Cytokinetics

Discovery of *Aficamten (CK-274)*: Next-in-class Cardiac myosin inhibitor (CMI) for obstructive Hypertrophic Cardiomyopathy (oHCM)

Drug Discovery Nexus San Diego 2024

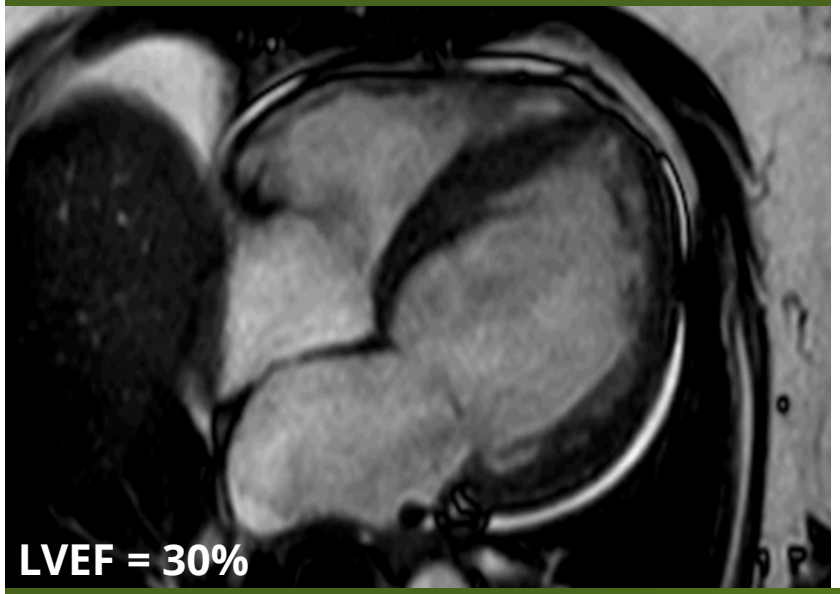
May 8th, 2024

Grace Chuang

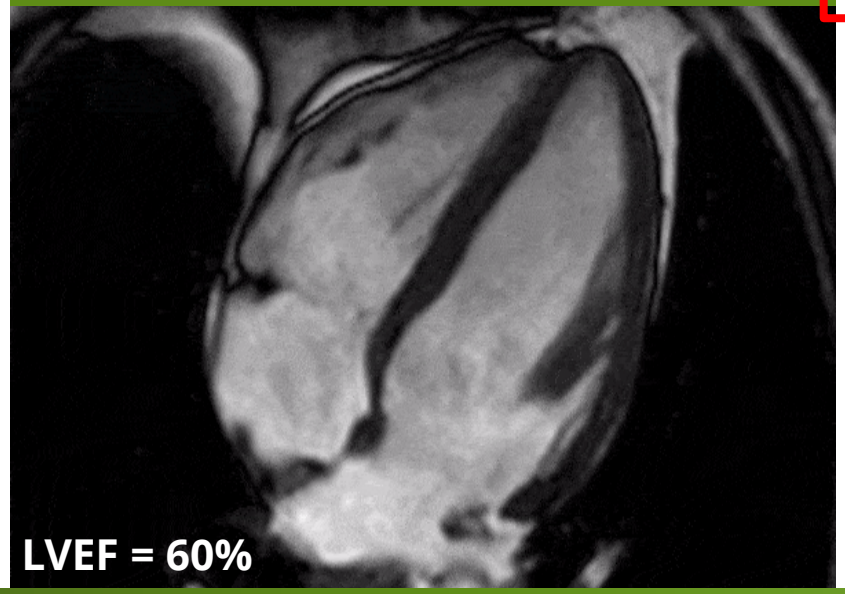
Diseases Associated with Reduced and Increased Cardiac Contractility

Hypothesis: Normalization of Contractility May Treat the Root Cause of the Disease

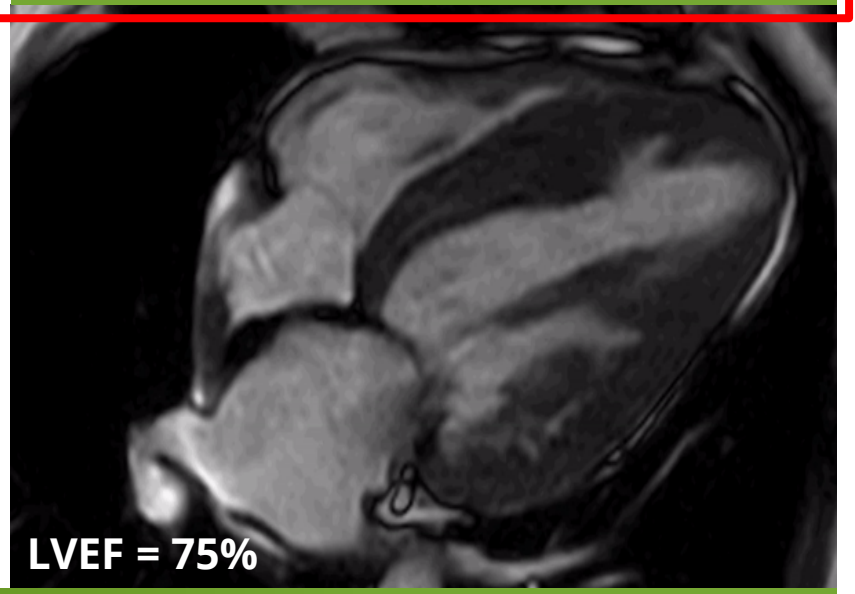
HFrEF (Low Contractility)



Normal Contractility



HCM & HFpEF (subset) (Hypercontractility)

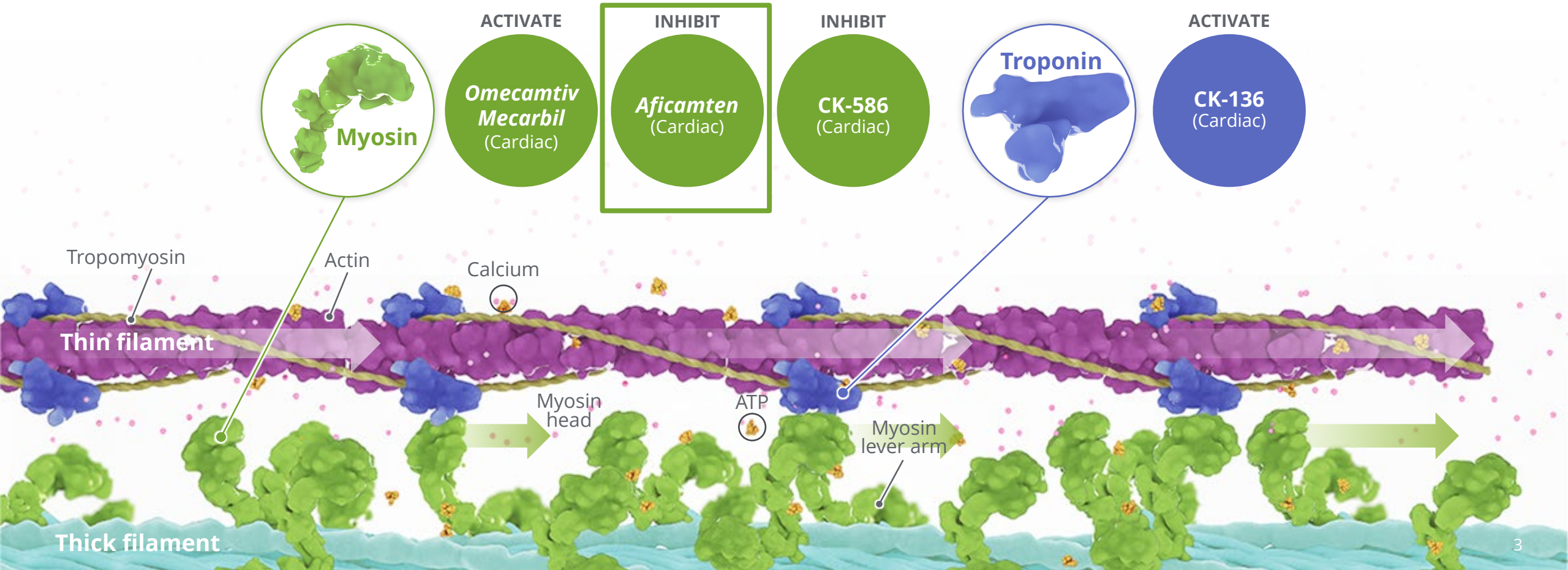


HFrEF = Heart Failure with Reduced Ejection Fraction
HFpEF = Heart Failure with Preserved Ejection Fraction
HCM = Hypertrophic Cardiomyopathy

