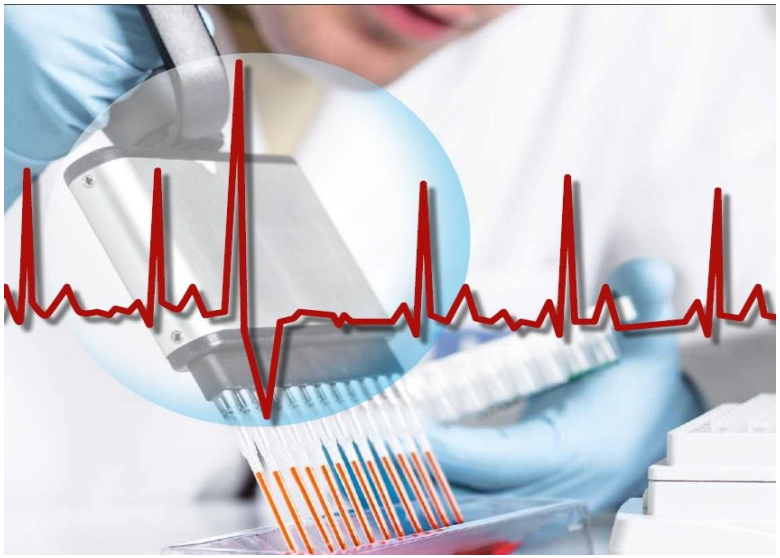
Current status of the implementation of new methods in cardiotoxicity safety testing



22nd European Congress
on Alternatives to Animal Testing
Linz, Austria, October 12, 2019

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Das Portal für tierversuchsfreie Forschung

Menschen für Tierrechte
Bundesverband der Tierversuchsgegner e.V.

What's this about?

There is a valid guideline S7B of the International Conference on Harmonization (ICH) for tests on cardiotoxicity of medicinal products, set into practice in 2005, but it has dangerous deficiencies.

ICH harmonisation for better health

ICH HARMONISED TRIPARTITE GUIDELINE

THE NON-CLINICAL EVALUATION OF THE POTENTIAL FOR DELAYED VENTRICULAR REPOLARIZATION (QT INTERVAL PROLONGATION) BY HUMAN PHARMACEUTICALS

S7B

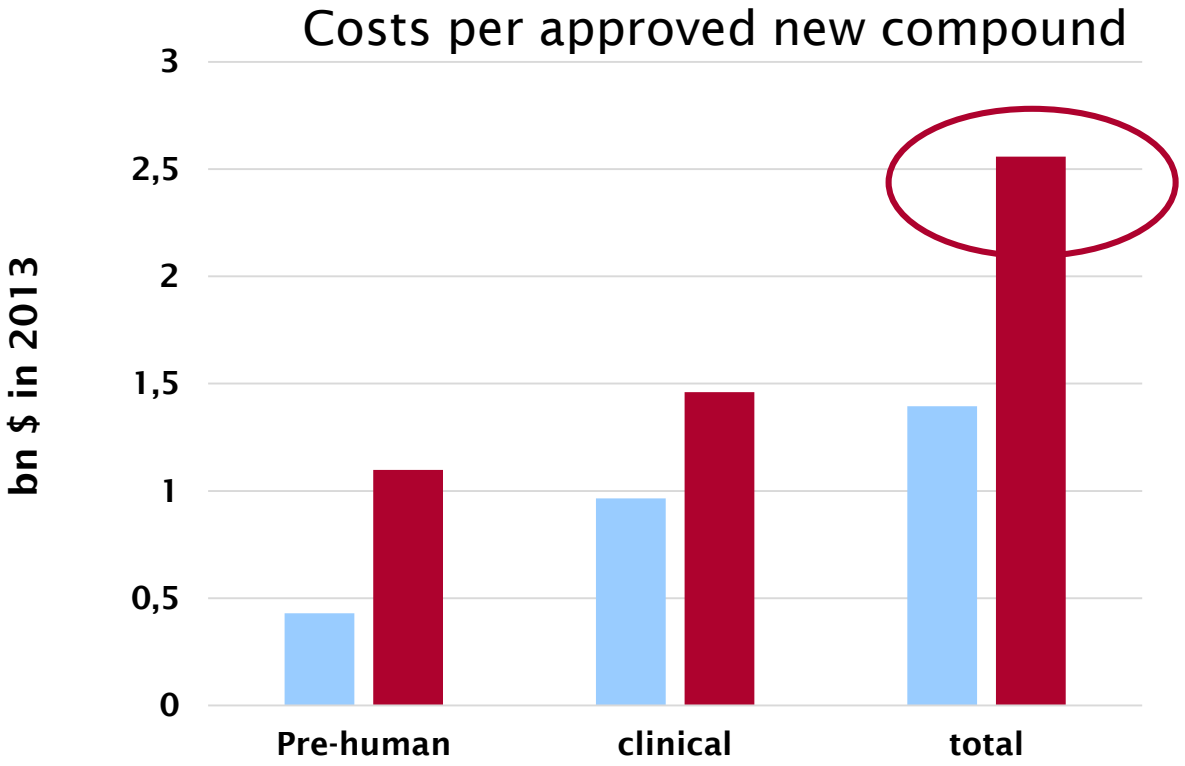
Substantial better animal-free methods, integrated into a draft of a new test strategy, developed by the *Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative.*

