P404 Application of a High-Throughput Human Stem Cell Cardiomyocyte Assay for Predicting Drug-Induced Changes in ECG Parameters During Drug Discovery and Development

Karuna Sachdeva[1], Steve Jenkinson[2], and Dino Manca[1]

Presenting author

[1]Jazz Pharmaceuticals, Palo Alto, CA, [2]Metrion Biosciences, Cambridge, UK

Abstract

The FDA-supported Comprehensive In Vitro Proarrhythmic Assay (CiPA) initiative aims to develop clinically translatable models that provide an early prediction of a drug's arrhythmia potential. In recent years, the use of human-induced pluripotent stem cell-derived cardiomyocytes (hIPSC-CM) has emerged as an important screening platform for safety profiling of drug candidates. Jazz Pharmaceuticals has implemented the use of a high-throughput hIPSC-CM model to provide an earlier prediction of clinically relevant effects of our assets on ECG endpoints including changes in QTc and QRS intervals. The compounds are screened through a high throughput assay using a fluorescent voltage sensitive dye methodology. Measurements are taken at 30 minutes (acute exposure) and 24 hours (chronic exposure) to ensure that responses from compounds that may exhibit slow kinetics or affect surface ion channel expression (e.g., inhibitors of hERG trafficking) are adequately captured. Multiple endpoints including APD_{90}, rise time, and beat rate can be assessed and are used to predict exposures that could be associated with clinically significant increases in clinical QTc interval (+10 msec), QRS prolongation, or provide a potential indication of toxicity. This poster describes data generated from the hIPSC-CM screens for select compounds and how these data, when complemented with IC_{50} values from whole-cell patch clamp assays were used to inform on the cardiovascular liability during drug discovery and development. In Jazz's experience, this model represents an essential component in the assessment of cardiac safety and provides a robust platform for predicting clinical QTc and QRS changes.

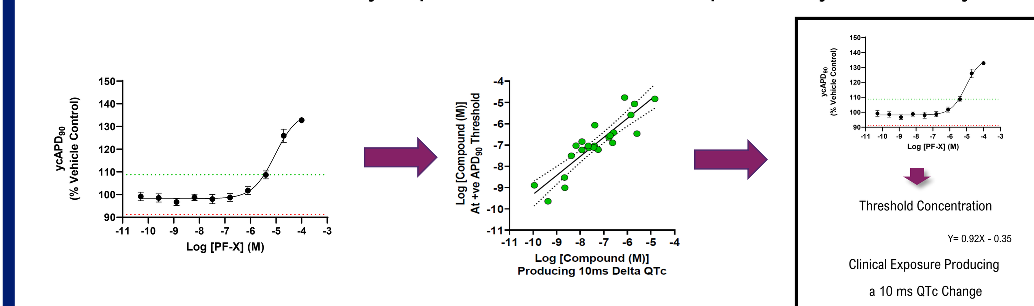
Background

- Traditional cardiac safety assessments for small molecule new chemical entities (NCEs) have relied on their ability to block the human ether-à-go-go related gene (hERG) channel in vitro; as well as prolong ventricular repolarization and the QTc interval of ECGs obtained in nonclinical studies and human clinical trials. While effective, this approach lacks specificity often deprioritizing candidates with low true proarrhythmic risk.
- The ICH S7B/E14 framework successfully reduced torsadogenic drug approvals but constrained drug development by overemphasizing hERG and QT effects as the primary determinants of risk.
- The Comprehensive In Vitro Proarrhythmia Assay (CiPA) paradigm provides a more predictive, mechanistic approach by integrating: 1) assessment of drug effects on critical ventricular ion channel currents, 2) in silico modeling of the ion channel to assess their effects on cardiac action potential changes, and 3) verification in integrated biological systems including hIPSC-CM and clinical EEGs.
- At Jazz, the cardiovascular safety evaluation follows a tiered, stage-specific strategy incorporating principles of the CiPA paradigm prior to first-in-human testing. These include: 1) ion channel/patch clamp screen; 2) hIPSC-CM assays (Metrion iCell[2] platform); and 3) in vivo non-rodent animal electrophysiology.
- Early integration of the hIPSC-CM and patch clamp data aid in early identification of potential cardiovascular liabilities and prioritizing NCEs.
- A weight-of-evidence (WoE) approach across the in vitro and in vivo tiers provide a refined, translational understanding of cardiac risk, aligning with CiPA-best practices.
- Using two specific case examples of small molecule NCEs (NCE 1 and NCE 2), we illustrate how integrated data from the hIPSC-CM and whole-cell patch clamp IC_{50} values were used to inform on the potential for clinical QTc and QRS changes (in concert with in vivo animal ECG data)