Sarcomere Directed Drug Development

The sarcomere is a molecular structure found in skeletal and cardiac muscle that enables myocytes to contract and generate force

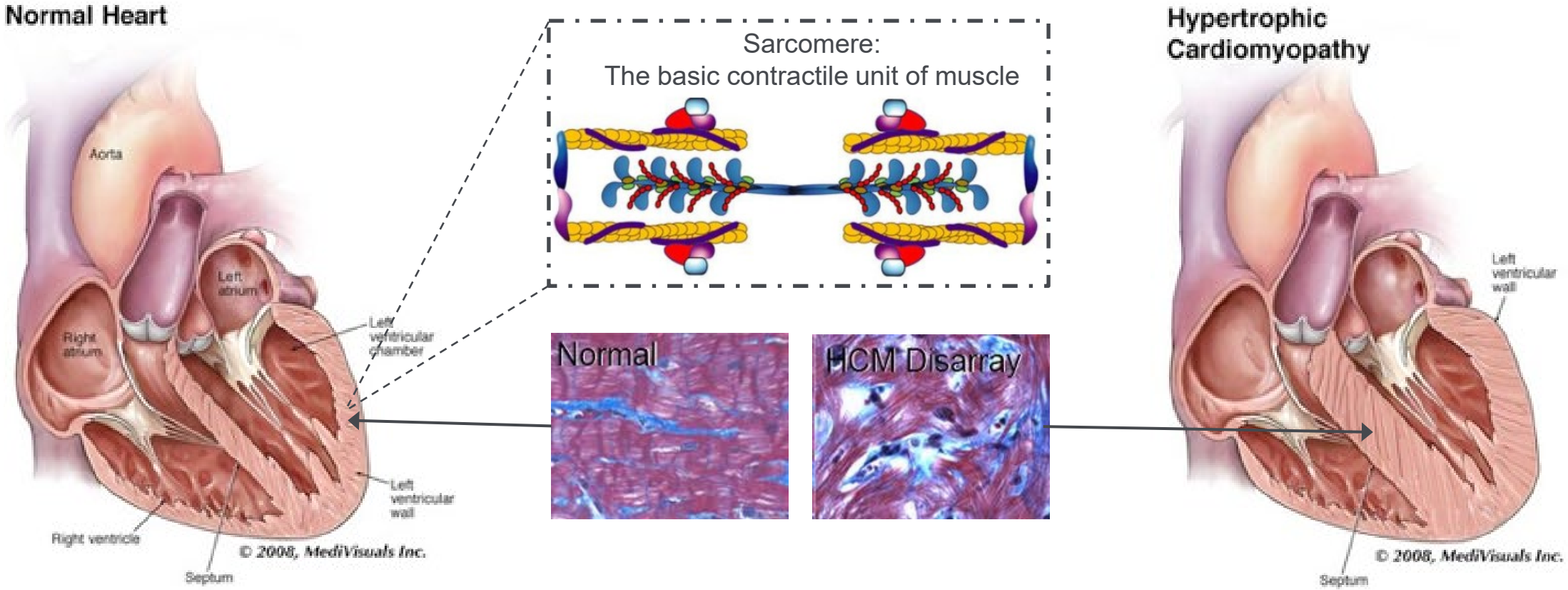
Cardiac Sarcomere Inhibition Hypothesis: Direct inhibition of cardiac contractility may be a viable approach to treat patients with HCM by restoring the proper degree of contractility within the sarcomere



Hypertrophic Cardiomyopathy (HCM)

A disease of sarcomere proteins

- Mutations in the sarcomere can cause hypercontraction, leading to abnormal growth (pathological hypertrophy) of the heart



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Significant Unmet Need in Hypertrophic Cardiomyopathies (HCM)

Current therapies do not target underlying disease

HCM is an inherited cardiovascular disease

- 1 in 500 have genetic mutation
- 1 in 3200 have HCM
- Subset of patients have progressive symptoms, atrial fibrillation, stroke, sudden death

Surgical intervention not permanent solution

- Invasive therapy to reduce septal thickness is effective to provide immediate symptom relief
- Surgical myectomy
  - Cutting away the thickened heart muscle
- Alcohol septal ablation
  - Alcohol induced muscle cell death

Current medical therapy does not target underlying disease

- Indirect mechanisms of action with systemic side effects
- Variable efficacy, often inadequate

Assay Systems for Muscle Contractility Lead Optimization

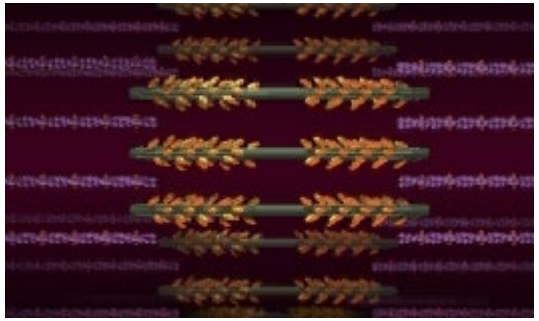
Muscle Contractility

Complexity

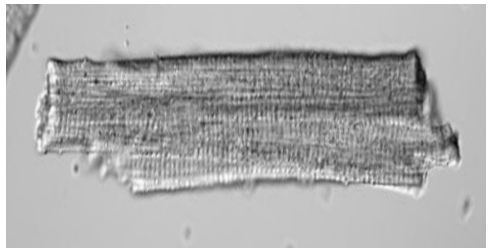
Throughput

Coherence

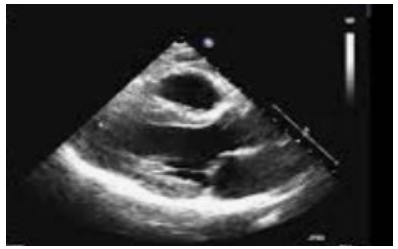
Myofibril suspension
Intact Sarcomere

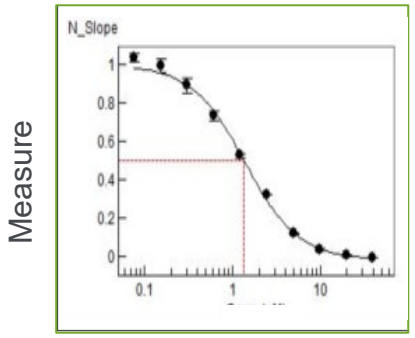


Cardiac Myocyte
Assay in Native Context



Organ and *in vivo*
Functional Outcome





Compound Conc.

