REAGAN-UDALL FOUNDATION FOR THE FDA

In Silico Alternative Methods Cluster Summary Report

February 2024

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Discussion Result – Summary of Findings from the In Silico Alternative Methods Cluster

To meet regulatory science goals and objectives that serve stakeholders in the FDA-regulated ecosystem for emerging technologies, the FDA's Office of the Chief Scientist's (OCS) Office of Regulatory Science and Innovation (ORSI), in partnership with the Reagan-Udall Foundation for the FDA (the Foundation), created the Regulatory Science Accelerator (RSA). The RSA creates collaboration space for sharing information regarding emerging technology that FDA centers will encounter in the near future.[1]

This report captures the discussion of the in silico alternative methods workgroup of the RSA. Experts presented proposals describing In Silico methods to replace animal models in the development of FDA-regulated products during the cluster workgroup meetings. Workgroup participants ranked the proposals from highest to lowest based on highest impact with lowest relative effort. Summaries of the proposal, ranked from highest to lowest impact, follow below. Full presentations are provided in Appendix D. The FDA Modeling & Simulation (M&S) Working Group and the FDA Alternative Methods Working Group, which include over 200 FDA scientists from across the Agency who support the implementation of M&S and in silico methods in the regulatory review process, then read the results of those discussions and provided an informal regulatory science perspective.

1. Proposal: Saying "I Do" to the Machine Learning / PBPK / QST Marriage

John Dibella, MS, President, SLP Division, Simulations Plus

The whole is greater than the sum of its parts… and the marriage of machine learning, physiologically based pharmacokinetic (PBPK) modeling, and quantitative systems toxicology (QST) approaches brings high-throughput and mechanistic modeling methods together to reduce animal testing.

Recent validation and advancements with these modeling modalities have increased the confidence of using them in combination to rapidly screen compound libraries for exposure, translate results across species, and assist with animal/human risk assessment, all to support alternative approaches to animal testing. Extending this application to organ toxicity, through additional machine learning models informing QST inputs, will help the pharma industry identify safety liabilities and adverse outcome pathways earlier, with fewer animals, and design effective dose regimens for target patient populations.

Several factors are driving greater adoption of these approaches, including more educational opportunities for students and scientists, industry-government collaborations to advance research forward, and encouragement from regulatory agencies to incorporate this to help reduce R&D

[1] Institute of Medicine (US) Forum on Drug Discovery, Development, and Translation. Building a National Framework for the Establishment of Regulatory Science for Drug Development: Workshop Summary. Washington (DC): National Academies Press (US); 2011. 2, Defining Regulatory Science. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54399/ stated""[1]

costs, animal testing, and regulatory burden. Continued investment in these low effort areas will provide the high impact solution for which we are all striving.

From a regulatory standpoint, this approach may be appropriate for screening and early drug selection, as well as replacement of definitive toxicology studies.

2. Proposal: Large animal models vs implant design iterations

Ashley Peterson, PhD, Vice President, Applied Science, Thornton Tomasetti

Our proposed animal/method model uses the digital twin approach to replace some, and potentially eliminate all, large animal models currently used for cardiovascular implant device design. In the proposed digital large animal model, the engineering performance assessed in the physical large animal models can be wholly replicated in the digital twin. This approach facilitates human specific device designs, by removing the erroneous influence animal trial results may have on device design iteration. Furthermore, the physics-based digital twin models can be used to create machine learning models to rapidly iterate the device design. As demonstrated in the 3x5 slides, the technology and simulation experience are ready and available today, the gap to industry adoption to overcome the status quo is regulatory acceptance.

From a regulatory standpoint, additional validation of computational models of thrombosis is important, particularly in assessing device thrombogenicity.

3. Proposal: An in silico protocol to support weight-of-evidence assessments in the ICH S1B guideline

Kevin Cross, PhD, Vice President, Product Engineering, Leadscope

The new addendum of the ICH S1B carcinogenicity testing guideline describes an integrated approach to assess human carcinogenic risk of pharmaceuticals using weight-of-evidence (WoE) criteria. Six WoE factors are evaluated on a case-by-case basis to determine the value of conducting a two-year carcinogenicity study. Several of these factors can be supported using in silico protocols where predictions are combined with existing experimental data in a structured, transparent, and reproducible manner. Such an approach may be used to construct Carcinogenicity Assessment Documents for regulatory use as an alternative to the long-term rodent bioassay study thereby reducing the use of animals without compromising human safety.

For each of the six weight of evidence factors described in the ICH S1B addendum, different elements and supporting AI technologies are identified:

- Genotoxicity
    - *in silico protocol*
    - *machine learning (Q)SAR models including bacterial mutation*
    - *intellectually defined alerts*
    - *read-across (structural, substructural, biological)*
    - *regulatory acceptance*
- Target Biology
    - *several endpoints present in other factors*
    - *limited development*
- Secondary Pharmacology
    - *NLP for Target Cancer Assessment*
    - *machine learning (Q)SAR models for single receptor bonding*
    - *target/cancer-relevant pathways defined using AOPs*
- Histopathology and Chronic studies

    - *organ-specific toxicology models*

- Hormonal effects
    - *in silico protocol*
    - *machine learning (Q)SAR models*
- Immune Modulation
    - *limited data to support AI*

From a regulatory standpoint, this proposal describes a computational approach that integrates drug-specific data and predicts the potential for human carcinogenicity based on known carcinogenicity pathways. Such an approach could be of value.

4. Proposal: Preclinical Database

April Naab, MS, Associate Scientist, Pharmaceuticals and Medical Devices, PETA Science Consortium

We proposed a curated Preclinical Database as both a tool in itself and a prerequisite to many other in silico efforts. A model is only as good as its inputs, but pharma lacks high-quality, accessible data – in large part due to confidentiality challenges. The Work Group may be in a unique position to overcome the data sharing challenge and gather much-needed data from sources like FDA science projects, literature, and industry partners. A curated database would maximize the work invested into pharma-related in silico projects, and it would provide a powerful tool for assessing the value of animal studies. The trend discovery application has a notably high and immediate impact, since animal testing could be reduced or eliminated in areas where it does not inform human safety. With basic database queries, regulators and developers could better understand the testing that's working or failing in preclinical.

From a regulatory standpoint, collaboration on toxicology-related models could be of significant interest if confidentiality issues can be overcome.

5. Proposal: In Silico Method-Animal Model Recommendations- STopTox as a case study

Alexander Tropsha, PhD, Professor, UNC Eshelman School of Pharmacy, UNC-Chapel Hill

The most common type of animal assays for acute toxicity assessment of chemicals including pharmaceuticals is a "6-pack" battery of tests, including three topical (skin sensitization, skin irritation and corrosion, and eye irritation and corrosion) and three systemic (acute oral toxicity, acute inhalation toxicity, and acute dermal toxicity) endpoints. Recently, 125 NDA reviews in 2015-2018 identified almost 400 applications with acute toxicity "6-pack" studies. We compiled, curated, and integrated the largest publicly available datasets and developed an ensemble of predictive computational models for all six endpoints. All models demonstrated an external accuracy ranging from 70 to 77%. STopTox can reduce animal testing by predicting compounds as toxic/non-toxic with high accuracy and confidence, identify statistically significant chemical alerts, and propose testing chemicals with low prediction confidence only. We established a publicly accessible Systemic and Topical chemical Toxicity (STopTox) web portal (https://stoptox.mml.unc.edu/) integrating all developed models for 6-pack assays. We expect that SToPTox models may cut animal use by at least 75% in support of the 2022 FDA Modernization Act 2.0 that calls to restrict and, eventually, eliminate animal testing of medical and cosmetic products and integrate alternative New Approach Methods (NAMs) including computational tools into regulatory safety assessment programs.

From a regulatory standpoint, this approach is not recommended for drug product development at this time.

6. Proposal: In Silico PBPK-QSAR hybrid model-An approach to reduce animal testing

Sayak Mukherjee, PhD, Senior Research Scientist-Computational Toxicology/Biology, Battelle

QSAR-coupled PBPK model can be used to reduce repeat-dose systemic toxicity testing in rats and mice in short term. The framework combines physiology inspired models of varying complexity with AI/ML aided state-of-the-art QSAR tools. Other aggregate exposure forecasting models and population-based variability in several ADME parameters can be easily incorporated in this framework. The goal is to provide a solution that can estimate internal organ specific dose for a target group rapidly. Such an estimate of the site-specific internal dosimetry combined with BMD estimates from fit-for-purpose NAMs can establish internal thresholds for toxicological concern (iTTC). However, for this approach to be successful, a few roadblocks need addressing. Understanding the role of non-hepatic metabolism, particularly in gut can help in developing more accurate estimates of bioavailability. Moreover, nested modeling of the primary agent and potentially toxic secondary metabolites, especially phase I metabolites, can improve iTTC estimates. Regulatory bodies and model developers must also work in tandem to build a standard procedure for model validation. The general outline of this framework is highly flexible, and, in my opinion, stands the best chance of reducing animal testing.

