Over-expressing ion channel mutations in hiPSC-derived cardiomyocytes to model arrhythmogenic diseases

Marc Pourrier, Ph.D

Precision Medicine & Ion Channel Retreat

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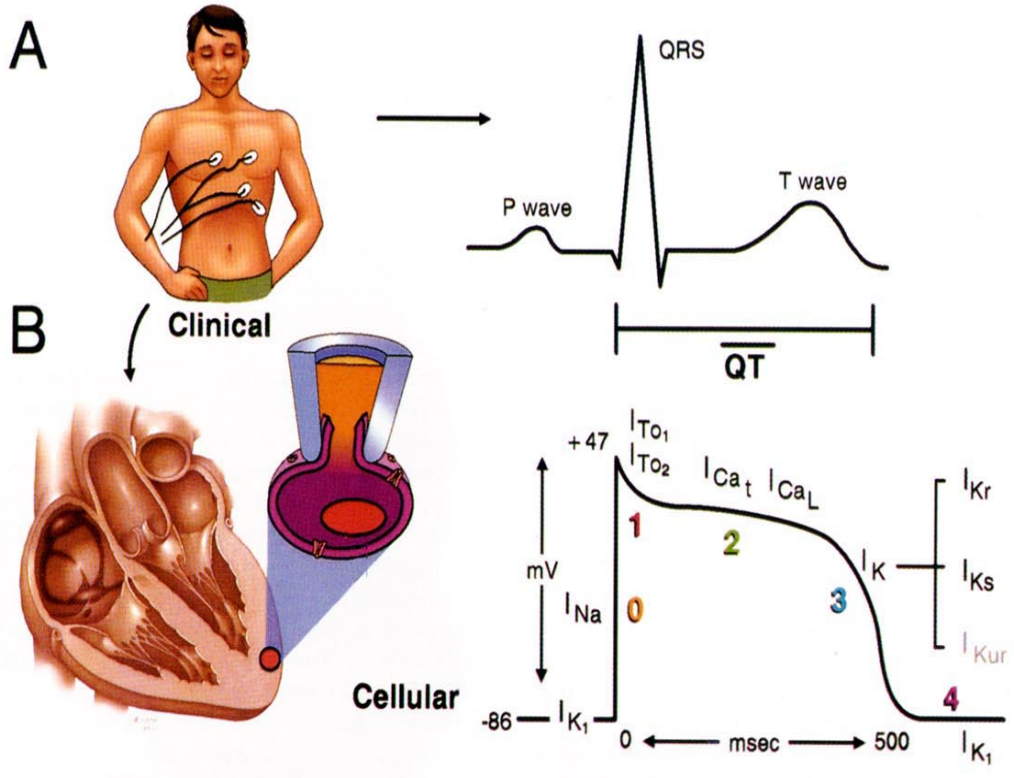
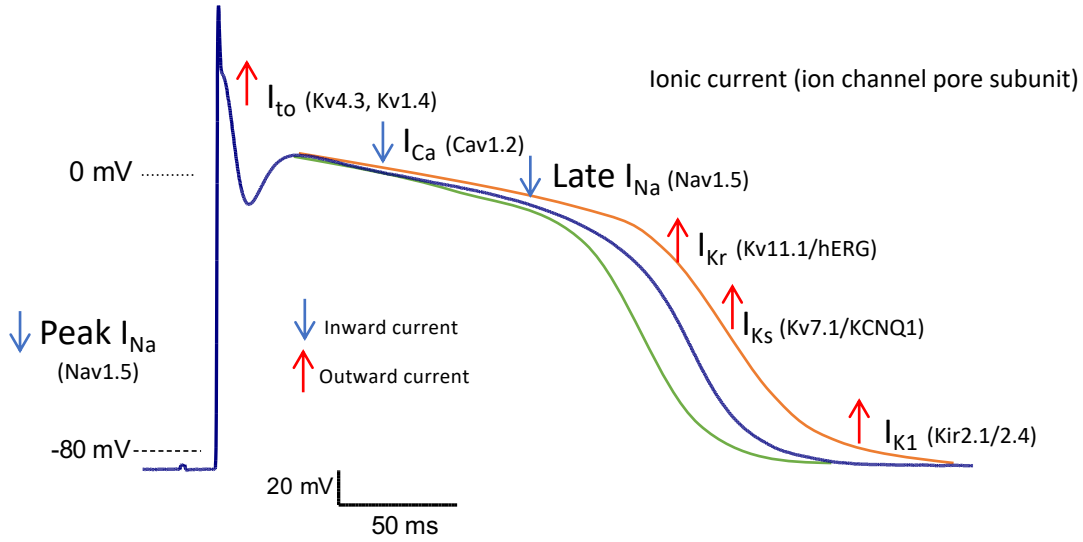
mpourrier@ionsgate.com

IonsGate Preclinical Services Inc.

From body surface to ion channels



Ackerman, *Mayo Clin Proc 1998, 73:250-269*



Ion current	Effect on action potential	Effect on ECG
I_K inhibition, enhanced late I_{Na} or $I_{Ca,L}$	Lengthens	↑ QT
Late I_{Na} inhibition $I_{Ca,L}$ inhibition, I_K opener	Shortens	↓ QT