Methods

hIPSC-Cardiomyocytes Assay

- Assay conducted using iCell[2] hIPSC-CM under serum free conditions
- High-throughput measurements of action potential (AP) morphology performed using voltage sensitive dye
  - 10 kHz measurement of action potentials
  - Action potential duration (APD) / rise time / beat rate measured
  - Assay amenable to extended incubation periods (30 min to 48 hours) and cellular viability assessments
- Correlation of threshold APD_{90} concentrations with clinical QTc data
- Linear fit allows estimation of exposure producing a 10ms change in QTc
- 4-fold cross validation analysis performed to understand predictivity of the assay



Potency Assessment of NCE 1 Against $hNa_v1.5$ in Resting and Inactivated States Using QPatch 48 Automated Electrophysiology Platform

- ***Electrophysiology Assay – voltage protocol***
  - *V0.5 determination protocol:* cells were stimulated at 0 mV for 20 ms (P1) and then held at a range of voltages (-120 mV to 0 mV in 10 mV steps, i.e. 13 sweeps) for 3 s before restimulation at 0 mV (P2). The QPatch II software calculates which voltage cause half-channel inactivation (V0.5 inactivation) and stores that value for use in the subsequent voltage protocol. Sweep-sweep interval: 15 s.
  - *V0.5 screening protocol:* cells were stimulated at 0 mV for 20 ms (P1) and then held at V0.5 inactivation for 3 s before restimulation at 0 mV (P2). P2 should be half the magnitude of P1. Sweep-sweep interval: 15 s.
- ***Electrophysiology Assay – application protocol***
  - The experiment commenced with the "V0.5 determination" voltage protocol performed in vehicle (0.3 % v/v DMSO) to estimate V0.5 inactivation for the individual cell.
  - Following V0.5 determination, the "V0.5 screening protocol" voltage protocol was applied to the cell. Cells were exposed to two vehicle additions (3 min / 12 sweeps each) followed by two compound additions (3 min / 12 sweeps each).

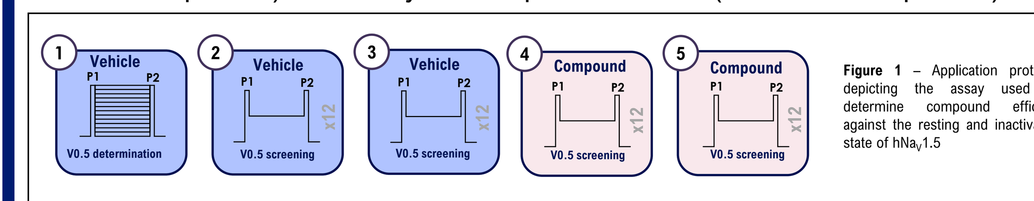


Figure 1 – Application protocol depicting the assay used to determine compound efficacy against the resting and inactivated state of $hNa_v1.5$

Results – NCE 1

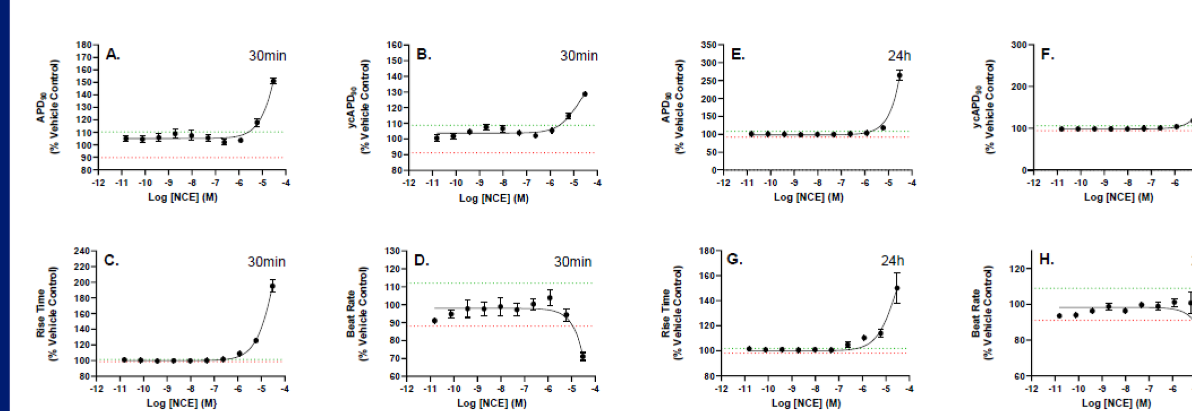
Table 1: Summary of In Vitro Electrophysiology Assays Conducted with NCE 1