From a regulatory standpoint, this proposal may be a realistic approach although there may be significant data shortages for the modeling components, as well at as technological challenges to execute the work.

7. Proposal: Virtual Assay software for pro-arrhythmic cardiotoxicity with the possibility of also targeting cardiotoxicity

Blanca Rodriguez, PhD, Professor of Computational Medicine, University of Oxford

(no summary provided by presenter)

8. Proposal: Combining 2D/3D Pharmacophore Modeling with Linear QSAR

Yuri K. Peterson, PhD, Associate Professor, Medical University of South Carolina

The combination of orthogonal computational methods can greatly improve the utility and accuracy of compound activity prediction. Both QSAR and pharmacophore modeling have advantages and disadvantages that can be improved or offset by combining the methodologies. Both of these mythologies provide improved predicted activity estimations over keyed molecular similarity comparisons (Tanimoto coefficient using MAACS or ECFP keys for example). A large reason why is pharmacophores and QSAR do not rely on connectivity, and therefore can make estimates for drugs that are dissimilar in terms of their chemical graph and organic chemistry. Linear QSAR modeling has the huge advantage of being able to predict rank order potency, but should only be applied to compounds that meet criteria derived from the training set. Pharmacophore modeling provides 4D chemical matching including X, Y, Z and atom type but only provides a goodness of fit parameter. In conclusion, using pharmacophore models as a discriminator for QSAR prediction is a workflow taking advantage of an orthogonal and independent process for increased confidence of compound activity to improve prioritization and estimate potency to help inform and reduce the overall need for in vivo experimentation.

9. Proposal: ONTOX – QIVIVE Framework

Alicia Paini, PhD, Principal Scientist, Lead Systems Toxicology, esqLABS GmbH

The ONTOX QIVIVE Framework allows extrapolation of in vitro effect concentration to relevant human exposure values, thus providing a means to establish points of departure for chemical safety assessment from in vitro toxicity data. The quantitative in vitro to in vivo extrapolation (QIVIVE) framework comprises several in silico models. Generic physiologically Based Kinetic (PBK) models coupled with quantitative structure-activity relationships (QSARs) allow the incorporation of toxicokinetic processes by simulating time-resolved tissue concentrations (forward dosimetry) and QIVIVE (reverse dosimetry). In addition, in silico models for simulating in vitro distribution kinetics are used to estimate cell-associated in vitro effect concentrations for potency ranking, input concentrations into the PBK models for QIVIVE, and quantitative Adverse Outcome Pathways (qAOP). In addition, the integration of these in silico models helps to identify relevant concentration ranges for testing in in vitro test batteries. Finally, measured urinary and blood concentrations will be compared with the source-to-dose calculations used for validation of the forward and reverse dosimetry of the framework. The validated ONTOX QIVIVE framework will be assessed and eventually applied for "next-generation risk assessment" solely relying on non-animal approaches, as a replacement of animal testing.

From a regulatory perspective, this project may be able to facilitate the adoption of an in vitro method for a particular endpoint prediction.

10. Proposal: In Silico Injection Modelling

Joel Gresham, Applied Sciences & Simulation Lead, Crux Product Design

Modern drugs (particularly biotherapeutics comprised of large protein molecules) often require formulations of high volumes and viscosities for subcutaneous injection, compared with traditional small molecule drugs. Risks of these new formulations include pain, tissue damage, leakage and absorption variability. These risks influence the design of injection devices, and optimisation opportunities arise from developing a physics-based understanding of the injection mechanics. Accurate computational modelling can provide insights that are not feasible to test experimentally. We present cutting edge digital models for the evaluation of subcutaneous injection device performance across a range of diverse digital patients. The modelling approach requires the inclusion of patient or animal-specific data e.g., anatomically-accurate geometry and mechanical properties acquired in a standardised way, suitable for parameterisation to represent the real-world variability of humans and animals.

From a regulatory perspective, this novel proposal may have promise.

Background and Processes

To meet regulatory science goals and objectives that serve stakeholders in the FDA-regulated ecosystem for emerging technologies, the FDA's Office of the Chief Scientist's (OCS) Office of Regulatory Science and Innovation (ORSI), in partnership with the Reagan-Udall Foundation for the FDA (the Foundation), created the Regulatory Science Accelerator (RSA). The RSA creates collaboration space for sharing information regarding emerging technology that FDA centers will encounter in the near future.[2]

Advancing Regulatory Science at FDA: Focus Areas of Regulatory Science (FARS)

The RSA, using the FARS report as its guide, represents opportunities for FDA to efficiently prepare for new science and technology that Agency staff will likely encounter in the regulatory process. In addition, RSA activities can positively influence the way science is conducted in the FARS by stakeholders in the FDA-regulated ecosystem. Outcomes from that science (applied and translational) can be efficiently vetted by FDA (i.e., qualified) and more readily implemented into the regulatory review process with minimal delay, while improving the quality and integrity of FDA's regulatory decisions.

The RSA is intended to provide additional insight into:

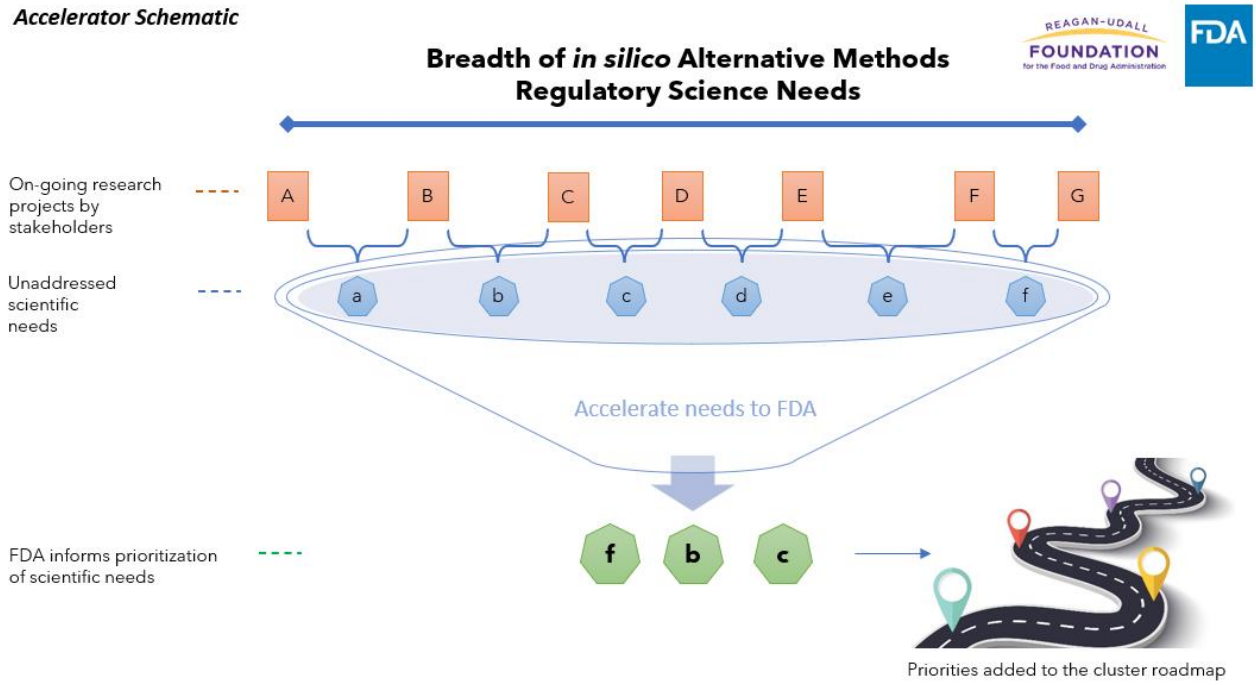
- emerging science and technology that centers need to provide future regulatory review,
- the opportunities and pitfalls associated with new science and technologies, and
- exploring potential next steps to meet the anticipated regulatory science to help speed innovation.

In this stage, the Foundation is convening the RSA to discuss in silico alternative methods. Figure 1 lays out the map for the RSA.

