Overview
Ion Channel Strategy

CSRC Meeting
December 6, 2016

Bernard Fermini
Ion Channel Working Group/Rapid Response team
HT Work Stream

Ion Channel Working Group (ICWG)
Deliverables

- Established in Dec 2013
  - sponsored by Safety Pharmacology Society

- Assemble a group of expert electrophysiologists to help guide the project

- Deliver robust, reliable and reproducible ion channel protocols to support *in silico* working group (ISWG) reconstruction of human ventricular AP
  - Which ion channels should be selected to support ISWG efforts
  - What properties should be studied: IC_{50} determination, kinetics, rate/use/voltage dependence, etc…?

- What requirements are needed to deliver robust, reliable and reproducible ion channel data in a high throughput screening (HTS) environment

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CiPA Ion Channel Selection

Selection based on:

- Fundamental role of ion channels in defining human action potential duration
- Information obtained from the Safety Pharmacology Society survey around ion channels routinely screening in Pharma for safety concerns
- Literature

Selected as initial working material for the CiPA assays

- Recombinant Human channels expressed in replicating cell lines
  - I_{Kr} = hERG
  - I_{Ca}(L-type) = Cav1.2
  - I_{Na} = Nav1.5 peak and late current – drug modified Nav1.5
  - I_{TO} =Kv4.3
  - I_{Ks} = KCNQ1+KCNE1
  - I_{K1} = Kir2.1

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Development Strategy

In silico strategy:

- To incorporate drug-channel kinetics and other factors into the O'Hara-Rudy human ventricular myocyte model as needed to reconstruct the human ventricular AP and identify mechanism-based metrics that can clearly separate drugs into 3 distinct TdP risk categories (high, intermediate, very low)

Ion channel pharmacology strategy:

- To produce consensus/simple IC_{50} protocol(s) for the different channels
- For hERG to get "well defined" IC_{50} data for 'training set' drugs
- To arrive at a dynamic block protocol that can reliably inform the *in silico* kinetic models of hERG block
- Data to be incorporated in the *in silico* AP model, as possible
- Initial data set to be obtained using manual patch technique at ambient temp and 37°C
- Potency on ion channels other than hERG may warrant dynamic block studies for these channels, as needed

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CiPA Training Set
Risk group based on Torsadogenic potential

HIGH	INTERMEDIATE	LOW
Quinidine	Chlorpromazine	Diltiazem
Bepridil	Cisapride	Mexiletine
Dofetilide	Terfenadine	Ranolazine
Sotalol	Ondansetron	Verapamil

Determination of Proarrhythmic risk challenged by several factors

- TdP risk is highly correlated with drug-independent properties such as patient

  Cardiovascular/TdP risk:

  - Left Ventricular function, heart failure, proclivity to have electrolyte abnormalities
  - Factors that may increase exposure
  - Risks conferred by concomitant medications to treat the disease state

