within ± 20.0% of the original results.	CA37831-03 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 75 days, within the established long-term stability limit of 501 days.	CA37831-03 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	3 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 27 datapoints) – no datapoints rejected	

ACS = American Chemical Society; CV = coefficient of variation; ESI = electrospray ionization; HPLC = high-performance liquid chromatography; IPA = isopropanol; LC-MS/MS = liquid chromatography-tandem mass spectroscopy; LLOQ = lower limit of quantification; LQC = low QC; MHQC = medium high QC; MLQC = medium low QC; MRM = multiple reaction monitoring; NA = not applicable; NR = not reported; QC = quality control; ULOQ = upper limit of quantification; UPLC = ultra-performance liquid chromatography.

Source: Appendix 3e, Module 2.7.1

Table 106. Summary of Method Performance for Determination of CK-3834282 and CK-3834283 in Human Plasma (CA29736-01)

Bioanalytical Method Validation Report Name, Amendments, and Hyperlinks	Validation of an LC-MS/MS Method for the Determination of CK-3834282 and CK-3834283 in Human Plasma (EDTA) Method Validation Report CA29736-01 Amendment 3
Method Description	An aliquot of human plasma (EDTA) containing the analyte and internal standard is extracted using a liquid-liquid extraction procedure. The extracted samples are analyzed by Waters Acquity UPLC I-Class System (SM-FTN) equipped with AB SCIEX Triple Quad 6500 triple quadrupole mass spectrometer using an ESI source. Positive ions are monitored in the MRM mode.
Materials Used for Standard Calibration Curve and Concentration	CK-3834282, Cytokinetics, Inc, Lot 04 CK-3834283, Cytokinetics, Inc, Lot 02 Calibration standards: 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 150, 420, and 500 ng/mL <u>Internal standard:</u> CK-3978133, Cytokinetics, Inc, Lot 04
Validated Assay Range	Both analytes: 1.00 to 500 ng/mL
Material Used for QCs and Concentration	CK-3834282, Cytokinetics, Inc, Lot 04 CK-3834283, Cytokinetics, Inc, Lot 02 QC concentration levels: 1.00 (LLOQ), 3.00 (LQC), 25.0 (MQC), and 375 (HQC) ng/mL <u>Internal standard:</u> CK-3978133, Cytokinetics, Inc, Lot 04
Minimum Required Dilutions	NR
Source and Lot of Reagents	(b) (4)
Regression Model and Weighting	Linear, weighted (1/concentration ²)

Validation Parameters	Method Validation Summary		Source Location
Standard Calibration Curve Performance During Accuracy and Precision Runs	Number of standard calibrators from LLOQ to ULOQ	9	CA29736-01 Amendment 3 Table 6 and Table 7
	Cumulative accuracy (%bias) from LLOQ to ULOQ	CK-3834282: -4.8% to 4.0% CK-3834283: -4.3% to 6.0%	
	Cumulative precision (%CV) from LLOQ to ULOQ	CK-3834282: ≤ 2.0% CK-3834283: ≤ 4.5%	
Performance of QCs During Accuracy and Precision Runs	<u>Cumulative accuracy (%bias) in 4 QC levels</u>	CK-3834282: 1.6% to 8.7% CK-3834283: -2.0% to 7.7%	CA29736-01 Amendment 3 Table 2 and Table 3
	<u>Interbatch %CV</u>	CK-3834282: ≤ 6.5% CK-3834283: ≤ 8.3%	
	<u>Total error</u>	NR	NA
Selectivity and Matrix Effect	<u>CK-3834282:</u> No significant matrix effect was observed in any of the 10 lots of plasma that were fortified with CK-3834282 at the LQC and HQC levels. Accuracy (%bias): -4.5% to -1.0% Precision (%CV): ≤ 6.2% Selectivity for CK-3834282 in a cocktail of dabigatran, carbamazepine, and itraconazole was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -6.7% to -1.3% Precision (%CV): ≤ 1.9% Selectivity for CK-3834282 in a cocktail of acetaminophen, atorvastatin, cetirizine, cotinine, ethinyl estradiol, famotidine, ibuprofen, norethindrone, omeprazole, pseudoephedrine, and salicylic acid was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -0.8% to 7.7% Precision (%CV): ≤ 2.9% Selectivity for CK-3834282 in a cocktail of caffeine, dextrophan, and metformin was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -7.0% to -4.3% Precision (%CV): ≤ 2.9% Selectivity for CK-3834282 in the presence of aficamten was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -7.7% to -0.7% Precision (%CV): ≤ 3.8% <u>CK-3834283:</u> No significant matrix effect was observed in 9 of 10 lots of plasma that were fortified with CK-3834283 at the LQC level or in 10 of 10 lots of plasma that were fortified with CK-3834283 at the HQC level. Accuracy (%bias): -4.7% to -3.2% Precision (%CV): ≤ 6.7% Selectivity for CK-3834283 in a cocktail of dabigatran, carbamazepine, and itraconazole was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -6.0% to -2.1% Precision (%CV): ≤ 2.1%		CA29736-01 Amendment 3 Table 18, Table 19, Table 26, Table 27, Table 28, Table 29, Table 30, Table 31, Table 32, and Table 33

Validation Parameters	Method Validation Summary	Source Location
	Selectivity for CK-3834283 in a cocktail of acetaminophen, atorvastatin, cetirizine, cotinine, ethinyl estradiol, famotidine, ibuprofen, norethindrone, omeprazole, pseudoephedrine, and salicylic acid was demonstrated at both the LQC and HQC levels. Accuracy (%bias): 0.0% to 4.7% Precision (%CV): ≤ 4.1% Selectivity for CK-3834283 in a cocktail of caffeine, dextrophan, and metformin was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -8.3% to 0.8% Precision (%CV): ≤ 4.5% Selectivity for CK-3834283 in the presence of aficamten was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -1.3% to 0.0% Precision (%CV): ≤ 3.8%	
Interference and Specificity	CK-3834282: No significant interference (0.0%) at the retention time and mass transition of CK-3834282 or internal standard was observed from endogenous components in any of the 10 lots of plasma screened. CK-3834283: No significant interference (0.0%) at the retention time and mass transition of CK-3834283 or internal standard was observed from endogenous components in any of the 10 lots of plasma screened.	CA29736-01 Amendment 3 Table 16 and Table 17
Hemolysis Effect	<u>CK-3834282:</u> No significant effect of hemolysis at the LQC and HQC levels. Accuracy (%bias): -4.0% to -3.7% Precision (%CV): ≤ 3.6% <u>CK-3834283:</u> No significant effect of hemolysis at the LQC and HQC levels. Accuracy (%bias): 0% to 3.5% Precision (%CV): ≤ 4.0%	CA29736-01 Amendment 3 Table 34 and Table 35
Lipemic Effect	<u>CK-3834282:</u> No significant effect of lipemia at the LQC and HQC levels. Accuracy (%bias): -8.5% to 1.7% Precision (%CV): ≤ 2.9% <u>CK-3834283:</u> No significant effect of lipemia at the LQC and HQC levels. Accuracy (%bias): -4.0% to 2.0% Precision (%CV): ≤ 3.5%	CA29736-01 Amendment 3 Table 36 and Table 37
Dilution Linearity and Hook Effect	<u>CK-3834282:</u> 25-fold dilution of 5000 ng/mL Accuracy (%bias): 1.4% Precision (%CV): 2.0% <u>CK-3834283:</u> 25-fold dilution of 5000 ng/mL Accuracy (%bias): 0.4% Precision (%CV): 3.1% Hook effect: NA	CA29736-01 Amendment 3 Table 72 and Table 73

Validation Parameters	Method Validation Summary	Source Location
Bench-top/ Process Stability	<u>Both analytes:</u> 83 hours in a polypropylene 96-well plate at 5°C for processed samples 24 hours in polypropylene tubes at ambient temperature under white light 50 hours in polypropylene tubes at ambient temperature under white light (total of all thaw cycles)	CA29736-01 Amendment 3 Table 45, Table 46, Table 47, Table 48, Table 49, and Table 50
Freeze-thaw Stability	Both analytes: 6 cycles at -20°C and -80°C in polypropylene tubes under white light	CA29736-01 Amendment 3 Table 45, Table 46, Table 47, and Table 48
Long-term Storage	Both analytes: 512 days in polypropylene tubes at -20°C and -80°C	CA29736-01 Amendment 3 Table 41, Table 42, Table 43, and Table 44
Parallelism	NA	NA
Carryover	CK-3834282: Carryover was evaluated by injecting a blank (zero sample) after high-level samples of CK-3834282 and internal standard. There was no significant carryover (< 0.04%) evident in the carryover blanks injected at the beginning and end of 3 batches. CK-3834283: Carryover was evaluated by injecting a blank (zero sample) after high-level samples of CK-3834283 and internal standard. There was no significant carryover (< 0.04%) evident in the carryover blanks injected at the beginning and end of 3 batches.	CA29736-01 Amendment 3 Table 14 and Table 15
Method Performance in CY 6012 Bioanalytical Report: CA40650-02		
Assay Passing Rate	CK-3834282: 20 of 21 runs (95%) met acceptance criteria CK-3834283: 20 of 20 runs (100%) met acceptance criteria	CA40650-02 Table 1
Standard Curve Performance	<u>CK-3834282:</u> Cumulative accuracy (%bias): -1.7% to 1.6% Cumulative precision (%CV): ≤ 3.6% <u>CK-3834283:</u> Cumulative accuracy (%bias): -0.5% to 0.6% Cumulative precision (%CV): ≤ 4.4%	CA40650-02 Table 5 and Table 7
QC Performance	<u>CK-3834282:</u> Cumulative accuracy (%bias): -2.9% to 0.7% Cumulative precision (%CV): ≤ 4.6% Total error: NR <u>CK-3834283:</u> Cumulative accuracy (%bias): -2.1% to -0.4% Cumulative precision (%CV): ≤ 6.1% Total error: NR	CA40650-02 Table 2 and Table 4
Method Reproducibility	CK-3834282: Incurred sample reanalysis was performed on 104 samples, and 95.2% of samples were within ± 20.0% of the original results. CK-3834283: Incurred sample reanalysis was performed on 104 samples, and 96.2% of samples were within ± 20.0% of the original results.	CA40650-02 Section 3.9

Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 62 days, within the established long-term stability limit of 512 days.	CA40650-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 20 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 180 datapoints) – 2 datapoints rejected CK-3834283: 20 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 180 datapoints) – 4 datapoints rejected	
Method Performance in CY 6013 Bioanalytical Report: CA37831-02		
Assay Passing Rate	CK-3834282: 7 of 7 runs (100%) met acceptance criteria CK-3834283: 7 of 7 runs (100%) met acceptance criteria	CA37831-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -3.6% to 3.5% Cumulative precision (%CV): ≤ 4.4% CK-3834283: Cumulative accuracy (%bias): -3.3% to 3.0% Cumulative precision (%CV): ≤ 4.3%	CA37831-02 Table 4 and Table 5
QC Performance	CK-3834282: Cumulative accuracy (%bias): 0.0% to 3.2% Cumulative precision (%CV): ≤ 3.4% Total error: NR CK-3834283: Cumulative accuracy (%bias): -1.7% to 1.9% Cumulative precision (%CV): ≤ 5.1% Total error: NR	CA37831-02 Table 2 and Table 3
Method Reproducibility	Both analytes: Incurred sample reanalysis was performed on 32 samples, and 71.9% of samples were within ± 20.0% of the original results.	CA37831-02 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 41 days, within the established long-term stability limit of 512 days.	CA37831-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 7 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 63 datapoints) – 1 datapoint rejected CK-3834283: 7 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 63 datapoints) – 1 datapoint rejected	
Method Performance in CY 6014 Bioanalytical Report: CA38190-02		
Assay Passing Rate	CK-3834282: 35 of 36 runs (97%) met acceptance criteria CK-3834283: 35 of 36 runs (97%) met acceptance criteria	CA38190-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -1.5% to 1.6% Cumulative precision (%CV): ≤ 3.5% CK-3834283: Cumulative accuracy (%bias): -1.1% to 1.8% Cumulative precision (%CV): ≤ 3.9%	CA38190-02 Table 6 and Table 8

QC Performance	CK-3834282: Cumulative accuracy (%bias): -0.7% to 0.8% Cumulative precision (%CV): ≤ 4.1% Total error: NR CK-3834283: Cumulative accuracy (%bias): -1.7% to 1.1% Cumulative precision (%CV): ≤ 4.1% Total error: NR	CA38190-02 Table 2 and Table 4
Method Reproducibility	Both analytes: Incurred sample reanalysis was performed on 125 samples, and 99.2% of samples were within ± 20.0% of the original results.	CA38190-02 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 128 days, within the established long-term stability limit of 512 days.	CA38190-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 35 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 315 datapoints) – 2 datapoints rejected CK-3834283: 35 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 315 datapoints) – 2 datapoints rejected	
Method Performance in CY 6017 Bioanalytical Report: CA39610-02		
Assay Passing Rate	CK-3834282: 10 of 10 runs (100%) met acceptance criteria CK-3834283: 10 of 10 runs (100%) met acceptance criteria	CA39610-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -1.7% to 2.0% Cumulative precision (%CV): ≤ 3.9% CK-3834283: Cumulative accuracy (%bias): -1.5% to 2.0% Cumulative precision (%CV): ≤ 4.3%	CA39610-02 Table 4 and Table 5
QC Performance	CK-3834282: Cumulative accuracy (%bias): -2.4% to -1.6% Cumulative precision (%CV): ≤ 3.8% Total error: NR CK-3834283: Cumulative accuracy (%bias): -2.4% to -2.0% Cumulative precision (%CV): ≤ 4.7% Total error: NR	CA39610-02 Table 2 and Table 3
Method Reproducibility	Both analytes: Incurred sample reanalysis was performed on 54 samples, and 100% of samples were within ± 20.0% of the original results.	CA39610-02 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 97 days, within the established long-term stability limit of 512 days.	CA39610-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 10 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 90 datapoints) – no datapoints rejected CK-3834283: 10 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 90 datapoints) – no datapoints rejected	

Method Performance in CY 6019 Bioanalytical Report: CA39506-02		
Assay Passing Rate	CK-3834282: 17 of 18 runs (94%) met acceptance criteria CK-3834283: 17 of 17 runs (100%) met acceptance criteria	CA39506-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -3.3% to 5.0% Cumulative precision (%CV): ≤ 5.0% CK-3834283: Cumulative accuracy (%bias): -2.1% to 3.0% Cumulative precision (%CV): ≤ 6.0%	CA39506-02 Table 5 and Table 7
QC Performance	CK-3834282: Cumulative accuracy (%bias): -2.7% to 0.3% Cumulative precision (%CV): ≤ 4.6% Total error: NR CK-3834283: Cumulative accuracy (%bias): -4.5% to -2.4% Cumulative precision (%CV): ≤ 4.9% Total error: NR	CA39506-02 Table 2 and Table 4
Method Reproducibility	Both analytes: Incurred sample reanalysis was performed on 88 samples, and 100% of samples were within ± 20.0% of the original results.	CA39506-02 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 175 days, within the established long-term stability limit of 512 days.	CA39506-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 17 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 153 datapoints) – no datapoints rejected CK-3834283: 17 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 153 datapoints) – no datapoints rejected	
Method Performance in CY 601-10 Bioanalytical Report: CA41800-02		
Assay Passing Rate	CK-3834282: 56 of 59 runs (95%) met acceptance criteria CK-3834283: 55 of 60 runs (92%) met acceptance criteria	CA41800-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -3.2% to 2.4% Cumulative precision (%CV): ≤ 4.0% CK-3834283: Cumulative accuracy (%bias): -2.6% to 2.2% Cumulative precision (%CV): ≤ 4.0%	CA41800-02 Table 6 and Table 8
QC Performance	CK-3834282: Cumulative accuracy (%bias): -2.7% to 1.7% Cumulative precision (%CV): ≤ 5.6% Total error: NR CK-3834283: Cumulative accuracy (%bias): -4.0% to -0.8% Cumulative precision (%CV): ≤ 4.6% Total error: NR	CA41800-02 Table 2 and Table 4

Method Reproducibility	CK-3834282: Incurred sample reanalysis was performed on 163 samples, and 97.5% of samples were within $\pm 20.0\%$ of the original results. CK-3834283: Incurred sample reanalysis was performed on 163 samples, and 99.4% of samples were within $\pm 20.0\%$ of the original results.	CA41800-02 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C . Samples were analyzed within a maximum time from sample collection of 145 days, within the established long-term stability limit of 512 days.	CA41800-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 56 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 504 datapoints) – 4 datapoints rejected CK-3834283: 55 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 495 datapoints) – 2 datapoints rejected	
Method Performance in CY 6021 Bioanalytical Report: CA29455-02		
Assay Passing Rate	CK-3834282: 40 of 46 runs (87%) met acceptance criteria CK-3834283: 40 of 46 runs (87%) met acceptance criteria	CA29455-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -1.5% to 3.0% Cumulative precision (%CV): $\leq 5.2\%$ CK-3834283: Cumulative accuracy (%bias): -1.2% to 1.4% Cumulative precision (%CV): $\leq 5.3\%$	CA29455-02 Table 6 and Table 8
QC Performance	CK-3834282: Cumulative accuracy (%bias): 1.7% to 2.8% Cumulative precision (%CV): $\leq 7.3\%$ Total error: NR CK-3834283: Cumulative accuracy (%bias): -1.0% to 1.6% Cumulative precision (%CV): $\leq 4.9\%$ Total error: NR	CA29455-02 Table 2 and Table 4
Method Reproducibility	CK-3834282: Incurred sample reanalysis was performed on 182 samples, and 92.9% of samples were within $\pm 20.0\%$ of the original results. CK-3834283: Incurred sample reanalysis was performed on 180 samples, and 92.8% of samples were within $\pm 20.0\%$ of the original results.	CA29455-02 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C . Samples were analyzed within a maximum time from sample collection of 512 days, within the established long-term stability limit of 512 days.	CA29455-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 40 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 360 datapoints) – 4 datapoints rejected CK-3834283: 40 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 360 datapoints) – 3 datapoints rejected	

Method Performance in CY 6022 Bioanalytical Report: CA34180-02		
Assay Passing Rate	CK-3834282: 13 of 14 runs (93%) met acceptance criteria CK-3834283: 13 of 14 runs (93%) met acceptance criteria	CA34180-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -2.9% to 2.0% Cumulative precision (%CV): ≤ 5.2% CK-3834283: Cumulative accuracy (%bias): -1.9% to 1.3% Cumulative precision (%CV): ≤ 5.3%	CA34180-02 Table 6 and Table 8
QC Performance	CK-3834282: Cumulative accuracy (%bias): -1.6% to 2.4% Cumulative precision (%CV): ≤ 10.5% Total error: NR CK-3834283: Cumulative accuracy (%bias): -2.9% to 0.0% Cumulative precision (%CV): ≤ 10.9% Total error: NR	CA34180-02 Table 2 and Table 4
Method Reproducibility	CK-3834282: Incurred sample reanalysis was performed on 41 samples, and 95.1% of samples were within ± 20.0% of the original results. CK-3834283: Incurred sample reanalysis was performed on 40 samples, and 95.0% of samples were within ± 20.0% of the original results.	CA34180-02 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 482 days, within the established long-term stability limit of 512 days.	CA34180-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 13 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 117 datapoints) – 2 datapoints rejected CK-3834283: 13 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 117 datapoints) – 2 datapoints rejected	
Method Performance in CY 6031 Bioanalytical Report: CA34895-02		
Assay Passing Rate	CK-3834282: 80 of 82 runs (98%) met acceptance criteria CK-3834283: 81 of 83 runs (98%) met acceptance criteria	CA34895-02 Table 1
Standard Curve Performance	CK-3834282: Cumulative accuracy (%bias): -1.6% to 1.0% Cumulative precision (%CV): ≤ 4.1% CK-3834283: Cumulative accuracy (%bias): -1.5% to 1.2% Cumulative precision (%CV): ≤ 4.4%	CA34895-02 Table 6 and Table 8
QC Performance	CK-3834282: Cumulative accuracy (%bias): -2.7% to 1.2% Cumulative precision (%CV): ≤ 9.1% Total error: NR CK-3834283: Cumulative accuracy (%bias): -2.4% to 2.0% Cumulative precision (%CV): ≤ 17.6% Total error: NR	CA34895-02 Table 2 and Table 4

NDA 219083
MYQORZO (aficamten)

Method Reproducibility	CK-3834282: Incurred sample reanalysis was performed on 264 samples, and 96.6% of samples were within $\pm 20.0\%$ of the original results. CK-3834283: Incurred sample reanalysis was performed on 264 samples, and 97.0% of samples were within $\pm 20.0\%$ of the original results.	CA34895-02 Section 3.9
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C . Samples were analyzed within a maximum time from sample collection of 441 days, within the established long-term stability limit of 512 days.	CA34895-02 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	CK-3834282: 80 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 720 datapoints) – 4 datapoints rejected CK-3834283: 81 calibrator sets of 1 replicate each of 9 concentration levels from 1.00 to 500 ng/mL (total 729 datapoints) – 4 datapoints rejected	

ACS = American Chemical Society; CV = coefficient of variation; EDTA = ethylenediaminetetraacetic acid; ESI = electrospray ionization; HPLC = high-performance liquid chromatography; HQC = high QC; LC-MS/MS = liquid chromatography-tandem mass spectroscopy; LLOQ = lower limit of quantification; LQC = low QC; MQC = middle QC; MRM = multiple reaction monitoring; NA = not applicable; NR = not reported; QC = quality control; ULOQ = upper limit of quantification; UPLC = ultra-performance liquid chromatography.

Source: Appendix 3h, Module 2.7.1

Table 107. Summary of Method Performance for Determination of Moxifloxacin in Human Plasma (ZZ00945-02)

Bioanalytical Method Validation Report Name, Amendments, and Hyperlinks	Validation of an LC-MS/MS Method for the Determination of Moxifloxacin in Human Plasma (EDTA) Method Validation Report ZZ00945-02 Amendment 3		
Method Description	An aliquot of human plasma (EDTA) containing the analyte and internal standard is extracted using a solid phase extraction procedure. The extracted samples are analyzed by an HPLC equipped with an AB SCIEX API 4000 triple quadrupole mass spectrometer using an ESI source. Positive ions are monitored in the MRM mode.		
Materials Used for Standard Calibration Curve and Concentration	Moxifloxacin hydrochloride, USP, Lot G1L290 Calibration standards: 25.0, 50.0, 100, 250, 400, 900, 1400, 2500, 4000, and 5000 ng/mL <u>Internal standard:</u> d ₄ -Moxifloxacin, (b) (4) Lot CD-MDSPSRs-IV-81		
Validated Assay Range	25.0 to 5000 ng/mL		
Material Used for QCs and Concentration	Moxifloxacin hydrochloride, USP, Lot G1L290 QC concentration levels: 25.0 (LLOQ), 75.0 (LQC), 350 (MQC), and 3750 (HQC) ng/mL <u>Internal standard:</u> d ₄ -Moxifloxacin, (b) (4) Lot CD-MDSPSRs-IV-81		
Minimum Required Dilutions	NR		
Source and Lot of Reagents	(b) (4)		
Regression Model and Weighting	Linear, weighted (1/concentration ²)		
Validation Parameters	Method Validation Summary		Source Location
Standard Calibration Curve Performance During Accuracy and Precision Runs	Number of standard calibrators from LLOQ to ULOQ	10	ZZ00945-02 Amendment 3 Table 4
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.1% to 3.8%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 3.7%	
Performance of QCs During Accuracy and Precision Runs	<u>Cumulative accuracy (%bias) in 4 QC levels</u>	-2.1% to 2.0%	ZZ00945-02 Amendment 3 Table 2
	<u>Interbatch %CV</u>	≤ 5.9%	
	<u>Total error</u>	NR	NA

Validation Parameters	Method Validation Summary	Source Location
Selectivity and Matrix Effect	No significant matrix effect was observed in any of the 6 lots of plasma that were fortified with moxifloxacin at the LLOQ and HQC levels. Accuracy (%bias): -2.8% to -1.3% Precision (%CV): ≤ 7.0% Selectivity for moxifloxacin in a cocktail of acetaminophen, atorvastatin, caffeine, cetirizine, dextromethorphan, ethinyl estradiol, famotidine, ibuprofen, levonorgestrel, metformin, omeprazole, ondansetron, pseudoephedrine, and salicylic acid was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -1.9% to 2.9% Precision (%CV): ≤ 5.3%	ZZ00945-02 Amendment 3 Table 10 and Table 13
Interference and Specificity	No significant interference (0.0%) at the retention time and mass transition of moxifloxacin or d4-moxifloxacin (internal standard) was observed from endogenous components in any of the 6 lots of plasma screened.	ZZ00945-02 Amendment 3 Table 9
Hemolysis Effect	No significant effect of hemolysis in any of 3 lots of plasma at the LLOQ and HQC levels. Accuracy (%bias): -6.0% to 1.1% Precision (%CV): ≤ 6.5%	ZZ00945-02 Amendment 3 Table 14
Lipemic Effect	No significant effect of lipemia in any of 3 lots of plasma at the LLOQ and HQC levels. Accuracy (%bias): -4.8% to 0.3% Precision (%CV): ≤ 2.4%	ZZ00945-02 Amendment 3 Table 15
Dilution Linearity and Hook Effect	10-fold dilution of 15000 ng/mL Accuracy (%bias): -1.3% Precision (%CV): 0.9% Hook effect: NA	ZZ00945-02 Amendment 3 Table 28
Bench-top/Process Stability	171 hours in a polypropylene 96-well plate at 5°C for postpreparative samples 152 hours in a polypropylene 96-well plate at ambient temperature for postpreparative samples 128 hours in a polypropylene 96-well plate at 5°C for processed samples 125 hours in a polypropylene 96-well plate at ambient temperature for processed samples 24 hours in polypropylene tubes at ambient temperature under white light 49 hours in polypropylene tubes at ambient temperature under white light (total of all thaw cycles)	ZZ00945-02 Amendment 3 Table 20, Table 21, and Table 22, Table 29, and Table 30
Freeze-thaw Stability	5 cycles at -20°C in polypropylene tubes under white light	ZZ00945-02 Amendment 3 Table 20
Long-term Storage	407 days in polypropylene tubes at -20°C	ZZ00945-02 Amendment 3 Table 19
Parallelism	NA	NA

Validation Parameters	Method Validation Summary	Source Location
Carryover	Carryover was evaluated by injecting a blank (zero sample) after high-level samples of moxifloxacin and d4-moxifloxacin (internal standard). There was no significant carryover (< 0.1%) evident in the carryover blanks injected at the beginning and end of 3 batches.	ZZ00945-02 Amendment 3 Table 8
Method Performance in CY 6019 Bioanalytical Report: CA39506-03		
Assay Passing Rate	7 of 10 runs (70%) met acceptance criteria	CA39506-03 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -4.8% to 3.2% Cumulative precision (%CV): ≤ 3.8%	CA39506-03 Table 4
QC Performance	Cumulative accuracy (%bias): 1.3% to 4.4% Cumulative precision (%CV): ≤ 5.1% Total error: NR	CA39506-03 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 40 samples, and 100% of samples were within ± 20.0% of the original results.	CA39506-03 Section 3.8
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 109 days, within the established long-term stability limit of 407 days.	CA39506-03 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	7 calibrator sets of 1 replicate each of 10 concentration levels from 25.0 to 5000 ng/mL (total 70 datapoints) – no datapoint rejected	

ACS = American Chemical Society; CV = coefficient of variation; EDTA = ethylenediaminetetraacetic acid; ESI = electrospray ionization; HPLC = high-performance liquid chromatography; HQC = high QC; LC-MS/MS = liquid chromatography-tandem mass spectroscopy; LLOQ = lower limit of quantification; LQC = low QC; MQC = middle QC; MRM = multiple reaction monitoring; NA = not applicable; NR = not reported; QC = quality control; ULOQ = upper limit of quantification; USP = United States Pharmacopeia.

Source: Appendix 3m, Module 2.7.1

Table 108. Summary of Method Performance for Determination of Free Dabigatran in Human Plasma (ZZ55164-01)

Bioanalytical Method Validation Report Name, Amendments, and Hyperlinks	Validation of an LC-MS/MS Method for the Determination of Dabigatran (Free) in Acidified Human Plasma (EDTA) Method Validation Report ZZ55164-01 Amendment 2		
Method Description	An aliquot of acidified human plasma (EDTA) containing the analyte and internal standard is extracted using a solid phase extraction procedure. The extracted samples are analyzed by an HPLC equipped with an AB SCIEX API 4000 triple quadrupole mass spectrometer using an ESI source. Positive ions are monitored in the MRM mode.		
Materials Used for Standard Calibration Curve and Concentration	Dabigatran, Cayman Chemical, Lot 0464319-31 Calibration standards: 0.500, 1.00, 3.00, 6.00, 15.0, 45.0, 90.0, 200, and 250 ng/mL <u>Internal standard:</u> ¹³ C ₆ -Dabigatran, (b) (4) Lot CC-8022		
Validated Assay Range	0.500 to 250 ng/mL		
Material Used for QCs and Concentration	Dabigatran, Cayman Chemical, Lot 0464319-31 QC concentration levels: 0.500 (LLOQ), 1.50 (LQC), 12.5 (MQC), and 185 (HQC) ng/mL <u>Internal standard:</u> ¹³ C ₆ -Dabigatran, (b) (4) Lot CC-8022		
Minimum Required Dilutions	NR		
Source and Lot of Reagents	(b) (4)		
Regression Model and Weighting	Linear, weighted (1/concentration ²)		
Validation Parameters	Method Validation Summary		Source Location
Standard Calibration Curve Performance During Accuracy and Precision Runs	Number of standard calibrators from LLOQ to ULOQ	9	ZZ55164-01 Amendment 2 Table 4
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.8% to 2.8%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 4.6%	

Validation Parameters	Method Validation Summary		Source Location
Performance of QCs During Accuracy and Precision Runs	<u>Cumulative accuracy (%bias) in 4 QC levels</u>	-2.0% to 1.6%	ZZ55164-01 Amendment 2 Table 2
	<u>Interbatch %CV</u>	≤ 8.0%	
	<u>Total error</u>	NR	NA
Selectivity and Matrix Effect	No significant matrix effect was observed in any of the 10 lots of acidified plasma that were fortified with dabigatran at the LLOQ and HQC levels. Accuracy (%bias): -11.9% to -5.6% Precision (%CV): ≤ 7.8% Selectivity for dabigatran (free) in a cocktail of acetaminophen, atorvastatin, caffeine, cetirizine, dextromethorphan, ethinyl estradiol, famotidine, ibuprofen, levonorgestrel, metformin, omeprazole, ondansetron, pseudoephedrine, and salicylic acid was demonstrated at both the LQC and HQC levels. Accuracy (%bias): -10.8% to -10.7% Precision (%CV): ≤ 4.3%		ZZ55164-01 Amendment 2 Table 10 and Table 13
Interference and Specificity	No significant interference (0.0%) at the retention time and mass transition of dabigatran (free) or ¹³ C ₆ -dabigatran (internal standard) was observed from endogenous components in any of the 10 lots of acidified plasma screened.		ZZ55164-01 Amendment 2 Table 9
Hemolysis Effect	No significant effect of hemolysis in any of 3 lots of acidified plasma at the LLOQ and HQC levels. Accuracy (%bias): -3.2% to 2.6% Precision (%CV): ≤ 6.6%		ZZ55164-01 Amendment 2 Table 15
Lipemic Effect	No significant effect of lipemia in any of 3 lots of acidified plasma at the LLOQ and HQC levels. Accuracy (%bias): -5.4% to -3.4% Precision (%CV): ≤ 5.1%		ZZ55164-01 Amendment 2 Table 16
Dilution Linearity and Hook Effect	5-fold dilution of 750 ng/mL 20-fold dilution of 2000 ng/mL Accuracy (%bias): -12.9% to -8.5% Precision (%CV): ≤ 3.8% Hook effect: NA		ZZ55164-01 Amendment 2 Table 32
Bench-top/Process Stability	197 hours in a polypropylene 96-well plate at 5°C for postprocessed samples 228 hours in a polypropylene 96-well plate at 5°C for processed samples 6 hours in polypropylene tubes in an ice water bath under white light 22 hours in polypropylene tubes in an ice water bath under white light (total of all thaw cycles)		ZZ55164-01 Amendment 2 Table 21, Table 22, and Table 33
Freeze-thaw Stability	4 cycles at -20°C in polypropylene tubes under white light		ZZ55164-01 Amendment 2 Table 21
Long-term Storage	465 days in polypropylene tubes at -20°C		ZZ55164-01 Amendment 2 Table 20
Parallelism	NA		NA

Validation Parameters	Method Validation Summary	Source Location
Carryover	Carryover was evaluated by injecting a blank (zero sample) after high-level samples of dabigatran and ¹³ C ₆ -dabigatran (internal standard). There was no significant carryover (< 0.04%) evident in the carryover blanks injected at the beginning and end of 3 batches.	ZZ55164-01 Amendment 2 Table 8
Method Performance CY 6014 Bioanalytical Report: CA38190-03		
Assay Passing Rate	7 of 7 runs (100%) met acceptance criteria	CA38190-03 Table 1
Standard Curve Performance	Cumulative accuracy (%bias): -3.0% to 3.8% Cumulative precision (%CV): ≤ 4.8	CA38190-03 Table 3
QC Performance	Cumulative accuracy (%bias): -3.2% to -0.8% Cumulative precision (%CV): ≤ 4.1% Total error: NR	CA38190-03 Table 2
Method Reproducibility	Incurred sample reanalysis was performed on 66 samples, and 100% of samples were within ± 20.0% of the original results.	CA38190-03 Section 3.10
Study Sample Analysis Stability	Samples were received frozen on dry ice and stored at -20°C. Samples were analyzed within a maximum time from sample collection of 78 days, within the established long-term stability limit of 465 days.	CA38190-03 Section 2.2
Standard Calibration Curve Performance During Accuracy and Precision	7 calibrator sets of 1 replicate each of 9 concentration levels from 0.500 to 250 ng/mL (total 63 datapoints) – 1 datapoint rejected	

ACS = American Chemical Society; CV = coefficient of variation; EDTA = ethylenediaminetetraacetic acid; ESI = electrospray ionization; FCC = Food Chemicals Codex; HPLC = high-performance liquid chromatography; HQC = high QC; LC-MS/MS = liquid chromatography-tandem mass spectroscopy; LLOQ = lower limit of quantification; LQC = low QC; MQC = middle QC; MRM = multiple reaction monitoring; NA = not applicable; NR = not reported; QC = quality control; ULOQ = upper limit of quantification; USP = United States Pharmacopeia.

Source: Appendix 3n, Module 2.7.1

Overall, the bioanalytical method validation and performance in study sample analysis are in compliance with the ICH guidance for industry *M10 Bioanalytical Method Validation and Study Sample Analysis* ([November 2022a](#)) and are acceptable.

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

Not applicable.

14.5. Pharmacometrics Assessment

14.5.1. Summary of Applicant's Population PK Analysis

A PPK analysis was conducted to support the dose selection for the treatment of adults with symptomatic oHCM.

The **objectives** of the Applicant's modeling analysis were:

- To develop a PPK model from the pooled PK data in healthy subjects and subjects with HCM treated with aficamten.
- To identify the intrinsic and extrinsic factors impacting aficamten pharmacokinetics and assess the magnitude of covariates with significant effect.
- To provide individual PK parameters of aficamten for planned subsequent PK/PD and E-R analyses.

