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S7B: Best Practice Considerations for *In Vitro* Studies

hERG/Cardiac Ionic Currents
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Repolarization – Human Cardiomyocytes
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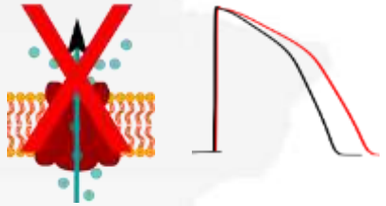
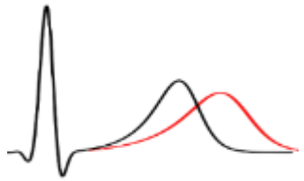
Abbvie Inc., United States
PhRMA

S7B Testing Strategy – Summary and Impact

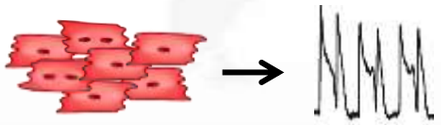
hERG channel block



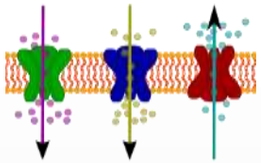
***In vivo* QT**



Human ventricular myocyte assay



Multi-ion channel assay

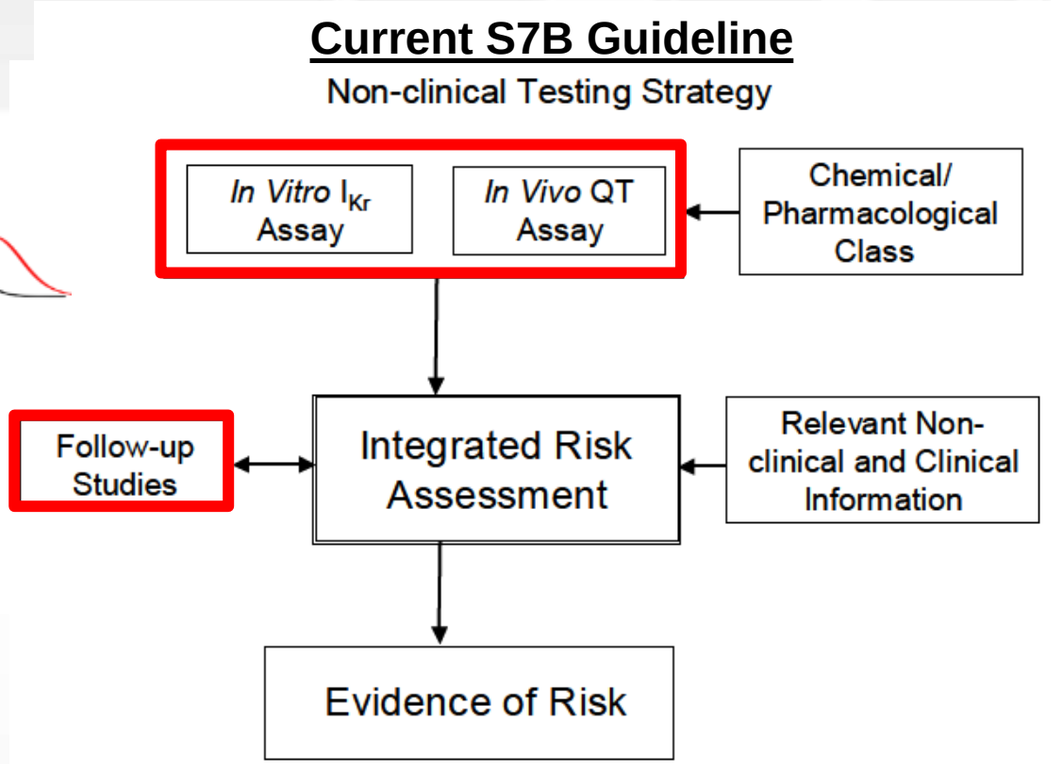


***In silico* myocyte model**

→ Validated Model Risk prediction

Current S7B Guideline

Non-clinical Testing Strategy



Successful in identifying drugs with large effects on ventricular repolarization that could impact clinical trial design

S7B Testing Strategy – Room for Improvement

- Lack technical details or best practice recommendations

Diverse protocols & practices

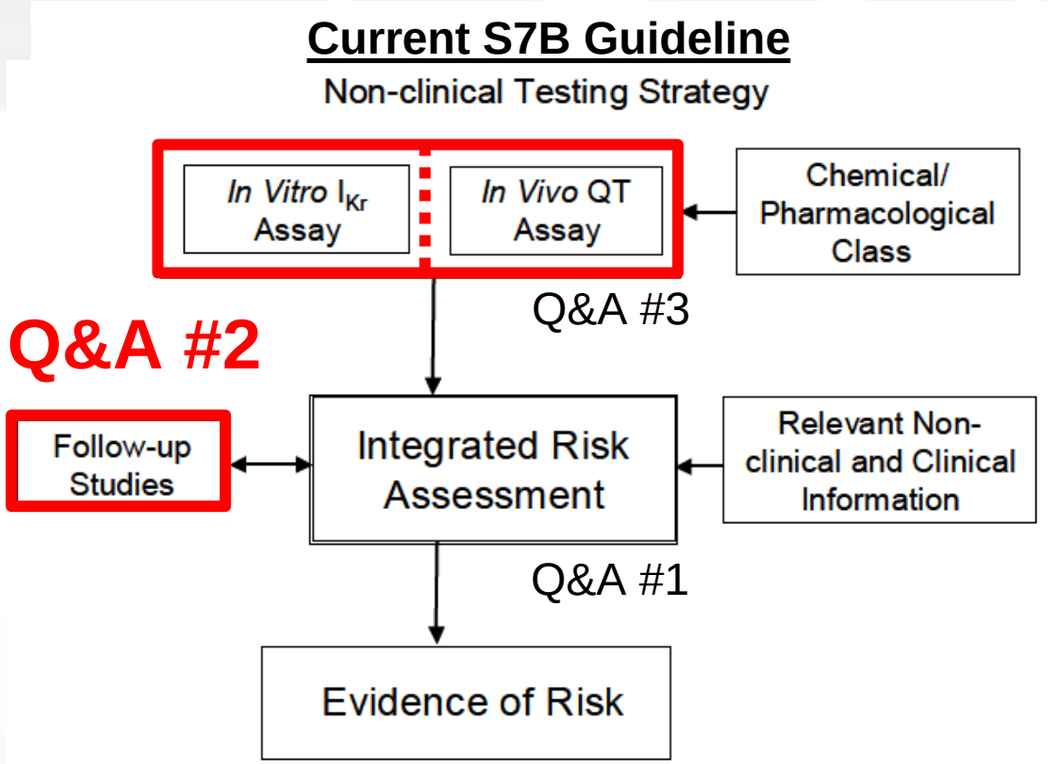
↓

Wide degree of data variability

hERG safety margin, ($\frac{IC_{50}}{free\ Cmax}$)

First-In-Human (FIH)

