Cytokinetics

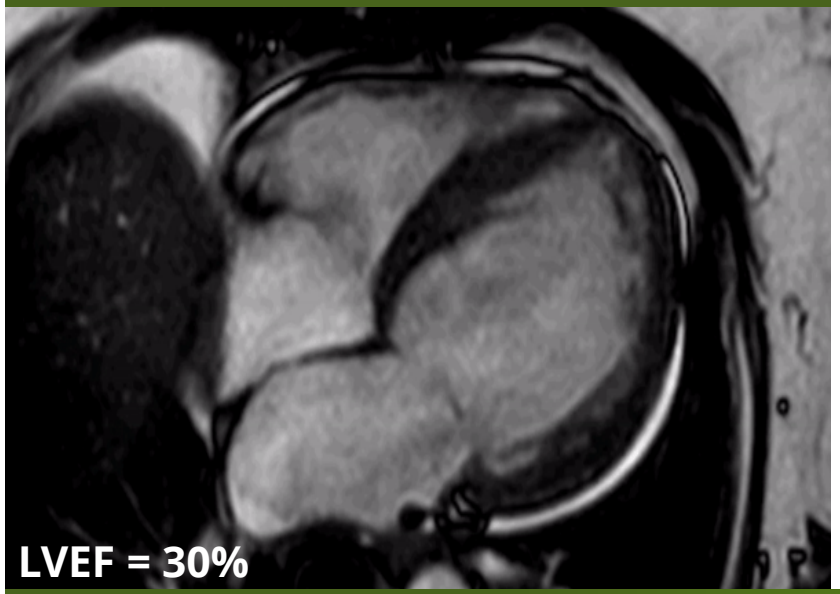
CK-4021586: A Novel Cardiac Myosin Inhibitor with An Alternative Mechanism of Action for the Treatment of HFpEF

Luke Ashcraft • ACS First Time Disclosures • 03.26.25

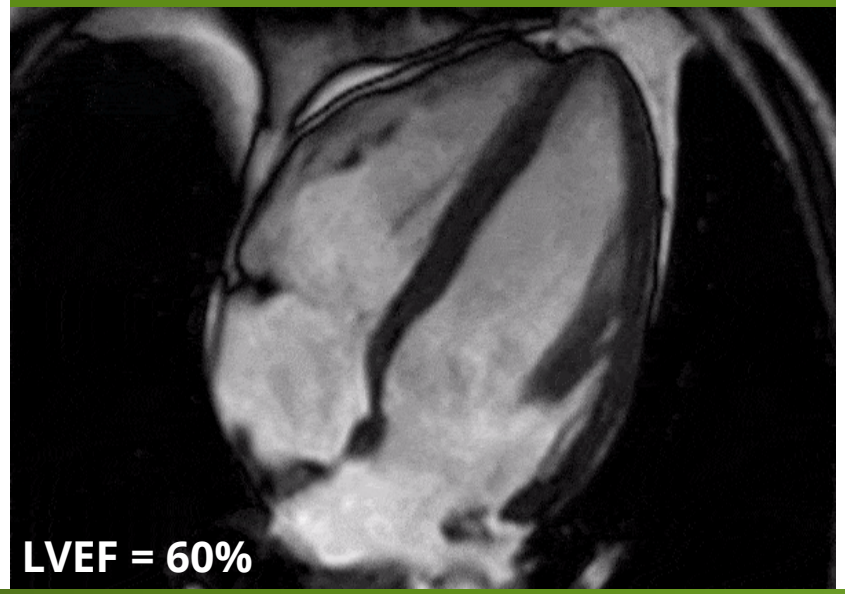
Diseases Associated with Reduced and Increased Cardiac Contractility

Normalization of Contractility May Treat the Underlying Cause of the Disease

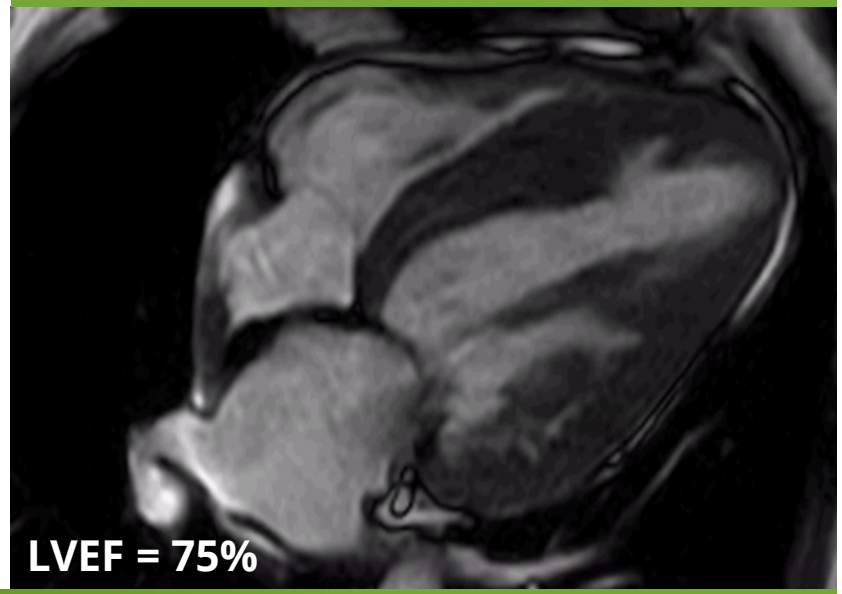
HFrEF (Low Contractility)



Normal Contractility



HCM & HFpEF (subset) (Hypercontractility)



Decreased Cardiac Contractility	Increased / Preserved Cardiac Contractility
HFrEF = Heart Failure with Reduced Ejection Fraction DCM = genetic Dilated Cardiomyopathies Pulmonary Hypertension w/ Right Ventricular Heart Failure	HCM = Hypertrophic Cardiomyopathies • Obstructive (oHCM) & Non-Obstructive (nHCM) HFpEF = Heart Failure with Preserved Ejection Fraction

