Evaluation of Cytochrome P450 2C9, 2C19, and 2D6 Inhibition on the Pharmacokinetics of Aficamten in Healthy Participants

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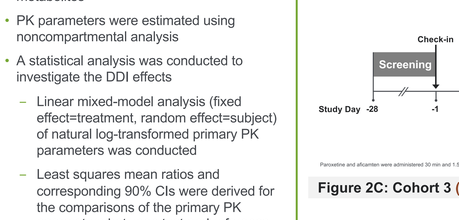
INTRODUCTION

- Aficamten is a next-in-class small molecule, selective cardiac myosin inhibitor in development for treating hypertrophic cardiomyopathy
- Aficamten stabilizes myosin in the released post-power stroke state, unable to hydrolyze adenosine triphosphate (Figure 1)
- In vitro phenotyping studies indicate contribution of cytochrome P450 (CYP) 2D6, 3A4, 2C9 and 2C19 in metabolism of aficamten
- Previous clinical studies indicated a small contribution of CYP3A (fraction metabolized [fm]=26%) and lack of meaningful effect of CYP2D6 (poor- versus normal-metabolizer analysis) to aficamten metabolism[1, 2]
- Carbamazepine (strong CYP inducer) decreased aficamten exposure by 51%, indicating a contribution from CYPs other than CYPs 2D6 and 3A[1]

Figure 1: Aficamten Mechanism of Action



AFI=aficamten; ADP=adenosine diphosphate.

This study aimed to:

1. Evaluate the "worst-case" scenario of multiple CYP-mediated inhibition on the metabolism of aficamten
2. Elucidate the contributions of CYP2C9 & 2C19
3. Definitively determine the level of CYP2D6 involvement

OBJECTIVES

Primary objectives

To evaluate the effect of

Fluconazole (strong CYP2C19 inhibitor & moderate CYP2C9 & CYP3A inhibitor)

Paroxetine (strong CYP2D6 inhibitor)

Fluoxetine (strong CYP2C19 & CYP2D6 inhibitor)

on the pharmacokinetics (PK) of aficamten

Secondary objectives

To evaluate the safety and tolerability of aficamten administered alone, and in combination with fluconazole, paroxetine, and fluoxetine

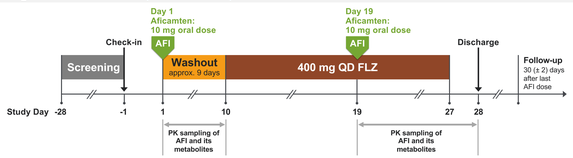
METHODS

Study Design

- This was an open label, fixed sequence, drug-drug interaction (DDI) study in healthy participants
- Plasma samples of aficamten and its pharmacologically inactive metabolites (CK-3834282 and CK-3834283) were collected predose and for 216 hours postdose
- A validated liquid chromatography-tandem mass spectroscopy method was used to analyze concentrations of aficamten and its metabolites[2]
- PK parameters were estimated using noncompartmental analysis
- A statistical analysis was conducted to investigate the DDI effects
  - Linear mixed-model analysis (fixed effect=treatment, random effect=subject) of natural log-transformed primary PK parameters was conducted
  - Least squares mean ratios and corresponding 90% CIs were derived for the comparisons of the primary PK parameters between test and reference treatments in each cohort
  - A Wilcoxon signed rank test of half-life ($t_{1/2}$) was performed, and the p-value was presented for the differences in $t_{1/2}$ between test and reference treatments
- Safety and tolerability were monitored throughout the study

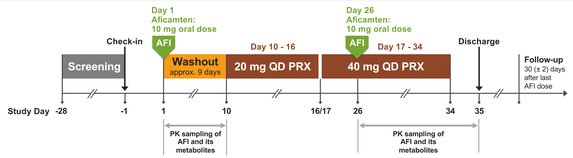
Treatment Cohorts

Figure 2A: Cohort 1 (Fluconazole)



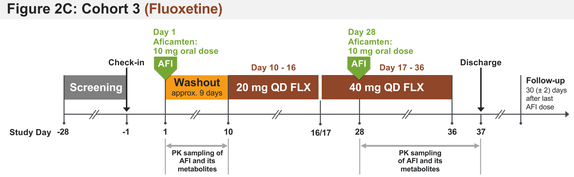
Fluconazole and aficamten were administered 30 min and 1.5 h post standardized breakfast, respectively. On Day 19, aficamten was dosed 1 hour after fluconazole dosing.