Later in clinical development



- Lack guidance on nonclinical integrated risk assessment strategy

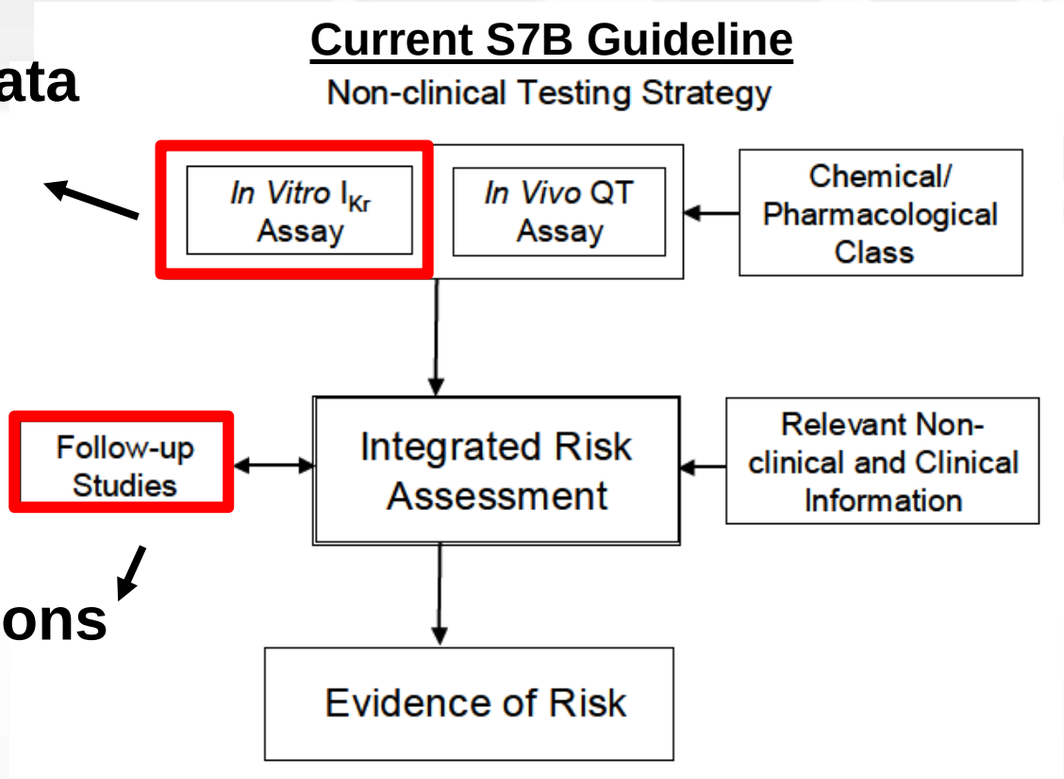
Objectives of S7B Q&A #2

1. **Harmonize approaches; reduce data variability; allow for better translation to clinical findings**

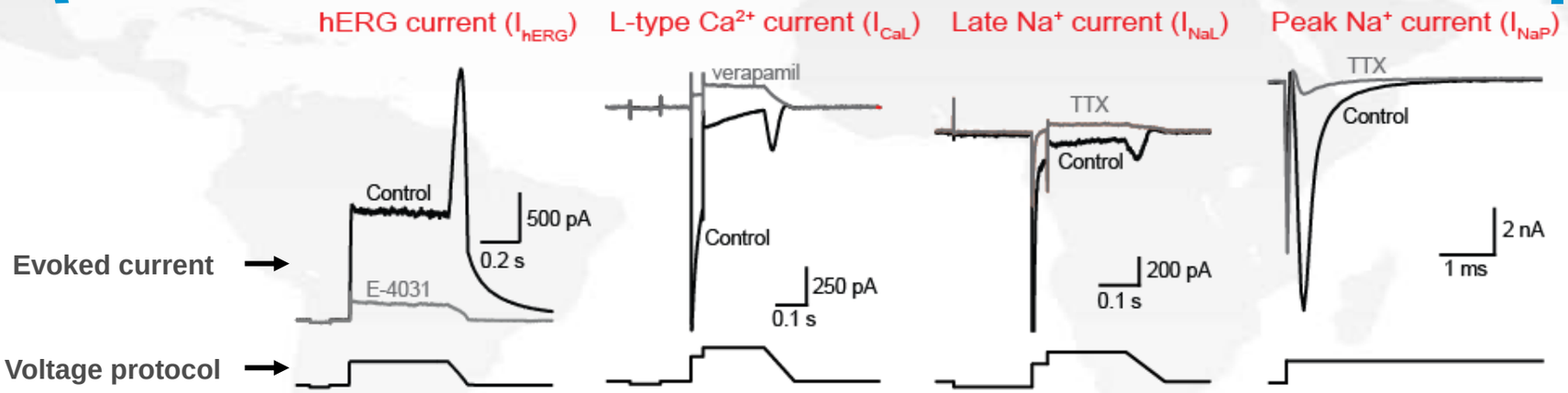
   Double negative findings from core assays can be used to support E14 Q&As 5.1 and 6.1

2. **To incorporate conventional and newer technologies and preparations to assess drug effect on repolarization**

   An option when there is a positive signal from core S7B assays to assist TdP risk assessment



Q&A 2.1 – Best Practices for Patch Clamp Studies



Recommendations for assay design:

- Recording temperature – physiological (35 – 37°C)
- Voltage protocol – should incorporate key elements of a ventricular action potential configuration applied at relevant rates

References for hERG: Walker *et al*., 1999; Kirsch *et al*., 2004; Guo *et al*., 2005; Yao *et al*., 2005; Alexandrou *et al*., 2006; Kauthale *et al*., 2015.

References: hERG - Stork *et al*., 2007
I_{CaL} - Nawrath *et al*., 1997
I_{NaP} - Weirich and Antoni, 1990

Q&A 2.1 – Test Article Concentrations

S7B guideline states that "Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration limiting."

Recommendation: Test multiple concentrations that cover the steep part of the concentration-inhibition graph (e.g., between 20 - 80% current inhibition)

S7B guideline states that adsorption to glass or plastic or nonspecific binding to the test matrix can reduce the concentration of the test substance in the incubation or perfusion medium.

Recommendation: Verify concentration using solution samples collected from the test apparatus

"Real experiment" or "Satellite Experiment"

Reference: Qu *et al*., 2011
Brimecombe *et al*., 2009
Goineau *et al*., 2013

Q&A 2.1 – Assay Sensitivity and Recording Quality

S7B guideline states that a sub-maximal concentration of a positive control substance should be used to demonstrate assay sensitivity

Recommendations:

- Positive control: Concentration-inhibition graph
- Negative control: Time course plots to demonstrate cell health and recording stability

S7B guideline did not provide recommendation on recording quality

Recommendation:

- Demonstrate voltage control, manageable background/leak currents, and recording stability

Q&A 2.1 – Data Summary (I)

S7B guideline did not provide recommendations on data summary

Recommendations:

- Provide both mass and molar concentrations (i.e., ng/mL and nM, respectively)
- Report % or fractional current inhibition at each tested concentrations for individual cells and group average (mean ± SEM)

