Novel, Potent, and Highly Selective Calcium/Calmodulin-Dependent Protein Kinase II (CaMKII) Inhibitors Reduce Substrate Phosphorylation in Rat Hearts and Prolong Survival in a Mouse Model of Severe Heart Failure

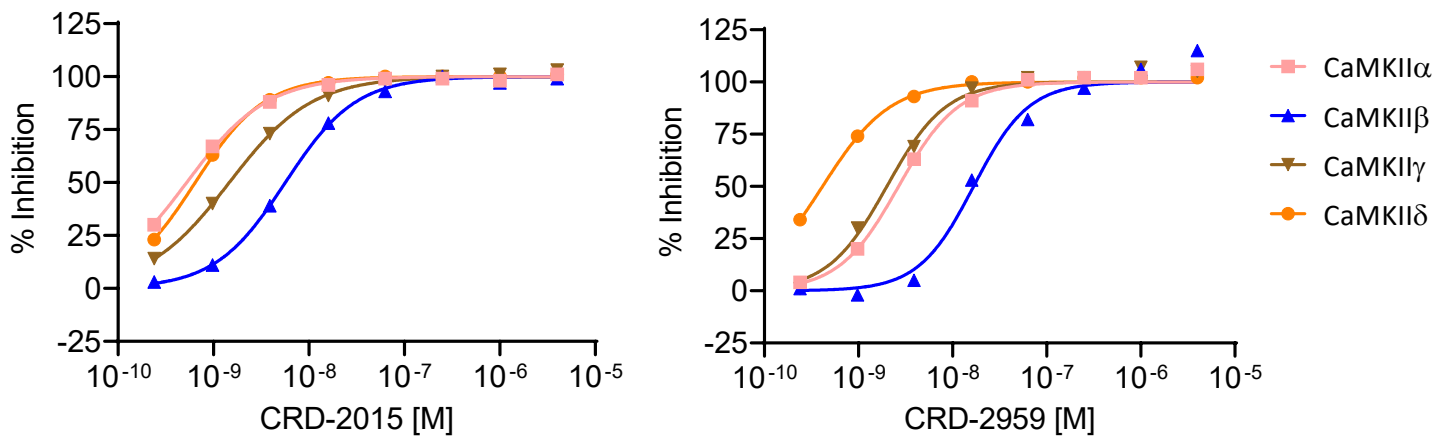
Shuichi Takagahara, Mioko Harada, Mari Asada, Masataka Harada, Masao Hirakata, Howard K. Surks, Michael E. Mendelsohn, Shota Ikeda

COI: The authors are all employees of Cardurion Pharmaceuticals.

Cardurion Has Developed Novel and Potent CaMKII Inhibitors

- CaMKII is a target of longstanding interest in cardiovascular drug development[1]
- CaMKII has been validated in multiple preclinical models of heart failure and arrhythmia, including atrial fibrillation and catecholaminergic polymorphic ventricular tachycardia (CPVT)[2,3]
- Through extensive, iterative structure-activity efforts by Cardurion's medicinal chemists, our team has discovered novel and potent ATP-competitive CaMKII inhibitors
- **CRD-2015 and CRD-2959** are orally active and CNS-excluded compounds with excellent selectivity. Another inhibitor that we have developed, **CRD-4730, is currently in early clinical trials**

Potency: In Vitro Kinase Inhibition (TR-FRET Binding Assay)



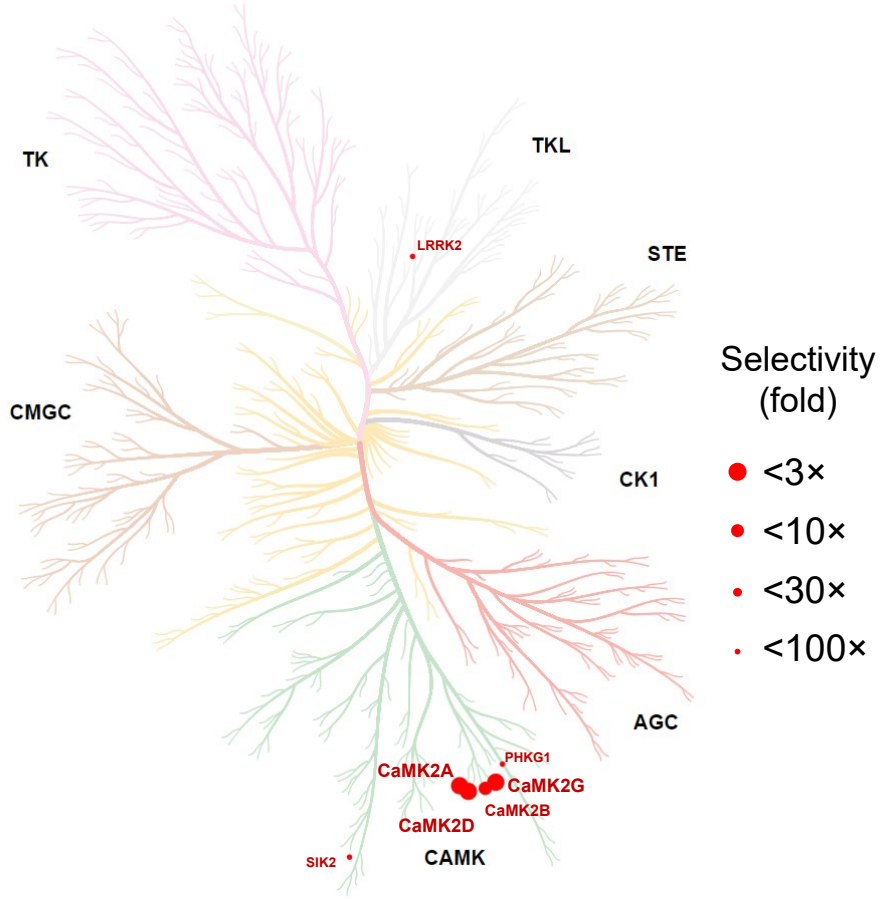
IC_{50} (Selectivity) for CaMKII		
	CRD-2015	**CRD-2959**
CaMKIIδ	0.62 nM	0.40 nM
CaMKIIα	0.53 nM (1×)	2.7 nM (7×)
CaMKIIβ	5.6 nM (9×)	17 nM (43×)
CaMKIIγ	1.5 nM (2×)	2.9 nM (5×)

