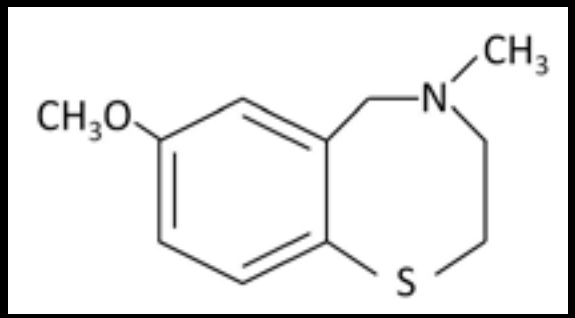
Rycals™ in Mouse Models of *Muscle Disease*



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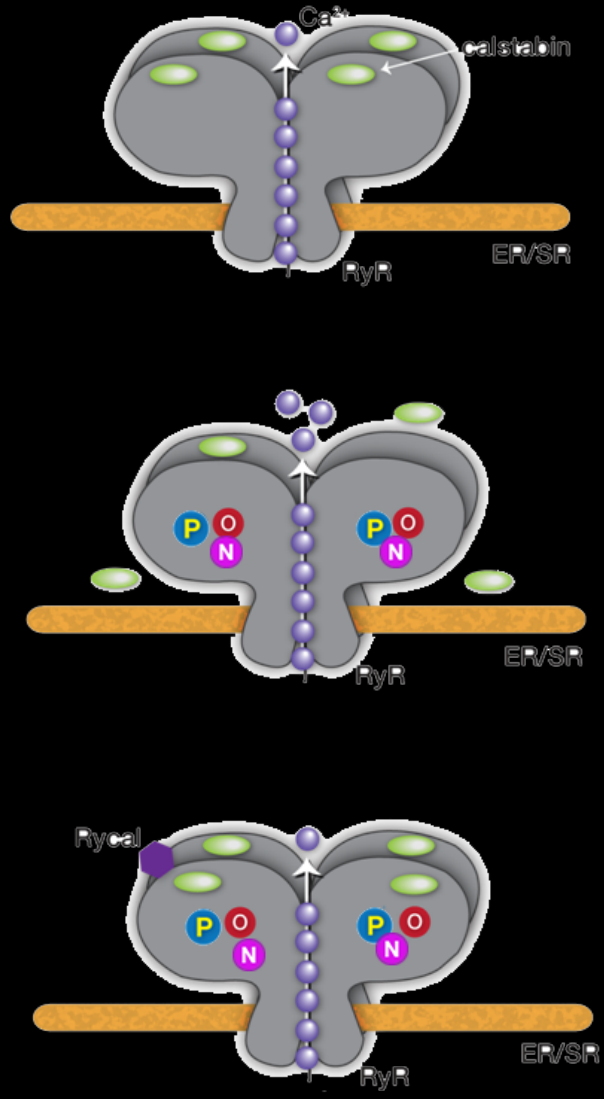
Columbia University
July 23, 2016

COI Disclosure: share holder and SAB Chair ARMGQ Pharma, Inc.

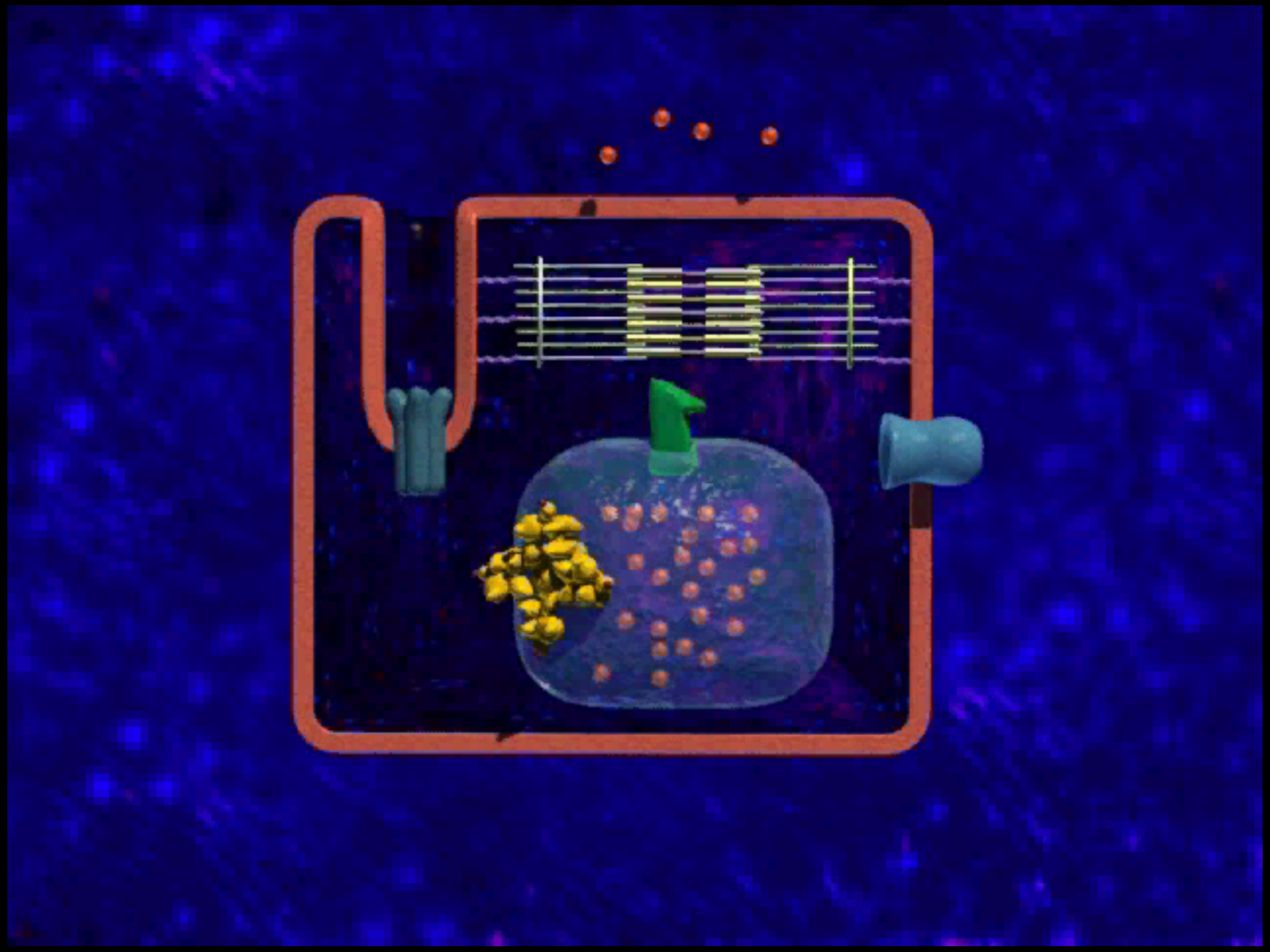
Target Overview – the Ryanodine Receptor

