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Presentation "Current status and future perspectives in cardiac safety assessment using human iPSC technology" by Yasunari Kanda, PhD, Division head, National Institute of Health Sciences Japan

Q: Your observation that SARS-CoV-2 spike protein can reduce oxygen consumption rate in iPSC-CM is very interesting. Why do you think this is happening?

A: Actually, I don't have a specific answer for that. One thing that we have observed is that mitochondria can be damaged by SARS-CoV-2 infections. When we see the iPSC cardiomyocyte with SARS-CoV-2, the shape of the mitochondria is going to go to the part of dotted structure. Mitochondria shows basically a fusion type, but in the case of SARS-CoV-2 infection, the shape has been totally changed. So I guess the mitochondria function should be important to understand the possible mechanism by which SARS-CoV-2 infection caused cardiac damage.

Q: You mentioned a late Nav1.5 ion channel assay - are you using a pharmacological opener such as ATX-II, or a LQT3 genetic mutant?

A: Yes. We have used ATX-II to measure a late NaV1.5.

Q: Clomipramine exhibits significant poly-pharmacology. Have you been able to narrow down the possible mechanism(s) responsible for inhibiting internalization of Covid-19 spike protein by running Structure activity relationship studies with a variety of chemical series targeting different MOAs?

A: BY using a docking simulation, we are trying to identify the structure of clomipramine which inhibit the ACE2 internalization.

Presentation "From iPSC Banking to Drug Development & Cardiac Safety" by Ralf Kettenhofen, PhD, Senior Scientist and Business Development, Fraunhofer Institute for Biomedical Engineering IBMT

Q: Will the QA amendments from 2.2 to 2.5 allow to submit iPSC-derived cardiomyocytes data to the FDA for the approval of clinical studies?

A: That's a really good question because the current status is that it is supposed to be used for follow-up studies so if the double negative are not occurring in the preclinical core studies or in the clinics, so it's not supposed to be directly but I've heard from with direct communication with scientists from the FDA that the stem cell recognized that assay data is welcome to be submitted as well so I think this will not be a problem since they are validated and they are now in the guidelines, there shouldn't be a big issue to submit data from stem cells.

Q: Which assays with iPSC-derived cardiomyocytes can be used to asses acute drug induced effects on repolarization?

A: I think we are going to hear quite a lot of stems cells in the next talk so I won't go into deep here but, actually, I can refer to the publication by Gary Junton in 2020 where the best practices are all mentioned and they propose to have transmember and potential assays for instance patch clamp of stem cell refication myocytes or microelectrode based transmembrane measurements like the leap assay, for instance, that can be used few potential recordings. Impedance and contraction assays can be used as well as optical recordings like voltage-sensitive thighs as well as calcium transmission recordings.

Presentation "Advanced cardiac contractility assessment with integrated mechanical and optical stimulation of human iPSC-derived cardiomyocytes" by Bettina Lickiss, PhD, Scientific Communications Manager, innoVitro GmbH

Q: We know how many cells we added/well - any recommendations to perform cell counting/well at the end of the experiment?

A: Regarding cell numbers after the experiment, I would suggest either cell staining or trypsinisation and then simple cell counting.

Q: How long caPn we keep the cells in the Flexcyte plates?

A: Since we are a contract research provider, we are culturing the cells usually for no longer than 14 days to perform acute or chronic measurements, but we also know from collaboration projects that it is indeed possible to culture them for much longer.

Q: Was the medium also protein free since that is a major concern with regards to compounds that are highly protein bound?

A: Yes, I know that there is always a concern regarding protein in the buffer. The medium used for the FCDI serum-free project was protein free. The data where we have shown the Frank Starling mechanism was also conducted with a reduced assay buffer without protein.

Q: Can you comment on the evolution of contractility over maturation and the differences with human myocytes? Is the amplitude very different and duration velocities?

A: Ribero et al has recently published a summary of contractility parameters such as amplitude, passive tension and sarcomere lenght which are all bigger in mature cardiomyocytes compared to hiPSC-derived cardiomyocytes. It is also known that beat rates in mature cardiomyocytes are lower than in juvenile cardiomyocytes.