ATP, adenosine triphosphate; CNS, central nervous system; IC_{50}, half maximal inhibitory concentration; TR-FRET, time-resolved fluorescence energy transfer.

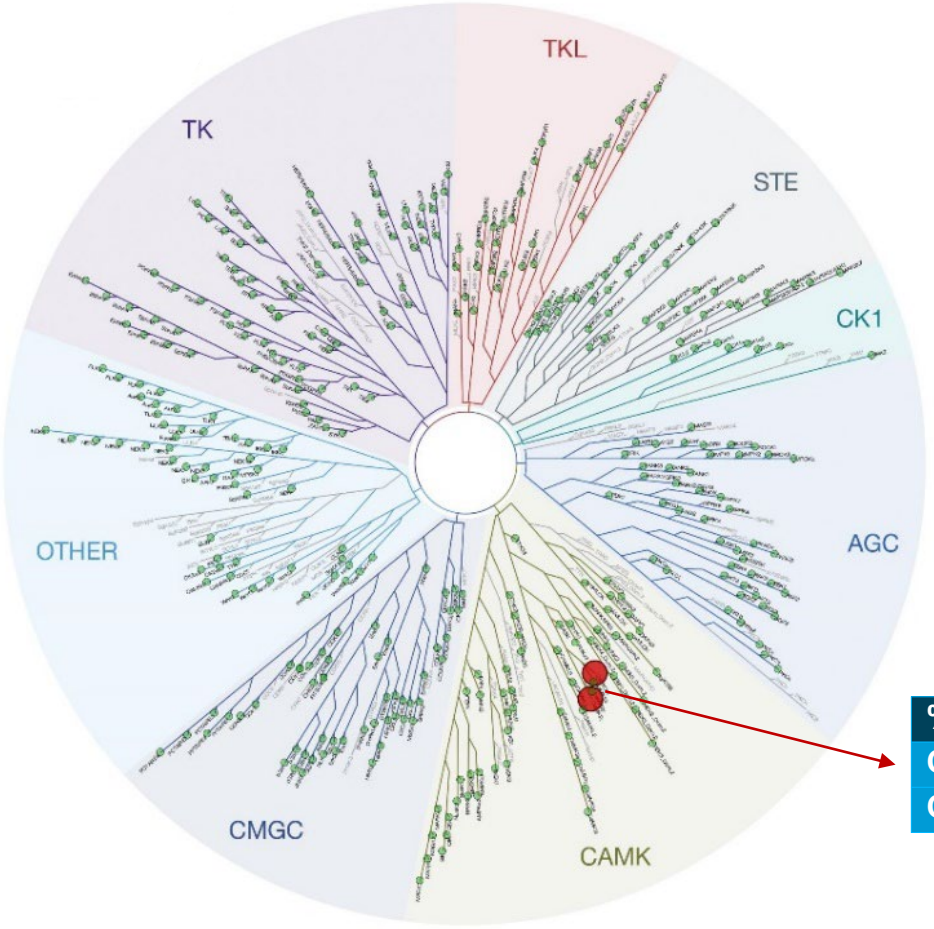
1. Nassal D, et al. *Front Pharmacol.* 2020;11:35. 2. Bezzerides VJ, et al. *Circulation.* 2019;140(5):405-419. 3. Liu Z, et al. *Heart Rhythm.* 2019;16(7):1080-1088.