Ion Channel	Mean IC_{50} (µM)
hERG	50
$hNa_v1.5$	70
$hCa_v1.2/\beta2/\alpha2\delta1$	>60

- The IC_{50}s of NCE 1 on the hERG, $hNa_v1.5$, and $hCa_v1.2$ channels were ≥200-fold higher than unbound plasma levels anticipated at the proposed highest efficacious dose

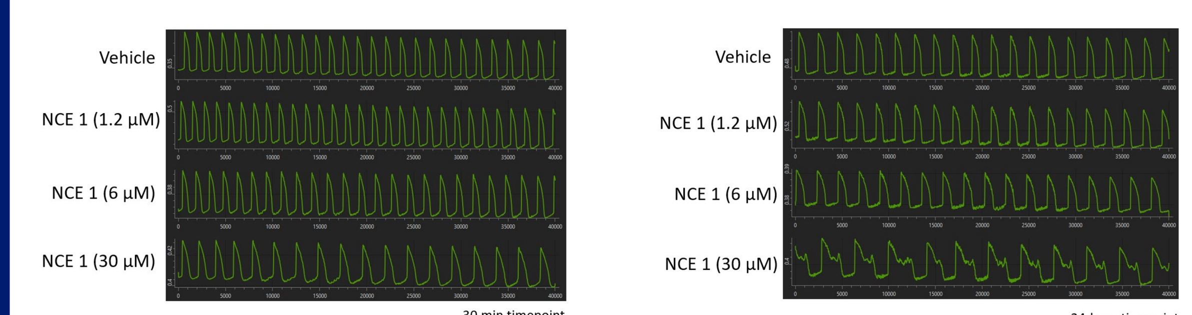
Results – NCE 1 (cont.)

Figure 1: Effect of NCE 1 on Various hIPSC-CM Action Potential Parameters following 30 minute and 24-hour Incubation Periods



Endpoint data represent mean ± SEM of triplicate samples. Green and red dashed lines represent the threshold levels for an increase or decrease in response, respectively.

Figure 2: Effect of NCE 1 on hIPSC-CM Waveform Morphology following 24-hour Incubation Period



- NCE 1 produced a concentration-dependent increase in APD_{90} and Yamamoto corrected APD_{90} ($ycAPD_{90}$) at 30 minute and 24-hour timepoints (Figure 1), suggesting a potential for hERG blockade.
  - The increase in parameters suggested that NCE 1 may produce a 10 ms change in QTc at ≥1 µM free clinical exposures.
- Furthermore, the potency of $Na_v1.5$ effect, as measured using AP rise time, was also evident. A concentration-dependent increase in AP rise time was observed at 30 minute and 24-hour timepoints (Figure 1), suggesting that NCE 1 may produce an increase in QRS at free clinical exposures around 0.5 µM.
- A concentration-dependent decrease in beat rate was observed at 30 minute and 24-hour timepoints (Figure 1).
- Waveform morphology, as evaluated in the hIPSC-CM model at the 30-minutes and 24-hour timepoint, demonstrated evidence of prolongation action potential duration and early after depolarization (EAD).
- Additional follow-up investigations were conducted to determine whether the $Na_v1.5$ current block was state-dependent (Table 2).

Table 2: Summary of Potency Values of NCE in the $hNa_v1.5$ State-Dependency Assay[a]

Compound	IC_{20} (µM)			IC_{50} (µM)		
	P1	P2	P1/P2 ratio	P1	P2	P1/P2 ratio
NCE	120	30	4.0	350	160	2.2

Abbreviations: IC_{20} = 20% maximum inhibitory concentration; IC50 = 50% maximum inhibitory concentration; P = NCE inhibition on $hNa_v1.5$ resting channel; P2 = NCE inhibition on $hNa_v1.5$ inactivated channel.
[a]Preferential P2 inhibition over P1 inhibition is characteristic of state-dependent inhibitor.

- To determine whether the block of $Na_v1.5$ current was state-dependent, the potency assessment of several concentrations of NCE 1 were evaluated against $hNa_v1.5$ channel under conditions where the channel was in the resting state (holding potential of -120 mV) or in a half-inactivated state using a 3-second pre-pulse at V0.5 inactivation (i.e., the voltage that elicits 50% inactivation).
- Under these conditions, NCE 1 demonstrated modest state-dependency, inhibiting P1 (inhibition on $hNa_v1.5$ resting channel) and P2 (inhibition on $hNa_v1.5$ inactivated channel) with IC_{50}'s of 350 µM and 160 µM, respectively, with a P1/P2 IC_{50} ratio of 2.19.
- The IC_{20} for P1 and P2 inhibition was 120 µM and 30 µM, respectively (P1/P2 IC_{20} ratio of 4.0), thus suggesting that state-dependency was more evident at lower NCE 1 concentrations.

