FUNDING
Austrian Science Fund (FWF) (grant no. KLI 694-B30).

CONFLICT OF INTEREST
The authors declared no competing interests for this work.

1. Schinkel, A.H. *et al.* Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**, 491–502 (1994).
2. Kannan, P. *et al.* Imaging the function of P-glycoprotein with radiotracers: pharmacokinetics and in vivo applications. *Clin. Pharmacol. Ther.* **86**, 368–377 (2009).
3. Tournier, N., Stieger, B. & Langer, O. Imaging techniques to study drug transporter function in vivo. *Pharmacol. Ther.* **189**, 104–122 (2018).
4. Muzi, M. *et al.* Imaging of cyclosporine inhibition of P-glycoprotein activity using ^{11}C-verapamil in the brain: studies of healthy humans. *J. Nucl. Med.* **50**, 1267–1275 (2009).
5. Kalvass, J.C. *et al.* Why clinical modulation of efflux transport at the human blood-brain barrier is unlikely: the ITC evidence-based position. *Clin. Pharmacol. Ther.* **94**, 80–94 (2013).
6. Wanek, T. *et al.* Factors governing p-glycoprotein-mediated drug-drug interactions at the blood-brain barrier measured with positron emission tomography. *Mol. Pharm.* **12**, 3214–3225 (2015).
7. Pottier, G. *et al.* Imaging the impact of the P-glycoprotein (ABCB1) function on the brain kinetics of metoclopramide. *J. Nucl. Med.* **57**, 309–314 (2016).
8. Hsin, C.H., Fuhr, U. & Trueck, C. Response to "Impact of acute alcohol exposure on P-glycoprotein function at the blood-brain barrier assessed using ^{11}C-metoclopramide PET imaging". *Clin. Pharmacol. Ther.* **105**, 814–814 (2018).
9. Tournier, N. *et al.* Impact of P-glycoprotein function on the brain kinetics of the weak substrate ^{11}C-metoclopramide assessed with PET imaging in humans. *J. Nucl. Med.* (2019). https://doi.org/10.2967/jnumed.118.219972. [e-pub ahead of print].
10. Syvänen, S., Xie, R., Sahin, S. & Hammarlund-Udenaes, M. Pharmacokinetic consequences of active drug efflux at the blood-brain barrier. *Pharm. Res.* **23**, 705–717 (2006).

"Thorough QT/QTc in a Dish": Can Human Induced Pluripotent Stem Cell–Derived Cardiomyocytes Predict Thorough QT Outcomes?

Hugo M. Vargas[1,*]

The emergence of human induced pluripotent stem cells–derived cardiomyocyte (iPSC-CM) has sparked interest in this tool for cardiovascular safety assessment during preclinical safety testing. The use of multiple iPSC-CM cell lines in parallel to identify drug-induced corrected QT (QTc) prolongation risk resembles the execution of a clinical thorough QT (TQT) (i.e., "TQT/QTc study in a dish"). This article highlights the potential and challenges of using a human iPSC-CM model for predicting the outcomes of a TQT study, as proposed by Blanchette *et al.*[1]

BACKGROUND

The concept of "clinical trials in a dish" (CTiD) is gaining attention as a new approach to assess the risk, and efficacy, of candidate drugs during preclinical drug development given the emergence of human-derived induced pluripotent stem cell (iPSC) models for experimental evaluation and drug testing.[2,3] The ability to create multiple iPSC lines derived from a range of healthy or diseased human donors enables the notion of CTiD. The use of multiple human cells lines mimics the interindividual variability seen in clinical trials and may enhance the detection of drug-induced effects rather than using a single organotypic cell line from one donor.

In specific regard to preclinical cardiovascular safety assessment, a well-known battery of *in vitro* (e.g., human ether-a-go-go (hERG) channel function, etc.) and *in vivo* (dog or primate telemetry, etc.) assays have been used routinely to identify QTc prolongation risk, a known electrophysiological biomarker for polymorphic ventricular tachycardia (Torsades de pointes (TdP)).

[1]Comparative Biology & Safety Sciences, Amgen, Inc., Thousand Oaks, California, USA. *Correspondence: Hugo M. Vargas (hvargas@amgen.com)

Received December 7, 2018; accepted January 23, 2019. doi:10.1002/cpt.1384

The standard battery of cardiovascular safety pharmacology models has been effective in identifying clinical candidates with low QTc/TdP risk, but new models are becoming available. Human cardiomyocytes derived from iPSC (iPSC-CM) approaches have the potential to improve the quality of early-stage risk assessment, the clinical relevance of findings, and reduce animal use during preclinical testing. Theoretically, iPSC-CM represents the opportunity to include a human-centric cardiac functional evaluation of drug-induced risk ahead of first-in-human clinical trials. This idea is appealing, especially if the sensitivity, specificity, and predictive performance characteristics of the iPSC-CM assay and end points can be established, as has been done for the hERG function and *in vivo* QTc assays used currently. For example, we know that drug candidates with high potency to block hERG function have higher risk for QTc prolongation and TdP[4] and that the preclinical *in vivo* QT assay has high negative predictive value.[5]

