Notes from Martha Wang, Fischell Department of Bioengineering PhD Student

Fourth AIMBE/NIH Workshop on Validation and Qualification of New In Vitro Tools and Models for the Pre-clinical Drug Discovery Process
March 6 - 7, 2014
NIH Campus, Lister Hill Auditorium
Friday 7 March 2014

Session 3: Development of Draft Validation Guidelines

Anne Plant, Ph.D., Division Chief, Biosystems and Biomaterials Division, National Institute of Standards and Technology (NIST), Moderator

- Requirements to consider in validating a new technology?
    - Context?
    - Justification for tech vs. existing tech?
    - Endpoints of the tech?
    - Limitations?
    - Comparison to the gold standard?
    - Is tech robust and transferrable?
    - Does test method allow for standardization?
    - Is the tech cost effective?

NCATS Perspective on Validation of In Vitro Microphysiological Systems and the Tox 21 Program

Christopher P. Austin, M.D., Director, National Center for Advancing Translational Sciences, NIH

Three areas of importance:

- Medical Technologies
- Patient Engagement

Drug development – toxicity is a main reason why drugs fail
A grand challenge: predicting toxicity is a difficult task

Expose an animal or human to certain dosages to see what is toxic and then set the drug dose based on that. Therefore could we breakdown all the pathways into a virtual system of cellular pathways and then test each one of those pathways individually, then combine to create the "virtual" human/rabbit model. This is important since there are over 80,000 chemical pathways.

- Tox21 program – ID patterns of biological response to:
    - Characterize toxicity, disease pathways
    - Facilitate cross species extrapolation
    - Model low dose extrapolation (important for environmental impacts)
    - **combine to a create a predictive model for biological response in humans**
    - Regulatory decisions can be made from this set of data so they have been extremely vigorous validation for all the testing methods used through Tox 21.
    - Validation for compound purity and identification – critical for all compounds. Don't just rely on the certificate of analysis from the company
    - Uses a robotic screening system – flexible

    - Phase II – 2009 – present
        - EPA ToxCast ~700 compounds, 700 assays, ~1000 compounds in endocrine activity assays (3726)
        - NCGC qHTS Phase II: 10k compound library, focusing on nuclear receptor activation or inhibition, induction of cellular stress response pathways (3526)
        - NTP: Industrial chemicals (3194)
    - 88 single sourced compounds in duplicate on each plate - they overlapped all groups to be able to compare results
    - Limitations:
        - Extent of pathway coverage
        - Focus on the use of reporter gene assays using immortal cell lines
        - Extent of chemical coverage
        - Focus on single compounds
        - Limited capability for xenobiotic metabolism
        - Focus on simple biological systems
        - Limited to acute exposure scenarios
    - PubChem Data: 66 assayIDs, 33 million data points
    - EPA released data on 1800 chemicals with more in-depth information
    - Phase III: High Content Assays and High Throughput gene Expression
    - Using cells capable of xenobiotic metabolism
    - ES/iPSC derived differentiated cell populations (cardiomyocytes, neurocyte, hepatocytes…)
    - Native cell lines (many of which area are already in use)
- *Tox21 focuses on secondary screening needed to bridge HTS to in vivo toxicology* -> lab on a chip
- qHTS ~10.000 compounds -> 100cpds lab on a chip -> 5-10 cpds in a human
- Advancing Regulatory Science: FDA + NIH, improving the science behind regulatory decisions
- MPS (Microphysiological Systems Program) / Tissue Chip / Organs on Chips
    - Goal: develop an in vitro platform that uses human tissues to evaluate the efficacy, safety and toxicity of promising therapies
    - All 10 human physiological systems:
    - Circulatory, endocrine, GI, immune, integumentary, musculoskeletal, nervous, reproductive , respiratory, urinary,
    - Physiologically relevant, genetically diverse and pathologically meaningful
    - Modular, reconfigurable platform
    - Tissue viability for at least 4 weeks
    - Community-wide access
    - Five year program: currently between year 2 and year 3. At end want 5 – 10 viable MPS chips
    - MPS from Common Building Blocks:
    - Scaffold, cells, structure, spatial/temporal patterning, perfusion, bioreactors, innervation, host response, functional readout, computation design. All are worked on individually and are significantly complex and so this technology
    - Current projects: lung on a chip, heart, liver, muscle and vasculature, capillaries from iPS into tumor organoid
    - 100+ known toxic compounds compiled from MPS project members,
    - Want to move to a “body-on-a-chip” link up all the individual organoids w/each has a read out.