> *Our regulatory scientists must be able to understand therapies that are being developed using the most recent scientific advances, they must have the right tools to evaluate these therapies, and they must be a partner with the greater scientific community as they work to bring these therapies to people." In addition, former Commissioner Hamburg voiced that "Outreach and collaboration are central to regulatory science efforts. When successful, these collaborative efforts will help predict which discoveries will succeed or fail as actual products, thereby reducing product development costs and getting better products to patients faster.*
>
> Margaret Hamburg, former FDA Commissioner[1]

[2] Institute of Medicine (US) Forum on Drug Discovery, Development, and Translation. Building a National Framework for the Establishment of Regulatory Science for Drug Development: Workshop Summary. Washington (DC): National Academies Press (US); 2011. 2, Defining Regulatory Science. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54399/ stated ""[2]

Figure 1: Regulatory Science Accelerator/ In Silico Alternative Methods



Clusters

Guided by the 2022 update to the Advancing Regulatory Science at the FDA: Focus Areas of Regulatory Science Report,[3] the ORSI/Foundation collaboration identified two discrete cross-cutting issues (clusters) stemming from the FARS report warranting continued investment – In Silico Alternative Methods and Good Simulation Practice. The top line shows the scope of In Silico Alternative Methods Regulatory Science needed by FDA. The rectangle boxes are current and past research that meets the FDA's Alternative Methods Regulatory Methods. The remainder of the diagram and Figure 2 depict identified gaps in research, which FDA can prioritize to meet regulatory science needs.

A cluster is a subset of the RSA convened to discuss a discrete topic, here In Silico Alternative Methods. In Silico Alternative Methods are methods which can be used to replace traditional animal testing with non-invasive methods or substitution, using in silico (computational) approaches.

This report is a summary of the activities of the first cluster, In Silico Alternative Methods.

[3] Commissioner of the FDA. Focus Areas of Regulatory Science Report. U.S. Food and Drug Administration. Accessed June 19, 2023. https://www.fda.gov/science-research/advancing-regulatory-science/focus-areas-regulatory-science-report.

Figure 2: Focus Areas of Regulatory Science (FARS) Framework

I. Modernize development and evaluation of FDA-regulated products

A. Alternative Methods
B. Advanced Manufacturing Approaches
C. Analytical and Computational Methods
D. Biomarker Tools
E. Clinical Outcome Assessment
F. Complex and Novel Clinical Trial Design
G. Methods for Assessing Behavioral, Economic, or Human Factors
H. Approaches to Incorporate Patient and Consumer Input
I. Methods to Assess Real-World Data to serve as Real-World Evidence
J. Methods to Assess Data Source Interoperability

II. Strengthen post-market surveillance and labeling of FDA-regulated products

A. Methods to Assess Real-World Data to Support Regulatory Decision-Making
B. Using and Validating Artificial Intelligence Approaches
C. Novel Clinical Trial Design, Statistical and Epidemiologic Methods
D. Automated Reporting Tools for Adverse Events and Active Surveillance
E. Methods to Improve Communication About Risk to Patients and Consumers
F. Approach to Expand Data Capacity, and Increase Data Quality and Use
G. Efforts to Harmonize Existing and Emerging Data Standards

III. Invigorate public health preparedness and response of the FDA, patients, and consumers

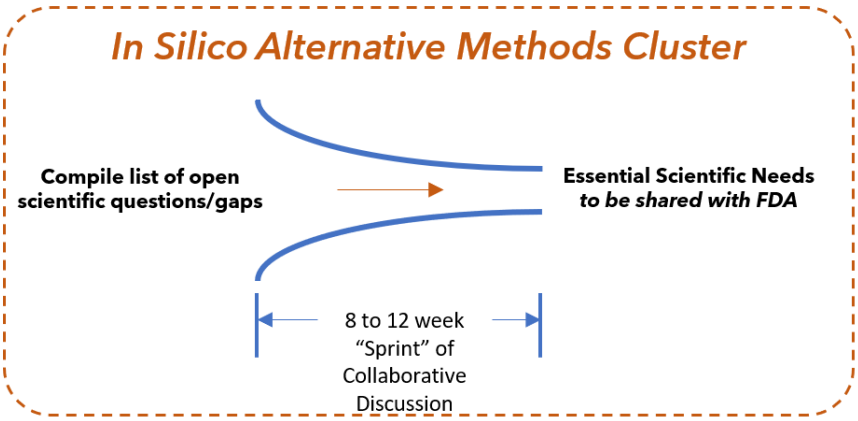
A. Reinforce Medical Countermeasures Initiative (MCMi)/Increase preparedness and response for emerging public health threats.
B. Mitigate Antimicrobial Resistance
C. Strengthen Patient and Consumer Engagement and Communication
D. Understand Substance Use and Minimize Misuse
E. Apply Population Approaches to Precision Medicine
F. Expand One Health Approaches
G. Identify and Harness Relevant Emerging Sciences & Technologies
H. Strengthen Global Product Safety Net

In Silico Alternative Methods Cluster

The mission of the In Silico Alternative Methods (ISAM) Cluster of the RSA was to identify open regulatory science gaps and prioritize the critical gaps (or essential scientific methods) that might be closed within the next three to five years (Figure 3).

Subject matter experts were identified to serve as an Advisory Group for the cluster (Appendix A). Membership for the cluster was selected using a questionnaire seeking input about ISAM methodology and applications (Appendix C). Four interactive webinars were held to identify in silico method-model pairs to replace an animal model in research and establish the groundwork to achieve the mission of the ISAM Cluster.

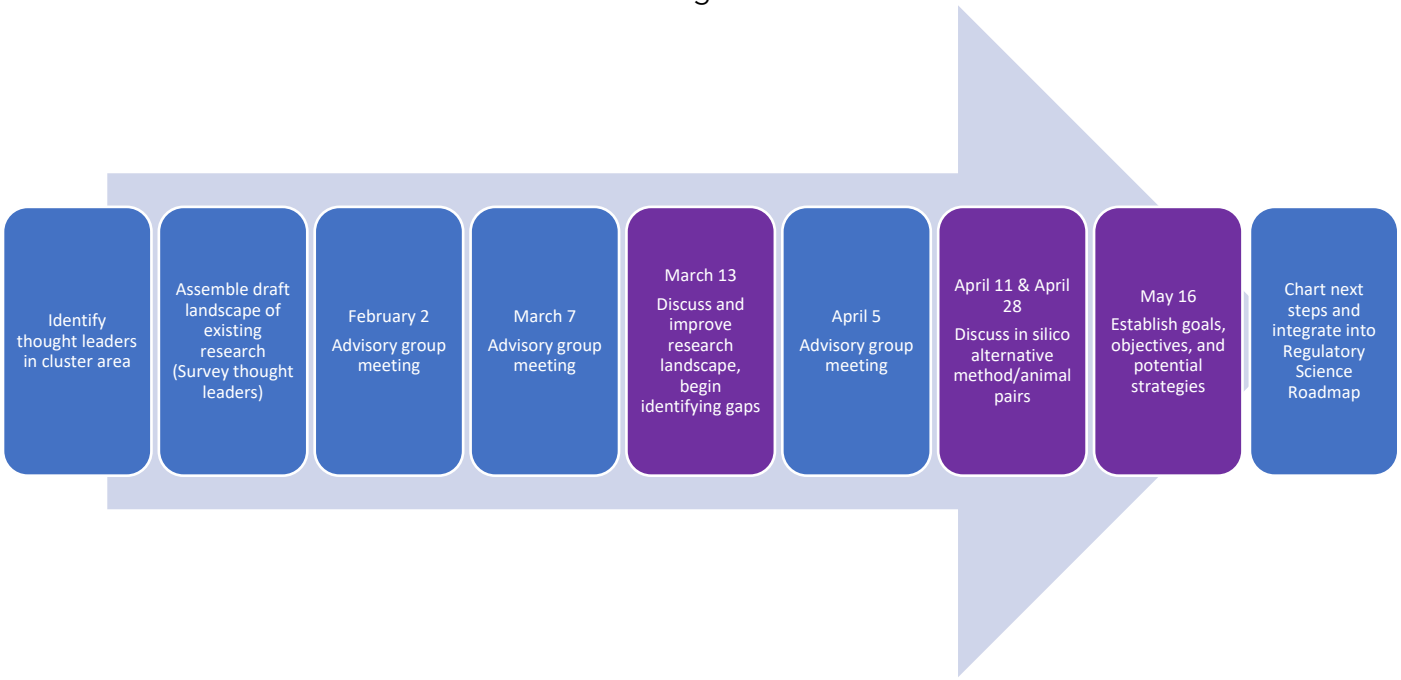
Figure 3: In Silico Alternative Methods



Timeline

Figure 4 provides the timeline for the In Silico Alternative Methods cluster. The advisory group met three times prior to and in between the four cluster workgroup sessions.

Figure 4



Membership and Registration Questionnaire

Fifty-nine respondents completed the registration questionnaire (Appendix C). Respondents resided primarily in the United States (83%) and represented academia (36%), FDA-regulated industry (24%), governmental/public service (10%), non-FDA-regulated industry (3%), not-for-profit (7%), and other organizations (20%).