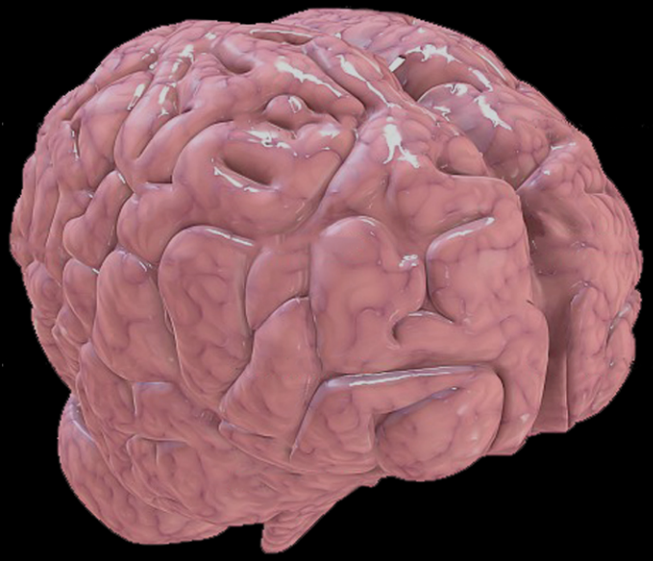
- RyR is the largest known ion channel, located on the endoplasmic or sarcoplasmic reticulum
- Responsible for regulating intracellular calcium flow, from the ER/SR into the cytoplasm. Present in heart muscle (RyR2), skeletal muscle (RyR1), neurons (RyR1, RyR2, RyR3) and other tissues
- RyR maintains calcium homeostasis essential for muscle contraction and neuronal signaling, normally alternating between a resting (closed) and excited (open) state.
- In certain disease states RyR becomes "leaky", resulting in muscle weakness (heart failure, muscular dystrophy) or cognitive impairment (PTSD, AD, HD).
- A "leaky" RyR state is caused by hyper-phosphorylation, oxidation and nitrosylation, which results in dissociation of calstabin (FKBP) and persistent channel opening. **Similar findings in Duchenne Muscular Dystrophy and in RyR1 myopathy**
- Rycals promote the rebinding of calstabin, returning the RyR to a "non-leaky" state. Rycals do not block RyR, but rather restore cycling of normal open and closed states.



What is RYANODINE?

