An Update from the Department of Defense for the Interagency Coordinating Committee on the Validation of Alternative Methods

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Tasking

- Provide an annual update on the Department's activities related to alternative methods for toxicology testing for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Public Forum

Mission

- The Animal Protection Program's mission is to ensure compliance with Department of Defense Instruction 3216.01, "Use of Animals in DoD Programs"

- This Instruction states that alternatives to animal use shall be used whenever possible to obtain the objectives of testing if such alternative methods produce scientifically valid or equivalent results

Implementation

- Participating in ICCVAM Acute Toxicity Working Group
- Designing out hazards from materials early in the process and leveraging ongoing material science and synthetic chemistry research that occurs throughout the DoD
- Tri-Services Toxicology Consortium
  - Representatives from relevant DoD organizations
  - Share knowledge and ideas, collaborate on projects, and implement best practices
- One Health Initiative
  - Facilitates communication and collaboration across disciplines where the health of humans, animals, and the environment intersect
- Alternative animal models
  - Relative replacements of one species for another – i.e. - selecting species with lower neurophysiological development, when feasible

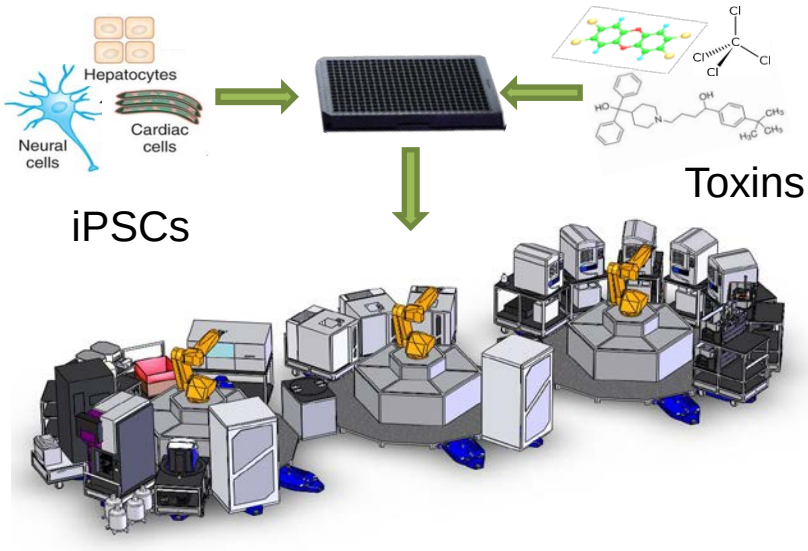
Implementation

- "Application of Modern Toxicology Approaches for Predicting Acute Toxicity for Chemical Defense"
  - A report that resulted from a DoD-funded study performed by the National Academies of Sciences, Engineering, and Medicine, Committee on Toxicology
  - Task: Consider modern approaches for predicting toxicity and suggest an overall conceptual approach for using such information to help the DoD in its efforts to prevent debilitating, acute exposures to deployed personnel
  - http://dels.nas.edu/Report/Application-Modern-Toxicology-Approaches/21775

Cellular Sentinels of Toxicity Platform

Phenotypic Assays for Assessing Toxicity
A cost effective tipping point
Combining throughput with relevance

Unlimited Throughput	Ultra High uHTS > 500,000/day	High HTS/HCS > 50,000/day	Medium MTS > 5,000/day	Low LTS >500s/Yr	Highly Limited



Tier 1: Phenotypic Screen

Neurons
- Neurite Morphology
- Mitochondrial Membrane Potential (MMP)
- ROS
- Cytotoxicity (nuclear # & morphology)

Cardiomyocytes
- Apoptosis
- MMP
- Cytotoxicity

Hepatocytes
- Apoptosis
- MMP
- Lipidosis
- CYP P450
- Cytotoxicity

Tier 2: Physiologic Screen

Neurons
- Spontaneous electrical activity
- Synchronous electrical activity

Cardiomyocytes
- Contractility (beat, amplitude, arrhythmia)
- Cellular respiration (oxygen consumption)
- NADH redox (energy production)