The first question asked, "Which in silico alternative ***method*** do you think holds the most promise for advancing regulatory science?" Method domains identified most frequently included: computational modeling & simulation, mechanistic and/or biological relevance & prediction, data analysis, and model integration methods. From the responses, the top methods emerging as holding the most promise for advancing regulatory science included: QSAR (quantitative structure–activity relationship and PBPK (physiology-based pharmacokinetic) models, AI & machine learning, and simulation of devices, physiologic or biologic processes.

Question two addressed application of the in silico method, "Which ***application*** of that in silico alternative method do you think holds the most promise in advancing regulatory science?" Responses to this question also spanned a wide spectrum, but one theme stood out – using in silico methods to generate evidence supporting medical products – primarily medical devices and drugs/pharmaceuticals. Other emerging themes regarding application of in silico methods included toxicity evaluation, conducting risk assessments, and determining product efficacy. Areas of application included drugs and medical devices, food products and cosmetics.

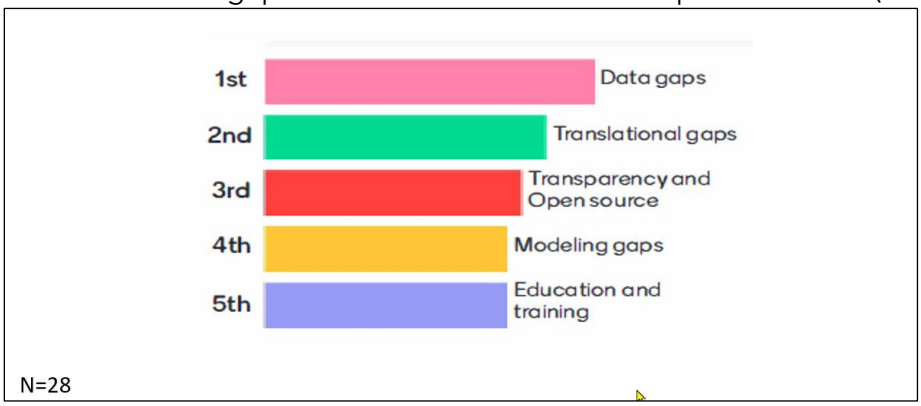
Data quality and validation, followed by physical/biological or mechanistic relevance were the top responses to the third question, "Which do you think are the most critical needs to fully realize in silico alternative methods to advance regulatory science?"

The final question asked respondents to list what science gaps remain for fully harnessing in silico alternative methods to advance Regulatory Science.
Primary themes included:

- Guidelines for good in silico practice,
- Data gaps (availability (generation), quality (standardization, reliability, validation), analysis, integration),
- Transparency and open source needs (how data/models are generated, utilized and analyzed),
- Modeling gaps (mechanistic and biological relevance, model accuracy), and
- Translational gaps.

Questionnaire results were reviewed and discussed at the first In Silico Alternative Methods workgroup cluster meeting. Workgroup participants were asked which of these gaps could be addressed in a short period of time (3-5 years). Ranked responses are provided in figure 5.

Figure 5: Which of these gaps can be addressed in a short period of time (3-5 years)?



Workgroup Meetings

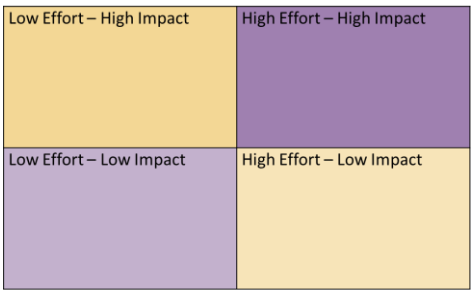
Four workgroup meetings were held in 2023 on March 13, April 11, April 28, and May 16. In addition to the advisory group and FDA observers, 52 to 59 community members attended each session (Appendix B). The first meeting provided a project overview and reviewed the results from the membership questionnaire. Workgroup members identified priority gaps that need to be addressed regarding application of in silico alternative methods to advance regulatory science (Figure 5).

Other key takeaways from the first meeting included:

- A "transparent analytic process" is an essential piece to validation and a key attribute for regulatory acceptance.
- Model advancement over time should be considered.
- Uncertainty quantification will be critical to good simulation practice.
- The discussion also exposed an overarching question for a future discussion: How can artificial intelligence (AI) and machine learning support in silico alternative methods?

During the second and third workgroup meetings, scientists presented proposals describing potential in silico methods that might replace animal models. Five proposals were presented at each meeting (Appendix D). Working group participants provided input on where they believed the method proposed fit on an effort/impact scale (Figure 6).

Figure 6: Effort/Impact Scales used to rate the method/model pair as low effort-high impact, high effort-high impact, low effort- low impact, or high effort-low impact.



*used 4/11/2023

*used 4/28/2023

Next Steps

The In Silico Alternative Methods Cluster is the first step in the development of a roadmap cross-cutting regulatory science that is driven by the regulatory science community.

The RSA will continue working toward the roadmap and identification of the critical gaps. Next steps include:

1. Select the methodology/proposal with the most promise. The proposal rank order will go to FDA for consideration.
2. Conduct a workshop to identify needs, parameters, and performance metrics of the methodology.
3. Challenge modelers to develop tools for method prediction and provide feedback.
4. Incorporate feedback into the model and finalize the software.
5. Demonstrate performance metrics.
6. Submit performance metrics to a regulatory body and publish tools as accepted.

Future clusters will continue to focus on critical gaps, an implementation strategy to close identified gaps in the next three to five years, and the public health impact of this process.

Appendices

Appendix A: Advisory Group

Joel Bercu, PhD, MPH, DABT, Executive Director in Nonclinical Safety and Pathobiology, Gilead Science

Fabio Broccatelli, PhD, Associate Director DMPK Group Leader at Bristol Myers Squibb

Catrin Hasselgren, PhD, MS, Senior Director Predictive Toxicology, Safety Assessment, Development Sciences, Genentech Inc.

Paul Watkins, MD, Howard Q Ferguson Distinguished Professor in the schools of Medicine, Pharmacy, and Public Health and Director of the Institute for Drug Safety Sciences at the University of North Carolina – Chapel Hill

Chihae Yang, PhD, Managing Director and CEO, Molecular Networks GmbH and Altamira LLC (MN-AM)

Appendix B: In Silico Alternative Methods Working Group Participant List

Russ Altman, Stanford University
Michael Ambrose, US Pharmacopeia
Lennart Anger, Genentech, Inc.
Arianna Bassan, Innovatune
Jeff Bischoff, Zimmer Biomet
John Buse, University of North Carolina, Chapel Hill School of Medicine
Tejas Canchi, ResMed Ltd
Judy Cannon, University of New Mexico
Arindam Chakraborty, Vias3d
Suman Chakravarti, MultiCASE Inc
Helen Chow, Bigfoot Biomedical
Yaroslav Chushak, Henry M Jackson Foundation
Murat Cirit, Javelin Biotech
Donna Clemons, AbbVie
Edward Croom, Haemonetics
Kevin Cross, Instem
Brendan Cunniffe, Prelude Medical
Kristian Debus, Thornton Tomasetti Inc.
Lane Desborough, Nudge BG
Luca Emili, InSilicoTrials LLC
Jean Feng, University of California, San Francisco
Whitney Fies, ICF
Ronald Fortunato, Bayer Healthcare
Alejandro Frangi, University of Leeds
Simon Funnell, UK Health Security Agency
Robert D Gibbons, The University of Chicago
James Giordano, Georgetown University Medical Center
Joseph Gormley, Tufts Medical Center and CTSI (Clinical and Translational Sciences Institute)
April Green, The Ohio State University
Nigel Greene, AstraZeneca
Joel Gresham, Crux Product Design
Vasant Honavar, Pennsylvania State University
Gary Kobinger, GNL/UTMB
Jakub Kostal, GWU/ToxFix
Nynke Kramer, Wageningen University
Steven Kreuzer, Exponent
Christopher Long, Hesperos, Inc.
Enrique Morales Orcajo, Ambu
Sayak Mukherjee, Battelle
Glenn Myatt, Leadscope
April Naab, PETA Science Consortium International e.V.
Andrew Nguyen, PETA Science Consortium International e.V.
Denice O'Connell, AbbVie
Alicia Paini, esqLABS GmbH
Abhijeet Patil, Amneal Pharmaceuticals Pvt. Ltd.
Ashley Peterson, Thornton Tomasetti
Yuri Peterson, MUSC
Elsje Pienaar, Purdue University
Blanca Rodriguez, University of Oxford
Ehsan Samei, Duke University Medical Center
Gabriela Silveira, Lhasa Limited
Stuart Sundem, Legacy Health Capital
Lisa Sweeney, UES, Inc.
Rachael Tennant, Lhasa Limited
Alexander Tropsha, UNC Chapel Hill
Shannon Valenti, University of Pittsburgh
Terry R Van Vleet, AbbVie
Leo Volakis
Jun Yang, UTMB
Jun (Vivien) Yin, Mayo Clinic

Advisory Group

Joel Bercu, Gilead Science
Fabio Broccatelli, BMS
Catrin Hasselgren, Genentech
Paul Watkins, University of North Carolina, Chapel Hill
Chihae Yang, Molecular Networks GmbH and Altamira LLC (MN-AM)

FDA Observers

Jason Aungst
Omari Bandele
Reema Goel
Xing Jing
Saniya Rattan
Michael Santillo

Appendix C: Membership Questionnaire

Question 1: Which in silico alternative ***method*** do you think holds the most promise for advancing regulatory science? (Regulatory science is the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of all FDA-regulated products.)

