心不全治療を目指した新規低分子化合物

心室ミオシン調節軽鎖の選択的アップレギュレーター
特にHFrEF患者に効果的

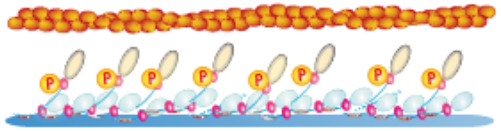
Dr. Osamu Tsukamoto
Department of Medical Biochemistry Osaka University
Department of Biochemistry, Hyogo Medical University

Tech Manage Corp.
Keiji FUJITO

Concept

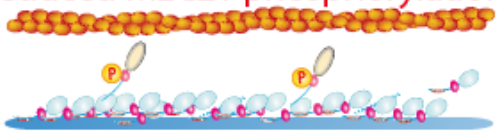
cMLCK活性化因子：カルシウム非依存性メカニズムを介してサルコメアタンパク質を直接活性化することにより心臓収縮を増強する

healthy myocardium



failing myocardium (DCM)

(reduced MLC2v phosphorylation)

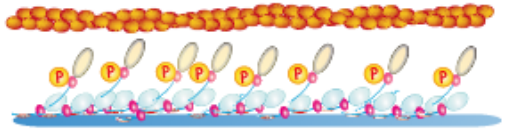


reduced contractility

disorganized sarcomere

our compound
(cMLCK activator)

(normalized MLC2v phosphorylation



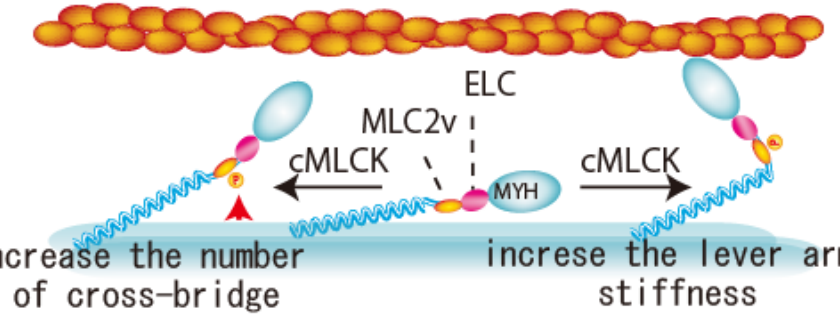
improved contractility

restored sarcomere structure

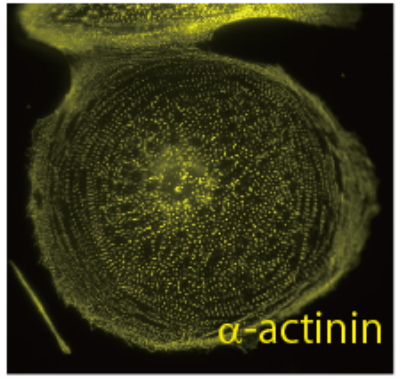
Action mechanism of cMLCK activator

①myotropic effect （functinal improvement）

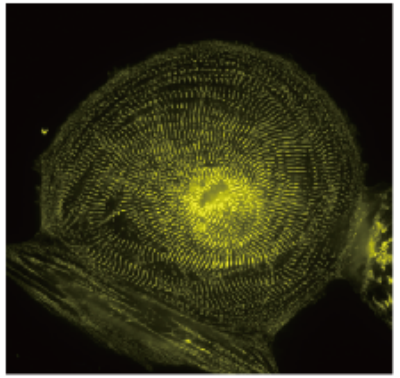
(improve the contractility without affecting Ca^{2+} transient)



②promote sarcomere organization (structual improvement)



cardiomyocyte with reduced MLC2v phosphorylation



cMLCK activation

③improve cardiac remodeling

Tech Manage

Background-1　効果的で信頼性の高い強心薬が強く求められています

◆ Heart Failure

- 1,200,000 人（日本）, 6,500,000人米国,　患者数には更に増加の見込み.
- 約40% が駆出率の減少による心不全 (HFrEF)

◆ Inotrope Treatment for HFrEF Stage C/D

Modulation of calcium transients: PDEIII inhibitor, catecholamine, beta-adrenergic stimulator

◆ Problems of Traditional Inotropes (Calcinotrope)