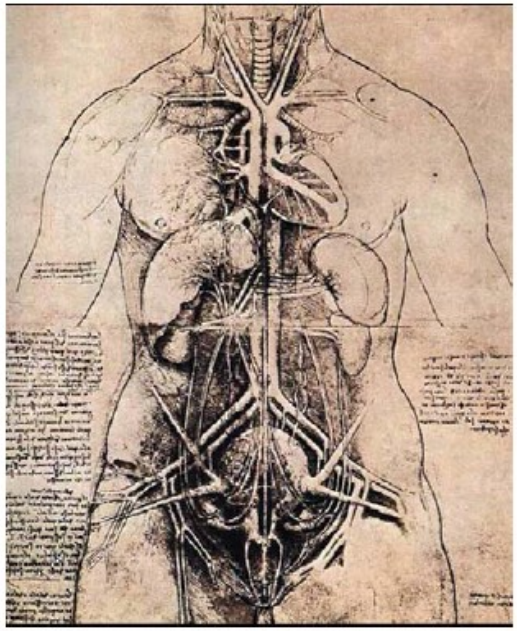
Hepatocytes
- Cellular respiration
- NADH redox

Greatly Reduced

DARPA Microphysiological Systems Program

Develop an *in vitro* platform that uses human tissues to evaluate the efficacy and toxicity of medical countermeasures.

In other words, build a human-on-a-chip:



- All ten human physiological systems will be functionally represented by human tissue constructs:
  - Circulatory
  - Endocrine
  - Gastrointestinal
  - Immune
  - Integumentary
  - Musculoskeletal
  - Nervous
  - Reproductive
  - Respiratory
  - Urinary
- Tissue viability for at least 4 weeks.
- Commercialization plan.

Metrics/Milestones

Status: 5 year program, in middle of 4th year

- MIT LL- Lung, Liver, Gut & Microbiome, Endometrium Complete, Heart, Pancreas, CNS in Future
- Harvard / Wyss Institute

Ex vivo Countermeasure Evaluation and Licensure (XCEL) Programs

XCEL: Development of Integrated-multi-organs-on-a-chip platforms to revolutionize assessment and evaluation of medical countermeasures and threat agents for chemical and biological defense and beyond

ATHENA – Los Alamos National Laboratory	ECHO – Wake Forest Institute of Regenerative Medicine
Liver and Cardiac Organoids (working on lung and kidney)	Liver and Cardiac Organoids
Modular microfluidics	Functional Assessment (Reactivity) and long-term viability
Universal Media Development	Bioprinting – augments function and controls spatial distribution
Ion Mobility – Mass Spectroscopy analysis of analytes/metabolites	Modular microfluidic system with rejuvenating in-line sensors

Rapid Threat Assessment (RTA) Program

Problem: It takes many years to figure out how drugs work

RTA Goal: In 30 days, figure out how a chemical, threat agent, drug, or biologic exerts its effects on biological systems

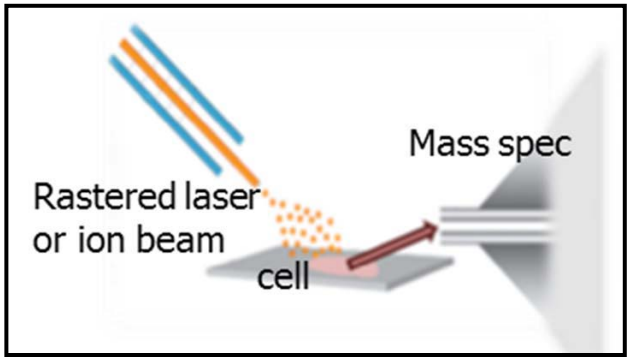
Potential Impact to ICCVAM: Significant decrease in time to understand mechanism of action, decrease in need for animal studies throughout process