CRD-2015 and CRD-2959 Are Highly Selective Inhibitors for CaMKII

CRD-2015
(Axcelead GKP Panel: 338 Kinases)



CRD-2959
(Eurofins: KINOMEscan® 468 Kinases)



% Inhibition at 10 nM	
CaMKIIA	93%
CaMKIID	91%

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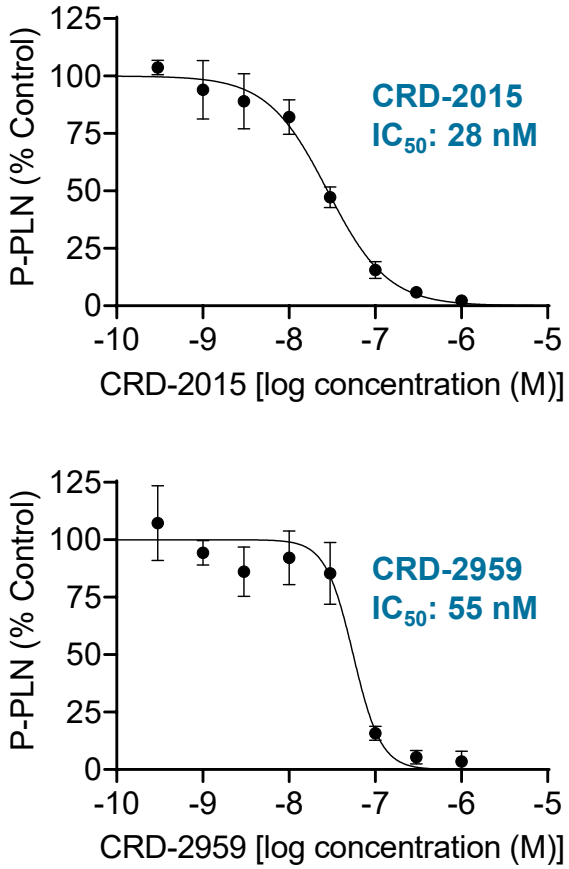
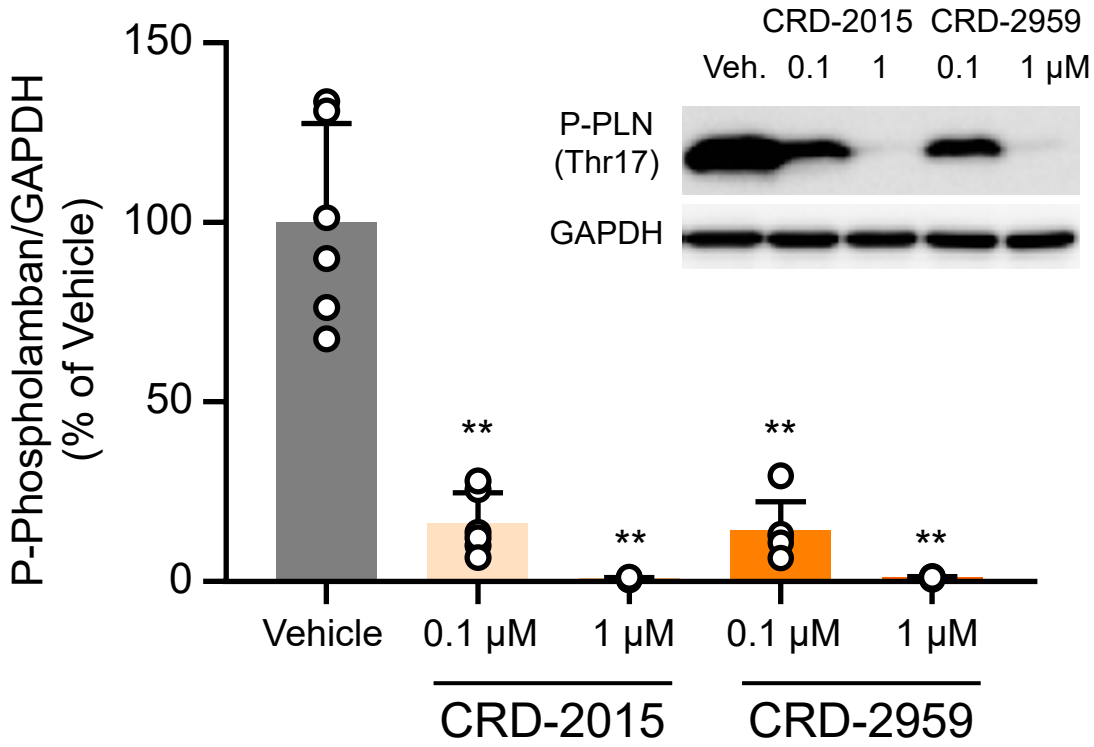
CRD-2015 and CRD-2959 Reduced Phosphorylation of CaMKII-Specific Targets

CaMKIIδ/Phospholamban (PLN)-Overexpressing HEK293 Cells



4-h treatment, mean±SD (n=4).

Neonatal Rat Cardiomyocytes (NRCMs)