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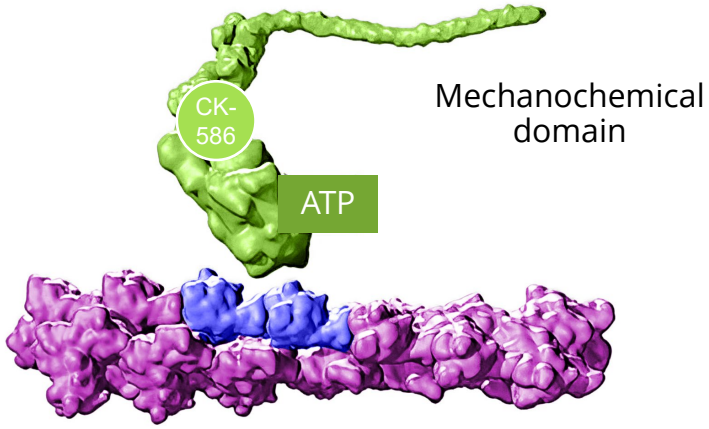
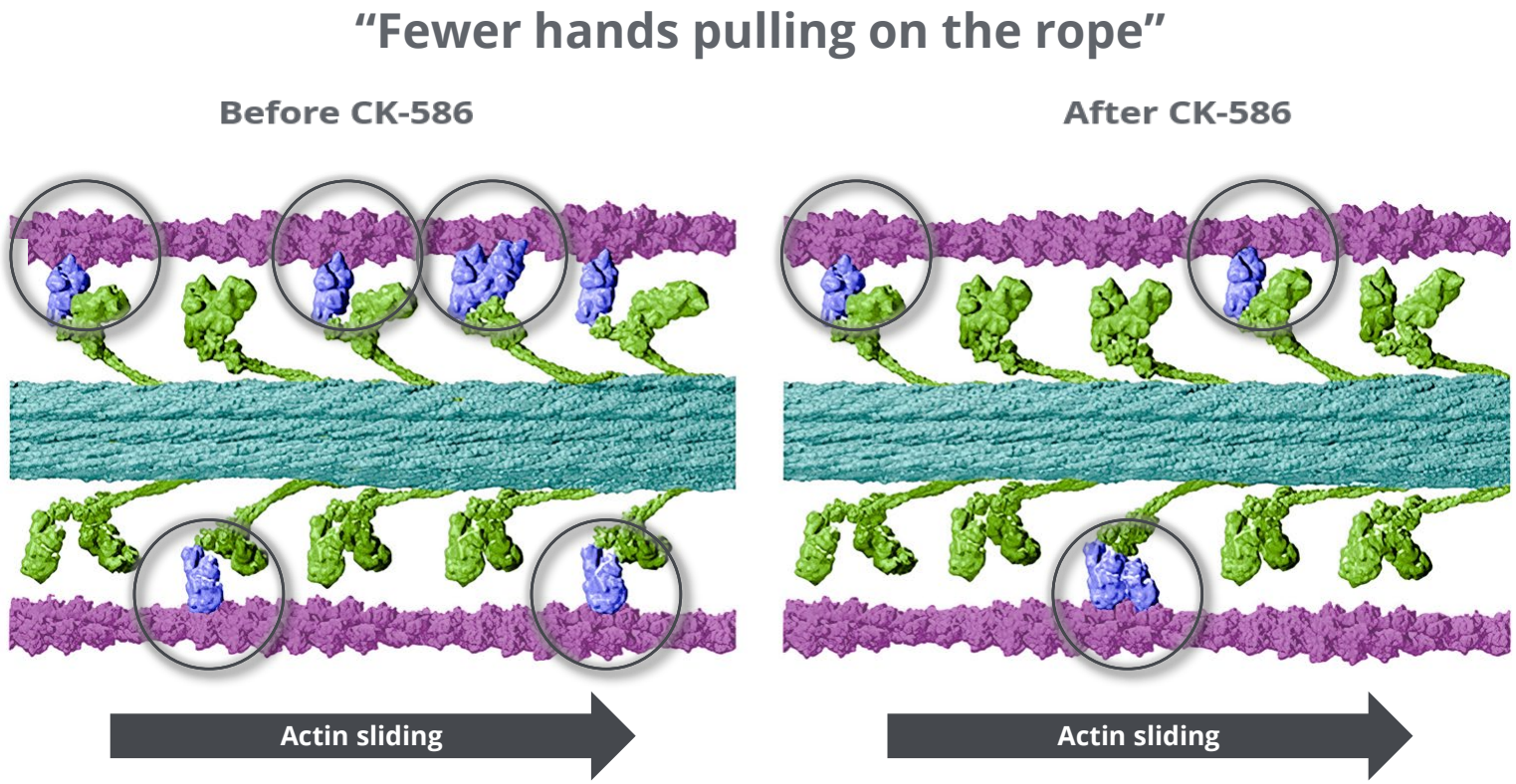
CK-586 Reduces Actin-Myosin Cross-Bridges in the Cardiac Sarcomere

Distinct mechanism of action from other cardiac myosin modulators





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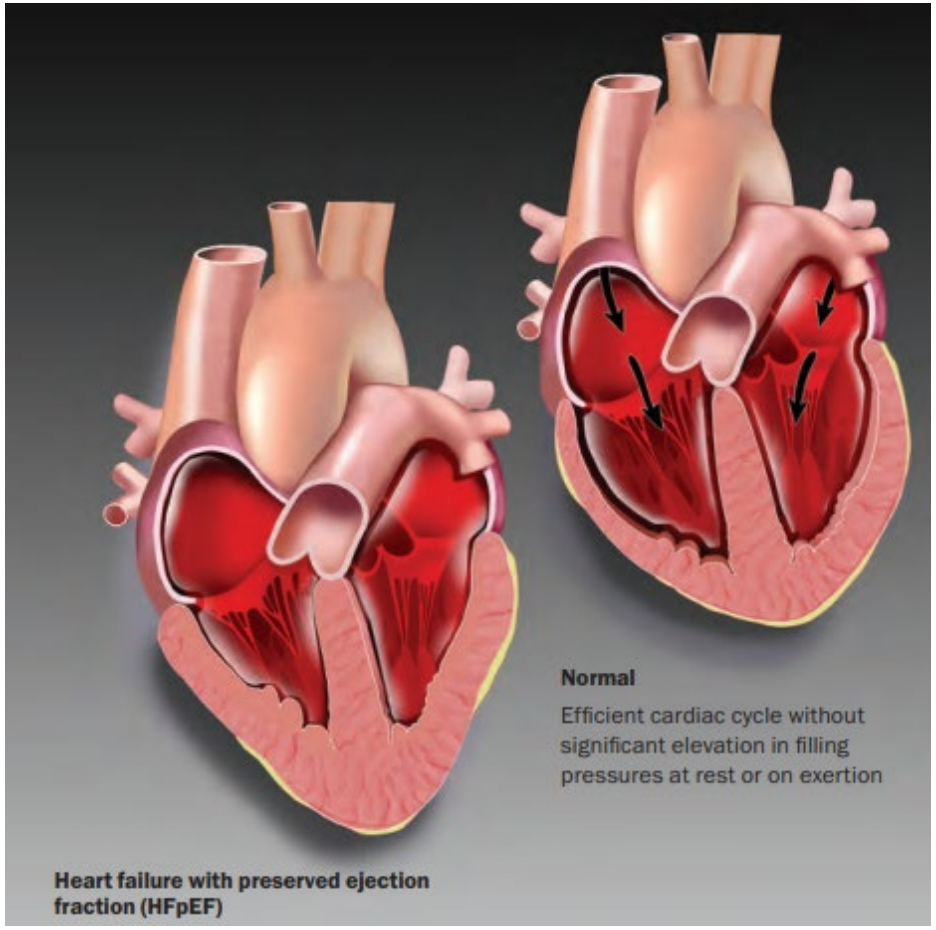
HFpEF: Impaired Relaxation with Normal or Increased Ejection Fraction

Reducing the number of actin-myosin cross-bridges should improve diastolic function

HFpEF Characteristics:
- Impaired cardiac relaxation / decreased compliance
- Often with cardiac structure and functional abnormalities
  - Left atrial dilation & thickened left ventricular walls
  - Compromised tissue mechanics
  - Reduced stroke volume at rest
    - inability to augment with exercise

Cardiac Myosin Inhibition:
- Reduces the number of actin-myosin cross-bridges
- Enhances myocardial compliance and LV relaxation in HCM
- Improves resting myocardial energetics

Improving Diastolic Function Should:
- Reduce LV filling pressures and volume
- Enhance cardiac reserve and exercise capacity
- Improve symptoms and promote positive remodeling