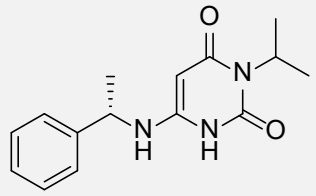
- Potency measures
  - Myofibrils: ATPase inhibition = IC_{50}
  - Cardiac Myocyte: %FS @ conc.
  - Cardiac Myocyte Ca^{2+} transient
  - *In vivo*: Rat echocardiogram = window assessment IC_{50}/IC_{10}*

*IC_{50}/ IC_{10}= conc for 50% reduction in fractional shortening (FS) / conc for 10% reduction in fractional shortening (FS)

A Next-in-Class Cardiac Sarcomere Inhibitor (CSI)

Finding a next-in-class compound to expand the utility of this MOA

First-in-Class CSI inhibitor, *mavacamten*, has a long human $t_{1/2}$



Mavacamten

- Demonstrated clinical benefit in oHCM patients
- Observe CYP3A4 induction (human hepatocytes)[1]
- Exhibits $t_{1/2}$ of 7-9 days in humans[2,3]
  - Reach steady-state around 6 weeks

Origin of Initial Hit

CK compound libraries: 1-7

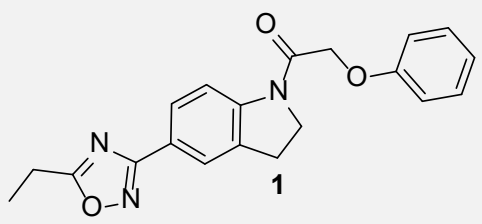
Primary Optimization Objectives

- ✓ Predicted human $t_{1/2}$ for *qd* dosing and reach steady state within 2 weeks
- ✓ No substantial CYP induction or inhibition (human hepatocytes / microsomes)
- ✓ Large therapeutic index *in vivo* with clear PK/PD relationship
  - ✓ Echocardiography to assess the relationship of conc. vs fractional shortening (FS)
  - ✓ Shallow dose response curve *in vivo* with larger PD window (IC_{50}/IC_{10})[4]

CK compound libraries: 8 and beyond

1. MP Grillo, *et.al. Xenobiotica,* 2019; *49*, 718-733; . 2. Myokardia S-1 SEC filling September 28, 2015. 3. SB Heitner, *et.al*. *Ann. Intern. Med.* 2019, Jun4; *170 (11*), 741-748.
4. IC_{50}/ IC_{10}= conc. 50% reduction in fractional shortening (FS) / conc. 10% reduction in fractional shortening (FS) measured in rat models

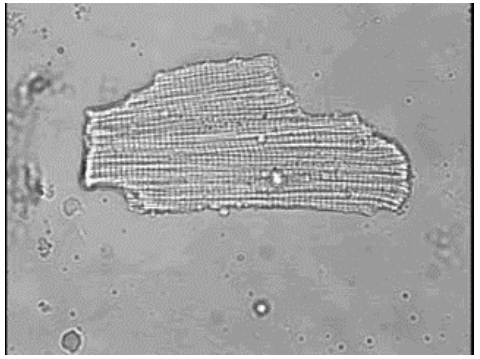
Screening Hit 1: Properties and Direct Inhibition of Cardiomyocyte Contractility

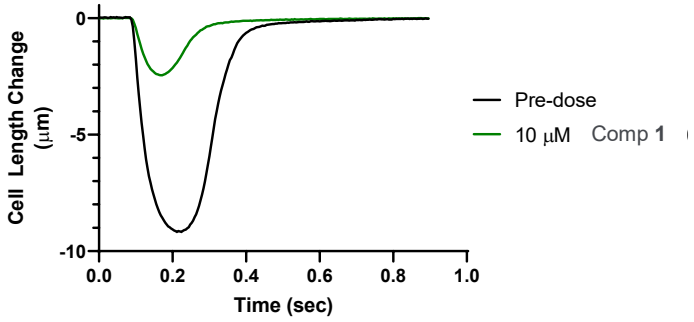


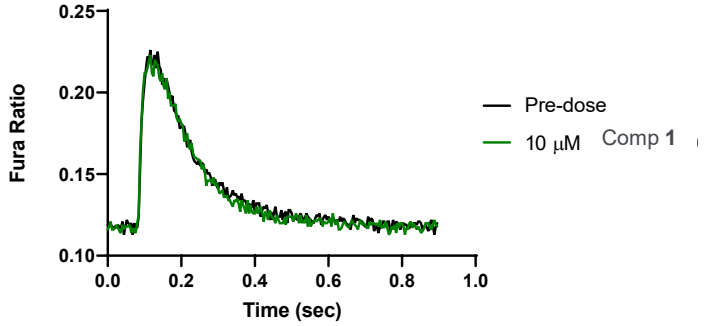
HTS hit	
CDMF[1] Ca75 IC_{50}	2.7 µM
SMM[2] IC_{50}	> 39 uM
LE[3]	0.28
cLogP[4]	3.5
ER[5] (r); ER (h)	>0.69; >0.76
MW	349

- Singleton hit
  - Reasonable potency and ligand efficiency
  - "Drug-Like" cLogP and MW
  - No SMM inhibition observed
- Metabolic hot spots to address
  - Phenyl rings and the glycolic linker
  - Indoline

Adult Rat Ventricular Cardiomyocyte





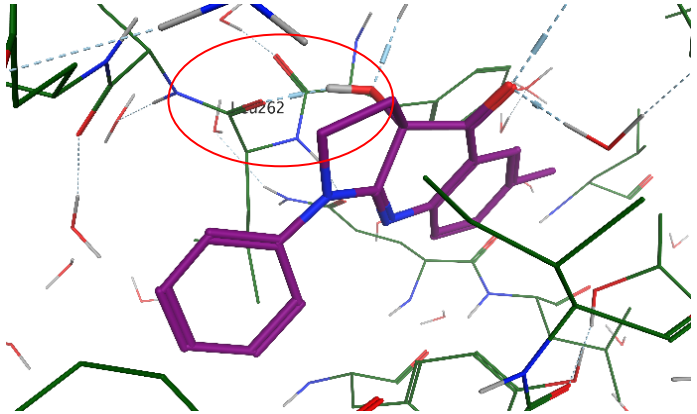


❖ Strong contraction reduction observed without affecting the Ca^{2+} concentration

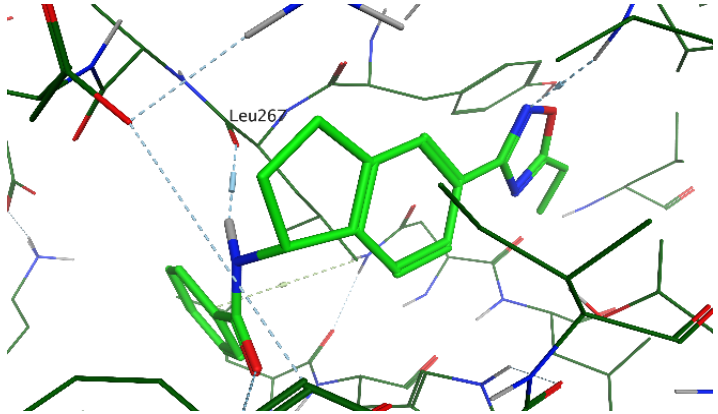
1. CDMF Ca75: cardiac myofibril ATPase assay with Ca concentration @ 75% of the maximum contraction
2. SMM: smooth muscle myosin
3. LE: ligand efficiency based on number of heavy atoms
4. cLogP: determined using ACD calculator
5. ER microsomal extraction ratio (ER = (Clint)/(Clint + hepatic blood flow), h=human r=rat

Breakthrough Modification to Incorporate H-Bond interaction

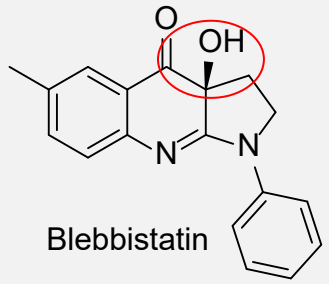
X-ray Co-crystal of Blebbistatin reveals a key H-bond interaction



Blebbistain with myosin II from dictyostelium

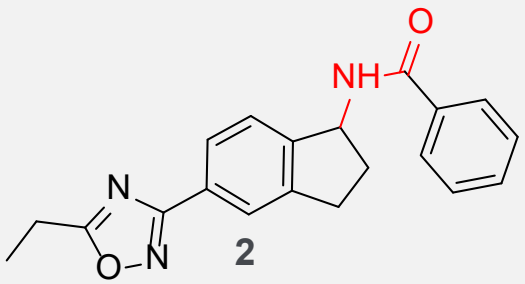


Comp **2** docked into a similar binding pocket with cardiac myosin



Blebbistatin

Blebbistain, a pan myosin inhibitor
- Inhibits myosin ATPase activity
- Binds in allosteric site of ATP domain
- Tool compound used as control



2

Reframe the Linker
- Improved cardiac potency (IC_{50} = 1.0 μM)
- Removed aniline moiety