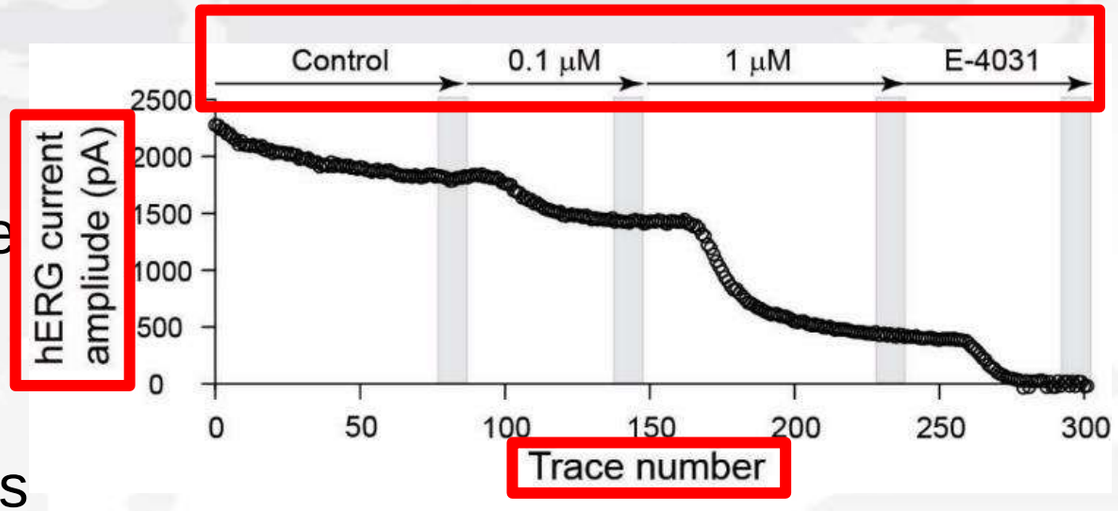
	Fractional inhibition of hERG current																
[Drug] μM	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12	Cell 13	n	Mean	SD	SEM
0.1	0.25	0.13	0.20	0.20				0.31	0.29	0.22				7	0.23	0.06	0.02
0.3					0.49	0.48	0.44				0.52	0.49	0.40	6	0.47	0.04	0.02
1	0.73	0.71	0.73	0.73				0.80	0.74	0.77				7	0.74	0.03	0.01
3					0.89	0.90	0.89				0.91	0.91	0.87	6	0.89	0.02	0.01

Q&A 2.1 – Data Summary (II)

S7B guideline states that the duration of exposure should be sufficient to obtain steady-state electrophysiological effects.

Recommendation for graphics: Time course plots for individual cells

- Ionic current-of interest



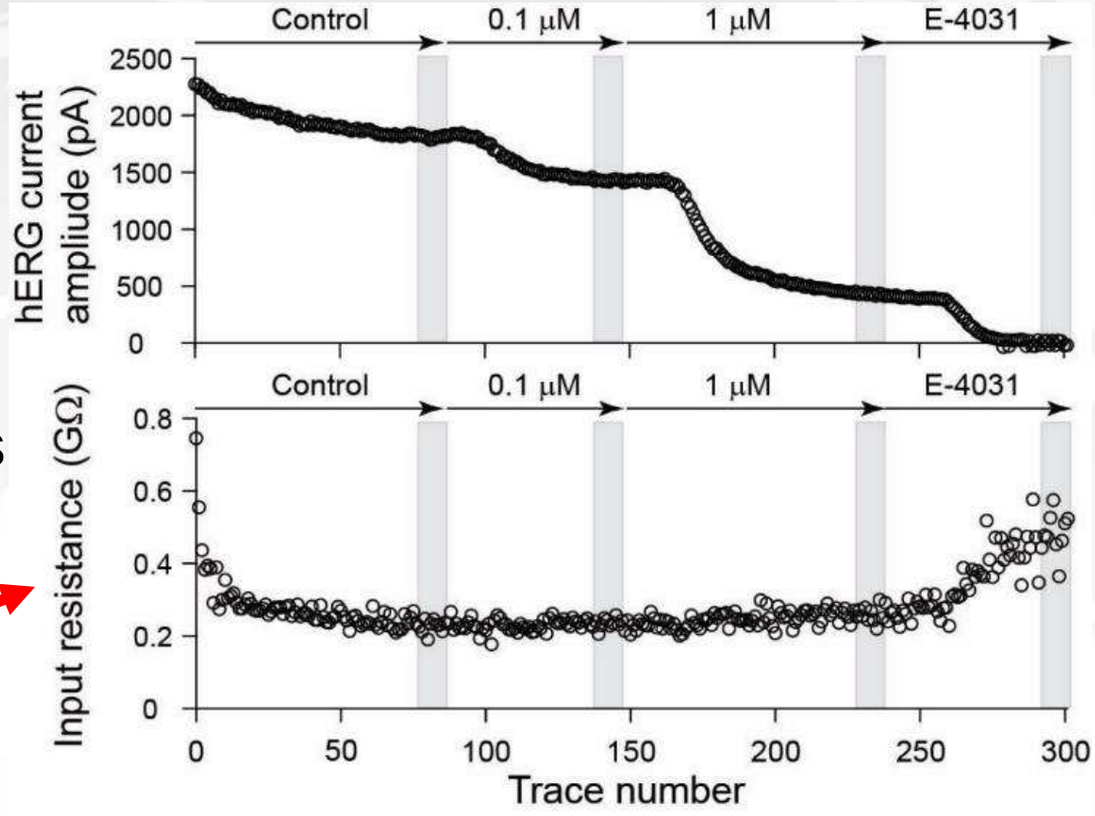
Q&A 2.1 – Data Summary (II)

S7B guideline states that the duration of exposure should be sufficient to obtain steady-state electrophysiological effects.