Question 2: Which ***application*** of that in silico alternative method do you think hold the most promise in advancing regulatory science? (e.g., quantify toxicities from tobacco products, food products, and/or generate evidence about medical products)

Question 3: Which do you think are the most critical needs to fully realize in silico alternative methods to advance regulatory science? (Select up to three) [Responses: data quality, standardized data elements, transparent analytic process, centralized data repository, generalizable/accessible methods, intellectual property, physically or biologically relevant, mechanistically relevant, repeatability/reproducibility, reliability, independent review/assessment, validation, other (please specify)]

Question 4: What science gaps remain for fully harnessing in silico alternative methods to advance Regulatory Science?

Additional Information
In developing a road map to address gaps in in silico advance methods, we are building a catalog of resources. Please share links to journal articles, pre-publication research, and other relevant resources that you authored.

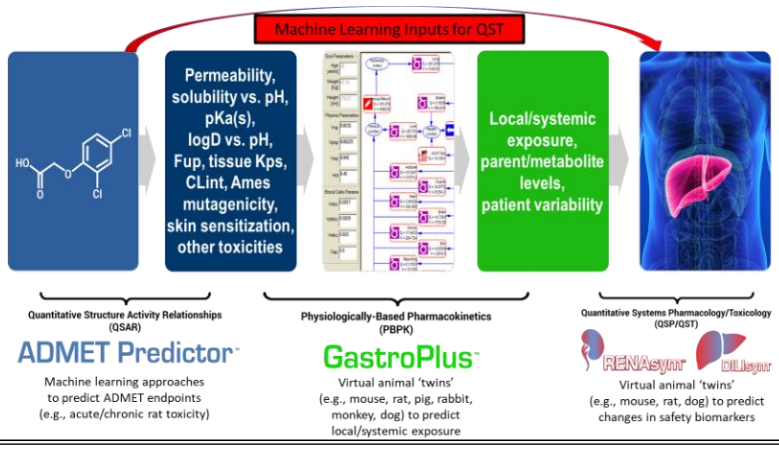
If it is convenient for you, please upload your CV, copies of recent articles, or other relevant resources you would like us to review. (You may email documents to regsci@reaganudall.org.)

Contact information and Type of organization (academia, FDA-regulated industry, governmental/public service, non-FDA-regulated industry, not-for-profit, other)

Appendix D: Presentations

1. Saying "I Do" to the Machine Learning / PBPK / QST Marriage
John Dibella, MS, President, SLP Division, Simulations Plus, Inc.

The In Silico Method- Animal Model Pair



Gaps to be Navigated

- *Industry-government collaborations (e.g., data generation) to drive in silico research forward*
- *Guidance/harmonization from agencies to accept in silico strategies*
- *Will drive changes to industry SOPs reflecting the availability of these technologies*
- *Training and education of the next generation of scientists*

Impact of Proposed Method

- *Combination of machine learning, PBPK modeling, and QST approaches + in vitro data collection will provide a holistic, mechanistic understanding of the drug's exposure and safety profiles, leading to increased confidence in our ability to reduce animal testing*
- *BTW: this is already happening today!* ☺

2. Large animal models vs implant design iterations

Ashley Peterson, PhD, Vice President, Applied Science, Thornton Tomasetti
Kristian Debus, PhD, Vice President, Life Sciences, Thornton Tomasetti

Thornton Tomasetti

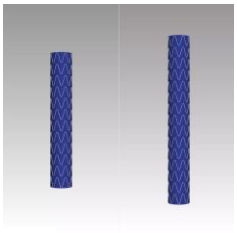
In Silico Method- Animal Model Pair:
Large animal models vs implant design iterations

- *Proposed method-model pair*
  - **METHOD**: virtual geometries, virtual deployment to assess the mechanical performance of a new device (Imaging, CFD/FEA/FSI)
  - **ANIMAL MODEL**: large animal (pigs, cows, sheep) preclinical studies typically assess deployment sequences, thrombogenicity, toxicity, and device performance assessment
- *How does the proposed in silico alternative method (or methods) replace an animal study model?*
  - It's a **1-1 replacement** for the animal study

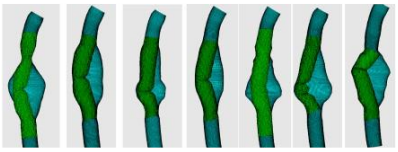
Gaps to be Navigated

- *What gaps need to be navigated to yield replacing the animal model in the next 3-5 years? I.e., What else needs to be done?*
  - **Technology is there– Regulatory acceptance is the GAP**
    - 3-5 years to get the technology accepted
    - Status Quo is hard to displace
  - **V&V and VVUQ methods are established– need to action these in a transparent way**
    - **V&V study required**
    - Depending on **model accuracy**, increased **# of benchtop test** might be required for validation

Impact of Proposed Method



- *How will the proposed in silico method-animal model pair reduce the use of animal models?*
  - Implantable devices require **many preclinical** studies
  - **Design related** preclinical studies can be **eliminated** (or reduced to a minimum)
  - Typical preclinical study: Example Thoracic Aortic Aneurysm
    - Often require several or many preclinical studies, used to demonstrate performance
    - Animal studies often only yield "**feel good**" results
    - "Design" by deploying in animals can lead to an **animal specific device**



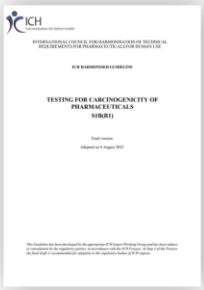
3. Carcinogenicity prediction and weight-of-evidence factors supporting animal reduction and use of animal models

Kevin Cross, PhD, Vice President of Product Engineering, Leadscope

Carcinogenicity prediction and weight-of-evidence factors supporting animal reduction and use of animal models

Rodent Carcinogenicity

- The recently introduced addendum to the ICH S1B guideline includes 6 weight of evidence factors to determine whether performing a 2-year rat study would add value
- With sufficient information, a 2-year rat study may not need to be performed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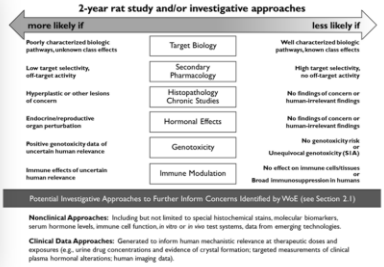
TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS
S1B(R1)



Goal: Establish a protocol to support weight-of-evidence assessment for the S1B guideline

Regulatory Science Accelerator Questions

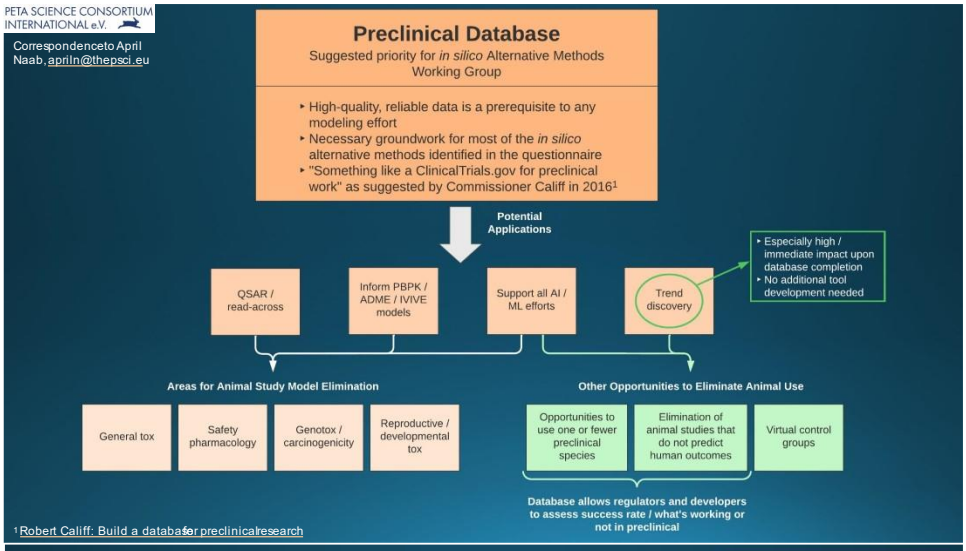
1. Gaps to be Navigated
   - *Fit-for-purpose models need to be identified for several factors*
   - *Data for supporting development are needed*
2. Impact of Proposed Method
   - *Reduction in rats used for carcinogenicity studies*