- Example issue: different tissues need different tissue culture environments
- **Translation is a team sport**

- Cross agency team : NCATS, NTP/NIEHS, EPA/ FDA (strong collaboration)
  - Brookings Medical Device: FDA Orphan Products and NCATS Center for Rare Disease Research collaboration

Questions:

John: Common fund program – Links (Library of Integrated Cellular…) program, does it overlap in the compound ID? Can they dovetail with Tox21?

Yes, we are deeply involved w/them at this point of the process. Originally focused only on biological readouts in individual assays but overtime we have refocused and so have they to have greater overlap.

J Hickman: All the data that comes up in Tox21 then is putting back into animals for validation. Are there plans to go into lab on a chip/human on a chip in lieu of animal models for validation?

Yes, I hoped you wouldn't notice that. Animals have lots of disadvantages however as a regulatory response we are asked to compare our results to the gold standard, which are animal studies. Even though these are all human cells, it's hard to interpret the results when they are not very clear as to the results. How much of the data has supposed the Tox21 data? We're not that far enough along In the analysis to understand it. We can produce the data (a lot) but we lack the manpower to do all of the analysis.

What about the impact that there is no liver/metabolism in the model compared to a rat model?

Yes, good question.

Technologies Used by the FDA for Toxicology Research

Donna L. Mendrick, Ph.D., Associate Director of Regulatory Affairs, National Center for Toxicological Research (NCTR), FDA

- Technologies used by the FDA to test for toxicity

Feedback to Chris: There is not a disease pathway/toxicity pathway so we have to be careful since this leads us to think that there are biomarkers of disease/toxicity compared to biomarkers of just organ damage

How can we replace the "gold standard assay"? If our assay disagrees w/the gold standard, the psychological response is that the alternative is not accurate however we are moving away from the gold standard since it is not 100% predictive.

- Insufficient Predictivity of Current Methods:
  - <100% accuracy in predicting toxicity of even single compounds
  - Don't test enough humans to understand human to human variability
- Serious Adverse Drug Reactions (ADRs) Caused by Market Drugs
- Greater number of serious ADRs, deaths; growing faster than # of prescriptions
- Looks like its getting worse, we need new methods
- Focused on Translational Biomarker Approach
  - Rodent capability of what we see in humans
  - Body fluids/ imaging vs. histology in animals (we're looking at apples and oranges)
  - Best of all worlds would be to use in silico, even skip in vitro
- Organ Damage induced by FDA-regulated products

    - Areas of interest include: liver, heart, lung, brain, developmental bio, carcinogenicity
        - Ex: Primary hepatocytes, looking at mitochondrial integrity
        - iPS-Derived Human Cardiomyocytes
        - Differentiated air liquid interface human airway cultures; by day 28 you can get a representative of bronchial /air interface
        - Zebrafish work; lacks the ability to see impacts on later differentiation
        - OECD Fish Embryo Acute Toxicity (FET) Test for Chemicals
            - Zebrafish embryos,
            - Quantitative methods as well
            - Examples: Ketamine on ZE, anesthetics on embryos, Anesthetic Induced neurotoxicity with propanol.
- In vivo Imaging Capabilities
    - MRI, MRS, PET
- Nanotoxicology: cell-free and cell based assays
- Induction of oxidative damage by nanomaterials
- In silico Modeling: QSAR and QSDAR Approaches: Quant Spectral Data Activity relationship
    - Has been used to predict endocrine disruptors, environmental toxicants, drug efficacy and drug toxicity.
    - This has to be combine w/multiple approaches but have a significant place within toxicity testing
- Computational Approaches: can we match what we see in silico in *in vivo/in vitro*? Can you use screening with patients to identify HLA SNPs for trends.
- Psychological Challenges:
    - If people don't understand you, then you may have to make more data or put it into different viewpoint
    - If you Build It, They May Not Come
    - Motivated Reasoning, confirmation bias, disconfirmation bias – read to figure out how to refute it
    - How can we combat this?
    - Learn that the generation and dissemination of evidence alone are insufficient
    - Show how the new information will fit their beliefs/concerns vs. being threatening
    - Present experts on both sides of the argument
- Future: when new technology is oversold, it can burn the bridges
- Who are you selling it to? Tailor your sale to the audience.

Questions: How do we implement a process of change? What is our target audience, our regulators, what is an environment that is

Pharma is our real audience b/c if they can show that it is useful, regulators will see that and begin to become more familiar with the technologies. Is that accurate?

We have to work with not just the early adopters, but from project team to project team to convince each one that their compounds are also applicable. You need both governments to accept the approach but also the people who will be submitting the data to adopt the approach.