<u>Recommendation</u> for graphics: Time course plots for individual cells

- Ionic current-of interest
- Input resistance

Seal quality & membrane integrity



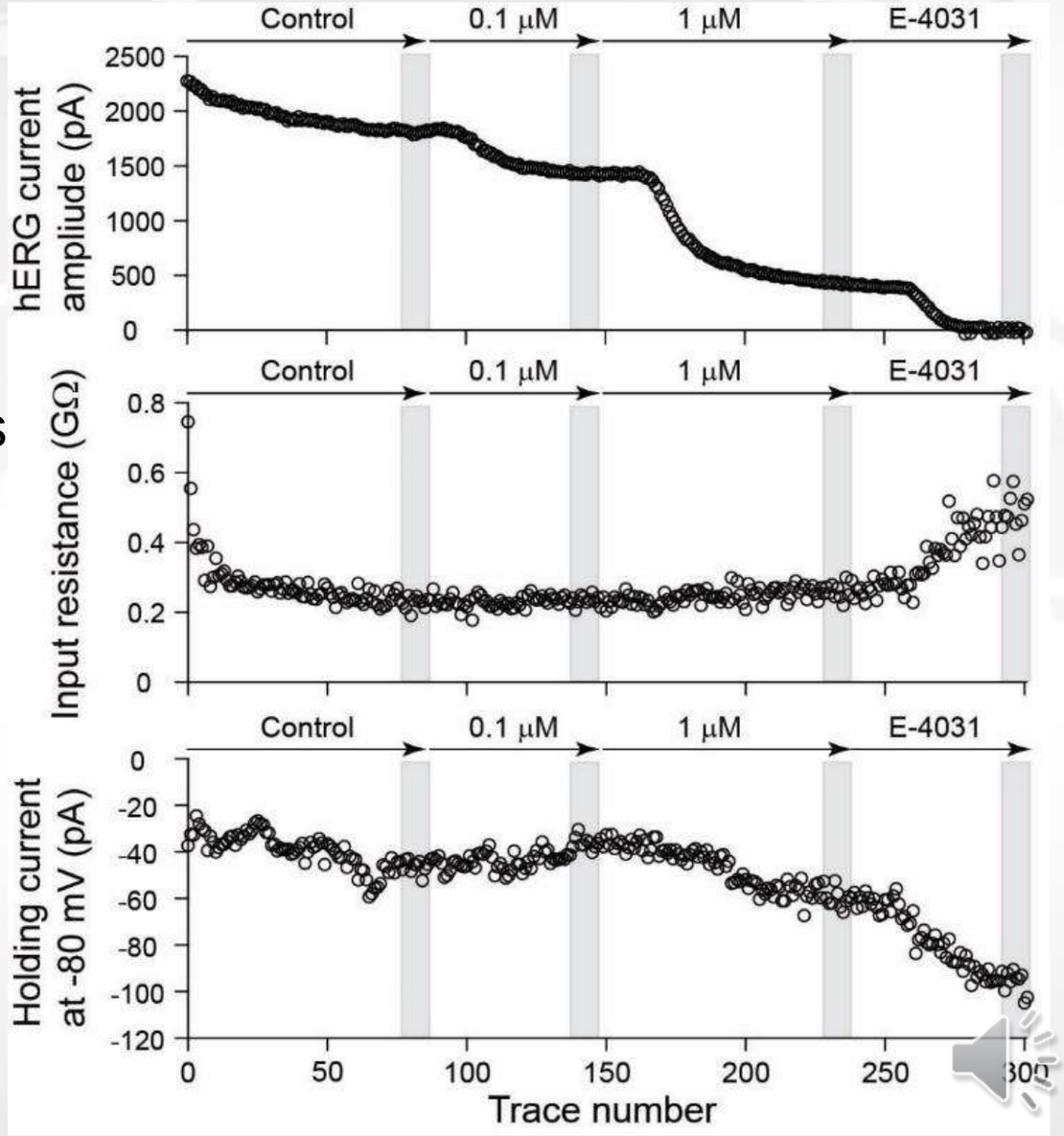
Q&A 2.1 – Data Summary (II)

S7B guideline states that the duration of exposure should be sufficient to obtain steady-state electrophysiological effects.

<u>Recommendation</u> for graphics: Time course plots for individual cells

- Ionic current-of interest
- Input resistance
- Holding current at rest

Cell health for the duration of recording



Q&A 2.1 – Data Summary (II)

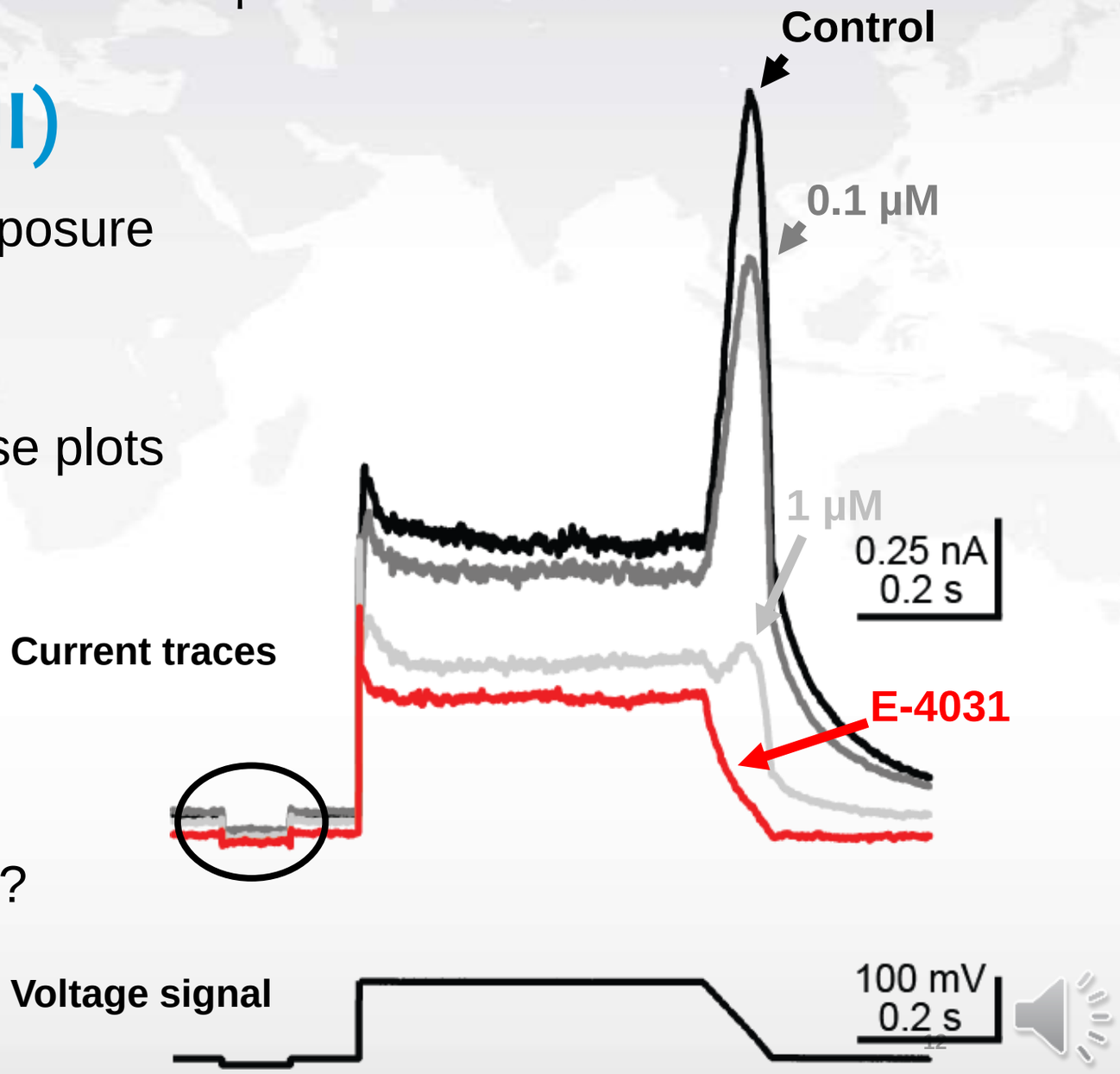
S7B guideline states that the duration of exposure should be sufficient to obtain steady-state electrophysiological effects.

<u>Recommendation</u> for graphics: Time course plots for individual cells

- Ionic current-of interest
- Input resistance
- Holding current at rest

Where and how measurements were made?

Background/leak current subtraction?