H-Bond donor incorporation

- lead to ~3X potency improve
- opportunity to optimize drug-like properties quickly

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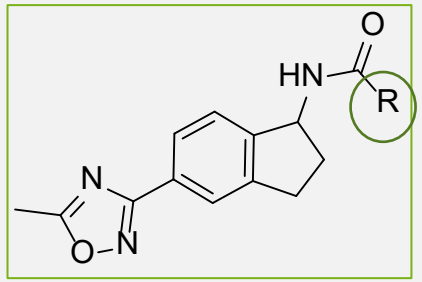
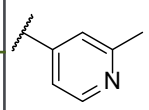
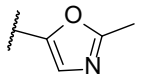
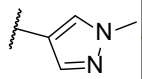
Subtle Differences in ADME Properties Linked to Stereochemistry

In vitro vs in vivo disconnect between enantiomers



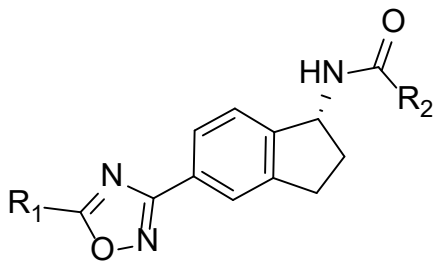
Cmpd	Chirality	R	CDMF IC_{50} (μM)	CDMF IC_{15} (μM)	CL_{int} (r)[1] (mL/min/kg)	CL_{int} (h)[1] (mL/min/kg)	CL (r)[2] (mL/min/kg)	CL_u (r)[3] (mL/min/kg)	cLog P[4]	PPB (r)[5] (% free)
3	*R*		3.5	1.0	< 21	<11	7.6	156.3	2.4	7.7
4	*S*		>39	>39	25.3	36.4	40.1	401	2.4	10
5	*R*		4.9	1.4	<21	<11	13.9	55.8	0.7	25
6	*S*		>39	>39	40.3	<11	83.3	391.8	0.7	21
7	*R*		4.4	1.2	<21	<11	2.1	32.3	1.5	6.5
8	*S*		>39	>39	<21	<11	57.7	395.2	1.5	15

1.Cl_{int} = Intrinsic clearance in rat (r) microsome, human (h) rat (r); 2. CL = *in vivo* rat clearance dosed at 0.5 mg/kg for **7** and **8** and dosed at 2.0 mg/kg for **3-6**; 3. CL_u = unbound *in vivo* clearance in rat (r) (*in vivo* rat clearance / free fraction in rats); 4. cLogP: determined using ACD calculator; 5. PPB = plasma protein binding, % unbound, rat (r).

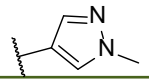
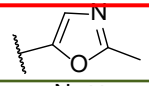
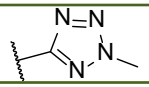
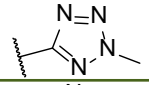
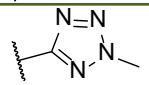
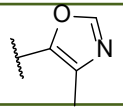
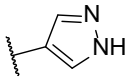
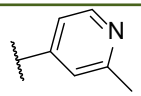
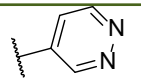
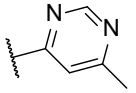
- Stereochemical preference
  - Preferred *R*-isomer confirmed through synthesis
  - No inhibition observed *with S*-isomer at high end of concentration (40 μM)
  - Eudysmic ratio is > 40x based in IC_{15}
  - Generally lower intrinsic CL observed in *in vitro rat* microsomal system with *R* isomer
  - *R* isomer demonstrated a much lower clearance in *in vivo* system

Fine Tuning Pharmacokinetic Properties and Potency

Pyrazole stands out with balance of in vitro and in vivo PK properties



- R_1 modification
  - Et, cPr, and iPr all within desired potency range
  - cPr lead to long $t_{1/2}$ observed in **21**
- R_2 modification
  - Tolerance for wide range of 5-and 6-membered heterocycles
  - Various Cl, $t_{1/2}$, and %F observed
  - Cmpd **11** has the best overall balance in PK profile

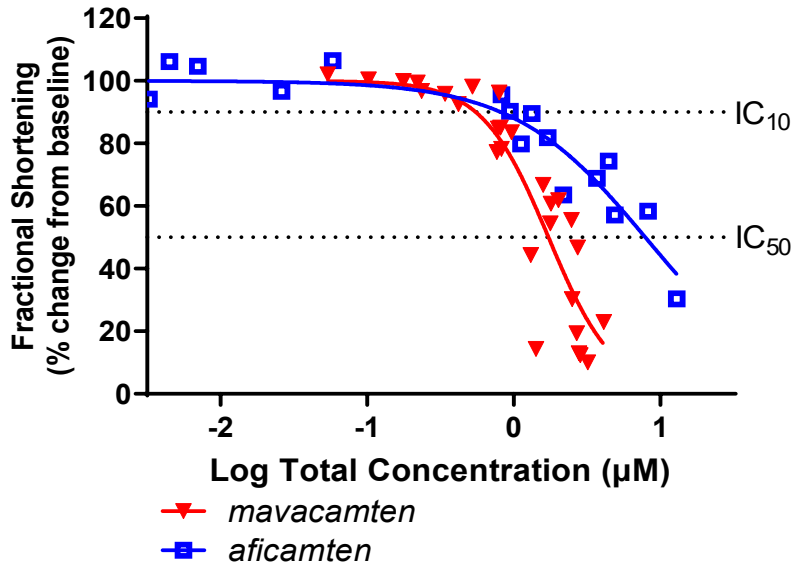
Cmpd	R_1	R_2	CDMF IC_{50} (μM)	Rat, CL[1] (mL/min/kg)	Rat, $t_{1/2}$[2] (hour)	Rat %F[3]	CL_{int} (r)[4] (mL/min/kg)	CL_{int} (h)[4] (mL/min/kg)	PPB (r , h)[5] (% free)
11	Et		1.4	3.1	2.5	59	<21	<11	98 / 90
19	Et		1.7	21.4	4.1	13	<21	<11	95 / 82
20	Et		0.9	19.5	5.9	53	<21	<11	89 / 71
21	cPr		1.0	5.2	17.8	100	<21	<11	91 / 83
22	iPr		1.9	15.7	4.6	52	<21	<11	93 / 84
23	Et		0.8	39.7	14.7	0	>124	<11	98 / 90
24	Et		1.1	62.5	1.4	10	66	<11	80 / 80
25	Et		1.2	13.0	3.5	32	0.3	<11	98 / 92
26	Et		1.2	43.8	6.5	16	-	<11	- / 69
27	Et		1.8	20.2	5.9	26	24	<11	95 / 91

1. Rat Cl = *in vivo* rat clearance dosed at 1 mg/kg; Cl_{int} = Intrinsic clearance in rat (r); 2. Rat $t_{1/2}$ = *in vivo* half-life in rat iv experiment; 3. Rat F = *in vivo* bioavailability for compounds dosed iv and po at 1 mg/kg except compound 11 and 20, which was dosed po at 2 mg/kg, and compound22, which was dosed po at 6 mg/kg. 4. Cl_{int} = Intrinsic clearance in rat (r) microsome, h=human r=rat; 5. PPB = plasma protein binding, % unbound, rat (r), human (h).

