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Exploring new technologies in biomedical research

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The Health Law, Policy & Ethics Project at Emory University School of Law and the Human Toxicology Project Consortium of the Humane Society of the United States co-sponsored a symposium on October 23, 2017, to showcase innovations using human-based in silico and *in vitro* models for drug and device discovery. The goal of the symposium was to introduce researchers and students to exciting new tools and possible future careers that will increase understanding of disease and improve the search for effective therapeutics, while reducing reliance on animal testing. The symposium concluded with a discussion between scientists and lawyers about the legal regulation of new biomedical research technologies.

Introduction

In 2017, the Human Toxicology Project Consortium of the Humane Society of the United States sponsored a symposium to showcase innovations using human-based *in silico* and *in vitro* models for drug and device discovery [1]. New biomedical technologies such as elucidating disease pathways, human-on-a-chip technology and computer modeling can support better science, faster development of medical treatments, cost savings and reduced animal use [2]. With respect to the need for better science, the FDA acknowledges that preclinical tests from animal models seldom translate into safe and effective drugs for humans [2]. More than 90% of drugs fail in clinical trials, largely owing to lack of efficacy or toxicity in humans that was not predicted by animal tests [3]. Despite a large increase in spending over the past 20 years, the success rate of new drugs has not improved [4]. There are several reasons for this: animal physiology is different; many factors of human life that cannot be covered by animal tests affect drug efficacy, including diet, age, health status and developmental state; and animals are often exposed to higher doses of chemicals than humans would be, making it difficult to predict how humans will respond to exposure [2].

Novel approach methodologies (NAMs) can also make development and testing of medical treatments faster and less costly. In chemical toxicity testing, for example, ``the same number of chemicals that have been tested over the last 20 to 30 years [are] being tested now in a single day'' [2]. NAMs could also be more efficient and have the potential to lower the time and other costs of drug development. Under the conventional drug development paradigm, the average cost of bringing a drug to market is US$2.6 billion and can take more than a decade [4,5]. In addition, animal research subjects must be purchased, housed, fed and provided with veterinary care [2]. NAMs avoid these inefficiencies of using animal research subjects. NAMs could spare the lives of millions of rodents and other species used in medical research each year [6].

The goal of the symposium was to introduce graduate, undergraduate and advanced-science high-school students as well as researchers in the biomedical and bioengineering sciences to exciting new tools and possible future careers that will increase our understanding of disease and improve our search for effective therapeutics. The speakers discussed new biomedical technologies and their implications for the advancement of scientific research and human medicine, with a focus on pathway-based approaches, microphysiological systems (human-on-a-chip and organoid models) and *in silico* tools (computer modeling). Simply stated,

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pathway-based approaches mine and organize biomedical research data for optimal use. Microphysiological systems are created by placing a small number of cells or organoids on a chip made of inert materials, allowing scientists to simulate how these 'organs' would interact *in vivo*. Mechanical force, for example the 'breathing' of the lung-on-a-chip, can be added to mimic human physiological activity. Organoids are simpler 3D tissues grown from cells. *In silico* tools use digital models used to predict chemical toxicity and medical device compatibility and efficacy.

The conference concluded with a roundtable discussion about the legal regulation of NAM. Importantly, Congress and the FDA recognize the significance of NAM and support its development. In 2016, Congress enacted the 21st Century Cures Act, which, in part, provides the FDA US$500 million to improve the drug approval process [7]. The most recent FDA Commissioner, Scott Gottlieb, proposed accelerating drug approvals during his tenure through the Medical Innovation Access Plan and other federal initiatives [7,8]. Each of these topics is examined in turn. The list of speakers and the topics of their presentations is provided in Table 1.