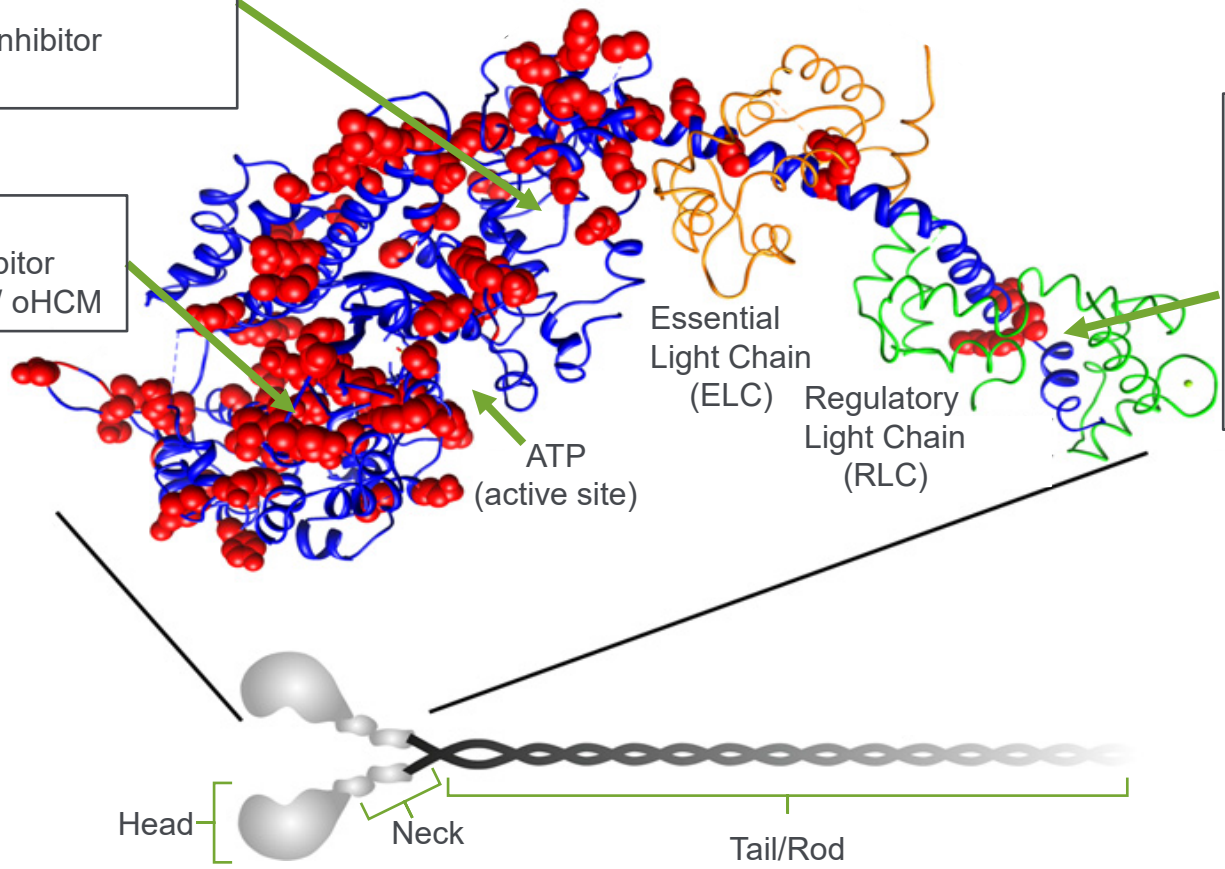
LV = Left Ventricle; EF = Ejection Fraction; HCM = Hypertrophic Cardiac Myopathy

Myosin Modulation to Normalize Contractility: Multiple Binding Sites to Activate / Inhibit Cardiac Myosin

Hypothesis: CK-586 Novel binding may induce novel pharmacology

X-Ray:
omecamtiv mecarbil – Cardiac Myosin Activator
- Phase 3 / HFrEF
mavacamten – Cardiac Myosin Inhibitor
-Market / oHCM

X-Ray:
aficamten – Cardiac Myosin Inhibitor
- PDUFA anticipated 9/26/2025 / oHCM



Probable Binding Site: CK-586
- RLC required for activity
- Isothermal titration calorimetry demonstrates direct, stereoselective interaction
- Binding requires RLC and Myosin Heavy Chain

Cardiac Myosin:
Hexameric protein with 2 myosin heavy chain polypeptides that self-associate via an α-helical coiled-coil tail domain

oHCM = obstructive hypertrophic cardiomyopathy; PDUFA = Prescription Drug User Fee Act

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Optimization Objectives for Follow-on Cardiac Myosin Inhibitor (CMI)

Expanding the utility of cardiac myosin inhibition to treat cardiac diseases

Goals for a follow-on candidate relative to *aficamten*

- New mechanism of action (MOA) that inhibits a different region of myosin
- A new chemical series distinct from known CMI chemical matter
- Once daily oral dosing with human half-life < 24 hours to enable faster time to reach steady state

Maintain the desirable qualities of *aficamten*

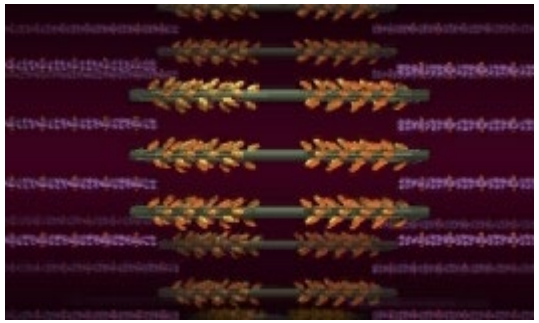
- Shallow and predictable PK/PD relationship

Provide a novel CMI for a different indication such as HFpEF

Assay Systems To Guide Muscle Contractility During Lead Optimization

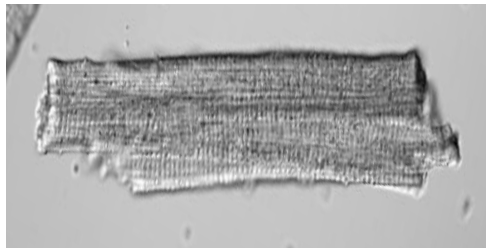
Muscle Contractility

Myofibril
Intact Sarcomere



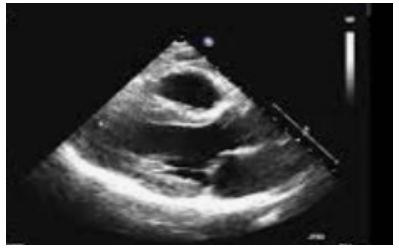
Biochemical

Cardiac Myocyte
Assay in Native Context

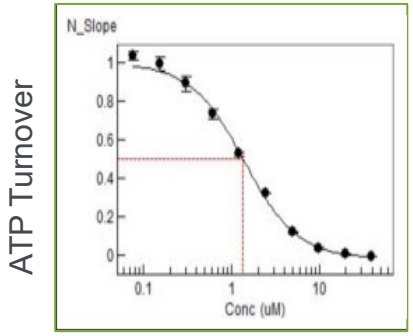


Cellular

Organ and *in vivo*
Functional Outcome



Echocardiography



Compound Conc.

- Potency measures
  - Myofibril 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)

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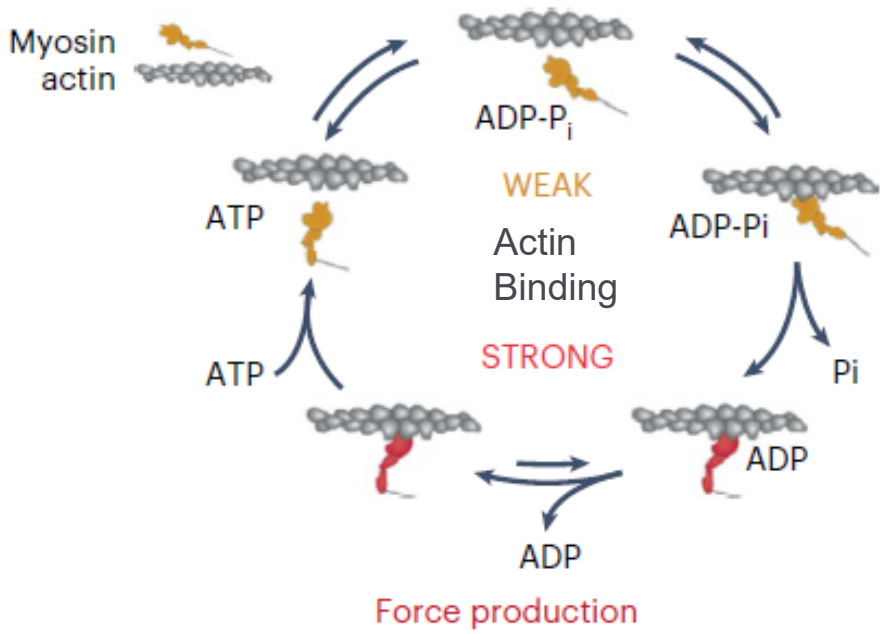
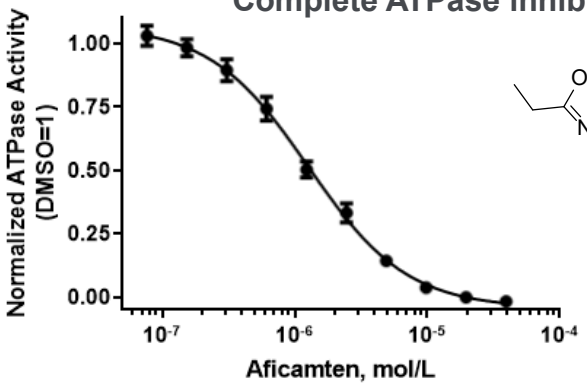
A Novel Hit Class With Biochemical Phenotype Characterized by Partial Inhibition of Bovine Cardiac Myofibrillar ATPase