Efficacy Demonstrated with *in vivo* Echocardiography Measurement

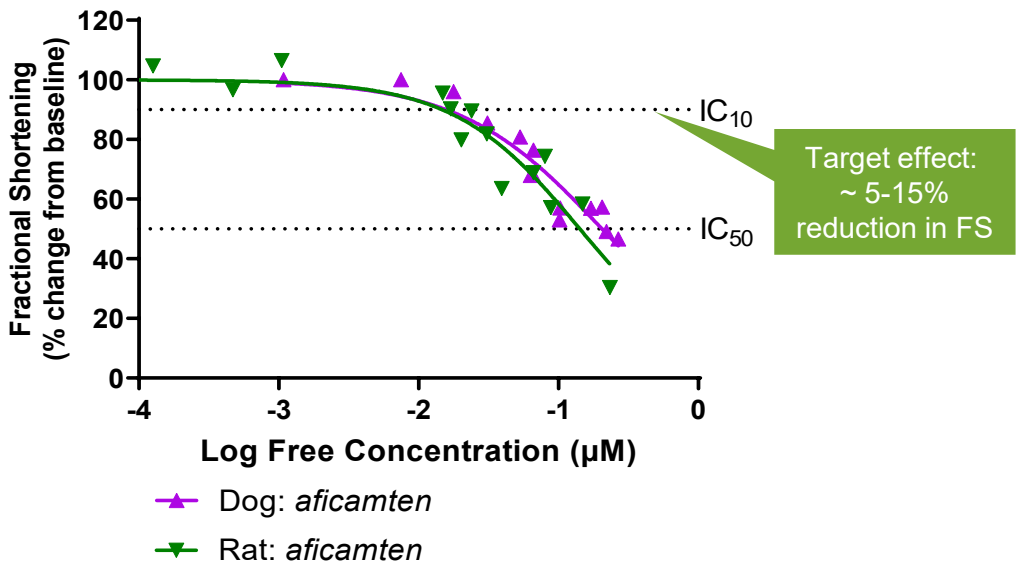
Wide PD Window in Healthy Rat and Dog Models with Aficamten

Rat Model: Conc. vs. FS



	IC_{10} (μM)$_{total}$ *	IC_{50} (μM) $_{total}$*	IC_{50}/IC_{10}*
mavacamten	0.6	1.7	2.8
aficamten	0.8	7.9	9.9

Rat vs Dog Model: Conc. vs. FS



	$IC_{10\text{-free}}$ (μM)	$IC_{50\text{-free}}$ (μM)	IC_{50}/IC_{10}*
Dog	0.0178	0.231	13.0
Rat	0.0144	0.142	9.9

* IC_{50}/ IC_{10}: Pooled concentration for 50%/10% reduction of FS (fractional shortening) in normal Sprague Dawley rat or Beagle Dog

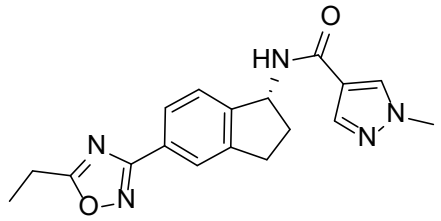
Shallow exposure-response relationship of *Aficamten* in rats and dogs

Aficamten Accomplished Optimization Objectives

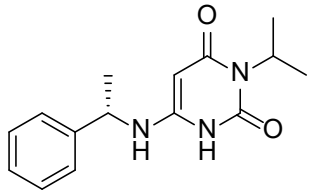
Objectives:

- ✓ Predicted human $t_{1/2}$ for *qd* dosing and reach steady state within 2 weeks
- ✓ No substantial CYP induction or inhibition (human hepatocytes / microsomes)
- ✓ Large therapeutic index across pre-clinical species with clear PK/PD relationship

	Assay	Parameters	
		Aficamten	***Mavacamten***
***in vitro* pharmacology**	Bovine Cardiac Myofibril Ca75 IC_{50} (μM)	1.26 (1.20-1.33)[1,2]	0.60 (0.54-0.67)[1]
***in vivo* pharmacology**	IC_{10} (μM)[3]	0.8	0.6
	IC_{50} / IC_{10}[3]	9.9	2.8
Human PK projection	Human $t_{1/2}$ projected (days) (Human $t_{1/2}$ actual)	2.8[8] (3.4)[7]	9[6] (7-9)[4,5]
CYP Profile	CYP450 IC_{50} (μM) (Time-dependent inhibition)	IC_{50} > 30[9] (None)	IC_{50} > 30[6,9]
	CYP450 Induction, EC_{50} (μM)[10]	No substantial induction up to 25 μM	2.2 ± 0.4 / CYP3A4[6] 5.1 ± 0.2 / CYP2B6[6]



Aficamten



Mavacamten

Aficamten showed no notable off-target effects with in vitro safety pharmacology screening

1. Cytokinetics data on file. Bovine cardiac myofibrils assayed at 75% of max Ca-dependent activation. Mean values (95% confidence intervals). 2. JJ Hartman, *et al.* Poster# 2912 presented at 64th Biophysical Society Annual Meeting, February 19th, 2020. 3. Compounds were dosed orally to Sprague Dawley rats and compound effect assessed by echocardiography. A reduction in a measure of cardiac function (fractional shortening [FS]) was quantitated alongside plasma concentrations to calculate the inhibitory concentration at a 10% (IC_{10}) and 50% (IC_{50}) reduction in FS, IC_{50}/ IC_{10} 4. Myokardia S-1 SEC filling September 28, 2015. 5. SB Heitner, *et.al. Ann. Intern. Med.* 2019, Jun4; *170 (11)*, 741-748. 6. MP Grillo, *et.al. Xenobiotica* 2019, *49,* 718-733. 7. LA Robertson, *et al.* Poster #210 presented at the 23rd HFSA Annual Scientific Meeting, September 13–16, 2019, Philadelphia, PA, USA. 8. P Cremin, *et al.* Poster # 887215 presented at AAPS annual meeting, Atlanta, Georgia, October 28-November 5, 2021. 9. Mavacamten include CYPs 1A2, 2B6, 2C9, 2C19, 2D6, 3A4 and 3A5; *aficamten* include 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 10. The potential to induce CYP1A2, CYP2B6 and CYP3A4 was tested *in vitro* in cryopreserved palatable human primary hepatocytes from three separate donors using mRNA level as the end point.

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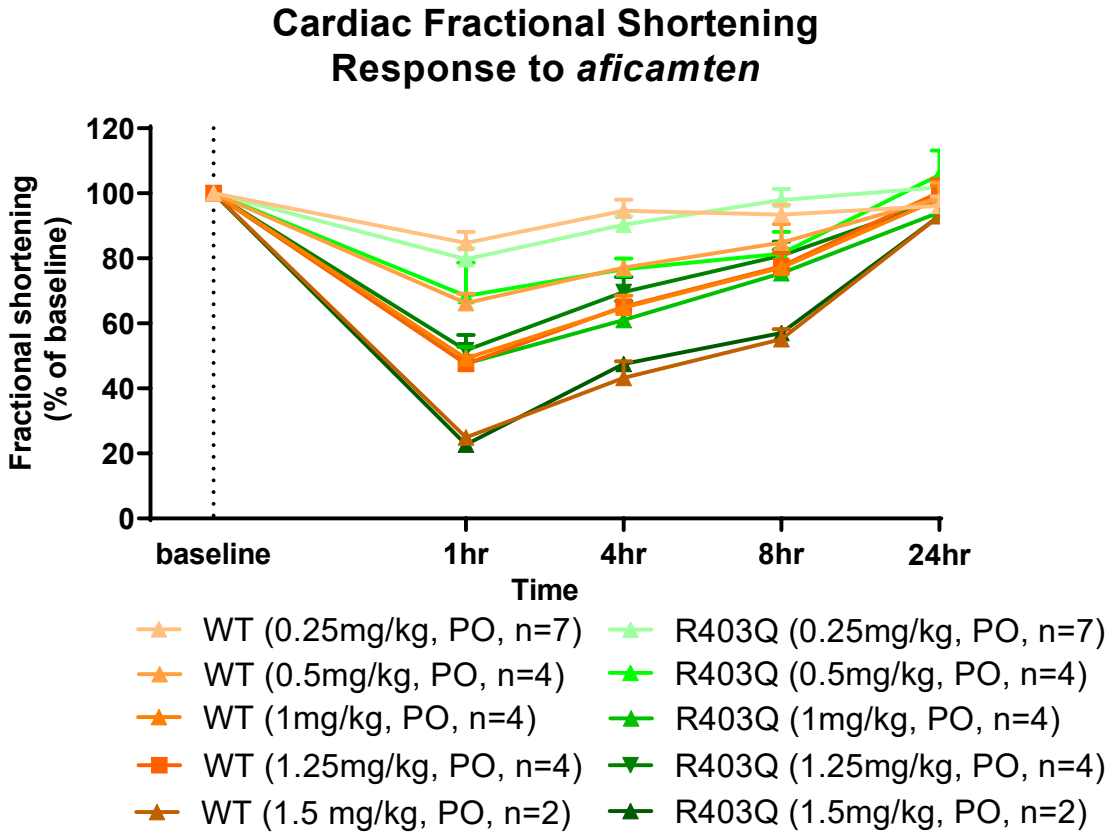
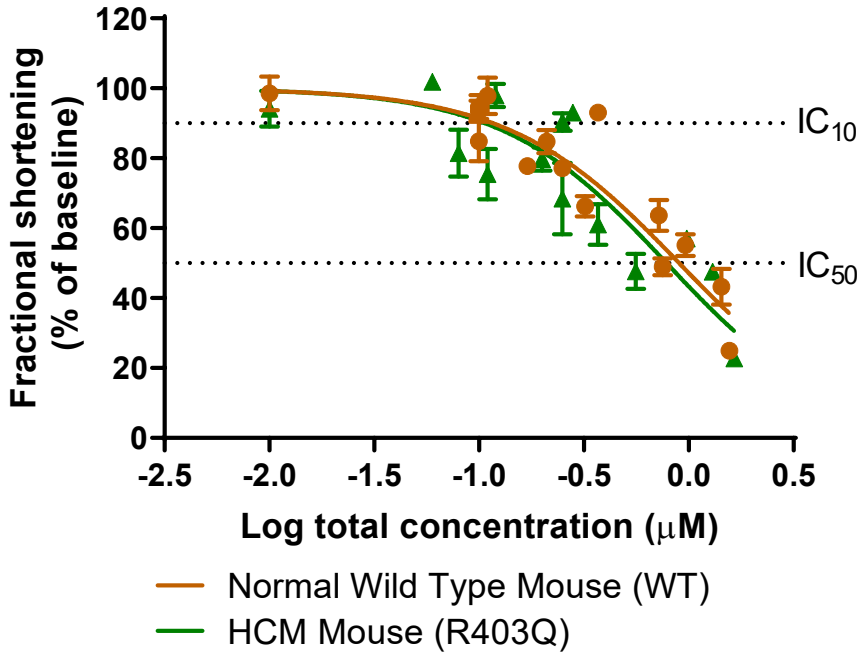
Aficamten Has a Similar PD Window in a Genetic HCM Mouse Model