Methods

Pooled data from Phase 1 to 3 studies including healthy subjects and subjects with oHCM after oral administration of aficamten were included in the analysis. Population PK modeling was performed with the first-order conditional estimation with etaepsilon interaction (FOCE-I) method in NONMEM® Version 7.5.0. Model covariates were selected through univariate screening followed by a stepwise backward elimination method based on statistical significance levels as of 0.01 and 0.001, respectively. Model evaluations were performed by inspection of model parameters and their confidence intervals, standard residual-based goodness-of-fit plots, and simulation-based diagnostics such as prediction corrected visual predictive checks. The impact of covariates on the PK exposures in subjects with oHCM was evaluated using boxplots and summary tables. After the model validation, the final PPK model was used to calculate the post hoc PK exposure metrics needed for subjects with oHCM and support for the subsequent E-R analyses.

Results

The PPK dataset included 9963 PK samples from 447 subjects, including 264 healthy subjects and 183 subjects with oHCM. [Table 109](#) summarizes the clinical studies included in this analysis. A two-compartment model with linear elimination was used to describe the pharmacokinetics of aficamten. Absorption of aficamten was modeled as 1st-order after a lag time. Among the evaluated covariates, body weight and sex had an impact on both the volume and clearance of aficamten. Other model-determined significant covariates (i.e., formulation and food status) demonstrated no-to-minimal impact on the PK exposure. The final PPK model parameter estimates are provided in [Table 110](#). The goodness-of-fit plots for the final PPK model are presented in [Figure 17](#). There is good agreement between the individual or population predicted aficamten concentrations and the observed concentrations. Prediction corrected visual predictive checks ([Figure 18](#)) show that the model adequately captures the central tendency and range of the PK results observed for all the studies. The final PPK model was used to conduct the simulations to evaluate the impact of selected statistically significant covariates on the PK exposures (AUC_{tau} , C_{max} , and C_{tau}) in subjects with oHCM given the 15-mg QD regimen at steady state. The results show that there was no impact of significant covariates on the overall PK variability in subjects with oHCM. No dose adjustments based on demographics is warranted since aficamten dose is titrated by echocardiography assessments to achieve desired efficacy ([Figure 19](#)).

Table 109. Summary of the Number of Subjects and PK Samples

Study	Overall		Included in the final model		PK samples excluded		
	N Subjects	N PK samples	N subjects	N PK samples	BQL	Outliers identified in exploratory plots	CWRES >5
CY 6011	91	2610	91	2500	86	-	24
CY 6012	18	1020	18	954	49	-	17
CY 6014	34	704	34	673	25	-	6
CY 6017	8	256	8	242	13	-	1
CY 6019	43	617	43	459	157	-	1
CY 601-10	49	1028	49	988	30	-	10
JX01001	21	648	21	646	-	-	2
CY 6021	41	719	41	713	5	-	1
CY 6031	142	2361	142	2348	8	4	1
Overall	447	9963	447	9523	373	4	63

Source: Applicant's Population PK Analysis Report, Table 5.1, Page 31

Abbreviations: BQL, below quantification limit; CWRES, conditional weighted residual; N, number of subjects or samples; PK, pharmacokinetic

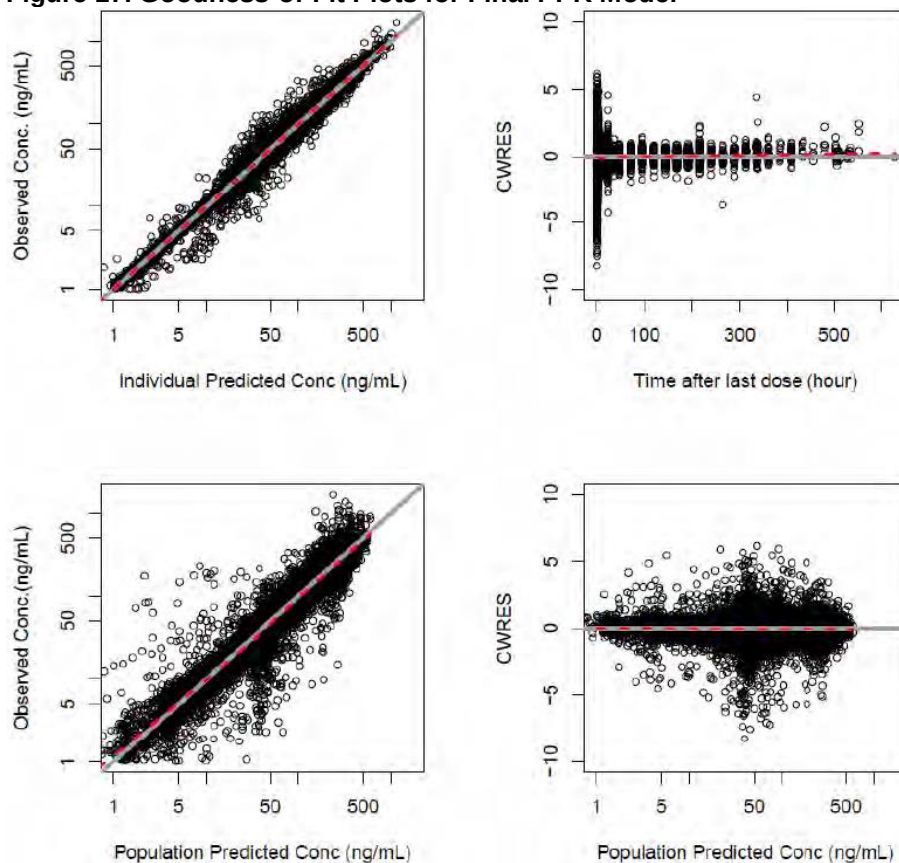
Table 110. Population Parameter Estimates for the Final PPK Model

Parameter	Unit	Estimate	RSE (%)	Shrinkage (%)
CL/F	L/hr	2.62	2.53	
Vc/F	L	18.1	12.6	
Q/F	L/hr	57.6	1.14	
Vp/F	L	295	4.37	
Ka	1/hr	0.337	5.03	
Tlag of Tablets	Hr	0.229	0.17	
Tlag of Capsule	Hr	0.248	0.0268	
Fasted on Ka		0.170	47	
WT on CL and Q		0.586	8.02	
WT on Vc and Vp		0.882	13.1	
Healthy Participants on CL		0.356	12.1	
Sex Female on CL		-0.128	22	
Healthy Participants on Vp		0.266	22.5	
Sex Female on Vp		-0.193	14.4	
High fat meal on Tlag		0.115	2.12	
High fat meal on F1		0.0687	17.1	
High fat meal on Ka		-0.365	12.3	
IIV (%CV)				
$\omega^2_{CL/F}$		0.0793 (28.7%)	6.4	6.9
$\omega^2_{Vc/F}$		2.35 (308%)	11.9	22.6

Source: Applicant's Population PK Analysis Report, Table 5.6, Page 41-42

Note: IIV was reported as variance (ω^2) and %CV ($\sqrt{\exp(\omega^2) - 1} \times 100$ (%)); RSE calculated as standard error/estimate $\times 100$ (%)
Abbreviations: CL, clearance; CL/F, apparent clearance; F, oral bioavailability; IIV, interindividual variability; Ka, first order absorption rate; PK, pharmacokinetic; PPK, population pharmacokinetic; Q/F, apparent intercompartmental clearance; RSE, relative standard error; Tlag, absorption lag time; Vc, volume of the central compartment; Vc/F, apparent volume of the central compartment; Vp, volume of the peripheral compartment; Vp/F, apparent volume of the peripheral compartment; WT, weight

Figure 17. Goodness-of-Fit Plots for Final PPK Model

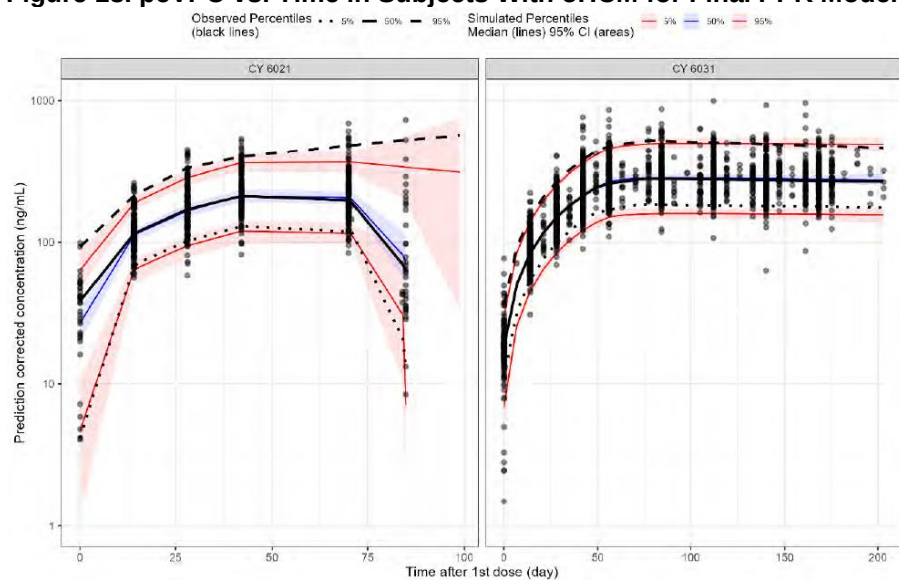


Source: Applicant's Population PK Analysis Report, Figure 5.5, Page 43

Note: Solid gray line is line of unity. Red dashed line is a locally estimated scatterplot smoothing line.

Abbreviations: Conc, concentration; CWRES, conditional weighted residuals; PPK, population pharmacokinetic

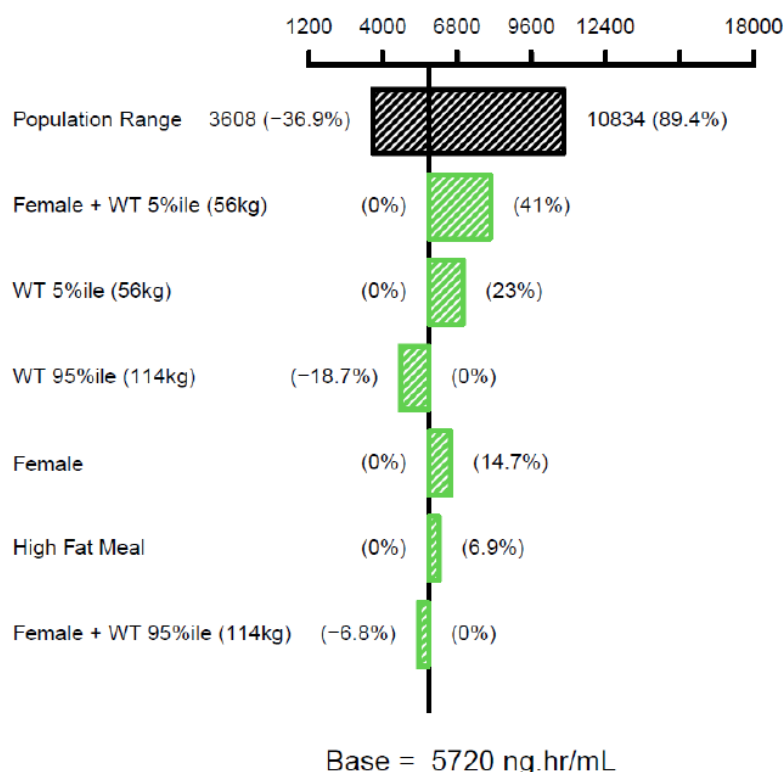
Figure 18. pcVPC vs. Time in Subjects With oHCM for Final PPK Model



Source: Applicant's Population PK Analysis Report, Figure 5.6, Page 44

Abbreviations: oHCM, obstructive hypertrophic cardiomyopathy; pcVPC, prediction corrected visual predictive check; PPK; population pharmacokinetic

Figure 19. Impact of Covariates on Steady-State AUC_{tau} in Subjects With oHCM
Steady State AUC_{tau} (ng.hr/mL)



Male patient with oHCM, WT=80kg, 15 mg tablet, with or without food

Source: Applicant's Population PK Analysis Report, Figure 5.8, Page 46

Note: Black bar represents 5th to 95th percentile of the exposure calculated using Empirical Bayes

Note: Estimates of the oHCM population for a dose regimen of 15-mg QD tablet with or without food. The impact of a covariate on the exposure was calculated using the parameter(s) incorporating the isolated effect of that covariate, with other unaffected parameters fixed to the typical values (estimated for a male oHCM patient with baseline body weight of 80 kg). Baseline body weight was evaluated at 5th to 95th percentile of the population

Abbreviations: AUC_{tau}, daily AUC; oHCM, obstructive hypertrophic cardiomyopathy; PK, pharmacokinetic; QD, once daily; WT, body weight

The Applicant's PPK model appears to describe the aficamten serum concentration-time profiles adequately following the administration of aficamten in subjects with oHCM. Therefore, the PPK model is acceptable for simulating post hoc exposure metrics, e.g., AUC_{tau}, C_{max}, or C_{tau} of aficamten for PK/PD and E-R analyses of efficacy or safety measurements.

Covariate analysis identified body weight (47 to 156 kg), population type (i.e., healthy versus HCM subjects) and sex (39.4% female) as statistically significant covariates on aficamten pharmacokinetics. The impact of food on the PK exposure of aficamten is minor.

14.5.2. Exposure-Response Analysis

The Applicant described the population PK/PD and conducted the E-R analysis of data from subjects with oHCM in the Phase 2 (Study CY 6021) and the Phase 3 (Trial CY 6031) programs to support the proposed commercial aficamten dose titration.

The **objectives** of the PK/PD and E-R analyses were:

- To develop PK/PD models characterizing longitudinal LVEF and LVOT-G change following aficamten treatment in subjects with oHCM in Phase 2 and 3 studies.
- To evaluate the proposed commercial aficamten dose titration regimen based on less frequent measurement of LVEF and LVOT-G, using PK/PD simulation.
- To quantify the E-R relationship for the primary, secondary, and key exploratory efficacy endpoints in subjects with oHCM in the Phase 3 study.
- To explore the E-R relationship for key safety endpoints in subjects with oHCM in Phase 3 study.

Methods

PD data collected in Study CY 6021 and Trial CY 6031 were used for PK/PD analyses. Efficacy and safety data collected in Trial CY 6031 were used for E-R analyses. PK/PD analysis was performed for LVEF and LVOT-G, based on core and site-read laboratory measurements collected in Studies CY 6021 and CY 6031. The selected exposure metric was the PPK model predicted average aficamten concentration 24 hr prior to the assessment ($C_{avg,24}$). To support the proposed commercial dose regimen, simulations explored alternate dose titration frequencies over three years, with the same criteria for dose titration used in the Phase 3 study. The alternate dose titration regimens included once every two-week (Q2W), once every four-week (Q4W), once every six-week (Q6W), and once every eight-week (Q8W) dose titrations with a starting dose of 5-mg QD and dose escalation options of 10 mg to 20-mg QD. Exposure-efficacy analysis was performed with the selected primary endpoint, which was the change in pVO₂ by CPET from baseline to Week 24. Exposure-safety analyses included incidence of selected TEAEs as the endpoints. Exposure metrics included in the E-R analyses were the PPK model predicted $C_{avg,24}$ (i.e., $C_{avg,ss}$ at Week 12 and Week 24 in the exposure-efficacy analysis), model predicted average aficamten concentration from Day 1 to the time of the event ($C_{avg,TOE}$), model predicted trough aficamten concentration at steady-state ($C_{min,ss}$), and model predicted maximum aficamten concentration at steady-state ($C_{max,ss}$).

Results

[Table 111](#) summarizes the 2880 LVOT-G and 2876 LVEF data records collected from 183 subjects for the PK/PD analysis. [Figure 20](#) shows the PK/PD relationship for both core and site-read measurements of post Valsalva LVOT-G as well as a declining trend with increasing exposure observed between the PK exposure metrics and the LVEF measurements.

Table 111. PK/PD Subjects and Data Summary

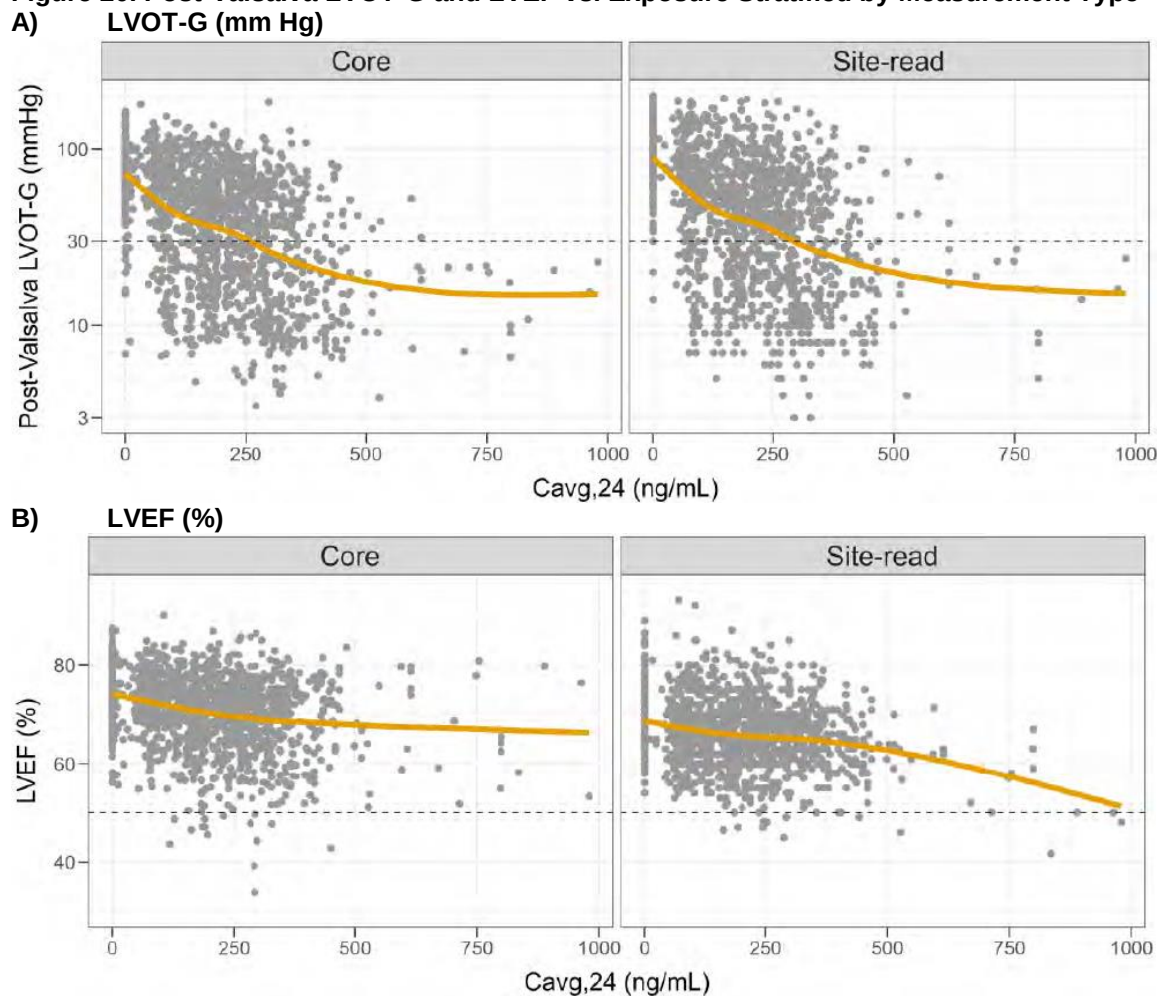
Participant/Data Category	CY 6021 Aficamten Cohort 1	CY 6021 Aficamten Cohort 2	CY 6021 Aficamten Cohort 3	CY 6031 Aficamten	Pooled Aficamten
Participants					
All Participants	14	14	13	142	183
PK-PD Participants	14	14	13	142	183
LVOT-G (site-read and core)					
All Data	156	161	143	2,992	3,452
Missing Baseline				5	5
Missing Value				9	9
Unscheduled Visit	30	35	26	320	411
End of Study Visit				138	138
Duplicate record				9	9
PK-PD Data	126	126	117	2,511	2,880
LVEF (site-read and core)					
All Data	170	175	156	2,978	3,479
Missing Baseline			7		7
Missing Value	2	5	2	48	57
Unscheduled Visit	30	34	25	314	403
End of Study Visit				136	136
PK-PD Data	138	136	122	2,480	2,876

Source: Applicant's Population PK/PD and Exposure Response Report, Table 4.1, Page 43

Note: The value indicates the number of subjects or the number of data points

Abbreviations: LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; PK/PD, pharmacokinetic/pharmacodynamic

Figure 20. Post-Valsalva LVOT-G and LVEF vs. Exposure Stratified by Measurement Type



Source: Applicant's Population PK/PD and Exposure Response Report, Figure 4.2 and 4.5, Page 48 and 56

Note: Points represent time-matched concentration and LVOT-G and LVEF pairs. The gold thick line is a loess smooth across the time points. In panel A, the horizontal dashed line is the titration cutoff, below which subjects are deemed to have adequate efficacy and not require up titration. In panel B, the horizontal dashed is the titration cutoff, below which subjects are deemed to have excessive LVEF lowering, and are down-titrated

The final PK/PD model estimations for LVOT-G and LVEF are listed in [Table 112](#) and [Table 113](#). The structure of both models comprised of a curve declining in a slightly exponential manner with increasing exposure. The model predictions are generally consistent with the data shown in exposure response visual predictive checks.

Abbreviations: $C_{avg,24}$, average concentration in 24 hours prior to measurement; CI, confidence interval; CV, coefficient of variation; LVOT-G, left ventricular outflow tract gradient; PK/PD, pharmacokinetic/pharmacodynamic; SE, standard error

Table 112. Post-Valsalva LVOT-G PK/PD Final Model Parameters

Name	Parameter	Estimate	SE	CI Low	CI High
Log Intercept	θ_1	4.49	0.02		
Intercept (mmHg)	Intercept _{Typical}	89.4		85.9	93.0
Log Slope	θ_2	1.44	0.05		
Slope (K_{Typical} , 1/(ug/mL))	K_{Typical}	4.23		3.87	4.62
Base on Intercept	θ_3	0.63	0.03		
Type on Log Intercept	θ_4	0.12	0.03		
Type on Intercept	TypeInt	1.13		1.08	1.19
Type on Log Slope	θ_5	0.07	0.03		
Type on Slope	TypeSlope	1.07		1.02	1.13
Between subject CV on slope (%)	ω	59.6		53.7	66.2
Within subject CV (%)	σ	49.6		48.5	50.8
Concentration to decline by 50%, Core (ng/mL)		164			
Concentration to decline by 50%, Site-read (ng/mL)		153			

Source: Applicant's Population PK/PD and Exposure Response Report, Table 4.4, Page 51

Note: The low and high CI values are 5th and 95th percentiles. The percent between-subject CV in slope is 59.6% using the approximation $\%CV = 100 \times \omega$. The percent within-subject CV is 49.6% using the approximation $\%CV = 100 \times \sigma$. Variability terms are the same for core vs. site-read values. Median baseline core LVOT-G was 82.1 mm Hg; median baseline site-read LVOT-G was 91.0 mm Hg

Abbreviations: $C_{\text{avg},24}$, average concentration in 24 hours prior to measurement; CI, confidence interval; CV, coefficient of variation; LVOT-G, left ventricular outflow tract gradient; PK/PD, pharmacokinetic/pharmacodynamic; SE, standard error

Table 113. LVEF PK/PD Final Model Parameters

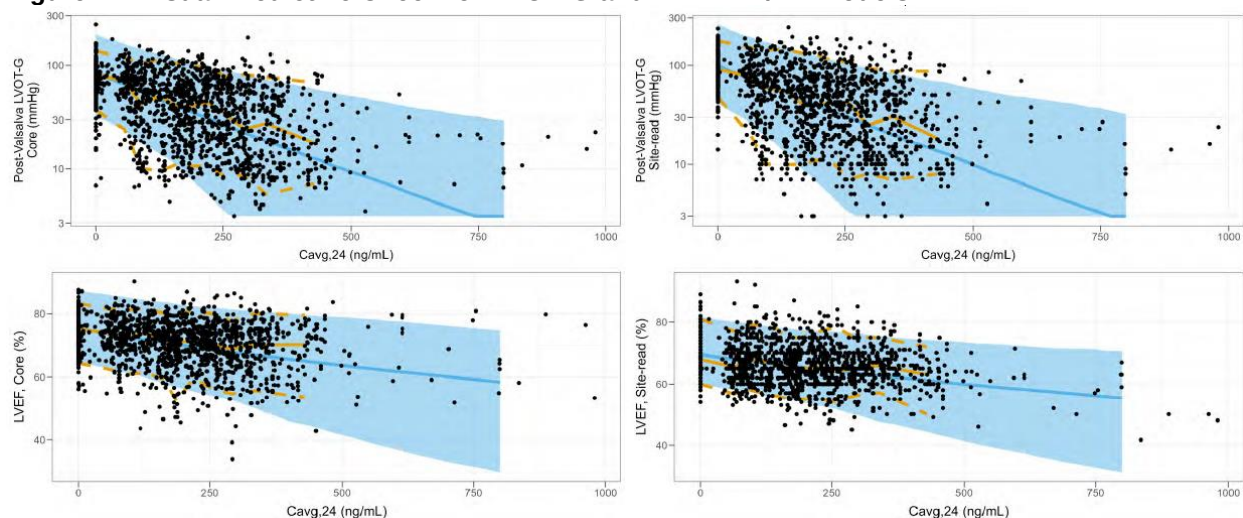
Name	Parameter	Estimate	SE	CI Low	CI High
Log Intercept	θ_1	4.32	0.00		
Intercept (%)	Intercept _{Typical}	75.5		75.0	75.9
Log Slope	θ_2	-1.16	0.08		
Slope (K_{Typical} , 1/(ug/mL))	K_{Typical}	0.31		0.27	0.36
Base on Intercept	θ_3	0.56	0.02		
Type on Log Intercept	θ_4	-0.08	0.00		
Type on Intercept	TypeInt	0.92		0.91	0.92
Type on Log Slope	θ_5	-0.12	0.05		
Type on Slope	TypeSlope	0.88		0.81	0.96
Between subject CV on slope (%)	ω	75.9		67.9	84.8
Within subject CV (%)	σ	7.6		7.5	7.8
Concentration to decline by 50%, Core (ng/mL)		2219			
Concentration to decline by 50%, Site-read (ng/mL)		2512			

Source: Applicant's Population PK/PD and Exposure Response Report, Table 4.7, Page 59

Note: The values are referenced to core LVEF. Type adjusts to site-read LVEF. The low and high CI values are 5th and 95th percentiles. The percent between-subject CV in slope is 75.9% using the approximation $\%CV = 100 \times \omega$. The percent within-subject CV is 7.6% using the approximation $\%CV = 100 \times \sigma$. Variability terms are the same for core vs. site-read values. The approximate reduction in LVEF with increasing exposure uses the approximation $\text{Change} = \text{Baseline} \times \text{Slope} \times C_{\text{avg},24}/1000$

Abbreviations: $C_{\text{avg},24}$, average concentration in 24 hours prior to measurement; CI, confidence interval; CV, coefficient of variation; LVEF, left ventricular ejection fraction; LVOT-G, left ventricular outflow tract gradient; PK/PD, pharmacokinetic/pharmacodynamic; SE, standard error

Figure 21. Visual Predictive Check for LVOT-G and LVEF PK/PD Models



Source: Applicant's Population PK/PD and Exposure Response Report, Figure S1, Page 8

Note: Blue shaded regions show the 5th to 95th percentile simulated range for 1000 subjects at the indicated concentration, and the solid blue line is the median. The dashed gold lines are the 5th, 95th, and solid gold line is the median concentrations for the data binned into 11 bins covering the x-axis

Abbreviations: LVEF, left ventricular ejection fraction; LVOT-G, post-Valsalva left ventricular outflow tract gradient; PK/PD, pharmacokinetic/pharmacodynamic

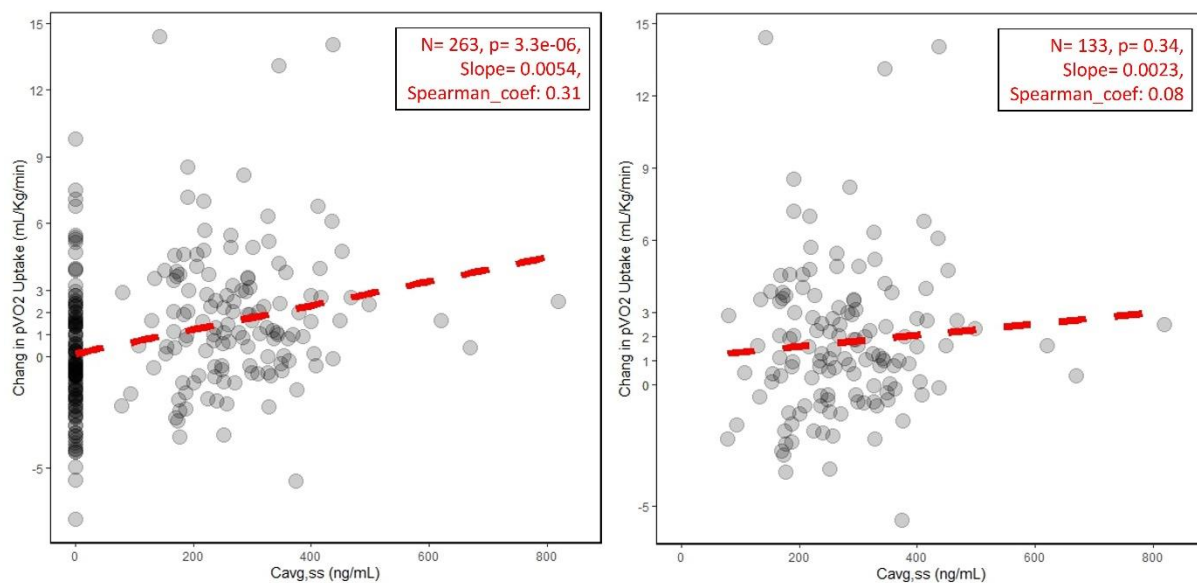
Exposure-Efficacy Relationship

The Exposure-efficacy population included 282 subjects, of which 140 received placebo and 142 received aficamten. The key findings from the Applicant's or reviewer's E-R analyses are as follows:

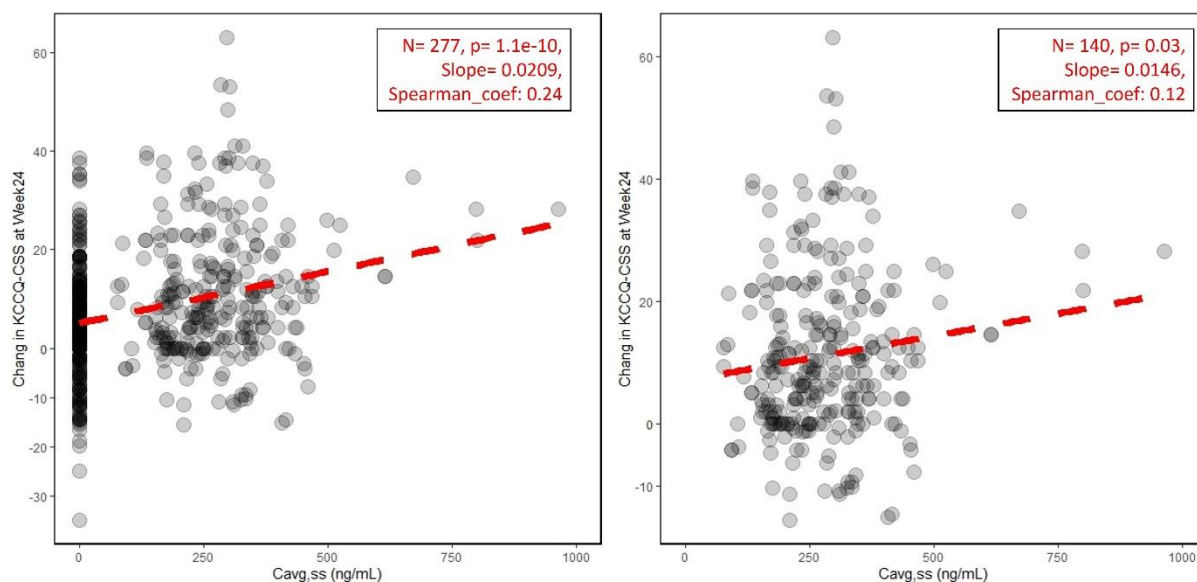
1. pVO₂ Uptake: in 263 placebo- and aficamten-treated subjects, higher aficamten C_{avg,ss} was associated (Spearman coefficient: 0.31) with greater improvements in pVO₂ from baseline at Week 24 ([Figure 22](#)).
2. NT-proBNP: in 266 placebo- and aficamten-treated subjects, there was a significant exposure response relationship (p<0.05) for probability of NT-proBNP change from baseline to Week 24, both with or without placebo.
3. KCCQ-CSS: in 117 placebo- and aficamten-treated subjects, increased response at Week 24 was associated with higher aficamten C_{avg,ss} (p<0.05) ([Figure 22](#)).
4. ≥1 NYHA Class Improvement: in 117 placebo- and aficamten-treated subjects, there was also a significant exposure response relationship (p<0.05) for probability of change from baseline to Week 24, both with or without placebo ([Figure 22](#)).
5. hs-cardiac-TnI: While large variability in changes in hs-cardiac-TnI from baseline was found, a relationship between this endpoint and aficamten exposure was not detected ([Figure 23](#)).