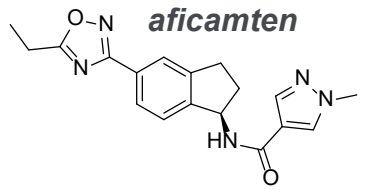
Myosin Mechanochemical Cycle



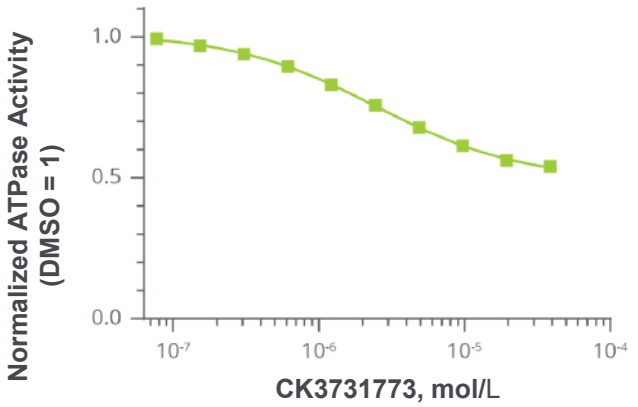
- Chemical energy in ATP-phosphate bond is translated into mechanical force

Complete ATPase inhibition

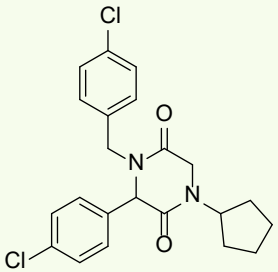




Partial ATPase inhibition



Screening Hit



(cluster of hits)
IC = 2.6 mM
IC_{50}^{15} >39 mM

RLC interface with myosin heavy chain identified as probable binding site

Chuang, C. et al. Discovery of Aficamten (CK-274), a Next-Generation Cardiac Myosin Inhibitor for the Treatment of Hypertrophic Cardiomyopathy *J. Med. Chem.* **2021**, 64, 14142−14152

Hartman, J. et al. Aficamten is a small-molecule cardiac myosin inhibitor designed to treat hypertrophic cardiomyopathy, *Nature Cardiovascular Research* volume 3, pages 1003–1016 (**2024**)

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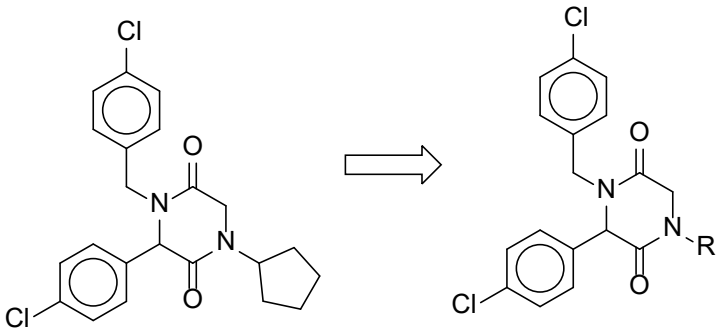
Defining the Minimum Pharmacophore and Stereochemical Requirements

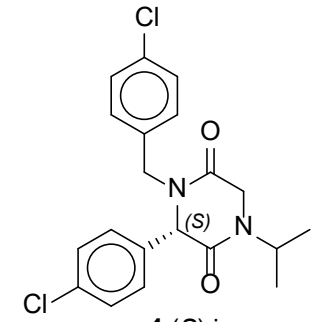
>39x eudismic ratio favoring S enantiomer observed



Screening Hit
IC_{15} = 2.6 µM
IC_{15} > 39 µM
IC_{50} µM

Cmpd	R	enantiomer	CDMF* IC_{15} (µM)	cLogP
1	Cyclopentyl	R	>39	4.7
2	Cyclopentyl	S	1.0	4.7
3	Isopropyl	R	>39	4.0
4	Isopropyl	S	0.8	4.0
5	Oxetane	R	>39	3.4
6	Oxetane	S	>39	3.4
7	Methyl	R	>39	3.2
8	Methyl	S	>39	3.2

*Bovine Cardiac Myofibril Biochemical Potency

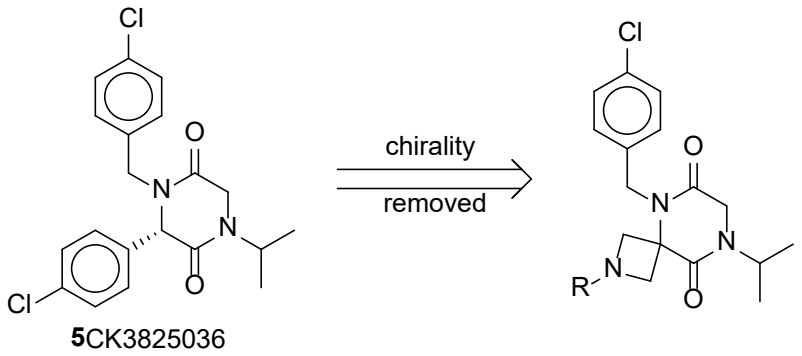


4 (*S*) isomer
Compound
IC_{15} = 0.8 µM
uSome, CL int,h (ml/min/kg) > 62
uSome, CL int,r (ml/min/kg) > 124
Rat PK:
CL = 41 ml/min/kg
T1/2 = 9.3 hours
Vss = 18 L/kg

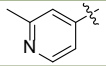
- Clear preference observed for “S” enantiomer of diketopiperazine
- Replacement of cyclopentyl with isopropyl decreased MW and improved cLogP
- Poor *in vitro* stability and *in vivo* rat PK characterized by excessive $T_{1/2}$ and high V_{ss} for compound **4**

Chirality Not Required for Biochemical Potency

Tolerance for quaternary carbon and N-substituted azetidines discovered

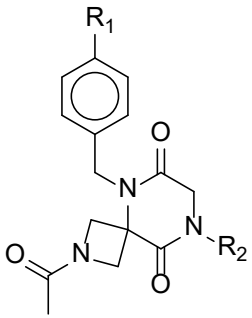


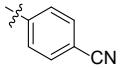
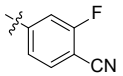
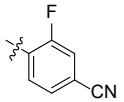
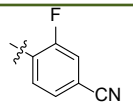
5CK3825036