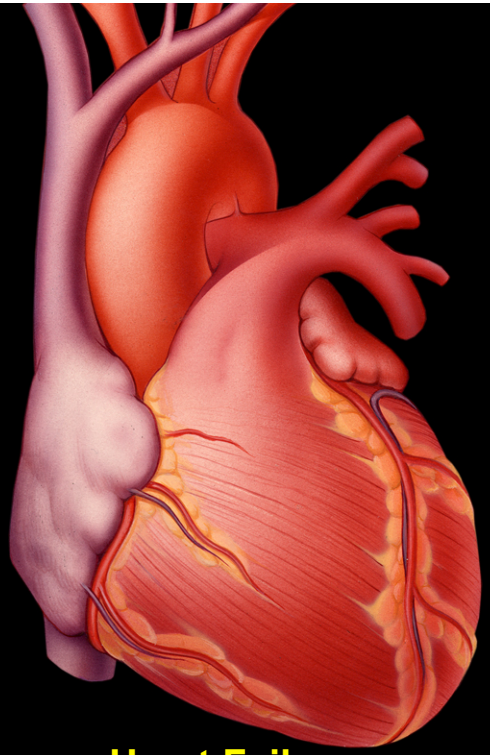
Ryania speciosa





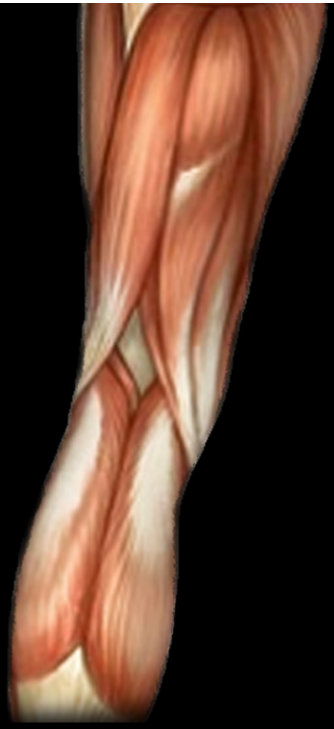
Seizures
Cognitive Dysfunction

Cell 150:1055, 2012



Heart Failure
Arrhythmias (CPVT, AF)

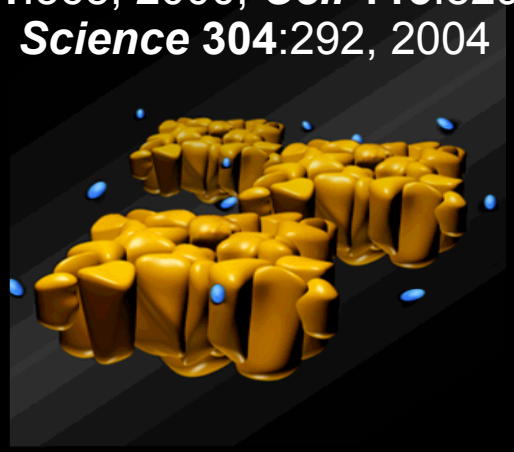
Cell **101**:365, 2000; ***Cell*** **113**:829, 2003
Science **304**:292, 2004



Muscular dystrophy
Sarcopenia
Cancer-associated muscle weakness

RyR1 myopathy (unpublished)

JCI 125:1968, 2015

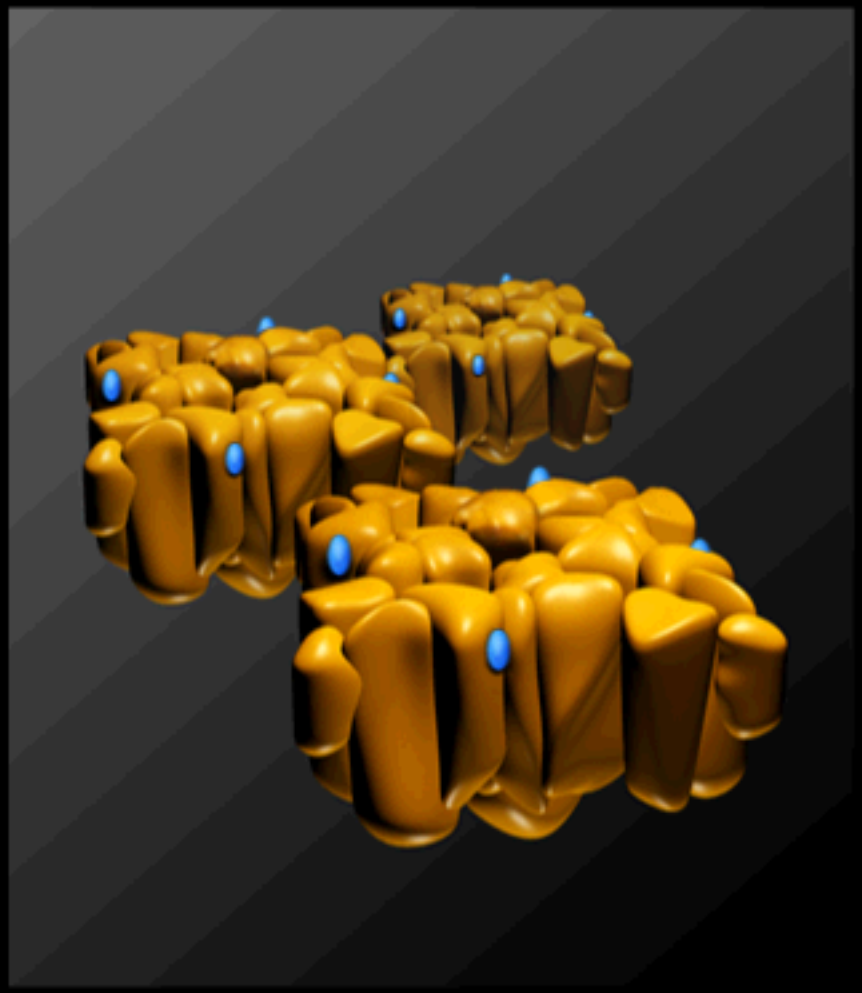


Nature Medicine **15:**325, 2009
Cell Metabolism 14:196, 2011
Nature Medicine 21:1262-71, 2015