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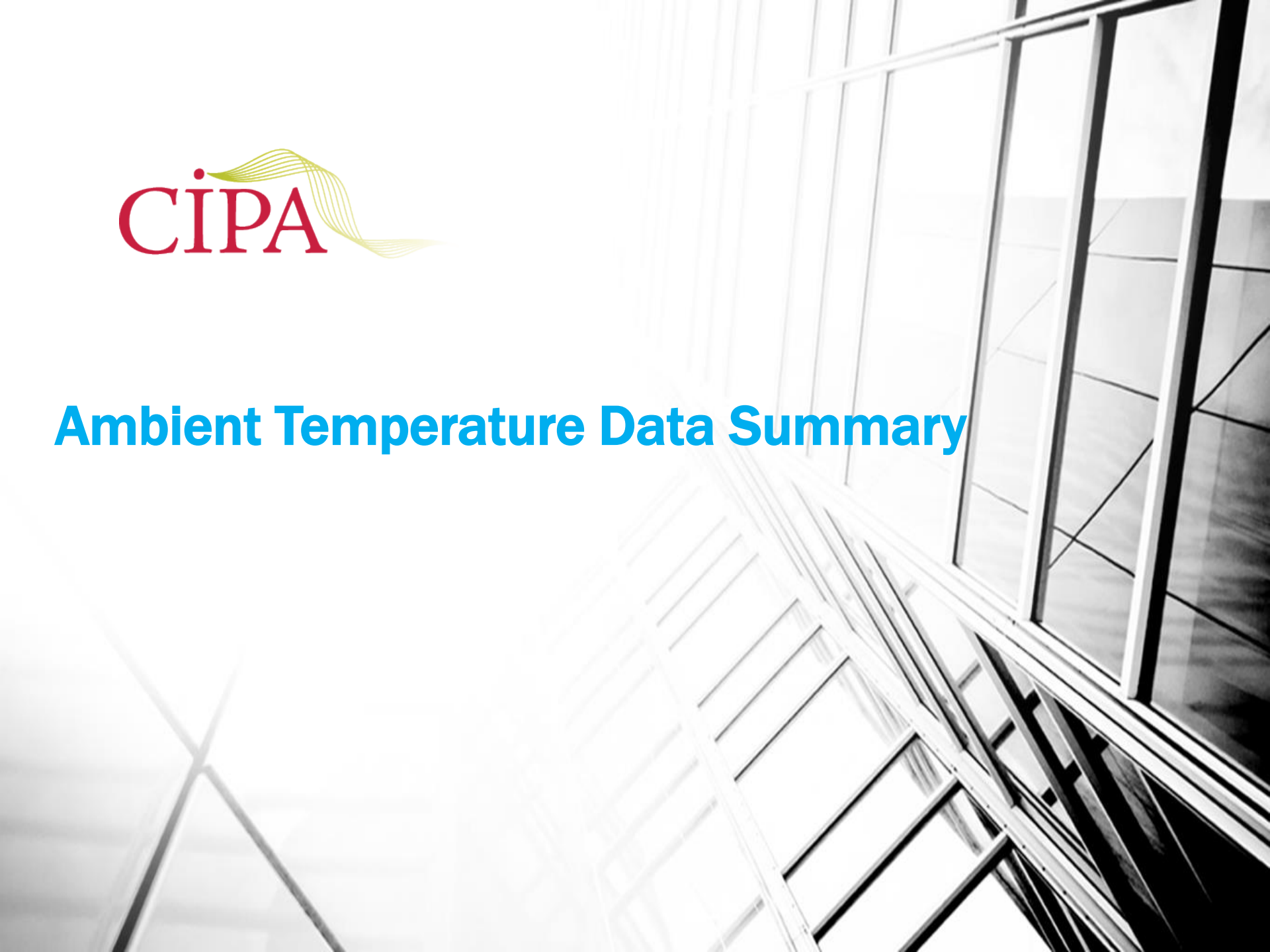
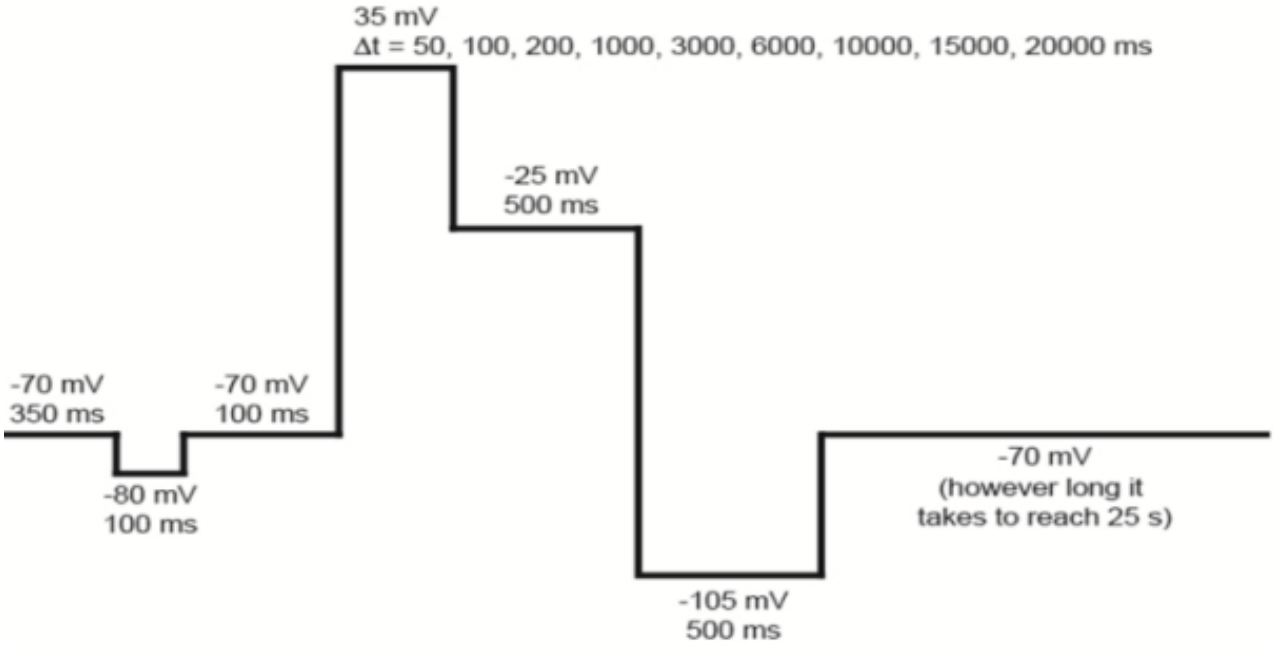
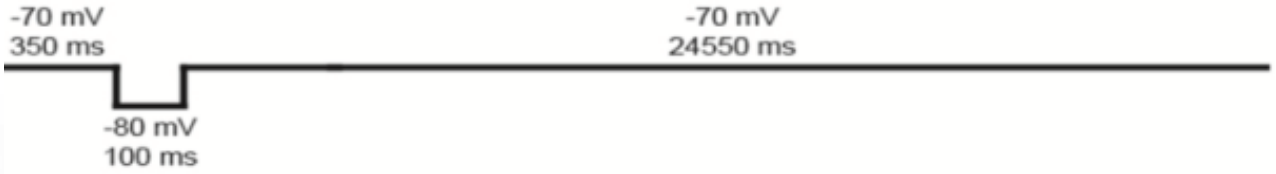