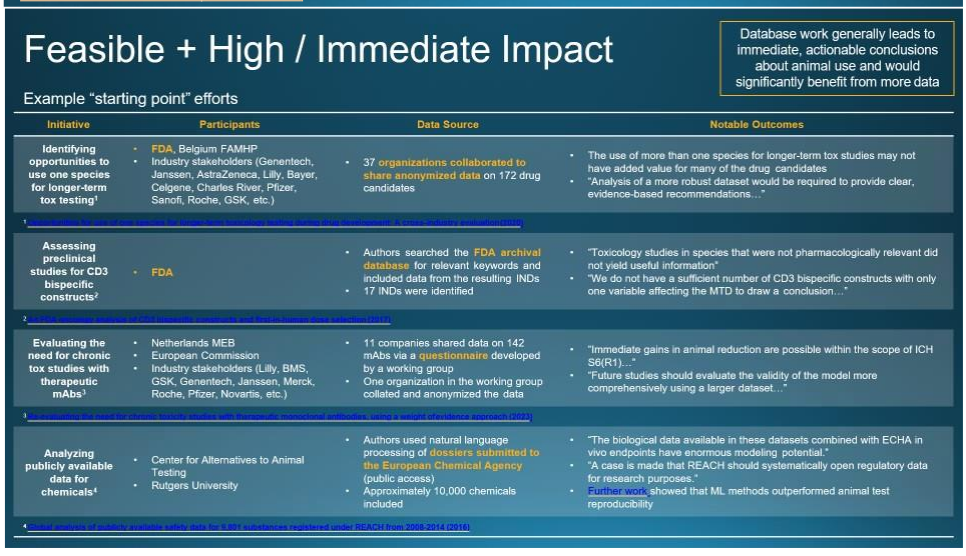
Weight-of-evidence Factors and use of various AI Methods

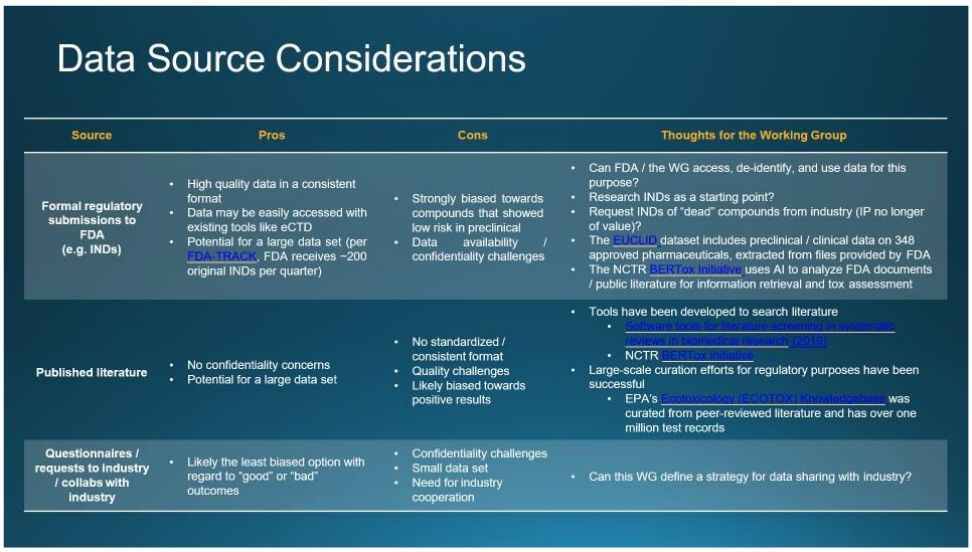
- Genotoxicity – technologies developed for predicting mutagenicity of impurities in pharmaceutical
  - *machine learning (Q)SAR models*
  - *Intellectually-defined alerts*
  - *Read-across (structural, substructural, biological)*
- Target Biology – some methods in development
- Secondary Pharmacology
  - *association defined between targets and cancer-relevant pathway using AOPs*
- Histopathology and Chronic studies – liver tox models, others limited
- Hormonal effects – machine learning models available
- Immune Modulation – little data available and poor relevance of rat studies

4. Preclinical Database

April Naab, Associate Scientist, Pharmaceuticals and Medical Devices, PETA Science Consortium

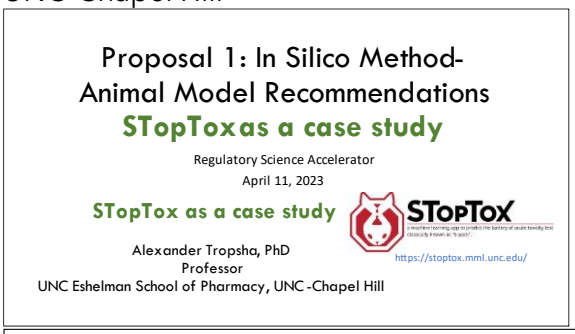


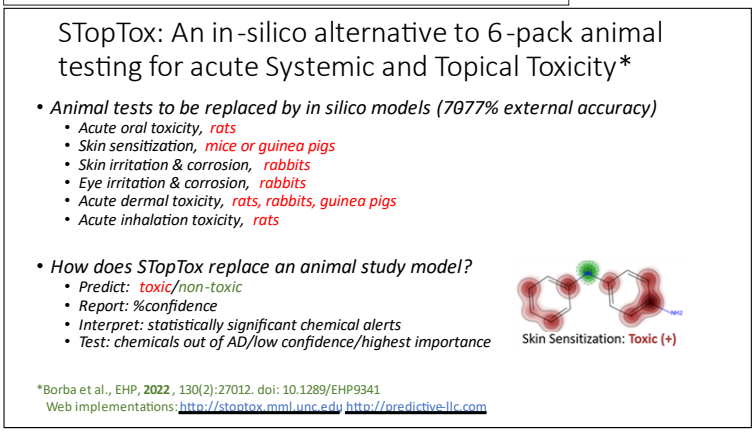




5. In Silico Method-Animal Model Recommendations

STopTox as a case study - Alexander Tropsha, PhD, Professor, UNC Eshelman School of Pharmacy, UNC-Chapel Hill





Gaps to be Navigated

- ***Ultimate objective*** *: Transform animal data into alternative regulatory tools for evidence -based in silico toxicity assessment*
- *Establish paths to regulatory acceptance of in silico models :*
  - *Steps to review, validate, and support the regulatory use of in silico tools (Ex: MDDT* or OECD** programs)*
  - *Protocols for assessing and accepting predicted toxicity by regulators*
- *Establish "good in silico practices" compliant with regulatory requirements (Ex: OECD QSAR model validation principles ***)*
  - *Size, diversity, quality, consistency and relevance of the training set data*
  - *Transparent protocols for data collection, curation, and validated model development*

*https://www.fda.gov/medical-devices/medical-device-development-tools-mddt
**OECD (2021), Guideline No. 497: Defined Approaches on Skin Sensitisation; https://doi.org/10.1787/b92879a4 -en
***https://www.oecd.org/chemicalsafety/risk-assessment/37849783.pdf

Impact of STopTox

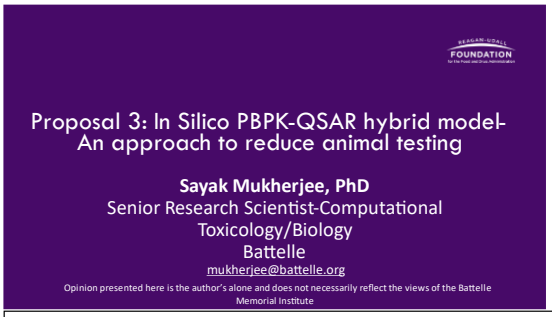
- *Currently, ca. 600K animals are sacrificed in Europe only for 6 -pack testing (comprising 55% of animal testing)**
- *125 NDA reviews in 2015-2018 identified almost 400 applications with acute toxicity "six-pack" studies***
- *Models can be developed using toxicity assessment of novel chemicals irrespective of the intended commercial use (see earlier study***)*