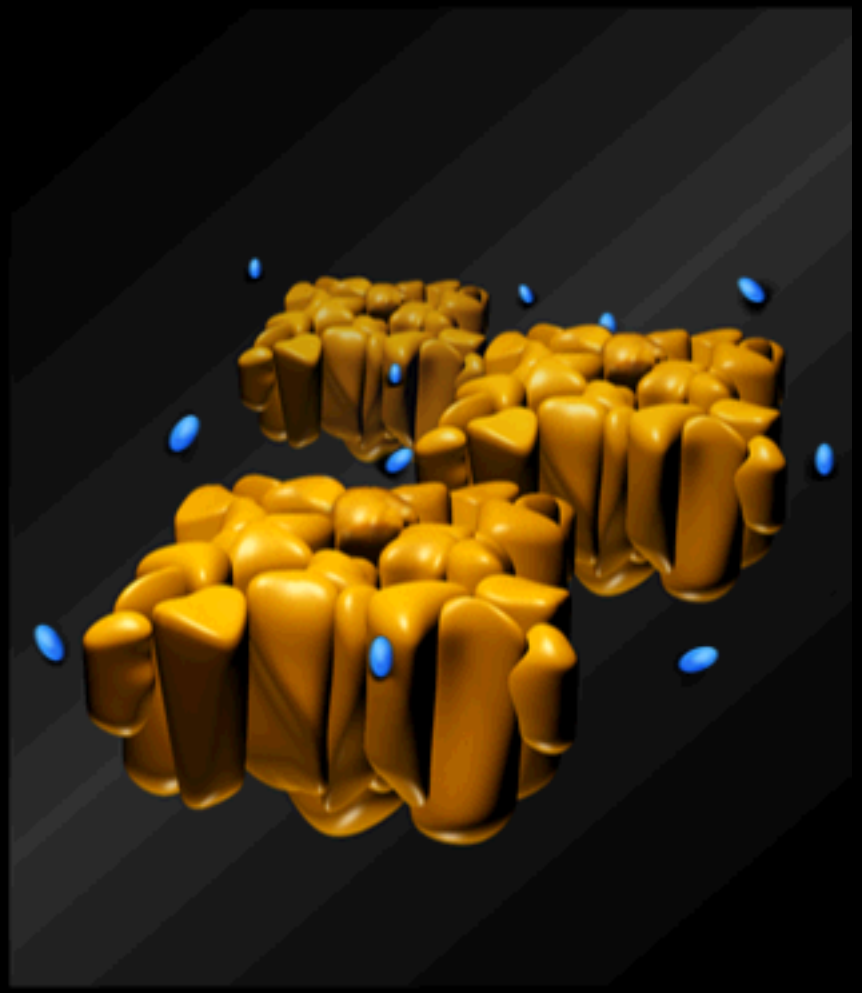
RyR "leak" shown to play a role in multiple disease states in animal models

Model/Disease	RyR Subtype/ Tissue	Rycal™ shown to be effective	Key Publication
Heart Failure	RyR2/cardiac	Yes	Marx, S.O., et al. *Cell*, (2000) Huang, F., et al., *PNAS (*2006)
Arrhythmia	RyR2/cardiac	Yes	Lehnart, S.E., et al., *PNAS* (2006)
CPVT	Mutant RyR2/ cardiac	Yes	Behrens, X.H., et al., *Science*, (2004) Lehn art , S.E., et, al., *JCI* (2008)
Sarcopenia	RyR1/skeletal muscle	Yes	Andersson, D. et al., *Cell Metab* (2011)
Muscular Dystrophy	RyR1/skeletal muscle	Yes	Bellinger, A.M., et al., *Nature Med, (*2009) Andersson, D. et al.., *Skel Musc* (2012)
PTSD Alzheimer's Disease Huntington's Disease	RyR2/neurons	Yes Yes (unpublished) TBD	Liu, X., et al., *Cell* (2012) Oulès, B., et al., *J. Neurosci* (2012) Chen, X., et al., *Mol Neuro (2011)* Suzuki, M., et al., *BBRC* (2012)
Cancer Cachexia	RyR1/skeletal muscle	TBD	Waning et al *Nature Medicine*, 2015 Nov; 21(11):1262-71