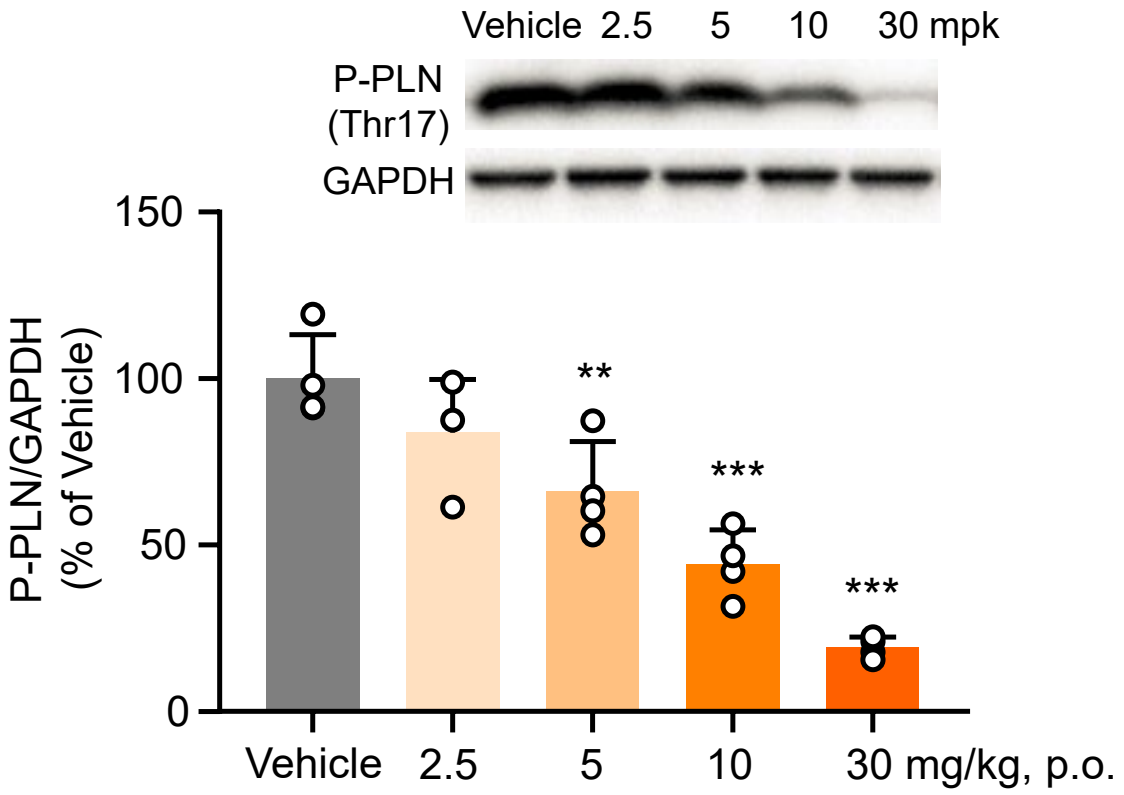


1.5-h treatment, mean±SD (n=6). ***P*<0.01 vs vehicle.

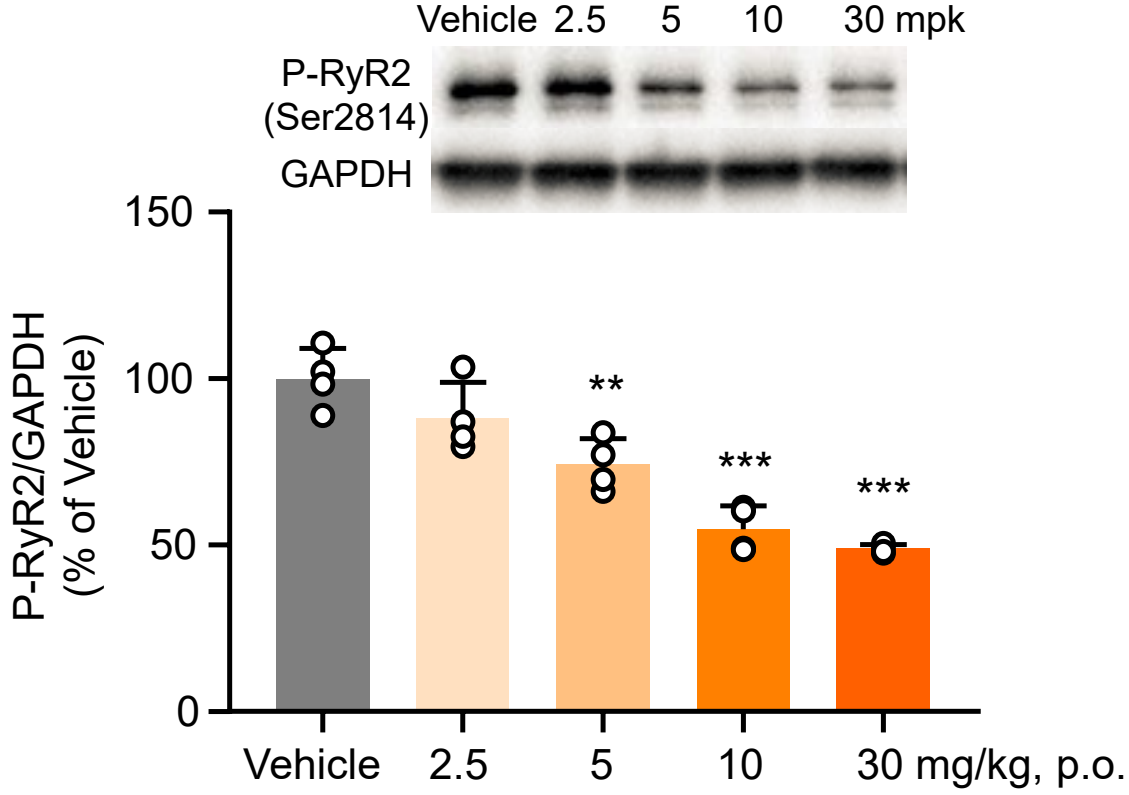
HEK, human embryonic kidney.