Presentation "In vitro cardiac safety pharmacology- considerations beyond hERG" by Khuram Chaudhary, PhD, Scientific Director, GlaxoSmithKline

Q: You showed so many assays, in your opinion, what would it take for an assay to be adopted in the pre-clinical drug discovery and safety paradigm?

A: Well, there's really two parts to that question. One, as an overview, I know recently there has been quite a bit of discussion in ICH on validating novel assays, so clearly that discussion is happening. But, on the one hand, it is important in the pharmaceutical industry for us to make the appropriate business decisions to develop the highest quality drug and that is more of a resource issue. We want to reduce costs, we want to efficiently get into the clinic, we want to predict these toxicities early on.

Secondly, there is a regulatory component. So, you know, you want to ensure that you're doing the right studies as part of your regulatory submission or your follow-up studies to reduce cardiac risk. And, so, it's really a matter of understanding both that early development issue and the business decision as well as reducing attrition late in development.

I think all of these technologies have utility. One thing that I haven't talked about are things like the use of primary myocytes, that's a hot topic as well these days. And again, it's really fit for purpose type of paradigm. Whatever you need to ensure that you're producing the highest quality drugs is that you should be implementing, I think.

Q: The experiments that you had done with mitochondria in the MEA and you tested rotenone, Did you also take a look at if there is some cellular toxicity directly on the cardiomyocytes, if the cells are affected? I mean, in neurons in Parkinson's disease for example rotenone is the number one toxin so to say.

A: There's a few ways to look at this. We have a more comprehensive way of addressing these issues that falls under investigative safety, where we look at high content imaging, cellular structure, mitochondrial toxicity using energetic assays, ATP assays. But what we found was that when we were studying company compounds and saw this very unique profile, and then looked at that investigative work to see if there were any links to either screening studies, off-target effects, or even some of these additional toxicities including mitochondrial toxicity. There was a link between mitotoxins and this fingerprint that we saw. That's when we went back and looked at some of the reference compounds and found that depending on which complex in the electron transport chain you tinker with, you get a very unique pattern in the MEA assay and so we're not suggesting that we're going to use the MEA to predict myotoxicity, but it is a surrogate that gives a signal that we

reproducibly see that related to myotoxicity. And so, it might be the trigger to do further studies downstream.

Q: Are you intending to add cardiomyocytes from disease models into you assay cascade?

A: The concern most people have had with disease modeling is variability and translation to clinical disease phenotypes. I've been a proponent of disease modeling in safety assessment, but it's also important to recognize that the first patient population to be exposed to novel drugs is a "normal" human volunteer in Phase I studies.

Q: For the experiment with DOX, have you measured other parameters for example cyto-toxicity?

A: We have evaluated cytotox in alternate assays. However, we do much of our e-phys/cardiotox screening early in development, and found it useful to identify phenotypes in the e-phys assays suggestive of non-ion channel off-target liabilities.

Q: There is a lot of discussion on 2d v 3d. What's your perspective on incorporation of both modalities into early drug safety studies?

A: There's utility in both 2D and 3D. Points to consider include throughput/screening early in lead optimization or definitive follow-up studies late in development. We also know issues related to Car-T for instance, only show up in complex models (EHTs).

Q: How can we measure structural cardiotoxicity in your system?

A: Structural cardiotox is exactly that, “structural” and not necessarily “functional”. The assays we described during my session are predominately functional. However, because the MEA platform as well as the plate-based assays discussed (e.g. BioWire and FlexCyte) can be cultured with compound for several days, there is the possibility to explore functional consequence of longer duration exposures of drug; and transfer of assay plates to imaging systems to measure, directly, structural endpoints.

Q: How does the pre-maturation with contraction affect ion channel expression? How does that correlate to increased beat rate?

A: We have not assessed ion channel expression (RNA or Protein) in our assays. Our assumption is that the function is supported by the appropriate inputs/proteins such as expression of ion channels and proteins found in the heart. The literature supports the functional maturation of hiPSc-CMs using defined stimuli such as electrical stimulation, extracellular matrix proteins, and media supplements. Ultimately, it is the researcher’s responsibility to define what is “fit for purpose”.