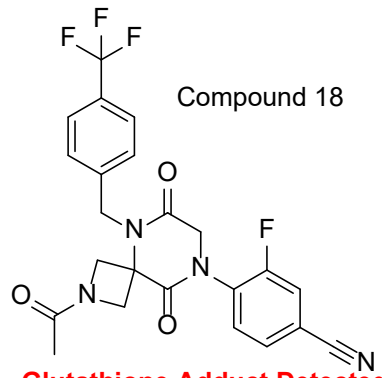
Cmpd	R	CDMF IC_{15} (μM)	Rat, CL (mL/min/kg)	Rat, $t_{1/2}$ (hour)	Rat %F	CL_{int} (r) (mL/min/kg)	CL_{int} (h) (mL/min/kg)
9		1.0	161.7	1.4		>124	>62
10		5.2	224.1	1.7		23.5	<11
11		2.4	84.3	0.5	9	25.6	<18

- Stereocenter successfully replaced with azetidines, however, *in vivo* PK properties are not optimal

- Right-hand side aromatic benzonitriles improve *in vitro* and *in vivo* pharmacokinetic properties



Cmpd	R_1	R_2	CDMF IC_{15} (μM)	Rat, CL (mL/min/kg)	Rat, $t_{1/2}$ (hour)	Rat %F	CL_{int} (r) (mL/min/kg)	CL_{int} (h) (mL/min/kg)
12	CF_3		15.8	8.9	2.8	98	<21	<11
13	CF_3		3.1	11.1	5.4	57	<21	<11
14	Cl		1.6	16.5	3.4	40	<21	<11
15	CF_3		**1.2**	**8.2**	**4.0**	**39**	<21	<11



Glutathione Adduct Detected

hERG liability (IC_{50} = 11.5 μM)

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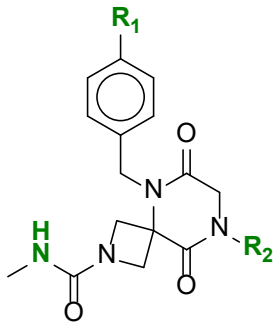
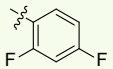
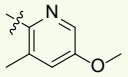
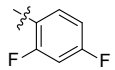
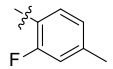
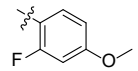
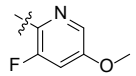
N-Methyl Ureas Provide Desirable Projected Human $T_{1/2}$



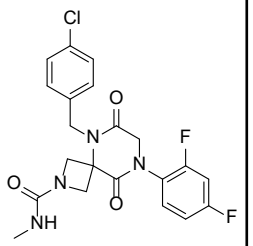
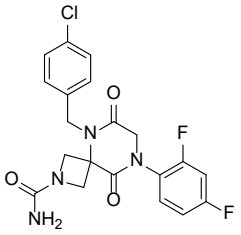
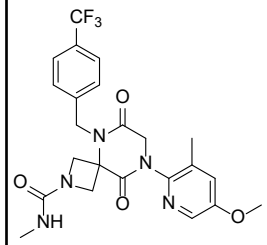
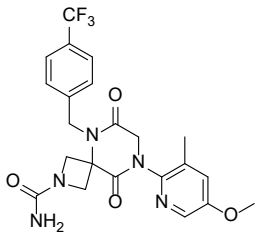
Cmpd	R_1	R_2	CDMF IC_{15} (μM)	CL (mL/min/kg) [1]r, d, m	$t_{1/2}$ (hour) r, d, m	Vss (L/Kg) r, d, m	cLogP	PSA	Projected Human $t_{1/2}$ [2] (hour)
16	Cl		1.0	36, 11, 13	5, 11, 13	11, 8, 11	3.0	73	40
17	CF_3		1.7	29, 15, 14	5,10,12	9, 9, 12	2.1	95	26
18	CF_3		0.70	34, 6, nd[3]	5, 19, nd	13, 8, nd	3.0	73	
19	CF_3		0.72	14, 28, nd	5, 10, nd	6, 19, nd	3.3	73	
20	CF_3		0.43	27,15, nd	8, 15, nd	15, 17, nd	2.8	82	
21	CF_3		0.44	22, 25, nd	12, 15, nd	20, 28, nd	1.7	95	

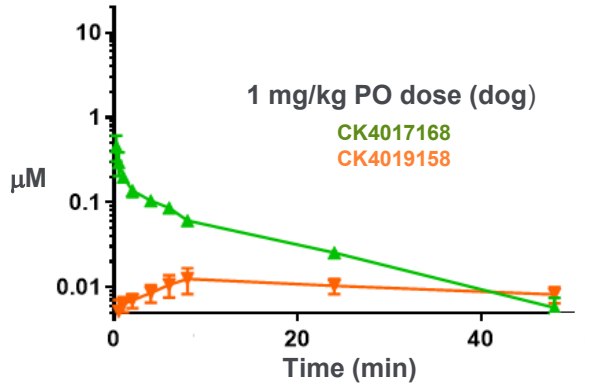
[1]r = rat, d = dog, m = cynomolgus monkey [2]determined using multispecies allometry [3]nd = not determined

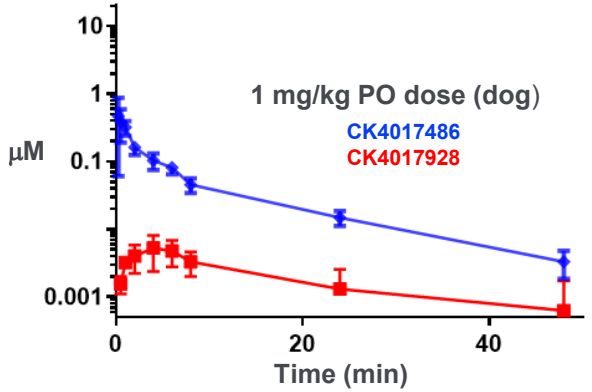
- Favorable *in vivo* pharmacokinetic properties and potency with reasonable projected human half-lives
- ***N-methyl ureas met most of our optimization parameters***

Cytokinetics

Identification of Primary Urea Active Metabolites in Preclinical PK Studies

Parent	Parent IC_{15}	Metabolite	Metabolite IC_{15}	Species	PO Dose (mg/kg)	Metabolite % of AUC	Parent $t_{1/2}$ (hours)	Metabolite $t_{1/2}$ (hours)
Compound **16** 	**1.3**	Compound **22** 	**1.7**	**Rat**	10	**20**	4	8
				Dog	1	**23**	11	>11
				Monkey	1	**6.5**	12	33
Compound **17** 	**1.3**	Compound **23** 	**2.9**	**Rat**	10	**2**	4	8
				Dog	1	**4.4**	11	<11
				Monkey	1	**2.5**	12	>12

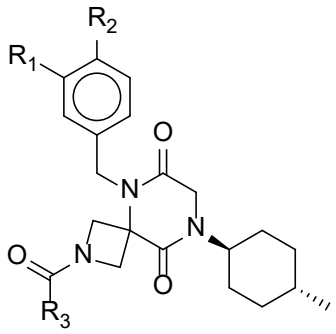




- Accumulation of an active metabolite in pre-clinical models precluded the advancement of N-methyl ureas
- *Primary urea active metabolites* revealed long half-lives and significant efflux
  - Compound **22** Caco-2 efflux ratio (ER) = 8.9

Cytokinetics

A Return to Aliphatic Right-Hand-Side with Removal of H-Bond Donors



Cmpd	R_1	R_2	R_3	CDMF IC_{15} (μM)	Dog Cl (mL/min/kg)	Dog $T_{1/2}$ (hours)	Dog V_{ss} (L/Kg)	cLogP
24	H	CF_3	CH_3	10.8				3.5
25	F	F	CH_3	26.6				3.0
26	F	Cl	H	1.2	15.0	6.7	6.5	3.2
27	H	Cl	H	2.0	13.8	5.6	4.8	3.1
28	CH_3	F	H	1.9	18.5	4.5	5.1	3.0
29	Cl	F	H	1.3	17.3	2.5	6.5	3.2
CK-4021586	F	F	H	1.5	5.1	11.8	4.5	2.5