Figure 2B: Cohort 2 (Paroxetine)



Paroxetine and aficamten were administered 30 min and 1.5 h post standardized breakfast, respectively. On Day 26, aficamten was dosed 1 hour after paroxetine dosing.

Figure 2C: Cohort 3 (Fluoxetine)



Fluoxetine and aficamten were administered 30 min and 1.5 h post standardized breakfast, respectively. On Day 28, aficamten was dosed 1 hour after fluoxetine dosing.
AFI=aficamten; Approx.=Approximate; FLZ=Fluconazole; FLX=Fluoxetine; PRX=Paroxetine; QD=once a day.

RESULTS

Demographics

- 17 subjects were enrolled in each cohort
  - All 17 subjects in Cohort 2, and 16 subjects in cohorts 1 and 3 completed the study

Table 1: Summary of baseline demographics

Demographics	Cohort 1 (N=17)	Cohort 2 (N=17)	Cohort 3 (N=17)
Sex (male/female), n/n	14/3	14/3	12/5
Age, mean (SD), years	32.4 (6.22)	35.9 (7.83)	33.4 (5.95)
BMI, mean (SD), kg/m²	25.1 (3.22)	24.7 (2.75)	25.3 (2.58)
Race, n (%)			
Asian	0 (0)	2 (11.8)	0 (0)
Black or African American	5 (29.4)	4 (23.5)	7 (41.2)
Black or African American, American Indian or Alaska Native	1 (5.9)	0 (0)	0 (0)
White	9 (52.9)	11 (64.7)	9 (52.9)
White, Black or African American	2 (11.8)	0 (0)	1 (5.9)
Ethnicity, n (%)			
Hispanic or Latino	5 (29.4)	2 (11.8)	6 (35.3)

BMI, body mass index.; SD, Standard Deviation.
1 participant each in Cohorts 1 and 3 terminated early from the study due to personal reasons.

Safety

- There were no deaths, serious adverse events, or discontinuations due to adverse events (AEs) across the 3 cohorts
- No safety concerns were identified from the evaluation of clinical laboratory reports, vital signs, electrocardiograms, or physical examinations in any cohort
- All treatment-emergent AEs reported for cohort 1 (n=6), cohort 2 (n=10) and cohort 3 (n=11) were of mild severity and considered unrelated to aficamten, except 1 event (fatigue) related to aficamten in cohort 3
- All AEs across the 3 cohorts were resolved by the end of the study

Pharmacokinetics

Figure 3. Aficamten mean (SD) concentration-time profile alone and with fluconazole

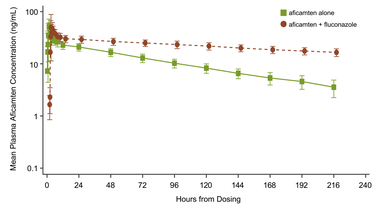


Table 2: Statistical PK comparisons for aficamten administered alone and with fluconazole

Analyte	PK parameter[a] (N=16[b])	Reference	Test	Test vs Reference
		10 mg AFI	**10 mg AFI + 400 mg FLZ QD**	**GLSM Ratio (%) (90% CI)**
Aficamten	$AUC_{0\text{-}t}$ (ng·h/mL)	2440 (15.6)	4980 (14.3)	204 (193, 217)
	$AUC_{0\text{-}inf}$ (ng·h/mL)	2860 (19.0)	10,900 (22.1)	378 (347, 411)
	C_{max} (ng/mL)	64.4 (30.0)	66.4 (44.4)	98.9 (86.6, 113)
	t_{max} (h)	1.53 (1.01, 2.00)	1.50 (0.99, 3.01)	–
	$t_{1/2}$ (h)	76.7 (65.8, 90.4)	226 (210, 273)	<0.0001[c]
CK-3834282	MR $AUC_{0\text{-}inf}$	0.71 (40.6)	0.17 (33.6)	24.4 (21.4, 27.8)
CK-3834283	MR $AUC_{0\text{-}inf}$	1.31 (30.8)	0.40 (25.0)	30.6 (27.8, 33.7)