Oral CRD-2015 Significantly Reduces Phosphorylated Phospholamban (P-PLN) and Ryanodine Receptor (P-RyR) In Vivo in Normal Rat Hearts

Phospholamban (PLN, Thr17)



Ryanodine Receptor 2 (RyR2, Ser2814)



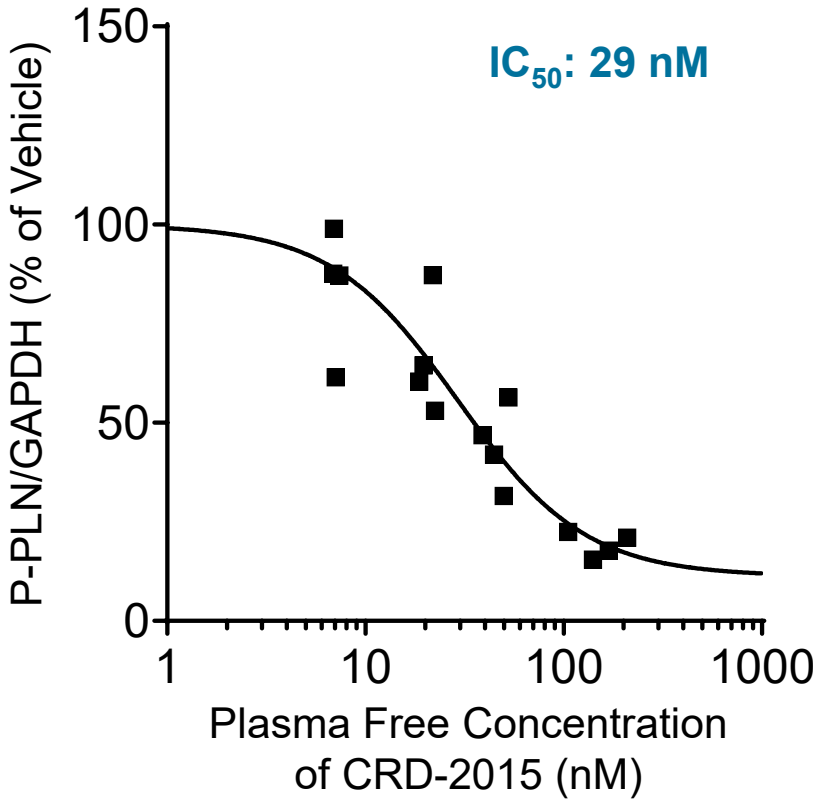
Male Ctrl:CD (Sprague–Dawley) rats at 6 weeks of age, sacrificed at 6 h after dosing vehicle (0.5% methylcellulose), CRD-2015 2.5, 5, 10, 30 mpk, p.o.

Mean±SD (n=4) and individual values. ***P*<0.01, ****P*<0.001 vs vehicle.

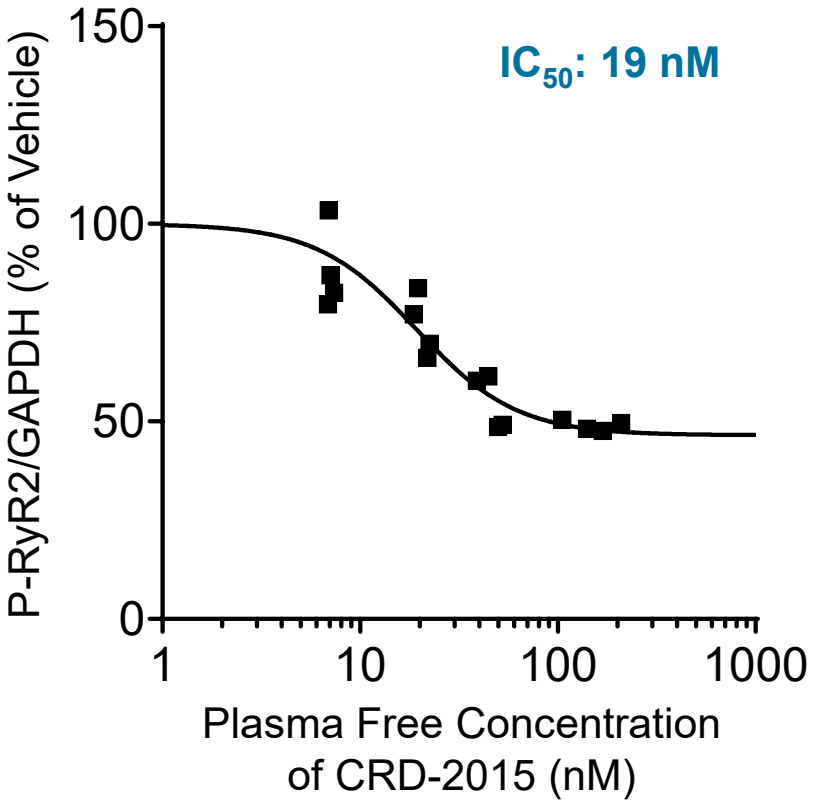
GAPDH, glyceraldehyde 3-phosphate dehydrogenase; p.o., oral; SD, standard deviation.