RyR1 can be leaky in RyR1 myopathies

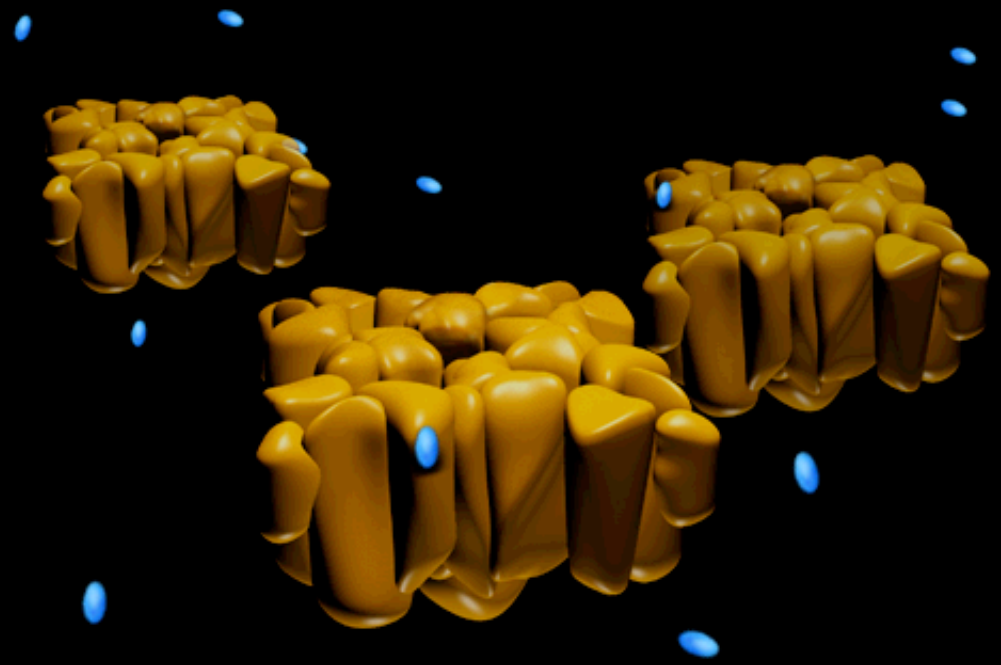


RyR1 from normal muscle



RyR1 from RyR1 myopathy muscle

Rycals fix the leak in RyR1 & RyR2, prevent HF progression, are anti-arrhythmic and improve exercise capacity in animal models



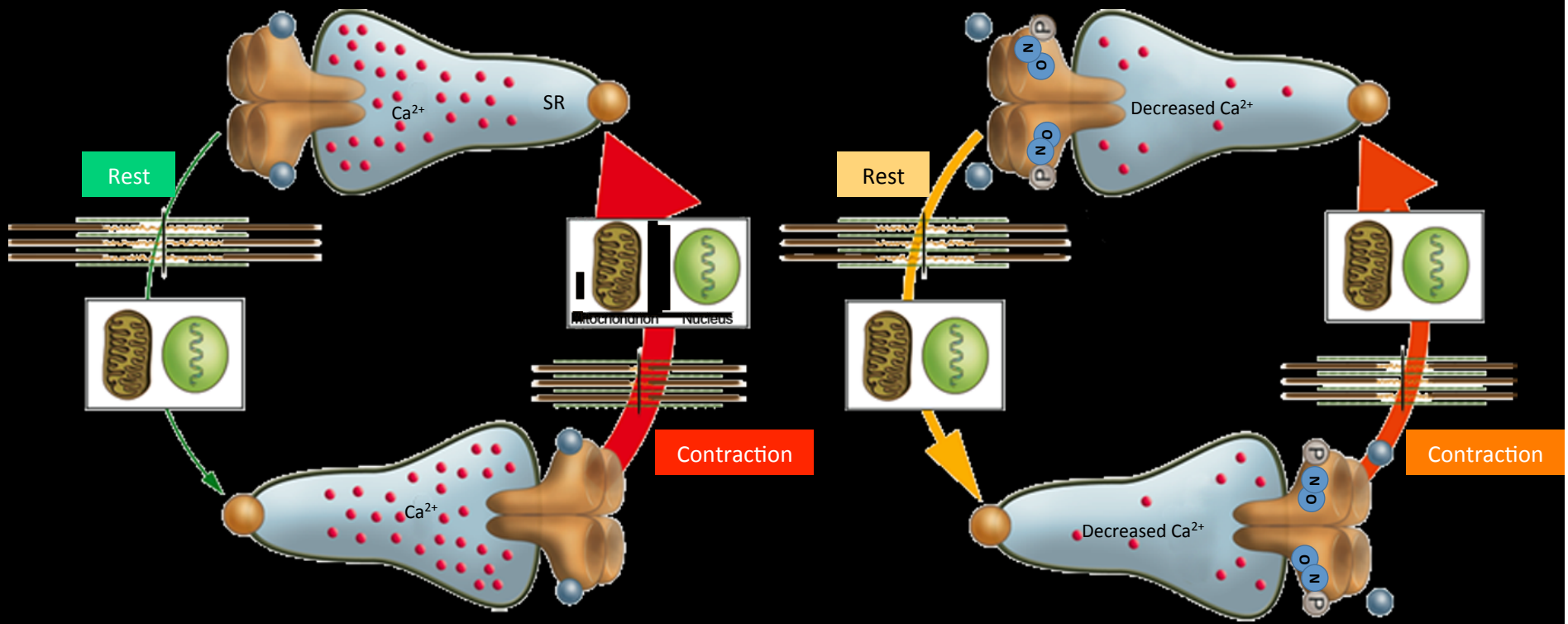
Rycals improve EF, reduce LV size and decrease BNP levels in heart failure patients in Phase IIa clinical trial: 2nd generation rycal (ARM210) started September 2015 (Phase I)

ARM107 (S107) in *Mouse Models of Muscle Disease*

- RyR1 in diseased skeletal muscle is remodeled (oxidized)
- RyR1 in diseased skeletal muscle is depleted of regulatory protein, Calstabin1