Technology Platform #1: Use of Regenerative Medicine and Bioprinting Techniques for Human Tissue Testing

Anthony Atala, M.D., Director, Wake Forest Institute for Regenerative Medicine, Presented by: Shay Stoker PhD

Want to do tissue engineering to replace damaged and non-functional tissues or organs.
Current "state of the art"; simple single cellular layer
Examples: cornea replacement: Development of a gel based scaffolds for cornea transplant. After 5 weeks in vivo the cornea clears and the eye looks as normal. (flat structure)

- Blood vessel engineering in a bioreactor to train the cells to respond to physiological environment; then put into an AVF in a sheep. Tested w/needle sticks, can see that tissue regrows. (tubular structure)
- Bladder tissue engineering (hollow structure)
- However real challenge is the solid organs: kidney, liver, pancreas, etc.
    - Using native tissue as a scaffold ECM (decellularized authentic structural support)
    - Leaves some of the native markers and vasculature in place
    - Acellular liver, recellularized shows liver functioning
    - If we take discs of tissue and put onto a chip, "acellular liver disc" can we use it for drug screening? An "organoid." Yes, they are functionally active (Albumin and urea) and better than cells in culture and can metabolize drugs as well.
    - From this idea we then expanded to a body on a chip program.
    - Bioprinter to print both scaffold material (e.g. PCL) as well as cells
        - Tried with: MTJ, muscles, testis,
        - Investigating: liver using a biogel to keep cells alive and active for almost 28 days vs. 14 days in basic cell culture.
    - Working with INGOTS, to make a 4 tissue chip. Liver, heart, lung and blood vessel

Technology Platform #2: Use of Stem Cell Derived Cardiomyocytes and Real-time Impedance-based Measurements to Accurately Predict Drug-Induced QT prolongation and arrhythmia

Kyle Kolaja, Ph.D., D.A.B.T., Fellow, A.T.S., Vice President, Business Development at Cellular Dynamics, International, consultant in vitro cardiac impedance measurements

Cellular Dynamics International
Overview of Technology: 3-party development project

- Cardiovascular Safety Pharmacology
    - Early cardiovascular toxicology; specifically QT prolongation evaluation
    - 10 drugs pulled from the market based on torsades de pointe
    - Regulatory guidance documents to require hERG screening
    - Two consequences: no drug induced torsades, a lot of beneficial drugs not marketed
    - Early screening relies on hERG screening - but this is not indicative of arrhythmia (can be independent of hERG)
    - There is a better approach – using HESI and CIPA – "Cardiovascular safety and regulations could
      be better"
- ACEA's technology:

- xCELLigence RTCA-Cardio System (see slide)
    - Why impedance vs. MEA?
        - Higher throughput, treat and collect data for days vs. hours, noninvasively
    - Look at cardiomyocytes contraction w/in a dish
    - Uses hIPs Cardiomyocytes
    - (My question: Phenotype of the cardiomyocytes/what about disease models for these hIP cells?)
- Uses blebbistatin – that attaches to myosin and doesn't allow for cardiomyocyte contraction; imprudence measures the physical presence in the dish vs. MEA which measures the electrical potential which still are present even when contraction does not occur (as seen when blebbistatin is present)
- Can quantitate the irregularity of the beats to predict the concentration that would cause a 20% arrhythmia at a given sampling timepoint. ("IB20")
- Demonstrated this using a range of drugs that create changes in arrhythmia *in vivo*
- Can use this to identify the drugs that use the hERG channel but don't induce arrhythmia; also can you use this to assess it over a long period of time which improves accuracy.
- Investigate potential confounding effects of cytotoxicity
- Compared response to internal Roche cardiomyocyte response – whether QT prolongation or arrhythmia; found good correlation
- Validation (although different meanings for different people); microelectrode array – looked at multiple doses and timepoints compared to results seen using MEA.
- Highly predictive model, doesn't require a lot of compound, quick turnaround, strong identification method for ; also context hERG data for early decisions during the development process

- Questions:
- Regarding IB20; did you try to look at other timepoints to help improve specificity? What about an IB30 or different. Yes, we picked it early but then we went back and reevaluated it and it still looked relevant.

Anne Plant: we can also talk about this during our breakout sessions.