Presentation “Predictive and Physiologically Relevant 3D Tri-culture Cardiac Microtissue with iCell® Cardiomyocytes, Endothelial Cells and Primary Cardiac Fibroblast” by Ravi Vaidyanathan, PhD, Product Manager - Cardiac - FUJIFILM Cellular Dynamics, Inc.

Q: With spheroids greater than 500 micrometers, did you observe a hypoxic/necrotic core?

A: No, so the maximum size of triculture spheroid we tried were with the 10k total cells. These spheroids had an average diameter of 500 micrometer and we didn’t see any necrotic core, at least from the experiments that we’ve done.

Q: With what kind of analyzer can we use the spheroids?

A: Any type of optical imaging system should work with Spheroid. I think with some modifications, it can be used on the Flexcyte and Nanion CardioExcyte, I think. There might be a little bit more work to be done but it if works on the CardioExcyte it can be worked on most MEA platforms too with some modifications. But I think the easiest analyzer is of course optical imaging and data I shared on the CardioExcyte.

Q: Can the spheroid be adapted for high throughput with 384 well plates?

On a 96 well plate we recommend anywhere from 5000 to 10000 total cells depending on the type of plate used, but if you’re going with a 384 well plate you can do down to 2000 to 2500 cells total and still keep the same of ratio of iCell cardiomyocytes, iCell endothelial cells and primary fibroblasts.

Q: Are the spheroids more relevant than 2-d systems in your opinion?

A: There are benefits to both systems. Physiologically other cell types do play a role in heart function and physiology and hence there are some advantages for 3D spheroids over the 2D.

Presentation “The use of stem cell cardiomyocyte models in predicting clinical cardiac endpoints” by Stephen Jenkinson, PhD, Director of In Vitro Pharmacology, Pfizer Inc.

Q: You mention using hERG binding data in your predictions.....does hERG patch-clamp data offer improved predictivity?? Binding hasn't really been considered in the CIPA paradigm

A: Yes, so it's kind of interesting. We'll be publishing this data shortly, but what we didn't show here is that if you actually ise just the hair binding data alone with regards to QT, the correlation is just as good as what you get from the stem cells. So the hair binding data at least in our hands, is equally as predictive as the stem cell data. We actually have less with patch clamp because of the high-throughput systems and some of the issues with the small volumes and sticking to the plates. We don't get as great correlation with the actual patch clamp data from those systems so the binding's actually been more robust for us.

Q: Which serum free media used?

A: It is + 20 mM HEPES

Q: Your stem cell APD model predicts the QTc changes very nicely. Just curious if your in vitro fluorescence APD data correlates very well in vitro E-field potential data sets for published and in-house compounds?

A: We haven't looked at some published data and compared it, to be honest. We haven't done any field potential, so our group does not run field potential duration studies. We just focused purely on looking at multi-sensitive dye endpoints because we find this is the most reproducible. It's a very robust assay, this system works extremely well so we're kind of focused on that.

Presentation “Challenging our understanding of iPSC-based cardiac safety with physiologically relevant and assay-ready cell culture media” by Jin Chang, BD & Alliance Manager, NEXEL Co. Ltd

Q: When you added calcium and magnesium to the medium was the reversal of the clarithromycin effect immediate or did this require longer incubation?

A: I expected it to take longer but it was immediate. This was an acute study where we would change the medium just as we would in normal settings, two or three hours before the assay, and that was enough to reverse the FPD shortening into a lengthening.

Q: how many cardiomyocytes per vial provide Nexel? is it 5 millions of cells/vial?

A: We have two different formats, the C-001 which contains 2.5 million cells/vial and the C-002 which contains 5 million cells/vial. You can find out more by either reaching out to me (jhchang@nexel.co.kr) or on our website (https://www.nexel.co.kr/products/ipsc-derived-cardiomyocytes)

Presentation “An automated, cloud-based platform for quality control, data analysis and risk prediction of inherited and drug-induced arrhythmias” by Adam Hill, PhD, Head Computational Cardiology, Victor Chang Cardiac Research Institute

Q: For those out there who are not hERG voltage-clamp protocol aficionados, can you explain why there is a critical need to implement the Milnes protocol, because it is clear that less complex protocols would vastly enhance the QC process in APC approaches So why handicap the APC approach by forcing the use of intractable voltage clamp protocols?