Aficamten decreased fractional shortening in a dose-related fashion in WT and R403Q HCM mice

- *A dose of 0.25-1.5 mg/kg is sufficient to determine an IC_{50}/IC_{10} window*
- *The fractional shortening response to aficamten is the same in WT and R403Q mice*

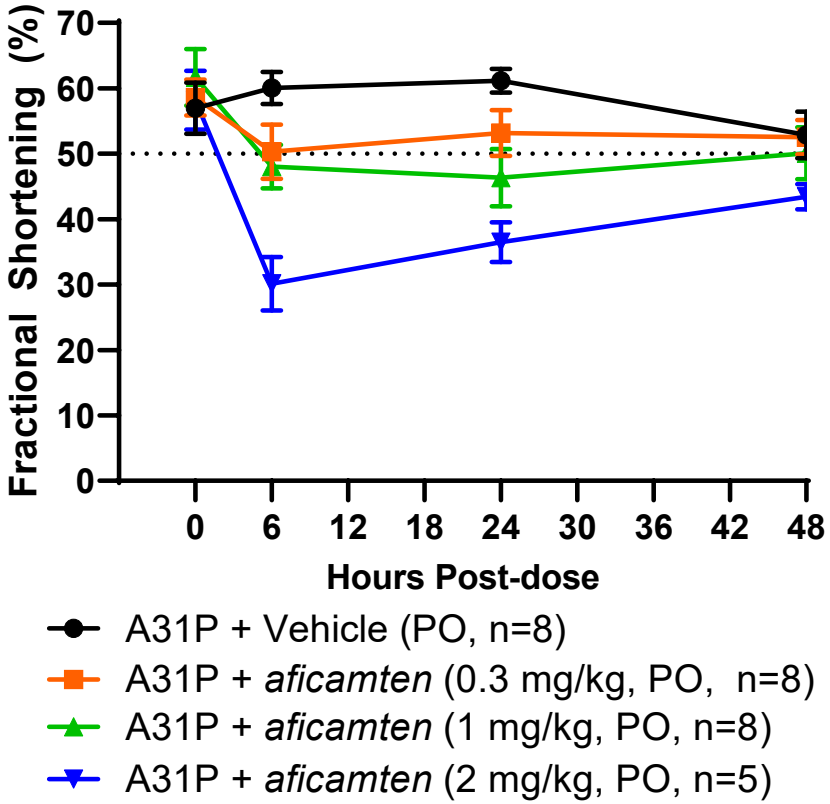


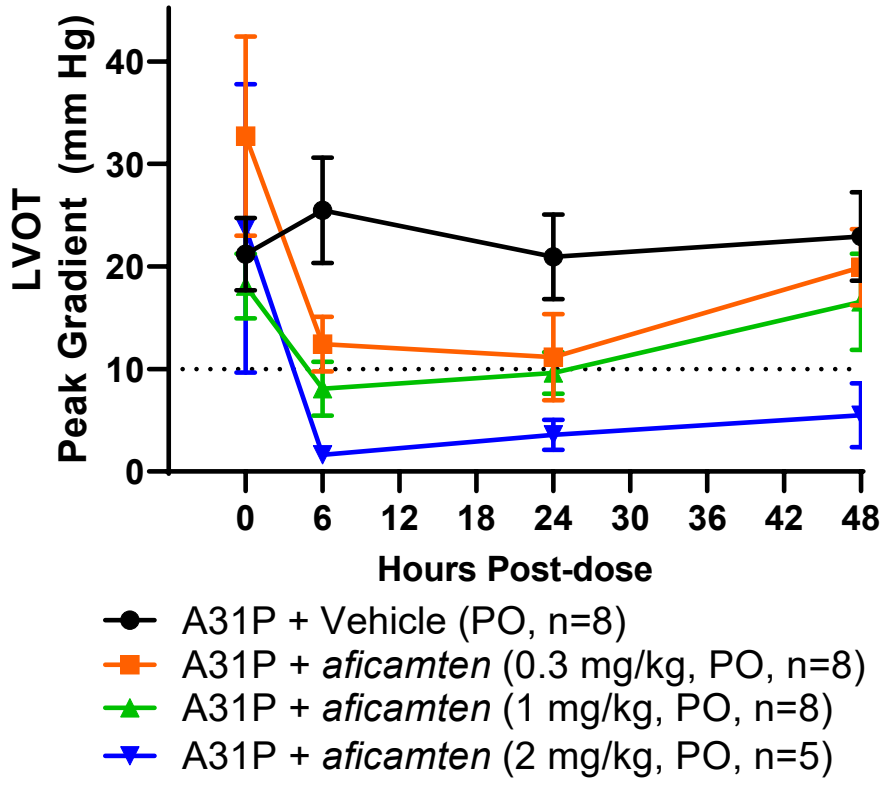


	IC_{10} (μM)	IC_{50} (μM)	IC_{50}/IC_{10}
WT	0.12	0.90	7.5
HCM (R403Q)	0.11	0.78	7.1

Aficamten Reduced FS* and LVOT* Obstruction in a Dose-dependent Manner in A31P HCM Maine Coon Cats[1]

- A31P HCM cats exhibit fractional shortening (> 50%) and LVOT peak gradient (>10 mm Hg) values that are above the normal range in cats