Figure 22. Relationship Between Aficamten PK Exposures ($C_{avg,ss}$) and Efficacy Endpoints Change From Baseline to Week 24 Using All Data (Placebo and Treatment Group, Left Side Figures) and Data from Treatment Group Only (Right Side Figures) Based on Trial CY 6031

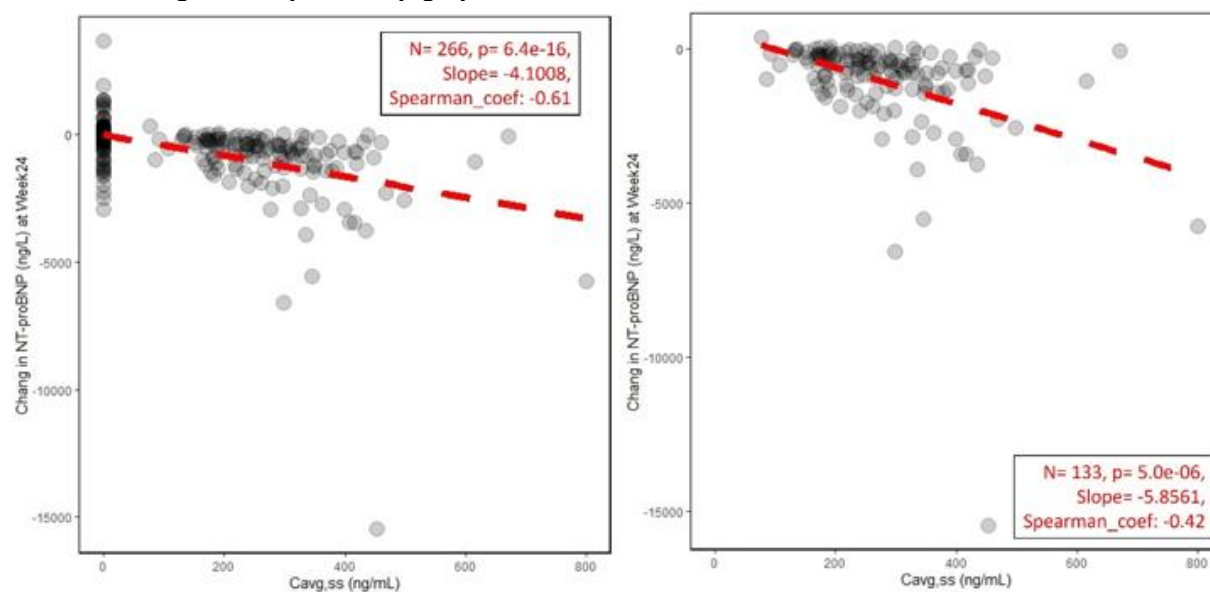
A. Change in pVO₂ Uptake (mL/Kg/min)



B. Change in KCCQ-CSS



C. Change in NT-proBNP (ng/L)



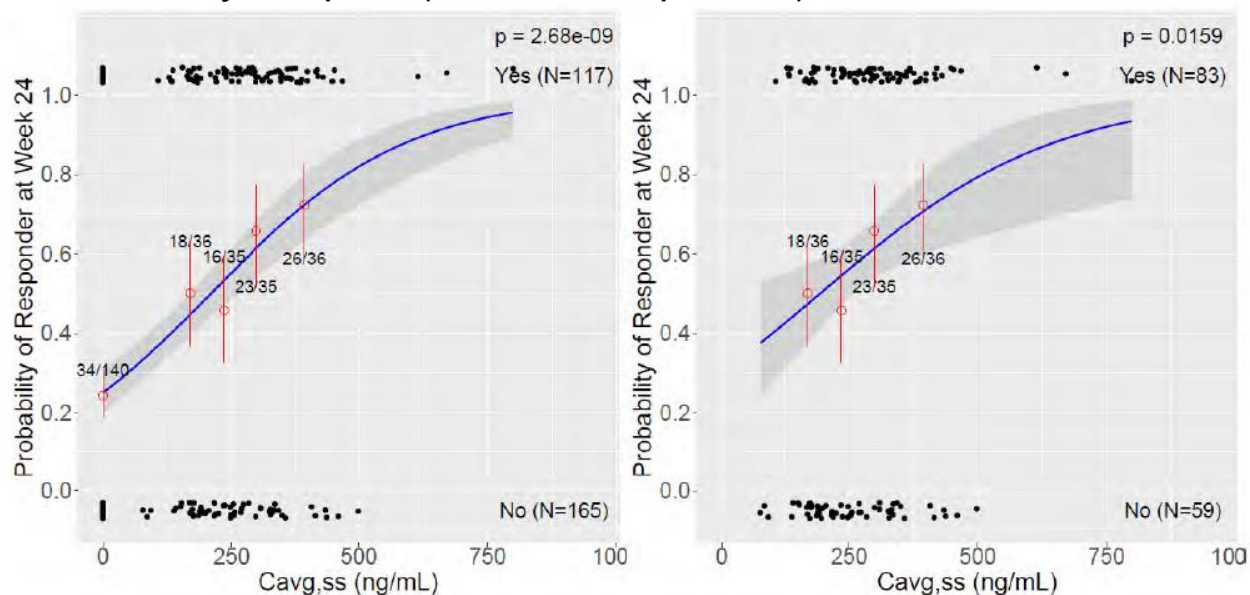
Source: Reviewer's analysis

Note: Red dashed line is a linear regression. Grey closed circles are the model predicted individual exposures. Placebo subjects shown at exposure =0 in the left panel.

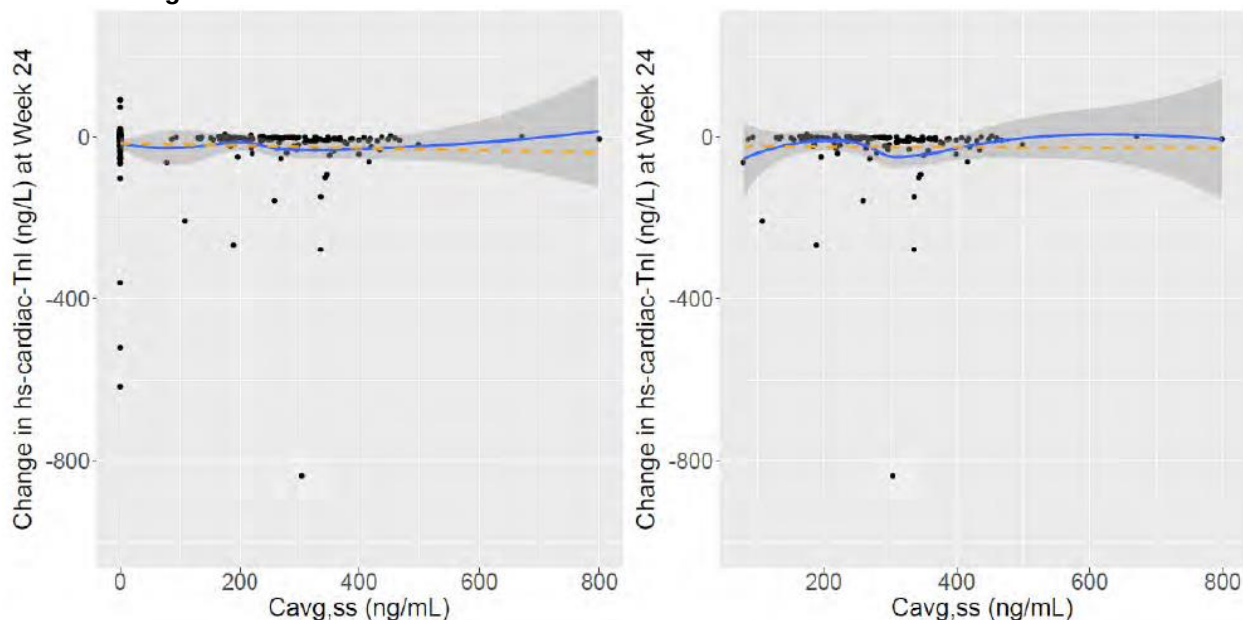
Abbreviations: $C_{avg,ss}$, model predicted average aficamten concentration at 24 hours prior to the assessment at Week 24; coef, coefficient; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire–Clinical Summary Score; N, number of subjects; NT-proBNP, N-terminal pro B-type natriuretic peptide; pVO_2 , peak oxygen consumption

Figure 23. Relationship Between Aficamten $C_{avg,ss}$ and (A) Probability of Being a Responder (≥ 1 NYHA Class Improvement From Baseline to Week 24) and (B) Change in hs-cardiac-TnI From Baseline for Placebo and Aficamten-Treated Subjects (Left Panel) and Aficamten-Treated Subjects Alone (Right Panel)

A. Probability of Responder (≥ 1 NYHA Class Improvement)



B. Change in hs-cardiac-TnI



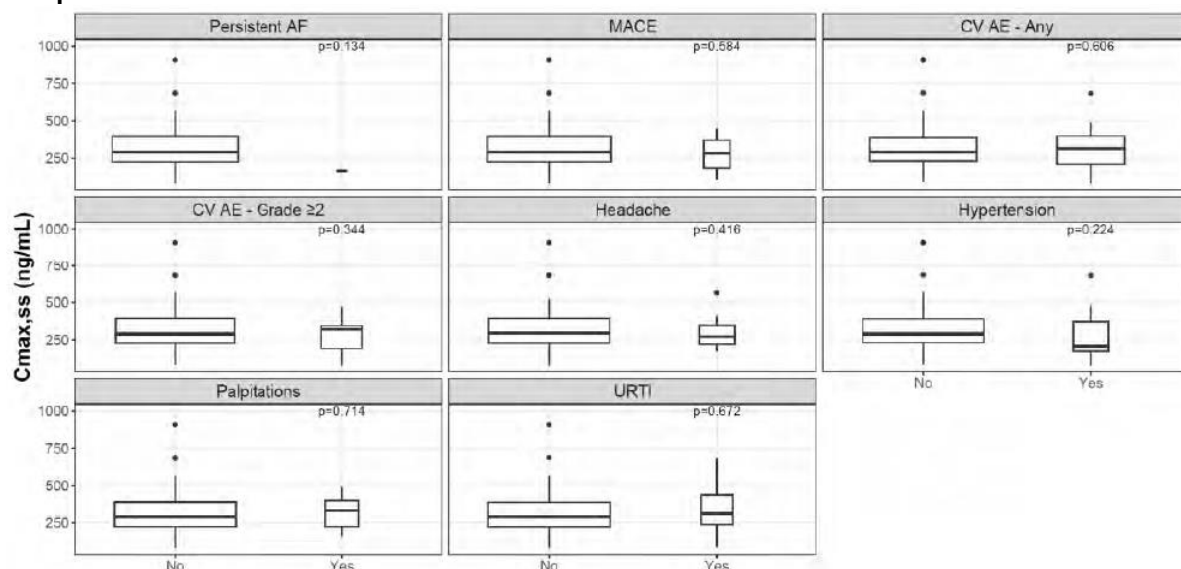
Source: Applicant's Population PK/PD and Exposure Response Report, Figure 4.15 and 4.17, Page 84 and 86

Note: In panel A, Yes and No refer to if subjects were or were not responders (≥ 1 NYHA class improvement from Baseline). Subjects were stratified into exposure quartiles. Red points are response rate and values are fraction of responders per exposure quartile plotted at the median exposure per quartile. Vertical red bars are 90% CIs of the response rate. Gray band represents the 5th to 95th percentile CI of a linear logistic regression fit. The p-value is the significance level of the slope of the logistic regression fit using a z-test. In panel B, the orange dashed line is a linear regression. Blue line and gray shaded area are a locally estimated smoothing function and 95% confidence interval, respectively. P-value for slope is 0.2433 (left panel) and 0.9253 (right panel). Abbreviations: $C_{avg,ss}$, model predicted average aficamten concentration at 24 hr prior to the assessment at Week 24; CI, confidence interval; hs-cardiac-TnI, high sensitivity troponin; NYHA, New York Heart Association; PK/PD, pharmacokinetic/pharmacodynamic

Exposure-Safety Analyses

The same population (282 subjects from Trial CY 6031, of which 140 received placebo and 142 received aficamten) was used in the exposure-safety analysis. A boxplot comparison ([Figure 24](#)) indicates that the rate of TEAEs was similar between placebo and aficamten-treated subjects, which included ICD discharge, persistent AF, major adverse cardiovascular event, Grade ≥ 2 cardiovascular AE, any Grade cardiovascular AE, headache, hypertension, palpitations, and upper respiratory tract infection as safety endpoints. [Table 114](#) shows that the rate of TEAEs did not increase with increasing aficamten exposures in the aficamten-treated group.

Figure 24. Boxplots of Aficamten $C_{max,ss}$ Grouped by Presence or Absence of Event for Safety Endpoints



Source: Applicant's Population PK/PD and Exposure Response Report, Figure 4.19, Page 89

Note: For boxplots, thick horizontal line represents median, box ends show the upper and lower quartiles of the data, whiskers show the range of points within 1.5 times the interquartile range, and points are data that lie outside the whiskers. The p-value is for a Kruskal-Wallis test of difference between distributions.

Abbreviations: AF, atrial fibrillation; Any, Any Grade; $C_{max,ss}$, steady-state maximum concentration; CV AE, cardiovascular adverse event; MACE, major adverse cardiovascular event; PK/PD, pharmacokinetic/pharmacodynamic; URTI, Any Grade upper respiratory tract infection

Table 114. Summary of Safety Endpoints by Treatment Group and by C_{max,ss} Quartiles in the Aficamten-Treated Group for the Exposure-Safety Dataset

Exposure/Safety Endpoint	Placebo	Aficamten	Aficamten C _{max,ss} Quartiles			
	All (N=140)	All (N=142)	Q1 (N=36)	Q2 (N=35)	Q3 (N=35)	Q4 (N=36)
Aficamten C_{max,ss}						
Median [Min, Max]	-	289 [81.2, 909]	189 [81.2, 223]	264 (223, 289]	349 (289, 394]	436 (394, 909]
ICD Discharge	1 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Persistent AF	1 (0.7)	1 (0.7)	1 (2.8)	0 (0)	0 (0)	0 (0)
MACE	9 (6.4)	6 (4.2)	2 (5.6)	1 (2.9)	2 (5.7)	1 (2.8)
CV AE - Any Grade	39 (27.9)	41 (28.9)	14 (38.9)	5 (14.3)	11 (31.4)	11 (30.6)
CV AE - Grade ≥2	16 (11.4)	15 (10.6)	6 (16.7)	1 (2.9)	5 (14.3)	3 (8.3)
Headache	10 (7.1)	11 (7.7)	4 (11.1)	3 (8.6)	2 (5.7)	2 (5.6)
Hypertension	3 (2.1)	11 (7.7)	6 (16.7)	0 (0)	2 (5.7)	3 (8.3)
Palpitations	4 (2.9)	10 (7)	3 (8.3)	1 (2.9)	3 (8.6)	3 (8.3)
URTI	12 (8.6)	9 (6.3)	2 (5.6)	2 (5.7)	2 (5.7)	3 (8.3)

Source: Applicant's Population PK/PD and Exposure Response Report, Figure 4.19, Page 88

Note: Values are number of events (%).

Abbreviations: C_{max,ss}, steady-state maximum aficamten concentration; CV AE, cardiovascular adverse event; ICD, implantable cardiac defibrillator; MACE, major adverse cardiovascular event; N, number of subjects; PK/PD, pharmacokinetic/pharmacodynamic; Q, quartile; URTI, upper respiratory tract infection

- The PK/PD model of aficamten is adequate to link aficamten exposure to LVEF and LVOT-G for aficamten-treated subjects from Study CY 6021 and Study 6031. The results of this analysis were used to support the proposed commercial dose regimen.
- The Pharmacometrics team has conducted independent analyses to evaluate the frequency of echocardiographic assessment during the titration and maintenance phases. Please refer to Section 6.1.
- Exposure-efficacy analyses demonstrated statistically significant relationships (p<0.05) between PK exposures and several efficacy endpoints, including the probability of ≥1 NYHA class improvement at Week 24, KCCQ-CSS change at Week 24, and NT-proBNP change at Week 24. However, exposure-efficacy analyses for pVO₂ change and hs-cardiac-TnI change at Week 24 did not demonstrate significant exposure-response relationships in the aficamten-treated group. The results of exposure-safety analyses did not show any trend between aficamten exposure (C_{avg,ss} and C_{max,ss}) and the evaluated safety endpoints.

14.5.3. Physiologically-Based PK Modeling

14.5.3.1. Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's PBPK analyses of predicting the DDI potential of aficamten as an object of strong, moderate and weak inhibitors or inducers of CYP2C9, CYP2C19, CYP2D6 and/or CYP3A in CYP2C9 normal and poor metabolizers (NM and PM).

The Division of Pharmacometrics has reviewed the PBPK analyses reports (cyt-11-b-report), the response to FDA's information requests submitted on February 24 (seq 0017), March 10, 2025 (seq 0021), and April 1, 2025 (seq 0026), and the modeling supporting files, and concluded as below:

- The strong CYP inducer rifampin was predicted to decrease aficamten AUC up to 87%.
- A moderate CYP inducer was predicted to decrease aficamten AUC up to 46%.
- A strong or moderate inhibitor of CYP3A or CYP2D6 was predicted to increase aficamten AUC up to 1.5-fold.
- The strong CYP2C9 inhibitor sulfaphenazole was predicted to increase aficamten AUC up to 2.6-fold.
- The prediction results for CYP inhibitors targeting multiple metabolic pathways involved in aficamten metabolism are complicated by the uncertainties regarding the contribution of CYP2D6 and CYP2C19 to aficamten metabolism. The effects of these inhibitors on aficamten exposure were predicted using an aficamten model without or with 3% or 21% contribution of CYP2D6 in aficamten metabolism incorporated, as summarized in [Table 124](#).

14.5.3.2. Background

Refer to Sections [5.2](#), [8.2](#), [14.1](#), and [14.2](#) for detailed information on aficamten regarding ADME properties, in vitro and clinical studies that was used in PBPK modeling.

14.5.3.3. Methods

Simulations related to evaluation of aficamten as an object of CYP inhibitors and inducers were performed using the PK/PD Profiles mode in the Simcyp® Simulator (Version 22, Certara, Sheffield, UK). Schemes of the PBPK simulation strategy are shown in [Figure 25](#), which summarizes the studies used for aficamten model development and verification, and model applications in DDI predictions. The final model input parameters are presented in [Table 115](#). The aficamten PBPK model consists of a first-order absorption model, a minimal PBPK model with a single adjusting compartment for distribution, and an enzyme kinetics model for elimination. The Simcyp library files itraconazole_Fed Capsule, itraconazole_Fasted soln, fluconazole, fluvoxamine, paroxetine, sulphaphenazole, verapamil, duloxetine, and voriconazole were used for DDI simulations without any modification. For the fluoxetine model, CYP3A4 inhibition parameters were excluded.

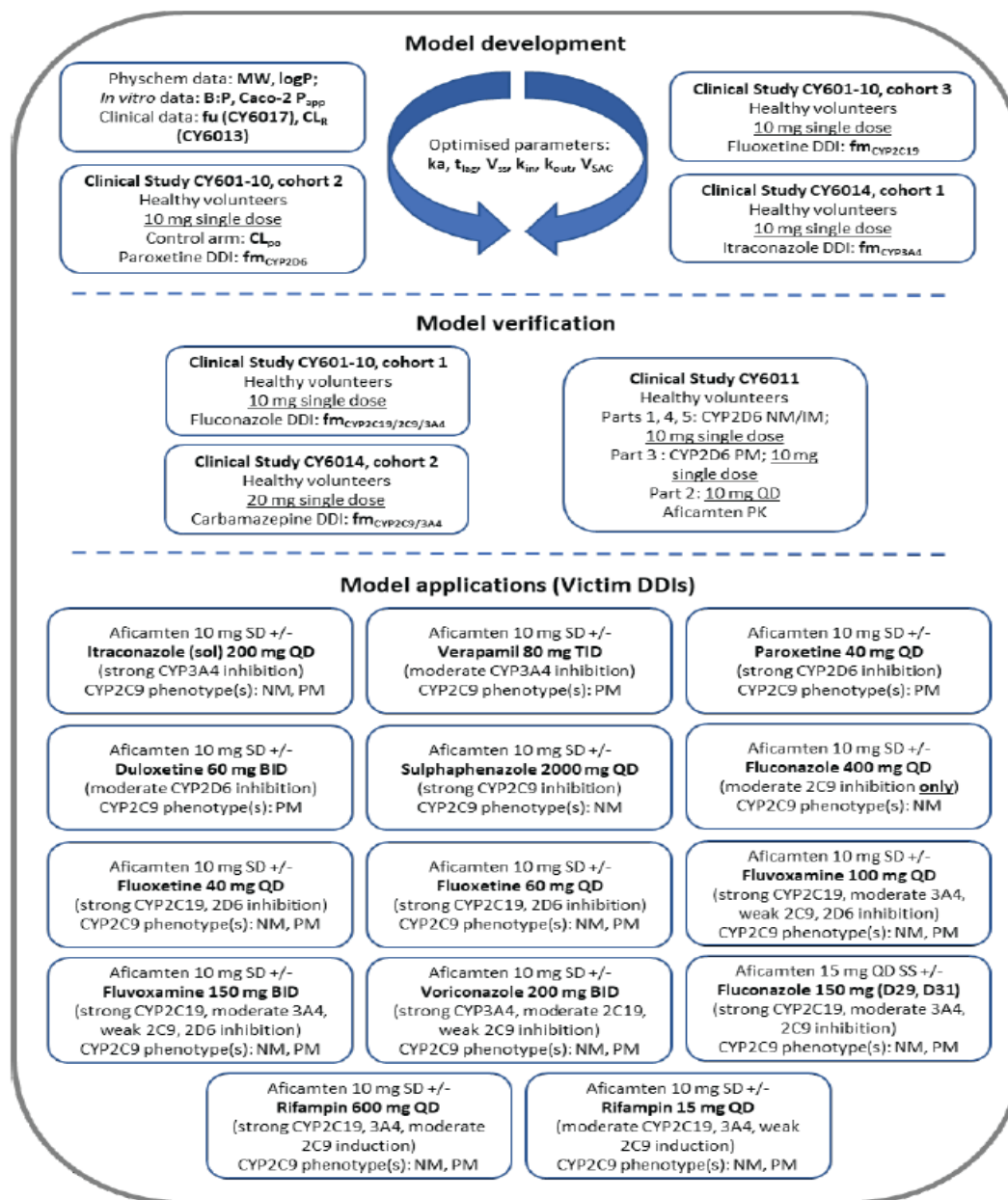
Additional Comments

6. The Applicant simulated interactions with carbamazepine using an updated carbamazepine model. In the updated model, CYP2C9 Ind_{max} and IndC_{50} values of 4.19 and 41.2 mM were incorporated in the model ([Endo-Tsukude et al. 2019](#)), and the $\text{Ind}_{\text{slope}}$ of CYP3A4 and CYP3A5 in the original model were replaced with their corresponding Ind_{max} and IndC_{50} values of 8.27 and 16 mM for CYP3A4 and 2.95 and 142 mM for CYP3A5 ([Stresser et al. 2016](#)). No changes were made to the model of its metabolite SV-Carbamazepine-10,11-epoxide. The performance of this updated carbamazepine model in predicting its induction effects on CYP3A substrates is presented in [Table 116](#). Since the CYP induction parameters in the carbamazepine model were updated specifically for this application, the Applicant was requested to provide additional verification of CYP2C9 induction by simulating the carbamazepine DDIs with S-warfarin and seladelpar,⁶ and the results are shown in [Table 117](#), [Table 118](#), and [Figure 26](#).
7. The rifampin-MD from version 23 was used for rifampin-related simulations. Rifampin 15-mg QD was used to simulate the effects of moderate CYP inducers on aficamten because of the lack of induction parameters of CYP2C19 and CYP2C9 in the commonly used efavirenz model. The Applicant was requested to provide evidence that rifampin 15-mg QD behaves as a moderate CYP3A and CYP2C19 and weak CYP2C9 inducer. The results are shown in [Table 119](#).
8. Upon the Agency's request, the Applicant provided the verification data for populations with CYP2D6 NM and CYP2D6 PM and populations with CYP2C9 NM and CYP2C9 PM. For the population models with different CYP2D6 phenotypes ([Figure 27](#)), although the majority of the AUC values of CYP2D6 substrates were predicted within 2-fold of the clinical observations, the predicted AUC differences between CYP2D6 NM and PM fell outside the 0.8 and 1.25. For the population models with different CYP2C9 phenotypes ([Figure 28](#)), the models could generally capture the observed plasma concentration-time profiles of S-warfarin and tolbutamide.
9. Fluconazole DDI with aficamten was used to verify the contribution of CYP3A, CYP2C9 and CYP2C19. The ability of the fluconazole model to predict its effects on substrates of these enzymes was examined and the results are shown in [Table 120](#).
10. Paroxetine was a reversible and time-dependent inhibitor of CYP3A4 in vitro. Although the CYP3A inhibition parameters were incorporated into the paroxetine model, it did not predict its interaction with quinidine, a moderately sensitive CYP3A substrate ([Table 121](#)). The reviewer updated the model by reducing the nonspecific binding parameter related to the time-dependent inhibition ($f_{u,\text{mic}}$) from 1 to 0.225 (Of note, similar optimization was done for the time-dependent inhibition parameters of CYP2D6 generated under similar experimental conditions [Simcyp paroxetine compound summary]). The modified model successfully reproduced the interaction between paroxetine and quinidine ([Table 121](#)) and thus was utilized in the subsequent analysis by the reviewer. Further details are provided in [Section 14.5.3.4](#). Of note, this modified paroxetine model predicted that paroxetine did not significantly increase the exposure of other CYP3A substrates ([Table 121](#)), which is consistent with clinical observations. In addition, the CYP2D6 time-dependent inhibition parameters from the same publication as the CYP3A inhibition parameters were reduced to

⁶ See FDA Summary Review for LIVDELZI (NDA 217899), dated December 14, 2023, available at https://www.accessdata.fda.gov/drugsatfda_docs/nda/2024/217899Orig1s000IntegratedR.pdf

reproduce the observed effects of paroxetine on CYP2D6 substrates (Simcyp paroxetine file summary).

Figure 25. Modeling and Simulation Strategy



Source: Figure 3 in the PBPK report (Cyt-11-b-report)

Abbreviations: BID, twice daily; B:P, blood to plasma ratio; CL_R , renal clearance; DDI, drug-drug interaction; f_m , fraction of drug metabolized; fu, fraction unbound in plasma; k_a , first order absorption rate constant; k_{in} , input rate constant; k_{out} , elimination rate constant; Log P, log of the octanol-water partition coefficient for the neutral compound; NM, normal or extensive metabolizer, P_{app} , apparent permeability coefficient; PBPK, physiologically-based pharmacokinetic; PK, pharmacokinetic; PM, poor metabolizer, QD, once daily; SD, single dose; TID, three times daily; t_{lag} , lag-time; V_{SAC} , volume of single adjusting compartment; V_{ss} , volume of distribution at steady state

Table 115. Final Input Parameters in the Aficamten Model

PARAMETER	Value	Reference
Physicochemical and Binding Parameters		
Molecular Weight (g/mol)	337.38	IB (V6.0)
Log P	2.7	CMC-0023
Compound type	Neutral	CMC-0023
B:P	0.94	Nonclin-0032
Fu	0.056	CY 6017
Main binding protein	HSA	Assumed
Absorption model – first order		
fa [CV]	1 [10%]	Assumed
ka (1/h) [CV]	2.25 [50%]	Manual optimisation (CY 601-10 Cohort 2, control arm)
t _{lag} (h) [CV]	0.25 [50%]	
f _{ugut}	1	Assumed
Caco-2 P _{app} (x10 ⁻⁶ cm/s)	41.4	Nonclin-0024
Calibrator P _{app} (x10 ⁻⁶ cm/s)	18.9	Nonclin-0024
P _{eff,man} (x10 ⁻⁴ cm/s)	8.1	Predicted from Caco-2 data
Q _{gut} (L/h)	17.5	Predicted from P _{eff,man}
F _G	0.99	Predicted from Q _{gut} model
Distribution model – minimal PBPK		
V _{ss} (L/kg)	4.66	Predicted, method 2 + K _p scalar
K _p scalar	4	Manual optimisation (CY 601-10 Cohort 2, control arm)
k _{in} (1/h)	0.3	
k _{out} (1/h)	0.2	
V _{SAC} (L/kg)	2.6	
Elimination parameters		
CYP3A4 CL _{int} (μL/min/pmol)	0.0243	See Table 2 . Total CL _{int,H} calculated using the retrograde model from CL/F (CY 601-10 Cohort 2, control arm) multiplied by estimates of fm determined from clinical DDIs (3A4: CY 6014 Cohort 1; 2D6: CY 601-10 Cohort 2; 2C19: CY 601-10 Cohort 3 and correct for hepatic abundance.
CYP2D6 CL _{int} (μL/min/pmol)	0.341	
CYP2C19 CL _{int} (μL/min/pmol)	0.0757	
CYP2C9 CL _{int} (μL/min/pmol)	0.113	
CL _R (L/h)	0.00263	CY 6013

Source: the PBPK report (Cyt-11-b-report)

Abbreviations: B:P, blood to plasma ratio; CL, clearance; CL/F, apparent oral clearance; CL_{int}, intrinsic metabolic clearance; CL_R, renal clearance; CYP, cytochrome P450; D50, median particle size; DDI, drug-drug interaction; f_a, fraction absorbed; F_G, intestinal availability; f_m, fraction of drug metabolized; fu, fraction unbound in plasma; f_{ugut}, fraction of blood unbound in the gut, i.e., within the enterocyte; k_a, first order absorption rate constant; k_{in}, input rate constant; K_p, tissue-plasma partition coefficient; k_{out}, elimination rate constant; Log P, log of the octanol-water partition coefficient for the neutral compound; P_{app}, apparent permeability coefficient; PBPK, physiologically-based pharmacokinetic; P_{eff,man}, effective human jejunum permeability; Q_{gut}, flow rate for overall delivery of drug to the gut (drug dependent); t_{lag}, lag-time; V_{SAC}, volume of single adjusting compartment; V_{ss}, volume of distribution at steady state

Table 116. Observed and Predicted Effects of Carbamazepine on Various CYP Substrates

Drug	Observed		Predicted		Predicted/Observed		Pubmed PMID
	C _{max} Ratio	AUC Ratio	C _{max} Ratio	AUC Ratio	C _{max} Ratio	AUC ratio	
Alprazolam	0.9	0.42	0.90(0.87 to 0.93)	0.48(0.41 to 0.53)	1	1..14	9536449
Omeprazole (a)	NA	0.7	0.83(0.78 to 0.87)	0.75(0.63 to 0.85)	NA	1.08	9278208
Omeprazole (b)	NA	0.77	0.81(0.71 to 0.91)	0.72(0.51 to 0.87)	NA	0.94	
Simvastatin	0.320	0.250	0.17(0.14 to 0.19)	0.15(0.12 to 0.16)	0.54	0.59	14691614
Zolpidem	0.590	0.430	0.70(0.69 to 0.71)	0.49(0.44 to 0.52)	1.19	1.15	21098143
Ethinylestradiol	0.81	0.58	0.68(0.62 to 0.75)	0.55(0.50 to 0.61)	0.84	0.94	12681003
Midazolam*\$	0.37	0.26	0.32(0.26 to 0.36)	0.23(0.18 to 0.27)	0.88	0.88	38864600
Midazolam*\$	0.318	0.211	0.31(0.29 to 0.38)	0.22(0.19 to 0.26)	0.99	1.03	29569712
Tolbutamide*\$	0.935	0.639	0.87(0.85 to 0.89)	0.64(0.61 to 0.67)	0.93	1.00	
Midazolam*\$	0.440	0.280	0.32(0.26 to 0.36)	0.22(0.18 to 0.26)	0.72	0.80	38105467
Midazolam*\$	0.460	0.320	0.32(0.26 to 0.38)	0.23(0.17 to 0.29)	0.70	0.71	

Source: Carbamazepine model summary in the appendix of the PBPK report (Cyt-11-b-report)

* Geometric mean

\$ AUC_{inf}

Abbreviations: AUC, area under the curve; AUC_{inf}, area under the curve extrapolated from time 0 to infinity; C_{max}, maximum plasma concentration; CYP, cytochrome P450; NA, not applicable; PBPK, physiologically based pharmacokinetic

Table 117. Observed and Predicted Median Clearance Ratios for Coadministration of Carbamazepine With S-Warfarin

Study	Dosing regimen		Test/Reference CL Ratio		
	Carbamazepine ^a	S-warfarin	Observed	Predicted	Predicted/ Observed
Herman <i>et al.</i> , 2006 ^b	100 mg BID Days 1-3, 200 mg BID Days 4-7, 300 mg BID Days 8-29	9 mg QD Day 16-29	2.13	1.88 (0.94 - 3.80)	0.89

^a Carbamazepine dosing regimen not provided, standard regimen assumed.

^b Parallel design – 2 simulations run with different demographics to capture interindividual variability.

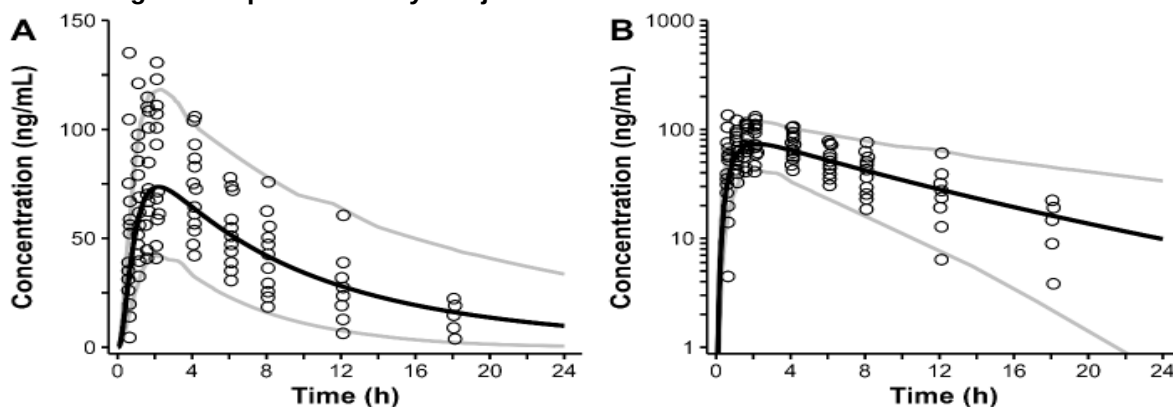
Source: response to NDA 219083 Aficamten_January 22, 2024, information request

Predicted Values Show Population Median and Trial Range (10 Trials).

[Herman et al. \(2006\)](#)

Abbreviations: BID, twice daily; CL, clearance; QD, once daily

Figure 26. Simulated and Observed Mean Plasma Concentration-Time Profiles of a Single Oral Dose of 10-mg Seladelpar in Healthy Subjects



Depicted are simulated (lines) and observed data (circles). The solid black line represents the mean and the grey lines the 5th and 95th percentiles for the simulated population (n = 100). Profiles are depicted on linear (A) and log-linear (B) axis.

Source: Response to NDA 219083 Aficamten_January 22, 2024, information request

Table 118. Observed and Predicted Seladelpar Exposure and Effects of CYP Precipitants on Seladelpar Exposure

Dosing Regimen		Baseline		With Perpetrator		GMR	
		C _{max} (ng/mL)	AUC _{inf} (ng/mL·h)	C _{max} (ng/mL)	AUC _{inf} (ng/mL·h)	C _{max} Ratio	AUC Ratio
Seladelpar 10 mg SD + Fluconazole 400 mg SD	Sim	66.3	727	105	1717	1.58	2.36
	Obs	78.1	697	110	1674	1.41	2.40
	S/O	0.849	1.04	0.952	1.03	1.12	0.984
Seladelpar 10 mg SD + Carbamazepine 300 mg BID	Sim	66.3	727	43.2	322	0.65	0.44
	Obs	79.7	675	60.6	381	0.76	0.56
	S/O	0.832	1.08	0.714	0.845	0.859	0.785

GMR = geometric mean ratio; Obs = observed; Sim = simulated; S/O = ratio of simulated to observed.

Source: response to NDA 219083 Aficamten_January 22, 2024, information request

Abbreviations: AUC_{inf}, area under the curve extrapolated from time 0 to infinity; BID, twice daily; C_{max}, maximum plasma concentration; CYP, cytochrome P450; SD, single dose

Table 119. Simulated Effects of Rifampin 15 mg Once Daily on the Exposure of CYP Substrates

Sensitive Substrate	Statistic	Baseline		With Perpetrator		GMR	
		AUC _{inf} (mg/L·h)	C _{max} (mg/L)	AUC _{inf} (mg/L·h)	C _{max} (mg/L)	AUC _{inf} Ratio	C _{max} Ratio
Midazolam (CYP3A4)	Simulated	0.0535	0.0194	0.0163	0.00757	0.304	0.390
	CV%	89.5	75.6	111	104		
	90% CI					0.281 - 0.328	0.364 - 0.417
Tolbutamide (CYP2C9)	Simulated	642	38.1	383	32.5	0.596	0.855
	CV%	75.4	37.3	76.1	39.7		
	90% CI					0.579 - 0.614	0.844 - 0.866
S-mephenytoin (CYP2C19)	Simulated	2.32	0.552	1.12	0.336	0.483	0.610
	CV%	171	56.3	193	72.6		
	90% CI					0.461 - 0.505	0.586 - 0.635

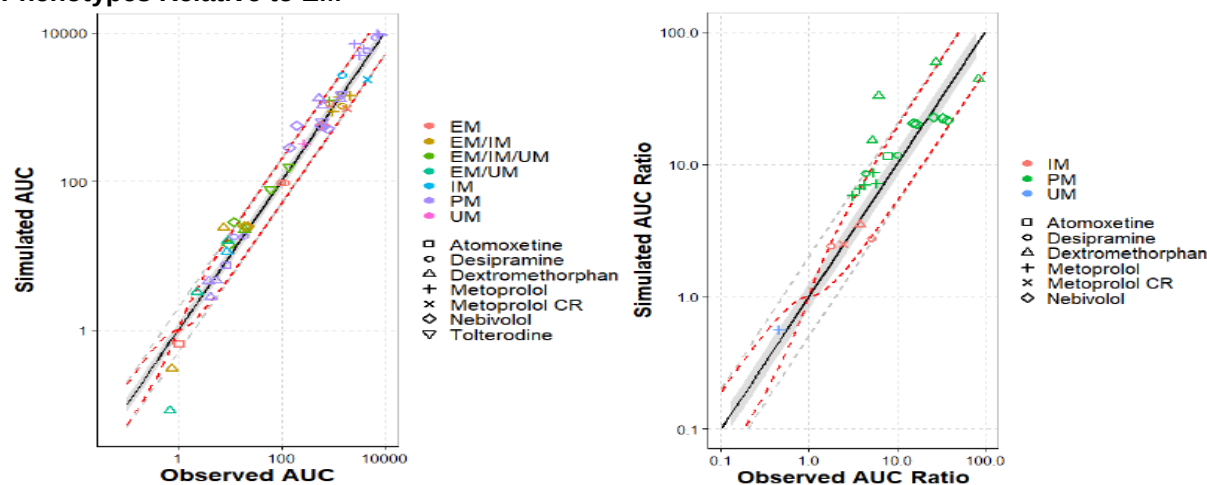
CV = coefficient of variation; CI = confidence interval; GMR = geometric mean ratio.