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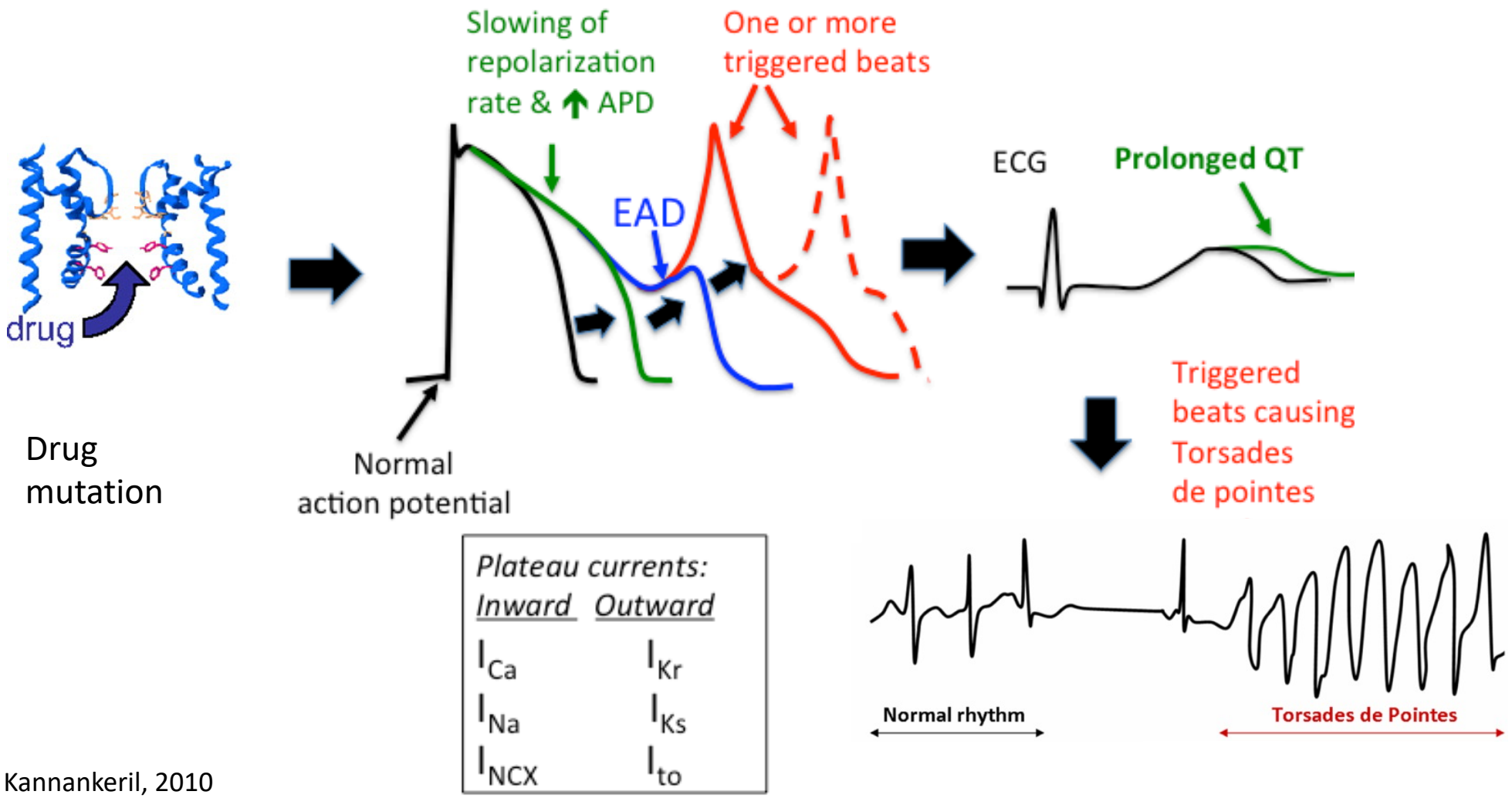
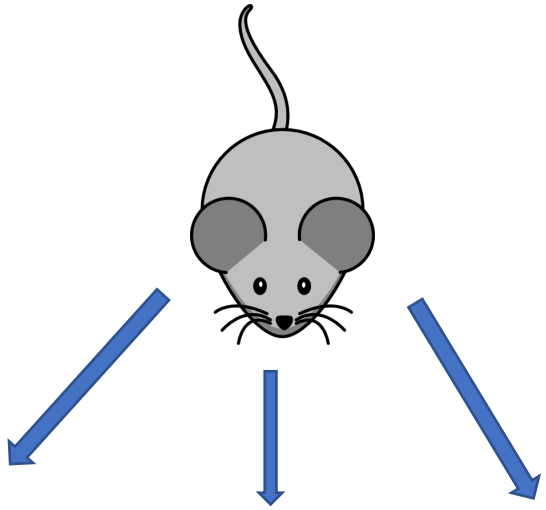
Mechanisms of Torsades de Pointe arrhythmias



Modified from Kannankeril, 2010

Disease modeling: There is a need for more predictive models



In Vivo:
Electrocardiogram (ECG)

Whole heart:
1- Cardiac function
1- Ca^{2+} wave
2- Voltage mapping

Isolated cardiomyocytes
1- Patch clamp
2- Ca^{2+} handling
3- Contraction

Modified from Bezzerides et al, Circ J. 2017

Limitations:
- Creating a transgenic mouse model is time consuming.
- Differences in ionic current between mouse and man.
- Difficult to scale up for drug screening.

Likewise, transgenic non-cardiac human cell lines, e.g. hERG-overexpressing HEK-293 cells, were shown to model cardiac diseases insufficiently since they do not recapitulate the complex cardiac phenotype, e.g. sarcomere organization, calcium handling, metabolism, and (electro)physiology.

Many human phenotypes fail to be successfully recapitulated in these models.