Using data more effectively

We are very good at generating data – so good that we have more data than we can effectively manage, let alone comprehend. And most of the data are in written papers or reports – not easily accessed or machine readable. Moreover, the databases that do exist are in different formats, using varying software that cannot communicate [9]. The NIH invested hundreds of millions of US$ in improving data mining from literature, for examples see [10] and [11], and in improving ways for databases to communicate with one another [12]. One example of a new way of organizing data is the Adverse Outcome Pathway (AOP) project, a global effort to organize, curate and evaluate biological pathway information as it relates to chemical toxicity. An AOP provides an organized framework for gathering and evaluating biological data according to causal relationships between a molecular initiating event (e.g., a chemical binding to a cellular receptor) and adverse outcomes. The AOP-based concept (Fig. 1) evolved from the concept of mode-of-action as it relates to chemical toxicity [13].

The AOP project is coordinated by the Organization for Economic Cooperation and Development [14] and consists of software packages being developed by a variety of member countries. A main element, the AOP Wiki, is a wiki-based crowdsourcing platform to collect, organize and evaluate all of the supporting information for individual biological pathways [15]. The software (and related guidance) is highly structured, and each pathway is reviewed by subject experts to ensure the quality and completeness of information at a given point in time. This tool has been designed specifically to support regulatory decisions about chemical safety; however, it also could be applied to drug safety and efficacy [16]. This specific tool has downsides: it is labor-intensive and time-consuming, and its success depends on broad adoption. But the benefits are substantial given transparent communication of the certainty of the information that underpins crucial safety decisions.

Additionally, for biomedical research, the capacity of AOP to provide a link between early molecular or cellular events with disease outcomes, or to reveal causal relationships between different elements within a pathophysiological pathway, could provide mechanistic understanding of human disease processes. AOPs have been used to demonstrate the mechanistic basis of neurotoxicity in Parkinson's disease [17], hypertension through perturbation of endothelial nitric oxide bioavailability [18], inflammation [19] and lung fibrosis [20]. However, to broaden the use of AOP, along with events such as these to introduce the AOP concept to a new audience, it is also important to acknowledge current shortcomings and offer recommendations with which to address their limitations [21]. Although the emphasis of AOPs lies in toxicology currently, it is likely that AOPs will become more-powerful and informative for biomedical research as more AOPs are submitted to the wiki.

Human-relevant tools

Sophisticated cell cultures and organs-on-a-chip

Organs-on-chips are generated using microscale engineering techniques that, when combined with cultured, living human cells, recreate the physiological, mechanical and biochemical microenvironment of the living organs in a reductionist yet complex, highly precise and controllable manner (Fig. 2) [22]. This technology enables the study of complex human physiology and pathology in an

TABLE 1

Schedule of events, speakers, affiliations and titles of presentations

Participant	Affiliation	Presentation title
Ani B. Satz PhD, JD Professor of Law and Project Leader	Emory University School of Law; Health Law, Policy & Ethics Project	Exploring new technolgies in biomedical research introduction
Catherine Willett PhD	Human Toxicology Project Consortium, The Humane Society of the United States/Humane Society International	Introduction and overview of a biological pathway-based approach to disease and drug discovery
Kambez Benam PhD	Division of Pulmonary Sciences and Critical Care Medicine, Departments of Medicine & Bioengineering, University of Colorado	Microengineered lung-on-a-chip technologies: novel human-derived experimental tools
Andre Kleensang PhD	Johns Hopkins Center for Alternatives to Animal Testing	Improved human relevance of neurobiology: study of omics in mini-brains
Zhang Qiang MD, PhD Associate Professor	Department of Environmental Health Rollins School of Public Health Emory University	Linking *in vitro* to *in vivo* with computer modeling
Michael Salmon PhD Vice President	Platform Translation and Development, Emulate	Organs-on-chips: creating a living system to emulate human biology and disease
Patricia Zettler JD Associate Professor	Georgia State University School of Law	Panel discussion and Q&A