Ambient Temperature Data Summary

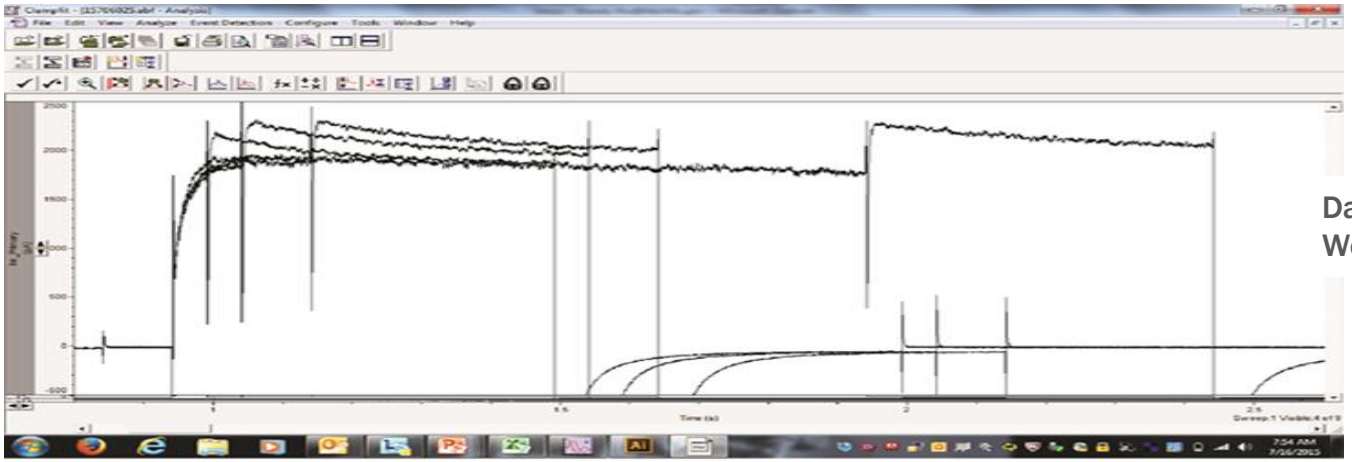
Dynamic Block Protocol V1.0



No depol. pulse protocol (during drug application)