Inspiration: New rapid mass spec imaging method

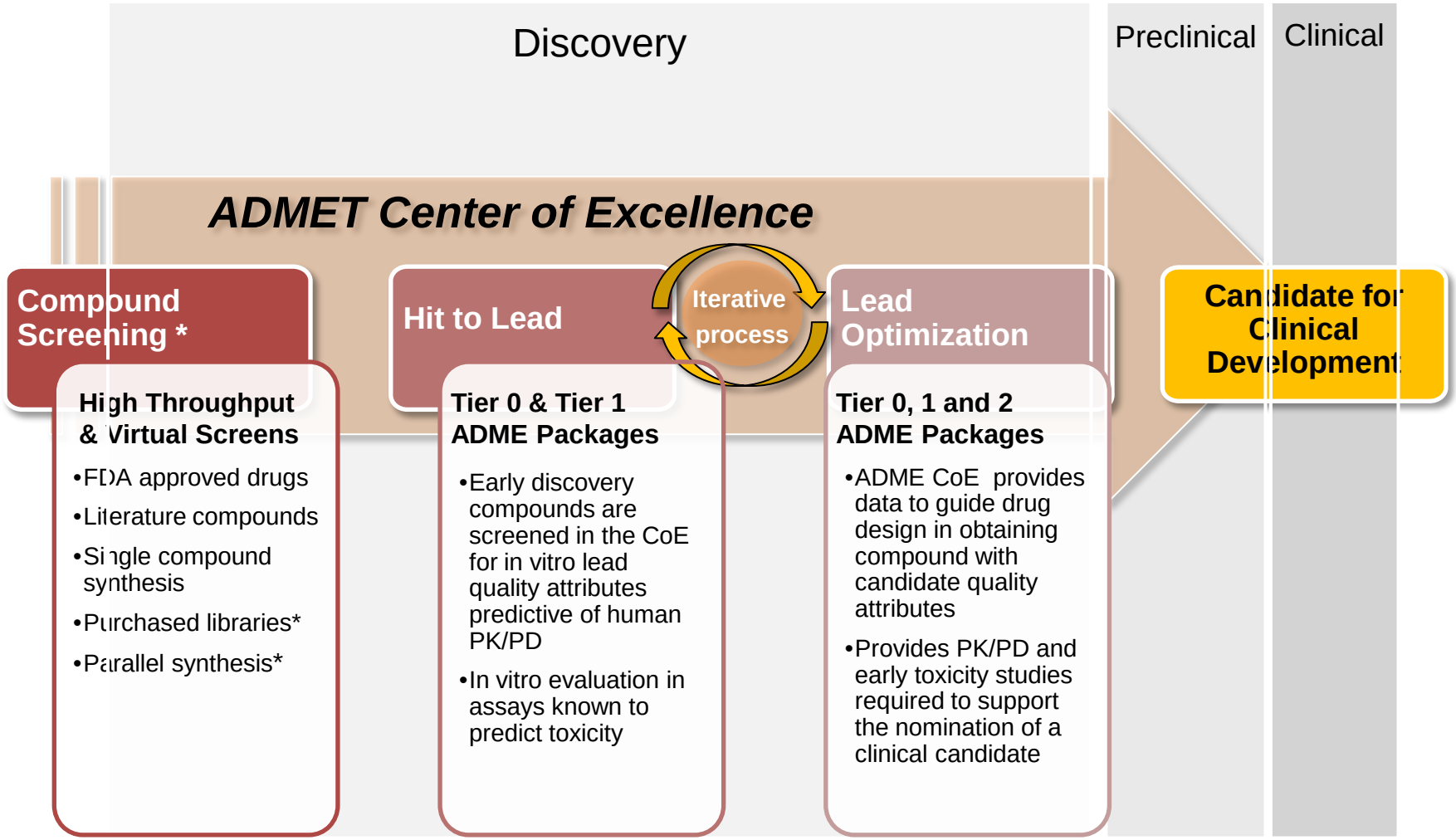
Status:

- 5 year program, currently in beginning of year 3
- 3 main performers – GWU, UC Bolder, Vanderbilt University
  - Proof of Concept Demonstration
    - Measured 100% proteins and metabolites in canonical mechanism of forskolin in 30 days

Mass Spectrometry Imaging



Absorption, Distribution, Metabolism, Elimination, and Toxicity (ADMET) Center of Excellence



* Includes High Throughput Screening, Virtual Screens, rational drug design and/or pharmacology screens

Summary

- In partnership with other Federal agencies, academia, and industry, the Department of Defense remains committed to refine, replace, and reduce reliance on animal models when scientifically valid