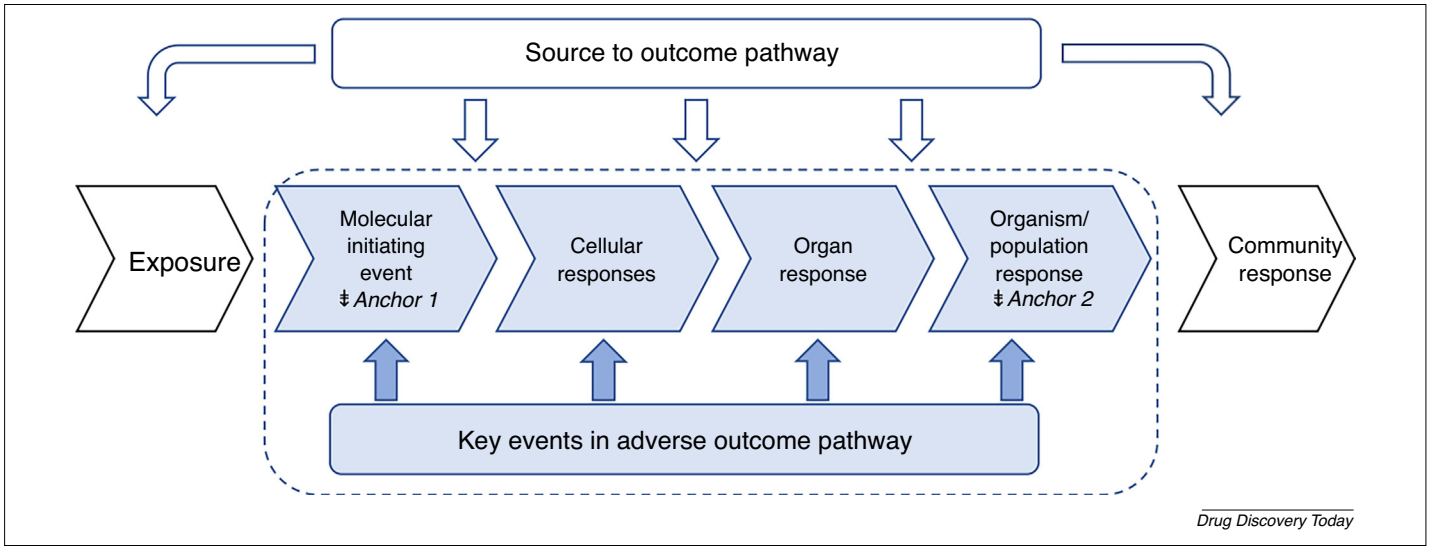

FIGURE 1

Simplified representation of key features of an adverse outcome pathway (AOP), linking two anchors [the molecular initiating event (MIE) and the adverse outcome at the level of an individual or population] together with intermediate 'key events'. Reprinted, with permission, from Ref. [40].

organ-specific context and offers a unique platform for developing specialized *in vitro* human disease models. Each organ-on-a-chip is approximately the size of an AA battery and is often composed of a transparent, flexible, biocompatible and gas-permeable material. Cells are cultured within continuously perfused microchannels that run through the chip, and the chip can be stretched or otherwise stimulated to recreate the physiological forces that cells experience in the body [23].

Organs-on-chips have been designed to reproduce the complex, dynamic state in which living cells function in a real human organ, which includes substrate (extracellular matrix), tissue–tissue interface, mechanical forces, immune cells and blood components, and biochemical surroundings [24]. One key benefit of the technology is that it allows scientists to gain