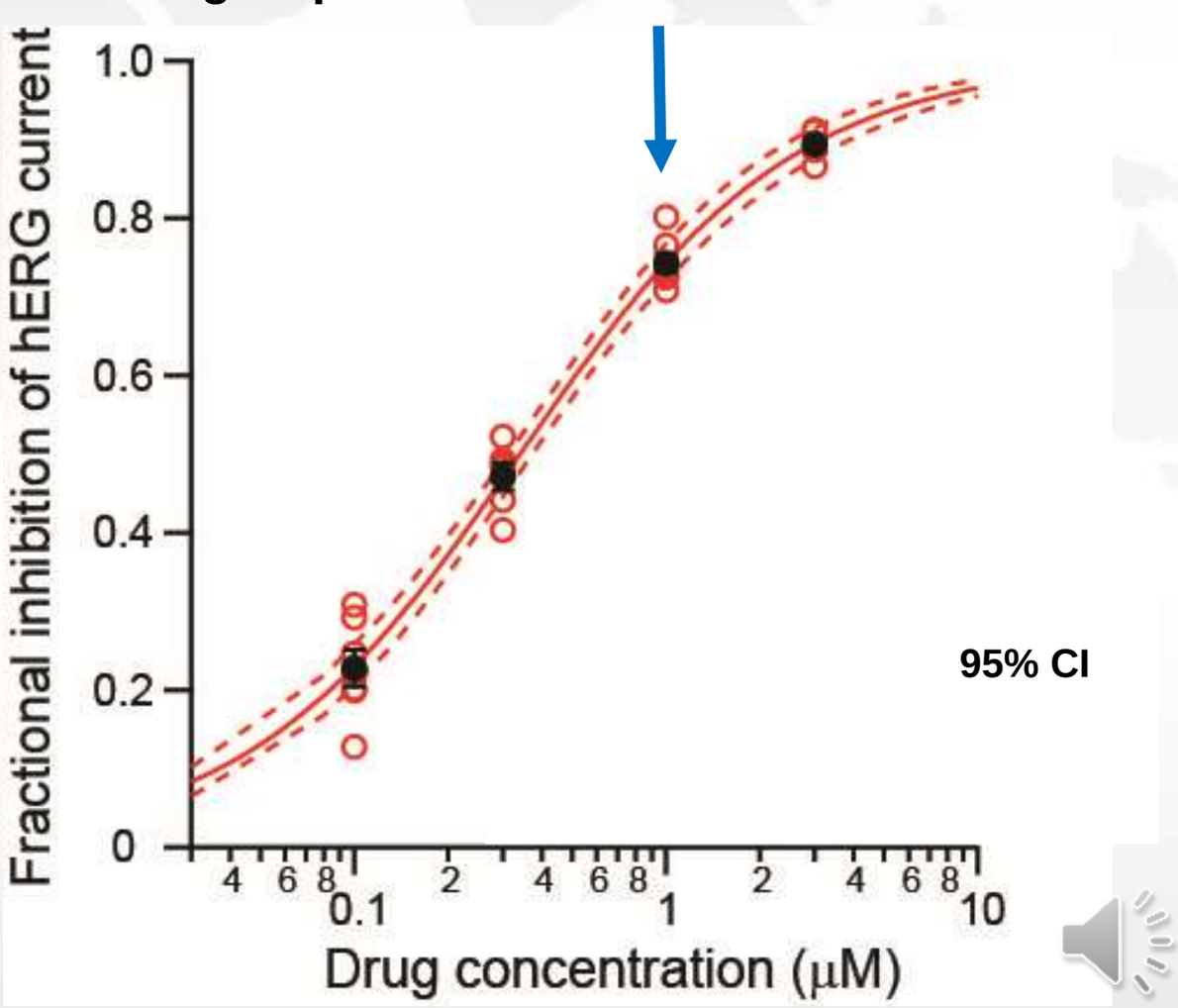


Q&A 2.1 – Data Summary (III)

The primary endpoint measurements are **IC_{50} and the Hill coefficient.**

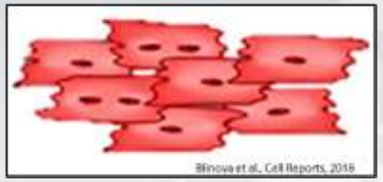
Recommendation for graphics: Plot concentration-inhibition relationship using individual data points and mean ± SEM

- Fit individual data with Hill equation
- State constraints placed on the fit
- Give measures of variability for IC_{50} and and the Hill coefficient estimates



Q&A 2.1 – Overall Summary

- **S7B guideline should continue to be followed to obtain nonclinical data to support first-in-human studies**
- **The new S7B Q&A 2.1 provides best practice recommendations for patch clamp studies including the core, *in vitro* hERG assay**
  - **Harmonize approaches to enhance data reproducibility; allow for better translation to clinical findings**
  - **Provide a robust hERG safety margin for use in revised ICH E14 Q&As 5.1 and 6.1**
- **Applying best practices is encouraged; prevent repeating assays later during clinical development**



Q&A 2.2-2.5 - Best Practices: *In Vitro* Human Myocyte Repolarization Assays

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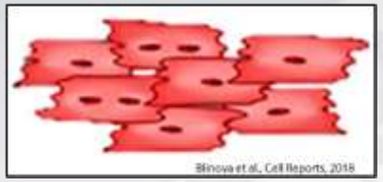
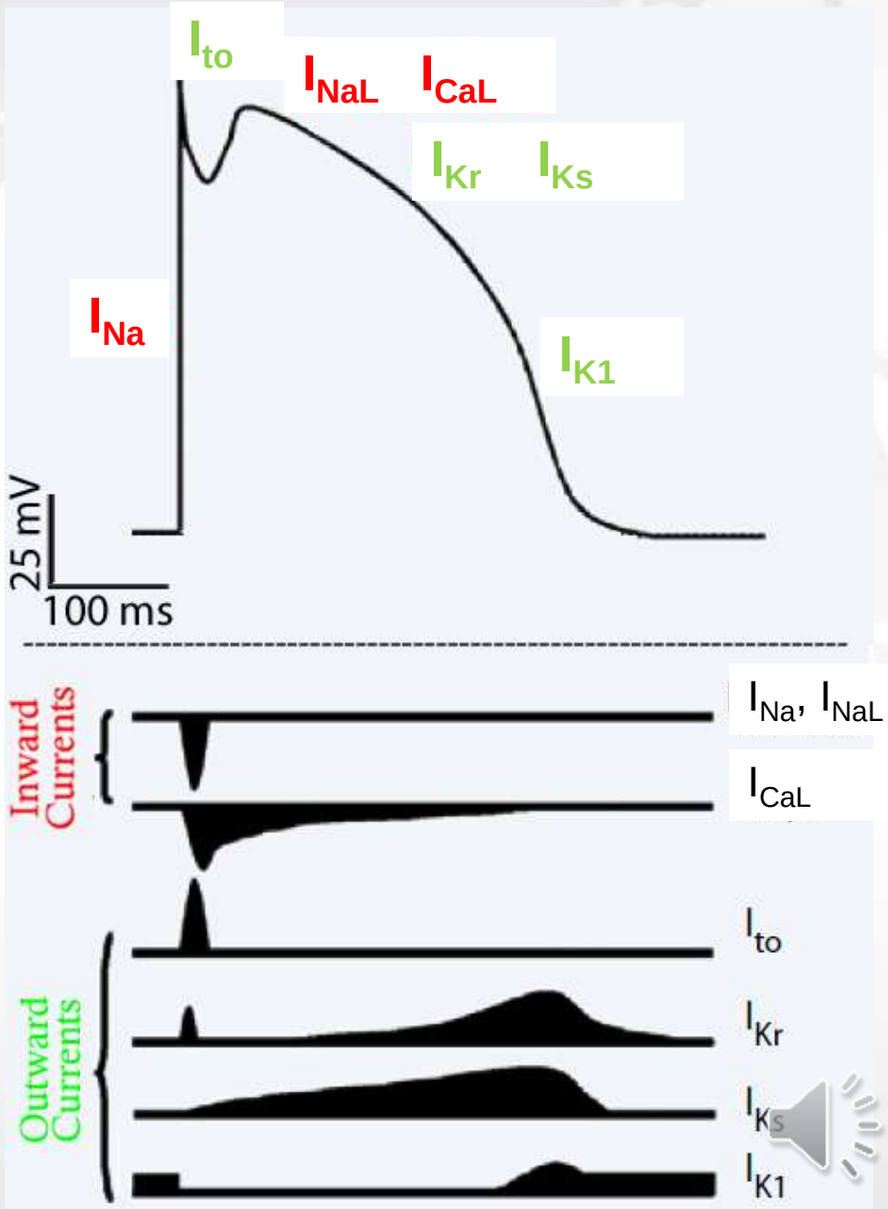
AbbVie, United States
PhRMA