[a]Arithmetic mean (%CV) presented; t_{max} and $t_{1/2}$ are presented as median (Q1, Q3). [b]One subject discontinued due to personal reasons and was excluded from PK analysis. [c]p-value presented for $t_{1/2}$. AFI, aficamten; $AUC_{0\text{-}t}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}t}$, area under the concentration-time curve, from time 0 to the last observed non-zero concentration; CV, coefficient of variation; FLZ, fluconazole; GLSM, geometric least squares means; LSM, least squares means; MR, metabolite to parent molar ratio; PK, pharmacokinetic; Q, quartile; QD, once daily; $t_{1/2}$, half-life; t_{max}, time to reach C_{max}.

- Concomitant administration of aficamten with fluconazole (strong CYP2C19 inhibitor and moderate CYP3A and CYP2C9 inhibitor) increased the $AUC_{0\text{-}inf}$ of aficamten by 278% with no change in C_{max}

Figure 4. Aficamten mean (SD) concentration-time profile alone and with paroxetine

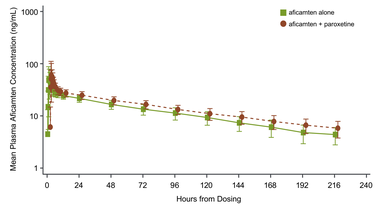


Table 3: Statistical PK comparisons for aficamten administered alone and with paroxetine

Analyte	PK parameter[a] (N=17)	Reference	Test	Test vs Reference[b]
		10 mg AFI	**10 mg AFI + 40 mg PRX QD**	**GLSM Ratio (%) (90% CI)**
Aficamten	$AUC_{0\text{-}t}$ (ng·h/mL)	2560 (21.0)	3090 (18.6)	121 (115, 127)
	$AUC_{0\text{-}inf}$ (ng·h/mL)	3100 (26.0)	3910 (24.9)	127 (119, 135)
	C_{max} (ng/mL)	65.3 (47.3)	79.5 (58.9)	120 (103, 141)
	t_{max} (h)	1.55 (1.00, 1.98)	1.00 (0.75, 2.02)	–
	$t_{1/2}$ (h)	86.1 (66.9, 94.8)	86.4 (81.5, 113)	0.0001[c]
CK-3834282	MR $AUC_{0\text{-}inf}$	0.85 (38.4)	0.70 (30.1)	85.0 (80.5, 89.8)
CK-3834283	MR $AUC_{0\text{-}inf}$	1.52 (44.1)	1.05 (22.4)	71.4 (66.0, 77.2)

[a]Arithmetic mean (%CV) presented; t_{max} and $t_{1/2}$ are presented as median (Q1, Q3). [b]p-value presented for $t_{1/2}$. AFI, aficamten; $AUC_{0\text{-}t}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}t}$, area under the concentration-time curve from time 0 to the last observed non-zero concentration; CV, coefficient of variation; GLSM, geometric least squares means; LSM, least squares means; MR, metabolite to parent molar ratio; PK, pharmacokinetic; PRX, paroxetine; Q, quartile; QD, once daily; $t_{1/2}$, half-life; t_{max}, time to reach C_{max}.