CRD-2015 Plasma Concentration (PK) and Reduction in Substrate Phosphorylation (PD) Are Well Correlated In Vivo in Normal Rats

Phospholamban (PLN, Thr17)



Ryanodine Receptor 2 (RyR2, Ser2814)

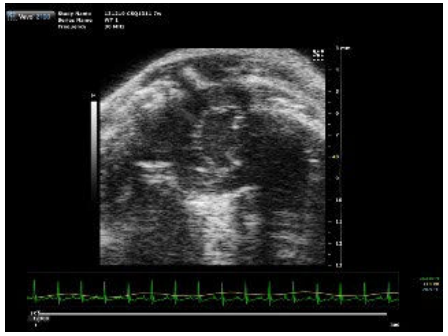


PD, pharmacodynamics; PK, pharmacokinetics.

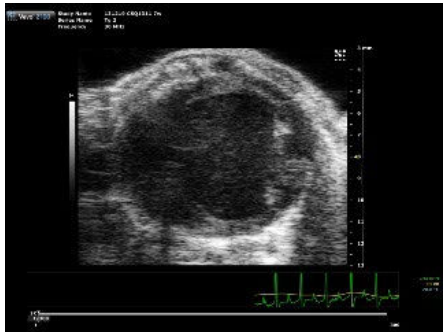
The Calsequestrin-Transgenic (CSQ-Tg) Heart Failure Mouse Model and Standard of Care Heart Failure Therapies

Severe Systolic Disfunction

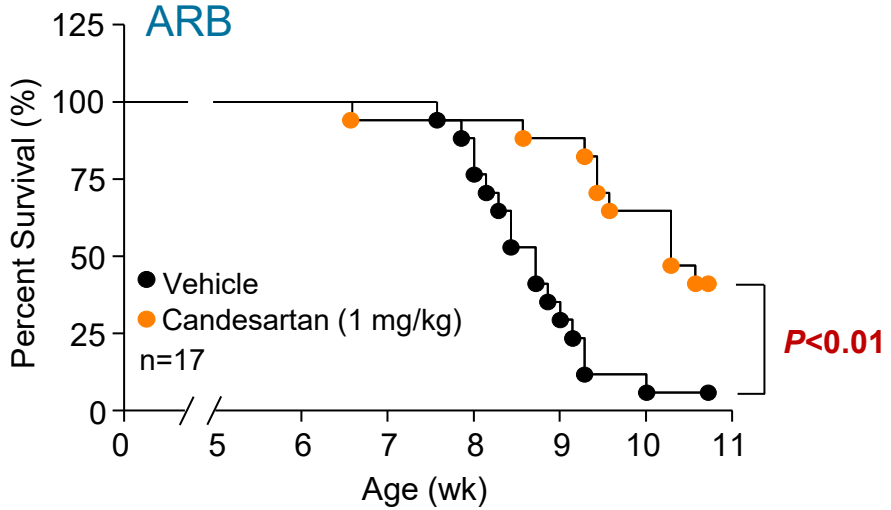
Wild Type (7-Week-Old)

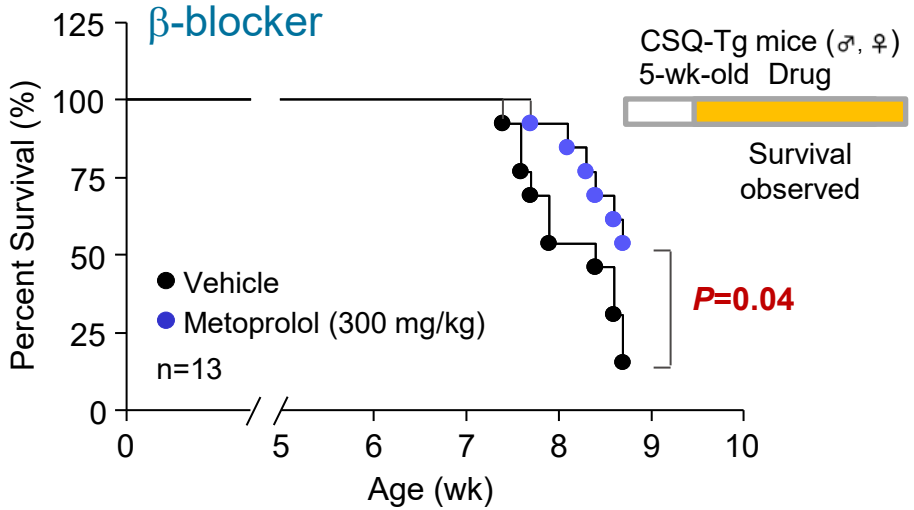


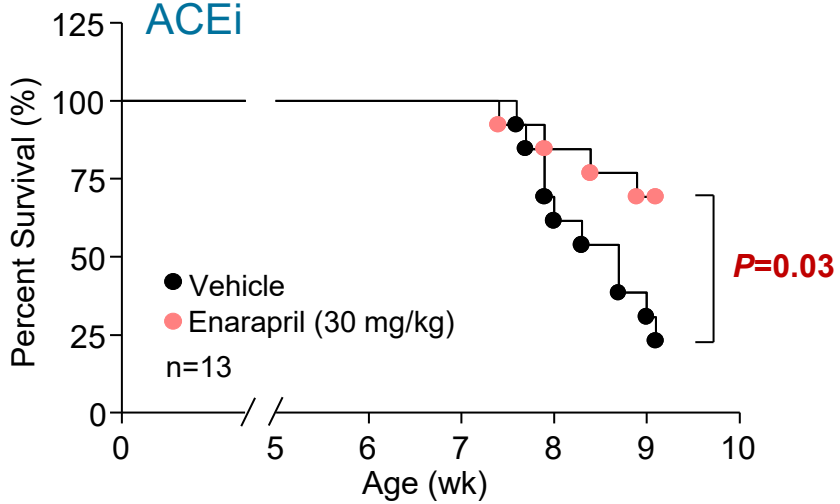
CSQ-Tg (7-Week-Old)