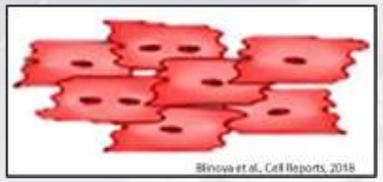


Q&A 2.2-2.5 - Follow-up Studies on Delayed or Altered Repolarization

- **Repolarization:** an integrated cellular response involving multiple overlapping inward (depolarizing [red]) & outward (repolarizing [green]) cardiac currents

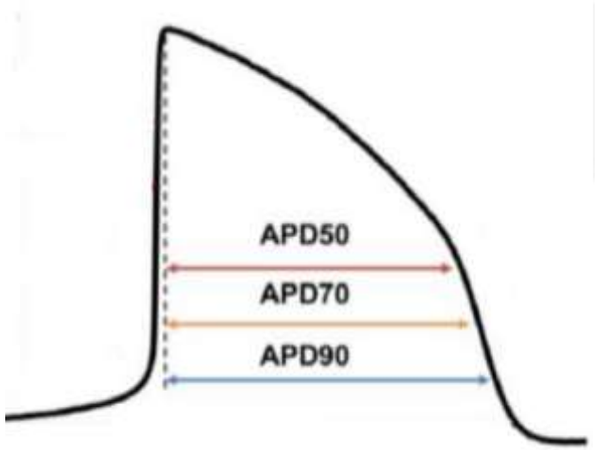
- **Preparations:** human induced pluripotent stem cell-derived cardiomyocytes or acutely isolated human ventricular myocytes





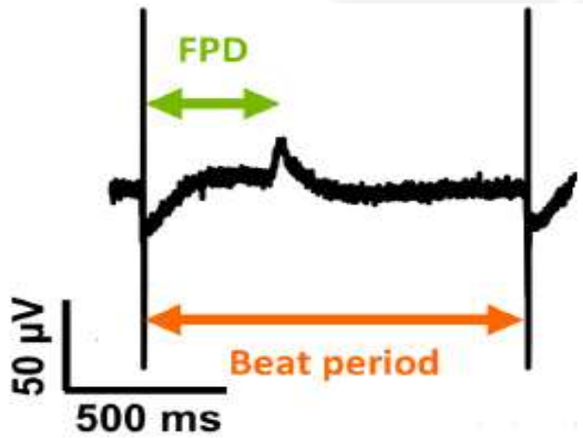
Q&A 2.2-2.5 - Electrophysiologic Study Approaches

Transmembrane Potential Recordings - APD



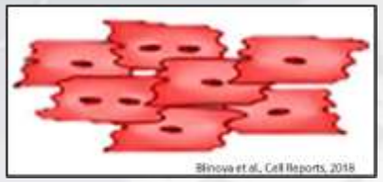
- Action Potential Duration (APD): Transmembrane potential measures (intra-cellular recordings or voltage-sensing dyes)

Extracellular Field Potential Recordings - FPD



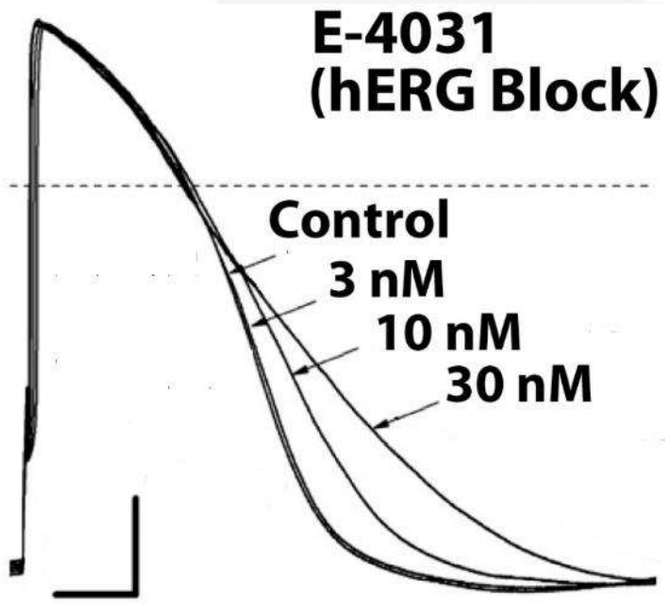
- Field Potential Duration (FPD): Multi-electrode array (MEA) recordings

- Recordings of electrical activity reflect cellular repolarization
- Calcium transients and contractility measures may provide surrogate evidence of repolarization changes downstream of electrical effects