- Models:
  - *mdx* mouse (dystrophin-deficient; Duchenne Muscular Dystrophy)
  - *Sgcb*-null mouse (β-sarcoglycan-deficient; Limb Girdle Muscular Dystrophy, type 2E)
  - Aged C57Bl/6 mouse (24 months-old; Sarcopenia)
- Rycal™ treatment prevents depletion of Calstabin1 from modified RyR1, reduces spontaneous Ca^{2+} sparks in both skeletal and cardiac muscle, reduces serum CK, reduces calpain activation, and improves muscle histology
- Rycal™ treatment increases exercise capacity, improves grip strength, reduces force deficit, decreases diaphragm pathology and increases EDL muscle specific force
- Rapid onset of beneficial Rycal™ effects

Leaky RyR1 Channels in Skeletal Muscle: Rycals™ Inhibit RyR1 Leak and Increase Exercise Capacity in Disease States



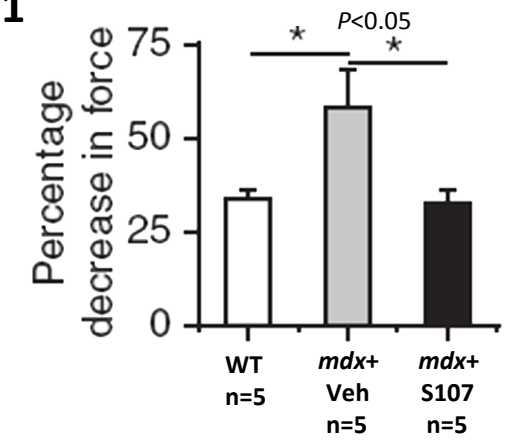
Normal RyR1

Leaky RyR1

Duchenne Muscular Dystrophy – *mdx* mouse model

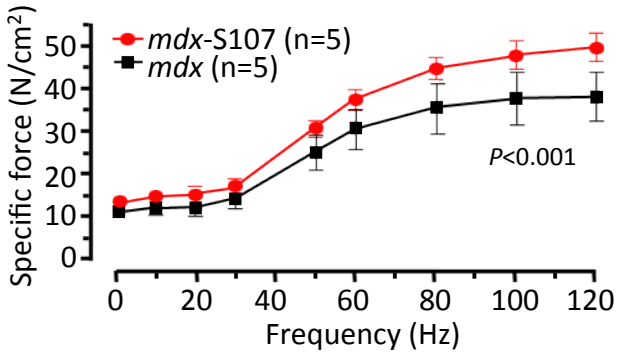
Rycal™ S107 (ARM107) Treatment Improves Muscle Function

1



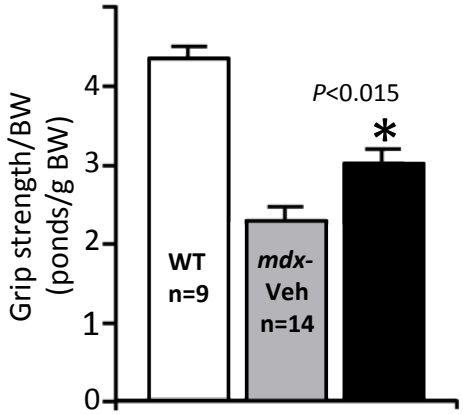
Reduces force deficit

2



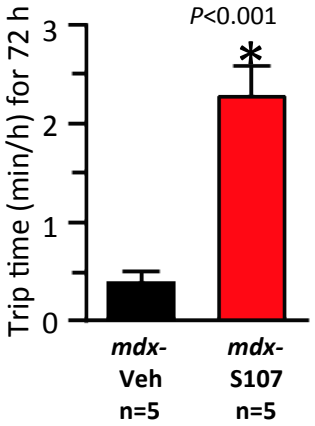
Increases EDL specific force

3



Improves grip strength

4



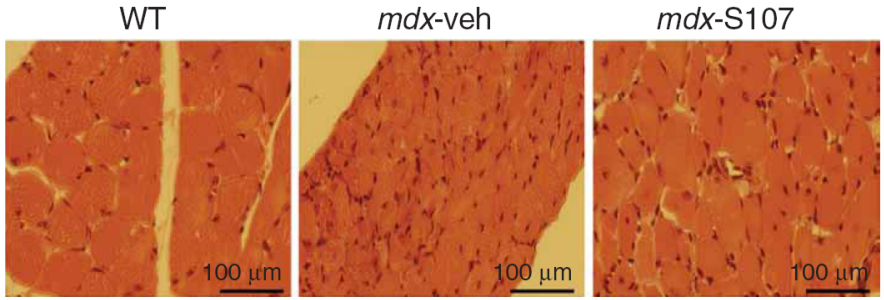
S107 ~37.5 mg/kg/d P.O.
Plasma concentration:
~35 +/- 21 ng/ml
10 days

Increases exercise capacity

Bellinger, et *al* (2009) *Nat Med* 15:325

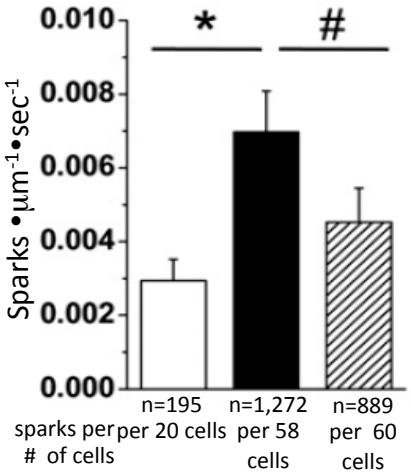
Duchenne Muscular Dystrophy – *mdx* mouse model

