This presentation reflects the views of the author and should not be construed to represent FDA’s views or policies.
On December 29, 2022, the Food and Drug Omnibus Reform Act of 2022 (“FDORA”) was signed into law

- **SEC. 3209. ANIMAL TESTING ALTERNATIVES.**
  
  (a) IN GENERAL.—Section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355) is amended
    
    (1) in subsection (i)—
      
      (A) in paragraph (1)(A), by striking “preclinical tests (including tests on animals)” and inserting “nonclinical tests”; and
      
      (B) in paragraph (2)(B), by striking “animal” and inserting “nonclinical tests”; and
    
    (2) by inserting after subsection (y) the following:
      
      “(z) NONCLINICAL TEST DEFINED.—For purposes of this section, the term ‘nonclinical test’ means a test conducted in vitro, in silico, or in chemico, or a nonhuman in vivo test, that occurs before or during the clinical trial phase of the investigation of the safety and effectiveness of a drug. Such test may include the following:
        
        “(1) Cell-based assays.
        
        “(2) Organ chips and microphysiological systems.
        
        “(3) Computer modeling.
        
        “(4) Other nonhuman or human biology-based test methods, such as bioprinting.
        
        “(5) Animal tests.”
FDA’s legal and regulatory framework for investigational drugs

• According to 21 CFR Part 312.23, an IND submission must include:

  • (8) **Pharmacology and toxicology information.** Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or in vitro, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations.

  • (ii) **Toxicology. (a)** An integrated summary of the toxicological effects of the drug in animals and in vitro. Depending on the nature of the drug and the phase of the investigation, the description is to include the results of acute, subacute, and chronic toxicity tests; tests of the drug's effects on reproduction and the developing fetus; any special toxicity test related to the drug's particular mode of administration or conditions of use (e.g., inhalation, dermal, or ocular toxicology); and any in vitro studies intended to evaluate drug toxicity.
What does FDA’s legal and regulatory framework say?

• FDA’s legal and regulatory framework *does not dictate* the type or design of studies used (whether animal, in vitro, or other tests) to support that a drug considered for clinical investigation is reasonably likely to be safe in humans.

• The areas where non-animal methods, would not be acceptable to support the safety of a drug are *not imposed by statute or regulations*, but instead by the availability and reliability of scientifically valid non-animal methods in existence able to produce the data needed.

• The provisions did, and still do, leave it up to FDA’s discretion what methods and tests are most appropriate under the circumstances.
Current safety testing paradigm (CDER)

Some Key Issues Addressed by Pharmacology and Toxicology Studies Supporting Pharmaceutical Development

- Pharmacological effects and mechanism(s) of action
- Attributes of drug ADME
- Safe “first in human” starting dose
- Safe maximum exploratory doses in early clinical trials
- Possible consequences of chronic exposure
- Risks for special populations (e.g., pediatrics)
- Specific parameters to monitor more closely in clinical trials
- Risks that are difficult or unethical to assess in humans
- Mechanistic understanding of an adverse biological change observed in animals or humans
## Extent of animal usage in drug development

### Table 8.1: Overview of the process of discovery and development of medicines

<table>
<thead>
<tr>
<th>Objective</th>
<th>Stage no.</th>
<th>Description</th>
<th>Average number of compounds entering each stage</th>
<th>Average use of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery and selection of potential new medicines</td>
<td>1</td>
<td>Target identification</td>
<td>–</td>
<td>5–15%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Identification of possible medicines</td>
<td>1,000,000</td>
<td></td>
</tr>
<tr>
<td>The characterisation of promising candidate medicines</td>
<td>3</td>
<td>Lead identification</td>
<td>1,000</td>
<td>60–80%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Lead optimisation</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Ensuring the safety of selected candidates</td>
<td>5</td>
<td>Selecting candidate medicines</td>
<td>17</td>
<td>10–20%</td>
</tr>
<tr>
<td>Clinical studies on humans</td>
<td>6</td>
<td>Concept testing</td>
<td>12</td>
<td>Generally none</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Development for launch</td>
<td>9</td>
<td>Generally none</td>
</tr>
</tbody>
</table>

Nuffield Council on Bioethics 2005: The ethics of research involving animals
Typical animal general toxicology study endpoints