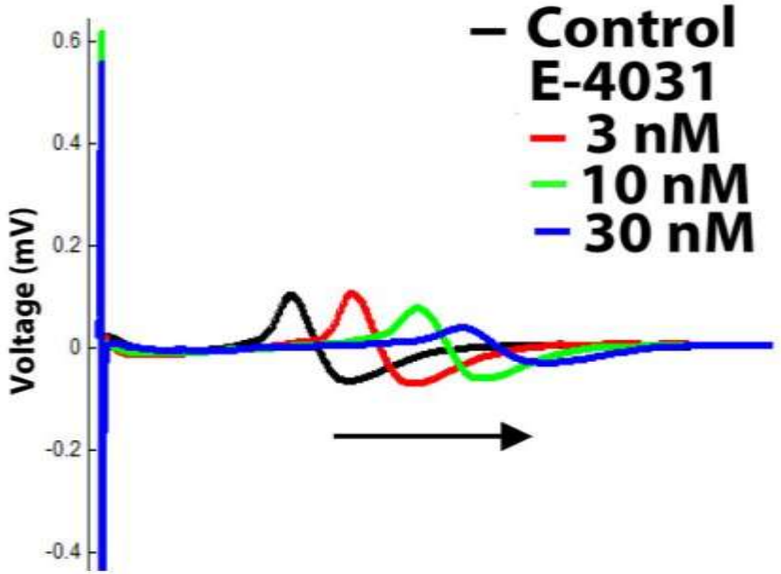


Q&A 2.2-2.5 - Examples of Delayed or Altered Cardiac Repolarization

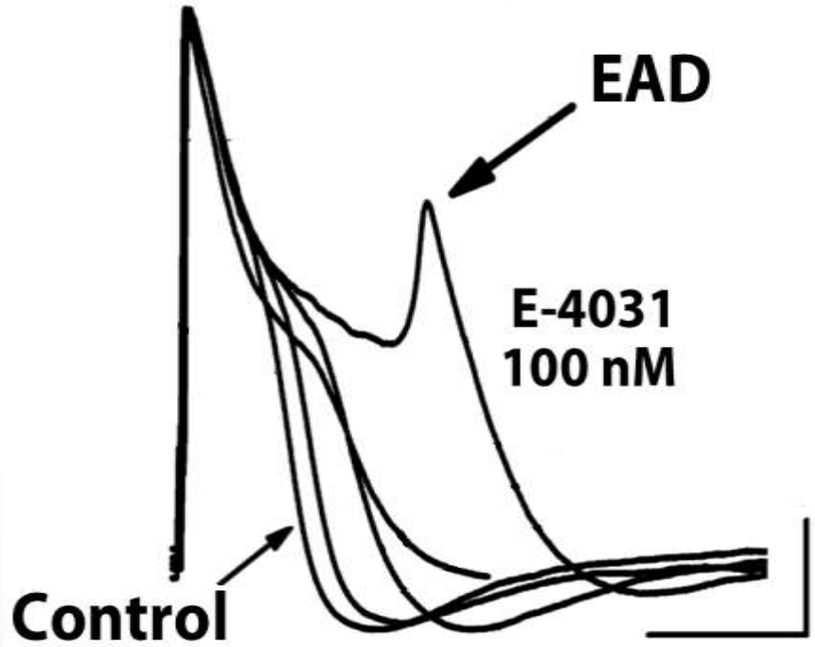
APD Prolongation



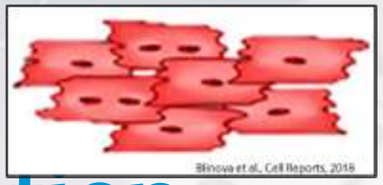
FPD Prolongation



Early Afterdepolarization



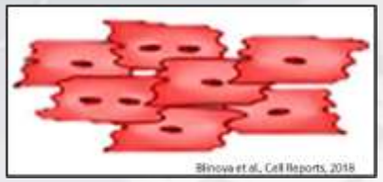
- hERG current inhibition reduces outward current and elicits delayed repolarization
- Excessive inhibition promotes altered and interrupted repolarization such as early afterdepolarizations [EADs]



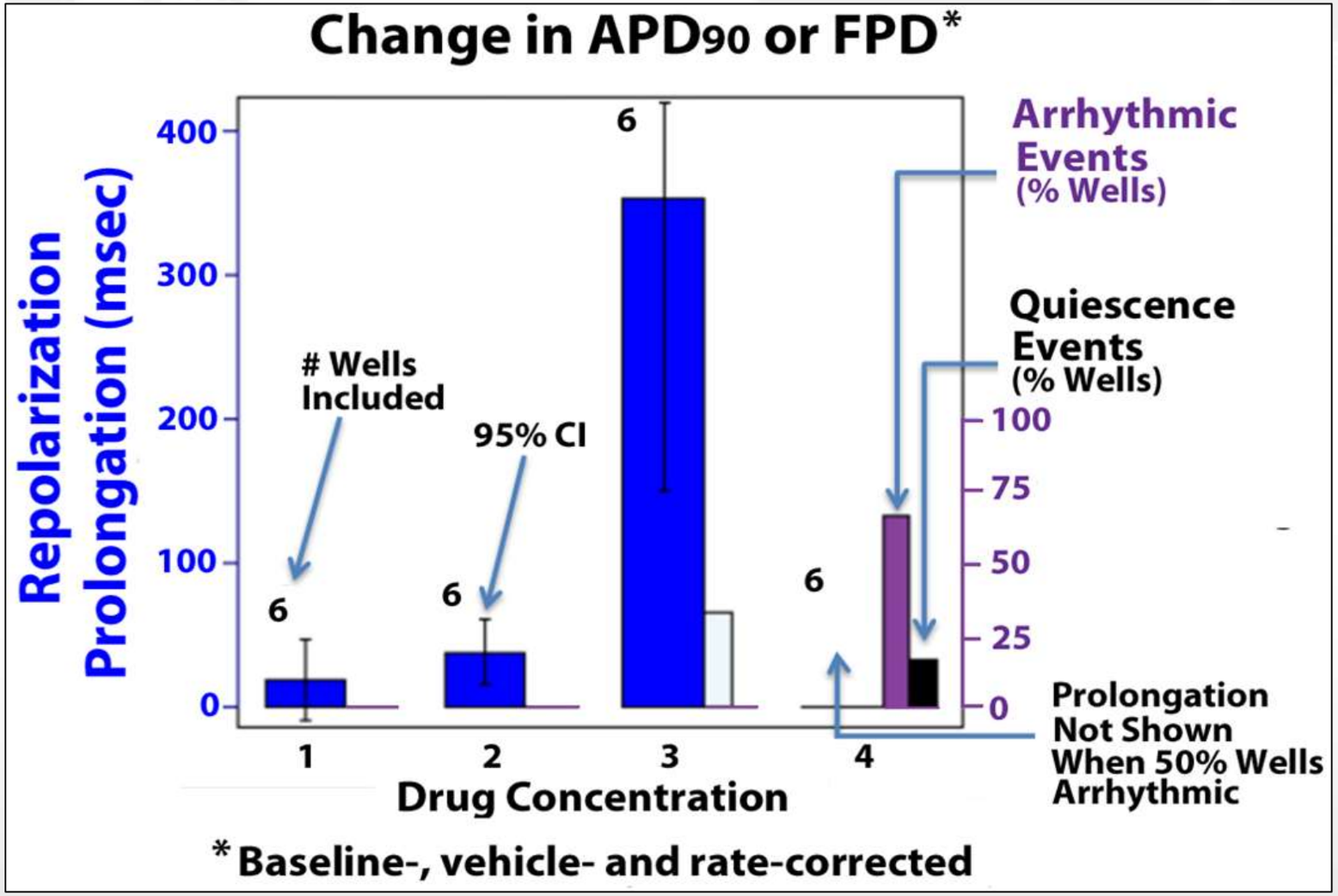
Q&A 2.2-2.5 - Best Practices: Primary Repolarization Endpoints

Experimental Approach	Primary Measure	Details
Micro/Patch Electrode or Voltage Sensing Dyes	Action Potential Duration (APD)	**Primary Endpoints:** a). APD_{90} (90% of terminal repolarization) from single micro/patch electrode or optical field (voltage-sensing dyes) b). Incidence of interrupted repolarization (Early afterdepolarizations, EAD's) **Secondary Endpoint:** Repolarization intervals ($APD_{50,70,90}$) inform on time course, suggest currents affected
Multi-Electrode Array (MEA)	Field Potential Duration (FPD)	FPD typically measured from peaks of depolarization and repolarization
		Recordings from either maintaned single electrode or averaged multiple electrodes
		EAD's more difficult to detect (may appear as extrabeats); report incidence/characteristics

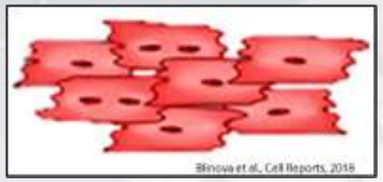
Vehicle, baseline, and rate-correct. Timing of post-dose sampling, sampling duration to be specified.
Justification of correction factors needed with agents affecting rhythmicity of spontaneously beating preparations.