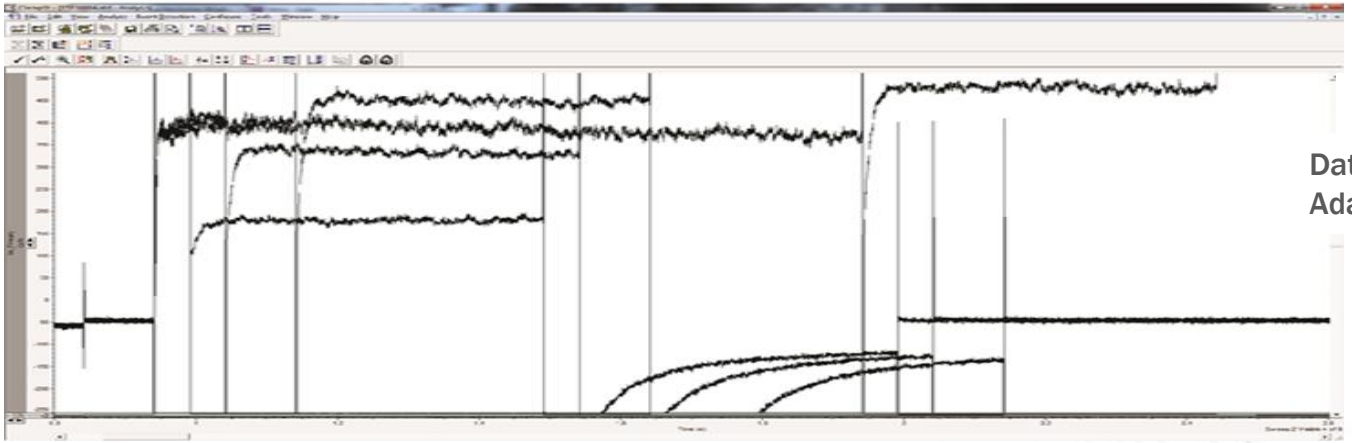


Dynamic Block Protocol V1.0
Temperature related challenges



37°C

Data provided by
Wendy Wu, US FDA

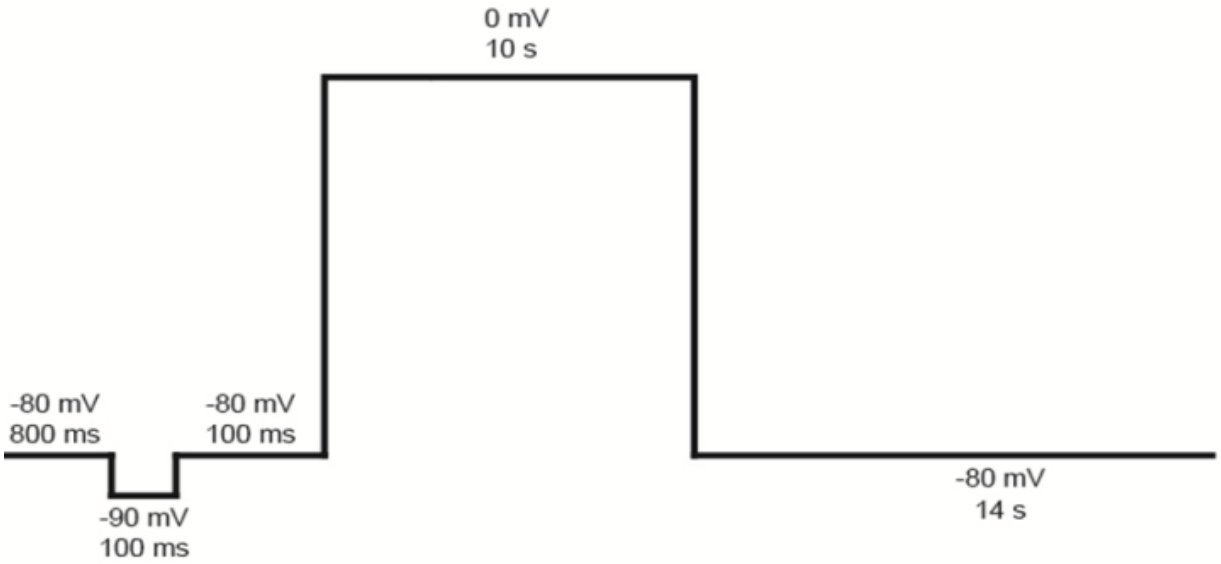


Ambient Temp

Data provided by
Adam Hill, VCCRI, AU

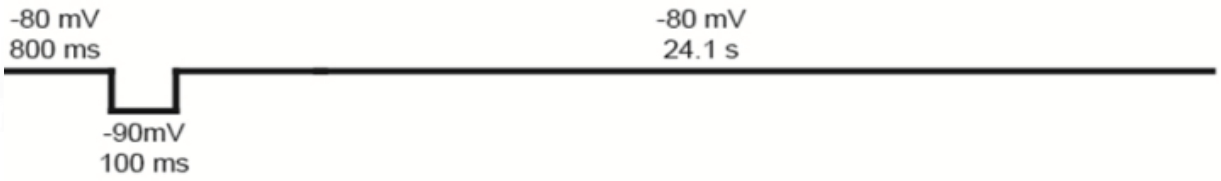
Issue: hERG kinetics or drug effects cannot be resolved for short pulse durations at ambient temp