長期使用により死亡リスクが高まることが問題

◆ 心筋ミオシン活性化剤: Omecamtive mecarbil (OM),

- 選択的心臓ミオシン ATPase 活性化剤, PIII development
- transientsに影響を与えることなく心臓の収縮性を改善します
- 心筋虚calcium血患者には注意が必要（相対拡張期が短縮するため）

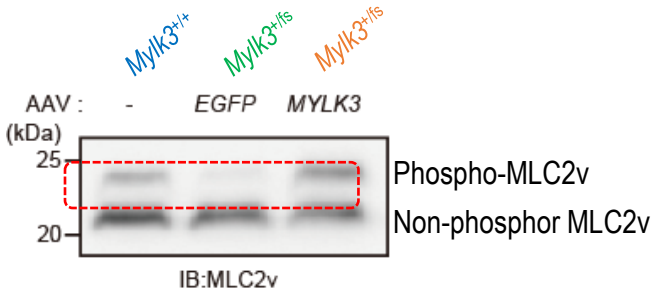
Background

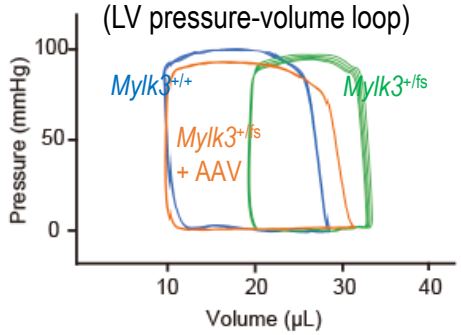
cMLCK 活性化剤 は 有望な治療薬候補

Functional analysis of *MYLK3* frame-shift mutation

Knock-in mice of the *MYLK3* frame-shift mutation

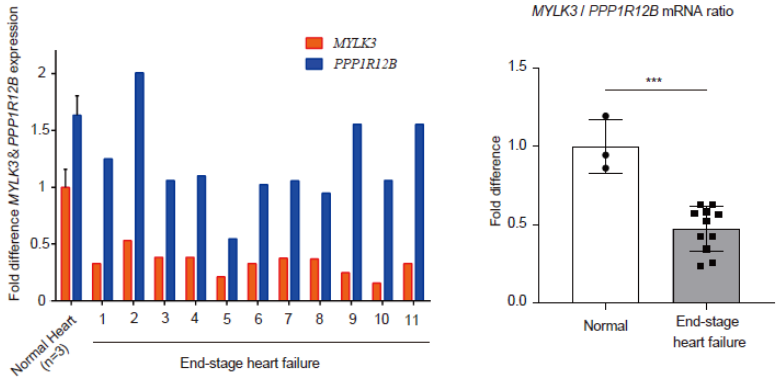
Reduced cMLCK expression → Reduction of MLC2v phosphorylation → Development of heart failure





Replenishment of cMLCK by AAV restores MLC2v phosphorylation and cardiac function.

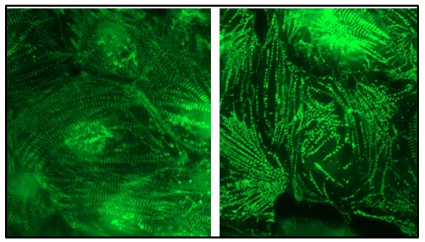
cMLCK activities in failing hearts from DCM patients

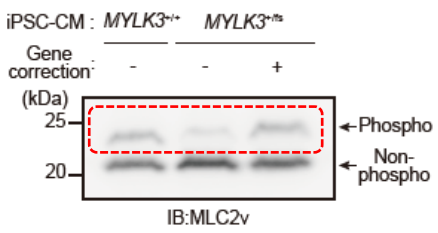


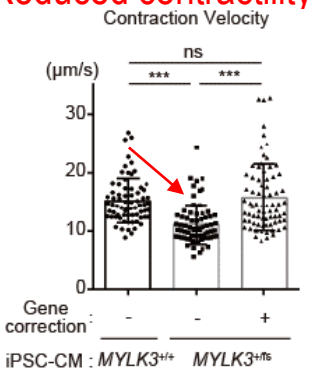
cMLCK activities were suppressed also in the hearts from DCM patients not caused by *MYLK3* mutations.

