I. Patch clamp protocol (Ion channel)

I.1. Have you already defined the manual patch clamp protocol?
I.1.a. Yes, the manual patch clamp protocols have been defined for all of the channel types to be assayed in the validation studies.

I.2. Have the completed 12 CiPA training compounds been done using the defined protocol?
I.2.a. Yes, all experiments using manual work for hERG have been completed for the 12 CiPA training drugs at both ambient and physiological temperatures.

I.3. If so, how did you define the appropriate protocol?
I.3.a. The final selection of the hERG protocol was based on the data obtained from a series of pilot experiments. The original hERG protocol proposed by the Ion Channel Working Group proved to be inadequate in determining kinetics of block for the training set of drugs. After careful review of the literature, and a series of preliminary experiments, the protocol from Milnes et al., (2010) was adopted. The Milnes protocol was originally developed to study the time-development of hERG inhibition by cisapride and dofetilide following channel gating. It was based on the protocol used previously by the same laboratory to study the effects of Lidoflazine on hERG (Ridley et al., 2004).

I.4. Is the protocol of high-throughput (HT) platforms the same as the manual patch clamp protocol?
I.4.a. The protocol of the HT platform is the same as the manual patch clamp protocol. While the majority of the HT experiments will be performed at ambient temperature, some data will also be obtained at physiologic temperature on the systems that allow temperature control. In addition, a second protocol described as “step-ramp” will also be used by the HT group to characterize the potency of hERG block of the same 12 compounds. The HT work is expected to eventually study all 28 CiPA compounds on all 7 ion channels on HT systems.

II. Temperature dependency (Ion channel/ In silico)

II.1. Have the completed experiments of the12 CiPA training compounds of the 7 cardiac channels been done at both ambient and physiological temperature in manual patch clamp?
II.1.a. For the dynamic hERG protocol, we have two sets of data: 37°C data from FDA and room temperature data from the ICWG. Differences are seen between the two data sets and we are still working on modeling the room temperature data. For other channels, physiological temperature IC50 data have been obtained (Crumb et al., 2016) while room temperature data still need to be generated.

II.2. Will you also develop temperature-dependent in silico models of the other six ion channels?
II.2.a. If necessary. The level of dynamic detail required to represent drug-channel interactions for channels other than hERG still needs to be assessed. IC50s can be temperature-dependent, so some accommodations are likely to be needed before using room temperature patch clamp data in the model (either additions to the model or changes to how IC50s are measured using patch clamp).

III. Modification of ORd model (In silico)

III.1. Have you evaluated the difference of cell type of ventricular layer (epi, m, endo) in O'Hara Rudy model using dynamic hERG model?
III.1.a. The endocardial cell model in ORd has been validated against experimental electrophysiology data from real human hearts. The mid-myocardial and epicardial models were derived by scaling ion channel conductance based only on gene expression data. While some exploratory simulations have been performed with other cell types, candidate proarrhythmic metrics will rely on the endocardial cell model because it is the model most closely aligned with actual electrophysiology data.

III.2. Which type of cellular model is suitable for evaluating the risk metrics?
III.2.a. As outlined above, the endocardial cell model is the only model that has been validated with experimental electrophysiology data with real human hearts. Thus the endocardial cell model will be the primary focus for evaluating candidate risk metrics.

IV. Inward current change metric (In silico)

IV.1. Is bradycardia also a key metric of inward current change? How about the result of inward current change and EAD with a cycle length of 1000 ms?
IV.1.a. Yes. Bradycardia is a key component of the metric. When simulating at 1000 ms, there is separation between Low and Intermediate/High risk drugs, but not between Intermediate and High risk compounds. It appears that High risk drugs that are trapped in the hERG channel exhibit reverse use dependency, where the APD prolongation and generation of EADs becomes more prominent at longer cycle lengths.

IV.2. Do we need to simulate drug effects with various cycle lengths and cellular model?
IV.2.a. In the evaluation of candidate metrics, various cycle lengths and cellular models, as well as various other physiological conditions, will be needed. The selected metric(s) may be linked to one particular condition (a particular cycle length, a particular cell model, etc.), like the current inward charge metric does. But it is also possible that the final metric will be a quantitative marker computed under various conditions.

V. MEA assay (Myocyte)

V.1. Have you already defined correction formula to correct FPD (field potential duration)? If so, how did you define the correction formula?
V.1.a. The original protocol for the MEA studies did not indicate any specific correction factor to use for the analysis. The FPD data are being collected as a raw or uncorrected value, thus allowing flexibility for correction factor(s) to be applied after experiments. There are various correction factors presently being employed in the literature, and Fridericia's appears to be more frequently used. However, we will have the capability of using this (or other(s)) correction factors based on group discussions.

V.2. Do you think that MEA assay need to be conducted with pacing?
V.2.a. It would be preferable to conduct MEA studies with paced myocytes (obviating correction factors). However, considering a reasonable rate for pacing (1 Hz), it would not be possible to stimulate some myocytes due to their intrinsically higher pacing rate. Consensus would have to be achieved for defining an acceptable "standard" rate for pacing. Further, the technologies for pacing 2D myocyte monolayers are still evolving, and presently not widely used or available to all laboratories. Given these limitations, the CiPA validation studies are being conducted on spontaneous, non-paced myocytes.

VI. Late sodium current (Phase 1 ECG)

VI.1. How did you define the concentration of lidocaine and mexiletine for appropriate block of late sodium current?
VI.1.a. The doses of lidocaine and mexiletine in Johannesen et al. (2016) were selected to match the expected exposure of previous studies showing QTc shortening (lidocaine: Rosero et al. 1997; mexiletine – Giardana et al. 1990).

VI.2. Did Lidocaine and Mexiletin prolong QRS at the concentration in multichannel block experiment?
VI.2.a. No QRS prolongation was observed or expected at the clinical concentrations studied. This is consistent with both lidocaine and mexiletine being selective late sodium (vs. peak sodium) blockers at the studied concentrations. (Johannesen et al., 2016, Crumb et al., 2016).

VII. ICaL (Phase 1 ECG)

VII.1. Have you evaluated balanced effects of ICaL block on J-Tpeak? Is the effect of ICaL block on J-Tpeak similar to late sodium current block?
VII.1.a. We have studied a mixed hERG potassium and calcium channel blocker (verapamil) a combination of a hERG potassium channel blocker (moxifloxacin) and a calcium channel blocker (diltiazem), and drugs from prior thorough QT studies with approximately equipotent hERG, calcium and late sodium block.
First, for verapamil, there was no prolongation of the J-Tpeakc interval, as expected (Johannesen et al., **(a)** 2014). Second, the addition of diltiazem to moxifloxacin did not result in a shortening of J-Tpeakc, however, that analysis was confounded by the potential accumulation of a moxifloxacin metabolite when diltiazem was co-administered and potential autonomic response that could increase the effect of IKs block (Johannesen et al., 2016). Third, two drugs from prior thorough QT studies that block hERG, calcium and late sodium prolonged QTc, but did not prolong J-Tpeakc (Johannesen et al., **(b)** 2014), as expected. Simulations also suggest that L-type calcium block shortens QTc by shortening the J-Tpeakc interval (Johannesen et al., **(b)** 2014), similar to late sodium block.

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