In this journal, Blanchette *et al.*[1] described a novel *in vitro* cardiovascular safety pharmacology evaluation in which they attempted to recapitulate the clinical QTc findings of 13 (10 = increase; 3 = no effect) marketed drugs using a population of iPSC-CM lines from many human donors ($N = 27$), hence a "TQT/QTc study in a dish." In the iPSC-CM lines, drug-induced changes in the calcium flux decay-rise ratio were used as the *in vitro* surrogate for prolongation of the QTc interval, based on prior experimentation with a single QTc prolonging agent. Most (9/10) of the positive control drugs known to cause QTc prolongation increased the decay-rise ratio (of the calcium signal) in a concentration-dependent manner, whereas two of the three agents with no human QTc effect (cabazitaxel and mifepristone) had no impact on the calcium decay/rise ratio at clinically relevant exposures. Notably, a third of the QTc prolongers exhibited a "notch" or plateau in the declining phase of the calcium signal, which could reflect a proarrhythmic calcium trigger, but none of the clean agents exhibited this phenomenon. Subsequent concentration-response modeling of the *in vitro* potency-response (decay/rise) data, and correlation analysis with the *in vivo* (human) QTc interval findings, demonstrated that the iPSC-CM–derived calcium flux data could predict the clinical concentration–QT effect for 85% (11/13) of the agents. One of the QTc prolongers (moxifloxacin; false negative) and one of the drugs with no QTc effect (lamotrigine; false positive) failed an accurate prediction.

The use of multiple iPSC-CM lines to reflect human population–based diversity and susceptibility to drug response avoids the bias and risk of using a single cell line, which could exhibit high or low sensitivity to repolarization changes and misinform a cardiovascular (QTc prolongation risk) safety decision, which is a concern of the single cell line approach.[3] An added advantage of this approach is the ability to study genotype-specific effects on drug-induced cellular changes. As a proof of concept study, Blanchette *et al.*[1] clearly indicates that using a single human iPSC-CM line has limitations and reinforces the need to use multiple cell lines to gain a more comprehensive understanding and interpretation of drug-induced effects on cardiac cell repolarization. From the practical point of view of laboratory resource utilization and screening throughput needed (high or low volume of compounds), testing new drug candidates in 27 cells lines and crunching calcium image analysis data is a large and resource-intensive task and begs the question: What is the minimum number of iPSC-CM cell lines that should be included in a "TQT/QTc study in a dish" to have confidence in the results? The authors characterized and used 27 iPSC-CM cell lines derived from healthy donors from a broad demographic (15 men and 12 women; 24 white and 4 African American ancestry), but the optimal number of cell lines needed for hazard identification based on the calcium flux decay-rise ratio vs. predicting the outcome of a TQT study could be different. Another consideration would be to use iPSC-CM that demonstrates genetic susceptibility to detect QTc prolongation. The range of drug concentrations tested in the cell lines is another key factor, as only 3 fixed concentrations of drug were applied to all 13 agents evaluated. Three concentrations may be inadequate for describing a concentration-response curve *in vitro*, especially for novel compounds with unknown or mixed pharmacological properties.

Regarding validation of the "TQT/QTc study in a dish" approach, the authors tested a small number of drugs (e.g., 10 QTc = positive and 3 QTc = no effect). The number of compounds tested is too low to reach a conclusion about the accuracy of prediction, especially for understanding the false-positive rate. Furthermore, no information about the cardiac multi-ion channel profile, especially hERG inhibitory potency, was provided for the 13 agents evaluated; this information may have influenced the choice of test concentrations used in their study. To truly understand the potential value of an *in vitro* TQT approach, a validation study (conducted in a blinded fashion) that uses the same set of 28 clinical reference compounds identified through the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative[6] needs to be conducted to understand the mechanistic link(s) between inhibition of various cardiac ion channels and effects on the calcium flux end points measured in the iPSC-CM lines. One of the QTc prolongers (moxifloxacin; false negative) and one of drugs with no QTc effect (lamotrigine; false positive) failed accurate prediction, but the reasons for this are unclear and highlight the need for additional studies. It is interesting to note that a recent study of moxifloxacin in a panel of 10 human iPSC-CM lines demonstrated consistent prolongation of the field potential (an indicator of delayed repolarization) that correlated well with QTc interval prolongation in the clinic,[7] which suggests that different iPSC-CM cell lines, test concentrations, or the end point influence the ability to detect drug-induced repolarization changes. Another complication of the Blanchette *et al.*[1] analysis was the inclusion of mifepristone as a negative (no QTc) control, given conflicting clinical evidence. For example, Darpo *et al.*[8] conducted a follow-up QTc study with mifepristone and did not observe any effect upon ventricular repolarization at high clinical exposures, a finding that conflicted with a prior TQT study that demonstrated an increase in the QTc interval at lower exposure levels. Thus, more work needs to be done with a broader set of compounds to better understand the performance characteristics of this particular iPSC-CM-based approach.