Q&A 2.2-2.5 - Endpoints: Visual Summary Example

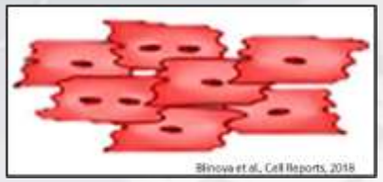


- ***Left axis***: Delayed repolarization effects with increasing concentrations
- ***Right axis***: Altered repolarization effects with increasing concentrations
- Data to be provided in tabular & graphic formats



Q&A 2.2-2.5 - Best Practices: Preparations and Experimental Conditions

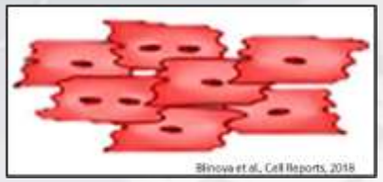
- **Origins, culture conditions and baseline electrophysiological characteristics of myocytes should be well characterized and described**
  - Cell(s) sources, time in culture, substrate, media, technology platform/software
  - Criteria for acceptance of preparations/recordings (APD_{90} or FPD values [means and variability], spontaneous beat rate [for non-paced preparations])
- **Details of experimental approaches**
  - Conditions: temperature, pacing/spontaneously beating myocytes, platform
  - Stability of vehicle controls, time course of drug equilibration
  - Correction factors used (or duration of pacing), number of replicates
  - Analysis software, statistical plan
  - Validation of drug exposures in testing chambers



Q&A 2.2-2.5 - Best Practices: Characterizing Assay Sensitivity

- **Sensitivity to hERG/IKr inhibition should be demonstrated with non-saturating concentrations of selective blockers (e.g. E-4031, dofetilide)**
  - Concentration-dependent prolongation of positive control

- **Sensitivity of inhibition of depolarizing inward currents (I_{CaL} and I_{NaL}) provided when multi-channel block is suspected**
  - Repolarization shortening with I_{CaL} inhibition (e.g. nifedipine or nisoldipine)
  - Repolarization shortening with I_{NaL} inhibition (e.g. mexiletine or lidocaine)
  - Selectivity of blocking agents and potential confounding changes in spontaneous rate should be discussed



Q&A 2.2-2.5 - Overall summary: *In Vitro* Human Myocyte Repolarization Assays

- Updated best practices for *in vitro* "Follow-up Studies" (as in original ICH S7B) using human derived ventricular preparations should:
  - Guide their evolving role in comprehensive electrophysiological studies of ventricular repolarization
  - Guide information submitted to regulatory authorities for human-derived cardiomyocytes

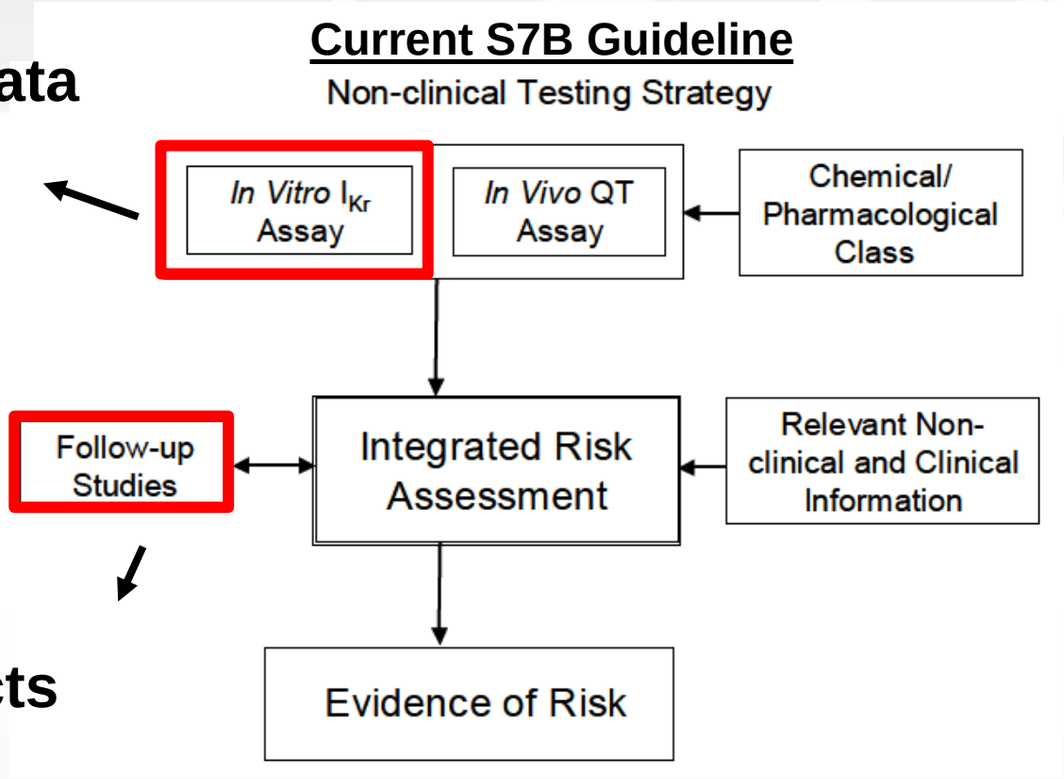
Objectives of S7B Q&A #2

1. **Harmonize approaches; reduce data variability; allow for better translation to clinical findings**

   Double negative findings from core assays can be used to support E14 Q&As 5.1 and 6.1.

2. **To incorporate conventional and newer technologies and human preparations to assess drug effects on repolarization**

   Optional to clarify positive signals from core S7B assays within Integrated Risk Assessment

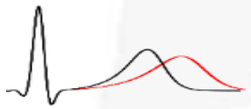


Day 2 Schedule

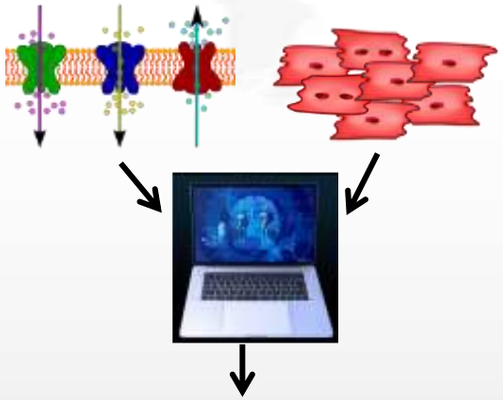
Best Practice Considerations

***In vitro* studies**

***In vivo* studies**



Principles of Proarrhythmia Models



Model Risk prediction

- ✓ **Recap of Day 1 and Introduction to Day 2**
  - ✓ **Derek Leishman, *PhRMA***
- ✓ **Best Practice Considerations for *In vitro* Studies Q&As**
  - ✓ **Wendy Wu, *FDA, United States* and Gary Gintant, *PhRMA***
- ➢ **Best Practice Considerations for *In vivo* QT Studies Q&As**
  - o **Satoshi Tsunoda, *MHLW/PMDA, Japan***
- ➢ **Principles of Proarrhythmia Models Q&As**
  - o **Takashi Yoshinaga, *JPMA***
- ➢ ***Discussion of Questions Received from the Q&A Pod***
  - o **Facilitators: Derek Leishman, *PhRMA* and David Strauss, *FDA, United States***
  - o **All Speakers and Xiaodong Zhang, *NMPA, China*; Eva Rached, *Swissmedic, Switzerland*; and Yu-Chung Chiao, *TFDA, Chinese Taipei; Katsuyoshi Chiba, JPMA***

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harmonisation for better health

Thank you!

International Council for Harmonisation of Technical Requirements
for Pharmaceuticals for Human Use

References

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