Dynamic Block Protocol V2.0
Milnes et al., J Pharmacol Toxicol (2010) 66: 178-191



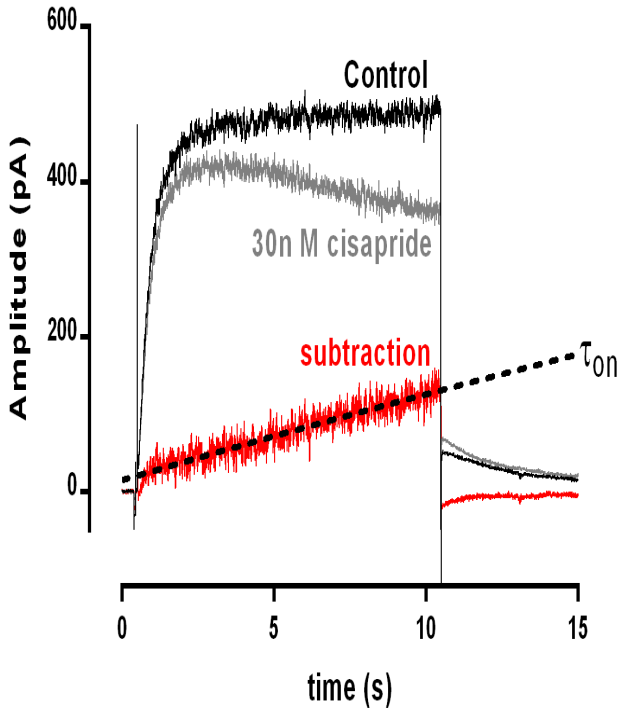
- 10 episodes; each is 25 s long
- Each recording period is ~4 minutes

No depol. pulse protocol (during drug application)



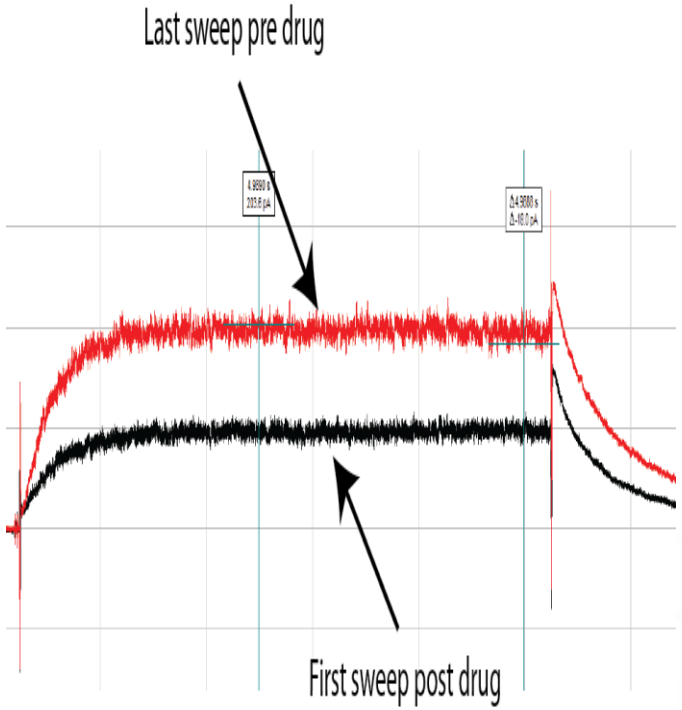
Dynamic Block Protocol V2.0
Temperature related challenges