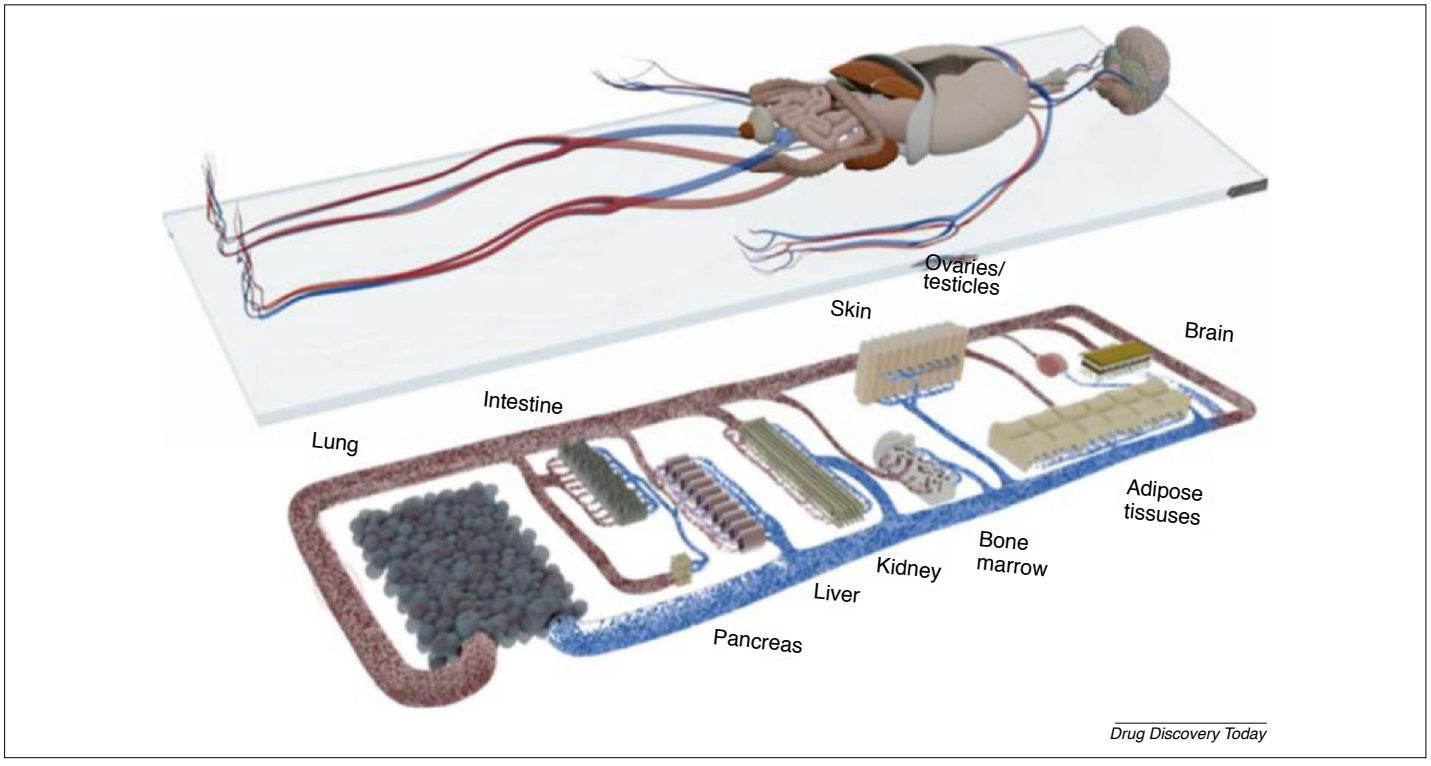

FIGURE 2

Representation of one possible iteration of the human-on-a-chip. Integration of different organs onto a single chip, linked by microfluidic flow, can be used to model drug absorption by ingestion (gut and skin models), distribution (adipose tissue model), metabolism (liver model) and excretion (kidney model). Image adapted, with permission, from https://www.tissuse.com/en/ (TissUse GmbH).