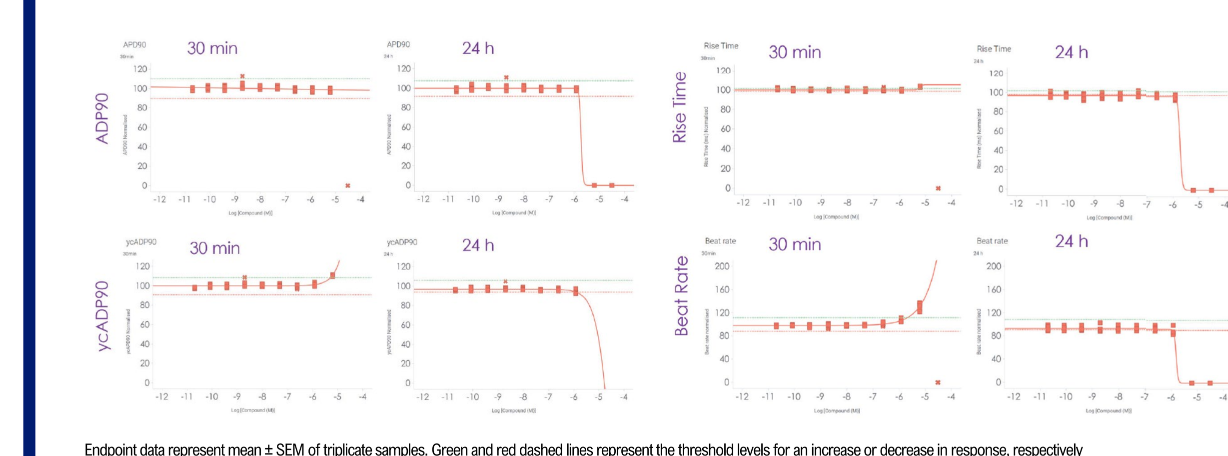
Results – NCE 2

Table 3: Summary of In Vitro Electrophysiology Assay Conducted with NCE 2

Ion Channel	Mean IC_{50} (µM)
hERG	2.3

- The IC_{50} of NCE 2 on the hERG channel was projected to be ~ ≥ 34-fold higher than unbound plasma levels anticipated at the proposed minimum efficacious human dose. This margin was below 30-fold at higher efficacious doses.

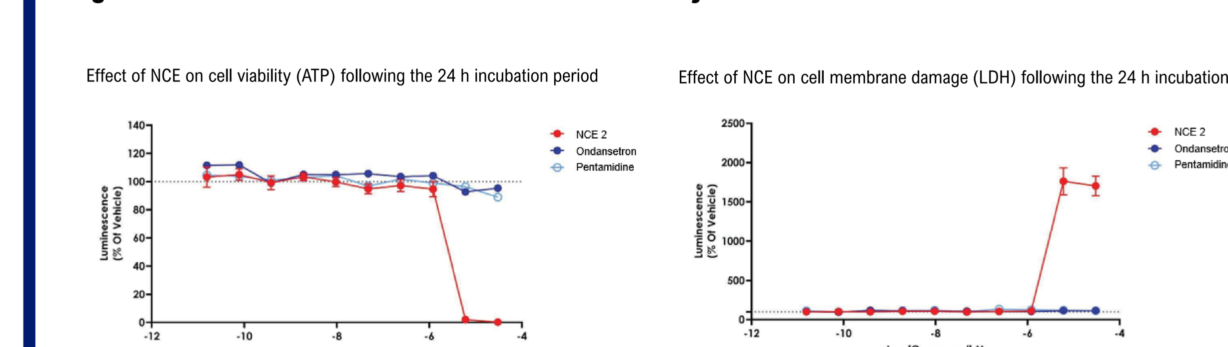
Figure 3: Effect of NCE 2 on Various hIPSC-CM Action Potential Parameters following 30 minute and 24-hour Incubation Periods



Endpoint data represent mean ± SEM of triplicate samples. Green and red dashed lines represent the threshold levels for an increase or decrease in response, respectively

- NCE 2 produced a modest concentration-dependent increase in $ycAPD_{90}$ at the 30 min timepoint only.
  - The increase in the APD parameters suggested NCE 2 may produce a 10 ms change in QTc at free clinical exposures in the range of 663 nM (0.663 µM).
- A concentration-dependent increase in beat rate occurred at 30 min at 6 µM, followed by quiescence at 30 µM.
  - At 24 h quiescence occurred at test concentrations of ≥ 1.2 µM.
- A projected 10-fold margin was estimated based on the 663 nM concentration in hiPSC-CM relative to unbound plasma levels anticipated at the proposed minimum efficacious human dose. This margin was reduced at higher efficacious doses.