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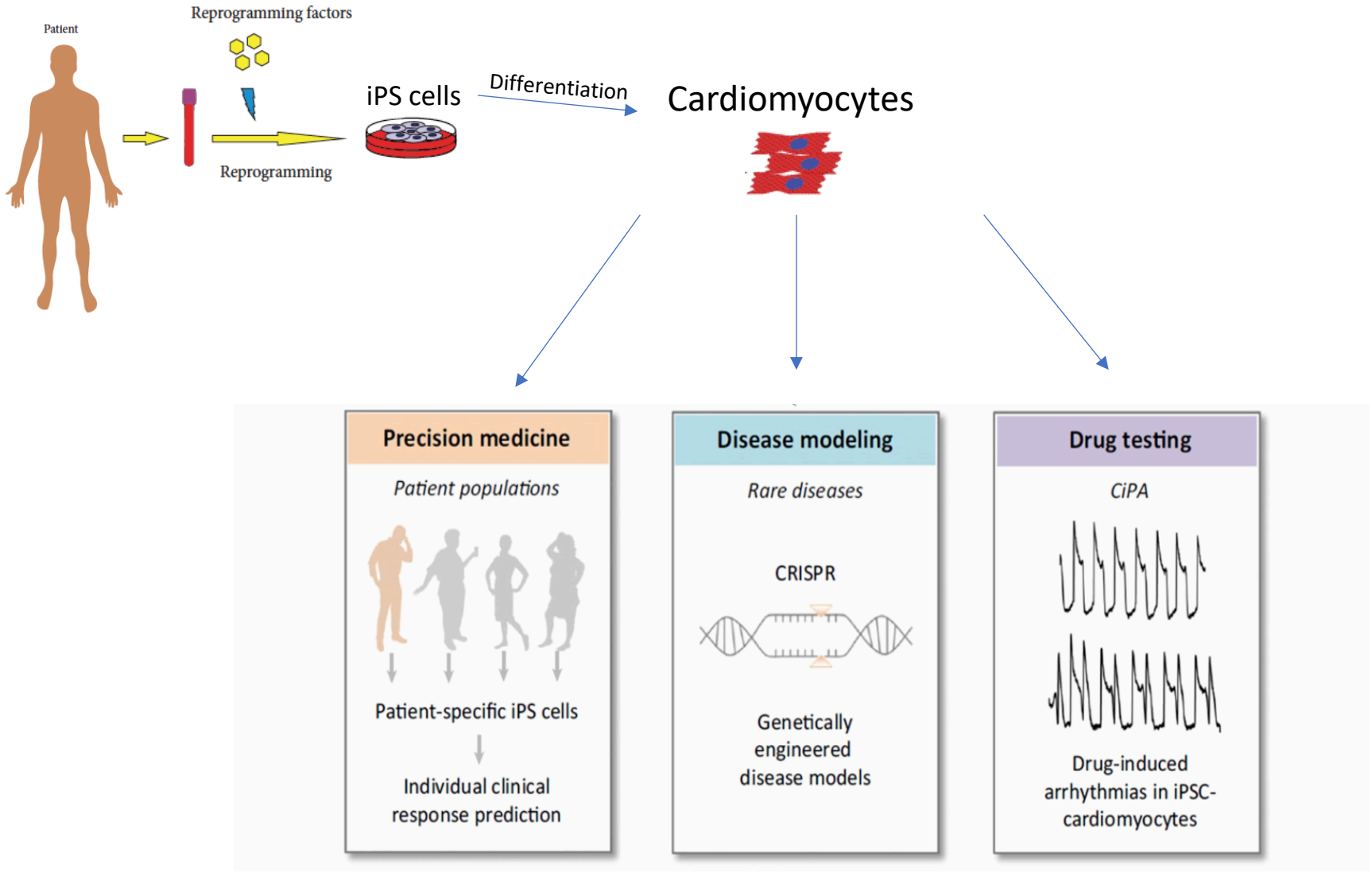
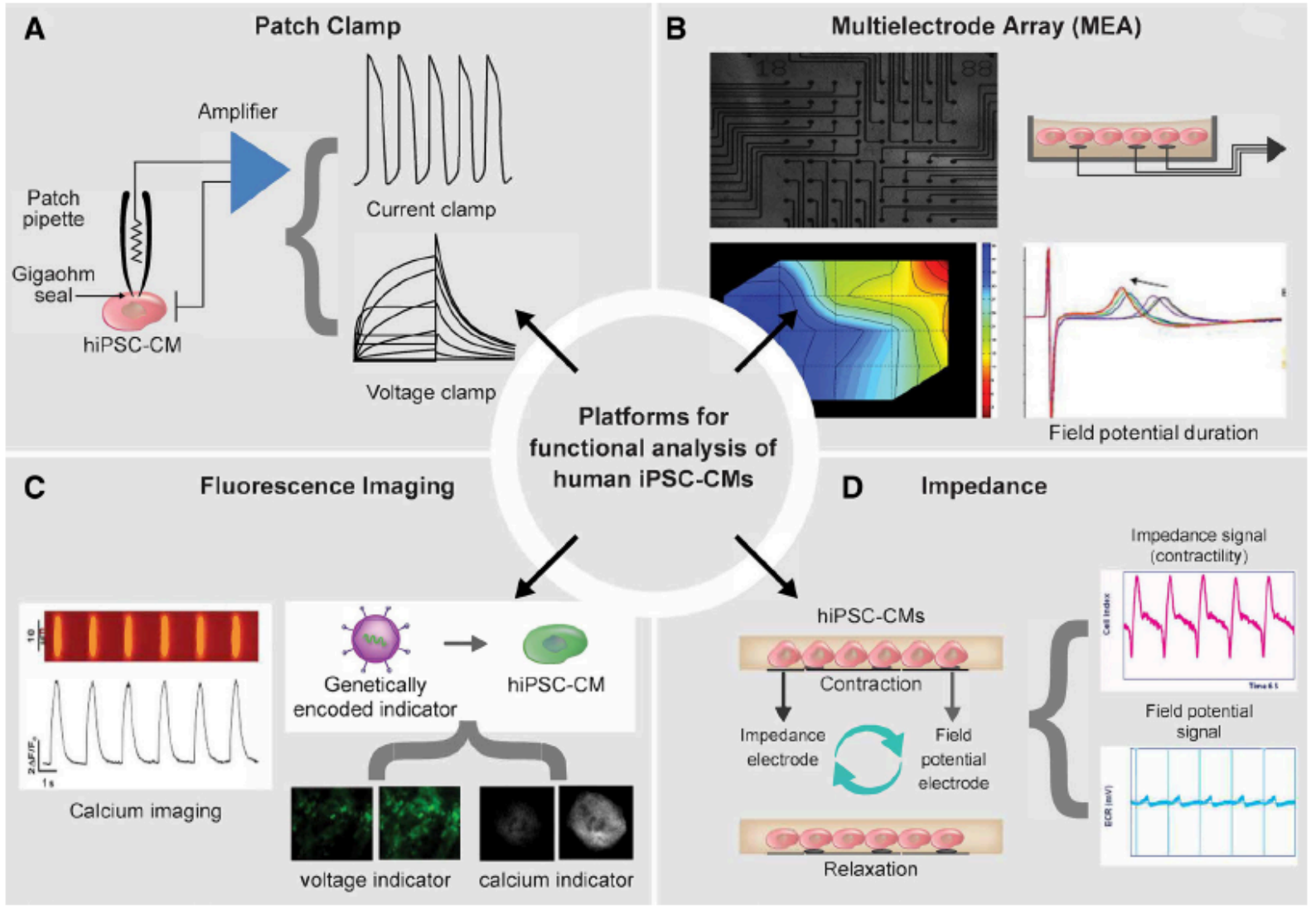
The excitement of Induced Pluripotent Stem (iPS) cells