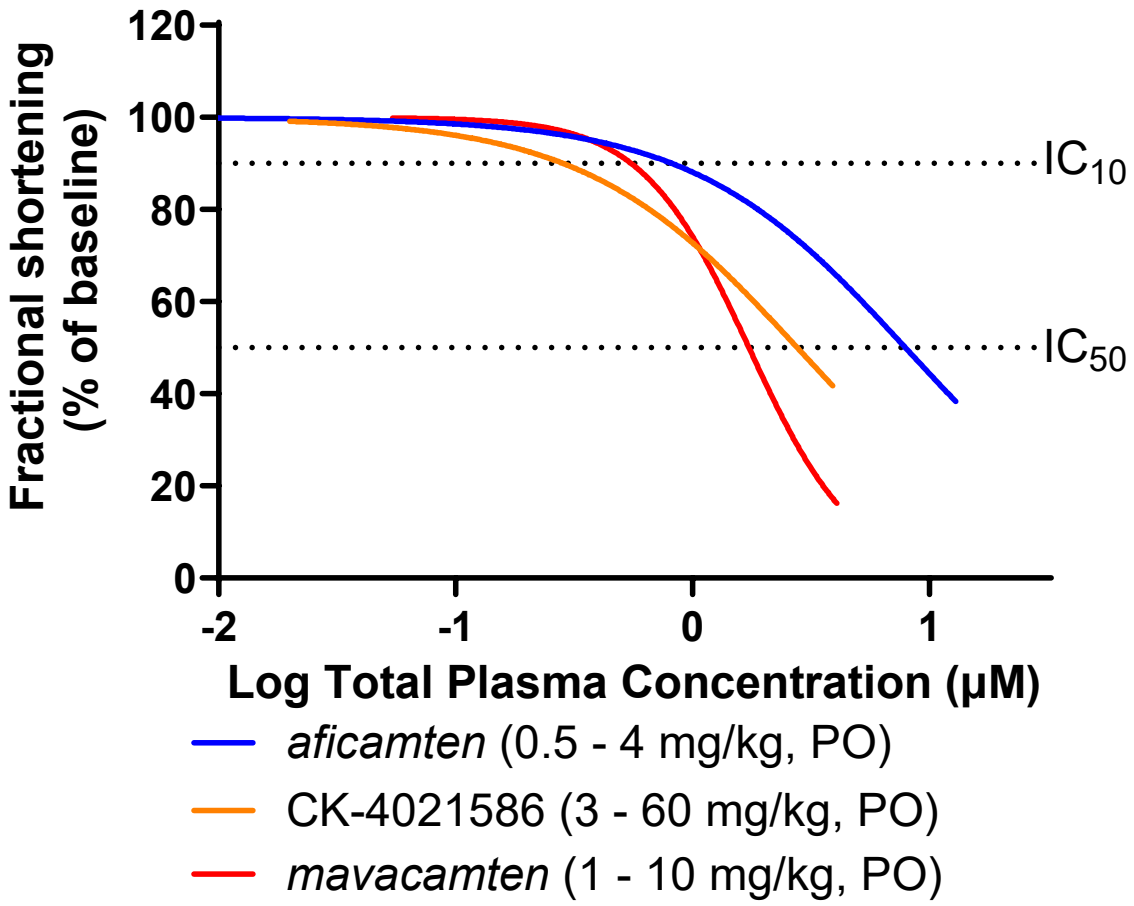
- Potency vector with identified with formamides
- Pharmacokinetic properties optimized with benzyl substitution

CK-4021586 provides a desirable balance ADME properties, potency, and solubility

- Metabolically stable across preclinical species
- Orally bioavailable and permeable with no efflux observed (Caco-2)
- Shake flask solubility > 1000 μM

Cytokinetics

CK-4021586 Maintains the Shallow Exposure Response in Healthy Rats



Pharmacodynamic window Fractional shortening IC_{50}/IC_{10} ratio			
	IC_{10} (µM)	IC_{50} (µM)	IC_{50}/IC_{10}
aficamten	0.8	7.9	9.9x
CK-586	0.3	2.8	9.3x
mavacamten	0.6	1.7	2.8x

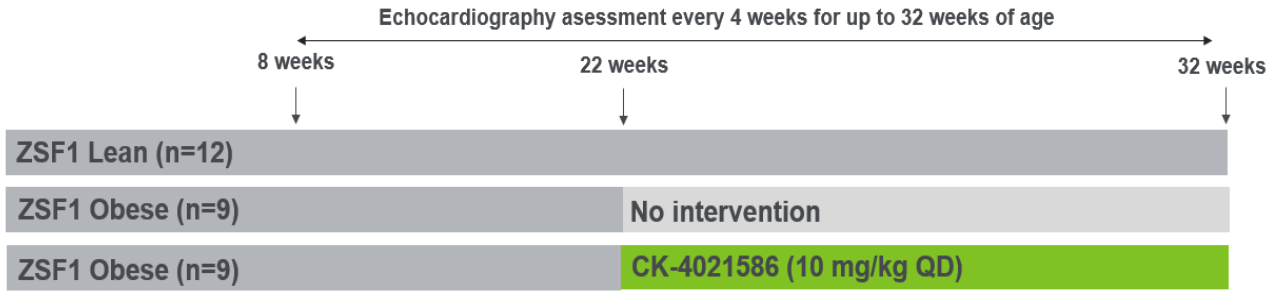
IC_{10}: plasma concentration at 10% relative reduction in fractional shortening
IC_{50}: plasma concentration at 50% relative reduction in fractional shortening

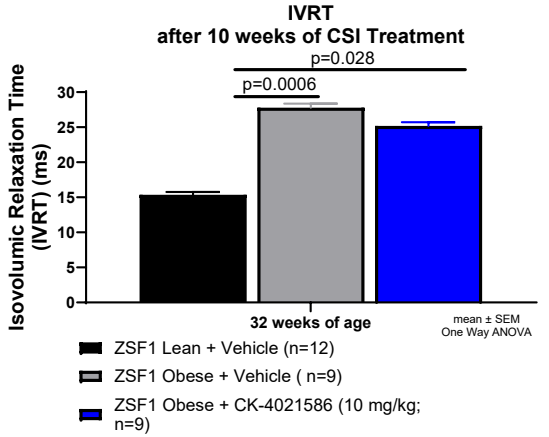
Compound half-life in humans	Actual	Projected
aficamten	~3 days	2.8 days
CK-586	14-17 hours	16 hours
mavacamten	~9 days	~9 days

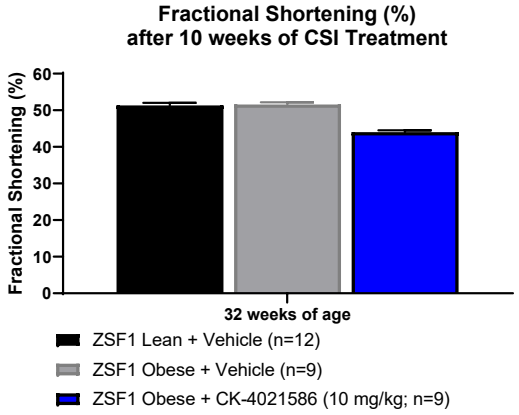
- CK-4021586 has shorter predicted human $t_{1/2}$ than *aficamten* and similar exposure response relationship

CK-4021586 Improves Diastolic Function and Decreases Fibrosis in ZSF1[1] HFpEF Rat Model

- As a measure of diastolic dysfunction, CK-4021586 (10 mg/kg, PO QD) mitigates increases in isovolumic relaxation time (IVRT) in ZSF1 obese rats which correlate to a small decrease in fractional shortening