A: Well I certainly think that if we’re going to undertake any sort of high throughput screening for drug kinetics which we probably need to be working towards if we are building to any of the next phases of the CiPA piplines, then the Milnes protocol is very difficult one to undertake – it just doesn’t lend itself to these automated platforms because there’s this requirement; they’re really long protocols and essentially the problem with stability is that you can be comparing your post drug sweep which may be several minutes after your pre drug sweep and just a few pico amps of change can really give you all that noise and throw out your potential to measure the kinetics importantly so I think we’ve probably got to be looking at alternatives, it’s probably a challenge for some of the modelists to say is the kinetic model of drug buying that we are using correct? Is there a simpler one that we could use, where we could use a much simpler protocol whether that be a step amp or an action potential with a simpler model and have enough depth of the data to constrain that model in terms of the ion channel kinetics so yes, I think the Milnes Protocol is very difficult to manage.

Presentation: "Multi-parametric Drug Safety Assessment in Bioreactor-produced Ncyte hiPSC-derived Cardiomyocytes" by Jessica Veenman-Koepke, Scientist & Project leader at Ncardia

Q: Is the RMP recorded by traditional current clamp, or were you using dynamic clamp injection with IK1?

A: We used traditional current clamp recordings.

Q: Did you look for expression of any atrial-specific markers in your iPSC-CMs?

A: We have RNA-seq data for the entire transcriptome, including atrial markers.

Presentation "Examining temperature dependence of hERG1a gating" by Gary Mirams, Professor of Mathematical Physiology, University of

Nottingham

Q: Any possibility to incorporate the voltage offset you measured with the reverse ramp into the Nanion system in real time ... i.e., reset the offset for each well by running the reverse ramp at the beginning of an experiment?

A: At the moment would be tricky as that reversal ramp was applied to post-processed data (after leak and E4031 subtraction). We would have to think carefully about how to do that sort of thing in real time, would probably only work with realtime leak subtraction and cells without any other endogenous currents at that moment.

Q: What is the biggest hurdle to embed modelling into safety assessments in your view?

A: I think it probably has been, working out how to deal with the data and the best ways to do that. We've done quite a lot with GSK where we've tried to embed stimulations in-house for them using their screening databases so IC50 and things and the most tricky things really were coming up with a quality control pipeline for their IC50 data. You know, if the hillslope was too low maybe it was down in the experiment rather than real drug effects, there's variability in those IC50's even when you do trust the hillslope vision is good. Yeah, it was building that pipeline of when do we think it's good data and then we know there is some variability in the data – how do we map it through the model predictions?

Q: Do professional statisticians have answers or at least hypotheses as to why highly skilled electrophysiologists all applying the same voltage-clamp and experimental protocols generate such variable IC50 values for hERG and other ion channel inhibition? Communities of practice have attempted to standardize their experimental paradigms, yet there still seems to be significant variability. So the question is; are we really standardized, and doing the same things when we make hERG measurements?

A: With a combination of the effects Jim has described across sites (compound stability, temperature, protocol, steady state.) and patch artefacts varying every cell, doesn't surprise me very much! We have an examination of exactly what the distribution is at a single site for a various positive controls https://pubmed.ncbi.nlm.nih.gov/23651875/ with >12,000 repeats for hERG-Cisapride at AZ! Follows the +/- 0.5 log unit rule of thumb for pIC50s

Presentation "Building hybrid biological-digital twins of cardiomyocytes using dynamic clamping" by Teun de Boer, Associate Professor, Dept. of Medical Physiology, UMC Utrecht

Q: By applying digital dynamic conductance's in the right amount and right time, did you get a HEK cell to fire action potentials?

A: That's exactly what we're doing with the dynamic action potential clamp, you could say you're adding the current from the HEK cell to a stimulated cardiomyocyte, but you could also say exactly the opposite, we're adding all the other ion channels to this HEK cell so that the whole ensemble is firing action potentials.