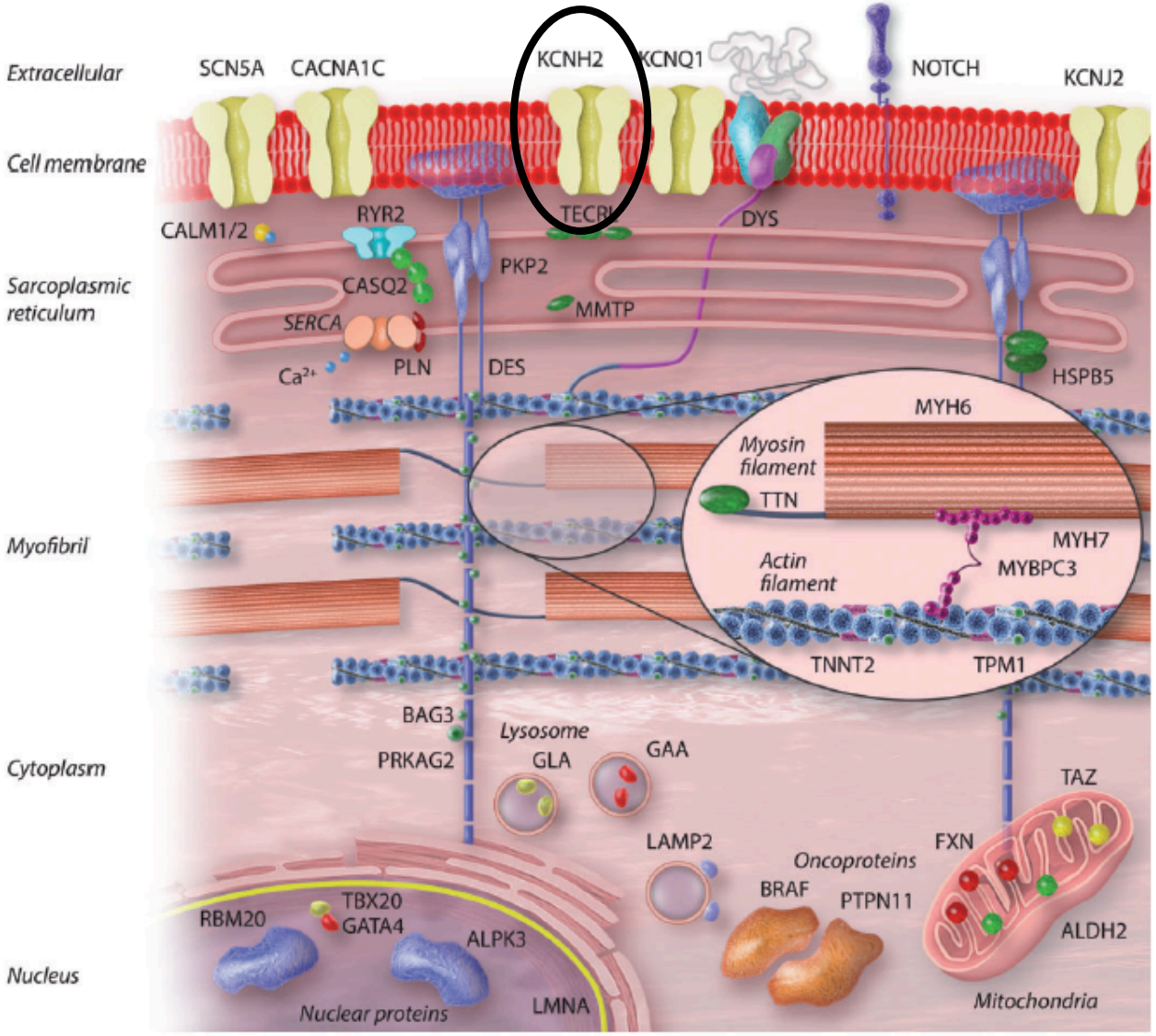
Modified from Strauss and Blinova. **Clinical Trials in a Dish.** *Trends in Pharmacological Sciences* 2017; 38: 4-7

Functional analysis of hiPSC-CMs



Garg et al, Circ Res. 2018

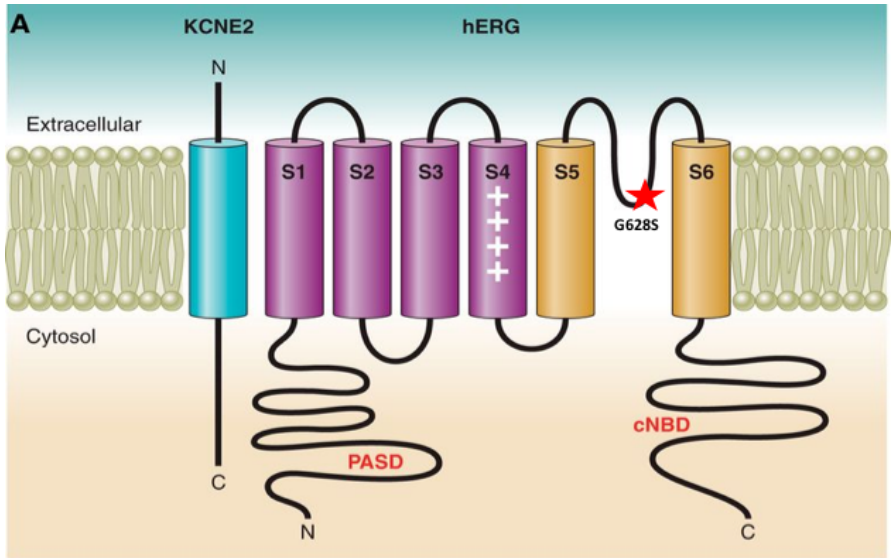
Overview of congenital cardiac diseases that have been modeled using hiPSC-CM