Simulated values listed are the geometric means.

Source: Table 4 in response to NDA 219083 Aficamten January 22, 2024, information request (seq0017)

Abbreviations: AUC_{inf}, area under the curve extrapolated from time 0 to infinity; C_{max}, maximum plasma concentration; CYP, cytochrome P450

Figure 27. Simulated vs. Observed AUC for CYP2D6 Substrates and AUC Ratios for CYP2D6 Phenotypes Relative to EM

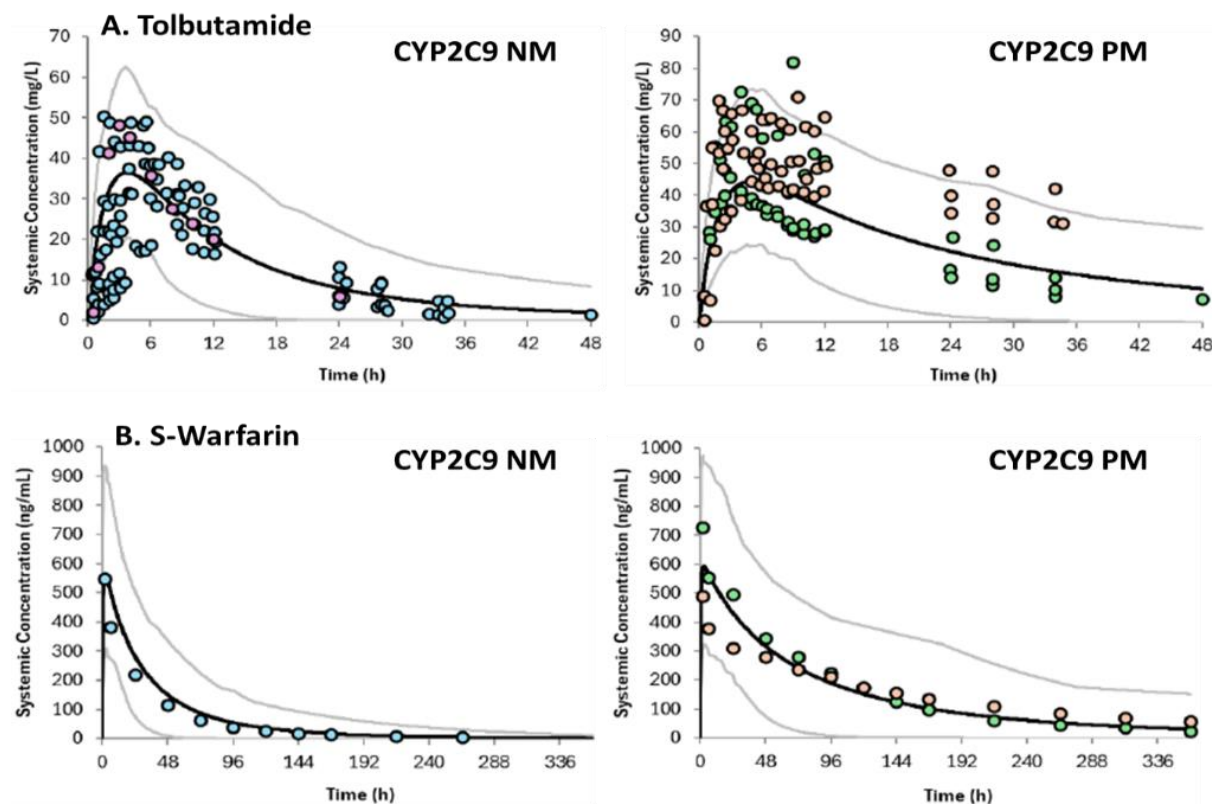


Source: Quantitative prediction of CYP2D6 phenotype pharmacokinetics and ratios relative to extensive metabolizer phenotype (seq0017)

Lines represent unity (solid black), predicted/observed fold error between 0.8 to 1.25 (solid grey) or within 2-fold (dashed grey) and according to the criteria described by (Guest et al. 2011) (red).

Abbreviations: AUC, area under the concentration-time curve; CR, controlled release formulation; CYP, cytochrome P450; EM, Extensive metabolizer; IM, Intermediate metabolizer; PM, poor metabolizer; UM, Ultra metaboliser

Figure 28. Simulated and Observed Plasma Concentration-Time Profiles of Tolbutamide and S-Warfarin Following a Single Oral Dose in CYP2C9 NM and PMs



Source: Responses to information request received on March 10, 2025 (seq 0021) and April 1, 2025 (seq 0026)
Depicted are simulated (lines) and observed data (circles). The black lines represent the mean and the grey lines the 5th and 95th percentiles of the simulated population (n=100). A. Data points represent either mean values (n=5; purple) or individual values (n=6, blue, CYP2C9*1*1; n=3, green, CYP2C9*2*3; n=3, orange, CYP2C9*3*3). B. Data points represent mean values (n=8, blue, CYP2C9*1*1; n=3, green, CYP2C9*2*3; n=4, orange, CYP2C9*3*3).
Abbreviations: CYP, cytochrome P450; NM, normal or extensive metabolizer, PM, poor metabolizer

Table 120. Predicted and Observed Fluconazole Interactions With Substrates of CYP3A, CYP2C9, and CYP2C19

Study	Observed		Predicted		Predicted/Observed	
	C _{max} Ratio	AUC Ratio	C _{max} Ratio	AUC Ratio	C _{max} Ratio	AUC ratio
Ahonen et al. 1997 (Midazolam)	2.30	3.73	1.92(1.78 to 2.12)	3.00(2.53 to 3.41)	0.83	0.80
Bavisotto et al. 2011 (S-Warfarin) *	1.15	2.56	1.06(1.04 to 1.08)	2.57(2.09 to 3.07)	0.93	1.00
Black et al. 1996 (S-Warfarin)	NA	2.92	1.06(1.04 to 1.09)	2.72(2.33 to 3.12)	NA	0.93
Lazar et al. 1990 (a) (Tolbutamide)	1.09	1.43	1.09(1.08 to 1.10)	1.31(1.28 to 1.35)	1.00	0.92
Lazar et al. 1990 (b) (Tolbutamide)	1.11	1.59	1.13(1.12 to 1.15)	1.51(1.45 to 1.58)	1.02	0.95
Sahai et al. 1994 (Zidovudine) †	1.79	1.75	1.30(1.28 to 1.34)	1.50(1.47 to 1.54)	0.73	0.86
Touchette et al. 1992 (Phenytoin) ‡	NA	1.33	1.09(1.07 to 1.11)	1.38(1.28 to 1.50)	NA	1.04
Varhe et al. 1996 (Triazolam) (a)	1.47	1.63	1.27(1.22 to 1.33)	1.52(1.44 to 1.64)	0.86	0.93
Varhe et al. 1996 (Triazolam) (b)	1.40	2.05	1.45(1.37 to 1.56)	2.01(1.86 to 2.24)	1.04	0.98
Varhe et al. 1996 (Triazolam) (c)	2.33	4.42	1.72(1.61 to 1.88)	3.03(2.71 to 3.49)	0.74	0.69
FDA 1998 (Celecoxib)	1.60	2.30	1.29(1.24 to 1.32)	1.85(1.80 to 1.92)	0.80	0.81
Derungs et al. 2016 (Omeprazole) §	3.43	13.50	3.46(3.18 to 3.76)	10.49(9.58 to 11.93)	1.01	0.78
Elbe et al. 2022 (Omeprazole)	3.25	8.22	3.54(3.16 to 4.20)	13.11(10.60 to 15.35)	1.09	1.60

Source: Simcyp V22 fluconazole summary report

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CYP, cytochrome P450; NA, not applicable

Table 121. Predicted and Observed Effects of Paroxetine on CYP3A Substrates

Paroxetine	CYP3A substrates	Observed		Predicted		Predicted/Observed		PMID
		C _{max} Ratio	AUC Ratio	C _{max} Ratio	AUC Ratio	C _{max} Ratio	AUC Ratio	
20 mg QD 12d	Quinidine 10 mg BID	1.30	1.38	1.06‡	1.08‡	0.78	0.81	22283559
				1.17	1.27	0.90	0.92	
20 mg QD 15d	Alprazolam 1 mg QD	1.00 (0.94, 1.07)	0.99 (0.93, 1.05)	1.10	1.20	1.10	1.20	15118480
20 mg SD	Midazolam 2 mg* SD		0.77	1.03	1.04†			32060866

Source: reviewer's analysis

* 30 mg was given in the clinical DDI study and AUC_{2-4h} was reported

† AUC_{0-inf}

‡ Predicted using Simcyp default CYP3A K_{app}. Others were simulated using CYP3A K_{app}×0.225.

Abbreviations: AUC, area under the concentration-time curve; AUC_{inf}, area under the concentration-time curve extrapolated from time 0 to infinity; BID, twice daily; C_{max}, maximum plasma concentration; CYP, cytochrome P450; DDI, drug-drug interaction; K_{app}, apparent rate constant; QD, once daily; SD, single dose

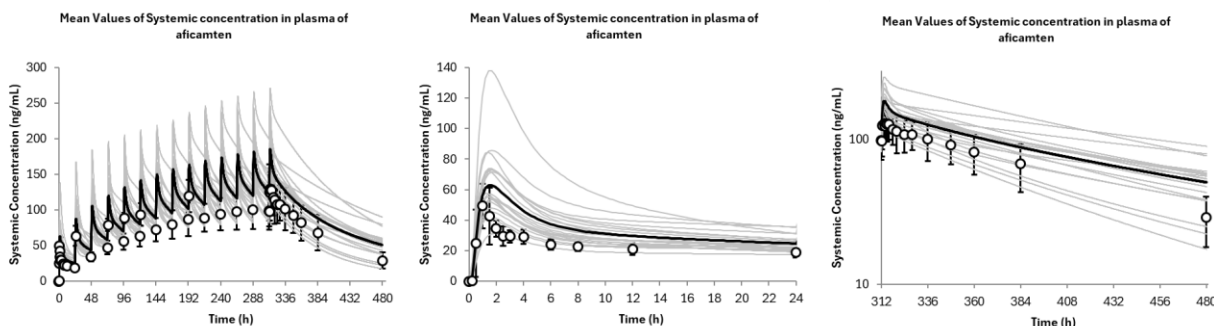
14.5.3.4. Results

Can the PBPK Model Adequately Describe the PK Profiles of Aficamten?

The aficamten PBPK model simulated plasma concentration-time profiles and PK parameters following administration of single and multiple doses of aficamten in healthy subjects are shown in [Figure 29](#), [Figure 30](#), [Table 122](#), and [Table 123](#). The model overpredicted the mean plasma concentration-time profiles compared to the observed mean profiles, but the predicted geometric mean PK parameters were mostly within the 0.8 to 1.25 bounds of the observed values. The discrepancy between the mean simulated and mean observed profiles may be attributed to

relatively large variability in the simulated trials. However, this may not be the case for aficamten pharmacokinetics in CYP2D6 PMs, where the variability in aficamten C_{max} and AUC between the simulated and the observed values is comparable ([Table 123](#)). The overprediction of aficamten pharmacokinetics in subjects with CYP2D6 PM will be further discussed later.

Figure 29. Simulated and Observed PK Profiles Following Oral Administration of 10-mg Aficamten Once Daily for 14 days in Healthy Subjects



Source: Figure 23 in the PBPK report (cyt-11-b).

Depicted are simulated (lines) and observed data (circles, mean of $n=6$; Clinical Study CY 6011, part 2). The grey lines represent mean values of simulated individual trials ($n=20 \times 6$) and the solid black line the mean data for the simulated population ($n=120$) [Source simulated data: cyt11b-ver-05].

Abbreviations: h, hour; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic

Table 122. Simulated and Observed PK Parameters Following Oral Administration of Aficamten in Healthy Subjects

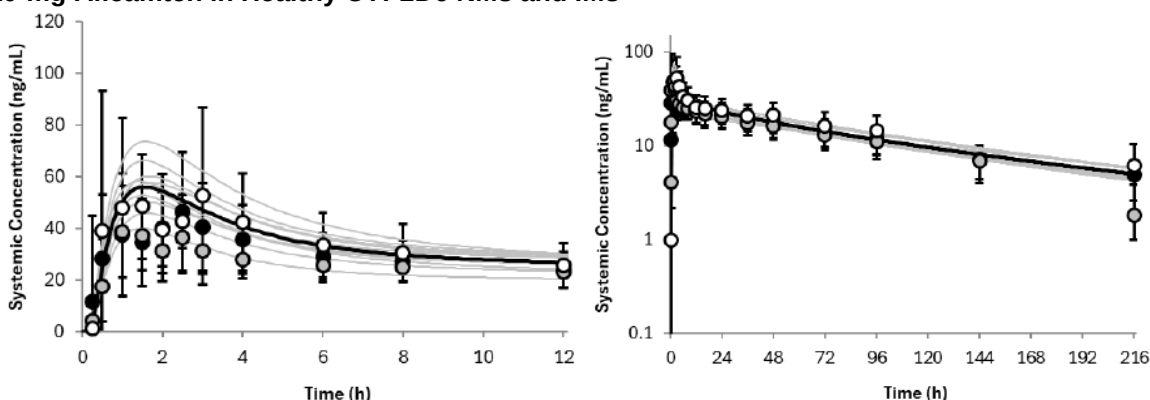
	Day 1			Day 14			
	AUC ₀₋₂₄ (h.ng/mL)	C _{max} (ng/mL)	t _{max} [*] (h)	AUC _{tau} (h.ng/mL)	C _{max} (ng/mL)	t _{max} [*] (h)	RA, AUC
Simulated	661	50.9	1.50	3193	171	1.45	4.83
GeoCV%	62	70	0.90 – 2.85	45	44	0.90 – 2.70	53
Observed	547	54.4	1.01	2632	141	2.51	4.82
GeoCV%	11	25	1.00 – 2.51	23	20	0.52 – 3.00	19
S/O	1.21	0.93	1.49	1.21	1.21	0.58	1.00

^{*}t_{max}: median, min, max; GeoCV: Geometric Coefficient of Variation; S/O: Simulated/Observed; Source observed data: Clinical Study [CY6011](#), part 2; Source simulated data: cyt11b-ver-05

Source: Table 14 in the PBPK report (cyt-11-b).

Abbreviations: AUC₀₋₂₄, area under the concentration-time curve from 0 to 24 hours; AUC_{tau}, area under the curve during dosing interval; C_{max}, maximum plasma concentration; h, hour; PK, pharmacokinetic; RA, accumulation ratio; t_{max}, time to maximum plasma concentration

Figure 30. Simulated and Observed Plasma Concentration-Time Profiles of a Single Oral Dose of 10-mg Aficamten in Healthy CYP2D6 NMs and IMs

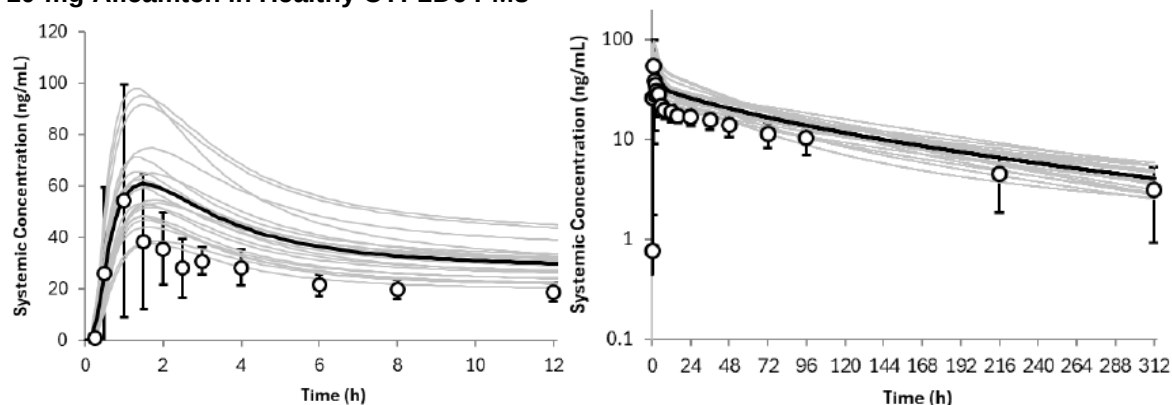


Source simulated data: cyt11b-ver-03.

Depicted are simulated (lines) and observed data (circles, white: mean of n=6; Clinical Study CY 6011, part 1; black: mean of n=10; Clinical Study CY 6011, part 4; grey: mean of n=0; Clinical Study CY 6011, part 5). The grey lines represent mean values of simulated individual trials (n=10x26) and the solid black line the mean data for the simulated population (n=260)

Abbreviations: CYP, cytochrome P450; h, hour; IM, intermediate metabolizer; n, number of subjects; NMs, normal metabolizer

Figure 31. Simulated and Observed Plasma Concentration-Time Profiles of a Single Oral Dose of 10-mg Aficamten in Healthy CYP2D6 PMs



Source: Figures 21 and 22 in the PBPK report (cyt-11-b)

Depicted are simulated (lines) and observed data (circles, mean of n=7; Clinical Study CY 6011, part 3). The grey lines represent mean values of simulated individual trials (n=20x7) and the solid black line the mean data for the simulated population (n=140) (Source: Simulated data, cyt11b-ver-04)

Abbreviations: CYP, cytochrome P450; h, hour; n, number of subjects; PBPK, physiologically based pharmacokinetic; PM, poor metabolizer

Table 123. Simulated and Observed PK Parameters Following Oral Administration of a Single Dose of 10-mg Aficamten in Healthy Subjects With Different CYP2D6 Phenotypes

Phenotypes	CYP2D6 NMs and IMs				CYP2D6 PMs			
PK parameters	C _{max} (ng/mL)	t _{max} [*] (h)	AUC _{0-inf} (h.ng/mL)	t _{1/2} (h)	C _{max} (ng/mL)	t _{max} [*] (h)	AUC _{0-inf} (h.ng/mL)	t _{1/2} (h)
Simulated	46.5	1.50	3394	89.8	50.4	1.53	4349	105
GeoCV%	63	0.85 – 3.35	55	77	67	0.90 – 2.80	56	82
Observed	50.0	2.50	3043	82.7	57.3	1.00	2966	103
GeoCV%	54	0.51 – 6.00	32	23	55	0.50 – 4.00	46	41
S/O	0.93	0.60	1.12	1.09	0.88	1.52	1.47	1.02

Source: Tables 12 and 13 in the PBPK report (cyt-11-b).

*t_{max}: median, min, max

Source observed data: Clinical Study CY 6011, parts 1, 3, 4 and 5; Source simulated data: cyt11b-ver-03, cyt11b-ver-04

Abbreviations: AUC_{0-inf}, area under the curve extrapolated from time 0 to infinity; C_{max}, maximum plasma concentration; CYP, cytochrome P450; GeoCV: Geometric Coefficient of Variation; h, hour; IM, intermediate metabolizer; max, maximum; min, minimum; NM, normal metabolizer; PK, pharmacokinetic; PM, poor metabolizer; S/O; Simulated/Observed; t_{1/2}, half-life; t_{max}, time to maximum plasma concentration ½

Can PBPK Analyses Predict the Effects of CYP Inhibitors and Inducers on the Pharmacokinetics of Aficamten?

The prediction results were complicated by the uncertainties about the contribution of CYP2D6 and CYP2C19 to aficamten metabolism. [Table 124](#) summarizes the predicted effects of both CYP inhibitors and inducers on the exposure of aficamten following single- or multiple-dose administration of aficamten, using an aficamten model with or without CYP2D6 metabolism incorporated. Similar effects of these precipitants on steady-state exposure of aficamten were predicted (data not shown, Response to information request (seq0021)). A detailed discussion follows.

Table 124. Predicted Drug Interactions of a Single Dose Aficamten as a Victim of CYP Inhibitors and Inducers in Healthy Subjects

CYP precipitants	Precipitant dosing regimen	Aficamten dosing regimen	Primary model ($f_{m,CYP2D6}$ 21%, $f_{m,CYP2C19}$ 3%)		Exploratory model ($f_{m,CYP2D6}$ 0%, $f_{m,CYP2C19}$ 24%)		Reviewer's model ($f_{m,CYP2D6}$ 3%, $f_{m,CYP2C19}$ 21%)			CYP2C9/ CYP2C19 phenotype
			AUC ratio	C _{max} ratio	AUC ratio	C _{max} ratio	AUC ratio	C _{max} ratio	Total AUCR	
Strong CYP3A inhibitor	Itraconazole * 200 mg QD 12d	10 mg SD D4	1.54	1.02						CYP2C9 PM
					1.45	1.02				CYP2C9 NM/ CYP2C19 PM
Moderate CYP3A inhibitor	Verapamil 80 mg TID 17d	10 mg SD D7	1.39	1.01						CYP2C9 PM
					1.28	1.01				CYP2C9 NM/ CYP2C19 PM
Strong CYP2D6 inhibitor	Paroxetine 40 mg QD 25d	10 mg SD D17	1.39	1.01						CYP2C9 PM
Moderate CYP2D6 inhibitors	Duloxetine 60 mg BID 12d	10 mg SD D4	1.16	1.00						CYP2C9 PM
Strong CYP2C9 inhibitor	Sulfaphenazole 2000 mg QD 15d	10 mg SD D7	2.13	1.03						CYP2C9 NM
					2.58	1.03	2.26	1.03	2.9	CYP2C9 NM/ CYP2C19 PM
Strong CYP2D6 & CYP2C19 inhibitor	Fluoxetine 40 mg QD 44d	10 mg SD D35	1.21	1.01	1.19	1.01				CYP2C9 NM
	60 mg QD 43d		1.22	1.01	1.21	1.01				
	40 mg QD 44d		1.33	1.01	1.30	1.01				CYP2C9 PM
	60 mg QD 43d		1.34	1.01	1.33	1.01				
Strong CYP2C19, weak CYP3A, 2C9 and 2D6 inhibitor	Fluvoxamine 100 mg QD 15d	10 mg SD D7	1.47	1.02	1.72	1.02				CYP2C9 NM
	150 mg BID 15d		2.16	1.04	2.51	1.04				
	100 mg QD 15d		1.39	1.01	1.72	1.02	1.66	1.02	3.5	CYP2C9 PM
	150 mg BID 15d		1.91	1.03	2.35	1.03	2.28	1.04	4.9	
Strong CYP3A, moderate 2C19 & weak 2C9 inhibitor	Voriconazole 400 mg BID D1 & 200 mg BID D2 -15	10 mg SD D7	1.94	1.04	2.66	1.05				CYP2C9 NM
					3.15	1.04	2.95	1.05	3.8	CYP2C9 NM/ CYP2C19 PM
			2.24	1.03	3.88	1.04	3.54	1.05	7.5	CYP2C9 PM
Strong CYP2C19, moderate CYP3A & CYP2C9 inhibitor	Fluconazole 400 mg QD 18d	10 mg SD D10			3.96	1.05				CYP2C9 NM
Moderate CYP2C19 inh					4.10	1.04	3.59	1.05	7.6	CYP2C9 PM
			1.57**	1.02**						CYP2C9 NM
Strong CYP2C19, moderate CYP3A & CYP2C9 inhibitor	Fluconazole 400 mg 2 doses 48 h apart***	15 mg QD	1.10	1.06	1.16	1.10				CYP2C9 NM
			1.08	1.05	1.13	1.09				CYP2C9 PM
Strong CYP3A & 2C19 & mod. CYP2C9 inducer	Rifampin 600 mg QD 37d	10 mg SD D29	0.21	0.83	0.16	0.77				CYP2C9 NM
			0.19	0.86	0.13	0.79				CYP2C9 PM
Mod. CYP3A & 2C19 & weak 2C9 inducer	Rifampin 15 mg QD 37d	10 mg SD D29	0.61	0.96	0.56	0.95				CYP2C9 NM
			0.60	0.97	0.54	0.96				CYP2C9 PM

Source: Table 40 and Table 67 in the PBPK report (cyt-11-b) and response to information request (seq0021)

Total AUCR, AUC in PMs in the presence of an inhibitor compared to AUC in NMs in the absence of the inhibitor was calculated by the product of AUCR in PMs in the presence of an inhibitor and difference in baseline AUC between NMs and PMs. For the worst case, the effect of sulfaphenazole on aficamten AUC in CYP2C9 NMs was considered as the difference in baseline AUC between CYP2C9 NMs and PMs. The difference in baseline aficamten AUC between CYP2C19 NMs and PMs was predicted to be 1.3. For reviewer's model, CL_{int} values (mL/min/mg) are 0.032 for CYP3A4, 0.101 for CYP2D6, 0.634 for CYP2C19, and 0.128 for CYP2C9

* SV-itraconazole_fasted soln was used for itraconazole simulations.

**considering CYP2C9 inhibition only.

***2 doses of fluconazole administered on Days 29 and 31 of steady state aficamten (15-mg QD) administration, AUC_{tau} for day 31 is presented.

Geometric mean ratios are presented.

Abbreviations: AUC, area under the concentration-time curve; AUCR, area under the concentration-time curve ratio; BID, twice daily; CL_{int}, intrinsic clearance; C_{max}, maximum plasma concentration; CYP, cytochrome P450; d or D, day; f_m, fraction metabolized; NM, normal metabolizer; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; PM, poor metabolizer; QD, once daily; SD, single dose; TID, three times a day

It should be noted that:

1. Contribution of each CYP in aficamten metabolism was a critical determinant of prediction accuracy. In vitro identification of CYP enzymes responsible for aficamten metabolism was conducted using recombinant CYP enzymes and chemical inhibition methods. Aficamten exhibited low turnover, with minimal changes in aficamten depletion except when incubated with recombinant CYP2D6. Notably, the major metabolite M1 formation occurred via recombinant CYP2C9, CYP2C19, CYP2D6 and CYP3A ([Figure 32](#)). However, isoform-selective inhibition of any single enzyme did not completely inhibit M1 formation ([Figure 32](#)).
2. The initial f_m values of CYP3A, CYP2D6 and CYP2C19 were determined using clinical DDI studies with itraconazole (Study CY 6014, cohort 1), paroxetine (Study CY 601-10, cohort 2) and fluoxetine (Study CY 601-10, cohort 3), respectively. The remaining metabolism was attributable to CYP2C9, based on the assumption that CYP2C9, CYP2C19, CYP2D6 and CYP3A collectively mediate all aficamten metabolism and that strong CYP inhibitors completely inhibit the corresponding CYP-mediated metabolism. For CYP2C19, its f_m value was derived by the difference between f_m values obtained from the DDI studies with fluoxetine (a strong CYP2D6 and CYP2C19 inhibitor) and paroxetine (a strong CYP2D6 inhibitor). The contributions of CYP3A and CYP2C9 were confirmed with the carbamazepine DDI study, which is a known inducer of CYP3A and CYP2C9. However, the aficamten model with 21% contribution of CYP2D6 in its metabolism (the primary model) resulted in overprediction of aficamten exposure in CYP2D6 PMs by 1.47-fold and overestimation of the exposure difference between PMs and non-PMs by 1.32-fold (data not shown). Additionally, this primary model underpredicted the interaction between aficamten and fluconazole ([Table 125](#)). Replacing the CYP2D6 contribution with CYP2C19 (the exploratory model) improved the prediction of aficamten exposure in CYP2D6 PMs and interaction of aficamten with fluconazole but failed to capture the effect of paroxetine ([Table 125](#)). It is worth noting that these models' ability to reproduce CYP2D6 polymorphism data may not provide robust evidence for model selection, given the large prediction errors observed in the verification of population models with CYP2D6 NM and PM phenotypes ([Figure 30](#)). Consequently, uncertainties persist regarding the relative contributions of CYP2D6 and CYP2C19 to aficamten metabolism.
3. In vitro studies revealed that while CYP2D6 exhibited greater catalytic activity in M1 formation ([Figure 32](#)), CYP2C19 displayed kinetic characteristics comparable to those observed with human liver microsomes. In addition, the CYP2C19 inhibitor ticlopidine at 25 mM produced maximal inhibitory effect on M1 formation among all CYP inhibitors tested in human liver microsomes ([Figure 32](#)). Moreover, the exploratory model, having 24% contribution of CYP2C19 in aficamten metabolism, could reproduce the effect of fluconazole on aficamten. Notably, fluconazole exerted markedly greater impact on aficamten metabolism than paroxetine or fluoxetine, rendering the fluconazole DDI data more reliable for f_m verification. This is particularly relevant given that the effects of paroxetine and fluoxetine were close to 1.25, a value that falls within the prediction error margins of PBPK analyses. To investigate whether the underprediction of fluconazole interaction was due to an underestimated contribution of CYP2C9, the reviewer modified the primary model by replacing CYP2C19 clearance with CYP2C9 clearance, increasing the contribution of CYP2C9 from approximately 56% to 67%. This adjusted model predicted a 2.56-fold increase in aficamten AUC by fluconazole, still underestimating its actual effect. Taken

together, the prediction results using the aficamten model with increased CYP2C19 contribution warrant consideration.

4. The Applicant used the results from the primary model ([Table 125](#)) to inform the product label. Their main argument was that the exploratory model, which did not account for CYP2D6 metabolism, could not reproduce the paroxetine DDI results. The main assumption of this argument is that paroxetine only inhibits CYP2D6, and the observed effect of paroxetine on aficamten pharmacokinetics confirms the contribution of CYP2D6 in aficamten elimination. As mentioned in Section [14.5.3.3](#), paroxetine is also a reversible and time-dependent inhibitor of CYP3A in vitro. The reviewer optimized the $f_{u,mic}$ value in the paroxetine model so that the model could reproduce the observed interaction between paroxetine and quinidine. To explore whether the exploratory model's inability to predict the paroxetine DDI with aficamten was due to an underestimation of paroxetine's inhibitory effects on CYP3A, the modified paroxetine model was employed to simulate the paroxetine and aficamten DDI. The exploratory model, which has no CYP2D6 contribution, predicted an 11% increase in aficamten AUC. The reviewer further modified the exploratory model by assigning an $f_{m,CYP2D6}$ of approximately 3% and an $f_{m,CYP2C19}$ of approximately 21%. This reviewer's adjusted aficamten model (reviewer's model) successfully predicted all the changes in aficamten AUC observed in the clinical DDI studies, including paroxetine ([Table 125](#)). However, since quinidine is the only CYP3A substrate in the Certara DDI Database that showed statistically significant interaction with paroxetine in vivo, additional simulation was performed using the reviewer's model and the Simcyp default paroxetine model, the one without modification of CYP3A inhibition parameters. Paroxetine was predicted to increase aficamten AUC by 11%, which was within 1.14-fold of the observed effect of paroxetine on aficamten. Therefore, even without the modified inhibitory effect of paroxetine on CYP3A, the reviewer's aficamten model could explain all the observed DDIs with aficamten.
5. Taken together, the modeling results suggested an alternative model which assumed a higher $f_{m,CYP2C19}$ value is possible. The Applicant's primary model (21% CYP2D6 and 3% CYP2C19) may not fully address the clinical DDI concern especially for managing DDIs with drugs that inhibit CYP2C19 but not CYP2D6 (e.g., fluvoxamine, fluconazole, voriconazole). For these drugs, DDI management should be informed by the predictions using the reviewer's model (3% CYP2D6 and 21% CYP2C19) ([Table 125](#)). The effects of CYP2C9 or CYP2C19 phenotype on aficamten exposure were simulated and the results are shown in [Table 126](#) and [Table 127](#).
6. It should be noted that the model for the CYP2C9 PM phenotype is a combination of two distinct genotypes (*2/*3 and *3/*3), which exhibit different CYP2C9 protein metabolic activities. The data were also weighted according to population frequencies (*2/*3: 1.6% of total population, 84.2% of PM population; *3/*3: 0.3% of total population, 15.8% of PM population). Consequently, the CYP2C9 PM phenotype model retains 50% of the metabolic activity compared to the NM phenotype. Notably, the estimated difference in aficamten exposure between CYP2C9 phenotypes may be underestimated, as the strong CYP2C9 inhibitor sulfaphenazole predicted an approximate 2-fold increase in aficamten AUC.