- **In life observations:**
  - 1. Clinical observations
  - 2. Body weight measurements
  - 3. Food consumption
  - 4. Ophthalmology
  - 5. Electrocardiography
  - 6. Respiratory rate
  - 7. Echocardiography
  - 8. Cardiovascular analysis (heart rate, blood pressure, body temperature, activity data)
  - 9. Clinical laboratory measurements (hematology, coagulation, clinical chemistry, urinalysis)
  - 10. Toxicokinetics
  - 11. Antidrug antibody measurements

- **Post-mortem observations:**
  - 1. Collection of about 50-60 unique tissues
  - 2. Organ weight measurements
  - 3. Macroscopic tissue evaluation
  - 4. Microscopic tissue evaluation of 50-60 tissues

- Studies typically include 4 males and 4 females treated with 3 doses and one vehicle control.
New Approach Methodologies

In vitro models

In silico models

Alternative organisms

3 Rs
FDA data needs during initial drug development

Lead Compound Selected

Discovery

IND supporting studies

Starting Clinical Dose Selected

First in Human

IND submitted

Formal FDA involvement begins with review of IND supporting studies once IND is submitted

Capture the potential of the human genome industrialisation of drug discovery

- Huge array of molecular targets
- Vast, diverse chemical libraries
- Rapid identification of leads
- Extensive survey of biological function

Genetics
Genomics
Proteomics

High-throughput chemistry
Ultra-high-throughput screening
High-throughput biology

Novel technologies, including through partnerships

Figure 8.1: The industrialisation of drug discovery to capture the potential of knowledge of the human genome

Source: GlaxoSmithKline
Challenges for CIVM to overcome

• **Fundamental challenges to take into account:**
  1. The diversity of different tissues and cell types that make up a living organism; hundreds of different cell types at various stages of development may function and respond in different ways, or to different degrees.
  2. The ways in which cells and tissues interact, both locally and via the bloodstream and nervous system; immune reactions, germ cell development, metabolism and many other normal and disease-related processes involve extensive interaction between cells of different types and in various locations in the body.
  3. The influence of tissue organization on the cellular environment; oxygen levels, rate of nutrient supply, intercellular communication and barrier formation all affect how cells behave and respond to external stimuli.

• **Methodological and practical challenges to take into account:**
  1. Availability of reproducible reagents (cells, media, etc...)
  2. Lack of confidence in reproducibility and robustness of the CIVM
  3. Lack of standards for MPS device components as well as performance parameters.
Context of Use

A statement that fully and clearly describes the way the drug development tool will be used and the drug development-related purpose of the use.

What scientific question needs to be answered and for what purpose?

• How much “validation/qualification” is needed for a particular method will depend on the particular context of use.

• COU, applicability domain and limitations of method are interdependent and can be expanded over time
Qualification and Validation

- **Qualification** is a conclusion that within the stated context of use, the DDT can be relied upon to have a specific interpretation and application in drug development and regulatory review. Once qualified, DDTs will be publicly available to be used in any drug development program for the qualified context of use without needing FDA to reconsider and reconfirm its suitability.

- **Validation** is the process by which the reliability and relevance of a test method are evaluated for the purpose of supporting a specific use.

- **Principles of validation** (adapted from OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment):

1. Rationale for assay – need and purpose
2. Relationship of endpoint to in vivo effect of interest, also limitations
3. Detailed protocol
4. Intra-test variability, repeatability and reproducibility of the test method within and amongst laboratories
5. Test method’s performance must have been demonstrated using a series of reference chemicals
6. Performance of a test method should be evaluated in relation to existing relevant toxicity data
7. Data supporting the validity must be available for review
8. Data should be obtained in accordance with GLPs
More on qualification and validation

- Once a COU is defined for a specific NAM intended to inform regulatory decisions, the FDA must be able to evaluate the applicability, limitations, relevance, reliability, reproducibility, and performance standards of that NAM to confirm that the test or series of tests have been appropriately validated.

- In addition to the technical validation of a NAM, biological and toxicological models and assays can also be qualified.

- When a method will be to support the safety of a drug for use in clinical trials or marketing, the data submitted for qualification will need to be sufficiently robust to give high confidence in the method.