Rycal™ S107 (ARM107) Treatment Improves Diaphragm Histology and Cardiomyocyte SR Ca^{2+} Leak



H&E-stained images from **diaphragm** of WT mice, *mdx* mice treated with vehicle or with S107 for 4 weeks via implanted osmotic pump.
(Delivery 0.11 ul/h; S107 @ 80 ug/ul)

Bellinger, et *al* (2009) *Nat Med* 15:325



S107 @ 25 mg/100 ml P.O.

* *P* < 0.05 WT vs mdx
\# *P* < 0.05 mdx vs S107-mdx

- SR Ca^{2+} leak assessed by Ca^{2+} spark analysis in **cardiomyocytes** isolated from *mdx* mice.
- Diastolic SR Ca^{2+} leak is estimated by the average spark frequency.

Fauconnier et *al* (2010) *PNAS* 107:1559

ARM210 / S48168
Skeletal Muscle Program
Supported by Servier/ARMGO

ARM210 / S 48168: Overall Profile

- Well-characterized, orally available and water soluble small molecule with good activity on the primary target
- Distinct, but related, chemical structure compared to cardiovascular clinical candidate, ARM036
- Attractive pharmacokinetic properties, both *in vitro* and *in vivo,* including 3-fold higher skeletal muscle penetration and longer half-life compared to ARM036
- Clean Safety Pharmacology profile
- No alerts after dose-ranging Tox and TK in rat & dog
  - In-life start of 4-week GLP toxicity studies (rat, dog): early 2013.
- Active in the *mdx* mouse model
- Genus covering ARM210 and uses patented. Favorable patentability assessment on ARM210 molecule, selection patent filed.

In vivo Efficacy Study with ARM210 / S 48168 in *mdx* Mice

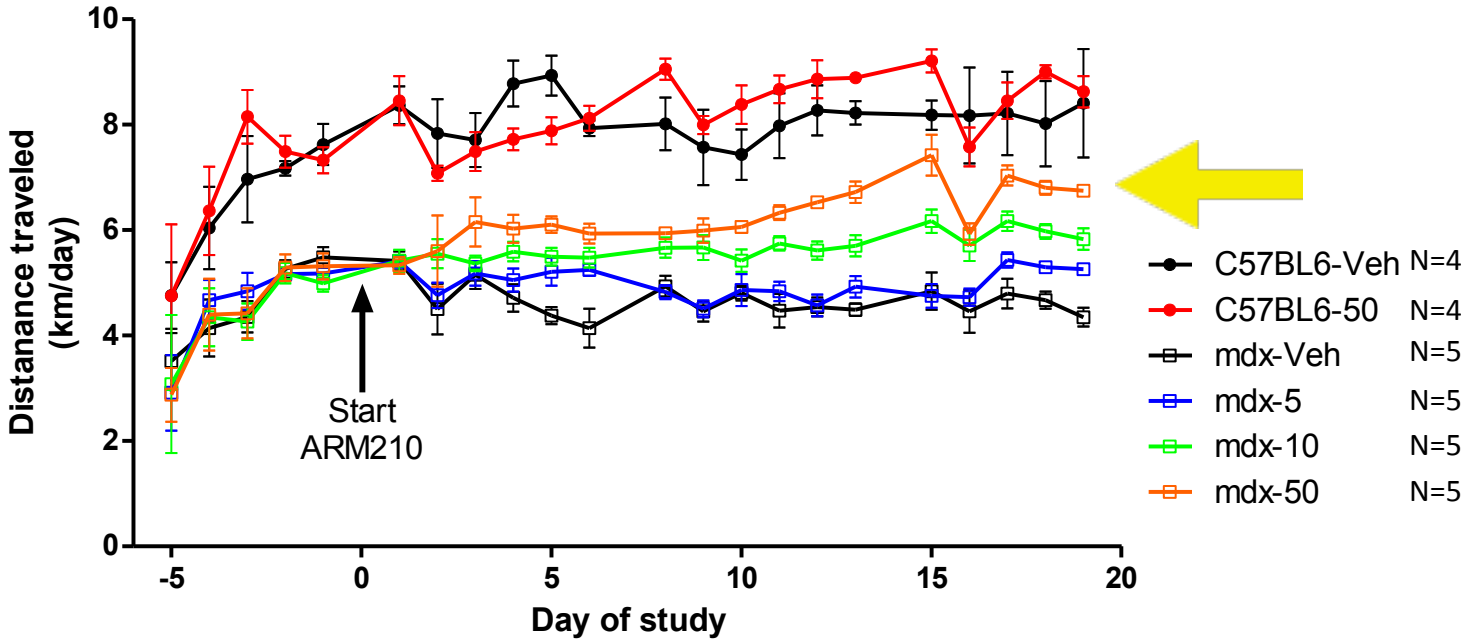
- Study NCP12-002-04: Identify Minimal Effective Dose
  - *mdx* mice: ARM210 @ 5, 10, 50 mkd (target doses*) and Vehicle
  - C57BL/6 mice: ARM210 @ 50 mkd (target dose*) and Vehicle

* ARM210 was dosed *ad lib* in drinking water. Actual doses were calculated based on weekly water consumption. For *mdx* mice: ~8, ~13 and ~62 mg/kg/day. For C57BL/6 mice: ~68 mg/kg/day.