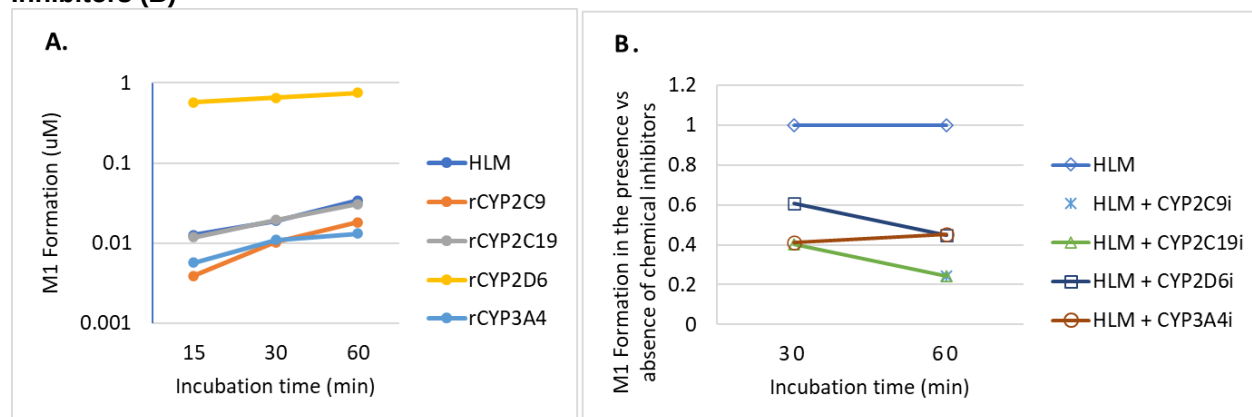
Table 125. Summary of Model Development and Verification of the Aficamten PBPK Model With or Without CYP2D6-Mediated Metabolism

Precipitant classification	Precipitant dosing Regimens	Aficamten dosing regimens	Aficamten models PK parameters	Primary model (f_m , CYP2D6 21%, f_m CYP2C19 3%)		Exploratory model (f_m , CYP2D6 0%, f_m CYP2C19 24%)		Reviewer's model (f_m , CYP2D6 3%, f_m CYP2C19 21%)	
				AUC Ratio	C_{max} Ratio	AUC Ratio	C_{max} Ratio	AUC Ratio	C_{max} Ratio
Strong CYP3A inhibitor	Itraconazole (N=16) 200 mg capsule QD 12 days	10 mg SD Day 4	simulated	1.27	1.01			1.3	1.02
			observed	1.26	0.93			1.26	0.93
			s/o	1.01	1.09			1.03	1.10
Strong CYP2D5 and weak CYP3A inhibitor	Paroxetine (N=17) 20 mg QD 7d 40 mg QD 18 days	10 mg SD Day 17	simulated	1.36	1.02	1.03	1.01	1.20*	1.01*
			observed	1.27	1.2	1.27	1.2	1.27	1.2
			s/o	1.08	0.84	0.82	0.83	0.94	0.85
Strong CYP2D6 and CYP2C19 inhibitor	Fluoxetine (N=17) 20 mg QD 7 days 40 mg QD 19 days	10 mg SD Day 19	simulated	1.29	1.01	1.24	1.01	1.3	1.02
			observed	1.32	1.55	1.32	1.55	1.32	1.55
			s/o	0.98	0.65	0.94	0.65	0.98	0.66
Strong CYP2C19, moderate CYP3A and CYP2C9 inhibitor	Fluconazole (N=16) 400 mg QD 18 days	10 mg SD Day 10	simulated	2.49	1.04	3.96	1.05	3.63	1.06
			observed	3.78	0.99	3.78	0.99	3.78	0.99
			s/o	0.66	1.05	1.05	1.07	0.96	1.07
Moderate to strong CYP3A and weak CYP2C9 inducer	Carbamazepine 100 mg BID 3 days, 200 mg BID 4 days, 300 mg BID 19days	10 mg SD Day 19	simulated	0.51	0.96			0.5	0.95
			observed	0.49	0.69			0.49	0.69
			s/o	1.03	1.39			1.02	1.38

Source: Tables 8 to 13 and 42 to 45 in the PBPK report (cyt-11-b) and reviewer's analysis

* Modified paroxetine model was used in this simulation, in which the nonspecific binding value for the CYP3A4 time-dependent inhibition K_{app} was set to 0.225 (see Section 14.5.3.3). For reviewer's model, CL_{int} values (mL/min/mg) are 0.032 for CYP3A4, 0.101 for CYP2D6, 0.634 for CYP2C19, and 0.128 for CYP2C9. SV-itraconazole_Fed Capsule was used for itraconazole simulations. Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; CL_{int} , intrinsic clearance; C_{max} , maximum plasma concentration; CYP, cytochrome P450; f_m , fraction metabolized; K_{app} , apparent rate constant; N, number of subjects; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; QD, once daily; SD, single dose

Figure 32. Depletion of Aficamten and M1 Formation Following Incubation of Aficamten With Human Liver Microsomes and Recombinant CYP Enzymes (A) or Isoform Selective Chemical Inhibitors (B)



Source: Generated by the reviewer-based on the data reported in Nonclin-0029

Note: the effect of the CYP2C9 inhibitor on M1 formation at 30 min was not determined.

Abbreviations: CYP, cytochrome P450; HLM, human liver microsomes; i, inhibitor; M, metabolite; r, recombinant enzyme

Table 126. Simulated Changes in CYP2C9 Expression and Aficamten AUC_{inf} in CYP2C9 NM and PM Populations

	CYP2C9 NMs	CYP2C9 PMs	GMR
CYP2C9 expression (pmol/mg)	57.2	22.6	0.396
Primary model AUC _{inf} (ng/ml.h)	3824	5624	1.47
Exploratory model AUC _{inf} (ng/ml.h)	3783	5523	1.46

Source: Table 69 in the PBPK report (cyt-11-b)

Abbreviations: AUC_{inf}, area under the curve extrapolated from time 0 to infinity; CYP, cytochrome P450; GMR, geometric mean ratio; NM, normal metabolizer; PBPK, physiologically based pharmacokinetic; PM, poor metabolizer

Table 127. Simulated Changes in CYP2C19 Expression and Aficamten AUC_{inf} in CYP2C19 NM and PM Populations

	CYP2C19 NMs	CYP2C19 PMs	GMR
CYP2C19 expression (pmol/mg)	3.94	0.00	-
Primary model AUC _{inf} (ng/mL·h)	4055	4131	1.02
Exploratory model AUC _{inf} (ng/mL·h)	3957	5152	1.30

CYP2C19 expression: median values, AUC_{inf}: geometric mean values.

Source: Table 9 in the response to IR (seq0021)

Abbreviations: AUC_{inf}, area under the curve extrapolated from time 0 to infinity; CYP, cytochrome P450; GMR, geometric mean ratio; NM, normal metabolizer; PM, poor metabolizer

14.6. Pharmacogenetics

Background

Aficamten is primarily eliminated via CYP2C9-mediated metabolism with contributions from CYP3A, CYP2D6, and CYP2C19. Aficamten is primarily metabolized to two pharmacologically inactive metabolites, CK-3834282 and CK-3834283, that circulate at approximately 56% and 103% of parent drug in plasma, respectively.

Multiple enzymes metabolizing aficamten are highly polymorphic (e.g., CYP2C9, CYP2C19, CYP2D6). In the DDIs study (Study CY 601-10), only subjects identified as normal or ultrarapid metabolizers of polymorphic enzymes were included. In addition, Study CY 6011 assessed the effect of CYP2D6 PMs on the pharmacokinetics of aficamten (see review Section [14.2](#) for additional study information and results).

The Applicant's proposed aficamten labeling includes sections **2 DOSAGE AND ADMINISTRATION** and **7 DRUG INTERACTIONS**, which describe the effect of coadministration of inhibitors of multiple enzymatic pathways on aficamten pharmacokinetics, efficacy, and safety, and provides recommendation for dosage adjustment. Due to lack of availability of genetic data for CYP2C9 and CYP2C19 PMs, effect of CYP2C9 and CYP2C19 PMs in combination with inhibition of multiple enzymatic pathways, is based on the results obtained from the clinical DDI studies and PBPK modeling. Similar increases in aficamten exposure are expected for CYP2C9 and CYP2C19 PMs as seen with coadministration of inhibitors of multiple enzymatic pathways that target CYP2C9 and CYP2C19. Dosage recommendations and management of DDIs for PMs of the primary metabolizing enzyme

CYP2C9, should follow general dosage recommendation and dosage modifications for drug-drug interactions. For additional study and PBPK modeling information refer to Section [14.2](#) and Section [14.5.3](#).

Aficamten dosage recommendation and management of DDIs, especially in situations when multiple metabolic pathways are inhibited, accounts for subjects who are CYP2C9 PMs with minimal enzyme activity. No additional dosage modifications are recommended to further adjust dosage of aficamten in subjects who are CYP2C9 PMs.

Translating CYP2C9 genotype-to-phenotype relies on categorizing alleles into functional status based on assigned “activity value” ranging from 0 to 1 (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for normal function) which are summarized into an “activity score (AS)” for each diplotype.

Table 128. Clinical Pharmacogenetics Implementation Consortium Standard for CYP2C9 Genotype-to-Phenotype Translation Using Activity Scores

Phenotypes	Activity Scores Definition	Examples of Diplotypes
Normal metabolizer	2	*1/*1
Intermediate metabolizer	1.5	*1/*2
	1	*1/*3, *2/*2
Poor metabolizer	0.5	*2/*3
	0	*3/*3

Source: Reviewer-generated table based on [Theken et al. \(2020\)](#)

Note: Other no function and decreased function alleles are more prevalent in Non-White population (e.g., *5, *6, *8, *11) than *2 and *3 alleles.

Abbreviations: CYP, cytochrome P450

The CYP2C9 phenotype frequencies differ across ancestrally diverse populations (see [Table 129](#)).

Table 129. Frequency of the CYP2C9 Phenotypes Across Racial/Ethnic Populations

Racial/Ethnic Populations	Phenotype Frequency (%) Based on Activity Scores		
	NM	IM	PM
African-American/Afro-Caribbean	76	24	0.1
American ¹	83	16	<0.1
Central/South Asian	60	36	3.8
East Asian	84	15	0.1
European	63	35	2.6
Latino	74	25	0.1
Near Eastern	61	36	3.0
Oceanian	91	9	0.1
Sub-Saharan African	73	26	0.1

Source: Reviewer-generated table based on [Theken et al. \(2020\)](#). Racial/Ethnic Population categorization based on [Huddart et al. \(2019\)](#)

¹The American genetic ancestry group includes populations from both North and South America with ancestors predating European colonization, including American Indian, Alaska Native, First Nations, Inuit, and Métis in Canada, and Indigenous peoples of Central and South America.

Abbreviations: CYP, cytochrome P450; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer

Labeling Recommendations

Draft labeling language for 12.5 Pharmacogenomics.

12.5 Pharmacogenomics

Aficamten is extensively metabolized by multiple highly polymorphic enzymes (e.g., CYP2C9, CYP2C19, CYP2D6) with primary metabolism mediated by CYP2C9 [see Clinical Pharmacology (12.3)]. The impact of CYP2C9 genetic variants on the pharmacokinetics of aficamten has not been directly evaluated, though, a similar effect on exposure is expected as seen with strong CYP2C9 inhibitors [see Drug Interactions (7.1)].

No additional dosage modifications are required for patients who are CYP2C9 poor metabolizers, as aficamten dosage titration and modifications account for CYP2C9 metabolizer status [see Dosage and Administration (2.2) and (2.3)].

Please refer to labeling for a final labeling recommendation.

Summary and Conclusions

Aficamten is extensively metabolized in the liver by multiple polymorphic CYP450 enzymes; CYP2C9 (major metabolizing enzyme), CYP3A4, CYP2C19, and CYP2D6. Results obtained from the DDI studies and from the PBPK model suggest that subjects with genetically decreased CYP enzyme activity are expected to have similar increase in exposure as those of corresponding CYP inhibitors. Based on the available information, aficamten labeling includes recommendations for dosage titration and dosage modification for DDIs, which accounts for subjects who are CYP2C9 PMs.

15. Study/Trial Design

The SEQUOIA-HCM trial (CY 6031) has been described in Section 6. [Table 130](#) summarizes the amendments to the protocol. None of the amendments jeopardized trial conduct or data integrity.

Table 130. Summary of Changes to the Protocol for SEQUOIA-HCM

Amendment	Description of Change
Amendment 01 August 17, 2021	• Revised eligibility criteria to include subjects with a wall thickness ≥ 13 mm in one or more wall segments and a known disease-causing gene mutation or positive family history of HCM. • Corrected exclusion criterion to state that subjects with an oxygen saturation $< 90\%$ were not eligible for the study. • Clarified that eligibility criteria for the cardiac magnetic resonance (CMR) sub-study were exclusion criteria.

Amendment	Description of Change
Amendment 02 December 10, 2021	- Allowed enrollment of subjects taking disopyramide; clarified enrollment criteria for subjects taking disopyramide; and added enrollment cap for subjects taking disopyramide. - Increased the number of subjects in the CMR sub-study to ensure appropriate power; clarified CMR requirements; and clarified CMR testing window at the end of treatment. - Added lifestyle restrictions prior to CPET. - Modified the schedule of activities: removed PGI-C on Day 1 and Week 28, removed CGI at Day 1, added EQ-5D-5L timing to align with those of KCCQ and NYHA, removed medical/surgical history at Day 1, added more frequent pregnancy testing, added collection of plasma for future analysis, and added an option to split the Week 24 visit over 2 days.
Amendment 03 January 3, 2023	- Added endpoints to evaluate the duration of time that subjects were eligible for SRT. - Updated pVO₂ criterion to allow for subjects with higher predicted pVO₂. - Added the option to increase the sample size (based on pVO₂ variability and missing data rate) to maintain the intended power. - Clarified that designated site staff who were unmasked to echocardiogram results remained blinded to subject treatment assignments.
Amendment 04 December 8, 2023	- Added the safety endpoint of incidence of LVEF <50% with signs and symptoms of heart failure (concomitant AE of heart failure or dyspnea) and/or increase in NT-proBNP from baseline. - Updated the definition of the FAS to include all randomized subjects. - Simplified the testing hierarchy from a parallel gatekeeping method to a closed testing procedure.

Source: Applicant's CSR Table 8

Abbreviations: CGI, clinical global impression; CMR, cardiac magnetic resonance; CPET, cardiopulmonary exercise testing; EQ-5D-5L, Euro Qol 5-dimension 5-level; FAS, Full Analysis Set; HCM, hypertrophic cardiomyopathy; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PCI-C, Patient Global Impression of Change; pVO₂, peak oxygen consumption; SRT, septal reduction therapy

16. Efficacy

16.1. Additional Efficacy Results in CY 6031 (SEQUOIA-HCM)

Subgroup Analysis of the Primary Endpoint

Subgroup analyses for the primary endpoint 'Change in pVO₂ by CPET from baseline to Week 24' were performed for the following subgroups:

- Sex (male, female)
- Age group (<65, ≥65 years old)
- Race
- Ethnicity
- Baseline body mass index (<30 versus ≥30 kg/m²)

- Baseline NYHA Class (II, III)
- Baseline KCCQ-CSS ($\leq$ median and $>$ median)
- Baseline LVEF ($\leq$ median and $>$ median)
- Baseline NT-proBNP ($\leq$ median and $>$ median)
- CPET modality (treadmill, bicycle)
- Baseline pVO₂ ($\leq$ median, $>$ median)
- Beta Blocker (use, no use)
- Baseline resting LVOT ($\leq$ median and $>$ median)
- Baseline KCCQ-CSS ($\leq$ median and $>$ median)

Forest plots of results are presented in [Table 131](#) and [Table 132](#) below. Overall, the treatment effect in these subgroups was consistent with that of the overall population, except in Hispanic or Latino, and Not reported subpopulations. The largest positive treatment effect was in the Hispanic or Latino subpopulation. Due to the very small sample size, these estimates have wide confidence intervals and lack precision.

There were likely some random highs and random lows in sample estimates of subgroup treatment effects due to small sample size and large variability for some subgroups. Therefore, we also derived shrinkage estimates of subgroup treatment effects using a Bayesian hierarchical model based on summary sample estimates. This approach leads to improved precision and lower variability. The total variability in the sample estimates is the sum of the within subgroup variability of the sample estimator and the across subgroups variability in underlying/true parameter values. A shrinkage estimates of the subgroup treatment effect, which borrows information from the other subgroups while estimating the treatment effect for a specific subgroup, is a “weighted” average of the sample estimate and overall estimate. The weights are based on the ratio of the between subgroup variability to the within subgroup variability. The greater that ratio the smaller the weight on the overall estimate (the less the shrinkage). We used the same flat prior to derive shrinkage estimates for all subgroups. The Bayesian hierarchical model assumptions for change in pVO₂ by CPET from baseline to Week 24 are:

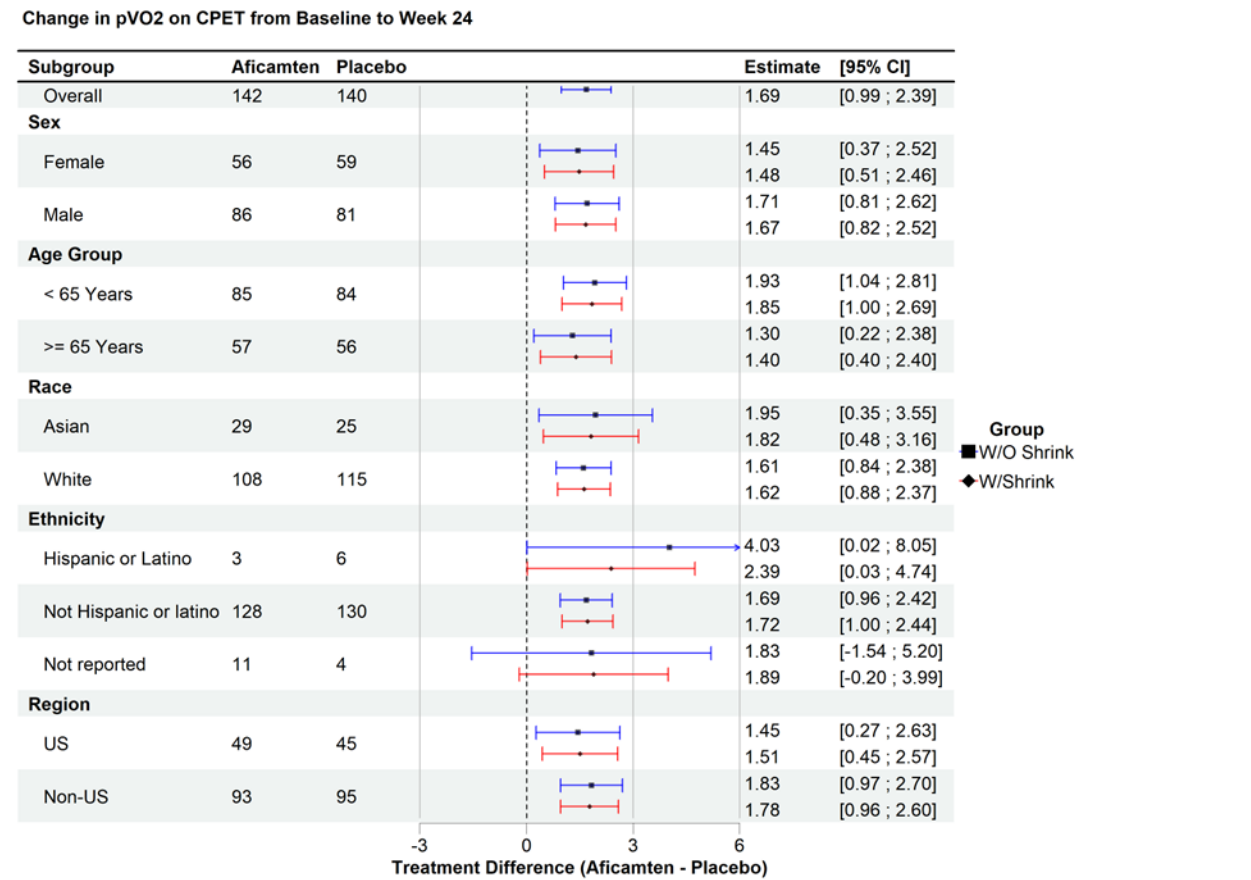
For $i = 1, 2, \dots$ Y_i represents the observed sample estimate of the treatment effect in a subgroup level i , assume $Y_i \sim N(\mu_i, \sigma_i^2)$ where:

- σ_i^2 are the observed variance for sample estimates
- $\mu_i \sim N(\mu, \tau^2)$
- $\mu \sim N(0, 16)$, $\tau \sim \text{Half Normal}(0, 4)$

We placed a prior distribution on overall mean $\mu \sim N(0, 16)$, reflecting a weakly informative belief that the average treatment effect is centered around zero, with most of the prior mass within ± 8 units. We used a Half-Normal (0,4) prior for the standard deviation of subgroup effects (τ). This reflects an assumption that subgroup variability is likely to be modest, based on the observed standard errors (ranging from 0.37 to 2.1), while still allowing for larger variation if supported by the data. [Table 131](#) compares the conventional subgroup analysis results of the sample estimate (in blue) and Bayesian shrinkage estimate (in red). The overall treatment effect

is 1.69 (95% CI: 0.99, 2.39). Subgroup analysis using Bayesian shrinkage estimate exhibits a narrower credible interval, and the shrinkage subgroup estimate is closer to the overall mean.

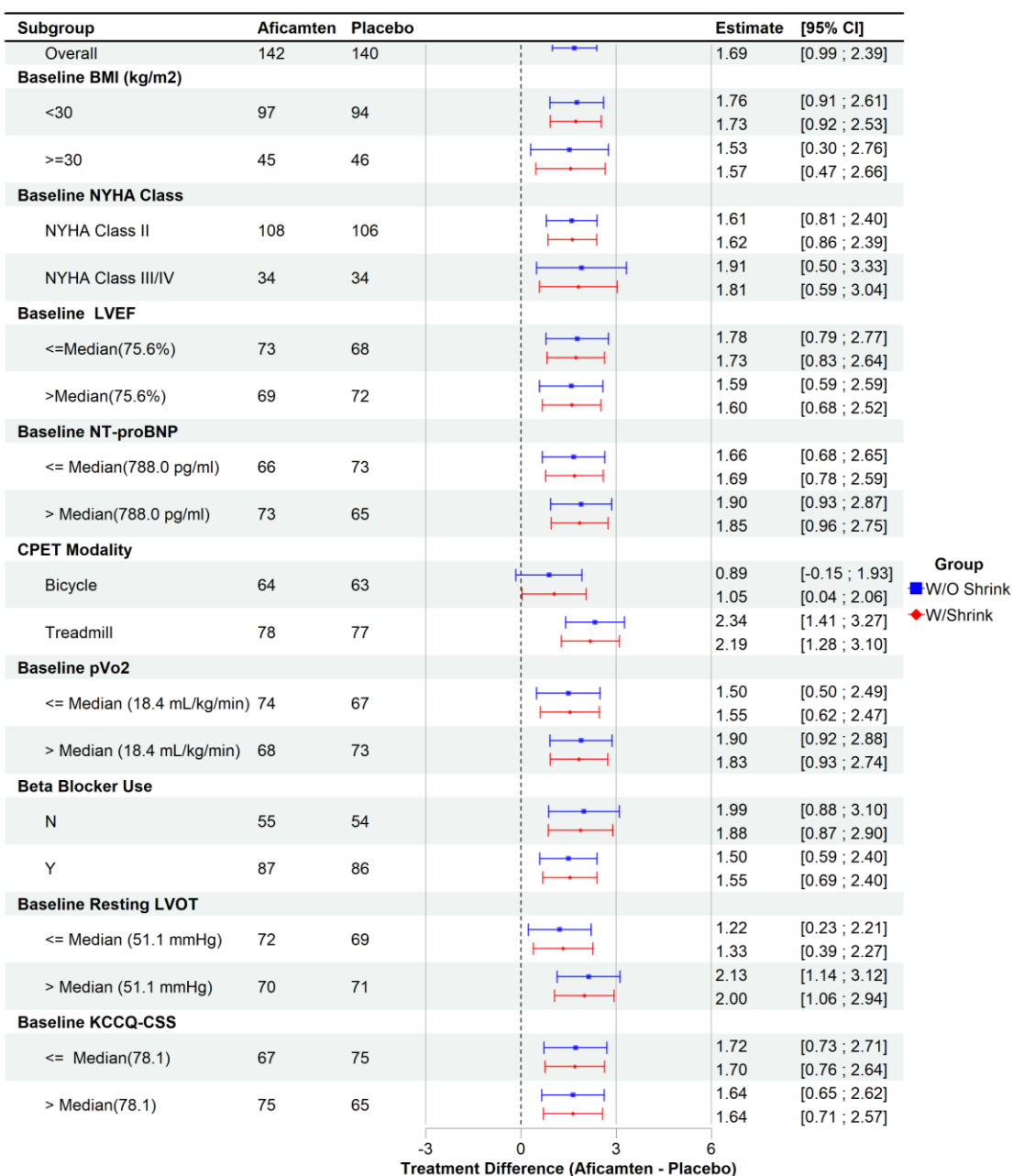
Table 131. Forest Plot of Change from Baseline to Week 24 in pVO₂ on CPET by Demographics Subgroups, Full Analysis Set, Trial CY 6031



Source: Statistical reviewer analysis
Abbreviations: CI, confidence interval; CPET, cardiopulmonary exercise testing; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; pVO₂, peak oxygen consumption; US, United States; W, with, W/O, without

Table 132. Forest Plot of Change From Baseline to Week 24 in pVO₂ on CPET by Baseline Characteristics Subgroups, Full Analysis Set, Trial CY 6031

Change in pVO₂ on CPET from Baseline to Week 24



Source: Statistical reviewer analysis

Abbreviations: BMI, body mass index; CI, confidence interval; CPET, cardiopulmonary exercise testing; LVOT, left ventricular outflow tract; pVO₂, peak oxygen consumption; W, with, W/O, without

Sensitivity Analyses of the Primary Endpoint

The statistical reviewer performed sensitivity analyses for the primary endpoint.

Tipping Point Analysis

For the tipping point analysis, missing pVO₂ values were imputed using placebo-based multiple imputation. Subsequently, imputed pVO₂ values for subjects with missing data in the aficamten group were systematically reduced (ranging from 90% to 26% of the imputed values) to identify the threshold at which the treatment effect would no longer remain statistically significant. For this analysis, imputed values in the placebo group were not shifted.

The results of this analysis ([Table 133](#)) showed that the p-value remained <0.01 through a shift to 50% of the imputed values; and it remained <0.05 through a shift to 30% of the imputed values. The amount by which the aficamten group shifted to lose significance seemed unlikely, thus the results appear to be statistically robust to underlying assumptions for missing data.

Table 133. Tipping Point Analysis: Change in pVO₂ on CPET From Baseline at Week 24, Full Analysis Set, Trial CY 6031

Tipping Point Shift Scale	LS Mean (SE)		LS Mean Difference vs. Placebo (95% CI)	p-Value
	Aficamten N=142	Placebo N=140		
100% (Primary analysis)	1.70 (0.255)	0.01 (0.257)	1.69 (0.98, 2.39)	<0.0001
90%	1.58 (0.256)	0.01 (0.256)	1.57 (0.86, 2.28)	<0.0001
80%	1.47 (0.259)	0.01 (0.264)	1.46 (0.74, 2.18)	<0.0001
70%	1.35 (0.266)	0.01 (0.272)	1.34 (0.60, 2.08)	0.0004
60%	1.23 (0.277)	0.00 (0.283)	1.22 (0.46, 1.99)	0.0019
50%	1.11 (0.288)	0.00 (0.295)	1.11 (0.31, 1.91)	0.0066
40%	1.00 (0.302)	-0.01 (0.309)	1.01 (0.17, 1.85)	0.0185
30%	0.90 (0.315)	-0.01 (0.323)	0.91 (0.03, 1.78)	0.042
26%	0.87 (0.319)	-0.00 (0.327)	0.88 (-0.01, 1.76)	0.0527

Source: Statistical reviewer analysis

Abbreviations: CPET, cardiopulmonary exercise testing; LS, least-squares; N, number of subjects in treatment group; pVO₂, peak oxygen consumption; SE, standard error

MMRM Analysis

The change from baseline in pVO₂ at Week 24 was analyzed using MMRM method with stratification factors (beta-blocker use/non-use and CPET modality), visit, stratification by visit and a numeric covariate, with 0 assigned to both treatment groups at baseline, 0 for the placebo group at Week 24, and 1 for the aficamten group at Week 24. The results of the analysis were consistent with the primary analysis: The treatment difference versus placebo was 1.75 mL/kg/min and nominally statistically significant (95% CI: 1.04, 2.45; p<0.0001)

16.2. Key Efficacy Results in CY 6021 (REDWOOD-HCM)

The results of the REDWOOD-HCM study, serving as confirmatory evidence to support the SEQUOIA-HCM trial, showed improvements in the LVOT-G (both resting and post-Valsalva) and NYHA functional class, considered to be clinically meaningful ([Table 134](#), [Table 135](#), and [Table 136](#)).

Table 134. Change From Baseline in Peak Resting LVOT-G, PD Analysis Set, Study CY 6021

	Pooled Placebo (N=12)	Cohort 1 Aficamten (N=14)	Cohort 2 Aficamten (N=14)	Cohort 3 Aficamten (N=12)
Resting LVOT-G (mmHg)				
Baseline, mean (SD) ^a	48.9 (25.6)	53.8 (25.2)	58.2 (36.3)	51.0 (26.1)
<i>On Treatment^b</i>				
Week 2				
LS mean change from BL (SE) [90% CI]	-11.2 (5.5) [-20.5, -1.9]	-29.7 (5.1) [-38.3, -21.1]	-39.5 (5.1) [-48.1, -30.8]	-21.3 (5.9) [-31.4, -11.1]
LS Mean Diff vs. Placebo (SE) [90% CI]		-18.5 (7.5) [-31.2, -5.8]	-28.2 (7.5) [-41.0, -15.5]	NA
p-value		0.019	0.0006	NA
Week 4				
LS mean change from BL (SE) [90% CI]	-9.1 (6.4) [-19.9, 1.7]	-26.6 (5.9) [-36.6, -16.6]	-38.9 (5.9) [-48.9, -28.9]	-29.2 (5.4) [-38.5, -19.9]
LS Mean Diff vs. Placebo (SE) [90% CI]		-17.5 (8.7) [-32.2, -2.8]	-29.8 (8.7) [-44.5, -15.0]	NA
p-value		0.052	0.002	NA
Week 6				
LS mean change from BL (SE) [90% CI]	-6.3 (3.4) [-12.1, -0.6]	-40.1 (3.1) [-45.4, -34.8]	-44.0 (3.1) [-49.3, -38.8]	-33.8 (2.6) [-38.4, -29.3]
LS Mean Diff vs. Placebo (SE) [90% CI]		-33.8 (4.6) [-41.5, -26.0]	-37.7 (4.6) [-45.5, -29.9]	NA
p-value		<0.0001	<0.0001	NA
Week 10				
LS mean change from BL (SE) [90% CI]	-15.2 (5.2) [-24.0, -6.4]	-40.6 (4.7) [-48.5, -32.6]	-39.9 (4.7) [-47.8, -31.9]	-29.7 (3.3) [-35.4, -24.0]
LS Mean Diff vs. Placebo (SE) [90% CI]		-25.4 (7.0) [-37.2, -13.6]	-24.7 (7.0) [-36.5, -12.8]	NA
p-value		0.0009	0.001	NA
<i>Washout</i>				
Week 12 ^a , mean change from baseline (SD)	-14.2 (25.4)	-6.5 (24.2)	-13.3 (40.5)	-3.1 (20.1)

ANCOVA = analysis of covariance; BL = baseline; CI = confidence interval; LS = least squares; LVOT-G = left ventricular outflow tract gradient; NA = not applicable; PDS = Pharmacodynamic Analysis Set; SD = standard deviation; SE = standard error

^a Summary statistics are provided for Baseline and Week 12 values (ie, means and SDs).

^b For Weeks 2–10, p-value and estimates were obtained from an ANCOVA model on change from baseline values with terms for baseline value, treatment, visit, and visit by treatment interaction.

Source: CY 6021 CSR Table 24

Table 135. Change From Baseline in Peak Post-Valsalva LVOT-G, PD Analysis Set, Study CY 6021

	Pooled Placebo (N=12)	Cohort 1 Aficamten (N=14)	Cohort 2 Aficamten (N=14)	Cohort 3 Aficamten (N=12)
Post-Valsalva LVOT-G (mmHg)				
Baseline ^a , mean (SD)	83.8 (21.8)	74.4 (25.2)	82.3 (36.5)	77.5 (27.8)
<i>On Treatment^b</i>				
Week 2				
LS mean change from BL (SE) [90% CI]	-11.7 (7.6) [-24.5, 1.1]	-27.2 (7.0) [-39.1, -15.4]	-48.4 (7.0) [-60.2, -36.6]	-18.0 (8.5) [-32.7, -3.4]
LS Mean Diff vs. Placebo (SE) [90% CI]		-15.5 (10.3) [-33.0, 1.9]	-36.7 (10.3) [-54.1, -19.3]	NA
p-value		0.14	0.001	NA
Week 4				
LS mean change from BL (SE) [90% CI]	-11.2 (7.8) [-24.4, 2.0]	-32.4 (7.3) [-44.6, -20.1]	-49.3 (7.2) [-61.5, -37.0]	-9.3 (7.3) [-22.0, 3.3]
LS Mean Diff vs. Placebo (SE) [90% CI]		-21.2 (10.7) [-39.2, -3.1]	-38.1 (10.6) [-56.0, -20.1]	NA
p-value		0.055	0.001	NA
Week 6				
LS mean change from BL (SE) [90% CI]	-8.5 (6.3) [-19.1, 2.1]	-41.4 (5.8) [-51.3, -31.6]	-50.4 (5.8) [-60.2, -40.6]	-30.5 (6.9) [-42.4, -18.6]
LS Mean Diff vs. Placebo (SE) [90% CI]		-32.9 (8.6) [-47.4, -18.4]	-41.9 (8.5) [-56.3, -27.5]	NA
p-value		0.0005	<0.0001	NA
Week 10				
LS mean change from BL (SE) [90% CI]	-5.1 (6.8) [-16.7, 6.4]	-40.4 (6.2) [-50.9, -30.0]	-50.9 (6.1) [-61.3, -40.5]	-26.9 (6.3) [-37.8, -16.0]
LS Mean Diff vs. Placebo (SE) [90% CI]		-35.3 (9.2) [-50.9, -19.7]	-45.8 (9.2) [-61.3, -30.3]	NA
p-value		0.0005	<0.0001	NA
<i>Washout</i>				
Week 12 ^a , mean change from baseline (SD)	-23.5 (30.8)	-6.4 (21.1)	-8.5 (26.1)	-9.8 (32.9)

ANCOVA = analysis of covariance; BL = baseline; CI = confidence interval; LS = least squares; LVOT-G = left ventricular outflow tract gradient; NA = not applicable; PDS = Pharmacodynamic Analysis Set; SD = standard deviation; SE = standard error

^a Summary statistics are provided for Baseline and Week 12 values (ie, means and SDs).

^b For Weeks 2–10, p-value and estimates are obtained from an ANCOVA model on change from baseline values with terms for baseline value, treatment, visit, and visit by treatment interaction.

Source: CY 6021 CSR Table 25

Table 136. Summary of Change in NYHA Class From Baseline to Week 10, PD Analysis Set, Study CY 6021

NYHA Class	Cohort 1		Cohort 2		Cohort 3 Aficamten (N=12)
	Aficamten (N=14)	Placebo (N=7)	Aficamten (N=14)	Placebo (N=5)	
Baseline					
Class II	10 (71.4)	6 (85.7)	7 (50.0)	4 (80.0)	5 (41.7)
Class III	4 (28.6)	1 (14.3)	7 (50.0)	1 (20.0)	7 (58.3)
Change at Week 10					
Worsening	0	0	0	0	0
Class II to Class III disease	0	0	0	0	0
Improvement	6 (42.9)	2 (28.6)	9 (64.3)	2 (40.0)	10 (83.3)
Class II to Class I disease	5 (35.7)	1 (14.3)	3 (21.4)	2 (40.0)	3 (25.0)
Class III to Class I disease	0	1 (14.3)	1 (7.1)	0	1 (8.3)
Class III to Class II disease	1 (7.1)	0	5 (35.7)	0	6 (50.0)
Maintenance	8 (57.1)	4 (57.1)	5 (35.7)	3 (60.0)	2 (16.7)
Class II disease	5 (35.7)	4 (57.1)	4 (28.6)	2 (40.0)	2 (16.7)
Class III disease	3 (21.4)	0	1 (7.1)	1 (20.0)	0

Source: CY 6021 CSR Table 29

Abbreviations: N, number of subjects in treatment group; NYHA, New York Heart Association

17. Clinical Safety

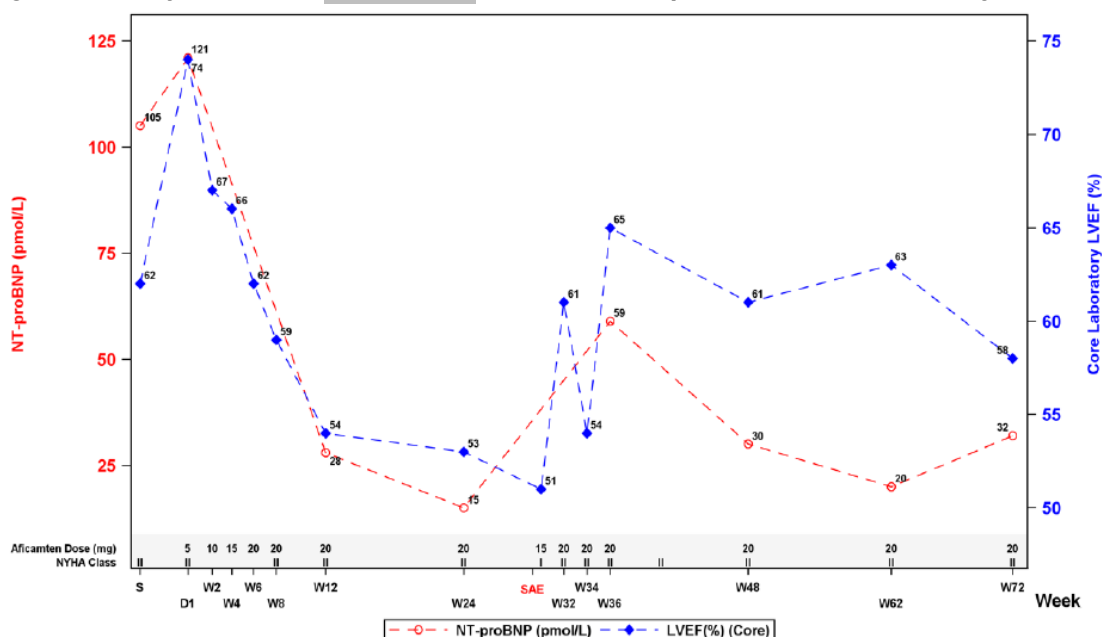
17.1. Narratives

Narratives for Aficamten-Treated Subjects With SAE of Systolic Dysfunction or Heart Failure

There were three SAEs of heart failure/systolic dysfunction in patients with nHCM in the OLE Study CY 6022 at the time of the NDA submission.

