Improving cardiac safety testing protocols

The International Conference on Harmonization (ICH) S7B and E14 regulatory guidelines were introduced in 2005 to evaluate the proarrhythmic liability of new drugs. They were implemented in response to the discovery that inhibition of a cardiac potassium channel, encoded by hERG, is associated with prolongation of the QT interval and a potentially deadly arrhythmia, Torsades de Pointes. The guidelines use hERG inhibition and QT interval prolongation as surrogate markers of proarrhythmic liability, which are highly sensitive and have proven effective at preventing proarrhythmic drugs from reaching the market.

However, these markers have low specificity, with only a modest correlation between hERG inhibition, QT prolongation and proarrhythmic liability. Indeed, hERG inhibition can be mitigated by concurrent inhibition of depolarising currents, indicating that screening against a wider panel of cardiac ion channels would provide better proarrhythmic liability profiling. This notion is supported by the observation that some drugs with low proarrhythmic liability, such as verapamil, are potent hERG blockers, but also inhibit depolarising currents such as ICa. Unfortunately, an over-reliance on hERG selectivity has potentially led to the removal of many efficacious and safe drugs from development pipelines. Consequently, this casts some doubt on hERG inhibition and/or QT prolongation as suitable markers for predicting proarrhythmic liability.

To address these limitations, the Comprehensive *in Vitro* Proarrhythmia Assay (CiPA) initiative was launched by the US Food and Drug Administration in July 2013. The CiPA initiative aims to improve the accuracy and reduce the cost of predicting cardiac liability using three 'pillars.' In the first instance, compounds will be profiled against a panel of human ventricular ion channels. In the second, this *in vitro* data will be incorporated into an *in silico* model of a human action potential (AP) to provide a proarrhythmic risk classification. In the third, compounds will be tested using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) to confirm the risk classification.

Three working groups composed of members from academia, pharmaceutical companies and contract research organisations were established to test and validate each of the CiPA pillars. A common toolbox of 28 compounds, separated into high, intermediate and low proarrhythmic risk groups, was used across all working groups. The toolbox was subdivided into sets of 12 training compounds and 16 validation compounds.

The Ion Channel Working Group (ICWG) was tasked with establishing robust and reproducible assays against a panel of seven ventricular currents. A large component of the work includes generating standardised assays and identifying the most applicable readout parameters to support the *In Silico* Working Group (ISWG). ICWG is exploring the variability of data obtained across research sites, and comparing data generated from conventional manual patch clamp and automated patch clamp platforms. An initial study was conducted using the 12 training compounds and standardised experimental methodologies to verify their accuracy, which was followed by blind testing of the 16 validation compounds. The training compound data collected for some ion channels revealed prominent variation in IC50 values across research sites. Some of this was related to intersite differences in experimental methods. Therefore, the ICWG continues its efforts to improve reproducibility across research sites.

The ISWG is developing an *in silico* model to allow prediction of proarrhythmic risk, focusing its efforts on the O'Hara-Rudy (ORd) model, which is based on human ventricular AP recordings. The model recapitulates early after depolarisations, which are crucial to accurately predict proarrhythmic risk. The FDA optimised the ORd model by incorporating the late component of NaV1.5 current, as well as a dynamic hERG blocking model that more accurately accounted for the higher proarrhythmic risk profile of compounds that become trapped within the hERG pore during repolarisation. The optimized ORd model was calibrated using IC50 data from the training set supplied by ICWG. The *in silico* readout includes a net charge metric 'qNet' and incorporates an uncertainty quantification method to account for experimental variability. The mean qNet value averaged across 1–4 fold of the Cmax drug plasma concentration is provided as the Torsade Metric Score (TMS). The ISWG has proposed two TMS thresholds that classify drugs into the three proarrhythmic risk categories. The calibrated model was then used to evaluate the validation compound set, showing that the model met all pre-specified measures for ranking, and classifying the drugs according to their clinical arrhythmia risk classification. The ICWG studies revealed that inhibition of hERG, CaV1.2 and late NaV1.5 current have the most significant impact on proarrhythmic risk prediction. This opens the possibility that the *in vitro* ion channel panel, which is the first pillar, may be reduced to these key currents.

The third pillar involves testing compounds in a complex human cellular system (iPSC-CM) that contains all of the ion channels in the CiPA ion channel panel, as well as additional ion channels and pumps that could affect a compound's proarrhythmic liability profile. The two favoured approaches for studying the effect of compounds on iPSC-CM include measuring extracellular field potentials (using multielectrode array platforms) and fluorescence signals from voltage-sensing dyes or genetically encoded voltage indicators. The CiPA cardiomyocyte consortium used the test and validation compounds across several iPSC-CM cell lines and plate-based readouts, revealing excellent specificity and sensitivity that matched, or exceeded, that of animal models (100% specificity and up to 79% sensitivity). There was greater variation between cell types than between different screening platforms, suggesting that further work is required to optimise the iPSC-CM reagents.

Additional testing and validation is ongoing for all three pillars to further improve the excellent sensitivity and specificity of these assays.

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