Lastly, Blanchette *et al.*[1] utilized the calcium flux decay-rise ratio as a surrogate for the *in vivo* QTc interval, with the assumption that the drugs that prolong cardiac myocyte action potential duration (e.g., inhibitors of hERG function) will also prolong the downward (decay) slope of the intracellular calcium signal and increase the decay-rise ratio. Cyclic elevations and reductions in calcium transients do reflect the orchestrated activity of multiple cardiac myocyte ion channels, but intracellular calcium signaling is regulated by cytosolic proteins (e.g., sarcoplasmic reticulum calcium ATPase) and the sodium-calcium exchanger. For example, inhibition of sarcoplasmic reticulum calcium ATPase by thapsigargin prolongs the duration to remove calcium from the cytosol,[9] which would manifest as an increase of the decay-rise ratio in the current study protocol. However, thapsigargin decreased action potential duration in the same myocyte that showed an increased duration of the calcium transient.[9] These findings suggest some agents that increase the calcium decay-rise ratio may not be associated with delayed cardiac repolarization, so the ratio may not be a specific indicator for QTc interval prolongation. Use of a voltage-sensitive dye would be a more useful and direct measure of membrane potential change and cardiomyocyte repolarization.

The ability to use human tissue to enable better preclinical safety testing is coming into practice, and the use of primary cardiac ventricular myocytes for proarrhythmia risk evaluation for new drug candidates is also possible.[10] The ability of iPSC-derived cardiomyocyte tissue platforms to perform preclinical drugs trials that can predict clinical outcomes (e.g., CTiD or "TQT/QTc studies in a dish") are innovative ideas. Such *in vitro* human platforms, and use of new safety end points (calcium-flux decay ratio), are in their infancy and only time and further testing will demonstrate the value of these new approaches to cardiovascular safety assessment and drug development.

FUNDING

No funding was received for this work.

CONFLICT OF INTEREST

The author is an employee of Amgen, Inc., a biopharmaceutical company.

1. Blanchette, A.D. *et al.* Thorough QT/QTc in a dish: an in vitro human model that accurately predicts clinical concentration-QTc relationships. *Clin. Pharmacol. Ther.* **105**, 1175–1186.
2. Strauss, D.G. & Blinova, K. Clinical trials in a dish. *Trends Pharmacol. Sci.* **38**, 4–7 (2017).
3. Fermini, B., Coyne, K.P. & Coyne, S.T. Challenges in designing and executing clinical trials in a dish studies. *J. Pharmacol. Toxicol. Methods* **94**, 73–82 (2018).
4. Pollard, C.E. *et al.* An analysis of the relationship between preclinical and clinical QT interval-related data. *Toxicol. Sci.* **159**, 94–101 (2017).
5. Park, E. *et al.* Can non-clinical repolarization assays predict the results of clinical thorough QT studies? Results from a research consortium. *Br. J. Pharmacol.* **175**, 606–617 (2018).
6. Blinova, K. *et al.* International multisite study of human-induced pluripotent stem cell-derived cardiomyocytes for drug proarrhythmia potential assessment. *Cell Rep.* **24**, 3582–3592 (2018).
7. Shinozawa, T. *et al.* Recapitulation of clinical individual susceptibility to drug-induced QT prolongation in healthy subjects using IPSC derived cardiomyocytes. *Stem Cell Reports* **8**, 226–234 (2017).
8. Darpo, B., Bullingham, R., Combs, D.L., Ferber, G. & Hafez, K. Assessment of the cardiac safety and pharmacokinetics of a short course, twice daily dose of orally-administered mifepristone in healthy male subjects. *Cardiol J.* **20**, 152–160 (2013).
9. Antoons, G. *et al.* Calcium release near L-type calcium channels promotes beat-to-beat variability in ventricular myocytes from the chronic AV block dog. *J. Mol. Cell. Cardiol.* **89**, 326–334 (2015).
10. Qu, Y., Page, G., Abi-Gerges, N., Miller, P.E., Ghetti, A. & Vargas, H.M. Action potential recording and pro-arrhythmia risk analysis in human ventricular trabeculae. *Front. Physiol.* **8**, 1109 (2018).