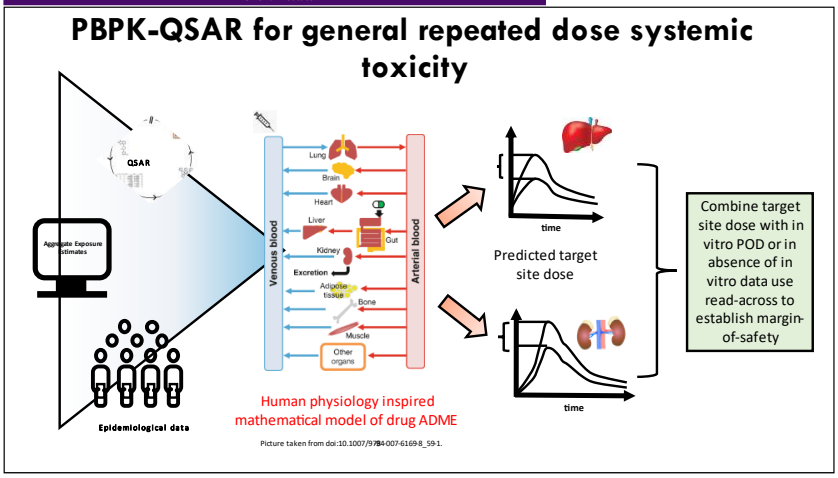
- ***In silico models (may be combined with in vitro models) are expected to cut animal use by at least 75%***

*https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2019.e170710
Mannuppello et al, Regul Toxicol Pharmacol., **2020;114:104666. doi: 10.1016/j.yrtph.2020.104666
***Alves et al., Food Chem Tox, **2018**, 112, 526 -534

6. In Silico PBPK-QSAR hybrid model-An approach to reduce animal testing

Sayak Mukherjee, PhD, Senior Research Scientist-Computational Toxicology/Biology, Battelle





Gaps to be Navigated

- Better understanding of bio -availability through inhalation, oral, & dermal routes
  - Understanding non-hepatic metabolism and role of transporters
- Modeling bio -distribution of primary as well as secondary metabolites, especially phase I metabolites
  - Integrated metabolic module that can predict hepatic as well as non-hepatic CYP mediated turnover rates, likely toxic downstream metabolites, and their ADME
- Re-validating endpoints for chemical battery with either 3D organoids or MPS systems
  - Throughput may pose a challenge
- Model validation

Impact of Proposed Method

- A validated high -throughput QSAR coupled PBPK model can be used prospectively to estimate target-site dose. Such an estimation, when compared with cell type specific in vitro PODs, can establish a more reliable internal margin of exposure based on which waivers can be issued. In the least, internal margin of safety estimates can be used to prioritize chemicals for further testing (reduce not replace)
- Case study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to Caffeine (OECD Series 321)

7. Virtual Assay software for pro-arrhythmic cardiotoxicity with the possibility of also targeting cardiotoxicity

Blanca Rodriguez, PhD, Professor of Computational Medicine, University of Oxford

Virtual Assay Software

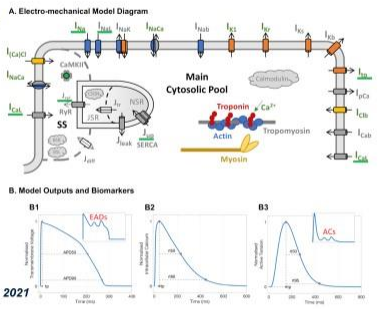
In Silico Drug Trials for Cardiac Contractility and Electrophysiology

HUMAN ELECTROPHYSIOLOGY
{Tomek et al. eLife 2019}

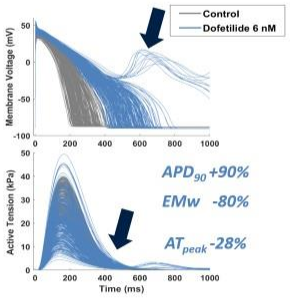
CALCIUM

HUMAN CONTRACTILITY
{Land et al. JMCC 2017}

Margara et al. Prog Biophys Mol Biol. 2021



***Dofetilide* (I_{Kr} block)**

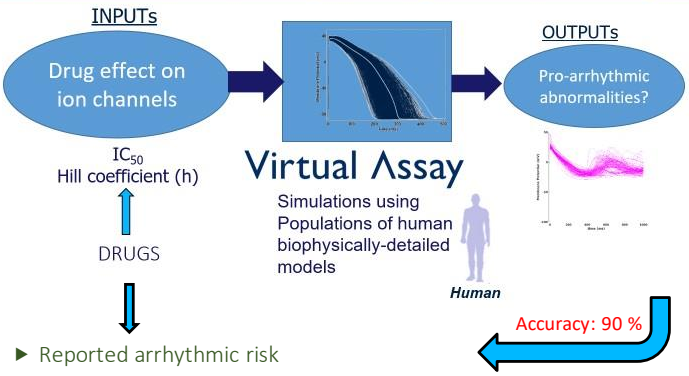


First scientific question of interest: Would the drug result in risk of developing Torsades de Pointes in the human population, even in the context of positive hERG assays and multichannel effects?

Professor Blanca Rodriguez, University of Oxford. Blanca@cs.ox.ac.uk

Virtual Assay Software

In Silico Trials for Drug Pro-arrhythmic Cardiotoxocity



Validation Studies:

- ✓ Passini et al. 2017 janssen
- ✓ Passini et al. 2019 MSD
- ✓ Zhou et al. 2020 AMGEN
- ✓ Delaunois et al. 2021 ucb
- ✓ Paci et al. 2021 janssen
- ✓ Trovato et al. *Frontiers, 2023* SANOFI

▶ Reported arrhythmic risk in human population: (https://crediblemeds.org/)

Accuracy Animal Experiments: 75-85%

Professor Blanca Rodriguez, University of Oxford. Blanca@cs.ox.ac.uk

Virtual Assay Software

In Silico Trials for Drug Pro-arrhythmic Cardiotoxocity

KEY ADVANTAGES OF IN SILICO MODEL
- Human pathophysiology
- Identification of sub-populations at risk (disease)

ANIMAL EXPERIMENTS
- Isolated cell experiments
  - Purkinje fibres
- In vivo experiments – additional endpoints
  - (contractility, heart rate

HUMAN EXPERIMENTS (CiPA initiative
- A Thorough QT study in healthy volunteers (ICH E14)
- Human stem cell derived cardiomyocytes

GAPS
- Integration in existing preclinical pipelines
- Variety of endpoints/experiment

Scientific Question of interest

Would the drug result in risk of developing Torsades de Pointes in the human population, even in the context of positive hERG assays and multichannel effects?

Regulatory impact	**High regulatory impact**: modelling and simulation results constitute the key source of evidence to answer the question of interest, *i.e.* replacing data traditionally generated in a clinical trial
Risk based analysis of decision consequence	**High clinical influence** given the new Q&A Guidelines: **impact on the decision to accept phase 1 to 3 trial designs**, and also based on this model, **waiver of intensive monitoring** of electrocardiogram (ECG) in confirmatory trials. This is also crucial for the evaluation of **cardiotoxicity in cancer drugs**. Wrong model prediction/simulation could expose patients to risk of lethal arrhythmias, in following clinical trials due to cardiotoxic drugs.
Credibility activities results	**The credibility factors** (as described in Section) were evaluated with **overall satisfactory results**. Details and results of model verification activities have been previously published.

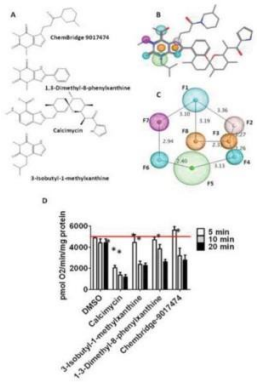
Scientific and regulatory evaluation of mechanistic in silico drug and disease models in drug development: Building model credibility.
Flora T. Musuamba, et al. 2021, CPT Pharmacometrics Syst Pharmacol . 2021 Aug;10(8):804-825. doi: 10.1002/psp4.12669. Epub 2021 Jul 13.