The new paradigm is not implemented, although the procedure should have been completed by the end of 2017.

Content:

- Why cardiotoxicity testing?
- Action potential (recorded by an ECG)
- Cardiac arrhythmias
- The old guideline
- In vitro hERG patch clamp test
- Ex vivo experiments controversial
- New ideas for cardiotoxicity testing: *Comprehensive In Vitro Proarrhythmia Assay (CiPA) Initiative*
- In silico modelling
- Pilot study with hiPSC derived cardiomyocytes
- Example: hiPSC-CM on an integrated cardiac multifluidic module
- Conclusion

Why cardiotoxicity testing?



According to DiMasi, Grabowski and Hansen, *J. Health Econ.* 2016, *47*, 20.

Cardiotox major cause
- **drug withdrawals** from the marketplace
- **End of the development** during late clinical phase

Development time:
10 - 15 years

(Cross et al. Br J Pharmacol. 2015 Feb; 172(4): 957–974.
doi: 10.1111/bph.12979)

Some examples:

Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes

Steven E. Nissen, M.D., and Kathy Wolski, M.P.H.

June 14, 2007
N Engl J Med 2007; 356:2457-2471
DOI: 10.1056/NEJMoa072761

Effect of Sibutramine on Cardiovascular Outcomes in Overweight and Obese Subjects

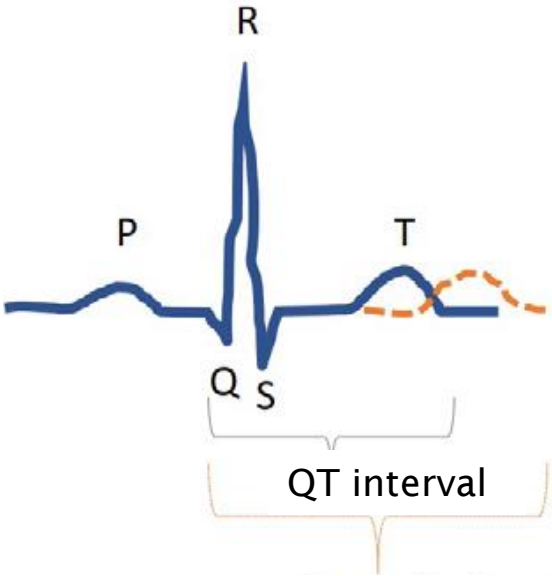
W. Philip T. James, M.D., D.Sc., Ian D. Caterson, M.D., Ph.D., Walmir Coutinho, M.D., D.Sc., Nick Finer, M.B., B.S., Luc F. Van Gaal, M.D., Ph.D., Aldo P. Maggioni, M.D., Christian Torp-Pedersen, M.D., Ph.D., Arya M. Sharma, M.D., Ph.D., Gillian M. Shepherd, B.Sc., Richard A. Rode, Ph.D., and Cheryl L. Renz, M.D. for the SCOUT Investigators*