- Concomitant administration of aficamten with paroxetine (strong CYP2D6 inhibitor) increased the $AUC_{0\text{-}inf}$ and C_{max} of aficamten by 27% and 20%, respectively

Figure 5. Aficamten mean (SD) concentration-time profile alone and with fluoxetine

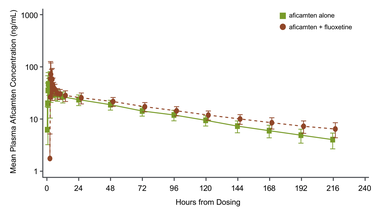


Table 4: Statistical PK comparisons for aficamten administered alone and with fluoxetine

Analyte	PK parameter[a] (N=16[b])	Reference	Test	Test vs Reference[c]
		10 mg AFI	**10 mg AFI + 40 mg FLX QD**	**GLSM Ratio (%) (90% CI)**
Aficamten	$AUC_{0\text{-}t}$ (ng·h/mL)	2750 (20.0)	3310 (18.7)	121 (116, 125)
	$AUC_{0\text{-}inf}$ (ng·h/mL)	3220 (21.5)	4260 (22.8)	132 (125, 140)
	C_{max} (ng/mL)	69.4 (58.5)	104 (55.0)	155 (128, 188)
	t_{max} (h)	1.53 (0.99, 2.77)	1.03 (0.88, 1.53)	–
	$t_{1/2}$ (h)	74.5 (65.0, 91.2)	95.4 (85.9, 104)	<0.0001[d]
CK-3834282	MR $AUC_{0\text{-}inf}$	0.73 (21.2)	0.60 (17.6)	81.8 (76.8, 87.1)
CK-3834283	MR $AUC_{0\text{-}inf}$	1.20 (13.7)	1.01 (17.9)	83.7 (78.9, 88.7)

[a]Arithmetic mean (%CV) statistics presented; t_{max} and $t_{1/2}$ are presented as median (Q1, Q3). [b]One subject discontinued due to personal reasons and was excluded from PK analysis. [c]p-value presented for $t_{1/2}$. AFI, aficamten; $AUC_{0\text{-}t}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}t}$, area under the concentration-time curve, from time 0 to the last observed non-zero concentration; CV, coefficient of variation; FLX, fluoxetine; GLSM, geometric least squares means; LSM, least squares means; MR, metabolite to parent molar ratio; PK, pharmacokinetic; Q, quartile; QD, once daily; $t_{1/2}$, half-life; t_{max}, time to reach C_{max}.

- Concomitant administration of aficamten with fluoxetine (strong CYP2D6 and CYP2C19 inhibitor) increased the $AUC_{0\text{-}inf}$ and C_{max} of aficamten by 32% and 55%, respectively. The fluoxetine-mediated increase in aficamten exposure was comparable to paroxetine, suggesting only a minor contribution of CYP2C19

Table 5: Estimation of the fraction of aficamten metabolized by P450s

CYP enzyme	fm (%)[a]
CYP2C9	50
CYP3A	26
CYP2D6	21
CYP2C19	3

[a]Estimates of the fraction metabolized were refined using physiologically-based pharmacokinetic modeling.
CYP, cytochrome P450; fm, fraction metabolized.

- Across all cohorts, CK-3834282 and CK-3834283 metabolite ratios (MR $AUC_{0\text{-}inf}$) decreased upon coadministration of aficamten with a CYP inhibitor
- These results confirmed that all evaluated perpetrator drugs acted by altering the systemic CYP-mediated metabolism of aficamten
- The estimated contribution of P450s to the metabolism of aficamten are shown in **Table 5**

CONCLUSIONS

- Aficamten is metabolized via multiple CYP enzymes (CYP2C9 [fm=50%], CYP3A [fm=26%; historical data], CYP2D6 [fm=21%], and CYP2C19 [fm=3%]) rendering it susceptible to only a limited number of uncommonly encountered drug interactions.
- Only weak DDIs (1.5- to < 2-fold) are likely from strong inhibition of any one pathway and only moderate (2- to < 5-fold) impact on aficamten exposure is expected with strong multi-pathway inhibitors (e.g. high-dose fluconazole, voriconazole and fluvoxamine) or inducers (e.g. rifampin, carbamazepine, phenytoin).

References:
1. D. Xu, et al. *American Society for Clinical Pharmacology* (ASCPT); 2024;PII-III.
2. Malik, F. et al. *JACC Basic Transl Sci.* 2022;7:763–75.

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