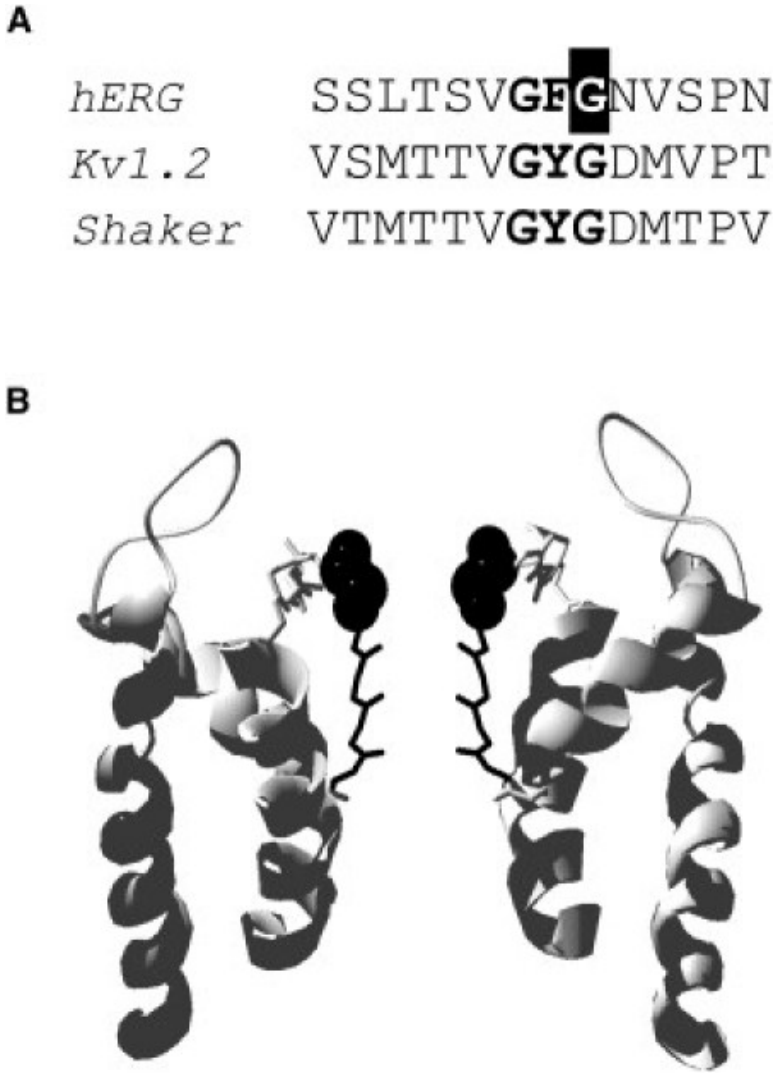
Van Mill et al, Cardiovasc Res. 2018

Hypothesis: Overexpressing mutated hERG channels in hiPSC-CM can reproduce the LQT2 phenotype.

hERG G628S (LQT2)

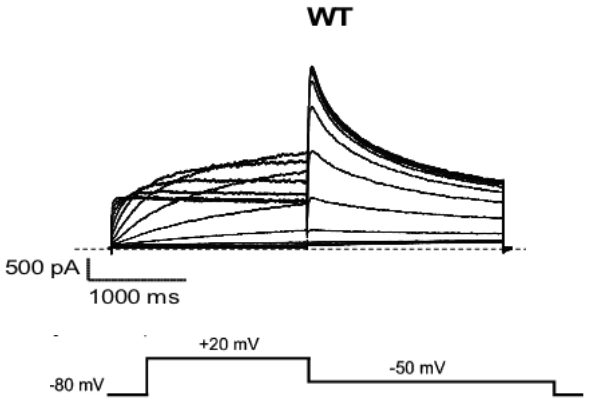


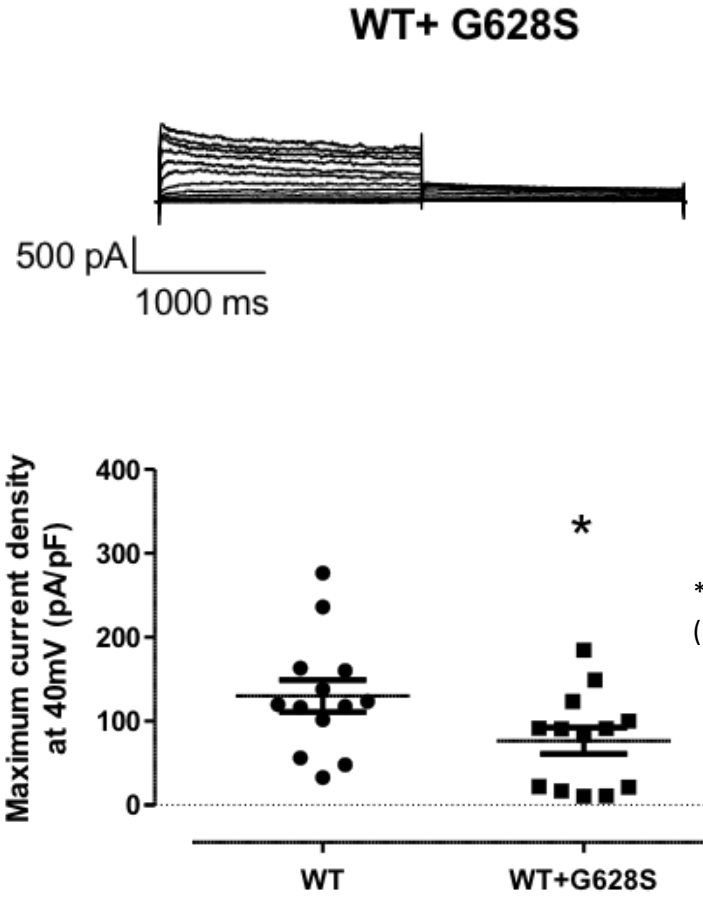
Modified from Bohnen et al, Physiol Reviews, Jan 2017