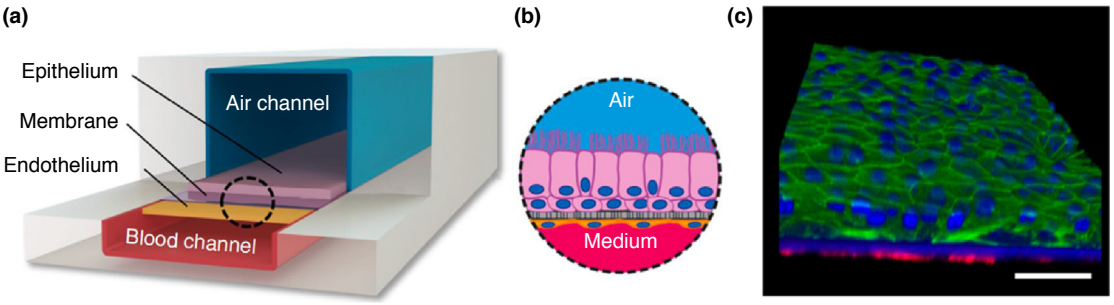

FIGURE 3

The human small airway-on-a-chip. **(a)** Schematic diagram of a cross-section showing the location of the air channel, lined with epithelial cells, and the blood channel, lined with endothelial cells. The dashed circle indicates the area depicted in **(b)**. **(c)** 3D reconstruction to illustrate the physiological nature of airways tissue – demonstrating fully differentiated, pseudostratified cells (green) overlying human pulmonary microvascular endothelial cells (red). Nuclei are stained blue; scale bar is 30 μm. Reprinted, with permission, from Ref. [28].

mechanistic insight into human biology and human response to drugs; and it has the potential to be used in many fields, including drug discovery, food and chemical research, as well as precision and regenerative medicine. However, the technology is still new and not yet fully mature – the added value of organs-on-chips compared with more-traditional 2D and 3D tissue culture models has yet to be confirmed empirically, and scientists currently believe that, although organs-on-chips definitely have the potential to bridge the translational gap that exists in preclinical testing, the technology is not yet capable of the absolute replacement of animals and should remain complementary to studies on animals for the near future [25].

Organs-on-chips for lung disease

Development of functional living human lung-related organs-on-chips has revolutionized preclinical research in respiratory medicine (Fig. 3) [26–33]. Development of new therapeutics for pulmonary disorders and advancement in our understanding of inhalational toxicopathology were hindered by challenges to the study of organ-level complexities *in vitro*. Moreover, clinical relevance of widely used animal models of respiratory diseases such as chronic obstructive pulmonary disease (COPD), which poses an enormous public health burden, is questionable [34]. Importantly, there are significant ethical concerns about the use of animals in biomedical research. In this presentation, Dr Benam demonstrated that, by applying tissue microengineering technology, his team successfully created a 'human lung small airway-on-a-chip' that supports full differentiation of a pseudostratified mucociliary airway epithelium from normal or diseased donors, precisely as seen in human lungs [28].

Using this robust *in vitro* approach, new biomarkers of COPD exacerbation (flare-up) were identified, and responses to anti-inflammatory compounds that inhibit cytokine-induced recruitment of circulating neutrophils were measured [27]. Importantly, by connecting the small airway chip to a custom-designed electromechanical instrument that 'breathes' cigarette smoke in and out of the chip microchannels (breathing-smoking human lung-on-a-chip), the team successfully recreated smoke-induced oxidative stress, identified new ciliary micropathologies and discovered unique COPD-specific molecular signatures. Thus, the small airway-on-a-chip and breathing-smoking lung-on-a-chip technologies offer potentially powerful alternatives to animal models for the study of human lung pathophysiology. For more information, please visit Dr Benam's lab webpage (https://benamlab.net/).

Organoids for brain research

Human 'mini-brains' replicate biology closely enough to study normal brain functions and diseases in a petri dish [35]. Rat cells were used to illustrate that 2D and 3D tissues grown in culture mimic certain features of normal and developing brains. 'Organotypic cultures' are by definition multicellular structures representing at least some of the characteristics and functions of the tissue they model. The rat primary aggregating brain cell organotypic cultures are spheroids of about 200 μm in diameter and consist of all cell types of the developing brain – namely, many subtypes of neurons, astrocytes, oligodendrocytes and microglia [36]. It shows synapse formation and axon myelination and is therefore suitable to model neurodevelopmental processes and to study xenobiotic perturbations. This model has been used to study changes in gene expression as a result of exposure to the flame-retardant isopropylated phenyl phosphate (IPP). Careful evaluation of the significant gene groups revealed four main mechanisms: (i) downregulation of neurotransmitter receptors and associated intracellular signal transduction; (ii) upregulation of macrophages MHC-I, FC IGg-R and AIF; (iii) upregulation of cell cycle; and (iv) changed fatty acid metabolism and transportation.