UC Davis / U Arizona

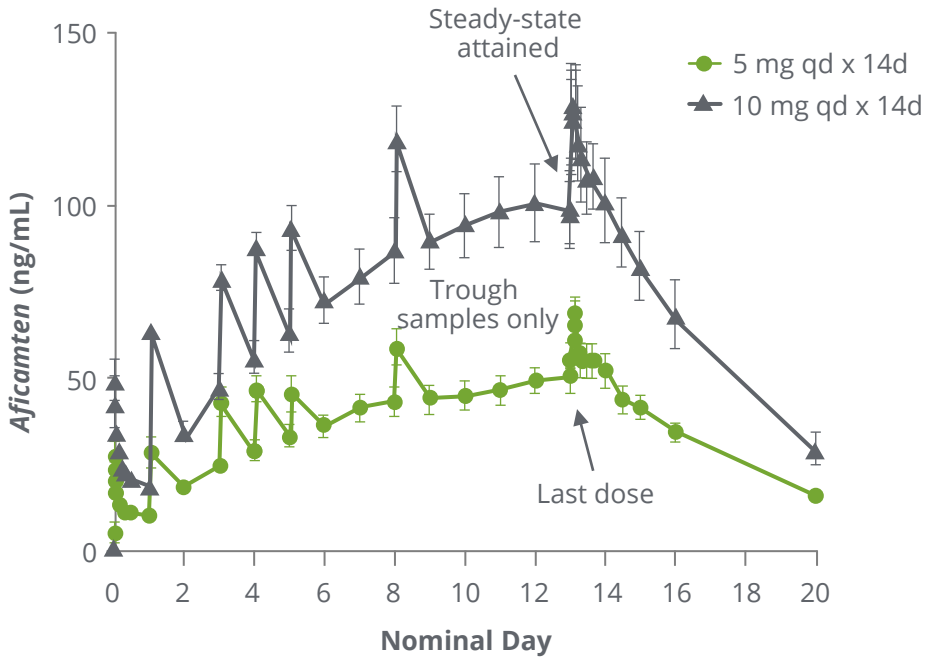
* FS= fractional shortening; LVOT left ventricular outflow tract

1. MS Oldach, et al. Poster presented at AHA Scientific Sessions 2020, Virtual Congress November 13-17th, 2020.

SAD & MAD Results Support Progression to Phase 2

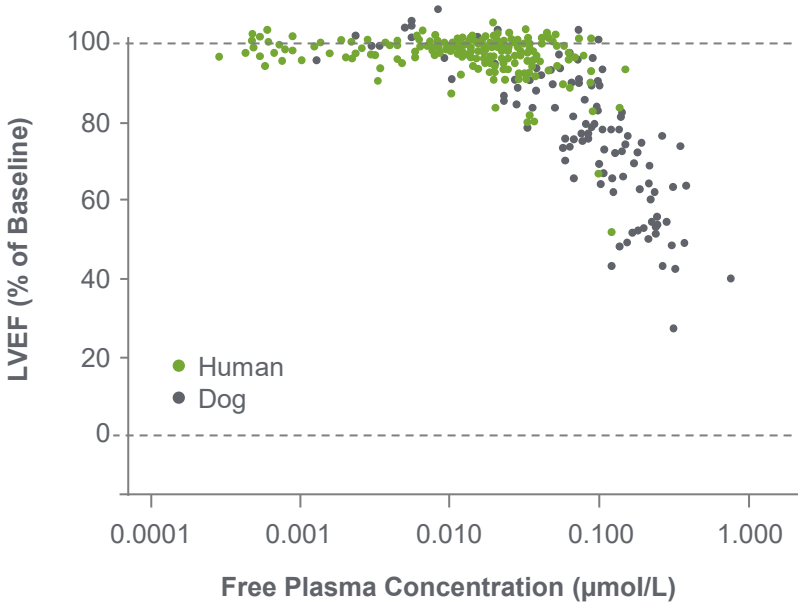
Preclinical data translated to healthy participants

MAD PK: Steady-State Achieved After 14 Days of Dosing



Shallow Exposure-Response Relationship Observed Pre-clinically Appears to Have Translated to Humans, May Enable Flexible Dose Optimization in Humans

PK/PD Relationship of *Aficamten* for Ejection Fraction (LVEF)



Graphs show LVEF as a function of exposure; data points represent observed values in dogs and humans.

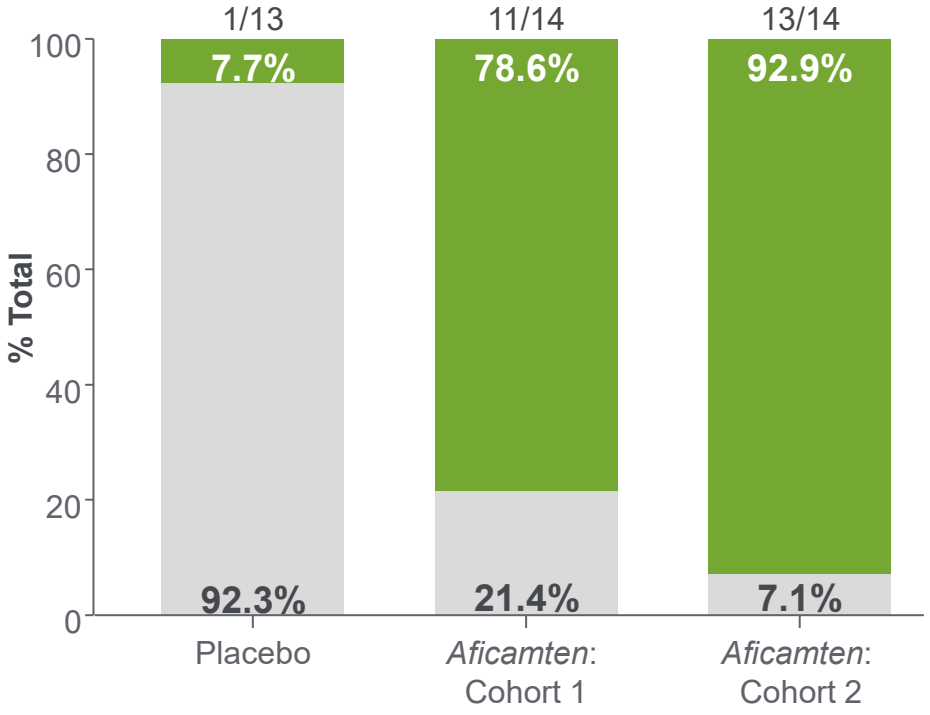
Decrease in LVEF as function of exposure is similar in humans and dogs.

Data points represent mean ± standard error of the mean.
d, day; qd, once daily.

LA Robertson, et al. Poster #210 presented at the 23rd HFSA Annual Scientific Meeting, September 13–16, 2019, Philadelphia, PA, USA.

Topline Results of Phase 2 REDWOOD-HCM Trial

High response rates on treatment with aficamten as compared to placebo, Cohorts 1 & 2



Responder # / Total #

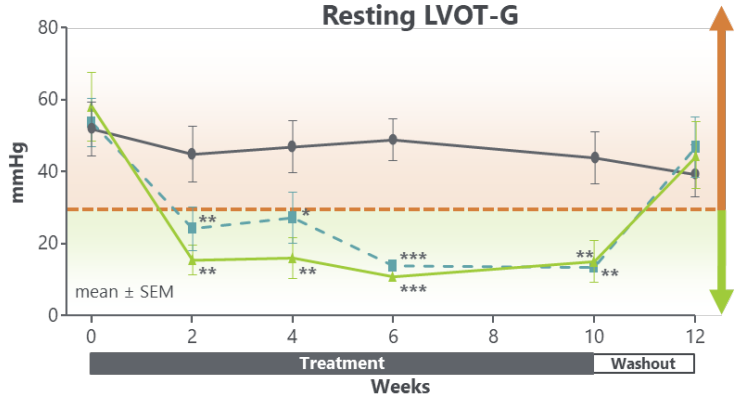
Not Responder
Responder

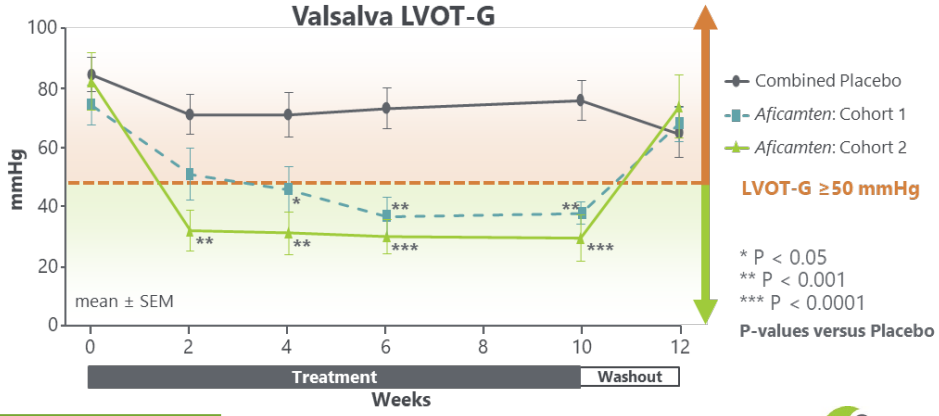
Responder Definition: Resting LVOT-G <30 mmHg and post-Valsalva LVOT-G <50 mmHg at Week 10