September 2, 2010
N Engl J Med 2010; 363:905-917

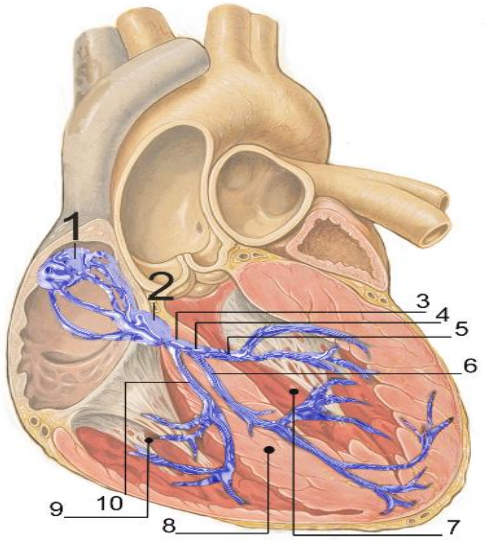
29. November 2013

Iclusig® ▼ (Ponatinib): aktualisierte Informationen bezüglich des Risikos für das Auftreten von Gefäßverschlüssen

■ Action potential (recorded by an ECG)



QT interval prolongation



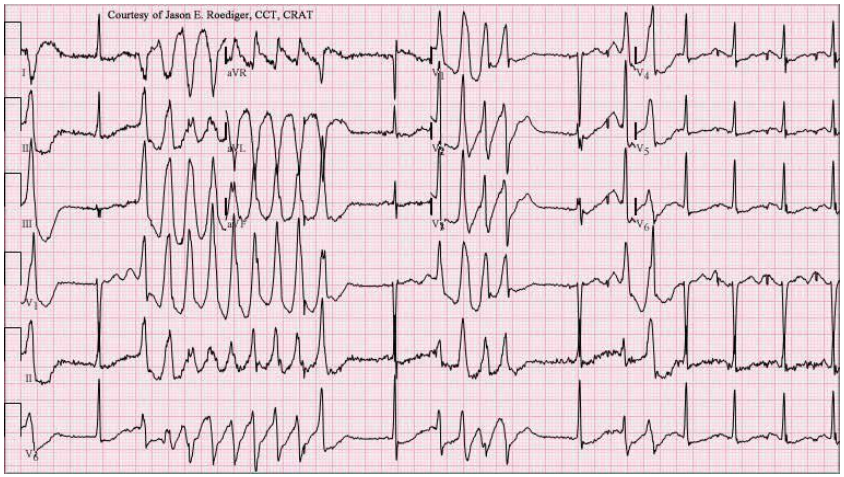
Wikipedia

Heart; conduction system. 1. SA node. 2. AV node. 3. Bundle of His. 8. Septum

- P wave: excitation propagation (atrial), propagation via AV node to ventricles
- QRS complex: excitation propagation in the ventricles
- T-wave: excitation regression in the ventricles
- QT interval: start excitation propagation ventricles (Q) to the end of the regression

→ QT interval prolongation: indicates delayed repolarization

Cardiac arrhythmias



Cardiogram of a torsade de pointes tachycardia.

Ion currents

Human ventricular membrane potential:

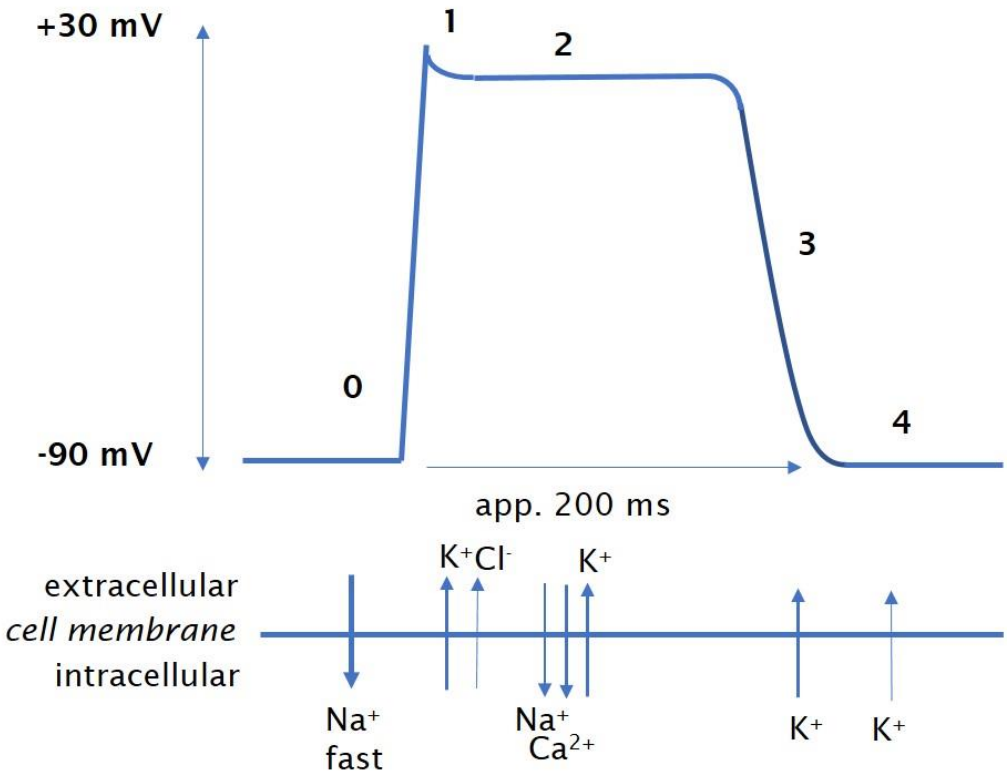
Phase 0: rapid influx of Na^+

1: inactivation of the Na^+ channels, efflux of K^+

2: equilibrium inflow Ca^{2+} (via L-type channels) and outflow of repolarizing K^+ currents.

3: late repolarization phase by outflow of K^+ (via delayed rectifier K^+ channels).

4: Rest potential by K^+ current outflow.



Membrane potential of a heart cell.,
scheme according to Ikonnikov & Wong, MPR, 2019.
http://www.pathophys.org/physiology-of-cardiac-conduction-and-contractility/

The old guideline

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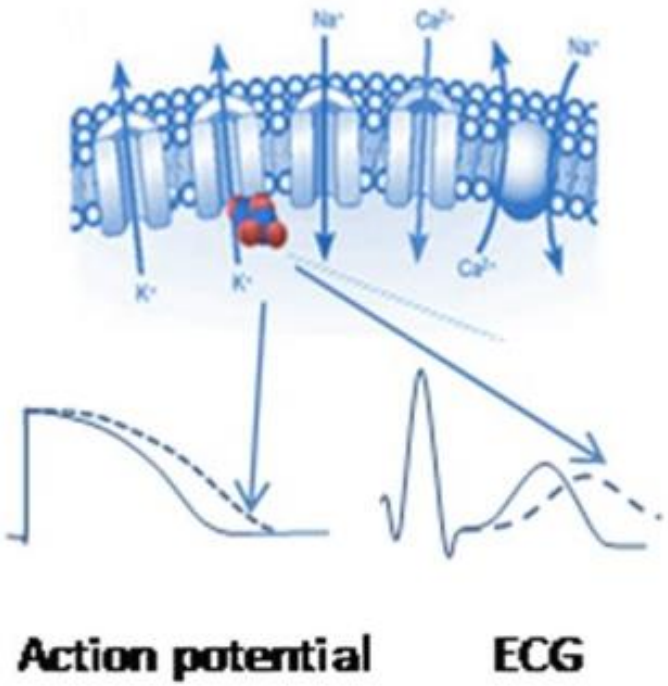
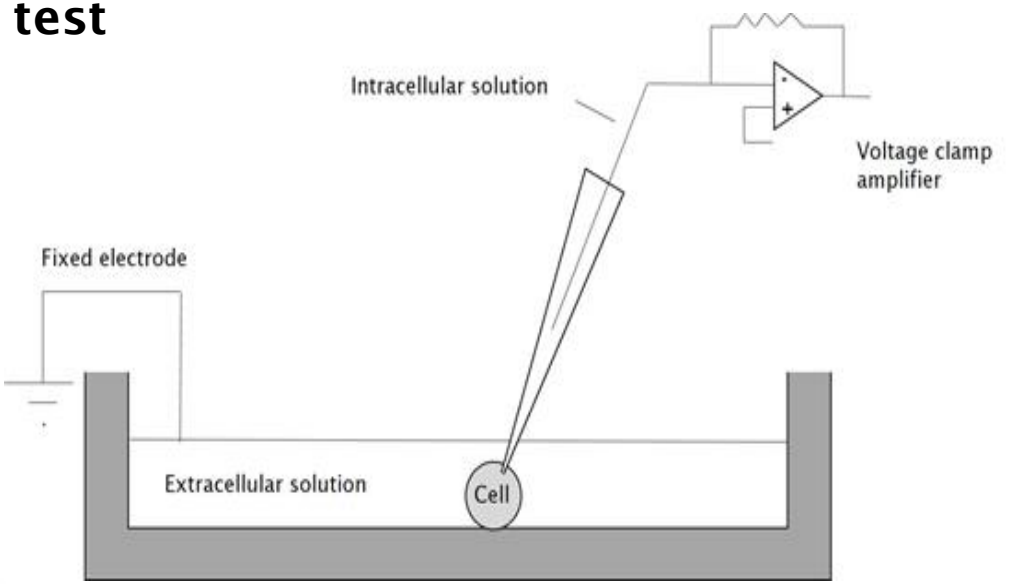
S7B