Mouse voluntary exercise testing: can a Rycal improve exercise capacity in mice with muscular dystrophies?

Study NCP12-002-04: Dose-Dependent Increase in Voluntary Wheel Activity in ARM210-treated *mdx* Mice

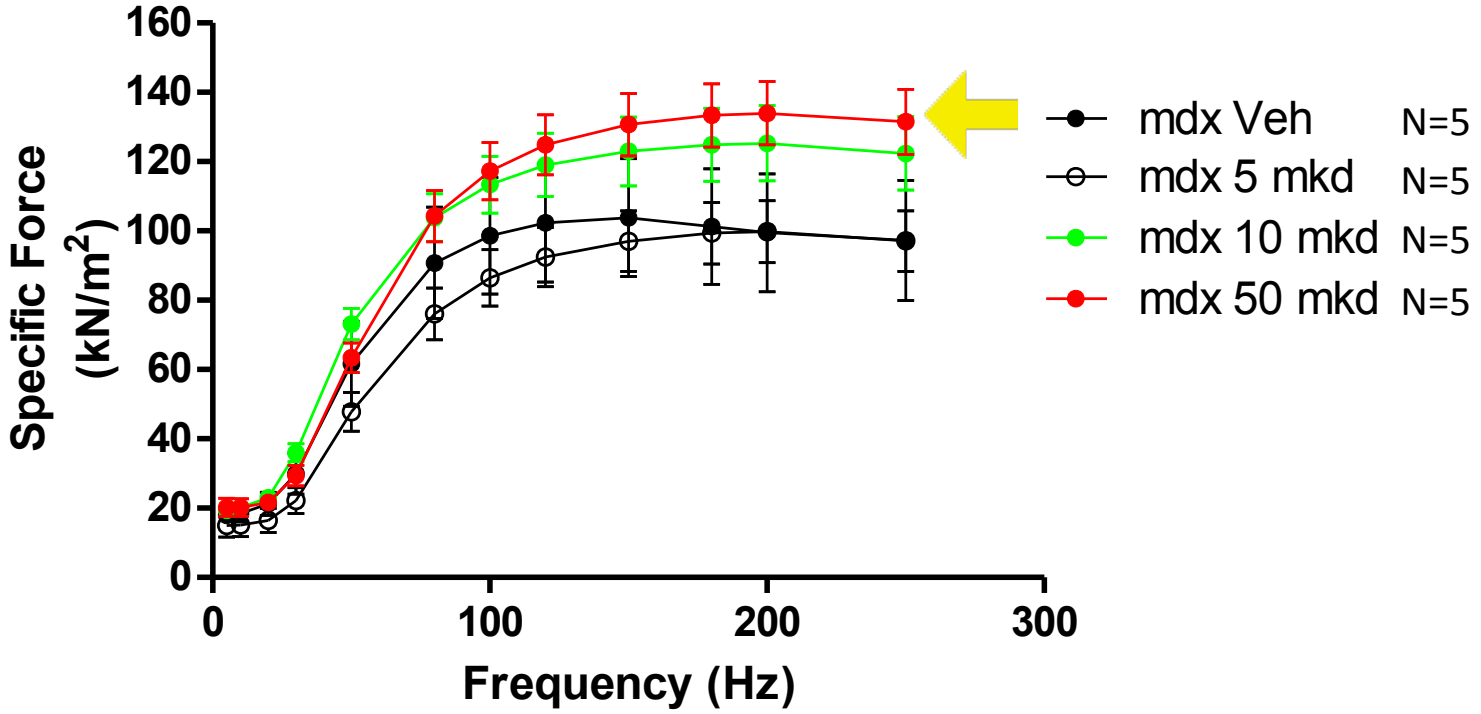
(preliminary unaudited data)



- Activity of *mdx* mice treated with ARM210 at 10 and 50 mg/kg/day is significantly greater than activity of vehicle-treated *mdx* mice ($p<0.0001$ for daily distance traveled, days 3 -19)
- No significant effect of ARM210 treatment on activity of C57BL/6 mice

Study NCP12-002-04: ARM210 Shows a Dose-Dependent Increase in EDL Muscle Specific Force

(preliminary unaudited data)



* EDL muscle specific force peaks at 150 Hz. Specific force in the vehicle-treated group declined rapidly after 150 Hz due to one EDL muscle (of five tested).

Skeletal Muscle Rycal Candidate: ARM210 / S 48168

Compound profile is favorable for continued development

Minimum effective dose established in *mdx* mice

Next Steps: complete Phase I safety trial – if all goes well start Phase II trial in DMD (run by Servier) and in RyR1 myopathy in 2017 at the NIH directed by Katy Meilleur with ARMGO.

Patient voluntary exercise testing: can a Rycal improve exercise capacity in humans with muscular dystrophies?