Subject CY-6022- (b) (6): This subject was a 44-year-old female with a history of nHCM and atrial fibrillation. While on a stable 20-mg dose of aficamten, they underwent an elective pulmonary vein ablation procedure for AF at Week 28. Approximately 2 weeks later, they presented with heart failure with LVEF of 49% (reported term: worsening heart failure and LVEF) by site assessment and 51% by core laboratory assessment. This event was classified as a SAE because it qualified as other medically important serious event (no hospitalization). The aficamten dose was subsequently down-titrated to 15 mg, and their LVEF recovered to over 60% at the next visit. While the investigator assessed the event as related to aficamten, the Applicant attributed the cardiac failure to the recent ablation procedure, noting that heart failure is a known complication of this intervention. The reviewer agrees that the event was confounded by the procedure; however, the role of aficamten cannot be ruled out given that the temporal relationship between LVEF reduction and dose up-titration (from Week 2 to Week 12) was observed prior to the ablation procedure. It should be noted that the subject was later up-titrated to 20 mg and continued treatment for another 40 weeks with LVEF $\geq 55\%$.

Figure 33. Subject CY-6022 (b) (6): LVEF and NT-proBNP Over Time, Study CY 6022

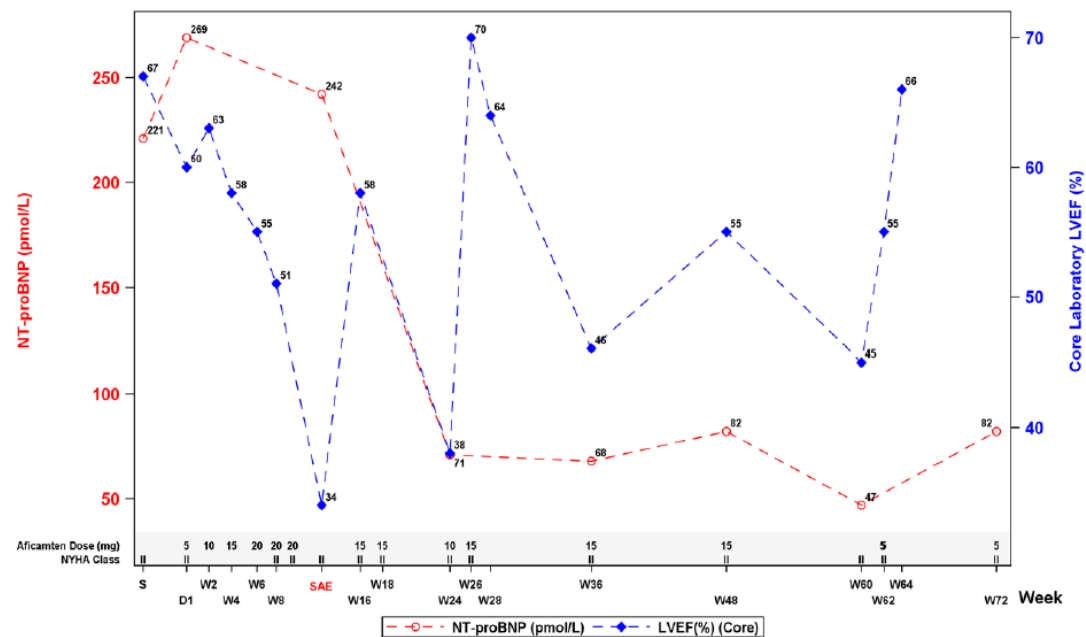


Source: Response to the Agency's request, seq0014

Abbreviations: LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SAE, serious adverse event; W, week

Subject CY-6022 (b) (6): This subject was a 39-year-old female with a history of nHCM and syncope. While on a 20-mg dose of aficamten, the subject suffered an acute thromboembolic stroke at Week 6, followed by an SAE of persistent atrial fibrillation at Week 6 and clinical deterioration. At Week 12, a scheduled echocardiogram revealed a significant drop in LVEF to 35% (site) and 34% (core lab), prompting an SAE report of ventricular dysfunction and the interruption of aficamten treatment. Following a cardioversion procedure, their atrial fibrillation resolved and their LVEF recovered to over 58%, and aficamten was restarted at a lower dose of 15 mg at Week 16. Subsequently, their clinical course was complicated by recurrent episodes of atrial fibrillation, which caused their LVEF to fluctuate, dropping as low as 38 to 45% during these paroxysms. The subject ultimately underwent pulmonary vein ablation around Week 53 to manage the arrhythmia. Aficamten was interrupted at Week 60 with site LVEF of 38% with a reported non-serious AE of ejection fraction (severe intensity). Following this interruption, the aficamten dose was restarted and reduced to 5 mg daily from Week 62 through Week 72, during which their LVEF remained stable in the normal range between 55% and 66%. Both the investigator and the Applicant assessed the ventricular dysfunction as being related to the subject's atrial fibrillation and not to the study drug.

Figure 34. Subject CY-6022- (b) (6) : LVEF and NT-proBNP Over Time, Study CY 6022

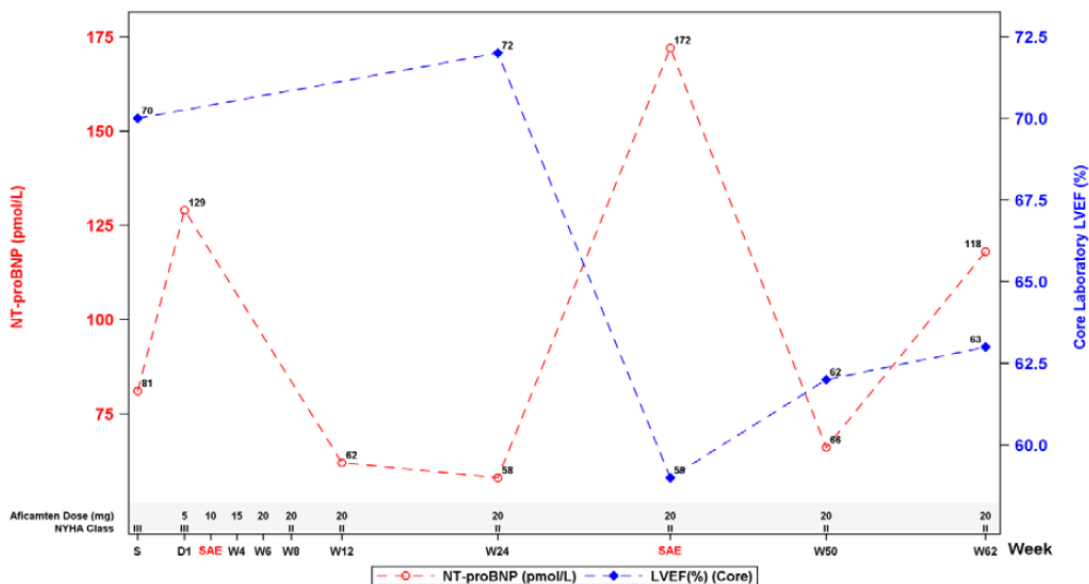


Source: Response to the Agency's request, seq0014
Abbreviations: LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SAE, serious adverse event; W, week

Subject CY-6022- (b) (6): This case was a 77-year-old male with an extensive medical history including nHCM, permanent atrial fibrillation, and congestive heart failure. The subject was hospitalized twice for acute heart failure while on a stable 20-mg dose of aficamten at Week 3 and Week 42. During both hospitalizations, his LVEF remained preserved at over 50% (65% and 75%, respectively), and no dose modification of aficamten was required. The subject had a known history of poor compliance with his prescribed diuretic therapy. Both the investigator and the Applicant concluded that the SAEs were not related to aficamten, attributing the episodes of

fluid overload to the subject's pre-existing chronic heart failure and suboptimal adherence to his diuretic regimen.

Figure 35. Subject CY-6022- (b) (6) : LVEF and NT-proBNP Over Time, Study CY 6022



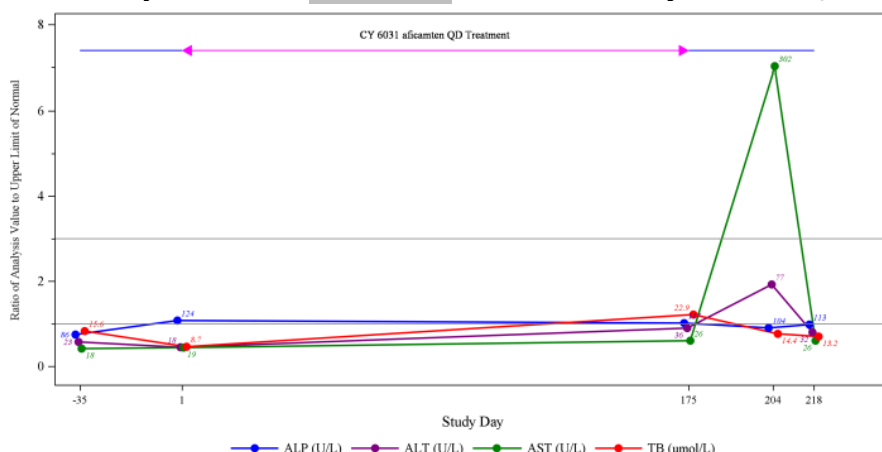
Source: Response to the Agency's request, seq0014

Abbreviations: LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SAE, serious adverse event; W, week

Narratives for Aficamten-Treated Patients With Elevated Transaminases

Subject CY-6031- (b) (6) : This subject was a 23-year-old White male who was on a stable aficamten dose titrated to 10 mg by Week 8. The subject had normal liver function tests at baseline and throughout 24 weeks of aficamten treatment. There were no AEs reported during the treatment period. The subject experienced a transient elevation in AST ($7\times$ ULN) with mildly elevated ALT ($1.9\times$ ULN), along with significantly elevated creatine phosphokinase ($>79\times$ ULN) and lactate dehydrogenase ($>3\times$ ULN) during the washout period (29 days after discontinuation of aficamten). All other liver tests were within normal range. All abnormal parameters returned to within the normal reference range within 2 weeks without intervention. The subject opted not to participate in the OLE Study CY 6022. Due to the absence of a temporal relationship, the absence of increased ALP and bilirubin, and the concurrent increase of AST with CPK and lactate dehydrogenase as well as the overall pattern of $AST > ALT$, this transient increase in AST is likely of non-hepatic origin.

Figure 36. Subject CY-6031- (b) (6) : Liver Laboratory Over Time, Trial CY 6031



ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; QD = once a day; TB = total bilirubin; ULN = upper limit of normal.

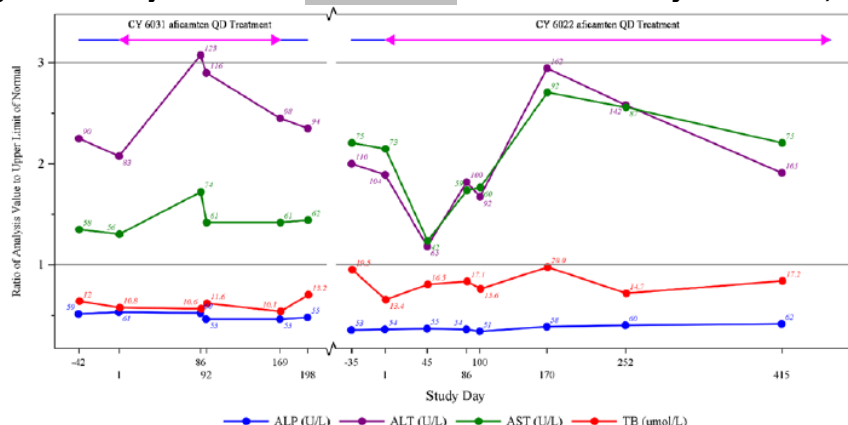
Data labels are observed values.

Pink and blue top line sections indicate on and off aficamten treatment period.

Source: Response to the Agency's request, seq0014

Subject CY-6031 (b) (6) : This subject was a 66-year-old White male who received aficamten and was titrated to a dose of 20 mg by Week 8 and remained on that dose until the end of the treatment period. The subject had mildly elevated ALT and AST at baseline ($2.1 \times \text{ULN}$ and $1.3 \times \text{ULN}$ respectively), which remained stable and above ULN but $<3 \times \text{ULN}$ during aficamten treatment except for an occurrence of a transient increase of ALT to $3.1 \times \text{ULN}$ (123 U/L) on Study Day 86. There was no associated AEs or changes in concurrent medications. His other liver tests were within normal reference range during the study. The subject opted to participate in the OLE Study CY 6022 and experienced another episode of a mild transient increase of ALT and AST ($2.9 \times \text{ULN}$ and $2.7 \times \text{ULN}$, respectively). All occurrences of mild ALT and AST elevations returned toward baseline levels while continuing aficamten.

Figure 37. Subject CY-6031 (b) (6) : Liver Laboratory Over Time, Trial CY 6031



ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; QD = once a day; TB = total bilirubin; ULN = upper limit of normal.

Studies included are CY 6031 (completed) and CY 6022 (data cutoff date 31AUG2024).

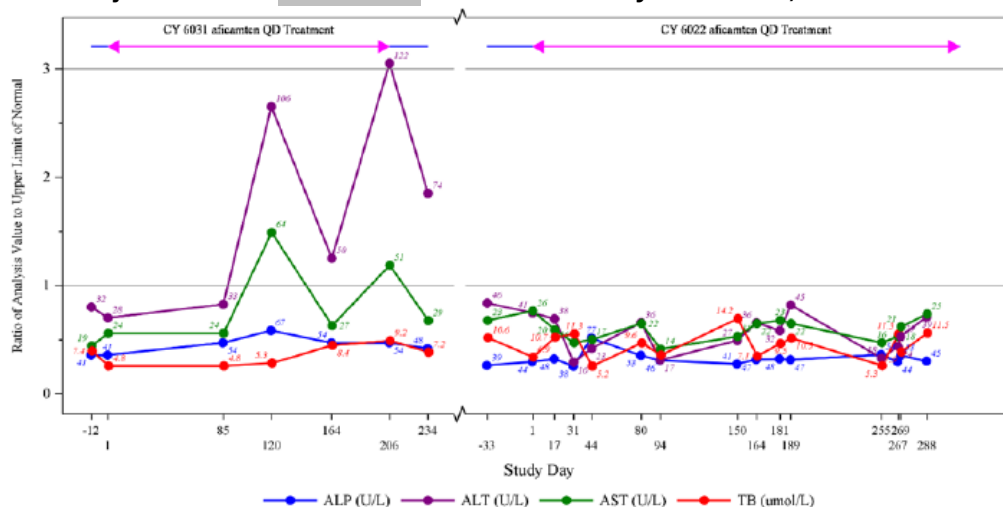
Data labels are observed values.

Pink and blue top line sections indicate on and off aficamten treatment period.

Source: Response to the Agency's request, seq0014

Subject CY-6031- (b) (6): This subject was a 41-year-old White male who received aficamten and was titrated to a dose of 20 mg by Week 8 and remained on that dose until the end of the treatment period. The subject had normal liver tests at baseline. The subject experienced two transient elevations in ALT (2.7x ULN and 3.1x ULN) and AST (1.5x ULN and 1.2x ULN) during the study. The first event was reported in the context of an ongoing non-serious AE of cardiac failure and there was no aficamten dose interruption or modification. ALT and AST levels returned to near normal after 1-month with continuous treatment of aficamten. The second event was reported on the same day when they completed the aficamten treatment and entered the washout period. There was no associated increase in bilirubin and alkaline phosphatase for either event. The subject enrolled in the OLE Study CY 6022, during which there was no observation of elevated transaminases during 41 weeks of continuous treatment with aficamten (through August 31, 2024, the data cutoff date).

Figure 38. Subject CY-6031- (b) (6): **Liver Laboratory Over Time, Trial CY 6031**

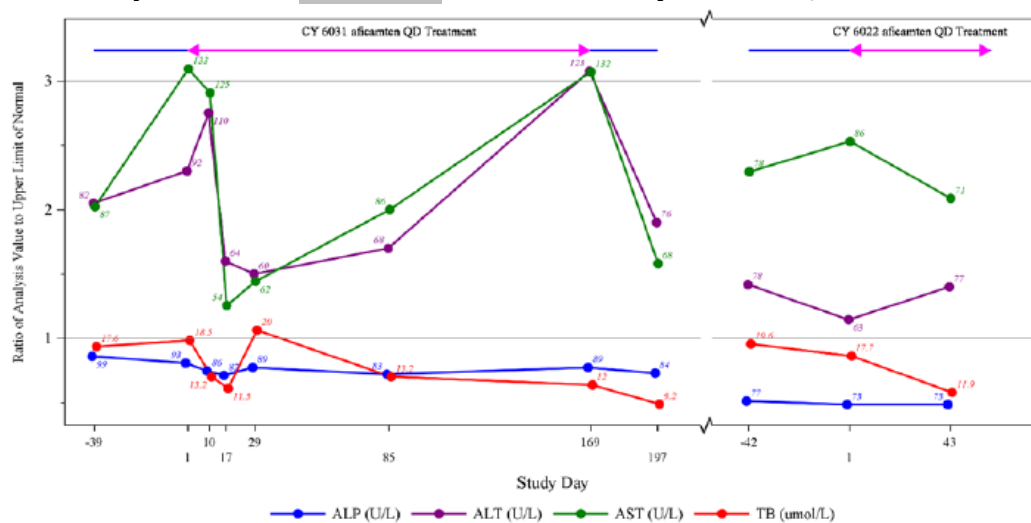


ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; QD = once a day; TB = total bilirubin; ULN = upper limit of normal.
Studies included are CY 6031 (completed) and CY 6022 (data cutoff date 31AUG2024).
Data labels are observed values.
Pink and blue top line sections indicate on and off aficamten treatment period.

Source: Response to the Agency's request, seq0014

Subject CY-6031- (b) (6): This subject was a 61-year-old White male who received aficamten and was titrated to a dose of 20 mg by Week 8 and remained on that dose until the end of the treatment period. The subject had mildly elevated ALT and AST at baseline (2.3x ULN and 3.1x ULN, respectively). The subject experienced fluctuations in liver enzymes throughout the study, with two episodes of ALT and AST reaching 3.1x ULN. These elevations returned to baseline levels despite continued aficamten treatment. The subject enrolled in the OLE Study CY 6022, during which a similar pattern of mild and stable elevations <3x ULN of both ALT and AST was observed through the cutoff date of August 31, 2024, for the 120-Day Safety Update.

Figure 39. Subject CY-6031- (b) (6) : Liver Laboratory Over Time, Trial CY 6031



ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; QD = once a day; TB = total bilirubin; ULN = upper limit of normal.

Studies included are CY 6031 (completed) and CY 6022 (data cutoff date 31AUG2024).

Data labels are observed values.

Pink and blue top line sections indicate on and off aficamten treatment period.

Source: Response to the Agency's request, seq0014

18. Clinical Virology

Not applicable.

19. Clinical Microbiology

Not applicable.

20. Mechanism of Action/Drug Resistance

Not applicable.

21. Other Drug Development Considerations

Not applicable.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

The Office of Scientific Investigations (OSI) conducted inspections covering the pivotal Phase 3 Trial CY 6031. Please refer to the OSI review dated July 29, 2025, for details. A summary of the review is provided below:

OSI inspected two clinical investigator sites and the Applicant, Cytokinetics Inc. Dr. Ahmad Masri's site (Site 1001) in Portland, Oregon, screened 29 subjects and randomized 20 subjects, while Dr. Michael Nassif's site (Site 1009) in Kansas City, Missouri, screened 18 subjects and randomized 12 subjects. All randomized subjects at both sites completed the study. The inspections verified the primary efficacy endpoint data (pVO₂) and found no discrepancies between the Applicant's data listings and the sites' source documents.

The overall assessment concluded that the study appears to have been conducted adequately, and the data generated by the clinical investigator sites and submitted by the Applicant appear acceptable in support of the proposed indication. The Applicant's conduct and oversight of the study were found to be adequate and compliant with Good Clinical Practice and FDA regulations.

23. Labeling: Key Changes

This PI review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the Applicant's draft PI ([Table 137](#)). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

Table 137. Key Labeling Changes and Considerations

Full PI Sections ¹	Rationale for Major Changes to Finalized PI ² Compared to Applicant's Draft PI
BOXED WARNING	The Boxed Warning was revised for consistency with current labeling practices to note that MYQORZO is available through a restricted program under a REMS.
1 INDICATIONS AND USAGE	The indication statement was revised to include the demonstrated benefits, 'to improve functional capacity and symptoms.'
2 DOSAGE AND ADMINISTRATION	This section was revised to improve clarity regarding recommendations as to when to perform echocardiogram assessments. Instructions regarding concomitant use with Fluconazole, Voriconazole, Fluvoxamine, Strong CYP2C9 Inhibitors, and moderate to strong CYP3A inducers. See Section 8.2 .
4 CONTRAINDICATIONS	Contraindication for use with (b) (4) was removed.

Full PI Sections ¹	Rationale for Major Changes to Finalized PI ² Compared to Applicant's Draft PI
5 WARNINGS AND PRECAUTIONS	A subsection for the MYQORZO REMS was added. In the cytochrome P450 interactions warning, CYP2C19 was included as contributing to the metabolism of MYQORZO. Instructions were also added to assess LVEF 2 to 8 weeks after initiation of inhibitors or discontinuation of inducers and to adjust the dose of MYQORZO accordingly.
6 ADVERSE REACTIONS	A description of effects on blood pressure was added to Section 6.1.
7 DRUG INTERACTIONS	Instructions for mitigation of drug interactions with Fluvoxamine, Fluconazole, Voriconazole, Strong CYP2C9 inhibitors and CYP inducers were revised. See Section 8.2 A proposed subsection regarding interactions with drugs with negative inotropic effects was deleted as no clinically significant interactions were noted.
8 USE IN SPECIFIC POPULATIONS (e.g., Pregnancy, Lactation, Females and Males of Reproductive Potential, Pediatric Use, Geriatric Use, Renal Impairment, Hepatic Impairment)	The Pregnancy and Lactations subsections were revised for consistency with current Pregnancy Lactation and Labeling Rule, (PLLR) practices. Language describing the pregnancy safety study was added.
9 DRUG ABUSE AND DEPENDENCE	N/A
10 OVERDOSAGE	This section was revised to note potential signs and symptoms of overdosage and to add language advising contacting the Poison Help line.
12 CLINICAL PHARMACOLOGY	The Mechanism of Action subsection, 12.1 was revised to focus the description of the mechanism determined most relevant to the indication. In the Pharmacodynamics subsection, p-values for described effects were deleted. In the Pharmacokinetics subsection, the predicted time to steady state was added. Descriptions of drug interactions sections were revised, please refer to Section 8.2 of this review. The Pharmacokinetics subsection was also generally revised for consistency with current labeling practices.
13 NONCLINICAL TOXICOLOGY	A pharmacogenomics sections was added to address CYP2C9 genetic variants. See Section 14.6 . The applicant proposed subsection for Animal Toxicology and/or Pharmacology was deleted, this section was determined to not be clinically relevant.
14 CLINICAL STUDIES	Clinical Studies was revised to clearly describe the data supporting the indication. Analyses and presentation of the data were revised consistent with the Team's review, see Section 6.3.2 .
17 PATIENT COUNSELING INFORMATION	(b) (4) [REDACTED] was removed. Language regarding the MYQORZO REMS and the presence of the Pregnancy Safety Study was added.

Full PI Sections ¹	Rationale for Major Changes to Finalized PI ² Compared to Applicant's Draft PI
Product Quality Sections (i.e., DOSAGE FORMS AND STRENGTHS, DESCRIPTION, HOW SUPPLIED/STORAGE AND HANDLING)	These sections were revised for accuracy and current labeling practices.

¹ Product quality sections (Sections 3, 11, and 16) are pooled under the last row in this table; Section 15 (REFERENCES) is not included in this table.

² For the purposes of this document, the finalized PI is the PI that will be approved or is close to being approved.

Abbreviation(s): PI, Prescribing Information

23.1. Approved Labeling Types

Upon approval of this [application/efficacy supplement], the following labeling documents will be FDA-approved:

- Prescribing Information
- Medication Guide

24. Postmarketing Requirements and Commitments

- **Descriptive Pregnancy Safety Study:** Conduct a worldwide descriptive study that collects prospective and retrospective data in women exposed to aficamten during pregnancy to assess risk of pregnancy and maternal complications, adverse effects on the developing fetus and neonate, and adverse effects on the infant. Infant outcomes will be assessed through at least the first year of life.
- **Lactation Study:** Perform a milk-only lactation study in lactating women who have received aficamten to measure concentrations of aficamten in breast milk using a validated assay. Assess the effects on the breastfed infant, if available, based on study population.

25. Financial Disclosure

Table 138. Covered Clinical Studies: Trial CY 6031

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: approximately 400 investigators		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 4		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 4 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 2		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Abbreviation: FDA, Food and Drug Administration

26. References

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NDA 219083
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Guidances for Industry

ICH Guidance for Industry *M12 Drug Interaction Studies* (August 2024)

FDA Draft Guidance for Industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products* (December 2019)

FDA Guidance for Industry *Clinical Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions* (January 2020)

FDA Guidance for Industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products* (May 1998)

ICH Guidance for Industry *M10 Bioanalytical Method Validation and Study Sample Analysis* (November 2022a)

ICH Guidance for Industry *S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals* (November 2022b)

FDA Draft Guidance for Industry *Demonstrating Substantial Evidence of Effectiveness With One Adequate and Well-Controlled Clinical Investigation and Confirmatory Evidence* (September 2023)

27. Review Team

Table 139. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory project manager	Maryam Changi
Nonclinical reviewer	Andrew Jabob
Nonclinical team leader	Xuan Chi
OCP reviewer(s)	Chongwoo Yu/ Katarzyna Drozda/ Ying-Hong Wang/ Hui Wei
OCP team leader(s)	Elly Moon/ Jeffrey Kraft/ Yuching Yang/ Vishnu Sharma
Clinical reviewer	Tzu-Yun McDowell
Clinical team leader	Fortunato Senatore
Biometrics reviewer	Sapna Thakur
Biometrics team leader	Jennifer Clark
Cross-discipline team leader	Fortunato Senatore
Division director (pharm/tox)	Calvin Elmore
Division director (OCP)	Shirley Seo
Division director (OB)	Jialu Zhang
Division Deputy Director (clinical)	Charu Gandotra
Office director (or designated signatory authority)	Hylton Joffe

Abbreviations: OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology

Table 140. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Theodor Carver
Microbiology	N/A
OPDP/ PLT	Tierra Butler/ Susan Redwood
OSI	Soyoung Tina Chang/ Phillip Korenstein
OSE/DEPI	Margie Goulding/ Natasha Pratt
OSE/DMEPA	Robbie Kattappuram/ Nicole Iverson
OSE/DRISK	Christopher Booze/ Yasmeen Abou-Sayed
DPV	Courtney Suggs/ Daniel Woronow

Abbreviations: DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations

27.1. Reviewer Signatures

Table 27-141 Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Secondary Reviewer	Xuan Chi OCHEN DPTCHEN	Sections: 5, 7, 8 and 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Xuan Chi Digitally signed by Xuan Chi Date: 12/19/2025 9:34 AM EST GUID: 20251219143423				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Primary Reviewer	Chongwoo Yu OCP DCEP	Sections: 5.2, 6.1, 8.1, 8.2, 8.3, 14.1, 14.2, 14.3	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Chongwoo Yu Digitally signed by Chongwoo Yu Date: 12/19/2025 9:35 AM EST GUID: 20251219143540				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Tertiary Reviewer	Selena DeConti OCHEN DCN	Sections: Section 7.7	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Selena DeConti		Digitally signed by Selena DeConti Date: 12/19/2025 9:36 AM EST GUID: 20251219143648		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Primary Reviewer	Tzu-Yun McDowell OCHEN DCN	Sections: Section 2, 3, 6, 7,8.4, 19, 16.2, 17, 22, 25	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Tzu-Yun McDowell		Digitally signed by Tzu-Yun McDowell Date: 12/19/2025 9:37 AM EST GUID: 2025121914379		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Genomics Reviewer Discipline Primary Reviewer	Katarzyna Drozda OCP DTPM	Sections: 14.6. Pharmacogenetics	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Katarzyna Drozda Digitally signed by Katarzyna Drozda Date: 12/19/2025 9:37 AM EST GUID: 20251219143759				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director for Labeling Discipline Secondary Reviewer	Michael Monteleone OCHEN DCN	Sections: 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Michael Monteleone Digitally signed by Michael Monteleone Date: 12/19/2025 9:40 AM EST GUID: 2025121914406				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director for Labeling Discipline Primary Reviewer	Michael Monteleone OCHEN DCN	Sections: 23	Based on my assessment of the application: ✓ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Michael Monteleone Digitally signed by Michael Monteleone Date: 12/19/2025 9:40 AM EST GUID: 20251219144033				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non- clinical Discipline Tertiary Reviewer	Calvin Elmore OCHEN DPTCHEN	Sections: 5.1, 7.1, 8.4, 13	Based on my assessment of the application: ✓ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Calvin Elmore Digitally signed by Calvin Elmore Date: 12/19/2025 9:42 AM EST GUID: 20251219144241				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline Primary Reviewer	Maryam Changi ORO DROCHEN	Sections: 8-12	Based on my assessment of the application: ✓ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Maryam Changi Digitally signed by Maryam Changi Date: 12/19/2025 9:43 AM EST GUID: 20251219144326				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Primary Reviewer	Sapna Thakur OB DBII	Sections: 6.2.1, 6.3, 16	Based on my assessment of the application: ✓ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Sapna Thakur Digitally signed by Sapna Thakur Date: 12/19/2025 9:43 AM EST GUID: 20251219144348				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
PBPK Reviewer Discipline Secondary Reviewer	Yuching Yang OCP DPM	Sections: 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Yuching Yang Digitally signed by Yuching Yang Date: 12/19/2025 9:48 AM EST GUID: 20251219144813				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non- clinical Discipline Primary Reviewer	Andrew Jacob OCHEN DPTCHEN	Sections: 5.1, 7.1, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Andrew Jacob Digitally signed by Andrew Jacob Date: 12/19/2025 9:48 AM EST GUID: 20251219144853				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
PBPK Reviewer Discipline Primary Reviewer	Yuching Yang OCP DPM	Sections: 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Yuching Yang		Digitally signed by Yuching Yang Date: 12/19/2025 9:49 AM EST GUID: 20251219144942		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline Secondary Reviewer	Alexis Childers ORO DROCHEN	Sections: 8-12	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Alexis Childers		Digitally signed by Alexis Childers Date: 12/19/2025 9:52 AM EST GUID: 2025121914522		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Genomics Reviewer Discipline Secondary Reviewer	Jeffrey Kraft OCP DTPM	Sections: 14.6. Pharmacogenetics	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Jeffrey Kraft Digitally signed by Jeffrey Kraft Date: 12/19/2025 9:54 AM EST GUID: 20251219145436				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Tertiary Reviewer	Shirley Seo OCP DCEP	Sections: 5, 6, 8, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Shirley Seo Digitally signed by Shirley Seo Date: 12/19/2025 9:58 AM EST GUID: 20251219145811				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Shirley Seo OCP DCEP	Sections: 5, 6, 8, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Shirley Seo Digitally signed by Shirley Seo Date: 12/19/2025 9:59 AM EST GUID: 20251219145953				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharmacometrics Reviewer Discipline Secondary Reviewer	Vishnu Sharma OCP DPM	Sections: 6.1, 14.5	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Vishnu Sharma Digitally signed by Vishnu Sharma Date: 12/19/2025 10:13 AM EST GUID: 20251219151354				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Primary Reviewer	Theodore Carver OPQAI DPQAI	Sections: 9, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Theodore Carver Digitally signed by Theodore Carver Date: 12/19/2025 10:14 AM EST GUID: 20251219151420				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharmacometrics Reviewer Discipline Primary Reviewer	Hui Wei OCP DPM	Sections: section 6, 8 and 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Hui Wei Digitally signed by Hui Wei Date: 12/19/2025 10:16 AM EST GUID: 20251219151610				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Secondary Reviewer	Theodore Carver OPQAI DPQAI	Sections: 9, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Theodore Carver		Digitally signed by Theodore Carver Date: 12/19/2025 10:27 AM EST GUID: 20251219152717		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Secondary Reviewer	Jennifer Clark OB DBII	Sections: 6.2.1, 6.3, 16.1	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Jennifer Clark		Digitally signed by Jennifer Clark Date: 12/19/2025 11:03 AM EST GUID: 2025121916352		

NDA 219083
MYQORZO (aficamten)

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Secondary Reviewer	Fortunato Senatore OCHEN DCN	Sections: 1, 2, 3, 4, 6, 7	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Fortunato Senatore Digitally signed by Fortunato Senatore Date: 12/19/2025 11:32 AM EST GUID: 20251219163242				

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/s/

FORTUNATO F SENATORE
12/19/2025 11:34:57 AM

CHARU GANDOTRA
12/19/2025 11:55:49 AM

HYLTON V JOFFE
12/19/2025 11:59:53 AM

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

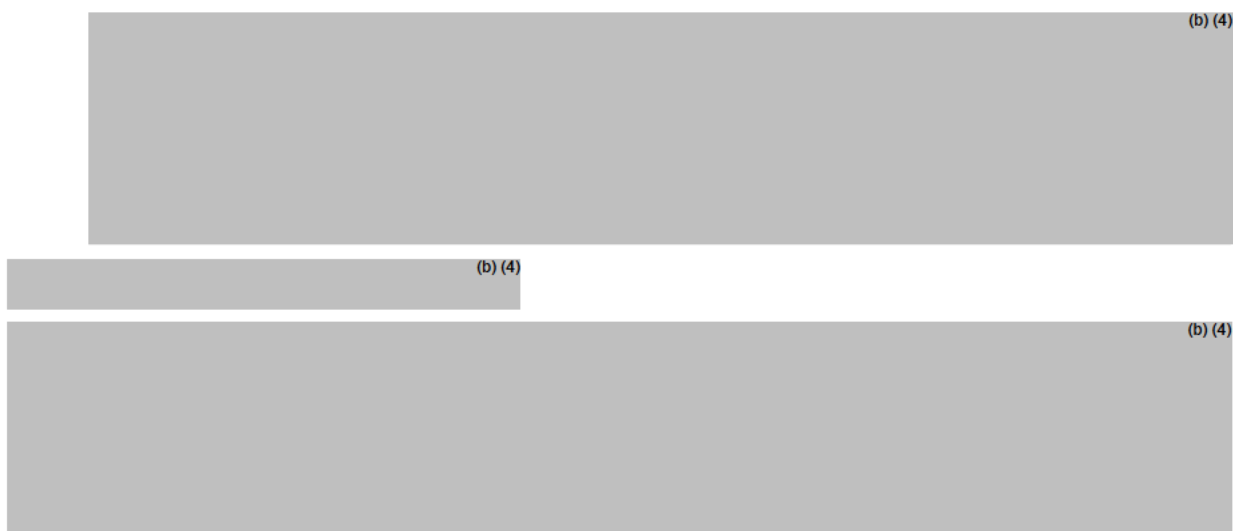
PHARMACOLOGY/TOXICOLOGY IND ASSESSMENT AND EVALUATION

Application Number*: NDA-219083
Supporting Document Number/s: 0042
CDER Receipt Date: 08/08/2025
Sponsor: Cytokinetics
Product: Aficamten
Pharmacologic Class: Cardiac Myosin Inhibitor
Indication: Treatment of symptomatic obstructive
hypertrophic cardiomyopathy
Therapeutic area: Cardiovascular Disease
Clinical Review Division: Division of Cardiology and Nephrology
(DCN)
Pharm/Tox Division: Division of Pharm/Tox for Cardiology,
Hematology, Endocrinology, and
Nephrology (DPT-CHEN)
Reviewer: Andrew Jacob, Ph.D.
Supervisor/Team Leader: Xuan Chi, M.D., Ph.D.
Project Manager: Maryam Changi
Purpose of Review: Study Report Review
Reviewer Completion Date: August 12, 2025

Background

Four impurities were identified by the Applicant which had structural alerts for mutagenicity ((b) (4)). The impurities and the corresponding structural alert are highlighted in the Applicant's diagram below:

Figure 1: ((b) (4)) **Intermediate and Related Impurities**



The Applicant's position was that the structural alerts were out-of-context and the impurities could be classified as not mutagenic (ICH M7 Class 4). A table summarizing the discrepancies between the Applicant's and the Agency's mutagenicity QSAR interpretation is shown below:

No.	Chemical Name	Applicant's Bacterial Mutagenicity Overall Prediction ¹	Agency's Bacterial Mutagenicity Expert Prediction ¹
1	((b) (4))	-	+
2		-	+
3		N/A	+
4		N/A	+

¹ + = positive; - = negative; N/A = no prediction available.