Although the rat cell-derived model is relatively easy to use in the lab, its value is limited because of rat–human interspecies differences. A human iPSC-derived 3D neural spheroid model was also created that displays some cell types of the developing brain – many subtypes of neurons, astrocytes and oligodendrocytes but no microglia [37]. This model also shows spontaneous electrical activity and myelination of axons by oligodendrocytes. Advantages of this model are that it is human-cell-based; but challenges include that it is not primary-cell-derived and not all cell types are present, it is difficult and takes significant time to produce, it is cost intensive and special user-training is needed. Further advantages and current limitations of brain organoid models are summarised in Table 2.

Computational modeling tools for *in vivo* extrapolation

Although human cell and organoid assays for toxicity testing are improved to better emulate *in vivo* milieus, they remain as isolated 'parts' being interrogated outside living biological systems. As a result, there is a disconnect between *in vitro* observations and the actual effects *in vivo* for human health outcomes. Bridging the gap requires the application of multiple computational approaches to toxicity testing utilizing *in vitro* assay data (Fig. 4) [38]. Two major computational modeling techniques are emerging. The first one is concerned with extrapolating the point-of-departure (POD) concentrations of the tested chemicals obtained *in vitro* to the external dose. Physiologically based pharmacokinetic (PBPK) modeling,

TABLE 2

Advantages and current limitations of brain organoid models [41]

Advantages	Current limitations
Gene-editing for precise disease models can be tailored for individual patients	Immaturity of cell types – models are often better suited to study neurodevelopment
Personalized models for drug efficacy and testing drug combinations	Heterogeneity of models – different cultures have differently sized brain regions – standardization will be required before models can be validated for reproducible use
Adaptation to high-throughput assays for early drug screening	Lifespan – lack of a vasculature to deliver oxygen and nutrients reduces culture life
Human cell-based systems (species relevance)	Partiality – organoids do not represent the entire brain, difficult to stimulate complex neurological disorders

which has been traditionally used to predict tissue dosimetry given an exposure condition, is used. In this *in-vitro–in-vivo* extrapolation application, PBPK models will be used in a reverse fashion, where, based on the exposure scenario, a range of exposure doses will be explored to predict the dose level that leads to a predefined tissue concentration. In this way, exposure conditions and doses resulting in POD perturbations, at least *in vitro*, can be predicted [39]. The second computational technique required to complement *in vitro* testing is quantitative AOP modeling, which can convert *in vitro* data into *in vivo* effects. This approach is particularly necessary for evaluating endocrine disruptors, where endocrine organs and tissues function collectively as a homeostatic system. This normally involves feedback regulations between the hypothalamus, pituitary and the target endocrine organs such that, within a limit, perturbation of the hormone levels will be compensated. As a result, *in vitro* data obtained with isolated endocrine cells or tissues need to be interpreted for their *in vivo* impact. Computational modeling of the endocrine feedback axes has a number of benefits. It will allow us to predict: nonlinear *in vivo* health effects resulting from feedback regulations by mechanistically linking toxicological and epidemiological data; *in vivo* mixer effects by mechanistically integrating individual molecular initiating events (MIEs) along toxicity pathways; and population effects in adverse outcomes by incorporating interindividual variabilities into toxicity pathways. Finally, linking PBPK models for tissue dosimetry and these quantitative AOP models will provide a more complete computational tool for risk assessment of a specific chemical exposure [38].

Panel discussion: regulatory implications of emerging biomedical research technologies

The panelists and discussants generally agreed that current FDA regulation does not appear to limit the potential application of the discussed emerging technologies to biomedical research. The speakers raised several issues that warrant further exami-