Presentation: "ICH S7B Q&As - Best Practices for Ion Channel Assays" by James Kramer, Principal Scientist, Charles River Laboratories

Q: With respect to the temperature and running at 37 degrees from your experience does increasing incubation time at lower temperature produce a similar result to that say at 37 degrees?

A: For these examples that I showed for erythromycin I think yes it would have eventually reached at room temperature it would have eventually reached the level that we achieved at higher temperature but would have required I think at least - I haven't looked at this data in a long time sorry this was many years ago but it would have taken I think almost 30 to 40 minutes of exposure to reach the levels that we saw at higher temperature and of course you know it's tough to do that, especially in a manual patch clamp - I've had a few that have lasted 30 minutes but that's not the form.

Q: Have you guys implemented any in-silico modelling already or this is not the plan?

A: No not yet, unfortunately we just have not done that I think the other experts are filling that void. Gary Miriam's is helping out with that and as well as some other groups the FDA of course has their own modelling effort. I'm kind of waiting to see how that all, especially with the FDA model how that all plays out I know that the kinetic, that at least the FDA model needs some kinetic information of hERG block that's on-rate and that was using the Milne's protocol it was very difficult and even for us it was difficult and we have done some work with that but I'm just waiting to see if that's modified, and I'll let the experts handle that!

Presentation “Automated Patch Clamp to Assess Sodium Channel Blocker Effects on hiPSC-derived Cardiomyocytes” by Al George, Professor and Chair of Pharmacology, Northwestern University

Q: Did you succeed to reach low Rs with your cell?

A: We observe seal resistance > 0.25 gigaOhm in approximately 50% of iPSC-CMs

Q: Do you see contractility of iPSC-CM as an issue for automated clamp?

A: We haven't perceived any issues related to contractility of the iPSC-CM. However, lately we have been interested in recording action potentials from these cells using automated patch clamp, and they cells will need to be contracting. We may need to suppress contraction when we measure APs. This is work in progress.

Q: Do you think that Fluoride can also interfere with inactivation properties?

A: We have not perceived this but do not have an internal solution without fluoride to compare with. Fluoride is an issue for K channels in our hands, but we have typically studied Nav channels with internal F also using manual patch clamp so it would be hard for me to say there are differences related to fluoride.

Q: Can you imagine that you have huge endocytosis of Nav channels because you have to use high external Na+ concentration right ?

A: Your question about endocytosis is interesting, but we haven't considered this.

Q: Did you analyse the expression of the sodium channel subunits in the stem cells?

A: By RNA-seq, Nav1.5 is by far the most highly expressed pore-forming subunit. Among the beta subunits, beta-1 is expressed 2-3 times higher than the others with beta-4 being lowest.

Q: Have the drug experiments been conducted with cardiomyocytes derived from a single hiPS line?

A: Yes, the data presented were from iPSC-CMs from one line.

Q: Is the sodium current in hiPSC-CMs nicer to work with than a HEK/CHO cell line? More cardiomyocyte-like in terms of subunits etc.?

A: I would say that Na current is still easier to work with in HEK cells, because the cell

culture is simpler. We like CMs because there are opportunities to study currents in various knock out lines, and eventually the ability to record action potentials (work in progress).

Presentation "A 20 Year Journey through the Continuously Evolving Role of Safety Pharmacology in Drug Safety Evaluation" by Michael K. Pugsley, PhD, FBPhS, DSP, Cytokinetics

Q: Do you have an idea of cardiomyocyte non-cardiomyocyte ratio?

A: So in the cultures I didn't emphasize this but these cultures from Dr. Burridges lab are from IPS Cells that have stable integration of a selectable marker and so these cells are placed under selection to get rid of all the non-cardiomyocyte or at least enrich in cardiomyocytes and so we don't have a number but by flow cytometer readings it looks like there's well over 90% of the cells are cardiomyocytes.

Q: Have the drug experiments been conducted with cardiomyocyte derived from a single IPS cell line?

A: Yes, they were, so these were all derived from a single IPS cell line, it's a control line that's used widely in the Burridge lab and that was what we started with and if I'm not mistaken, it's derived from a male - not a very diverse array of cardiomyocytes.