Issue 1
Onset of binding too slow to measure



Time constant can't be extracted for slow drugs with original 10 sec pulse

Issue 2
Onset of binding too fast to measure



100 µM Mexiletine

Block appears immediate
Onset of block at least as fast as current activation

Data provided by Adam Hill, Victor Chang Cardiac Research Institute, AU

Summary

- Data of highest quality generated in Dr. Adam Hill's laboratory (Victor Chang Cardiac Research Institute, AU)
- Data are key component to the CiPA initiative
- Data used to:
  - Determine potency of CiPA compounds using "gold standard" approach
  - Determine potential temperature effects on potency
  - Provide data to ISWG to parametrize human AP model
  - Determine how well hERG data obtained at ambient temp can predict effects at 37°C in AP model
  - Contribute to the development of a modified hERG model that can reproduce temperature-induced changes in channel gating processes
- Joint manuscript initiated in collaboration with ISWG (FDA)
- Data presented at the SPS meeting
  - Abstract selected for a talk at SPS
  - Dr. Monique Windley (Hill's lab) received a "Young Investigator Award" from SPS
- No additional manual work required at ambient temperature
- Experience and expertise gained is helping develop HESI-sponsored High Throughput (HT) Work Stream Initiative

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Next Step: High Throughput Work Stream

- Sponsored by HESI
- Provide critical data on multiple ion channels, including hERG, allowing for the calibration and validation of the in silico action potential (AP) model
- Assess the variability and reproducibility of high throughput screening platforms/sites for defining drug effects on cardiac ionic currents across and between platforms and sites
  - Not intended to endorse or recommend any single platform
- Contribute to the standardization of voltage clamp protocols for all ion selected channels
- Contribute to the establishment of data quality standards
- 20 different centers contributing to effort
- Studies include all 7 channels at ambient, and 37°C
- Initial test set of 12 drugs for optimization (Phase 1) followed by a set of 18 drugs for validation (Phase 2)
- Protocols used developed by ICWG, and includes Milnes et al., for hERG
- Ongoing work
  - Timeline for Phase 1 completion: 1Q17
  - Timeline for Phase 2 completion: 2Q17

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Staged approach/blinded compounds
Single distribution source (GSK/Khuram Chaudhary)

Phase I Calibration		Phase II Validation	
High		**High**	
Dofetilide	DI-Sotalol,	Azimilide	Ibutilide
Bepridil	Quinidine	Methadone	Vandenatib
Medium		**Medium**	
Cisapride	Terfenadine	Astemizole	Clarithromycin
Ondansetron	Chlorpromazine	Clozapine	Domperidone
		Droperidol	Pimozide
		Risperidone	Terfenadine
Low		**Low**	
Mexiletine	Ranolazine	Loratadine	Metoprolol
Verapamil	Diltiazem	Nifedipine	Nitrendipine
		Ranolazine	Tamoxifen

Acknowledgements

Rapid Response Team	Affiliation
Najah Abi Gerges	Anabios (co-Chair)
Thomas Colatsky	Formerly with FDA
Bernard Fermini	Coyne Scientific (co-Chair)
Jules C Hancox	Bristol University
Adam Hill	Victor Chang Cardiac Research Institute
Zhihua Li	FDA
David Strauss	FDA
Jamie Vandenberg	Victor Chang Cardiac Research Institute
Wendy Wu	FDA
HT Work Stream	**Affiliation**
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Tim Danker	NMSI
Melissa Gilden	HESI
Gary Gintant	AbbVie
Al George/Carlos Vanoye	Northwestern University
Sigma-Aldrich	Sigma-Aldrich
Simon Hebeisen	B'SYS GmbH
Herbert Himmel	Bayer Pharma AG
John Imredy	Merck & Co
Jim Kramer/Yuri Kuryshev/ Carlos Obejero-Paz	Chantest/CRL (co-Chair)
Paul Levesque/Jingsong Fan	BMS
Anders Lindqvist/Thomas Binzer	Biolin Scientific
Michael Morton	Apconix
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Naping Tang/Xijie Wang	NSCSER
Jeff Weber	Molecular Devices
Wendy Wu	FDA

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