Technology Platform #3: Arrhythmogenic Liability in Stem Cell-Derived Cardiomyocytes Early in Drug Discovery via Kinetic Image Cytometry (KIC)

Jeff Price, M.D., Ph.D., Founder, President and Chief Executive Officer, Vala Sciences, High-Content Imaging Systems; Adjunct Associate Professor, Sanford-Burnham Medical Research Institute, San Diego, CA

- Arrhythmogenic liability in stem cell-derived cardiomyocytes early in drug discovery via kinetic image cytometry (KIC) – Vala Sciences
- Functioning cell system, although not 3D, but highly predictive
    - Focus on validation
    - Average of all visible cells; not just one cell to have a better evaluation of the results
    - Measure rate of contraction stimulated and unstimulated for 10 seconds visually.
    - Less issues of hERG false positives
    - KIC can be used to detect multiple effects
    - 2 large validation studies:
        - Janssen study
        - 60 compounds, blinded, compounds w/mixed effects
        - QT prolonged drugs – detected them all

- QT shortening drugs – 5 of 6
- Ca2+ channel blocker – 5/5
- Sodium channel blockers – all but the 1b channels, but are picked up with changes in voltage with the KIC but don't see the change physically. This is caused by the change in potential shape but it's w/in the threshold.
- Chronotropic compounds – 6 of 6.
- DSEC Study:
  - Identify strength and weaknesses of the assay; wanted to evaluate those compounds that had passed in vitro but then failed in vivo. Especially compounds w/multi channel effects
  - Some false negatives and some false positives w/QT prolongation – so we set the threshold at this level to minimize the false positives.
  - Compounds which weren't identified: tricyclic antidepressants, grepafloxacin. But maybe we needed a longer incubation period to see if they have an effect w/a longer time period. Terfenadine – difficult compound to evaluate but not sure why.
- Beyond QT – in vitro detection of arrhythmia;
- You can fix and label the cells and then use the technology to get both immunofluorescent data and KIC data.

Questions: Elizabeth Ways:
Are there test article that are cardiotoxic to humans that have not been shown in humans, can it detect a cardiotoxicity that could not be seen in animals. – Went into humans, shown arrhythmia, but didn't show that during the animal testing?
They have not tried to validate vs. animal data since pharmaceutical companies are only focused on the human responses. Some were tested that were false positives and false negatives.

Technology Platform #4: An integrated in vitro model of perfused tumor and cardiac tissue
Steve George, M.D., Ph.D., Edwards Lifesciences Professor; Director, Edwards Lifesciences Center for Advanced Cardiovascular Technology, University of California, Irvine

Model System of Perfused Tumor and Cardiac

- Large group of collaborators
- UH2 grant
- Overall Strategy:
  - Patient specific type screening using iPS cells
  - Wanted to use this technology to screen for chemotherapies that would not have cardiac toxicity so they also want to model the tumor environment.

Creating three different systems:

- Integrated micro-organ system #1 - 3D w/blood supply
  - Patient derived hIPS-derived vessel system
- Integrated micro-organ system #2 – w/tumor organoids
- Integrated micro-organ system #3 – w/tumor organoids

Needed to create micro capillary w/blood flow
Use soft lithography w/in fluid channels w/two channels. One high pressure and one low pressure to provide the hydrostatic head to create flow across the cellular bed
Can use 1um fluorescent beads to watch flow through the device.
Three dimensional features differentiates it from other previously discussed systems
Cancer Tissue-Originated Spheroids (CTOS)

Uses primary human tumors – also colon cancer, recapitulate much of the original tumor structure. Wanted to see if they could use this as part of their system for tumor – endothelial cells included in the organoid system.

Thoughts on Commercialization methods:

- License it to pharma to screen libraries
- Personalized drug screening

Service: mfg and sell microtissue platform, heart tox screening

Discovery:

- Key ideas: use personalization – isolate cells from body, create and expand iPS cells, create EC, CM and Fib cells – add matrix and create 3D array of cells.

Tumor and cardiac microenvironments w/perfused human capillaries

Control of flow, dimensions, matrix cells and gradients

Patient specific modes (primary tumor, iPS cells)

Questions/Future:

Drug interaction with PDMS

Vessel remodeling (pruning, arterioles, venules), RBC/WBC/Platelets, reproducibility and validation

Audience Questions:

Can you use visualization of flows to compare to physiological values? Would this be helpful?

We use beads to look at average velocities?

Could you map beads for vector fields, pressures, etc to see how they compare physiologically?

Once we can determine (and titrate) the flow to make sure it supports the tissue, then we can look at those questions.

Have you transplanted cancer cells to see as the tumor grows that it develops vessels inside of it? Early tumor growth does it cause vessel growth vs. later tumor introduction?

Yes, we have added in tumor cells at different times of the tissue development to see the impact?

When you put them in an oxygen depleted/hypoxic environment do they "go nuts?"

We reduce the O2 and we grow most of them at 5% so it's important

J Hickman:

Really key part of the workshop will be during the breakout session, going through the idea of broad context of use, validation of these systems. So that we can come up with something useful to be the starting point for a regulatory document.