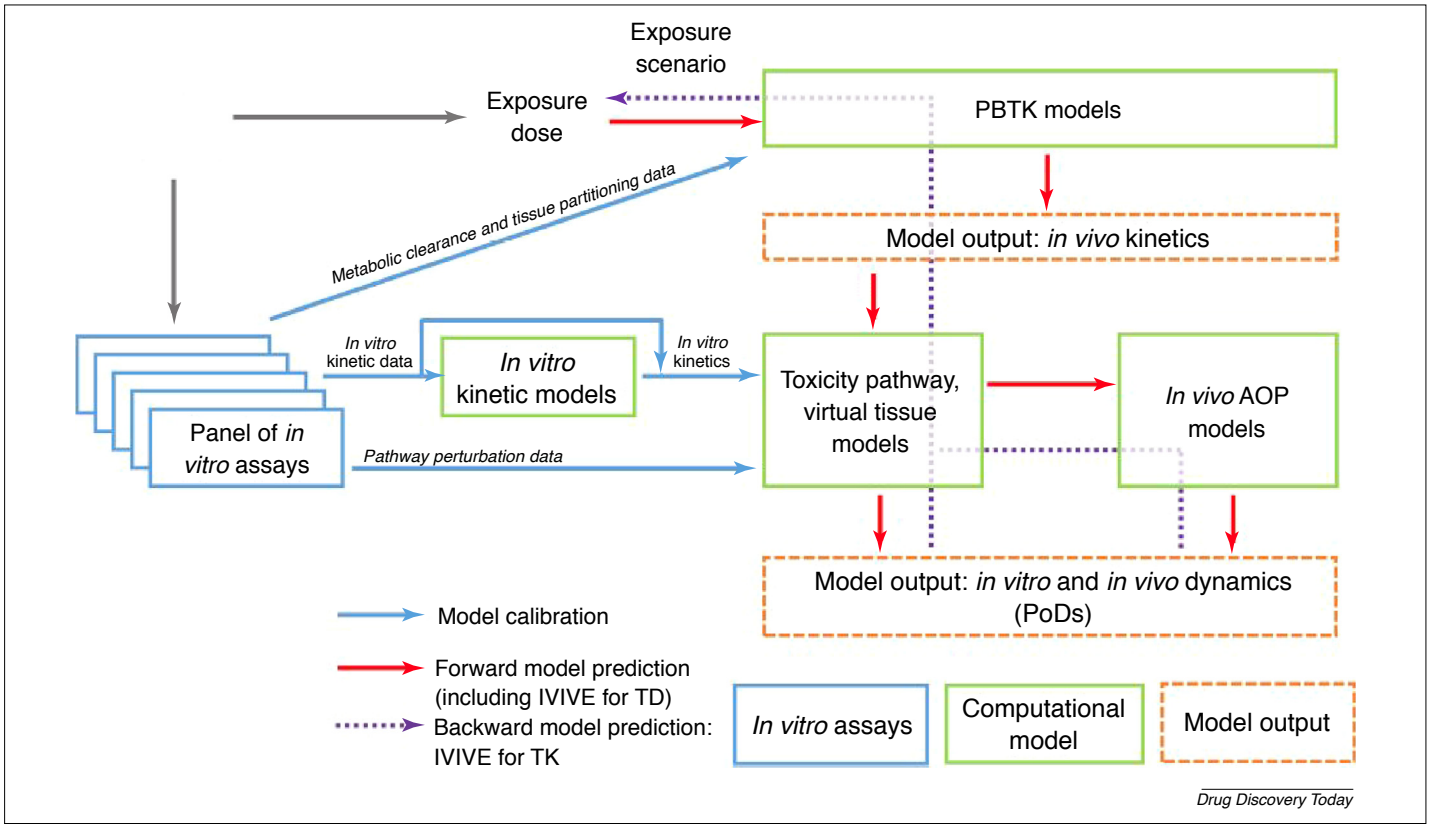

FIGURE 4

Schematic illustration of the possible roles for computational modeling within a workflow using *in vitro* assays and kinetic/toxicity pathway/AOP models to bridge the data gap between *in vitro* in *vivo* kinetics and PoD dose metrics. Reprinted, with permission, from Ref. [42] under CC BY.

nation elsewhere, including the timeline for implementing these new technologies for drug development, standardization across human organ chips (i.e., how many chips are required to validate results) and whether the cells need to be from more than one human cell line.

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