- Methods being used to explore the potential pharmacology of a drug or to screen out candidates with a potential safety liability may not need qualification by the Agency.

- The FDA is unlikely to accept a NAM for qualification with a COU that is only exploratory or screening in nature. While the FDA encourages submission of such data, these uses will be less pivotal in making regulatory safety decisions.

- The level of validation and qualification of NAMs will depend more on user needs than on those of the FDA and might be less rigorous in some circumstances.
Current mechanistic understanding of iDILI includes activation of the adaptive and innate immune response in part by danger-associated molecular pattern molecules (DAMPs) and drug/metabolite exposure, collectively leading to (1) mitochondrial dysfunction, (2) reactive metabolite accumulation and cholestasis, (3) bile salt export pump (BSEP) inhibition, and (4) lysosomal impairment.

A major risk factor identified for iDILI is the association with specific human leukocyte antigen (HLA) haplotypes.
When Immunity is involved

- The mechanisms combine two of the **worst predicted** drug-related adverse events by preclinical species: Metabolism-mediated hepatotoxicity and immunological reactions.

- The hepatic immune system involves an interplay between the innate and adaptive immunity including Kupffer cells (KCs), dendritic cells (DCs), natural killer (NK) cells, natural killer T (NKT) cells, CD4+ helper T cells and CD8+ T cells, coordinating a rapid and robust immune response.

- Early essential steps involved in this mechanism likely require the activation of antigen-presenting cells by DAMPs.

- KCs are essential to the first line of defense via recognition of pathogens and necrotic cells. KCs release diverse signaling molecules such as IL-6, tumor necrosis factor alpha (TNF), and chemokines, influencing cell viability, proliferation, and drug metabolizing enzyme activity.

- The underlying role of the innate and adaptive immune response in iDILI is unclear, but the extremely complex coordination of systems suggests that immune dysregulation influences the risk of iDILI development.
Considerations for CIVM in DILI assessment

• The drugs that have caused severe DILI in humans have not shown clear hepatotoxicity in animals

• When DILI is suspected, it is essential to gather mechanistic information to assess the risks of pursuing drug development.

• There is a need for predictive in vitro methods that can better evaluate the potential DILI and especially iDILI.
Examples of considerations to think about for CIVM that might be submitted for qualification to investigate immune mediated DILI

- What is the exact context of use: when and why would the model be used in drug safety assessment?

- What are the specific features of the model which make it equipped to accurately assess the mechanisms involving the immune system in DILI?

- Which drugs should be tested as positive and negative controls to measure model predictivity?

- What are the functional DILI endpoints to evaluate for each CIVM?

- What are the range of values for each endpoint being evaluated?
Opportunities for CIVM integration in drug development

• If any organ toxicity is seen in only one animal species but not in the other species, then the use of a relevant and justified human cell based CIVM might help to alleviate concerns about potential toxicity in humans.

• If CIVM from multiple species of animals were available, then these could help investigate which species are most representative of humans for a particular target organ toxicity. Ultimately, the goal would be to avoid doing studies in animal species which may not add value to a drug’s safety assessment. This may be useful to minimize the use of non-human primates.

• If a drug belongs to a class of drugs with a known safety problem, then CIVM may be helpful to demonstrate the lack of a known mechanism of toxicity.
  • If for example DILI is seen in humans, but the animal data failed to predict DILI, then CIVM for specific COUs could elucidate the mechanism of toxicity and identify possible ways to mitigate this toxicity in clinical trials.

• If a risk is identified in clinical studies, CIVM with specific COUs can help to understand the risk by:
  • Elucidating the mechanism of action/toxicity
  • Identifying sensitive populations
  • Providing more accurate information in package insert and to guide the monitoring of postmarket data

• If a Weight of Evidence (WoE) approach option to safety assessment is being considered (for example in the case of DART and carcinogenicity), then relevant and predictive CIVM can be useful.
Conclusion

- Prediction of Liver Toxicity
  - False Positive
  - False Negatives
  - Screening abilities

- Understanding of Risk
  - Mechanisms
  - Sensitive Population (Metabolism, DDI...)
  - Predictability/Detection

Avoid developing an Hepatotoxicant

Perform an appropriate Risk/Benefit Analysis