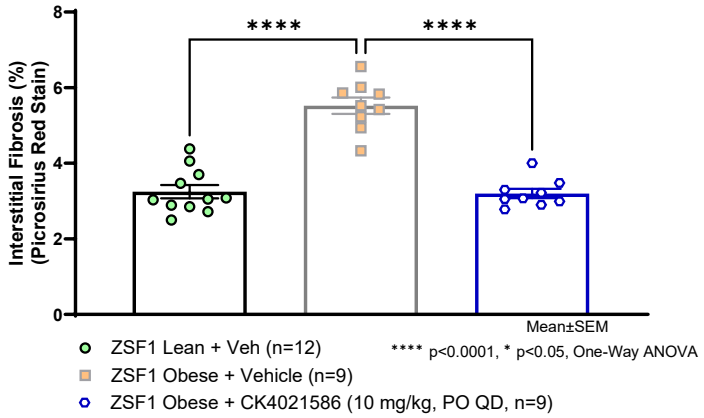


- CK-4021586 reduces cardiac fibrosis in ZSF1 rats with visualization using sirius red stain


ZSF1 Lean + Vehicle


ZSF1 Obese + Vehicle


ZSF1 Obese + CK-4021586



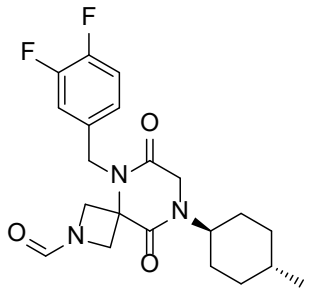
[1]ZSF1 = Zucker fatty and spontaneously hypertensive rat

Cytokinetics

CK-4021586: Optimization Objectives Achieved

- ✓ New mechanism of action requiring myosin regulatory light chain of cardiac myosin
- ✓ Exposure-response relationship similar to *aficamten*
- ✓ Predicted human $t_{1/2}$ for once daily (QD) oral dosing and reach steady state within 7 days

	Assay	*CK-4021586*
***in vitro* pharmacology**	Cardiac Myofibril Ca_{75} $IC_{15/50}$ (μM)[1]	1.5/>39
	Rat Cardiac Myocyte (%FS)	19 ± 9.9 @ 5 μM
***in vivo* pharmacology**	IC_{10} (μM)[2]	0.3
	IC_{50} / IC_{10}[2]	9.3
Human PK projection	Human $t_{1/2}$ projected[3] (Human $t_{1/2}$ actual)[4]	16 h (14-17 h)
***In vitro* profiling**	CEREP profiling	No significant findings @ 10 μM
	Cyp Inhibition IC_{50} (μM)	> 100, no TDI[5] observed
	Cyp Induction (PXR/AhR/CAR1)	No significant finding @ 10 μM
	hERG Inhibition IC_{50} (μM)	>100

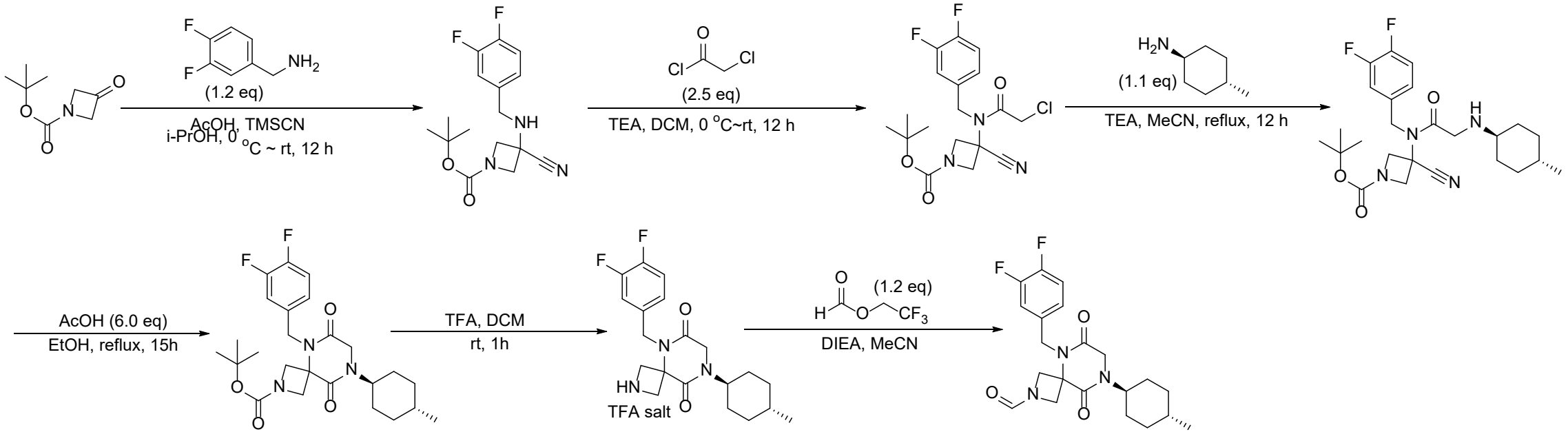


CK-4021586

1. Cytokinetics data on file. Bovine cardiac myofibrils assayed at 75% of max Ca-dependent activation. Mean values (95% confidence intervals). 2. 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 pooled plasma concentrations to calculate the inhibitory concentration at a 10% (IC_{10}) and 50% (IC_{50}) reduction in FS IC_{50}/ IC_{10} 3. Grillo, M.P. et al. Poster # M1330-10-68 American Association of Pharmaceutical Scientists (AAPS) 2023 PharmSci 360 October 22, 2023, Orlando, FL. 4. J.T. Lutz et al. Poster presented at American College of Clinical Pharmacology (ACCP24), September 8–10, 2024, Bethesda, MD. 5. Time Dependent Inhibition

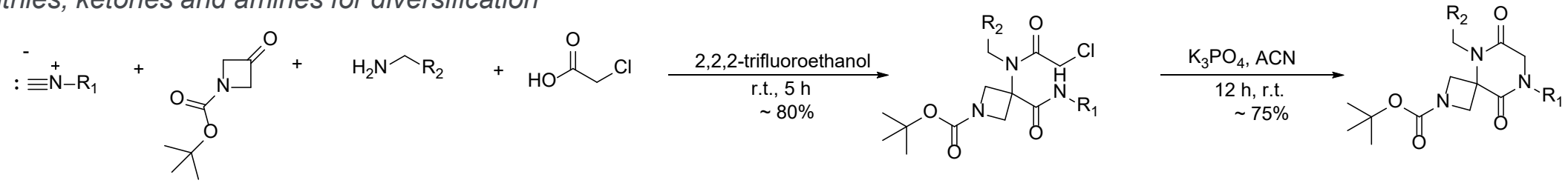
Medicinal Chemistry Synthetic Routes Used for Optimization