⇨ Tests are designed to determine:
- the degree of delay in re-polarization
- the action mechanism behind this event

Methods:
- in vitro tests (hERG patch clamp test)
- ex-vivo QT studies (heart tissue and whole hearts of rabbits)
- ECG tests on dogs

■ in vitro hERG patch clamp test

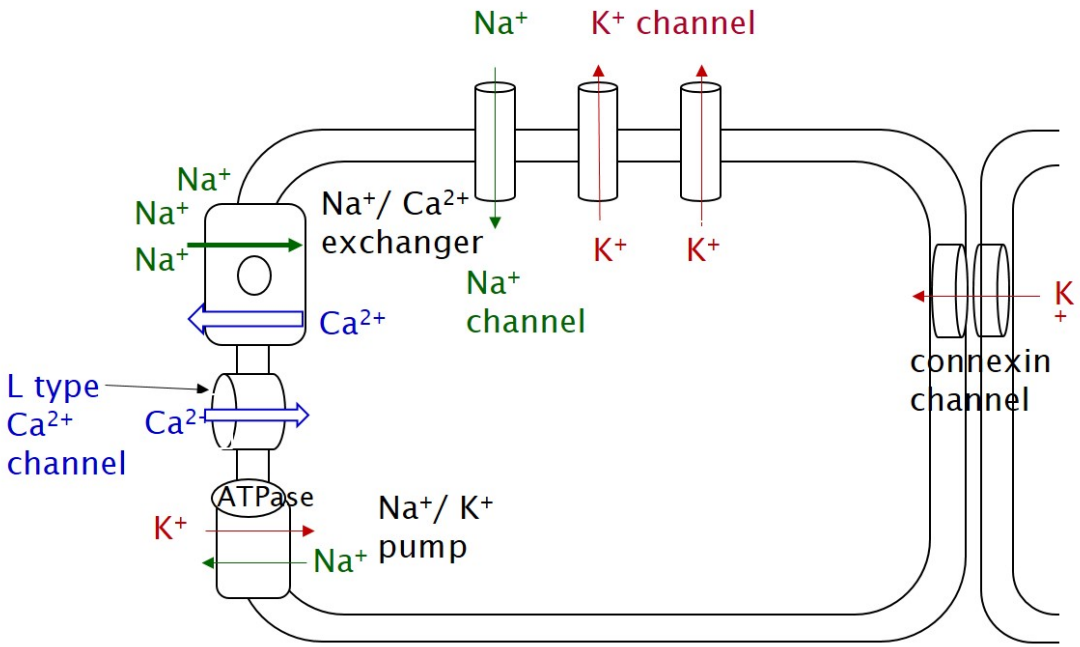




According to Priest, Bell & Garcia (2008).
Channels, 2:2, 87-93, DOI: 10.4161/chan.2.2.6004

A drug's interaction with ion channels may cause the heart's contraction to become irregular.
Source: https://www.fda.gov/drugs/science-research-drugs/impact-story-finding-better-test-predicting-risk-drugs-pose-heart