- Consistent, **clinically meaningful reductions in LVOT gradients** within two weeks
- **No treatment interruptions** or discontinuations
- No treatment-related SAEs
- **Reversibility of drug effect** demonstrated
- Statistically significant reductions in NT-proBNP
- Improvement in NYHA class

		Final Dose Achieved (N)				
		5 mg	10 mg	15 mg	20 mg	30 mg
N = 14	**Cohort 1**	4	5	5		
N = 14	**Cohort 2**		9		4	1

Maron M, Abraham T, Masri A, et al. "REDWOOD-HCM: A Randomized, Double-blind, Placebo-controlled, Dose-finding Trial of the Cardiac Myosin Inhibitor, *Aficamten*, In Obstructive Hypertrophic Cardiomyopathy"



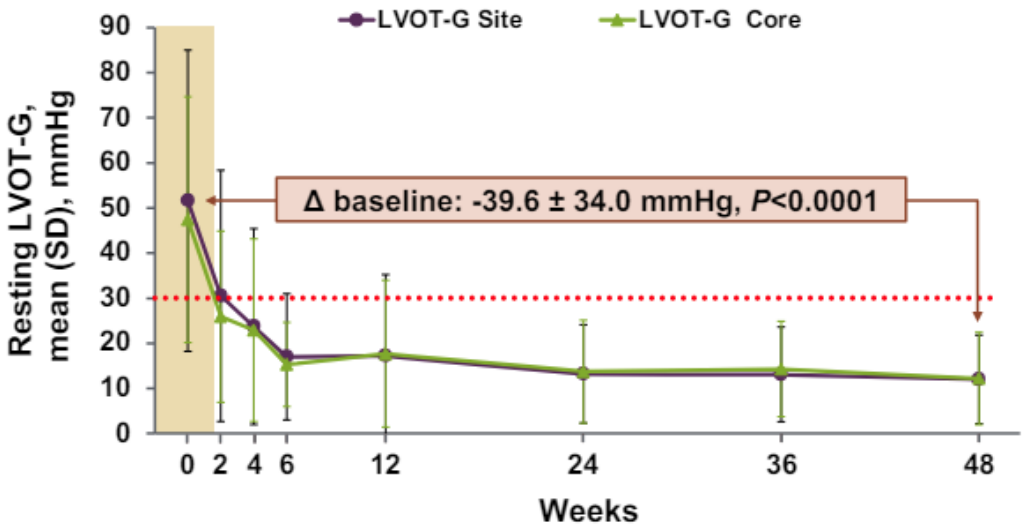


Cytokinetics®

Open Label Extension Study FOREST-HCM

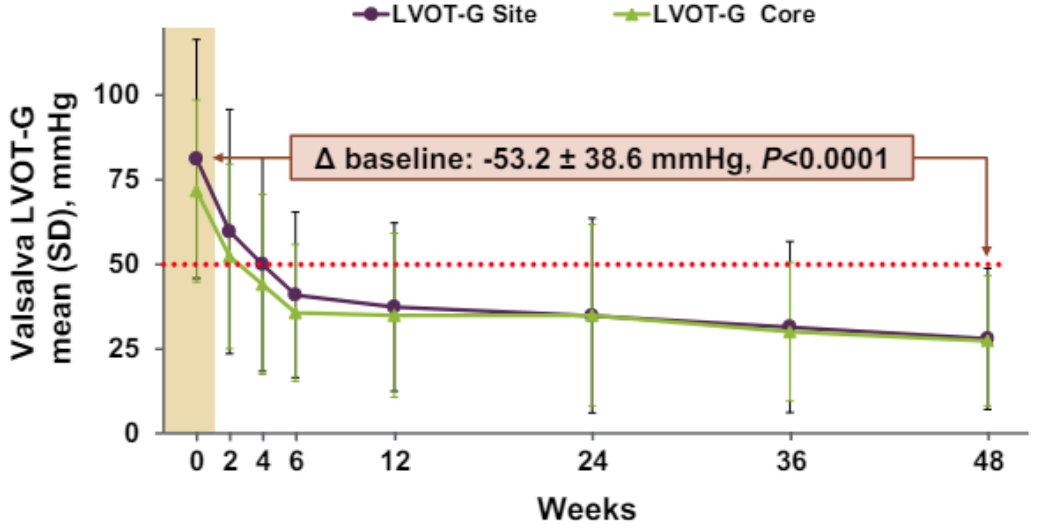
Substantial and sustained reduction in peak resting and Valsalva LOVT-G

Resting LVOT-G



Number of Patients and *P*-value compared with baseline							
Core	45	45‡	45‡	45‡	45‡	45‡	44‡	45‡
Site	45	45‡	45‡	44‡	44‡	45‡	43‡	45‡

Valsalva LVOT-G



Number of Patients and *P*-value compared with baseline							
Core	45	45‡	45‡	45‡	45‡	45‡	45‡	45‡
Site	45	45†	45‡	44‡	45‡	45‡	44‡	45‡

†*P*<0.001; ‡*P*<0.0001.

Core-laboratory and site-interpreted mean (SD) left ventricular outflow tract (LVOT) gradient (A) at rest and (B) with Valsalva. Boxed brown arrows represent change from baseline in site-read echo values. Red dashed line represents threshold for definition of obstruction or designation of severe obstruction.

- Treatment with *aficamten* was associated with rapid, substantial, and sustained improvements in echocardiographic hemodynamics paralleled by improvements in NYHA class and NT-proBNP

Sara Saberi, et al. Poster presented at the 73rd Annual American College of Cardiology (ACC) Scientific Session April6-8, 2024 in Atlanta, GA, Philadelphia, PA, USA.

Conclusion and Summary

- Our goal to provide a next-in-class cardiac sarcomere inhibitor was met
  - *Derived a unique NCE from singleton hit*
  - *Observed shallow exposure-response relationship in pre-clinical species (rat and dog) and in humans*
  - *Presented favorable ADME properties with no CYP induction or inhibition*
  - *Presented no notable off-targets effects with in vitro safety pharmacology screening*

- Human clinical trial status of *aficamten*
  - *Phase 1 data supported progression of aficamten to a placebo-controlled, double-blind Phase 2*
  - *Phase 2 trial, REDWOOD-HCM completed and high response rate observed with aficamten dose group*
  - *Open label long term extension trial, FOREST-HCM demonstrates a rapid, substantial, and sustained improvements in echocardiographic hemodynamic paralleled by improvement in NYHA class and NT-proBNP*
  - *Phase 3 trial, SEQUOIA-HCM met the primary and all secondary endpoints with p values <0.001*
  - *Additional Phase 3 trial, MAPLE-HCM is on-going to evaluate aficamten compared to metoprolol succinate in patients with symptomatic oHCM*
  - *Additional Phase 3 trial, ACACIA-HCM is on-going to evaluate aficamten in patients with symptomatic nHCM*

Acknowledgements

All current and former employees of Cytokinetics for the collaborative effort and patients for the inspiration

REDWOOD HCM

"Alone we can do so little; together we can do so much"

Attributed to Helen Keller