From Es-Salah-Lamoureux et al, Biophys J. 2011

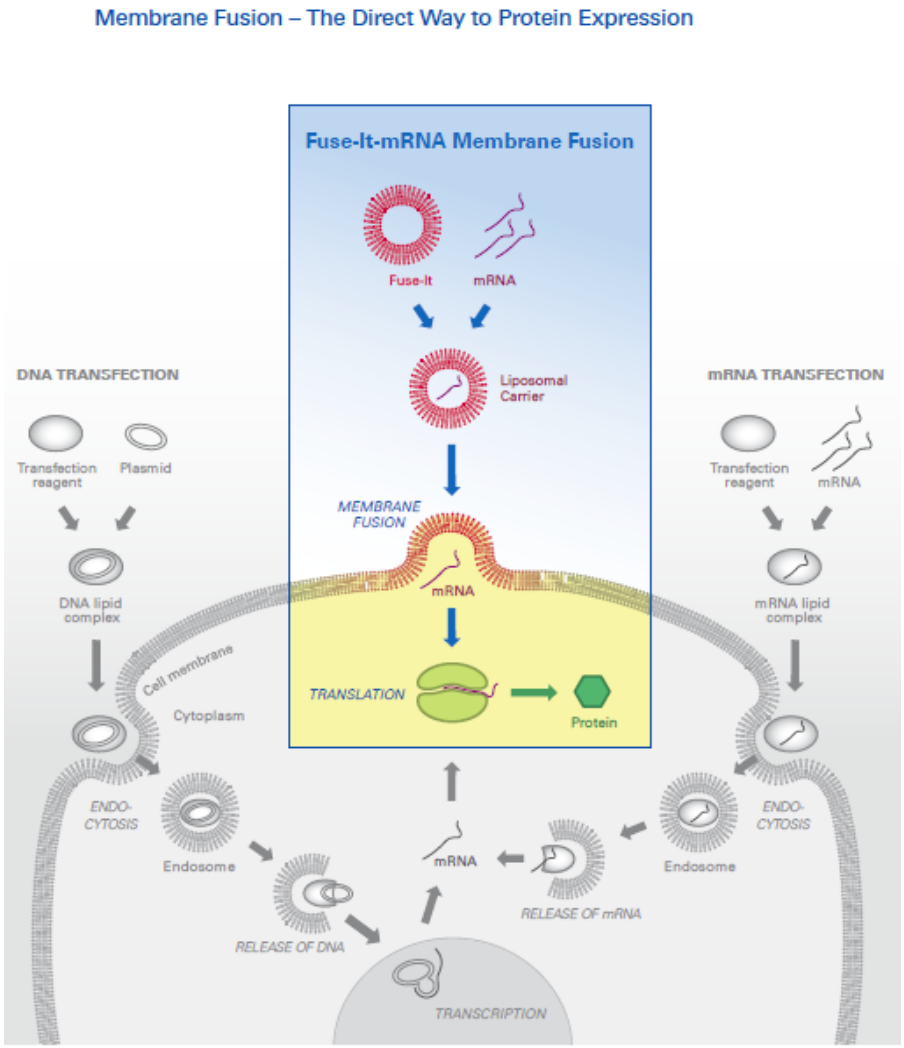
Co-expression of WT hERG with G628S decreases current amplitude of WT hERG channels in HEK cells





*p<0.05 unpaired t-test
(Mean ± SEM)

Transfecting hiPSC-CM using fusogenic liposomes.



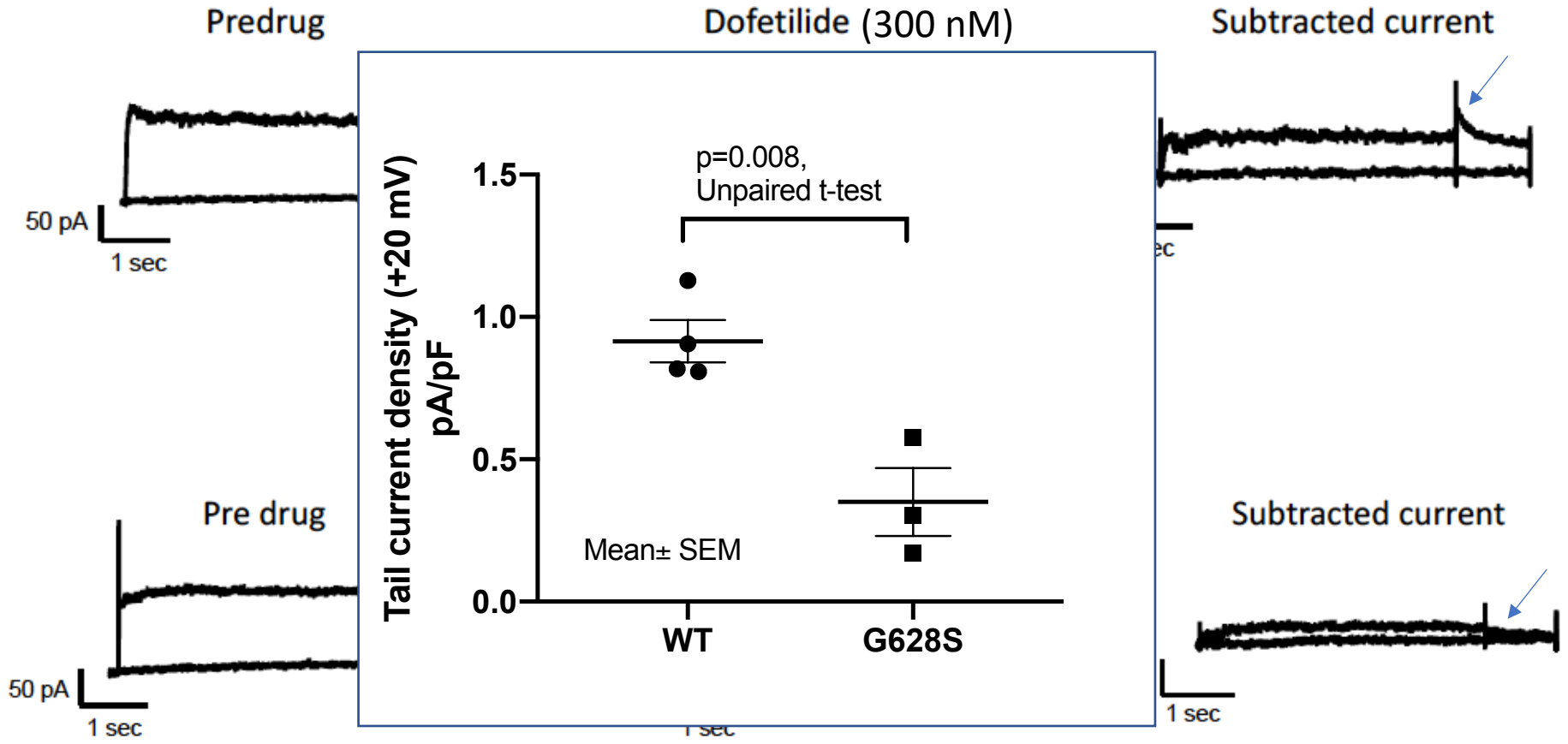
- Transfection competent vesicles are formed around RNA cargo
- Loaded vesicles fuse with plasma membrane of target cells
- Cargo is directly released into cytosol, bypassing the endo- / lysosomal pathway
- Instant bioavailability of cargo molecules in cytosol
- Fluorescent tracer molecule in vesicles allows for verification of successful transfection or cell sorting in flow cytometry