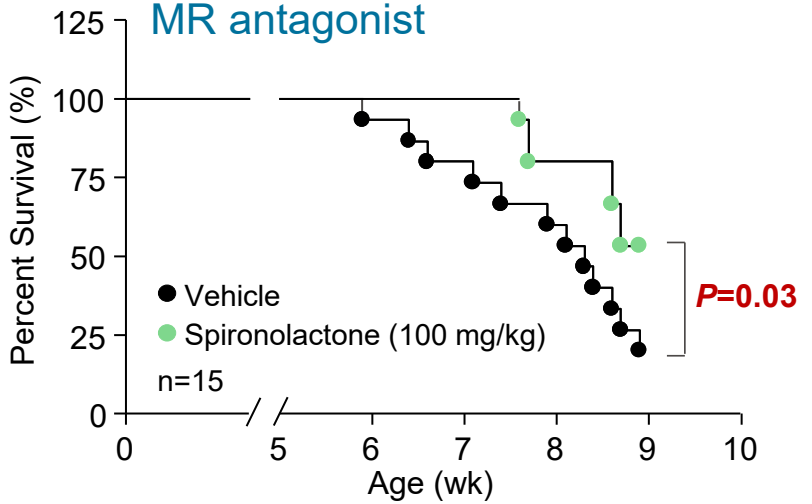


Efficacy of Current Therapies on Survival



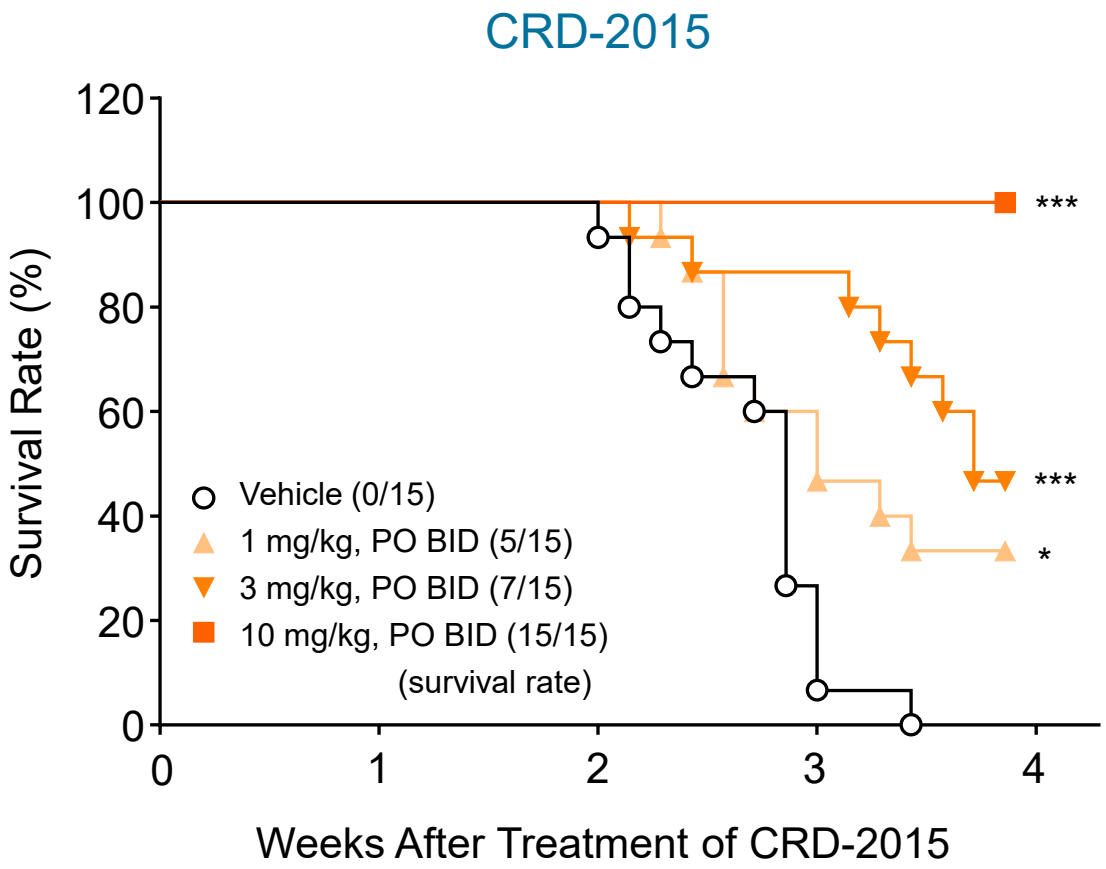


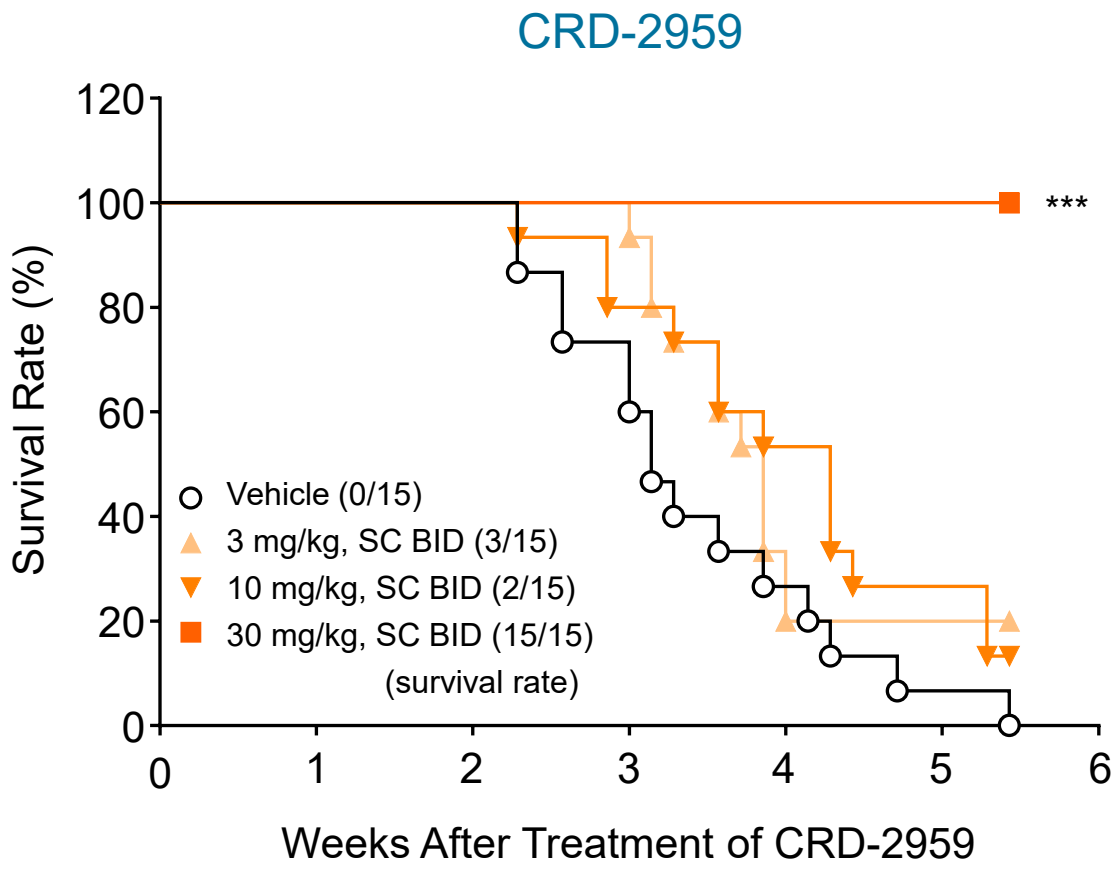




ACEi, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; MR, mineralocorticoid receptor.

Inhibition of CaMKII With CRD-2015 or CRD-2959 Demonstrated a Survival Benefit in the CSQ-Tg Mouse Model of Severe HFrEF





Female CSQ-Tg mice, drug administration started at 5 weeks of age. *P<0.05, ***P<0.001 vs vehicle (log-rank test).

BID, twice daily; HFrEF, heart failure with reduced ejection fraction; SC, subcutaneous.

Summary

- CRD-2015 and CRD-2959 are novel and potent CaMKII inhibitors with excellent selectivity against other kinases
- CRD-2015 significantly decreased levels of phosphorylated phospholamban and ryanodine receptor in neonatal rat cardiomyocytes in vitro and in rat hearts in vivo
- CRD-2015 showed a good correlation between plasma concentration (PK) and a reduction in the substrate phosphorylation (PD) in vivo
- CRD-2015 strongly prolonged survival in the CSQ-Tg mouse model of severe heart failure
- CRD-2959 showed similar pharmacological effects and prolongation of survival in CSQ-Tg mice
- These results demonstrate the successful creation of specific CaMKII inhibitors with efficacy in preclinical models and support clinical development of our CaMKII inhibitors for the treatment of heart failure and other cardiovascular diseases

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Cardurion Pharmaceuticals K.K. Medicinal Chemistry Team

Nobuyuki Matsunaga
Yasufumi Miyamoto
Junya Shirai
Zenyu Shiokawa
Tomohiro Okawa
Takashi Nakahata
Akito Shibuya
Akira Kawada

Consultants

Malcolm MacCoss
Senior Medicinal Chemist Consultant,
Visiting Professor of Chemistry for Medicine at
University of Oxford, Bohicket Pharma Consulting, LLC

Greg Stevens
Senior Drug Safety Consultant, GS Pharma
Solutions, LLC

Donavon McConn
Senior DMPK Consultant,
Lotura Therapeutics, XinThera, Inc.

Takeda Pharmaceutical Company Ltd.

Steve Hitchcock, CSO, Takeda Pharmaceuticals

Axcelead Drug Discovery Partners Inc.

Manami Kaneko
Tomonori Kitaura