iPSC-cardiomyocytes from DCM patient with the *MYLK3* frame-shift mutation

Reduced cMLCK expression → Reduction of MLC2v phosphorylation → Reduced contractility



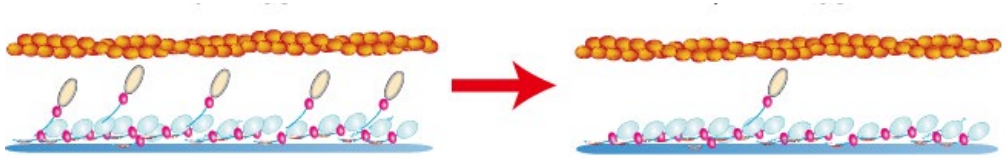




Gene correction by CRISPR/Cas9 restores MLC2v phosphorylation and cardiac function.

HYPOTHESIS

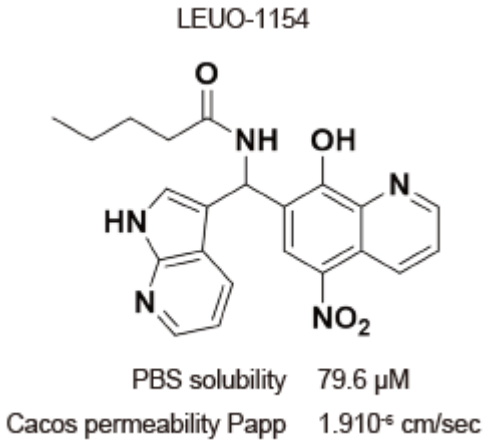
Normal heart → Failing heart



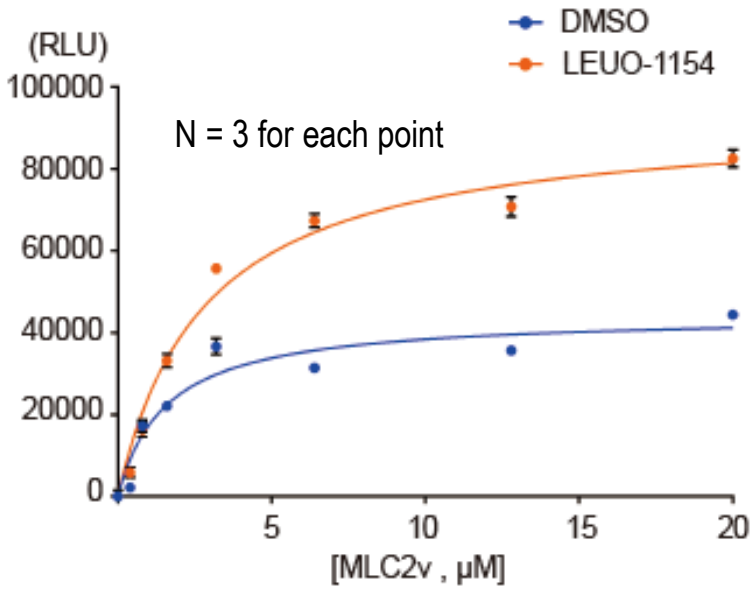
Reduction of MLC2v phosphorylation by depressed cMLCK activity was a common pathway for reduced cardiac contractility observed in advanced HFrEF.