Ion channel of interest is tagged with GFP or Cherry.
mRNA synthesized using the mMessage mMachine® kit

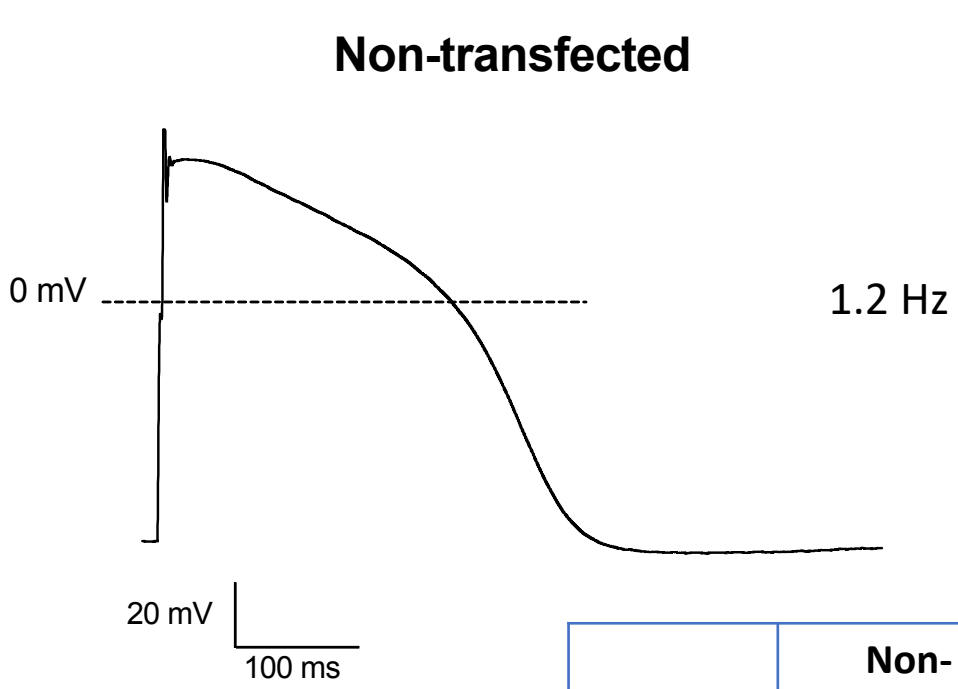
I_{Kr} is decreased in hiPSC-CM transfected with G628S.

Wild Type (Non-transfected)