Figure 4: Effect of NCE 2 on Various hIPSC-CM Viability at 24-hour Incubation Periods



- In order to investigate whether the quiescence seen in hIPSC-CM was due to NCE 2 mediated effect on ion channels (hERG inhibition) or a result of cellular toxicity, follow-up viability assessments were conducted.
- In these investigations, NCE 2, following incubation of different test concentrations for 24 hours, induced cardiomyocyte cell membrane damage (as indicated by increase in lactate dehydrogenase [LDH] activity) and cytotoxicity (indicated by decrease in adenosine triphosphate [ATP]) levels at ≥6.0 µM.
- These results suggested that the NCE2-related quiescence in cardiomyocytes following 24 hours of exposure was likely a result of direct cellular toxicity and not due to inhibition of ion channels.

Conclusions

- The iCell[2] hIPSC-CM assay (as developed by Metrion Biosciences) has been incorporated into Jazz's hazard identification and cardiac safety assessment strategy for novel drug candidates.
- Using two small molecule NCEs case examples, we illustrate how integrated data from the hIPSC-CM, whole-cell patch clamp IC_{50} values, and in vivo ECG data informed the assessment of potential cardiovascular liabilities during drug discovery and development.

NCE 1

- For NCE 1, whole-cell patch-clamp screening did not reveal any cardiovascular liabilities beyond hERG at the expected free clinical exposures (Table 1).
- The hIPSC-CM findings predicted ~10 ms QTc prolongation and potential QRS interval increase at free clinical exposures ≥1 µM and around 0.5 µM, respectively (Figures 1, 2).
- Relative to the initial patch-clamp profiling (Table 1), the hIPSC-CM model provided more robust clinically translatable evidence for the potency of NCE-mediated $Na_v1.5$ effect, as reflected by the prolongation of the AP rise time.
- The apparent disconnect between the whole-cell patch clamp ion channel profiling and the hIPSC-CM findings could potentially be explained by mixed ion channel activity observed for NCE 1.
  - Blockade of hERG by NCE 1 prolonged the hIPSC-CM action potential, maintaining the cell is in a more depolarized state for an extended period. This, in turn, may be expected to increase the fraction of $Na_v1.5$ channels in the inactivated state.
  - Follow-on patch clamp studies confirmed the state-dependent inhibition of peak $Na_v1.5$, with a preference for the inactivated state (Table 2).
- In repeat-dose toxicology studies in non-rodent species, QTc and QRS prolongations were observed at exposures exceeding the projected clinical range (data not shown).
- In summary, cardiac electrophysiological effects observed with NCE 1 in the hIPSC-cardiomyocytes demonstrated translational relevance, as ECG alterations at concentrations as predicted in the hIPSC-CM assay, were observed in humans.

NCE 2

- Whole-cell patch clamp conducted with NCE 2 revealed a potential CV liability (30-fold MHE) based on hERG IC_{50} relative to predicted clinical plasma exposures (Table 3).
- hIPSC-CM assay conducted with NCE 2 predicted 10 ms QTc prolongation after short-term exposure and potential for QRS interval increase at clinically relevant free exposures (Figures 3).
- NCE 2-related quiescence in cardiomyocytes was likely a result of direct cellular toxicity and not due to inhibition of ion channels.
- Collectively, these data indicate that NCE 2 may present a CV conduction liability at clinical drug concentrations.
- These data were used to inform preclinical and clinical risk assessment as well as clinical monitoring and management strategies.

References: 1. International Council for Harmaonisation (ICH) E14/S7B Implementation Working Group Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential. Questions and Answers. E14/S7B Q&As. Adopted 21 February 2022.
2. Kilfoil P, Feng SL, Bassyouni A, et al. Characterization of high throughput human stem cell cardiomyocytes assay to predict drug-induced changes in clinical electrocardiogram parameters. *European Journal of Pharmacology*. 2021; 912:174584.

Support and Acknowledgments: The authors thank Kevin Trouba, PhD, DABT (Jazz Pharmaceuticals) for their contributions to data on NCE 2.

Copies of this poster obtained are for personal use only and may not be reproduced without written permission of the authors.

Corresponding author: Karuna Sachdeva, Ph.D. DABT
karuna.sachdeva@jazzpharma.com

Poster presented at the American College of Toxicology Meeting; 17 Nov. 2025; Phoenix, AZ