Breakout Session #1: discussion of Technology Platform #2 (Use of Stem Cell Derived Cardiomyocytes and Real-time Impedance-based Measurements to Accurately Predict Drug-Induced QT prolongation and arrhythmia)

Federico Goodsaid, PhD, Vice President for Strategic Regulatory Intelligence, Vertex Pharmaceuticals Inc.

(15 attendees + moderator)

What do we need for this data for Phase I clinical trials?

- Comparative data w/known human set of compounds w/a wide range of responses, i.e. development of compound standards
  - Incremental/ report in addition of data to currently required test? – Everyone says NO;
  - Submit these data – this is not a discussion of submitting data in lieu of current data; submit these data to support conclusions from other tests
  - What would characterize the tests?
    - 5 – 1000 compounds
    - Assays should be run reproducibility
  - We want this to replace the animal, not in addition to it; so what do they need to do to make this the case?
    - For example cobalt/chromium to look at this question?
    - Didn't look at heavy metal cell death – see cell viability/proliferation changes due to toxicity
    - If we know that heavy metal causes toxicity, then see if there are similarities from the in vivo experience even post mortem
    - KIC hasn't looked at cardiomyopathy since it's harder to validate - not a tox path methodology since there are many routes of toxicity.
  - How much additional data will we need to determine how much will be replaced with these tests? Must be harmonized in the role out.
  - The community must sit down all together to discuss an important initiative and the work is done but then it's published and no one adopts it. If we make a decision, what sort of data does ICH need to make this decision? Will it be harmonized or put it in together?
  - How do we reduce risk for everyone involved – agency, patient, company
  - How does the sourcing of the cells impact the testing – different genetic makeup of cell sourcing? Make sure your cells are predetermined/resistant that you're testing.
  - First we need to show that it works w/the generic makeup then we can look at individual genotypes
  - Data into genetic liabilities, how do we mitigate this?
  - How much do genetic changes impact the cellular response when screening compounds?
  - In vitro test should be robust enough to derisk the really bad compounds from entering the clinical trials or even post market.
  - General concept: are these in vitro assays going to be able to reproduce animal assays or some part of the clinical trial? Mouse genetic inbreeding may not properly represent the human response therefore would a genetically single device.
    - Kyle – you don't see some things in mice since their CM repolarize differently so maybe the device is better.
  - How do we know if our test method is responsive? Would need a discussion on the knowledge regarding the target, the target distribution, where we expect to see it in the heart, potential cascades involved…
    - Is this relevant? For pharmacology it's not relevant vs. just wanting to know if it will impact the heart. You want to know if the drug is to target something in the heart since this may impact the results.

        - Do we need to know if the
- Assess QT prolongation, arrhythmia, etc after hERG screening
- Overall notes: breakout room could be improved for discussion if the tables were arranged into a round/rectangular table structure. 14 attendees at the end. High involvement with small group of people.

Breakout #2: Arrhythmogenic Liability in Stem Cell-Derived Cardiomyocytes Early in Drug Discovery via Kinetic Image Cytometry (KIC)
James J Hickman, Ph.D., AIMBE Fellow and Professor, NanoScience Technology Center, University of Central Florida

JH – can use for cardiac safety except for system toxicity

Need to use the correct terminology: DDT, EMA website how they define various parameters

Need to get plasma concentrations; work with the NIH to mine the data to find the Cmax # to do the proper validation of these systems. We may get all kinds of false results if we're not using physiologically relevant levels. Levels of predictability – ICH can set this; animals as ~70% for the indications that they are looking at.

Breakout #3, Technology Platform #4 (An integrated in vitro model of perfused tumor and cardiac tissue)
William Bentley, Ph.D., Robert E. Fischell Distinguished Professor, Department of Bioengineering, University of Maryland

Discussion of technology presented by Steve George today.

Human variability, number and types of organ systems – how do we determine which ones to choose?

Reproducibility, qualified in a mfg to help adoption for use in industry

Final Discussion of Workshop:
Further development of validation and qualification guidelines for microphysiological systems. These guidelines will be used by the steering committee to meet with the FDA to determine next steps based on FDA requirements and to set the agenda for the next workshop.

Did we accomplish our objectives?

Goals: FDA feedback: we need to create a community around these systems and listen to the community.

Great interaction w/regulatory toxicity people. Hopefully this will foster group interaction.

Next meeting will be on efficacy. Suggestions for topics to cover, etc. June 26th – 27th at same location.

Rosemarie- Want more pharma for next time!

Please contact with more suggestions for improvements.