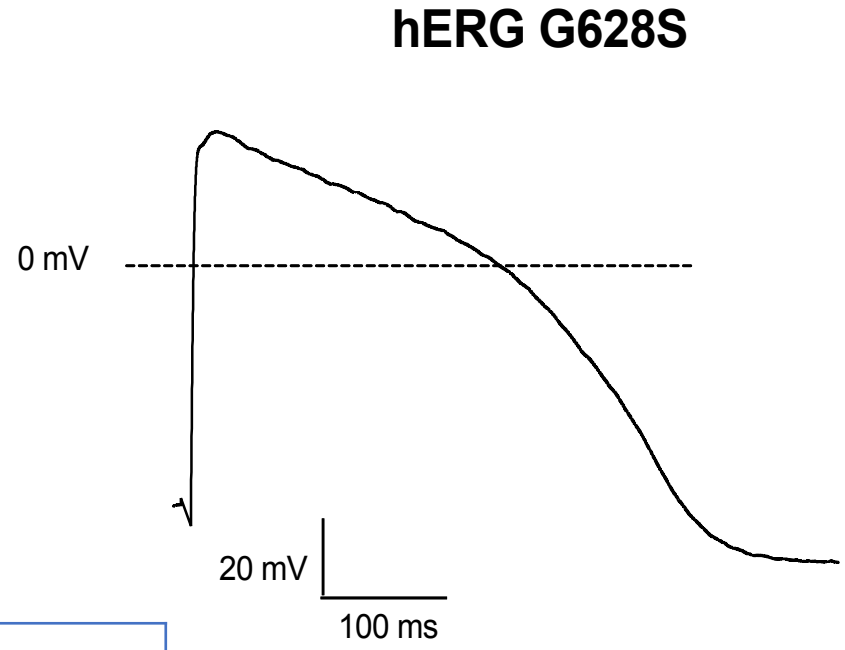
G628S



Effects of G628S on Action Potential parameters

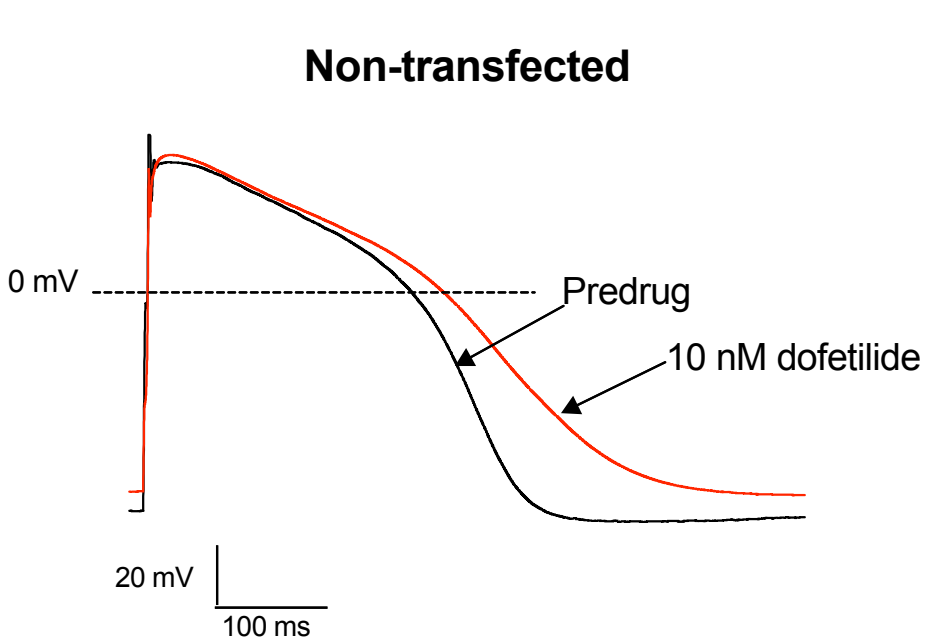


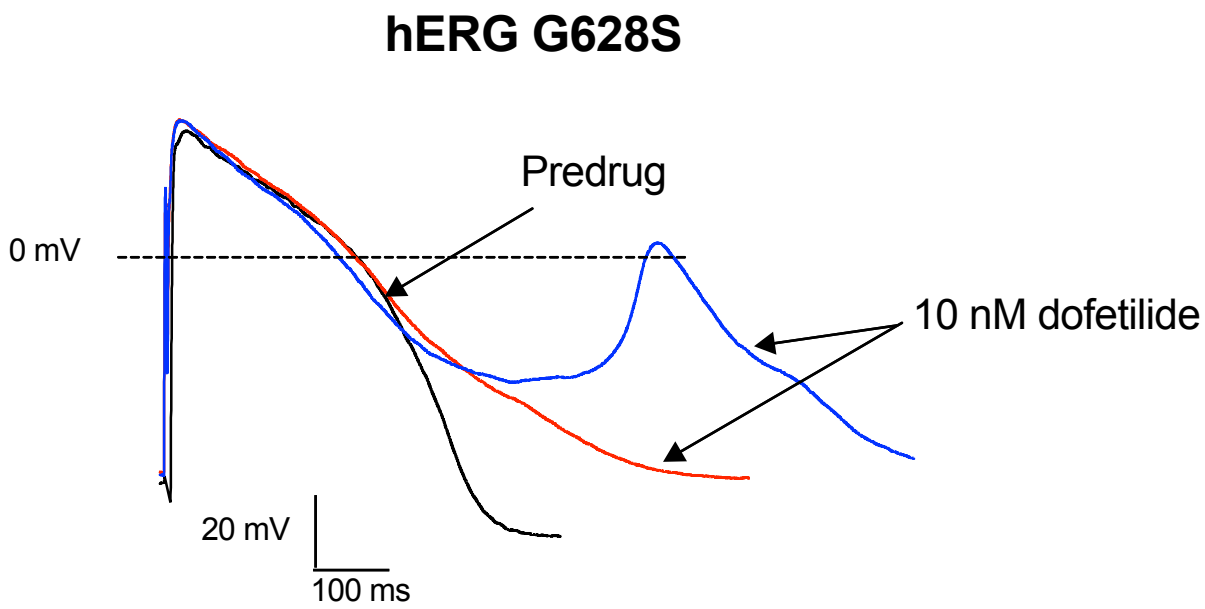
1.2 Hz



	Non-transfected	G628S
	Mean± SEM n=6	*Mean± SEM n=10*
MDP (mV)	-74±3	-74±2
APA (mV)	118±2	104±4
APD_{50} (ms)	286±20	291±19
APD_{90} (ms)	351±23	387±22

hiPSC-CMs overexpressing hERG G628S (LQT2) are more sensitive to dofetilide.





Data summary of the effects of dofetilide on non-transfected vs. G628S transfected cells.

	Non-transfected (n=5) **(Mean ± SEM)**			**hERG G628S (n=9)** **(Mean ± SEM)**		
	Pre-drug	*10 nM dofetilide*	*% change*	*Pre-drug*	*10 nM dofetilide*	*% change*
MDP (mV)	-76±3	-64±5	-15%	-75±2	-66±3	-12%
APA (mV)	118±2	107±5	-9%	104±4	98±5	-6%
APD_{50} (ms)	274±20	303±22	+11%	286±20	374±37	+31%
APD_{90} (ms)	345±27	449±48	+30%	381±24	661±74*	+74%

No EADs

EADs in 5 cells

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Conclusion

- Overexpressing hERG G628S in hiPSC-CM can reproduce some of LQT syndrome type 2 phenotypes.
  - Proof of principle for transfection of hiPSC-CM as a platform for small-molecule testing.
  - Follow up studies in patient-derived hiPSC-CM or using genome editing.

- Application to drug safety and personalized medicine
  - Responses to drug in “healthy” subject can be different from patients with inherited arrhythmogenic syndromes.
  - Increased drug sensitivity.
  - Predicting patient response to individualized therapy.

- Define patient-specific cellular mechanisms of inherited diseases

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