Result - 1　LEUO-1154：新規アロステリックcMLCK活性化因子

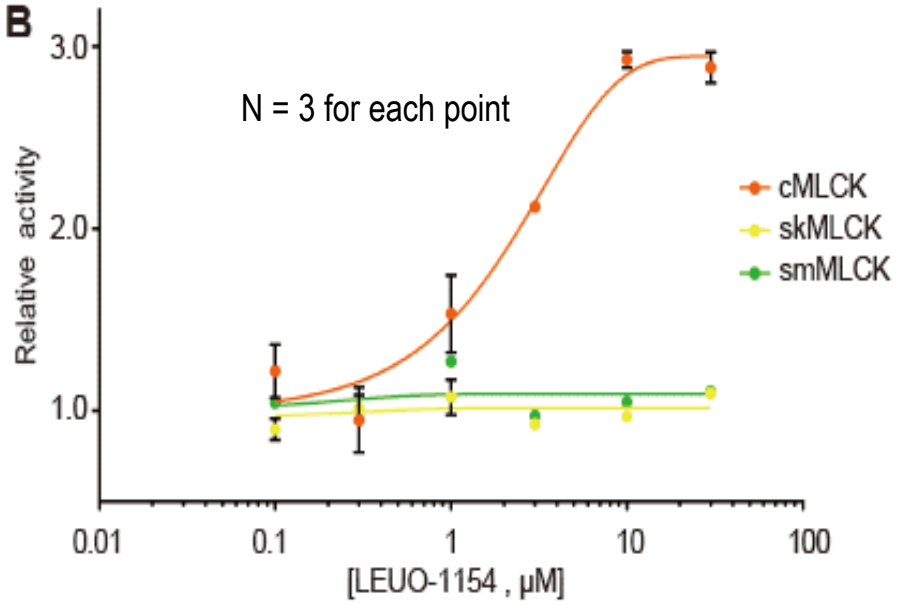
The chemical structure of LEUO-1154, a cMLCK activator



MLC2v dose-dependence of human cMLCK activities in the presence of LEUO-1154.



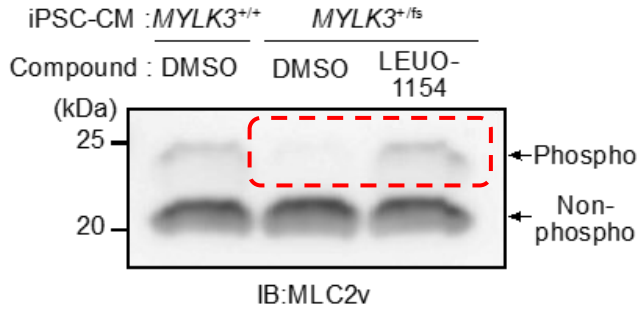
The dose-response effects of LEUO-1154 on the activities of human cMLCK, skMLCK, and smMLCK



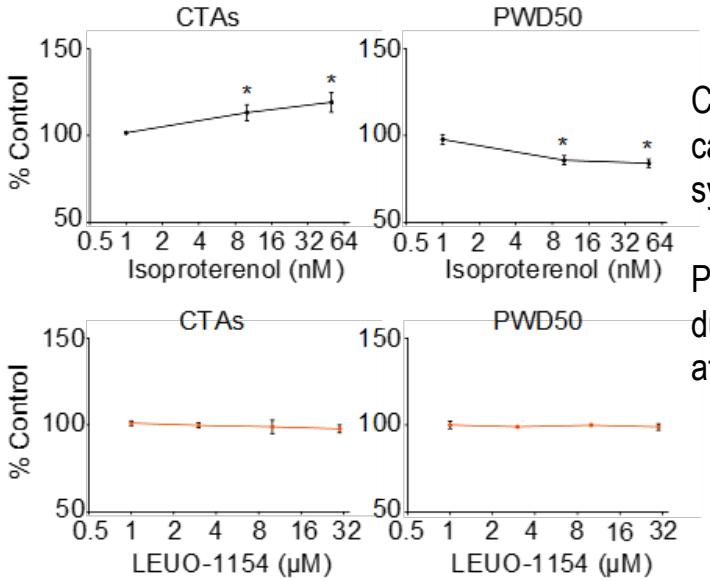
K_m(MLC2v) values of human cMLCK were 1.57 ± 0.58 or 2.81 ± 0.53 μM, and maximum luminescence values were 44,400 ± 4,400 or 92,800 ± 5,500 relative light unit (RLU) that corresponded to a V_{max} of 3.31 ± 0.19 or 7.10± 0.27 mol/min/mol kinase in the presence of DMSO or LEUO-1154 (10 μM), respectively.