Details regarding the Applicant's position can be found on DARRTS dated 02/13/2025. (Electronically linked here: <\\CDSESUB1\EVSPROD\nda219083\0015\m1\us\111-information-amendment\quality.pdf>)

In a mid-cycle communication dated 04/03/2025, the Agency suggested that the Applicant should either conduct Ames testing to assess mutagenic potential, or control the impurities as Class 3 per ICH M7. The Applicant agreed to conduct a GLP Ames assay for one of the four impurities ((b) (4)) and use the results as read-through

data for the others. The Applicant could not synthesize and isolate sufficient (b) (4) (b) (4) so the Ames assay was done using (b) (4) instead (see Quality Information Amendment checked into DARRTS on 05/30/2025).

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

7.1.1 Study Title: Study# Nonclin-0545: (b) (4) A Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*

Methods

This GLP-compliant Ames assay was conducted according to ICH-S2(R1) and OECD guidelines. The Applicant's table below summarizes the study parameters, including concentrations used, bacterial strains used, and positive and negative controls used:

Text Table 1
Main Test
Plate Incorporation Assay

Dose No.	Formulation Conc. (µg/mL) ^a	Dose Volume (µL/plate)	Final Conc. (µg/plate)	Number of Replicates		Strain
				-S9	+S9	
Negative Control	-	100	(b) (4)	3	3	all
(b) (4)	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	3	all
	(b) (4)	100	(b) (4)	3	-	TA1535, TA100
Positive Controls	(b) (4)	100	(b) (4)	3	-	TA1537
	(b) (4)	100	(b) (4)	3	-	TA98
	(b) (4)	100	(b) (4)	3	-	WP2 <i>uvrA</i>
	(b) (4)	100	(b) (4)	-	3	TA1535, TA1537
	(b) (4)	100	(b) (4)	-	3	WP2 <i>uvrA</i>
	(b) (4)	100	(b) (4)	-	3	TA98, TA100

^a Nominal concentrations; measured concentrations might be adjusted due to the limitations of the instruments used.

^b The OECD/ICH S2(R1) standard limit concentration (usually 5000 µg/plate).

- Not applicable.

-S9: In the absence of S9 mix; +S9: In the presence of S9 mix.

Negative Control: Dimethyl sulfoxide (DMSO).

(b) (4)

Results

(b) (4) was not mutagenic in any conditions used for this study. Revertant colony number slightly exceeded the 98% tolerance threshold for negative control data at 50 µg/plate and 158 µg/plate with S9 for strains TA98 and TA1537 respectively. However, revertant colony number did not exceed the ≥ 2 -fold increase cutoff above negative controls for any strain to be considered positive, and there was no dose-dependent increase in revertant colonies for any strain.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

ANDREW E JACOB
08/12/2025 02:22:59 PM
Ames study negative

XUAN CHI
08/13/2025 12:48:10 PM



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research

Office of Translational Sciences

Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number:	NDA219083
Drug Name:	aficamten (CK-3773274)
Indication:	Treatment of symptomatic obstructive hypertrophic cardiomyopathy
Applicant:	Cytokinetics, Incorporated
Test Facility Mice Study:	(b) (4) (b) (4)
Documents Reviewed:	Study report (Nonclin-0306) submitted on 07/30/2024 via NDA219083/0001 Data submitted on 09/10/2024 via NDA219083/0003
Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Zhuang Miao, Ph.D.

Concurring Reviewer: Rahman, Atiar Mohammad, Ph.D.

Reviewing Pharmacologist: Andrew Jacob Ph.D.

Keywords: Carcinogenicity, Dose response

Table of Contents

Summary	3
1. Background.....	4
2. Mouse Study	4
Table 1: Study Design in Mouse Study.....	4
2.1. Sponsor's analyses.....	4
2.1.1. Survival analysis.....	4
2.1.2. Tumor data analysis.....	4
Sponsor's findings	5
2.2. Reviewer's analyses.....	5
2.2.1. Survival analysis.....	5
Reviewer's findings	5
2.2.2. Tumor data analysis.....	5
Adjustment for multiple testing.....	6
Reviewer's findings	6
Table 2: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups -MaleMice	6
Table 3: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Positive Control -Male Mice	7
Table 4: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Positive Control-Female Mice	7
3. Appendix.....	9
Table A1: Intercurrent Mortality Rate -Male Mice.....	9
Table A2: Intercurrent Mortality Rate -Female Mice	9
Table A3: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control 1, Positive Control and Vehicle Control 1-Male Mice	9
Table A4: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control 1, Positive Control and Vehicle Control 1-Female Mice	9
Table A5: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Male Mice	10
Table A6: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups- Female Mice	11
Table A7: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control -Male Mice.....	12
Table A8: Tumor Rates and P-Values for Comparisons between Vehicle Control 1 and Positive Control - Female Mice	13
Figure 1: Kaplan-Meier Survival Functions for Male Mice	14
Figure 2: Kaplan-Meier Survival Functions for Female Mice.....	15
4. References.....	16

Summary

In this submission the sponsor included the report of one animal carcinogenicity study in transgenic mice. This study was intended to assess the carcinogenic potential of CK-3773274, when administered to hemizygous transgenic rasH2 mice by oral gavage daily for 26 weeks at appropriate dose levels.

Mouse Study:

Survival analysis:

1. The survival analyses did not show any statistically significant dose response relationships in mortality across the vehicle control group and treated groups in either male or female mice.
2. The survival analyses showed statistically significant increase in mortality across the vehicle control group and positive control group in both male and female mice.

Tumor analysis:

1. For female mice, the tumor analysis did not show any tumor types with statistically significant positive dose response relationships or statistically significant increases in any treated group compared to the vehicle control.
2. For male mice, the trend test showed statistically significant positive dose response relationships in the incidences of bronchioloalveolar adenoma, Lung (p-value=0.0056<0.05) and combined tumors of bronchioloalveolar adenoma and carcinoma (p-value=0.0212<0.05).
3. For male mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increases in the incidences of bronchioloalveolar adenoma, lung (p-value<0.001), bronchioloalveolar carcinoma, lung (p-value<0.001), combined tumors of bronchioloalveolar adenoma and carcinoma, lung (p-value<0.001), hemangiosarcoma, spleen (p-value<0.001) and combined tumors of hemangiosarcoma and hemangioma, whole body (p-value<0.001).
4. For female mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increases in the incidences of adenoma, glandular (p-value=0.0052<0.05), bronchioloalveolar adenoma, lung (p-value<0.001), bronchioloalveolar carcinoma, lung (p-value<0.001), combined tumors of bronchioloalveolar adenoma and carcinoma, lung (p-value<0.001),

hemangioma, spleen (p-value=0.0498<0.05), hemangiosarcoma, spleen (p-value<0.001) and combined tumors of hemangiosarcoma and hemangioma, whole body (p-value<0.001).

1. Background

In this submission the sponsor included the report of one animal carcinogenicity study in transgenic mice. This study was intended to assess the carcinogenic potential of CK-3773274, when administered by oral gavage daily for 26 weeks at appropriate dose levels.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as the dose increases.

2. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups, one vehicle control group, and one positive control group. Two hundred and fifty hemizygous transgenic rasH2 mice of each sex were randomly assigned to the treated groups vehicles and positive control groups in equal size of 25 mice per group. The mice in the vehicle control groups received 10% (w/w) Polysorbate 80 in Reverse Osmosis Deionized (RODI) Water. The mice in the water control group received RODI Water. The positive control mice were dosed by intraperitoneal injections with Urethane in 0.9% Sodium Chloride for Injection, USP, once on Days 1, 3, and 5. The study was designed to continue for up to 26 weeks for both sexes, however in accordance with study termination criteria, all surviving mice were sacrificed during Week 27. The overall study design and dose levels are shown in Table 1.

Table 1: Study Design in Mouse Study

Protocol Group No.	Dose Levels (mg/kg/day) Male/Female	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	25	25
2	0.5	Low	25	25
3	1.0	Mid	25	25
4	2.0	High	25	25
5	500	Positive	25	25

2.1. Sponsor's analyses

2.1.1. Survival analysis

The statistical analyses for mortality data will be performed separately for each sex and all statistical tests will be conducted at the 5% significance level. The survival function of each group will be estimated using the Kaplan Meier product limit method applied on daily intervals and graphs will be produced. Any animal with accidental injury that causes its death or its unscheduled sacrifice, or still alive at the day following the last day of the experimental period will be censored in the estimation.

The log rank test will be applied to all groups in order to assess the significance of the overall group effect on mortality data. If the result of this test is found to be significant at the 5% level, then, using dose level scores, the significance of an overall dose-related trend in mortality and pairwise comparisons between the reference item group and each test item treated group will be evaluated via a Peto's two-sided test (Peto, et al., 1980) at the 5% significance level.

Sponsor's findings: The sponsor's analysis showed that the numbers (percents) of survival were 25 (100%), 23 (92%), 23 (92%), 25 (100%), and 5 (20%) in male mice, and 23 (92%), 25 (100%), 25 (100%), 24 (96%), and 3 (12%) in female mice in vehicle control, low, mid, high dose groups and positive control group, respectively.

The sponsor's analysis results were not found to be statistically significant either for males or females.

2.1.2. Tumor data analysis

For tumor data, the statistically significant increase across doses was tested for an overall linear dose-related increase in tumor incidence rate and pairwise comparisons using the Cochran-Armitage's and the Fisher's one-sided exact tests, respectively.

Sponsor's findings: There were no neoplastic findings attributed to daily oral gavage administration of CK-3773274 to hemizygous transgenic rasH2 mice for 26 weeks at doses up to 2.0 mg/kg/day.

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses.

2.2.1. Survival analysis

The survival distributions of three treated groups, one vehical control group, and one positive control group were estimated using the Kaplan-Meier product limit method. The dose response relationship in survival was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1 and 2 in the appendix for male and female mice, respectively. The intercurrent mortality data are given in Tables A1 and A2 in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle and three treated groups are given in Tables A3 to A4 in the appendix for male and female mice, respectively.

Reviewer's findings: The reviewer's analysis showed that the numbers (percents) of survival were 25 (100%), 23 (92%), 23 (92%), 25 (100%), and 5 (20%) in male mice, and 23 (92%), 25 (100%), 25 (100%), 24 (96%), and 3 (12%) in female mice in vehicle control, low, mid, high dose groups and positive control group, respectively.

The survival analyses did not show any statistically significant dose response relationships in mortality across the vehicle control group and treated groups in either male or female mice.

The survival analyses showed statistically significant increase in mortality across the vehicle control group and positive control group in both male and female mice.

2.2.2. Tumor data analysis

The tumor data were analyzed for the positive dose response relationships and pairwise comparison between each of the treated groups control group for increased tumor incidence. Both the dose response relationship tests and pairwise comparisons were performed using the

Poly-K method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of $s_h = 1$. An animal that dies at week w_h without a tumor before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}} \right)^k$ < 1 . The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal with score $s_h = 1$ can be considered as a whole animal while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes were then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For animal carcinogenicity K=3 is generally suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used.

The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons between vehicle control and three treated groups, and between vehicle control and positive control are listed in Tables A7 to A8 in the appendix for male and female mice, respectively.

Adjustment for multiple testing: For the adjustment of multiple testing of dose response relationship for a submission with only one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels $\alpha = 0.05$ for both common tumors and rare tumors for the mouse study. For pairwise, the same guidance document suggests the use of test levels $\alpha = 0.05$ for both common tumors and rare tumors for the mouse study.

Reviewer's findings: The tumor types in Tables 2 to 4 below show p-values less than or equal to 0.05 in the trend test and pairwise comparisons between vehicle control and positive control group for males and females.

1. For female mice, the tumor analysis did not show any tumor types with

- statistically significant positive dose response relationships and statistically significant increases in any treated group compared to the vehicle control
2. For male mice, the trend test showed statistically significant positive dose response relationships in incidence of bronchioloalveolar adenoma, Lung (p-value=0.0056<0.05) and combined tumors of bronchioloalveolar adenoma and carcinoma (p-value=0.0212<0.05).
 3. For male mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increases in incidence of bronchioloalveolar adenoma, lung (p-value<0.001), bronchioloalveolar carcinoma, lung (p-value<0.001), combined tumors of bronchioloalveolar adenoma and carcinoma, lung (p-value<0.001), hemangiosarcoma, spleen (p-value<0.001) and combined tumors of hemangiosarcoma and hemangioma, whole body (p-value<0.001).
 4. For female mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increases in incidence of adenoma, gland harderian (p-value=0.0052<0.05), bronchioloalveolar adenoma, lung (p-value<0.001), bronchioloalveolar carcinoma, lung (p-value<0.001), combined tumors of bronchioloalveolar adenoma and carcinoma, lung (p-value<0.001), hemangioma, spleen (p-value=0.0498<0.05), hemangiosarcoma, spleen (p-value<0.001) and combined tumors of hemangiosarcoma and hemangioma, whole body (p-value<0.001).

Table 2: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle

Control and Treated Groups -Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	0.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	1.0 mg/kg/day Mid (N=25) P-value - Vehicle vs. Med	2.0 mg/kg/day High (N=25) P-value - Vehicle vs. High
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (25) 0.0056	0/25 (23) NC	1/25 (23) 0.4792	4/25 (25) 0.0549
Lung	C_bronchioloalveolar ADENOMA+CARCINOMA	1/25 (25) 0.0212	1/25 (24) 0.7449	1/25 (23) 0.7340	5/25 (25) 0.0947
& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.					

Table 3: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle

Control and Positive Control -Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (25)	23/25 (25) <0.001
	BRONCHIOLOALVEOLAR CARCINOMA	1/25 (25)	16/25 (22) <0.001
Lung	C_bronchioloalveolar ADENOMA+CARCINOMA	1/25 (25)	24/25 (25) <0.001
SPLEEN	HEMANGIOSARCOMA	1/25 (25)	20/25 (23) <0.001
Whold Body	C_Hemangiosarcoma+hemangioma	1/25 (25)	23/25 (24) <0.001

Table 4: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle

Control and Positive Control-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
GLAND, HARDERIAN	ADENOMA	0/25 (24)	5/25 (15) 0.0052

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (24)	20/25 (22) <0.001
	BRONCHIOLOALVEOLAR CARCINOMA	0/25 (24)	20/25 (22) <0.001
Lung	C_bronchioloalveolar ADENOMA+CARCINOMA	0/25 (24)	23/25 (23) <0.001
SPLEEN	HEMANGIOMA	0/25 (24)	3/25 (15) 0.0498
	HEMANGIOSARCOMA	2/25 (24)	15/25 (19) <0.001
Whold Body	C_Hemangiosarcoma+hemangioma	4/25 (24)	19/25 (22) <0.001

Zhuang Miao,

Ph.D.

Mathematical

Statistician

Concur:

Rahman, Atiar Mohammad, Ph.D.

Mathematical Statistician, Deputy Division Director, Biometrics-6

cc:

Yi Tsong, Ph.D.

Mathematical Statistician, Division Director, Biometrics-6

3. Appendix

Table A1: Intercurrent Mortality Rate -Male Mice

Week	Vehicle 0 mg/kg/day		Low 0.5 mg/kg/day		Mid 1.0 mg/kg/day		High 2.0 mg/kg/day		Positive 500 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 13	.	.	1	4.00	2	8.00	.	.	.	.
14 - 26	.	.	1	8.00	.	.	.	.	20	80.00
Ter. Sac.	25	100.00	23	92.00	23	92.00	25	100.00	5	20.00

Table A2: Intercurrent Mortality Rate -Female Mice

Week	Vehicle 0 mg/kg/day		Low 0.5 mg/kg/day		Mid 1.0 mg/kg/day		High 2.0 mg/kg/day		Positive 500 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 13	.	.	.	.	.	.	1	4.00	2	8.00
14 - 26	2	8.00	.	.	.	.	.	.	20	88.00
Ter. Sac.	23	92.00	25	100.00	25	100.00	24	96.00	3	12.00

Table A3: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control 1, Positive Control and Vehicle Control 1-Male Mice

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Dose-Response	Likelihood Ratio	.7338	.0935	.0935	.	<.0001
Homogeneity	Log-Rank	.2481	.1531	.1531	.	<.0001

Table A4: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control 1, Positive Control and Vehicle Control 1-Female Mice

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Dose-Response	Likelihood Ratio	.6225	.0935	.0935	.5678	<.0001

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Homogeneity	Log-Rank	.2947	.1531	.1531	.5717	<.0001

**Table A5: Tumor Rates and P-Values for Dose Response Relationship and
Pairwise Comparisons between Vehicle Control and the Treated Groups-Male
Mice**

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	0.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	1.0 mg/kg/day Mid (N=25) P-value - Vehicle vs. Med	2.0 mg/kg/day High (N=25) P-value - Vehicle vs. High
GLAND, HARDERIAN	ADENOMA	2/25 (25) 0.7381	0/25 (23) 1.0000	0/25 (23) 1.0000	1/25 (25) 0.8827
LARYNX	CARCINOMA	0/25 (25) 0.7423	1/25 (24) 0.4898	0/25 (23) NC	0/25 (25) NC
LIVER	HEMANGIOSARCOMA	1/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (23) 1.0000	0/25 (25) 1.0000
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (25) 0.0056	0/25 (23) NC	1/25 (23) 0.4792	4/25 (25) 0.0549
	BRONCHIOLOALVEOLAR CARCINOMA	1/25 (25) 0.5978	1/25 (24) 0.7449	0/25 (23) 1.0000	1/25 (25) 0.7551
Lung	C_bronchioloalveolar ADENOMA+CARCINOMA	1/25 (25) 0.0212	1/25 (24) 0.7449	1/25 (23) 0.7340	5/25 (25) 0.0947
MUSCLE, SKELETAL	HEMANGIOSARCOMA	1/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (23) 1.0000	0/25 (25) 1.0000
SKIN	PAPILLOMA	0/25 (25) 0.7396	1/25 (23) 0.4792	0/25 (23) NC	0/25 (25) NC
SPLEEN	HEMANGIOMA	0/25 (25) 0.7396	1/25 (23) 0.4792	0/25 (23) NC	0/25 (25) NC
	HEMANGIOSARCOMA	1/25 (25) 0.8121	0/25 (23) 1.0000	1/25 (24) 0.7449	0/25 (25) 1.0000
STOMACH	PAPILLOMA	0/25 (25) 0.2604	0/25 (23) NC	0/25 (23) NC	1/25 (25) 0.5000
THYMUS	THYMOMA, BENIGN	1/25 (25) 0.2091	0/24 (23) 1.0000	3/25 (23) 0.2730	2/25 (25) 0.5000
Whold Body	C_Hemangiosarcoma+hemangioma	1/25 (25) 0.8459	1/25 (23) 0.7340	1/25 (24) 0.7449	0/25 (25) 1.0000

**Table A6: Tumor Rates and P-Values for Dose Response Relationship and
Pairwise Comparisons between Vehicle Control and the Treated Groups- Female
Mice**

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	0.5 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	1.0 mg/kg/day Mid (N=25) P-value - Vehicle vs. Med	2.0 mg/kg/day High (N=25) P-value - Vehicle vs. High
GLAND, HARDERIAN	ADENOMA	0/25 (24) 0.7551	1/25 (25) 0.5102	0/25 (25) NC	0/25 (24) NC
HEMOLYMPHORET ICULA	LYMPHOMA, MALIGNANT	0/25 (24) 0.0618	0/25 (25) NC	0/25 (25) NC	2/25 (25) 0.2551
KIDNEY	HEMANGIOMA	1/25 (24) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (24) 0.4171	2/25 (25) 0.2551	1/25 (25) 0.5102	1/25 (24) 0.5000
Lung	C_bronchioalveolar ADENOMA+CARCINOMA	0/25 (24) 0.4171	2/25 (25) 0.2551	1/25 (25) 0.5102	1/25 (24) 0.5000
SKIN	HEMANGIOSARCOMA	1/25 (24) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000
	PAPILLOMA	0/25 (24) 0.7551	1/25 (25) 0.5102	0/25 (25) NC	0/25 (24) NC
SPLEEN	HEMANGIOMA	0/25 (24) 0.2449	0/25 (25) NC	0/25 (25) NC	1/25 (24) 0.5000
	HEMANGIOSARCOMA	2/25 (24) 0.9256	2/25 (25) 0.7110	2/25 (25) 0.7110	0/25 (24) 1.0000
THYMUS	MESOTHELIOMA, MALIGNANT	1/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/24 (23) 1.0000
	THYMOMA, BENIGN	1/25 (24) 0.9407	1/25 (25) 0.7653	0/25 (25) 1.0000	0/24 (23) 1.0000
UTERUS	ENDOMETRIAL STROMAL POLYP	0/25 (24) 0.2449	0/25 (25) NC	0/25 (25) NC	1/25 (24) 0.5000
	HEMANGIOMA	0/25 (24) 0.6263	1/25 (25) 0.5102	1/25 (25) 0.5102	0/25 (24) NC
	HEMANGIOSARCOMA	0/25 (24) 0.0581	0/25 (25) NC	0/25 (25) NC	2/25 (24) 0.2447
Whold Body	C_Hemangiosarcoma+hemangioma	4/25 (24) 0.8440	3/25 (25) 0.8084	2/25 (25) 0.9144	2/25 (24) 0.9059

Table A7: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control -Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
GLAND, HARDERIAN	ADENOMA	2/25 (25)	4/25 (17) 0.1680
HEMOLYMPHORET ICULA	LYMPHOMA, MALIGNANT	0/25 (25)	1/25 (17) 0.4048
	HEPATOCELLULAR ADENOMA	0/25 (25)	1/25 (16) 0.3902
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (25)	23/25 (25) <0.001
	BRONCHIOLOALVEOLAR CARCINOMA	1/25 (25)	16/25 (22) <0.001
LYMPH NODE, MANDIB	HEMANGIOMA	0/24 (24)	1/23 (15) 0.3846
Lung	C_bronchioloalveolar ADENOMA+CARCINOMA	1/25 (25)	24/25 (25) <0.001
MUSCLE, SKELETAL	CARCINOMA	0/25 (25)	1/25 (17) 0.4048
SPLEEN	HEMANGIOMA	0/25 (25)	3/25 (18) 0.0661
	HEMANGIOSARCOMA	1/25 (25)	20/25 (23) <0.001
STOMACH	SQUAMOUS CELL CARCINOMA	0/25 (25)	1/25 (17) 0.4048
Whold Body	C_Hemangiosarcoma+hemangioma	1/25 (25)	23/25 (24) <0.001

Table A8: Tumor Rates and P-Values for Comparisons between Vehicle Control 1 and Positive Control - Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
BONE MARROW	HEMANGIOMA	0/25 (24)	1/25 (14) 0.3684
BONE, STERNUM	MESOTHELIOMA, MALIGNANT	0/25 (24)	1/25 (15) 0.3846
CERVIX	SQUAMOUS CELL CARCINOMA	0/25 (24)	1/24 (15) 0.3846
GLAND, HARDERIAN	ADENOMA	0/25 (24)	5/25 (15) 0.0052
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/25 (24)	20/25 (22) <0.001
	BRONCHIOLOALVEOLAR CARCINOMA	0/25 (24)	20/25 (22) <0.001
	HEMANGIOSARCOMA	0/25 (24)	2/25 (15) 0.1417
Lung	C_bronchioloalveolar ADENOMA+CARCINOMA	0/25 (24)	23/25 (23) <0.001
MUSCLE, SKELETAL	HEMANGIOSARCOMA	0/25 (24)	1/25 (14) 0.3684
SKIN	HEMANGIOSARCOMA	1/25 (24)	3/25 (15) 0.1494
SPLEEN	HEMANGIOMA	0/25 (24)	3/25 (15) 0.0498
	HEMANGIOSARCOMA	2/25 (24)	15/25 (19) <0.001
UTERUS	ENDOMETRIAL STROMAL POLYP	0/25 (24)	1/24 (14) 0.3684
	HEMANGIOSARCOMA	0/25 (24)	1/24 (14) 0.3684
Whold Body	C_Hemangiosarcoma+hemangioma	4/25 (24)	19/25 (22) <0.001

Figure 1: Kaplan-Meier Survival Functions for Male Mice

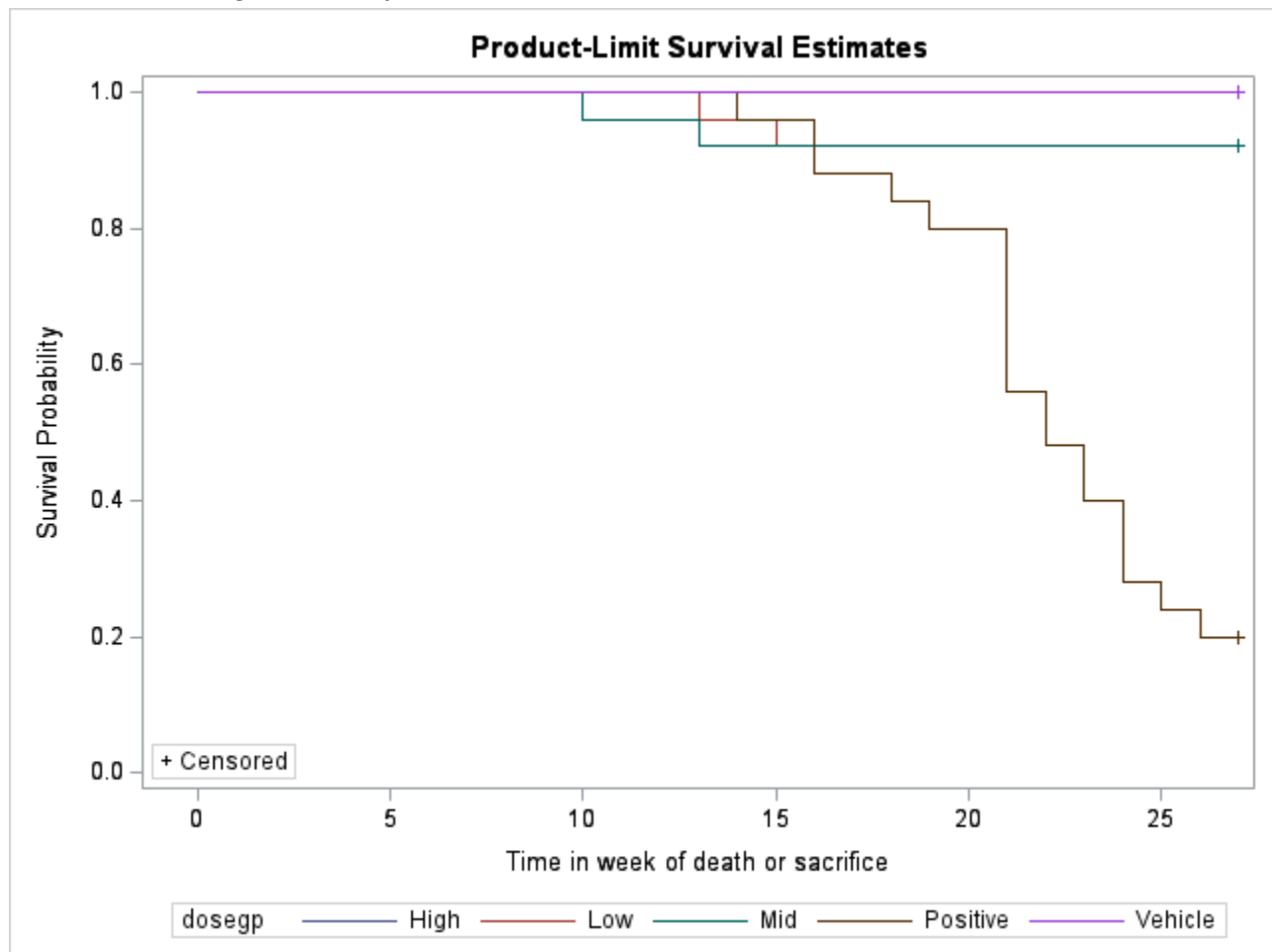
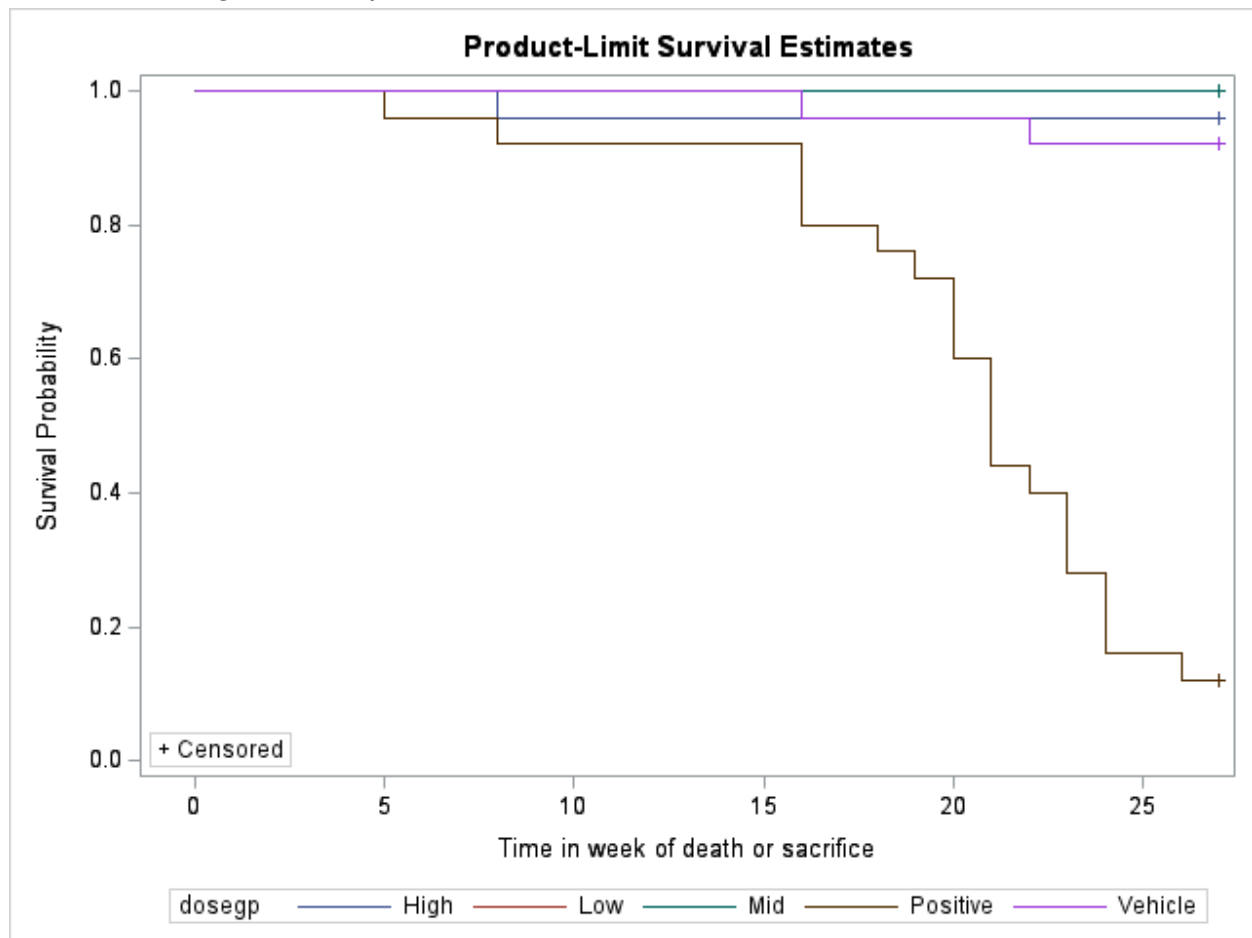


Figure 2: Kaplan-Meier Survival Functions for Female Mice



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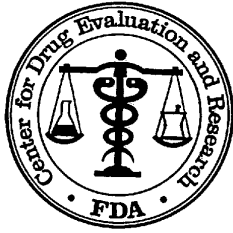
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ZHUANG MIAO
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MOHAMMAD A RAHMAN
03/11/2025 03:56:22 PM



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation
CARCINOGENICITY STUDY

IND/NDA Number: NDA219083

Drug Name: Aficamten (Myqorzo)

Indication: Treatment of symptomatic obstructive hypertrophic cardiomyopathy

Applicant: Cytokinetics, Incorporated
350 Oyster Point Boulevard, South San Francisco, CA 94080

Test Facility for Rats Study: (b) (4)
(b) (4)

Documents Reviewed: Study reports (Rat Study CK-3773274) and electronic data submitted via NDA219083/0007

Biometrics Division: Division of Biometrics -6

Statistical Reviewer: Zhuang Miao, Ph.D.

Concurring Reviewer: Rahman, Atiar Mohammad, Ph.D.

Reviewing Pharmacologist: Chi, Xuan, Ph.D.