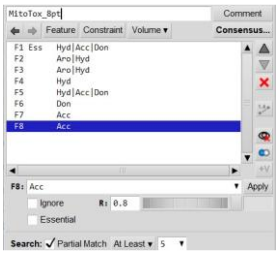
Professor Blanca Rodriguez, University of Oxford. Blanca@cs.ox.ac.uk

8. Proposal 4: Combining 2D/3D Pharmacophore Modeling with Linear QSAR

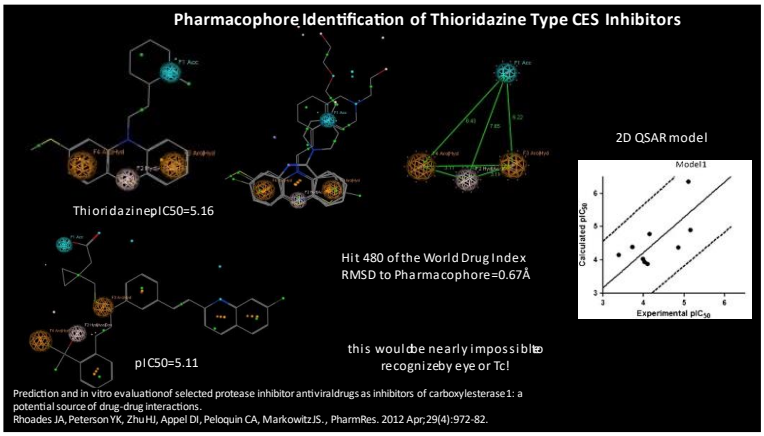
Yuri K. Peterson, PhD, Associate Professor, Medical University of South Carolina

Mitochondrial Toxicophore Generation: LOPAC OCR Screen with Chembridge Validation





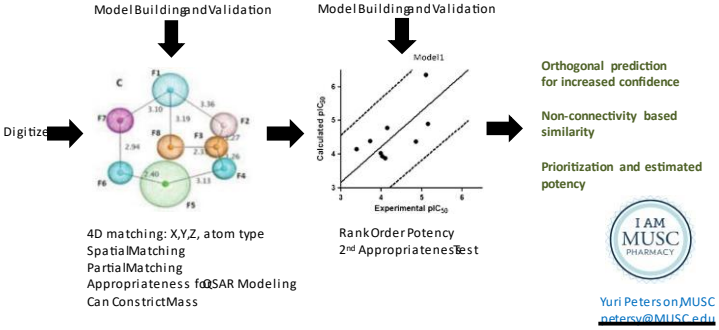
High-throughput respirometric assay identifies predictive toxicophore of mitochondrial injury.
Lauren P Wills, Gyda C Beeson, Richard E Trager, Christopher C Lindsey, Craig C Beeson, Yuri K Peterson, Rick G Schnellmann
Toxicol Appl Pharmacol. 2013 Oct 15;272(2):490-502. doi: 10.1016/j.taap.2013.06.014.



Prediction and in vitro evaluation of selected protease inhibitor antiviral drugs as inhibitors of carboxylesterase 1: a potential source of drug-drug interactions.
Rhoades JA, Peterson YK, Zhu HJ, Appel DI, Peloquin CA, Markowitz JS., PharmRes. 2012 Apr;29(4):972-82.

Combining 2D/3D Pharmacophore Modeling with Linear QSAR

MUSC Medical University of South Carolina



Yuri Peterson, MUSC
petersy@MUSC.edu

9. ONTOX – QIVIVE Framework

Alicia Paini, PhD, Principal Scientist, Lead Systems Toxicology, esqLABS GmbH

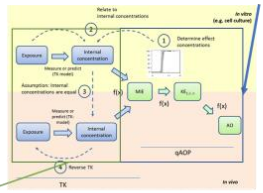
In silico tools for interpretation of in vitro effect data extrapolated to in vivo dose. EC - ONTOX – QIVIVE Framework

Maybe next round of pitch qAOP modeling

No single alternative method is able to provide a one-to-one replacement of in vivo assays for a specific toxicological endpoint, information from a combination of techniques is required!

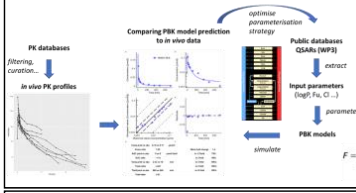
Underlying the exposure, the interactions between chemicals and biological systems, and the sequence of events that can lead to apical effects, will help to move forward the science of reducing and replacing animal experiments!

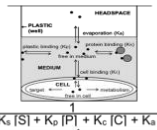
(Madden et al., 2020 DOI: 10.1177/0261192920965977)



Today´s ppt

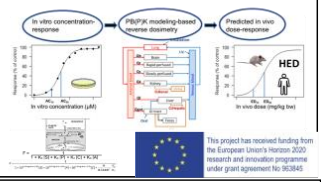
Models: QSAR + PB(P)K models + In vitro distribution models → reverse approach /QIVIVE Framework





$$F = \frac{1}{1 + K_s [S] + K_p [P] + K_c [C] + K_a [A]}$$

$$F = \frac{1}{1 + 10^{0.97 \log K_{ow} - 0.39} [S] + 10^{0.97 \log K_{ow} - 6.94} [P] + 10^{1.25 \log K_{ow} - 3.78} [C] + \frac{H}{8.3144} \frac{V_s}{V_w}}$$



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 963845

Gaps to be Navigated

- *What gaps need to be navigated to yield replacing the animal model in the next 3-5 years? I.e., What else needs to be done? Please be specific.*

The challenge is the env. chemicals (not drugs) to be assessed as they are:

- *High in amount*
- *different in chemical space with different kinetic properties*
- *mainly data-poor chemicals*

Dataset	No. Chemicals	No. chemicals with PBK model	% chemicals with PBK model
PBK	*1,187*	*1,187*	*100%*
Botanical	947	24	2.67
Pesticide	945	40	4.23
Cosmetic	5,554	123	2.41
Food	2,680	88	3.37
Drugs	6,712	382	5.80
REACH	75,633	620	0.85

Trust
- *Validation*
- *Guidance*
- *Education*

OECD Guidance on the characterisation and reporting of PB(P)K models used in the regulatory assessment of chemicals. Emphasis is placed on evolving applications in which the assessment relies on the use of in vitro and in silico (non-animal) approaches for toxicity testing, rather than in vivo data derived from animal studies.

Impact of Proposed Method

- How does your proposed in silico alternative method (or methods) replace an animal study model?
- How will the proposed in silico method -animal model pair reduce the use of animal models?

➢ *Reduces in vivo testing by filling data gaps refinement of UF and informing on chemical distribution in the body without additional in vivo testing.*

➢ *Refines in vitro concentrations from nominal to free/intracellular.*

➢ *Extrapolates from in vitro human to in vivo human relevant dose.*

➢ *Ultimately Replacing animal testing.*

Acknowledgements
René Geci & Alicia Paini & Huan Yang (esqLABS GmbH), Susana Proenca & Nynke Kramer (Wageningen University)
With **support** of Trine Husoy & Maria Kalyva (FHI), Domenico (Mirko) Gadaleta & Erika Colombo (IMN)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 963845

10.In Silico Injection Modelling

Joel Gresham, Applied Sciences & Simulation Lead, Crux Product Design

Animal Model Pair

Mechanical, Physics-Based → Drug Delivery

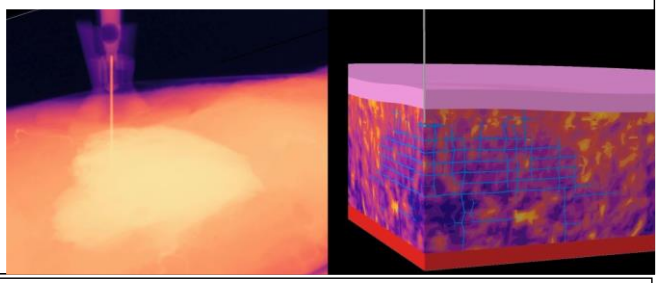
(Bio-)Chemical, Kinetics-Based → Drug PK

Industry Context – In Silico Injection Modelling:

Parenteral **delivery** of novel therapies (biologics & suspensions) is hindered by lack of knowledge on patient tolerability of large volume, high viscosity formulations which need to be delivered at fast injection rates.

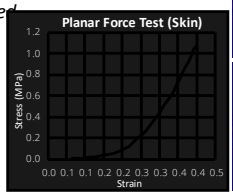
Physics-based models of 'mechanical' phenomena can improve delivery (and therefore therapeutic) performance, with potential to reduce, replace and refine animal testing by predicting e.g.:

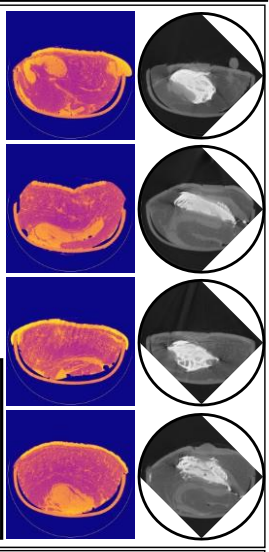
- Dispersion through various animal/human tissues
- Drug-device-tissue interactions
- Tissue damage & pain risks
- Needle blockage & leakage
- Likelihood of subcutaneous, intradermal or intramuscular delivery



Gaps to be Navigated

- *Physics-based models require accurate input data e.g.*
  - *Mechanical properties of tissues (stiffness, strength, permeability)*
  - *Animal and patient-specific anatomy (CT Scans or other imaging)*
    - *Including spatial characterization of vasculature for absorption routes to central compartment (link to PK)*
    - *Significant sample sizes to understand effects of biological variability*
- *Systems for banking and re-using knowledge*
  - *In Silico tools can be used across many projects*
  - *Design rules can be established and shared*
  - *Cross-department collaboration enabled*
    - *Device & formulation teams*





Impact of Proposed Method