Result - 2　LEUO-1154：患者iPS由来心筋細胞の機能を回復

MLC2v phosphorylation (phos-tag SDS-PAGE)



Profiles of calcium transients (FDSS)

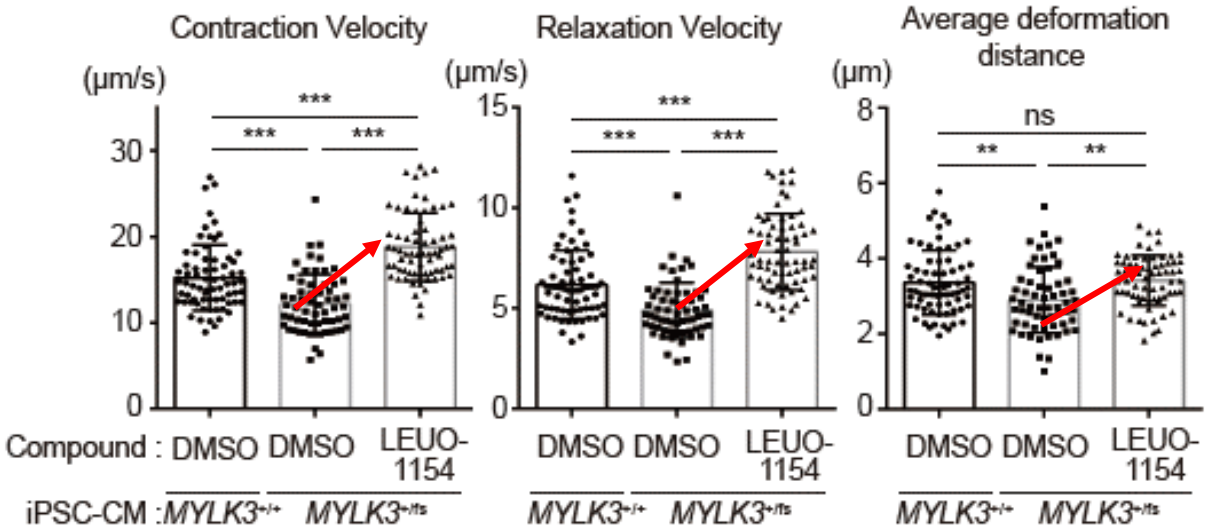


CTAs, amplitude of calcium transients at systole;

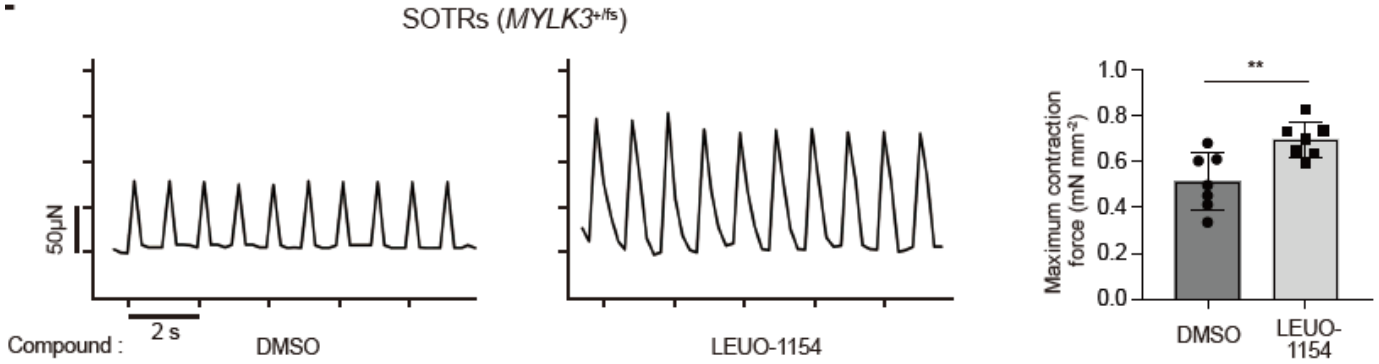
PWD50, pulse width duration measured at 50% CTAs

N = 5 for each point

Cardiomyocyte contraction and relaxation (SI8000)