CK-4021586 Strecker route



Ugi Multicomponent Reaction (MCR)

isonitriles, ketones and amines for diversification

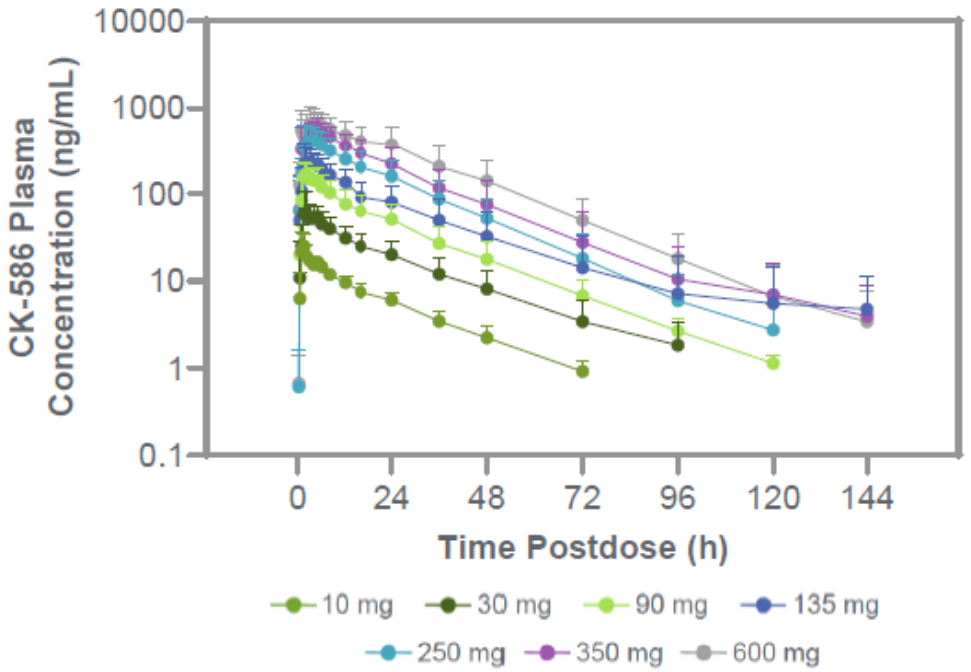


Cytokinetics

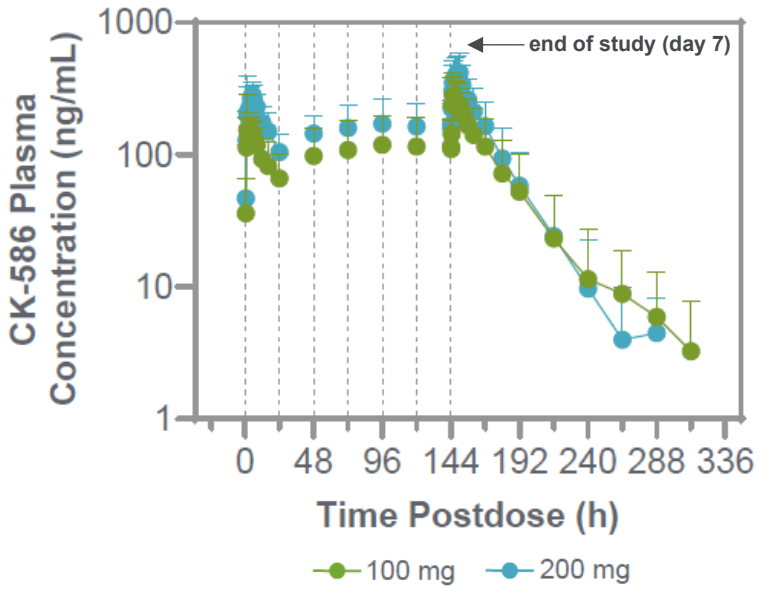
SAD and MAD Pharmacokinetics of CK-4021586 in Healthy Volunteers

CK-586 demonstrated dose linearity and reached steady upon 7 days dosing[1]

Plasma concentration after single ascending doses of CK-586



Plasma concentration after 2 ascending doses of daily CK-586 for 7 days

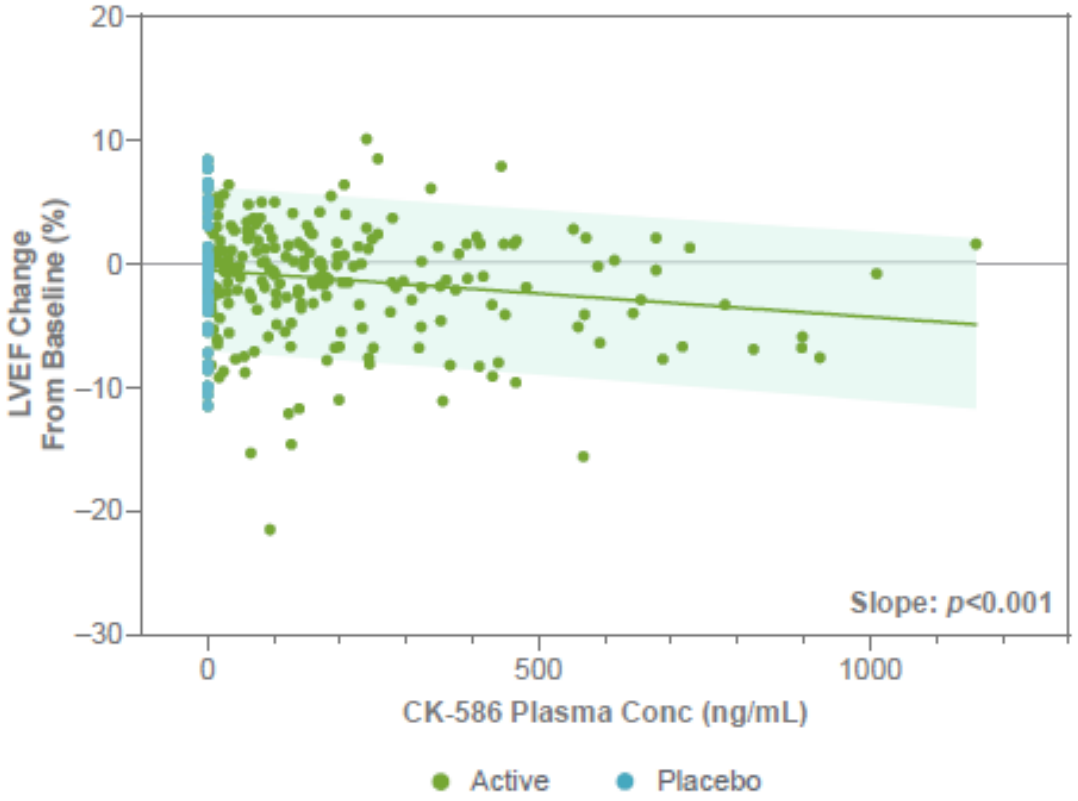


- ✓ Dose proportional and time-linear over a wide range of exposures
- ✓ Median plasma elimination half-life ($T_{1/2}$) of 14-17 hours
- ✓ Steady state achieved by day 7

[1]J.T. Lutz et al. Poster presented at American College of Clinical Pharmacology (ACCP24), September 8–10, 2024, Bethesda, MD.

PK-PD Relationship of CK-4021586 for Left Ventricular Ejection Fraction (LVEF) in Healthy Volunteers [1]

Change from baseline in LVEF as a function of plasma concentration[2]



- ✓ Plasma concentrations demonstrate shallow and predictable PK-PD relationship
- ✓ Well tolerated across all cohorts with no SAEs (serious adverse events) observed
- ✓ Ideal clinical pharmacologic properties for once-daily oral dosing
- ✓ Results provide key insights for phase 2 clinical trial design in HFpEF

[1]J.T. Lutz et al. Poster presented at American College of Clinical Pharmacology (ACCP24), September 8–10, 2024, Bethesda, MD.
[2]Line and shaded area are estimated mean 90% prediction interval, respectively

Summary and Conclusions

From > 2000 compounds synthesized and tested, a novel cardiac myosin inhibitor with a new MOA was discovered and advanced into clinical development

- Binding requires regulatory light chain and myosin heavy chain – novel MOA
- Human PK
  - $T_{1/2}$ < 24 hours and supportive of QD (once daily) dosing
  - Steady state achieved within 7 days
- Shallow exposure-response observed in healthy volunteers that may lessen or eliminate echocardiographic dose titration

Phase II clinical trial in HFpEF patients commenced January 2025

Acknowledgements

All current and former employees of Cytokinetics and Pharmaron for their collaborative effort

康龙化成
PHARMARON

Patients inspire us to do what we do!

Cytokinetics