■ in vitro hERG patch clamp test

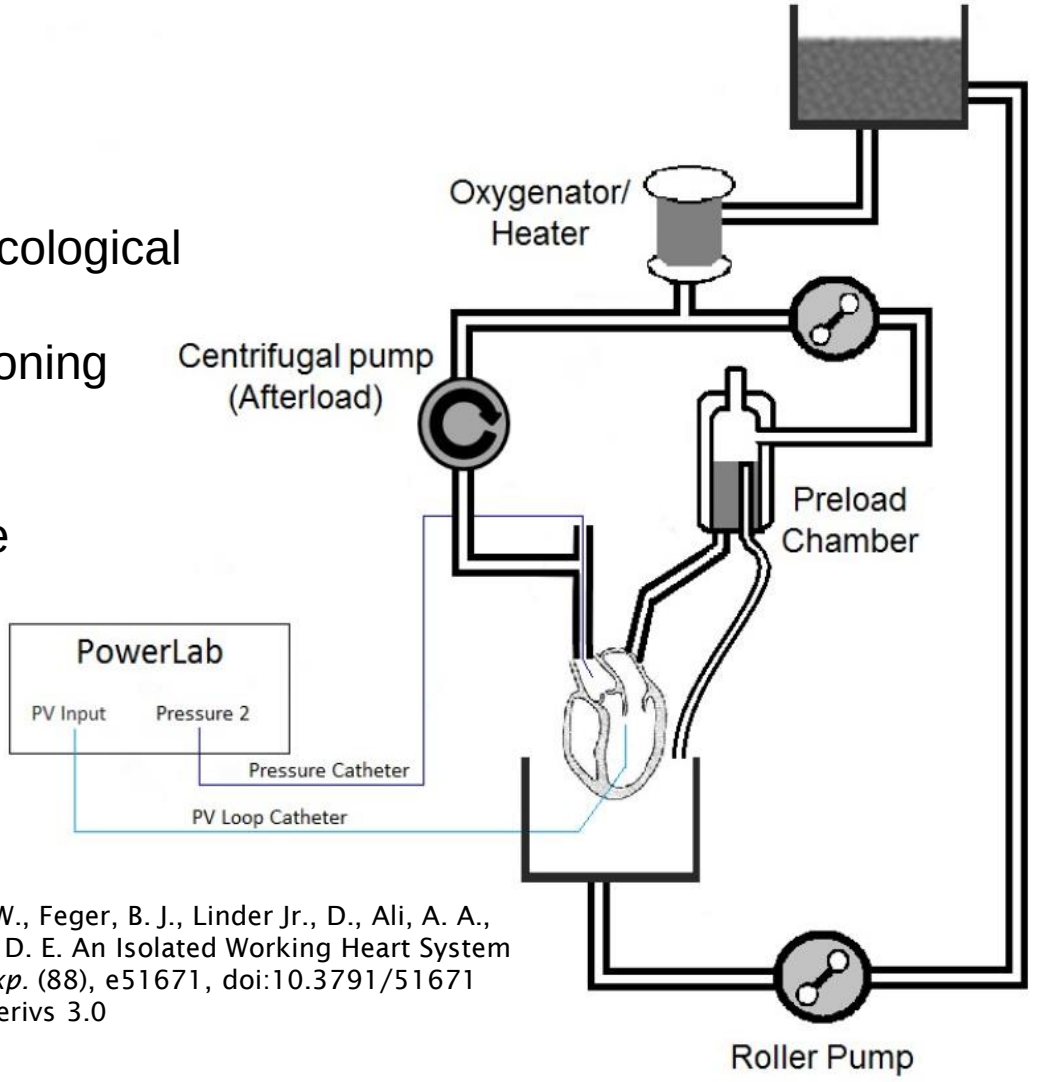


Several channels could lead to a prolongation

Can lead to incorrect results, because a prolongation of the action potential can be induced by ion currents

■ Ex vivo experiments not uncontroversial

Critique:
- no pathological/pharmacological conditions
- no autonomously functioning nervous system
- fluid accumulation
- only drug, no metabolite measurement



Schechter, M. A., Southerland, K. W., Feger, B. J., Linder Jr., D., Ali, A. A., Njoroge, L., Milano, C. A., Bowles, D. E. An Isolated Working Heart System for Large Animal Models. *J. Vis. Exp.* (88), e51671, doi:10.3791/51671 (2014). CCA-NonCommercial-NoDerivs 3.0

proband use according to guideline E14 in combination with the old S7B is questionable

Further points of criticism are:

- young, healthy animals do not represent the usually advanced age of the patients
- multifactorial pathologies cannot be modelled
- species differences in physiology

- **New ideas for cardiotoxicity testing: Comprehensive In Vitro Proarrhythmia Assay (CiPA) Initiative**