Keywords: Carcinogenicity, Dose response

Table of Contents

Summary	3	
1.	Background	4
2.	Rat Study	4
Table 1: Study Design in Rat Study		4
2.1. Sponsor's analyses		4
2.1.1. Survival analysis		4
2.1.2. Tumor data analysis		4
Sponsor's findings		5
2.2. Reviewer's analyses		6
2.2.1. Survival analysis		6
Reviewer's findings		6
Table 3: Trend test and pairwise comparison results with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups-Male Rats		6
Table 3: Trend test and pairwise comparison results with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups-Female Rats		7
2.2.2. Tumor data analysis		7
Adjustment for multiple testing		7
Reviewer's findings		8
Table 4: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups -Male Rats		8
Table 4: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups -Female Rats		8
3.	Appendix	9
Table 2: Intercurrent Mortality Rate -Male Rats		9
Table 3: Intercurrent Mortality Rate -Female Rats		9
Table 4: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Male Rats		9
Table 6: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Female Rats		9
Table 8: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Male Rats		10
Table 9: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Female Rats		14
Figure 1: Kaplan-Meier Survival Functions for Male Rats		17
Figure 2: Kaplan-Meier Survival Functions for Female Rats		18
4.	References	19

Summary

In this submission the sponsor included the report of one animal carcinogenicity study in Sprague Dawley rats. This study was intended to assess the carcinogenic potential of CK-3773274, when administered by oral gavage once daily for 104 weeks.

Rat Study:

Survival analysis:

1. For male rats, the survival analyses showed a statistically significant dose response relationship in mortality across the vehicle control group and treated groups (p-value of likelihood ratio test is <0.0001 and p-value of log-rank test is <0.0001). The pairwise comparison showed a statistically positive significant difference in mortality between the vehicle control group and the high dose group (p-value of likelihood ratio test is <0.0001 and p-value of log-rank test is <0.0001).
2. For female rats, the survival analyses showed a statistically significant dose response relationship in mortality across the vehicle control group and treated groups (p-value of likelihood ratio test is 0.0014 and p-value of log-rank test is 0.0014). The pairwise comparison showed a statistically positive significant difference in mortality between the vehicle control group and the high dose group (p-value of likelihood ratio test is 0.0288 and p-value of log-rank test is 0.0263).

Tumor analysis:

1. For male rats, the tumor analyses did not show any statistically significant dose response relationship in tumor incidence across the vehicle control groups and treated groups. The pairwise comparisons did not show any statistically significant differences in tumor incidence between the vehicle control groups and each of the treated groups.
2. For female rats, the tumor analyses did not show any statistically significant dose response relationship in tumor incidence across the vehicle control groups and treated groups. The pairwise comparisons showed a statistically significant difference in tumor incidence between the vehicle control groups and the low dose group (p-value=0.0221).

1. Background

In this submission the sponsor included the report of one animal carcinogenicity study in Sprague Dawley rats. This study was intended to assess the carcinogenic potential of CK-3773274, when administered by oral gavage once daily for 104 weeks.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as the dose increases.

Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one vehicle control group. Two hundred and sixty rats of each sex were randomly assigned to the treated groups and vehicle control group in equal size of 65 rats per group. The study was designed to continue for up to 104 weeks for both sexes.

Due to lower survival (n=20) in males and females administered 2 and 3 mg/kg/day, respectively, dose administration of all surviving males and females at these dose levels was suspended as of Days 474/475 (Week 68) and 635/636 (Week 91), respectively. Animals were maintained on study, without dosing and due to continued low survivability (n=15), all surviving males at 2 mg/kg/day were euthanized during Weeks 79 and 80, and surviving females at 3 mg/kg/day were euthanized during Week 96. Due to lower survival (n=20) in control males and females, all surviving males were euthanized during Weeks 100 and 101, and all surviving females were euthanized during Week 103.

Table 1: Study Design in Rat Study

Protocol Group No.	Dose Levels (mg/kg/day) Male/Female	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	65	65
2	0.5	Low	65	65
3	1	Mid	65	65
4	2/3	High	65	65

1.1. Sponsor's analyses

1.1.1. Survival analysis

Kaplan-Meier estimates of group survival rates were calculated by sex and shown graphically.

A log-rank dose response trend test of survival rates was performed utilizing ordinal coefficients. In addition, a log-rank test for survival was used to make pairwise comparisons of each treated group with the vehicle control group. Both the trend test and pairwise comparisons were conducted at the 0.05 significance level.

Survival times in which the status of the animal's death was classified as terminal euthanasia were considered censored values for the purpose of the Kaplan-Meier estimates and survival rate analyses.

Sponsor's findings: The sponsor's analysis showed that the numbers (percents) of survival were 19 (29%), 17 (26%), 16 (25%), and 15 (23%) male rats, and 20 (31%), 24 (38%), 20 (31%), and 15 (23%) in female rats in vehicle control, low, mid and high dose groups, respectively.

The sponsor concluded that there was a statistically significant lower survivability rate in males administered 2 mg/kg/day and females administered 3 mg/kg/day, compared with controls as well as a significant decreasing trend across dose groups.

1.1.2. Tumor data analysis

The incidence of tumours at different dose levels were analysed using a Peto analysis with unadjusted p-values, following the recommendations of OECD guidance document 116.

Tumours were distinguished by their finding (e.g., carcinoma) and their organ of occurrence. All of the tumours distinguished by finding and organ as contained in the data were analysed. A time stratum was used in the Peto analysis for the prevalence part: The experimental period was divided into the following time intervals : 0-52 weeks, 53-78 weeks, 79-92 weeks, over 92 weeks, and planned (terminal) sacrifice. These time intervals were applied to time to occurrence for incidental tumours. The occurrence part for fatal tumours of the Peto analysis used days as unit, using day of occurrence for fatal tumours. If day of occurrence was not available, day of death was used instead.

As described in, the methodology used normal approximations which may be unreliable if the number of tumours is small. For data sets with 10 tumours or fewer, a permutation test was used, as implemented in SAS Version 9.4 for PROC MULTTEST. The permutation test has also been used for data sets of 11 or more tumours.

Analyses were carried out by sex. Active treatment groups (i.e., Groups 2, 3, and 4) were compared with the control group (i.e., Group 1) using Petos one-sided test for trends, testing for higher incidence in the active treatment groups.

In addition to the linear trend test for all dose groups, pairwise comparisons of active treatment groups with the control group were carried out as well, using Peto one-sided test.

Sponsor's findings: In conclusion, there were no statistically significant tumor findings after CK-3773274

administration to rats by oral gavage once daily for up to 704 days at doses of up to 2 mg/kg/day in males and for up to 721 days at doses of up to 3 mg/kg/day in females.

1.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses.

1.2.1. Survival analysis

The survival distributions of three treated groups, one vehical control group, and one positive

control group were estimated using the Kaplan-Meier product limit method. The dose response relationship in survival was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1 and 2 in the appendix for male and female rats, respectively. The intercurrent mortality data are given in Tables 6 and 7 in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle control and three treated groups are given in Tables 8 and 9 in the appendix for male and female rats, respectively.

Reviewer's findings: The reviewer's analysis showed that the numbers (percents) of survival were 19 (29%), 17 (26%), 16 (25%), and 15 (23%) male rats, and 20 (31%), 24 (38%), 20 (31%), and 15 (23%) in female rats in vehicle control, low, mid and high dose groups, respectively.

For male rats, the survival analyses showed a statistically significant dose response relationship in mortality across the vehicle control group and treated groups (p-value of likelihood ratio test is <0.0001 and p-value of log-rank test is <0.0001). The pairwise comparison showed a statistically positive significant difference in mortality between the vehicle control group and the high dose group (p-value of likelihood ratio test is <0.0001 and p-value of log-rank test is <0.0001).

For female rats, the survival analyses showed a statistically significant dose response relationship in mortality across the vehicle control group and treated groups (p-value of likelihood ratio test is 0.0014 and p-value of log-rank test is 0.0014). The pairwise comparison showed a statistically positive significant difference in mortality between the vehicle control group and the high dose group (p-value of likelihood ratio test is 0.0288 and p-value of log-rank test is 0.0263).

Table 2: Trend test and pairwise comparison results with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups-Male Rats

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	<0.0001	<0.0001
Homogeneity	Log-Rank	<0.0001	<0.0001

Table 3: Trend test and pairwise comparison results with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups-Female Rats

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.0014	0.0288
Homogeneity	Log-Rank	0.0014	0.0263

1.2.2. Tumor data analysis

The tumor data were analyzed for the positive dose response relationships and the positive pairwise comparison increases between each of the treated groups with control group. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-K method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of $s_h = 1$. An animal that dies at week w_h without a tumor before the end of the study gets a score of

$s_h = \left(\frac{w_h}{w_{\max}} \right)^k < 1$. The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal

with score $s_h = 1$ can be considered as a whole animal while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes were then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and rat studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used.

The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons between vehicle control and three treated groups are listed in Tables 10, and 11 in the appendix for male and female rats, respectively.

Adjustment for multiple testing: For the chronic study in rats, the adjustment of multiple testing of the dose response relationship for a submission with one chronic rat study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels $\alpha = 0.005$ for common tumors and $\alpha = 0.025$ for rare tumors for the chronic rat study. For pairwise comparisons for the chronic rat study in the above type of submission with one chronic rat study, the same guidance document suggests the use of test levels $\alpha = 0.01$ for common tumors and $\alpha = 0.05$ for rare tumors for the chronic rat study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings:

For male rats, the tumor analyses did not show any statistically significant dose response relationship in tumor incidence across the vehicle control groups and treated groups. The

pairwise comparisons did not show any statistically significant differences in tumor incidence between the vehicle control groups and each of the treated groups.

For female rats, the tumor analyses did not show any statistically significant dose response relationship in tumor incidence across the vehicle control groups and treated groups. The pairwise comparisons showed a statistically significant difference in tumor incidence between the vehicle control groups and the low dose group (p-value=0.0221).

Table 4: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups -Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	2 mg/kg/day High (N=65) P-value - Vehicle vs. High
SKIN/SUBCUTIS	SARCOMA, MALIGNANT	0/8 (7) 0.0342	0/9 (7) NC	1/16 (12) 0.6316	1/3 (1) 0.1250
& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.					

Table 5: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Treated Groups -Female Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	3 mg/kg/day High (N=65) P-value - Vehicle vs. High
GLAND, PITUITARY	CARCINOMA, PARS DISTALIS, MALIGNANT	0/64 (40) 0.5359	5/55 (37) 0.0221	2/58 (38) 0.2341	1/65 (31) 0.4366
& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.					

Ph.D.

Statistician

Concur:

Rahman, Atiar Mohammad, Ph.D.

Mathematical Statistician, Deputy Director, Biometrics-6

cc:

Yi Tsong, Ph.D.

Zhuang Miao,

Mathematical

Appendix
Table 6: Intercurrent Mortality Rate -Male Rats

Week	Vehicle 0 mg/kg/day		Low 0.5 mg/kg/day		Mid 1 mg/kg/day		High 2 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	7	10.77	3	4.62	4	6.15	18	27.69
53 - 78	8	23.08	19	33.85	15	29.23	31	75.38
79 - 99	23	58.46	17	60.00	17	55.38	1	76.92
92 - 101	8	70.77	9	73.85	13	75.38	.	.
Ter. Sac.	19	29.23	17	26.15	16	24.62	15	23.08

Cum. %: Cumulative percentage except for Ter. Sac.

Table 7: Intercurrent Mortality Rate -Female Rats

Week	Vehicle 0 mg/kg/day		Low 0.5 mg/kg/day		Mid 1 mg/kg/day		High 3 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	5	7.69	6	9.23	4	6.15	6	9.23
53 - 78	19	36.92	8	21.54	13	26.15	25	47.69
79 - 99	8	49.23	9	35.38	14	47.69	14	69.23
92 - 104	16	67.69	17	60.00	14	67.69	5	76.92
Ter. Sac.	20	32.31	25	40.00	20	32.31	15	23.08

Cum. %: Cumulative percentage except for Ter. Sac.

Table 8: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Male Rats

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	<0.0001	0.6942	0.7697	<0.0001
Homogeneity	Log-Rank	<0.0001	0.6906	0.7660	<0.0001

Table 9: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Female Rats

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.0014	0.2100	0.7321	0.0288
Homogeneity	Log-Rank	0.0014	0.2040	0.7291	0.0263

Table 10: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	2 mg/kg/day High (N=65) P-value - Vehicle vs. High
BONE, STERNUM	CHONDROSARCOMA, MALIGNANT	0/64 (39) 0.1569	0/47 (22) NC	0/49 (25) NC	1/63 (16) 0.2909
BRAIN	GLIOMA, MALIGNANT	1/65 (40) 0.8544	1/48 (23) 0.6006	0/49 (25) 1.0000	0/65 (16) 1.0000
	GRANULAR CELL TUMOR, BENIGN	1/65 (40) 1.0000	0/48 (23) 1.0000	0/49 (25) 1.0000	0/65 (16) 1.0000
	MENINGIOMA, MALIGNANT	0/65 (40) 0.4000	0/48 (23) NC	1/49 (26) 0.3939	0/65 (16) NC
EPIDIDYMS	MESOTHELIOMA, MALIGNANT	0/65 (40) 0.1538	0/48 (23) NC	0/49 (25) NC	1/65 (16) 0.2857
GLAND, ADRENAL	ADENOMA, BENIGN	1/65 (40) 0.5941	1/52 (27) 0.6472	2/53 (30) 0.3920	0/65 (16) 1.0000
	PHEOCHROMOCYTOMA, BENIGN	12/65 (43) 0.8844	7/52 (28) 0.7043	5/53 (31) 0.9310	3/65 (17) 0.8786
	PHEOCHROMOCYTOMA, COMPLEX, MALIGNANT	1/65 (40) 1.0000	0/52 (26) 1.0000	0/53 (29) 1.0000	0/65 (16) 1.0000
	PHEOCHROMOCYTOMA, MALIGNANT	3/65 (40) 0.4184	2/52 (27) 0.6764	5/53 (31) 0.2224	1/65 (16) 0.7512
GLAND, MAMMARY	ADENOCARCINOMA, MALIGNANT	0/45 (28) 0.3836	0/32 (17) NC	1/36 (19) 0.4043	0/36 (9) NC
	FIBROADENOMA, BENIGN	1/45 (28) 0.8601	1/32 (18) 0.6348	0/36 (19) 1.0000	0/36 (9) 1.0000
GLAND, PARATHYROID	ADENOMA, BENIGN	2/58 (35) 1.0000	0/43 (20) 1.0000	0/45 (23) 1.0000	0/52 (12) 1.0000
GLAND, PITUITARY	ADENOMA, PARS DISTALIS, BENIGN	8/64 (41) 0.8373	9/48 (27) 0.1583	5/52 (30) 0.7284	2/65 (17) 0.8644
	ADENOMA, PARS INTERMEDIA, BENIGN	1/64 (39) 0.2743	0/48 (23) 1.0000	1/52 (29) 0.6747	1/65 (16) 0.5010
GLAND, PROSTATE	ADENOMA, BENIGN	5/65 (41) 0.9158	2/48 (24) 0.8127	0/48 (25) 1.0000	1/65 (17) 0.8889
	SCHWANNOMA, MALIGNANT	0/65 (40) 0.1744	1/48 (23) 0.3651	0/48 (25) NC	1/65 (17) 0.2982
GLAND, THYROID	ADENOMA, C-CELL, BENIGN	5/64 (40) 0.3146	4/46 (23) 0.4272	6/49 (28) 0.2562	3/65 (18) 0.4784
	ADENOMA, FOLLICULAR CELL, BENIGN	2/64 (39) 0.8614	4/46 (23) 0.1297	1/49 (26) 0.7908	0/65 (16) 1.0000
	CARCINOMA, C-CELL, MALIGNANT	1/64 (39) 1.0000	0/46 (22) 1.0000	0/49 (25) 1.0000	0/65 (16) 1.0000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	2 mg/kg/day High (N=65) P-value - Vehicle vs. High
	CARCINOMA, FOLLICULAR CELL, MALIGNANT	1/64 (39) 1.0000	0/46 (22) 1.0000	0/49 (25) 1.0000	0/65 (16) 1.0000
HEART	SCHWANNOMA, MALIGNANT	1/65 (40) 0.9138	1/65 (39) 0.7468	0/65 (40) 1.0000	0/65 (16) 1.0000
KIDNEY	NEPHROBLASTOMA, MALIGNANT	0/65 (40) 0.4127	0/60 (34) NC	1/60 (36) 0.4737	0/65 (16) NC
LIVER	CARCINOMA, HEPATOCELLULAR, MALIGNANT	0/65 (40) 0.4179	0/65 (38) NC	1/65 (40) 0.5000	0/65 (16) NC
LUNG	ADENOMA, BRONCHIOLOALVEOLAR, BENIGN	0/65 (40) 0.7037	1/65 (39) 0.4937	0/65 (40) NC	0/65 (16) NC
	HEMANGIOMA, BENIGN	1/65 (40) 1.0000	0/65 (38) 1.0000	0/65 (40) 1.0000	0/65 (16) 1.0000
	LYMPHOMA, MALIGNANT	0/65 (40) 0.3144	0/65 (38) NC	2/65 (41) 0.2531	0/65 (16) NC
PANCREAS	ADENOMA, ISLET CELL, BENIGN	2/65 (40) 0.5633	3/48 (24) 0.2684	1/50 (26) 0.7841	1/63 (16) 0.6436
	CARCINOMA, ISLET CELL, MALIGNANT	2/65 (40) 1.0000	0/48 (23) 1.0000	0/50 (26) 1.0000	0/63 (15) 1.0000
SKIN	CARCINOMA, BASAL CELL, MALIGNANT	1/64 (40) 1.0000	0/46 (22) 1.0000	0/48 (25) 1.0000	0/64 (16) 1.0000
SKIN/SUBCUTIS	ADENOMA, SEBACEOUS, BENIGN	0/8 (7) 0.4615	0/9 (7) NC	1/16 (12) 0.6316	0/3 (0) NC
	CARCINOMA, BASAL CELL, MALIGNANT	1/8 (7) 1.0000	0/9 (7) 1.0000	0/16 (11) 1.0000	0/3 (0) NC
	FIBROMA, BENIGN	0/8 (7) 0.5704	3/9 (8) 0.1231	1/16 (12) 0.6316	0/3 (0) NC
	FIBROSARCOMA, MALIGNANT	0/8 (7) 0.0978	0/9 (7) NC	3/16 (13) 0.2509	0/3 (0) NC
	HIBERNOMA, BENIGN	0/8 (7) 0.4615	0/9 (7) NC	1/16 (12) 0.6316	0/3 (0) NC
	KERATOACANTHOMA, BENIGN	2/8 (7) 0.2294	2/9 (7) 0.7203	4/16 (12) 0.6215	1/3 (1) 0.3750
	LIPOMA, BENIGN	1/8 (7) 1.0000	0/9 (7) 1.0000	0/16 (11) 1.0000	0/3 (0) NC
	PAPILLOMA, SQUAMOUS CELL, BENIGN	0/8 (7) 0.4615	0/9 (7) NC	1/16 (12) 0.6316	0/3 (0) NC
	SARCOMA, MALIGNANT	0/8 (7) 0.0342	0/9 (7) NC	1/16 (12) 0.6316	1/3 (1) 0.1250

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	2 mg/kg/day High (N=65) P-value - Vehicle vs. High
SMALL INTESTINE, DUODENUM	ADENOCARCINOMA, MALIGNANT	2/63 (39) 0.9360	1/42 (20) 0.7189	0/45 (23) 1.0000	0/56 (14) 1.0000
SMALL INTESTINE, ILEUM	ADENOCARCINOMA, MALIGNANT	1/54 (33) 1.0000	0/37 (17) 1.0000	0/38 (20) 1.0000	0/47 (13) 1.0000
SMALL INTESTINE, JEJUNUM	ADENOCARCINOMA, MALIGNANT	1/58 (37) 0.6767	0/37 (17) 1.0000	1/43 (22) 0.6108	0/54 (14) 1.0000
STOMACH	SARCOMA, MALIGNANT	0/65 (40) 0.4112	0/48 (23) NC	1/51 (28) 0.4118	0/65 (16) NC
TESTIS	LEYDIG CELL TUMOR, BENIGN	1/65 (40) 0.3342	0/50 (25) 1.0000	0/49 (25) 1.0000	1/64 (16) 0.4935
	MESOTHELIOMA, MALIGNANT	0/65 (40) 0.1509	0/50 (25) NC	0/49 (25) NC	1/64 (16) 0.2857
THYMUS	THYMOMA, BENIGN	2/60 (38) 0.8428	0/45 (22) 1.0000	1/45 (24) 0.7769	0/54 (14) 1.0000
URINARY BLADDER	CARCINOMA, UROTHELIAL, MALIGNANT	1/61 (36) 1.0000	0/47 (22) 1.0000	0/47 (24) 1.0000	0/62 (15) 1.0000

Table 11: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Female Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	3 mg/kg/day High (N=65) P-value - Vehicle vs. High
BRAIN	GLIOMA, MALIGNANT	0/63 (39) 0.2650	0/41 (23) NC	0/45 (24) NC	1/65 (31) 0.4429
CERVIX	CARCINOMA, SQUAMOUS CELL, MALIGNANT	1/65 (41) 0.4070	0/41 (23) 1.0000	1/44 (24) 0.6058	1/65 (31) 0.6792
	GRANULAR CELL TUMOR, BENIGN	0/65 (41) 0.1971	0/41 (23) NC	2/44 (24) 0.1327	1/65 (31) 0.4306
	KERATOACANTHOMA, BENIGN	0/65 (41) 0.6555	1/41 (23) 0.3594	0/44 (24) NC	0/65 (31) NC
GLAND, ADRENAL	ADENOMA, BENIGN	1/64 (40) 0.5433	1/44 (26) 0.6364	0/49 (28) 1.0000	1/65 (31) 0.6861
	PHEOCHROMOCYTOMA, BENIGN	2/64 (40) 0.4925	2/44 (26) 0.5168	1/49 (28) 0.8029	2/65 (31) 0.5907
	PHEOCHROMOCYTOMA, MALIGNANT	0/64 (40) 0.2480	0/44 (26) NC	0/49 (28) NC	1/65 (31) 0.4366
GLAND, MAMMARY	ADENOCARCINOMA ARISING IN FIBROADENOMA, MALIGNANT	2/62 (39) 0.3292	1/61 (43) 0.8968	3/56 (37) 0.4747	2/60 (31) 0.6013
	ADENOCARCINOMA, MALIGNANT	11/62 (42) 0.4360	17/61 (47) 0.2170	9/56 (38) 0.6965	11/60 (34) 0.3679
	ADENOMA, BENIGN	1/62 (38) 0.0874	1/61 (42) 0.7775	1/56 (36) 0.7397	3/60 (31) 0.2340
	FIBROADENOMA, BENIGN	39/62 (54) 0.9724	36/61 (50) 0.5967	36/56 (47) 0.3934	22/60 (41) 0.9814
GLAND, PARATHYROID	ADENOMA, BENIGN	0/52 (32) 0.5149	0/32 (17) NC	1/43 (23) 0.4182	0/61 (29) NC
GLAND, PITUITARY	ADENOMA, PARS DISTALIS, BENIGN	25/64 (47) 0.7003	29/55 (42) 0.0946	32/58 (45) 0.0596	19/65 (38) 0.6953
	ADENOMA, PARS INTERMEDIA, BENIGN	0/64 (40) 0.7241	1/55 (37) 0.4805	0/58 (37) NC	0/65 (31) NC
	CARCINOMA, PARS DISTALIS, MALIGNANT	0/64 (40) 0.5359	5/55 (37) 0.0221	2/58 (38) 0.2341	1/65 (31) 0.4366
	CRANIOPHARYNGIOMA, BENIGN	1/64 (40) 1.0000	0/55 (36) 1.0000	0/58 (37) 1.0000	0/65 (31) 1.0000
	GANGLIONEUROMA, BENIGN	2/64 (40) 1.0000	0/55 (36) 1.0000	0/58 (37) 1.0000	0/65 (31) 1.0000
GLAND, THYROID	ADENOMA, C-CELL, BENIGN	13/60 (41) 0.9926	9/39 (24) 0.4161	3/46 (26) 0.9882	4/65 (32) 0.9881

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	3 mg/kg/day High (N=65) P-value - Vehicle vs. High
	ADENOMA, FOLLICULAR CELL, BENIGN	1/60 (39) 0.5097	0/39 (21) 1.0000	0/46 (25) 1.0000	1/65 (31) 0.6932
	CARCINOMA, C-CELL, MALIGNANT	0/60 (39) 0.7111	2/39 (21) 0.1186	1/46 (25) 0.3906	0/65 (31) NC
KIDNEY	ADENOMA, BENIGN	0/65 (41) 0.2520	0/43 (25) NC	0/47 (26) NC	1/65 (31) 0.4306
	NEPHROBLASTOMA, MALIGNANT	0/65 (41) 0.6694	1/43 (26) 0.3881	0/47 (26) NC	0/65 (31) NC
LIVER	ADENOMA, HEPATOCELLULAR, BENIGN	1/65 (41) 0.4191	0/65 (46) 1.0000	0/65 (43) 1.0000	1/65 (31) 0.6792
LUNG	ADENOCARCINOMA, MALIGNANT	0/65 (41) 0.7469	1/65 (47) 0.5341	0/65 (43) NC	0/65 (31) NC
OVARY	GRANULOSA CELL TUMOR, BENIGN	1/65 (41) 1.0000	0/47 (29) 1.0000	0/51 (30) 1.0000	0/65 (31) 1.0000
	GRANULOSA CELL TUMOR, MALIGNANT	1/65 (41) 0.7641	0/47 (29) 1.0000	1/51 (30) 0.6700	0/65 (31) 1.0000
	HEMANGIOMA, BENIGN	0/65 (41) 0.4656	0/47 (29) NC	1/51 (30) 0.4225	0/65 (31) NC
PANCREAS	ADENOMA, ISLET CELL, BENIGN	1/64 (40) 0.8187	1/65 (46) 0.7866	1/65 (44) 0.7762	0/63 (30) 1.0000
SKIN/GLAND, MAMMARY	FIBROMA, BENIGN	1/65 (41) 1.0000	0/40 (22) 1.0000	0/45 (24) 1.0000	0/65 (31) 1.0000
SKIN/SUBCUTIS	CARCINOMA, BASAL CELL, MALIGNANT	0/5 (3) 0.1679	1/7 (6) 0.6667	1/7 (5) 0.6250	1/3 (2) 0.4000
	FIBROMA, BENIGN	3/5 (5) 0.9963	1/7 (5) 0.9762	0/7 (4) 1.0000	0/3 (1) 1.0000
	FIBROSARCOMA, MALIGNANT	0/5 (3) 0.1824	1/7 (5) 0.6250	1/7 (5) 0.6250	1/3 (2) 0.4000
	KERATOACANTHOMA, BENIGN	1/5 (3) 0.8551	2/7 (5) 0.7143	1/7 (5) 0.8929	0/3 (1) 1.0000
	SARCOMA, MALIGNANT	1/5 (3) 0.9670	1/7 (6) 0.9167	0/7 (4) 1.0000	0/3 (1) 1.0000
THYMUS	THYMOMA, BENIGN	0/60 (37) 0.5761	1/62 (45) 0.5488	1/60 (40) 0.5195	0/62 (29) NC
UTERUS	ADENOCARCINOMA, ENDOMETRIAL, MALIGNANT	1/65 (41) 0.4601	0/51 (32) 1.0000	0/58 (37) 1.0000	1/65 (31) 0.6792
	LEIOMYOMA, BENIGN	0/65 (41) 0.7113	1/51 (33) 0.4459	0/58 (37) NC	0/65 (31) NC
	POLYP, ENDOMETRIAL STROMAL, BENIGN	8/65 (42) 0.8070	7/51 (34) 0.5459	8/58 (39) 0.5443	4/65 (33) 0.8713

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=65) P-value - Trend	0.5 mg/kg/day Low (N=65) P-value - Vehicle vs. Low	1 mg/kg/day Mid (N=65) P-value - Vehicle vs. Med	3 mg/kg/day High (N=65) P-value - Vehicle vs. High
	POLYP, GLANDULAR, BENIGN	7/65 (42) 0.5026	3/51 (33) 0.9053	12/58 (40) 0.1212	4/65 (32) 0.7947
	STROMAL SARCOMA, ENDOMETRIAL, MALIGNANT	1/65 (41) 1.0000	0/51 (32) 1.0000	0/58 (37) 1.0000	0/65 (31) 1.0000
VAGINA	POLYP, VAGINAL, BENIGN	0/64 (40) 0.4783	0/37 (20) NC	1/46 (25) 0.3846	0/63 (30) NC

Figure 1: Kaplan-Meier Survival Functions for Male Rats

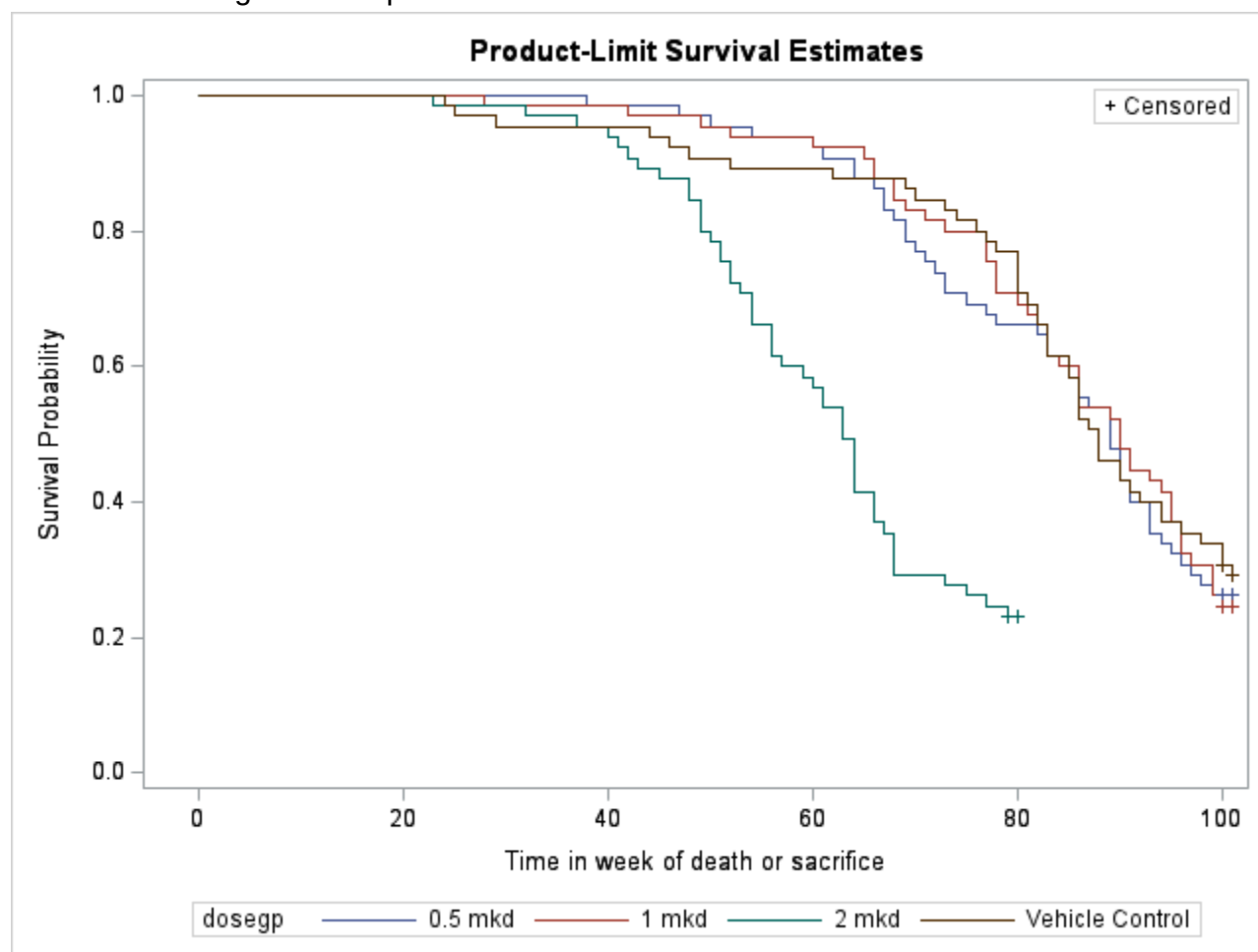
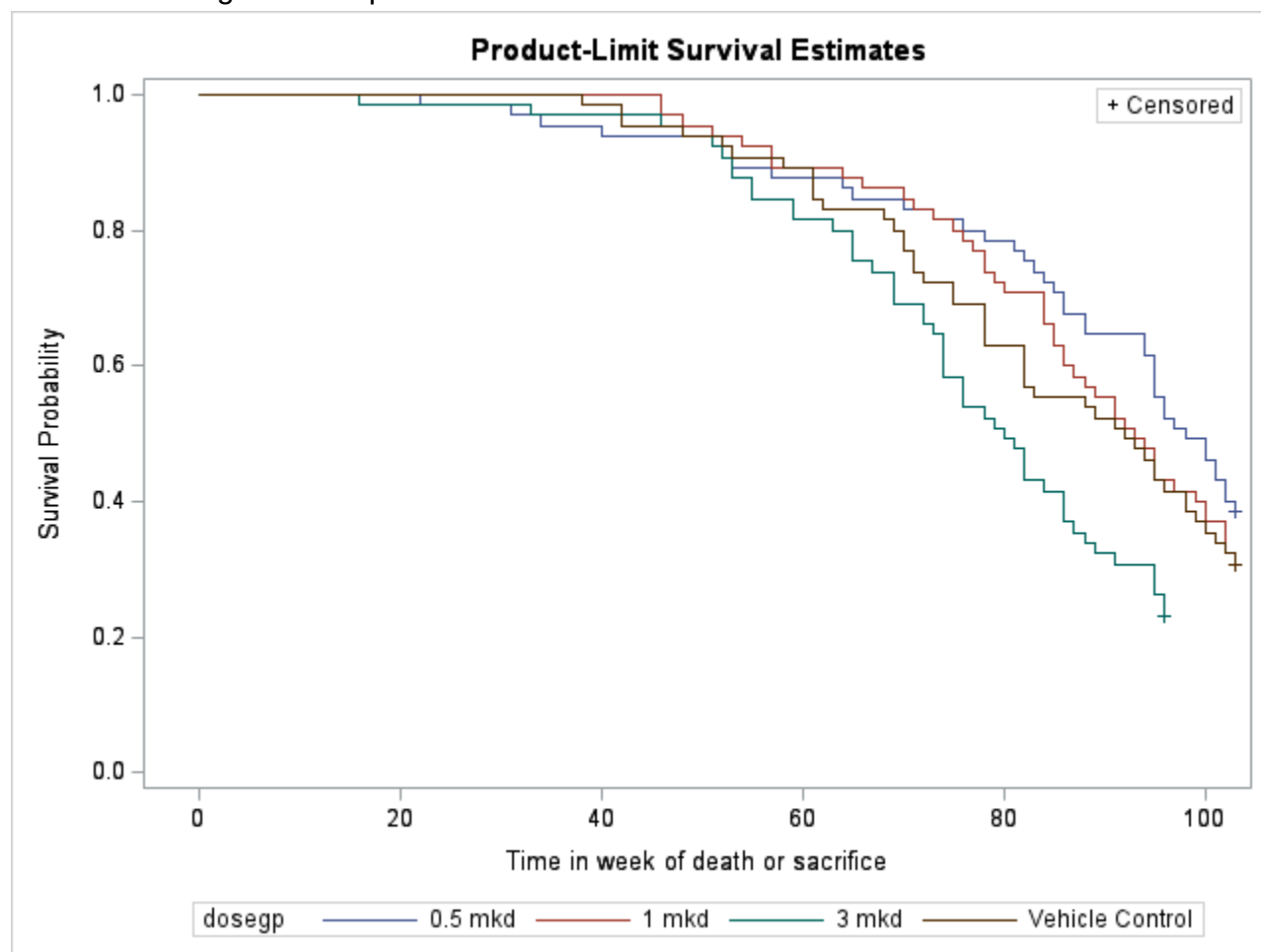


Figure 2: Kaplan-Meier Survival Functions for Female Rats



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MOHAMMAD A RAHMAN
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