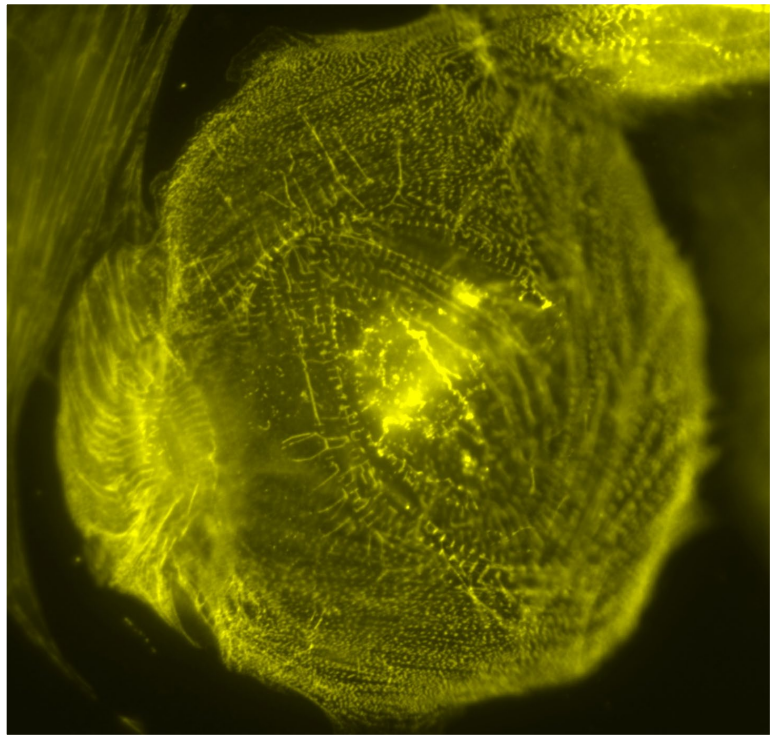
Force measurements using self-organized tissue rings (MicroTester G2)



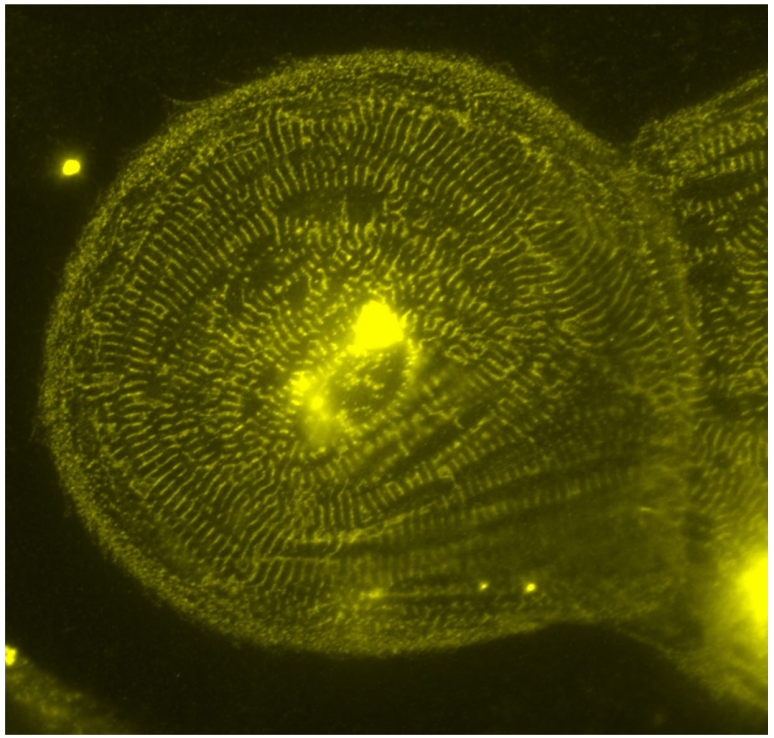
Result - 3　LEUO-1154：サルコメアの再構成を促進

The effect of LEU1154 on the sarcomere structure

DMSO



LEU-1154 (10 μM)



Not published

Summary

cMLCK 活性化剤：

- 心臓サルコメアを直接活性化することによる筋萎縮効果
- 機能不全の心臓における心臓サルコメアの回復効果あり
- カルシトロープに伴う副作用を伴わない心不全患者の血行動態の改善
- Omecamtive Mecambirとは異なるメカニズム

Publication

Circulation. 2023 May 2. doi:10.1161/CIRCULATIONAHA.122.062885

Patent

Filed, not published

Expectation

- **Future works**
  - Further optimization of LEUO-1154.
  - Exploring molecules with other backbone structures.
  - Identification of cMLCK inhibitors for treatment of HCM with hypercontraction.

Suggestions

- We are looking for companies who are interested in developing small molecule LEUO-1154 or its derivatives.
- Collaboration for identifying novel small molecules with our high throughput screening system is also welcome.
- We have other seed compounds, which need further evaluation.