CiPA

Steering Team: FDA, HESI, EMA, Health Canada, Japan NIHS and others

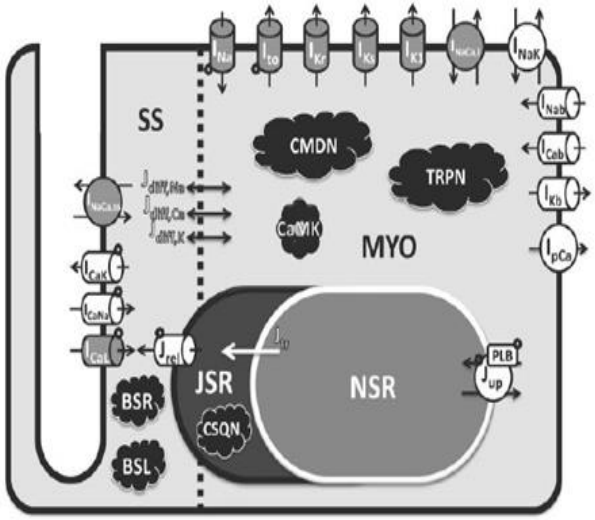
Goal: a new approach:

- ***7-channel tests*** with heterologous cell line (ionic current studies)
- Verification of the results with an **in silico program** that simulates the action potential of a human heart cell.
- **In vitro Tests** with ***human ventricular cardiomyocytes*** derived from ***hiPSC*** check and want to confirm the results of in silico simulation by real electrophysiologial investigations.
- clinical ECG assessments

■ In silico modeling

28 drugs tested with a CiPA AP standardized model

Type	High TdP risk	Intermediate TdP risk	Low TdP risk
Training	Bepridil	Chlorpromazine	Diltiazem
	Dofetilide	Cisapride	Mexiletine
	Quinidine	Terfenadine	Ranolazine
	d, l-Sotalol	Ondansetron	Verapamil
Validation	Azimilide	Astemizole	Loratadine
	Ibutilide	Clarithromycin	Metoprolol
	Vandetanib	Clozapine	Nifedipine
	Disopyramide	Domperidone	Nitrendipine
		Droperidol	Tamoxifen
		Pimozide	
		Risperidone	



Jin-Sol Park et al. (2019). Transl Clin Pharmacol 27/1: 12-18. https://doi.org/10.12793/tcp.2019.27.1.12

Schematic diagram of O'Hara-Rudy human ventricular myocyte model. Among various ion currents in the model, in silico studies **in CiPA** focus on the **7 major ion currents**: IKr, IKs, ICaL, INaL, INa, Ito und IK1.

→ results were reliable in comparison with the hiPSC-CM assays and the clinical ECG study

■ Pilot study with hiPSC derived cardiomyocytes (CMs)

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SOT | Society of Toxicology
www.toxsci.oxfordjournals.org
ToxSci 20 Years

TOXICOLOGICAL SCIENCES, 164(2), 2018, 550–562
doi: 10.1093/toxsci/kfy110
Advance Access Publication Date: April 27, 2018
Research Article

Cross-Site Reliability of Human Induced Pluripotent stem cell-derived Cardiomyocyte Based Safety Assays Using Microelectrode Arrays: Results from a Blinded CiPA Pilot Study

Daniel Millard,* Qianyu Dang,† Hong Shi,‡ Xiaou Zhang,§ Chris Strock,¶ Udo Kraushaar,‖ Haoyu Zeng,‖‖ Paul Levesque,‡ Hua-Rong Lu,‖‖ Jean-Michel Guillon,# Joseph C. Wu,** Yingxin Li,** Greg Luerman,†† Blake Anson,[a] Liang Guo,[a,b] Mike Clements,* Yama A. Abassi,§ James Ross,* Jennifer Pierson,[c,1] and Gary Gintant[d]

- 28 drugs classified as a risk for serious QT prolongation, studied in blinded experiments
- Two commercial human CM lines with 5 devices
- Repolarization effects evaluated using MEAs and voltage-sensing dyes

→ results demonstrated the applicability of hiPSC-CMs to detect drug-induced proarrhythmic effects as part of the evolving Comprehensive In Vitro Proarrhythmia Assay testing strategy.

→ an ongoing CiPA validation study will build upon the core protocol from this pilot study

Conclusion:

- Each component of the new testing strategy has been already developed by the CiPA participants
- With the new approach, more disturbances caused by molecules on heart cells can be investigated reliably and in detail
- This could lead to drug developments with substances that have been rejected earlier because of false-positive test results
- First validation studies for each of the CiPA components has been **already done** → base for further discussions
- Currently in discussion: ***How*** should the new approach be implemented? At the end, the old ICH guideline should be modified for more precise and quantitative evaluations.
- FDA has already recommended this approach to multiple drug developers

Thank you for your attention

Special thanks to my colleagues

- Dr. Claudia Gerlach
- Carolin Spicher
- Christina Ledermann

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